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Comparative study of application methods of biofertilizer for growth enhancement of cereals

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Abstract

The nutrient management plays a vital role in consistent agricultural production and maintenance of soil fertility. Due to price hikes, chemical fertilizers are going beyond the affordability of poor farming communities. Hence, agricultural scientists and farming communities are switching to other judicious and environment friendly substitutes. Use of biofertilizers is becoming popular day by day. Nevertheless, its popularity has to face some challenges like application methods. Farmers in Punjab have the practice to apply the biofertilizers on the growing crops either through spray or flooding or with irrigation water. This glass-house trial was designed to evaluate a more effective and efficient way for the application of biofertilizer to the cereals. Wheat and rice cereals were used as test crops. Three biofertilizer application methods viz. seed coating, flooding and foliar spray were tested employing eight treatments and following Completely Randomized Design (CRD) with three repeats. At maturity, yield data regarding wheat and rice crops were recorded. Statistical analysis of the data revealed that better yield was obtained in all treatments supplied with biofertilizer inoculation along with recommended dose of chemical fertilizers compared to the control with no inoculum application but only chemical fertilizer application. Moreover, integration of individual inoculum application techniques proved better than their individual application. © 2022 Department of Agricultural Sciences, AIOU

Keywords: Biofertilizer, Flooding, Foliar spray, Rice, Seed coating, Wheat

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Introduction

Developing countries like Pakistan are facing multifarious challenges and constraints in the way of increased crop yield. Maintenance and increase in soil fertility or to check infertility of lands is one of the major crushes among poor nations of the developing countries since better crop production depends on balanced supply of plant nutrients (Chen, 2006; Kumar et al., 2017; Jain et al., 2021b). This single factor may revolutionize the economic and cultural life of the farming community in third world countries. A quality soil is indispensable to sustain a quality agricultural system in any part of the world. A good quality soil is a good blend of organo-mineral components, which along with microorganisms collectively provides a favorable environment for biological, chemical and physical activities taking place in the rhizosphere environment (Preininger et al., 2018; Liu et al., 2022).

Extensive and unabated use of chemical agricultural inputs like fertilizers, herbicides and pesticides, has put at stake the health of land, farm workers and end users from human beings to different animals. This practice has multipronged ill-effects starting from the field to the human kitchen. Unluckily their harmful effects not only equaled their benefits but, in some instances, surpassed their beneficial effects. Resultantly, their noxious properties endangered the environment, plant, animal and human life. In this perspective agricultural researchers and the farming community have been seeking some more viable and persistent substitute since last century. Biofertilizers may partially be proven better alternatives being least hazardous for human and animal life and more environmentally friendly (Pirttilä et al., 2021). Their handling, storage and application could be laborious tasks for farmers, nevertheless, their use as natural composting of farm wastes and animal refuse were known to the farming community for centuries (AbdulHalim, 2009). Presently, such materials are being applied as seed inoculum or in either way to augment the microbial population required for the mineralization of nutrients in ready form for plants' uptake (Mohammadi et al., 2011; Mohammadi & Sohrabi, 2012; Jain et al., 2020; Mellidou & Karamanoli, 2022).

Soil microbes were explored in 18th century and since then these are part of agricultural farming in one or other way (Mahmood et al., 2016; Jain et al., 2021a). Term PGPR was first time surfaced by Kloepper & Scroth (1978). Similarly, Kloepper et al. (1989) also fabricated the term 'rhizobacteria'. Biofertilizers are the products containing cells of different types of beneficial microorganisms. Thus, biofertilizers can be significant components of integrated nutrients management. Organisms that are commonly used as biofertilizers. Biofertilizers are important components of integrated nutrients management. These potential biological fertilizers would play a key role in productivity and sustainability of soil and also protect the environment as ecofriendly and cost-effective inputs for the farmers. They are a cost effective, eco-friendly and renewable source of plant nutrients to supplement chemical fertilizers in a sustainable agricultural system (Etesami et al., 2017; Begum et al., 2019; Vocciante et al., 2022).

Biofertilizers are products containing living cells are nitrogen fixers (N-fixer), solubilizers (K-solubilizers) and phosphorus solubilizers (P-solubilizers), or with the combination of molds or fungi. With biological and organic fertilizers, a low input system can be carried out and it can help achieve sustainability of farms. After decades of research, a number of research workers inferred that beneficial microorganism streamline the nutrient supply to plants. Major functions of these beneficial microbes are supply of nutrients to crops, stimulation of plant growth producing phytohormones, biocontrol of phytopathogens, improving soil structure, bioaccumulation of inorganic compounds and bioremediation of metal contaminated soils (Subashini et al., 2007; Pattanayak et al., 2007; Abd-Alla et al., 2013; Fadiji et al., 2022). Nevertheless, a proper and efficient method of application of the biofertilizer decides the benefits of the biofertilizer (Habibzadeh et al., 2012; Aggani, 2013; Etesami et al., 2018; Abd El-Mageed et al., 2022).

Inconsistent response of biofertilizers is the main hindrance in the prevalence and number of reasons are reported in literature (Mercado-Blanco & Lugtenberg, 2014; Podolich et al., 2015; Fasusi et al., 2021; Qureshi et al., 2022). The inconsistency in results might be due to method of application, virulence of applied inoculants, enable to survive in the climatic conditions, competition of inoculating microbes to soil native population (Kumar et al., 2017). Several methods of biofertilizer application are reported in literature viz. seed treatment (seed coating), foliar spray, soil application either by dry or as flooding, dipping the seedlings in biofertilizer suspension (Mahmud et al., 2021). The methods/techniques used for biofertilizer application are based on crops, time of application, limitations of farmers, accessibility of farmers to biofertilizers, technical basis of end users and environmental conditions etc. (Mahmood et al., 2016). Some microbes used as biocontrol agents are applied at different growth stages of crops (Mohammadi & Sohrabi, 2012; Aasfar et al., 2021; Vocciante et al., 2022).

The application of microbial inoculants as seed inoculants is cost effective as compared to foliar spray that involves effort and sprayer. The method of application should be followed on the basis of natural microbiome in the rhizosphere and also the method ensures effective root colonization. The interaction with the native population and environmental impact should be considered while adopting the methods. The type of microbe used inoculation should be kept in mind while using method like, rhizosphere/endophytic bacteria need effective root colonization and within cells or internal plant tissues and mycorrhizal fungi usually colonized the root surface or rhizoplane, and germinating seeds etc. (Smith & Read, 2008; Pagnani et al., 2020). The performance of bacterial inoculation is inconsistent due to multiple factors viz. strain compatibility, culture content, method/time of application, plant species and soil & environmental conditions (Kaminsky et al., 2019; Mahmud et al., 2021). The biofertilizer technology has certain constraints that restrict its prevalence are carrier materials, technical hands, lack of awareness, time/method of application, storage conditions (Podolich et al., 2015; Mahmud et al., 2021). The storage problem can be handled by application of thermophilic and drought resistance strains (Brar et al., 2012; Gou et al., 2020). The inconsistent nature might be due to the lack of crop/region specific microbial inoculants (Alori et al., 2017; Ajmal et al., 2018; Odoh et al., 2020). Hence this study was designed for checking the efficiency of different methods of biofertilizer's application since different methods have various efficacy ratios.

Materials and Methods

Characterization of isolates

Pre-isolated strain of PGPR was used for the preparation of inoculum after characterization on the basis of different biochemical tests and IAA production activity (Sarwar et al., 1992). The procedure of Sarwar et al. (1992) was followed to analyze the production of bacterial auxin. Purposely, 25 mL of General-Purpose Media (GPM) media was autoclaved, then cooled and injected with bacterial isolates at the rate of 1.0 mL. The solution was incubated for 48 hours at temperature 28 ± 1ºC, and afterwards filtration was done through Whatman No. 2. Accordingly, 3 mL filtrate was mixed with 2 mL of Salkowski's reagent (98 mL $HClO_4$, 35% +2.0 mL $FeCl_3$, 0.5 M). The reading of samples was taken in a spectrophotometer at wavelength 535 nm. Control (un-inoculated control with GPM broth) was also prepared and run simultaneously for comparison.

Experimental design

Eight treatments with three repeats were devised for the pot trial following Completely Randomized Design (CRD). The treatments employed were T_1: Control (without inoculum), T_2: Seed coating, T_3: Flooding of inoculum, T_4: Foliar spray of inoculum, T_5: Seed coating + Flooding, T_6: Seed coating + Foliar spray, T_7: Flooding + Foliar spray and T_8: Seed coating + Flooding + Foliar spray.

Pot experiment

Pot experiment was conducted at Soil Bacteriology Section, Agri. Biotech. Research Institute, Faisalabad to devise a more convenient and adaptable method for farmers to apply biofertilizer to cereal seeds and seedlings. Sand clay loam soil was used for pot filling having pH 7.8, EC_e 2.4 dS m^{-1}, OM 0.67% and available P 8.1 mg kg^{-1}. Efficient isolates of Plant Growth Promoting Rhizobacteria (PGPR) were used for the preparation of inoculum. Seeds of approved varieties of rice (Shaheen Basmati) and wheat (Faisalabad-2008) were used for the trial. Ten saplings of rice and ten seeds of wheat were sown per pot. After getting a foothold, thinning of the pots was done and only four plants of rice and wheat were allowed to continue till maturity. Uniform fertilizer dose of NPK (110-90-70 kg ha^{-1} for rice and 150-100-60 kg ha^{-1} for wheat) was applied at sowing or transplanting. The PGPR were used in liquid broth media for application on wheat seed and rice nursery for one hour. Wheat and rice seeds were surface sterilized with sodium hypochlorite for 2 min and rinsed thoroughly in sterile distilled water. The surface sterilized seeds were then treated according to the treatment plan. Uniform cultural practices were carried out. At the tillering stage of both crops, the rhizobacterial microbial count was carried out to check the root colonization by the application methods of biofertilizers by standard dilution plate technique. The crops were harvested and threshed and data regarding biomass, grain yield, tillers, plant height and grains N & P content were recorded. The N & P were evaluated (Bremner & Mulvany, 1982; Olsen & Sommers, 1982).

Method of biofertilizer application

Three different biofertilizer application methods were used in this experiment. For seed treatment 20 g of biofertilizer is suspended in 30-40 mL of water with 100 g of seeds (per treatment) using sugar solution as an adhesive material. The seeds were then spread on a sheet under shade and let these dry and then used for sowing. Rice seedlings were dipped in biofertilizer slurry for 1.0 hour and then transplanted. For foliar spray of biofertilizer the same amount was mixed with 100 ml of water and sprayed on each treatment. A mixture of biofertilizer was applied to each pot with irrigation water as a third application method i.e., flooding. When more than one method is employed then the quantity of culture was applied in 1:1 or 1:1:1 according to the treatment plan.

Statistical analysis

The analysis of data was done by using analysis of variance technique (ANOVA) with CRD. For this purpose, Statistix v. 8.1 was used and arithmetic means were compared by using least significant difference (LSD) test (Steel et al., 1997).

Results

To explore a more feasible and effective method for biofertilizer inputs application on cereal crops regarding farmer's convenience, a pot trial was conducted at Soil Bacteriology Section, Agri. Biotech. Research Institute, Faisalabad. A sandy clay loam soil was used for pot filling. The pH of the soil used for the trial was 7.8 and electric conductivity was 2.4 dS m^{-1}. Available phosphorus in the soil was 8.1 mg kg^{-1} and estimated organic matter was 0.67%. Approved varieties of wheat and rice i.e., Faisalabad-2008 and Shaheen Basmati, respectively, were used as test crops. The inoculum/biofertilizer used for the trial was prepared from efficient isolates of Plant Growth Promoting Rhizobacteria (PGPR) prepared by Soil Bacteriology Section Agri. Biotech. Research Institute, Faisalabad.

Screening of PGPR isolates

The PGPR isolates (five of each) marked as (PGPR-1, PGPR-2, PGPR-3, PGPR-4 & PGPR-5) were screened for biochemical tests (methyl red, oxidase test & citrate utilization tests), IAA equivalents and P-Solubilization (Table 1). Results regarding biochemical tests (Table 1) evidently confirmed that rhizobacterial isolates produced IAA content and solubilized P. The rhizobacterial isolates produced IAA equivalents i.e., 2.7-3.9 and P-solubilization i.e., 2.10-2.45. The biochemical screening of rhizobacterial isolates also showed promising results.

Table 1 Characterization of PGPR isolates on the basis of biochemical tests, auxin production and solubilization index

Isolate	Biochemical tests			Solubilization index	IAA Equivalents (µg mL^{-1})
	Methyl red	Oxidase test	Citrate utilization		
PGPR-1	++	++	+	2.30	2.8
PGPR-2	++	++	++	2.35	3.1
PGPR-3	++	+	+	2.38	2.7
PGPR-4	+++	+++	++	2.45	3.9
PGPR-5	+	++	+	2.10	3.5

Effect on biomass yield

Biofertilizers methods of application (separate or in various combinations), increased the total biomass yield of rice over control (74.6 g pot^{-1}) where no inoculum was applied (Table 2). In our study the seed application method gave more biomass yield (90.0 g pot^{-1}) than flooding (80.6 g pot^{-1}) and foliar spray (78.6 g pot^{-1}). Moreover, the combination of seed coating + flooding (92.6 g pot^{-1}) surpassed seed coating + foliar spray (90.6 g pot-1) and flooding + foliar spray (82.0 g pot^{-1}). Similarly, biomass yield produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (93.6 g pot^{-1}) overshadowed the biomass yield obtained from the combination of any two application methods used for biofertilizer application. Almost the same trend was observed in case of wheat biomass data (Table 3). Application of biofertilizers inoculum, regarding three tested methods (single or in combinations), increased the biomass yield of wheat over control (58.6 g pot^{-1}) where no inoculum was applied. However, biofertilizer applied to seed gave higher biomass yield (65.0 g pot^{-1}) than the biofertilizer's flooding (61.0 g pot^{-1}) and its foliar spray (60.0 g pot^{-1}). Furthermore, the combination of seed coating + flooding (68.0 g pot^{-1}) exceeded seed coating + foliar spray (67.0g pot^{-1}) and flooding + foliar spray (62.0 g pot^{-1}). Likewise, biomass yield produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (70.0 g pot^{-1}) outshined the biomass yield obtained from the combination of any two application methods used for biofertilizer application.

Table 2 Effect of biofertilizer's application methods on growth improvement of rice

Rice (2020)					
Treatments	Biomass yield (g pot^{-1})	Grain yield (g pot^{-1})	No. of tillers pot^{-1}	Plant height pot^{-1} (cm)	Microbial count at tillering (Log10)
T_1: Control	74.6 c	35.6 d	19.0 d	95.3 d	8.175 f
T_2: Seed coating	90.0 a	42.3 abc	22.3 ab	100.0 abcd	9.079 a
T_3: Flooding	80.6 bc	38.0 cd	20.0 cd	97.3 cd	8.827 d
T_4: Foliar spray	78.6 bc	37.3 d	19.6 d	97.0 cd	8.736 e
T_5: $T_2 + T_3$	92.6 a	44.0 a	23.3 abc	103.0 ab	8.973 b
T_6: $T_2 + T_4$	90.6 a	43.6 ab	23.0 ab	101.0 abc	8.962 b
T_7: $T_3 + T_4$	82.0 b	39 bcd	21.0 bcd	98.0 bcd	8.884 c
T_8: $T_2 + T_3 + T_4$	93.6 a	45.0 a	24.0 a	103.6 a	9.117 a
LSD	7.084	4.832	2.448	5.083	0.047

Effect on grain yield

A clear increase in case of grain yield production (Table 2) was observed by the application of biofertilizers, in single or integrated application methods. Biofertilizers inoculum application, increased the grain yield of rice over control (35.6 g pot^{-1}) where no inoculum was applied. However, the seed application method gave more grain yield (42.3 g pot^{-1}) followed by flooding (38.0 g pot^{-1}) and foliar spray (37.3 g pot^{-1}). Moreover, the combination of seed coating + flooding (44.0 g pot^{-1}) surpassed seed coating + foliar spray (43.6 g pot^{-1}) and flooding + foliar spray (39.0 g pot^{-1}). Similarly, grain yield produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (45.0 g pot^{-1}) overshadowed the grain yield obtained from the combination of any two application methods used for biofertilizer application. In the case of wheat trials (Table 3) almost the same trend of increased grain yield was experienced. Biofertilizers increased grain yield of wheat over control (28.0 g pot^{-1}) where no inoculum was applied. Seed application method also caused a difference in grain yield where seed application gave more grain yield (34.3 g pot^{-1}) than flooding (29.6 g pot^{-1}) and foliar spray (30.0 g pot^{-1}). Moreover, the combination of seed coating + flooding (35.3 g pot^{-1}) surpassed seed coating + foliar spray (34.3 g pot^{-1}) and flooding + foliar spray (31.0 g pot^{-1}). Likewise, grain yield produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (36.0 g pot^{-1}) overshadowed the grain yield obtained from the combination of any two application methods used for biofertilizer application.

Table 3 Effect of biofertilizer's application methods on growth improvement of wheat

Wheat (2020-21)					
Treatments	Biomass yield (g pot^{-1})	Grain yield (g pot^{-1})	No. of tillers pot^{-1}	Plant height pot^{-1} (cm)	Microbial count at tillering (Log10)
T_1: Control	58.6 d	28.0 d	20.3 d	81.6 d	8.398 b
T_2: Seed coating	65.0 abc	34.3 abc	22.3 abc	86.6 abc	9.133 a
T_3: Flooding	61.0 cd	29.6 d	20.6 d	83.6 cd	8.844 ab
T_4: Foliar spray	60.0 cd	30.0 cd	21.0 cd	83.3 cd	8.777 ab
T_5: $T_2 + T_3$	68.0 a	35.3 ab	22.6 ab	89.3 a	8.996 a
T_6: $T_2 + T_4$	67.0 ab	34.3 abc	22.6 ab	87.6 ab	8.980 a
T_7: $T_3 + T_4$	62.0 bcd	31.0 bcd	21.3 bcd	84.3 bcd	8.392 b
T_8: $T_2 + T_3 + T_4$	70.0 a	36.0 a	21.3 a	88.0 ab	9.175 a
LSD	5.414	4.634	1.5401	3.6887	0.567

Effect on number of tillers pot^{-1}

Biofertilizers inoculum application, following all methods of application (separate or in various combinations), increased the No. of Tillers Pot^{-1} of rice plants over control (19.0 pot^{-1}) as shown in Table 2 where no inoculum was applied. However, the seed application method gave more No. of Tillers per Pot (22.3 pot^{-1}) than flooding (20.0 pot^{-1}) and foliar spray (19.6 pot^{-1}). Moreover, the combination of seed coating + flooding (23.3 pot^{-1}) surpassed seed coating + foliar spray (23 pot^{-1}) and flooding + foliar spray (21 pot^{-1}). Similarly, No. of Tillers Pot^{-1} produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (24 pot^{-1}) outshined the No. of Tillers Pot^{-1} obtained from the combination of any two application methods used for biofertilizer application. Almost the same trend was observed in case of No. of Tillers Pot^{-1} of wheat (Table 3). Application of biofertilizers inoculum, regarding three tested methods (single or in combinations), increased the No. of Tillers Pot^{-1} of wheat over control (20.3 pot^{-1}) where no inoculum was applied. Nonetheless, the biofertilizer applied to seed gave higher No. of Tillers per Pot (22.3 pot^{-1}) than the biofertilizers flooding (20.6 pot^{-1}) and its foliar spray (21 pot^{-1}). Furthermore, the combination of seed coating + flooding (22.6 pot^{-1}) exceeded seed coating + foliar spray (22.6 pot^{-1}) and flooding + foliar spray (21.3 pot^{-1}). Likewise, No. of Tillers per Pot produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (21.3 pot^{-1}) outperformed the No. of Tillers Pot^{-1} obtained from the combination of any two application methods used for biofertilizer application.

Effect on plant height

A clear increase in case of Plant Height Pot^{-1} (Table 2) was observed by the application of biofertilizers, in single or integrated application methods. Biofertilizers inoculum application increased the Plant Height Pot^{-1} of rice over control (95.3 cm) where no inoculum was applied. However, the seed application method gave more Plant Height Pot^{-1} (100 cm) than flooding (97.3 cm) and foliar spray (97.0 cm). Moreover, the combination of seed coating + flooding (103.0 cm) surpassed seed coating + foliar spray (101.0 cm) and flooding + foliar spray (98.0 cm). Similarly, Plant Height Pot^{-1} produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (103.6 cm) dominated the grain yield obtained from the combination of any two application methods used for biofertilizer application. In the case of wheat trials almost the same trend of increased Plant Height Pot^{-1} was experienced in table 3. Biofertilizers increased the Plant Height Pot^{-1} of wheat over control (81.6 cm) where no inoculum was applied. However, the seed application method gave more Average Plant height pot^{-1} (86.6 cm) than flooding (83.6 cm) and foliar spray (83.3 cm). Moreover, the combination of seed coating + loading (89.3 cm) surpassed seed coating + foliar spray (87.6 cm) and flooding + foliar spray (84.3 cm). Similarly, Plant Height Pot^{-1} produced by the combination of all employed application methods i.e., seed application + flooding + foliar spray (88.0 cm) surpassed the Plant Height Pot^{-1} obtained from the combination of any two application methods used for biofertilizer application.

Effect on microbial count

The microbial count (MC) or total viable count in the rhizosphere soil was determined by standard dilution plate technique on LB and nutrient agar medium just to check the microbial population rise in the rhizosphere soil at tillering stage. The application methods of biofertilizer significantly affected the MC in the rhizosphere soil of rice (Table 2). All application methods improved the microbial number presented as LOG 10 as compared to control i.e., 8.175. The highest microbial count was observed in seed coating i.e., 9.079 that was statistically at par with all employed application methods i.e., seed application + flooding + foliar spray i.e., 9.117. Almost similar trend was observed in the case of wheat presented in Table 3. The application methods employed improved the microbial number presented as LOG 10 as compared to control i.e., 8.398. The highest microbial count was observed in seed coating i.e., 9.133 that was statistically at par with all employed application methods i.e., seed application + flooding + foliar spray i.e., 9.175. The combination of methods in T_5 and T_6 were also statistically at par with seed coating.

Effect on grain N & P

The data regarding grain N & P of rice and wheat was presented in Table 4 revealed microbial inoculation methods increased the grain N& P content in both crops significantly. Biofertilizer inoculum application increased the grain N & P of rice over control (1.310 and 0.265%), respectively. However, high grain N & P (1.420 and 0.295%), respectively, was observed with seed coating than the rest of methods. However, when methods were combined together, higher values were observed. Moreover, maximum grain N & P produced by combination of all employed application methods i.e., seed application + flooding + foliar spray (1.435 and 0.325%), respectively. Similar findings were also observed in the case of wheat as mentioned in Table 4. The application of biofertilizer increased the grain N & P of wheat over control (81.525 and 0.295%), respectively. However, with the seed coating method, higher grain N & P (1.580 and 0.330%), respectively than the rest of the methods. However, when methods were employed together, higher values were observed. Moreover, maximum grain N & P produced by combination of application methods i.e., seed application + flooding + foliar spray i.e., 1.625 and 0.365%, respectively.

Table 4 Effect of biofertilizer's application methods on grain N&P content of rice and wheat

Treatments	Rice (2020)		Wheat (2020-21)	
	Grain N (%)	Grain P (%)	Grain N (%)	Grain P (%)
T_1: Control	1.310 e	0.265 e	1.525 c	0.295 e
T_2: Seed coating	1.420 ab	0.295 bcd	1.580 ab	0.330 bcd
T_3: Flooding	1.365 cd	0.275 de	1.560 cd	0.305 de
T_4: Foliar spray	1.340 de	0.285 cde	1.550 cd	0.315 cde
T_5: $T_2 + T_3$	1.375 bcd	0.305 abc	1.600 ab	0.340 abc
T_6: $T_2 + T_4$	1.380 bcd	0.315 ab	1.590 ab	0.355 ab
T_7: $T_3 + T_4$	1.390 abc	0.300 abcd	1.595 ab	0.335 abcd
T_8: $T_2 + T_3 + T_4$	1.435 a	0.325 a	1.625 a	0.365 a
LSD	0.0458	0.0251	0.0538	0.0336

Discussion

The PGPR are very well known for its growth promoting traits and characteristic feature of PGPR are the production of hormones, siderophores, antibiotics, primary/secondary metabolites, can compensate mineral nutrients and impart his role in plant growth promotion and ultimately the yields (Mehboob et al., 2011; Vocciante et al., 2022). The PGPR isolates (five isolates) were characterized for their IAA contents, mineral phosphate solubilization, production of hormones and other biochemical tests qualitatively like methyl red, oxidase test & citrate utilization tests. The rhizobacteria isolates produced IAA and P-solubilization index at varied degree and isolate (PGPR-4) was used in pot study due to its higher hormone production and solubilization index level. There are a number of reports in literature and confirm the role of microbial biosynthesis of IAA and phosphate solubilization potential (Akhtar et al., 2013). The present study illustrated that isolates produced IAA content i.e., 2.7-3.9 µg mL^{-1} and P-solubilization i.e., 2.10-2.45 and maximum values was obtained by PGPR-4 i.e., 3.9 µg mL^{-1} and P-solubilization i.e., 2.45 and other biochemical tests are also present in the isolate and reported by various researchers (Mohammadi et al., 2011; Farnia & Hasanpoor, 2015; Parthiban et al., 2016).

The present pot study emphasized the application methods and three methods with different combinations were checked viz. seed coating, foliar and flooding (soil application followed by irrigation) on rice and wheat. Results regarding yield parameters of rice and wheat clearly depicted the significant results (Table 2 & 3). The usage of biofertilizers provided an environmentally friendly, economic, and sustainable way to produce the yield on sustain basis and also improved the soil physical, chemical and biological conditions resultantly increased the crop yields (Hasanuzzaman et al., 2013; Kumar et al., 2017; Odoh et al., 2020). Also, the application of biofertilizers enables the plant to survive in adverse environmental conditions (Alori et al., 2017). The ultimate aim is to enrich the beneficial microbial population in the plant rhizosphere for better root colonization and antagonists to soil pathogens (Preininger et al., 2018; Odoh et al., 2020).

Results regarding physical parameters of rice and wheat clearly indicated that the application methods viz. seed coating, and flooding, foliar spray improved the yield attributes of rice and wheat and when these methods were combined showed promising significant results as compared to the un-inoculated control. The maximum biomass, grain yield, no. of tillers and plant height of rice were observed in T_8 where three methods have been combined might be attributed to plant growth promoting activities, root colonizing ability, production of hormones, other metabolites and application methods that provided the considerable number of microbes for root colonization (Mahmood et al., 2016; Mahmud et al., 2021; Liu et al., 2022). Literature confirmed the application of multiple methods and found that different methods performed differently with respect to the prevailing conditions (Dhar et al., 2007; Dal Cortivo et al., 2018; Mahmud et al., 2021). Each method had its significance, for the PGPR having the role of biocontrol agent, foliar spar was found superior to other methods (Habibzadeh et al., 2012; Bhardwaj et al., 2014). The PGPR-4 applied as seed coating was found superior to other methods due to its root colonizing capability and contributed to better nutrient supplies by solubilizing insoluble phosphates (Begum et al., 2019; Fadiji et al., 2022). The application of methods introduced higher numbers of microbes in the root zone/rhizosphere resultantly higher microbial count and better colonization was observed. The combination of methods imposed better root colonization by seed coating, and better colonization in intercellular plant tissues through foliar spray and flooding reported by numerous workers (Dal Cortivo et al., 2018; Pagnani et al., 2020). The wheat and rice inoculation as seed/seedling dipping has positive impact on native microbiome and resulted in enhanced microbial biomass due to the plant benefitting rhizobacteria (Zang et al., 2018; Fasusi et al., 2021; Liu et al., 2022).

The combined application of biofertilizer methods (T_8) provided a significantly higher number of beneficial bacteria in root/rhizosphere zones and ultimately improved the yield contributing factors (Afzal & Bano, 2008). Increased plant growth and yield was also reported by many workers (Tripathi et al., 2008; Tambekar et al., 2009; Mohammadi et al., 2011; Podolich et al., 2015; Singh et al., 2016; Pirttilä et al., 2021). The increased surface area of root/lateral roots encouraged better nutrient uptake. Increase in grain nutrient content owed to microbial mobilization of nutrients. The microbial inoculation in the root zone solubilized the non-available nutrients and better nutrient uptake in plant parts including grains (Singh et al., 2016; Mukhongo et al., 2017; Ullah et al., 2017b; Qureshi et al., 2022).

Conclusion

Present study concluded that biofertilizer application of methods had significant effect on growth and yield of crops, and if special conditions prevails then method should be opted carefully to get the maximum benefit and in normal conditions seed coating found to be superior and if farmer had not applied at sowing, then flooding might be carried out.

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