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RESEARCH ARTICLE

Impact of *Rhizobium* and phosphate solubilizing bacteria on soil phosphorus dynamics and enzyme activity in black gram (*Vigna mungo* L.)

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Abstract

The study aimed to investigate the impact of *Rhizobium* and phosphate solubilizing bacteria (PSB) on the transformation of soil phosphorus (P) fractions and the key soil enzymes involved in P cycling. A field experiment was conducted using black gram in a randomized block design (RBD) with 7 treatments consisting of control, recommended dose of fertilizer, *Rhizobium bangladeshense* and *Pseudomonas* sp. alone and combined inoculation with inorganic fertilizers. Each treatment was replicated thrice. The soil samples were collected to analyze P fractions (available P, soluble P, Al-P, Fe_1-P, Ca-P, Fe_2-P, residual P, labile organic P, moderately labile organic P and non-labile organic P) and soil biological activity using standard methods. Data were statistically analyzed to determine the treatment effects. Co-inoculation of *Rhizobium bangladeshense* at 10 mL kg^{-1} seed with *Pseudomonas* sp. at 10 mL kg^{-1} seed along with the application of 75 % nitrogen and phosphorus (NP) and 100 % potash (K) significantly enhanced the P fractions and soil biological property when compared with control and alone inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp. The combined inoculation of *Rhizobium bangladeshense* and Pseudomonas sp. (T7) significantly ($p \leq 0.05$) increased soluble, Al-, Fe- and Ca-bound P fractions, microbial counts and enzymatic (dehydrogenase and phosphomonoesterase) activities compared to control and single inoculations. These significant enhancements indicate superior P mobilization and soil biological activity under dual inoculation treatment. The combined inoculation of *Rhizobium* and PSB effectively mobilized fixed P forms and enhanced enzymatic activity, leading to improved P availability in soil while saving 25 % of N and P fertilizers.

Keywords: labile P; non-labile P; phosphorus fractionation; *Pseudomonas*; *Rhizobium*; soluble P

Introduction

Phosphorus (P) is the second most important macronutrient, essential for plant growth and involved in numerous biochemical processes in living organisms. Nevertheless, excessive or unavailable P can limit the growth and yield of different crops (1). The total concentration of P in the earth's crust ranges from 0.005 % to 0.15 %. The availability of P in soil is very much affected by the soil reaction, as it chemically precipitates with Fe or Al oxides when the soil pH drops below 5.5 (2). Simultaneously, in alkaline soil it precipitates as $CaCO_3$ with soil pH ranging from 7.5 to 8.5 which makes limited free phosphate ions (2, 3). The availability of P ions to the plants in soil may be increased through the solubilization of insoluble inorganic and organic P by different mechanisms performed by soil micro-organisms. It was reported that the inorganic P (Pi) is regarded as the most readily available form of soil P (4). The present experimental soil is alkaline in nature (pH 8.2), causing P to precipitate as $CaCO_3$ and resulting in low availability. It is suggested that availability of soil P may be enhanced through secretions of various microbial metabolites in the form of low molecular weight organic acids and protonation which leads to lowering the soil pH and solubilize P from inorganic insoluble and organic complexes (5). The application of potent P solubilizing bacteria is capable to increase P solubilization from insoluble inorganic as well as organic sources. A few strains of *Rhizobium* bacteroids, besides symbiotic nitrogen fixation, are involved in phosphate solubilization (6). However, the research on phosphate solubilizing activity of *Rhizobium* strains is limited (7, 8). The main advantage of using rhizobia as a phosphate-solubilizing microorganism will be their beneficial nutritional effect resulting both from phosphate mobilization and nitrogen fixation (9). The reviews are very scanty on the co-inoculation activity of *Rhizobium* and PSB. Thus, present study hypothesized that the inoculation of *Rhizobium* sp. and *Pseudomonas* sp. with various levels of nitrogen (N) and P would perform better over sole application of inorganics fertilizers with respect to obtaining different P fractions in low P soils.

Materials and Methods

The field experiment was conducted during summer season of 2023-24 at Acharya Narendra Deva University of Agriculture and technology, Kumarganj, Ayodhya, Uttar Pradesh, India. Kumarganj is situated in Eastern Plain agro-climatic zone of Uttar Pradesh, at 26.7068° N latitude and 82.1336° E longitude. Pre-sowing irrigation was applied. At optimum moisture conditions, the field was cultivated twice followed by planking. Prior to sowing, soil samples were collected from 0–15 cm depth of soil to analyze the initial physico-chemical properties. The soil was silt loam (Typic Ustochrepts) in texture. Soil samples were air dried and passed through a 2 mm IS sieve. Detailed soil characteristics are presented in Table 1.

Table 1. Initial characteristics of field experimental soil

Soil properties	Initial value
Soil texture	Silt loam
Sand	25.14
Silt	48.90
Clay	25.96
Soil bulk density	1.32
Soil particle density	2.60
pH	8.20
Electrical conductivity (ds m^{-1})	0.345
Oxidizable soil organic carbon (OC %)	0.42
Available N (kg ha^{-1})	130.58
Available P (kg ha^{-1})	10.98
Available K (kg ha^{-1})	245.70
Bacterial count (10^6 cfu g^{-1} of soil)	13.5
Fungal count (10^4 cfu g^{-1} of soil)	7.5
Actinomycetes count (10^5 cfu g^{-1} of soil)	10.5
Rhizobium (10^6 cfu g^{-1} of soil)	13.0
PSB (10^6 cfu g^{-1} of soil)	12.5
Dehydrogenase activity	70.68
Al bound phosphorus fraction (mg kg^{-1})	15.00
Fe_1 bound phosphorus fraction (mg kg^{-1})	35.82
Ca bound phosphorus fraction (mg kg^{-1})	79.18
Fe_2 bound phosphorus fraction (mg kg^{-1})	42.69
Residual phosphorus fraction (mg kg^{-1})	91.68
Labile organic phosphorus fraction (mg kg^{-1})	18.75
Moderately labile organic phosphorus fraction (mg kg^{-1})	14.25
Non labile organic phosphorus fraction (mg kg^{-1})	11.69

The experiment comprising 7 treatments with 3 replications were T_1-Control (no fertilizer); T_2-Recommended dose of fertilizers (RDF) i.e. 20:40:20 (N: P_2O_5: K_2O); T_3-*Pseudomonas* sp. at 10 mL kg^{-1} seed; T_4-*Rhizobium bangladeshense* at 10 mL kg^{-1} seed; T_5-*Rhizobium bangladeshense* at 10 mL kg^{-1} seed + 25 % N + 100 % PK; T_6-*Pseudomonas* sp. at 10 mL kg^{-1} seed + 25 % P + 100 % NK; T_7-*Rhizobium bangladeshense* at 10 mL kg^{-1} seed + *Pseudomonas* sp. at 10 mL kg^{-1} seed + 75 % NP + 100 % K. However, K was applied uniformly irrespective of all the treatments from T_2 to T_7. The treatments were arranged in a randomized block design (RBD) with triplicates of each treatment. Inorganic fertilizers were applied as urea for nitrogen (N), single super phosphate for P, muriate of potash for potassium (K). The seed inoculation was done with *Rhizobium bangladeshense* and *Pseudomonas* sp. at 10 mL kg seed with cfu 1.0 × 10^9 mL^{-1}. The cultures were procured from biofertilizer production unit, Bihar Agricultural University, Sabour, Bihar, India and the characteristics of inoculants used are presented in Table 2. All the phosphatic and potassic fertilizers were applied as basal application before sowing, 50 % of N was applied as basal application; 25 %, top-dressed at the tillering stage; and another 25 % top-dressed at the pod formation stage.

Table 2. Characteristics of used bacteria in the study

Parameters	*Rhizobium bangladeshense*	*Pseudomonas* sp.
Growth on media with 10 % NaCl	+	++
HCN production activity	+	++
Phosphate solubilization activity	+	++
Production of IAA (in ppm)	14.72	7.91
Production of ammonia (in ppm)	8.20	9.87
Gram staining	Ve^-	Ve^-
Morphology	Rod shape	Cylindrical shape

+: low, ++: medium.

Chemical analysis of experimental soil

The specimen soil samples were drawn from the field after harvest of crops then ground up to 0.25 mm in size to evaluate the different parameters by adopting standard methodology viz. oxidizable organic carbon (10), electrical conductivity and pH (11), available N (12), available P (13) and available K (14), respectively. The soil was found to be silt loam in texture.

Phosphorus (P) fractionation

Sequential fractionation of inorganic phosphorus (Pi)

The different fractions of soil P were extracted using the sequential extraction method as reported previously (15, 16). Soluble P (Sol-P) was recovered from a 3 g soil sample in a centrifuge tube. The soil sample was combined with 30 mL of 1.0 M ammonium chloride and agitated on a mechanical shaker for 30 min before being centrifuged for 10 min at 10000 rpm. The supernatant (5 mL) was collected and mixed with the reagent. The concentration of Sol-P was measured using a spectrophotometer (Genesys 180) at 660 nm wavelength.

Sequential fractionation of organic phosphorus (Po)

A stepwise approach was adapted for calculating all organically bound P fractions by digesting the samples with persulfate and sulphuric acid and quantifying at 882 nm wavelength using a calibrated spectrophotometer (14).

Soil biological parameters

Rhizospheric soil samples were collected from soil adhering to roots and root hairs at at flowering and at harvesting stage. 10 g of soil samples were placed in an Erlenmeyer flask containing 90 mL of sterilized distilled water and shaken for 30 min. Ten-fold series dilutions were prepared and appropriate dilutions were plated in specific media. For the isolation of bacteria, fungi and actinomycetes, *Rhizobium*, PSB, the plate count agar, Czapek-Dox agar (17), Kenknight and Munaier's medium, yeast extract mannitol agar medium and Pikovaskaya's medium respectively were used (18). The numbers of colony forming cells were determined in each plot by serial dilution pour plate method (19). The activities of soil enzymes: dehydrogenase activity (20), acid phosphatase and alkaline phosphatase (21) were determined.

Statistical analysis

Data was analyzed using IBM SPSS 20.0. The effects of the experimental factors studied were determined by one-way analysis of variance (ANOVA). Mean comparisons were carried out by the

Duncan ($p \leq 0.05$) test. The statistical analysis conducted in this experiment was followed by the standard protocol (22).

Results and Discussion

Inorganic phosphorus fractionation

The results presented in Table 3 and Fig. 1 showed that the combined inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp. (T7) significantly ($p \leq 0.05$) increased all inorganic and organic P fractions compared to control and single inoculations. The highest values of soluble P (36.23 mg kg^{-1}), Al-P (75.78 mg kg^{-1}), Fe_1-P (84.72 mg kg^{-1}), Ca-P (110.12 mg kg^{-1}), Fe_2-P (70.16 mg kg^{-1}) and residual P (116.40 mg kg^{-1}) were recorded under T7 treatment. Similarly, labile organic P (27.53 mg kg^{-1}), moderately labile organic P (25.19 mg kg^{-1}) and non-labile organic P (35.99 mg kg^{-1}) were also significantly higher in T7. These results indicate that the synergistic interaction between *Rhizobium* and *Pseudomonas* sp. effectively enhanced P solubilization and transformation across different fractions, improving overall P availability in alkaline soil.

Soluble P

Soluble P in soil showed a significant ($p \leq 0.05$) increase over control due to the application of mineral fertilizer and inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp (Table 3 and Fig. 1). Also, it was notably to note that treatments having 75 % NP, 100 % K and co-inoculation with *Rhizobium bangladeshense* and *Pseudomonas* sp. significantly ($p \leq 0.05$) increased soluble P when compared with control, 100 % application of chemical fertilizer, *Rhizobium bangladeshense* alone inoculation, *Pseudomonas* sp. alone inoculation and *Rhizobium bangladeshense* inoculation along with 75 % N and 100 % K application. This treatment was found to be statistically ($p \leq 0.05$) at par with the inoculation of *Pseudomonas* sp. + 75 % K + 100 % NP application.

The increment in the soil soluble P might be due to organic acids viz. oxalic and citric acids contain active functional groups in the form of -COOH (carboxyl) groups which liberates H^+ ions and secretion of acid phosphomonoesterase and alkaline phosphomonoesterase enzyme in the soil solution, helps in the release of P from inorganic insoluble P and organic P and increases the available P status (23, 24). The inoculation of *Pseudomonas* sp. had a significant ($p \leq 0.05$) positive impact in enhancing the phyto available P status in soil, over the uninoculated plots.

Al-P

The Al-P fraction was recorded from the experimentation and ranged from 15.13 to 75.78 mg kg^{-1} soil, while the Fe_1-P fraction ranged from 35.73 to 84.72 mg kg^{-1} soil, Fe_2-P ranged from 44.76 to 70.16 mg kg^{-1} soil and Ca-P fraction ranged between 80.53 - 110.12 mg kg^{-1} soil. In the present study, the Ca-P fraction was more dominant of inorganic P pool compared to the Al-P and Fe-P fractions (Table 3 and Fig. 1). The inoculation of *Rhizobium bangladeshense* + *Pseudomonas* sp. + 75 % NP +100 % K application significantly ($p \leq 0.05$) increased Al-P, Fe_1-P, Ca-P and Fe_2-P by 400.85 %, 137.11 %, 36.74 % and 56.75 % and 97.55 %, 29.11 %, 16.18 % and 10.94 %, respectively when compared with control (absolute) and application of 100 % RDF (recommended dose of fertilizer). Similar trends were observed for residual P. This might be due to the secretions of various low molecular weight organic acids by *Rhizobium bangladeshense* and *Pseudomonas* sp. and release of H^+ ions into the soil system which solubilize in insoluble inorganic P (25).

Organic phosphorus fractions

The statistical analysis of the observed data showed that among the organic P fractions, viz. labile organic P, non-labile P and the moderately organic labile P were found to be significantly ($p \leq 0.05$) increased under the application of *Rhizobium bangladeshense* + *Pseudomonas* sp. + 75 % NP +100 % K over the treatments control, 100 % RDF, *Rhizobium bangladeshense* at 10 mL kg^{-1} seed and *Pseudomonas* sp. at 10 mL kg^{-1} seed, respectively (Table 3 and Fig. 2). This treatment was found to be statistically ($p \leq 0.05$) at par with the

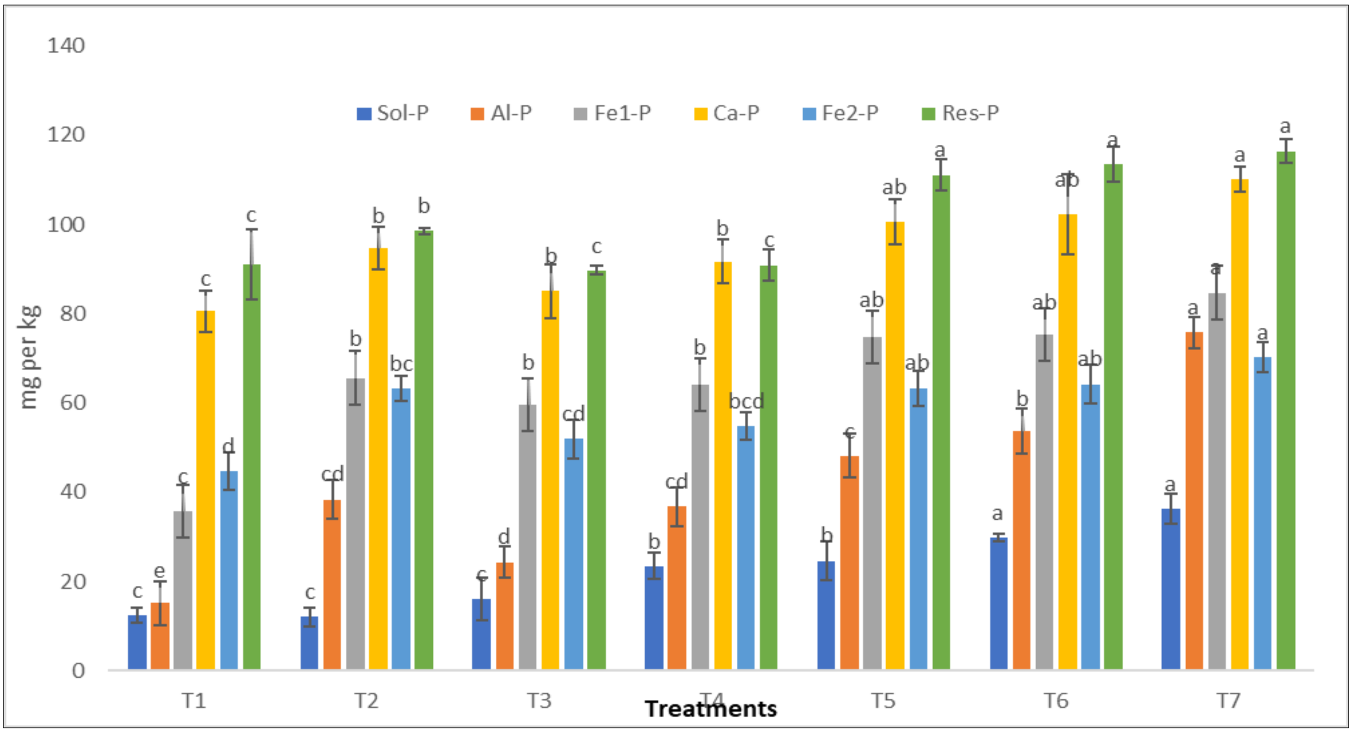

Fig. 1. Effect of the combined inoculation of *Rhizobium* and *Pseudomonas* sp. on the different inorganic fraction of phosphorus in soil. Values are means and bars represent S D. Different letters indicate significant ($p \leq 0.05$) differences between means.

Table 3. Influence of the inoculation of *Rhizobium* and PSB alone and in combination on different inorganic as well as organic soil phosphorus fractions

Treatments	Sol-P (mg kg^{-1})	Al-P (mg kg^{-1})	Fe_1-P (mg kg^{-1})	Ca-P (mg kg^{-1})	Fe_2-P (mg kg^{-1})	Residual P (mg kg^{-1})	Labile organic P (mg kg^{-1})	Moderately organic labile P (mg kg^{-1})	Non labile organic P (mg kg^{-1})
T_1- Control	12.39^{c}	15.13^{e}	35.73^{c}	80.53^{d}	44.76^{c}	91.04^{c}	19.19^{d}	15.80^{e}	22.10^{ab}
T_2- 100 % RDF	12.07^{c}	38.36^{cd}	65.62^{b}	94.78^{bc}	63.24^{ab}	98.54^{b}	22.58^{bcd}	21.51^{bc}	22.10^{ab}
T_3- *Rhizobium* alone at 10 mL kg^{-1}	15.95^{c}	24.29^{d}	59.58^{b}	85.08^{cd}	51.92^{b}	89.69^{c}	21.42^{cd}	18.16^{de}	19.62^{b}
T_4- PSB alone at 10 mL kg^{-1}	23.39^{b}	36.71^{cd}	64.17^{b}	91.73^{bcd}	54.75^{b}	90.84^{c}	23.25^{bc}	20.06^{cd}	20.84^{b}
T_5- *Rhizobium* at 10 mL kg^{-1}+ 75 % N+ 100 % PK	24.60^{b}	48.11^{c}	74.78^{ab}	100.65^{ab}	63.32^{ab}	111.11^{a}	24.53^{abc}	22.87^{ab}	24.03^{a}
T_6- PSB at 10 mL kg^{-1} + 75 % P+ 100 % NK	29.81^{a}	53.78^{b}	75.20^{ab}	102.34^{ab}	64.22^{ab}	113.56^{a}	25.64^{ab}	23.70^{ab}	24.38^{a}
T_7- *Rhizobium* at 10 mL kg^{-1} + PSB at 10 mL kg^{-1} + 75 % NP+ 100 % K	36.23^{a}	75.78^{a}	84.72^{a}	110.12^{a}	70.16^{a}	116.40^{a}	27.53^{a}	25.19^{a}	35.99^{a}
SeM(±)	1.19	1.75	2.43	3.32	1.19	2.25	1.17	0.81	0.85
C D (P = 0.05)	3.65	5.41	7.50	10.22	3.67	6.94	3.62	2.50	2.61

Values followed by the same small letters do not differ significantly (p <.05) between treatments

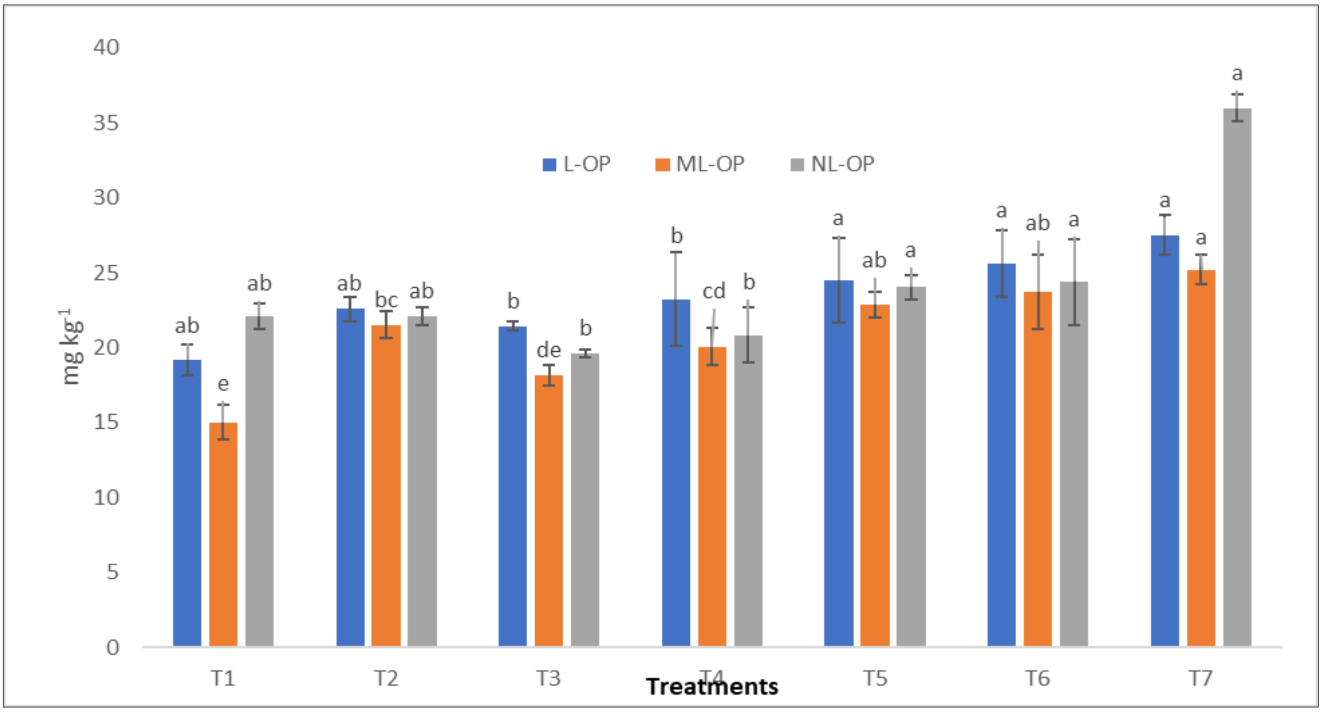

Fig. 2. Effect of the combined inoculation of *Rhizobium* and *Pseudomonas* sp. on the different organic fractions of phosphorus in soil. Values are means and bars represent S D. Different letters indicate significant ($p \leq 0.05$) differences between means.

application *Rhizobium bangladeshense* at 10 mL kg^{-1} seed +75 % N +100 % P and K, *Pseudomonas* sp. at 10 mL kg^{-1} seed + +75 % P + 100 % N and K and *Rhizobium bangladeshense* at 10 mL kg^{-1} seed + *Pseudomonas* sp. at 10 mL kg^{-1} seed + +75 % NP +100 % K, respectively. The effect of various treatments on non-labile organic P was found to be not significant. However, the non-labile organic P was ranged between 19.62 - 35.99 mg kg^{-1} soil. The increment in organic P fractions might be due to the fact that both the bacteria produce enzymes like phosphatase and phytase which helps in the dissolution of P from the organic pool, which then mobilized by microorganisms for their cell synthesis and increases the organic P fractions upon death of microbial cells. Similar findings have been reported previously, who conducted a pot experiment to evaluate the response of Nano-P and PSB for soil P fractions in wheat crop (26). They reported that the death of the microbes in the soil results in the release of microbial biomass P into the soil, increasing the labile pool of P acquired from organic sources (27).

Soil biological properties

It has been observed that the seed inoculation with *Rhizobium bangladeshense* and *Pseudomonas* sp. at the rate of 10 mL kg^{-1} seed and application of 75 % NP and 100 % potassium showed significant ($p \leq 0.05$) hike in the microbial population for all the microbes viz. bacterial, actinomycetes, fungal, *Rhizobium* and PSB population under alkaline soil (Table 4 and Fig. 3).

Microbial population

A decline in microbial population was observed post-harvest compared to the 50 % flowering stage (Table 4 and Fig. 3). This might be mostly due to decreased root exudation, which reduces the release of organic compounds, ultimately leading to a decreased source of energy for microorganisms (28). Hence, after the harvest of the crop, the population of microbes gets reduced. Similar findings were previously reported who conducted a field experiment to assess the effect of N levels and irrigation schedules on the soil biological activity (29). They reported that the microbial population,

Table 4. Effect of the combined and single inoculation of *Rhizobium* and *Pseudomonas* sp. on the microbial population in soil

Treatments	Bacteria ($\times10^6$ cfu g^{-1} of soil)		Actinomycetes ($\times10^5$ cfu g^{-1} of soil)		Fungi ($\times10^4$ cfu g^{-1} of soil)		*Rhizobium* ($\times10^6$ cfu g^{-1} of soil)		PSB ($\times10^6$ cfu g^{-1} of soil)	
	After 50 % flowering	After harvest	After 50 % flowering	After harvest	After 50 % flowering	After harvest	After 50 % flowering	After harvest	After 50 % flowering	After harvest
T_1- Control	20.33^{c}	10.33^{c}	16.67^{c}	9.67^{d}	12.33^{bc}	8.00^{c}	10.00^{c}	6.33^{d}	10.00^{e}	5.33^{d}
T_2- 100 % RDF	24.33^{b}	12.67^{c}	20.67^{bc}	12.00^{c}	12.33^{bc}	9.67^{bc}	10.33^{bc}	6.67^{cd}	11.00^{d}	5.67^{cd}
T_3- *Rhizobium* alone at 10 mL kg^{-1}	22.33^{bc}	12.00^{c}	18.67^{c}	11.67^{c}	11.67^{d}	8.33^{bc}	10.67^{bc}	7.33^{c}	11.33^{d}	6.67^{bcd}
T_4- PSB alone at 10 mL kg^{-1}	24.00^{b}	12.33^{c}	20.33^{bc}	13.00^{c}	12.67^{bc}	9.00^{bc}	11.00^{abc}	7.67^{c}	12.00^{c}	7.33^{bcd}
T_5- *Rhizobium* at 10 mL kg^{-1} + 75 % N+ 100 % PK	25.00^{b}	13.33^{bc}	23.33^{ab}	15.33^{b}	12.67^{bc}	10.33^{abc}	11.33^{ab}	8.67^{bc}	13.00^{b}	7.67^{bc}
T_6- PSB at 10 mL kg^{-1} + 75 % P+ 100 % NK	28.67^{a}	15.67^{ab}	25.33^{a}	17.67^{ab}	13.00^{b}	10.67^{ab}	11.33^{ab}	9.67^{ab}	13.67^{a}	8.67^{ab}
T_7- *Rhizobium* at 10 mL kg^{-1} + PSBat 10 mL kg^{-1}+ 75 % NP+ 100 % K	32.67^{a}	18.33^{a}	33.00^{a}	21.67^{a}	16.67^{a}	12.33^{a}	12.00^{a}	11.00^{a}	14.00^{a}	10.33^{a}
SeM(±)	0.95	0.90	1.39	0.56	0.87	0.74	0.36	0.36	0.17	0.62
C D ($P = 0.05$)	2.92	2.78	4.30	1.73	2.67	2.27	1.10	1.12	0.53	1.90

Values followed by the same small letters do not differ significantly ($p < .05$) between treatments.

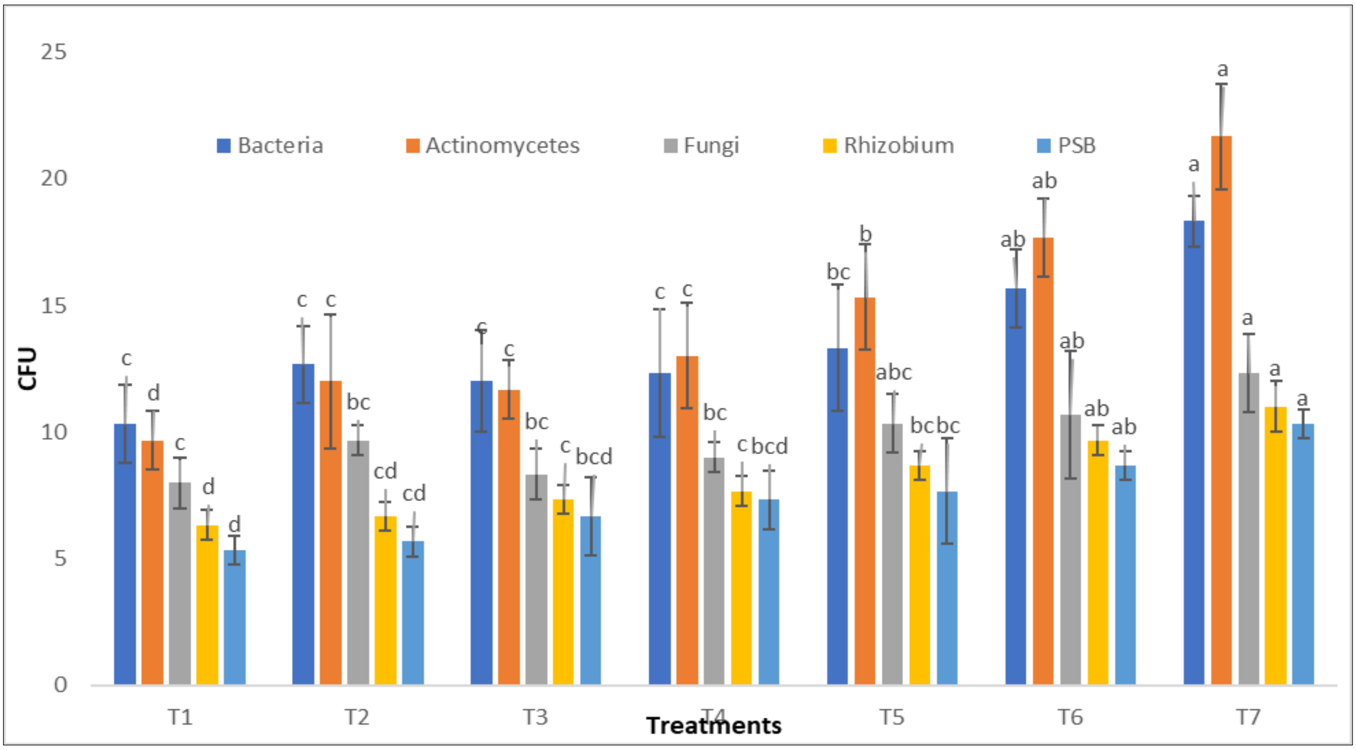

Fig. 3. Effect of the inoculation of *Rhizobium* and PSB in combination and alone on microbial population in soil at different stages of the crop growth. Values are means and bars represent S D. Different letters indicate significant ($p \leq 0.05$) differences between means.

i.e. bacteria, fungi and actinomycetes was the highest at the time of 50 % flowering. However, the co-inoculation of *Rhizobium* bangladeshense at 10 mL kg^{-1}+ *Pseudomonas* sp. at 10 mL kg^{-1} + 75 % NP+ 100 % K significantly increased the total bacterial count, *rhizobium* count and PSB count when compared with treatments viz., control, 100 % application of mineral fertilizers, inoculation of *Rhizobium bangladeshense* alone at 10 mL kg^{-1} seed and inoculation of *Pseudomonas* sp. alone at 10 mL kg^{-1} seed at 50 % flowering and harvesting stage.

The bacterial population (10^6 cfu g^{-1} soil) and actinomycetes population (10^5 cfu g^{-1} soil) exhibited a significant ($p \leq 0.05$) increment over control due to the application of chemical fertilizer along with the co-inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp. Additionally, it was observed that the treatment involving the application of 75 % NP and 100 % K application along with the co-inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp. showed significant results as compared to control, 100 % chemical fertilization, inoculation of *Rhizobium bangladeshense* alone, inoculation of *Pseudomonas* sp. alone and inoculation of *Rhizobium bangladeshense* along with 75 % N, 100 % P and K application, while sitting statistically at par with the treatment involving the inoculation of *Pseudomonas* sp., 75 % P, 100 % N and K application. The increased bacterial and actinomycetes population in the soil might be attributed to the ability of *Rhizobium bangladeshense* and *Pseudomonas* sp. to solubilize insoluble P compounds in the soil into soluble forms that are more readily accessible to plants, thus improving P availability in the soil. The increased P availability encourages plant development while additionally promoting the proliferation of soil microorganisms, increasing the overall bacterial and actinomycetes population (30). Similar findings were found previously, who conducted a field experiment to study the changes that occurred in biological properties which are influenced by the co-inoculation of different bacterial cultures with *Rhizobium phaseoli* in black gram grown Vertisols (31).

However, the fungal (10^4 cfu g^{-1} soil), *Rhizobium* (10^6cfu g^{-1} soil) and PSB (10^6cfu g^{-1} soil) population showed a significant ($p \leq 0.05$) increase over control due to the application of 75 % NP and 100 % N accompanied by the combined inoculation of *Rhizobium bangladeshense* as well as *Pseudomonas* sp. It was also observed that the treatment including the application of 75 % NP and 100 % N application along with the co-inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp. showed significant results over control, 100 % chemical fertilization, inoculation of *Rhizobium bangladeshense* alone, inoculation of *Pseudomonas* sp. alone, inoculation of *Rhizobium bangladeshense* along with 75 % N, 100 % P and K application and inoculation of *Pseudomonas* sp. along with the application of 75 % P, 100 % N and K. The increase in the fungal population may attributed to the growth promoting substances secreted by crops during growth period (32).

Soil enzymatic activity

Interestingly that the enzymatic activity of all 3 enzymes viz. dehydrogenase enzymatic activity, acid phosphomonoesterase activity and alkaline phosphomonoesterase activity engaged in the experimental investigation decreased after the crop was harvested, as opposed to the enzymatic activity reported during the crop's 50 % flowering stage (Table 5–7, Fig. 4–6). This might be due to the reduction in the microbial population after the harvest of the crop which leads to the loss of nutrient rich root zones and organic inputs, which ultimately causes a decline in the enzymatic activity in the soil (33).

Application of *Rhizobium bangladeshense* at 10 mL kg^{-1} seed + *Pseudomonas* sp. at 10 mL kg^{-1} seed+ 75 % NP+ 100 % K significantly increased dehydrogenase, acid phosphomonoesterase and alkaline phosphomonoesterase activities over control, 100 % application of recommended dose of fertilizers, inoculation of *Rhizobium bangladeshense* alone at 10 mL kg^{-1} seed and inoculation of *Pseudomonas* sp. alone at 10 mL kg^{-1} seed at 50 % flowering stage, respectively. This might be due to the secretions of more dehydrogenases, acid phosphomonoesterase and alkaline phosphomonoesterase with the inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp. The results are in the alignment of the early findings who conducted a study examining the effects of *Rhizobium* inoculation on the faba bean rhizosphere soil (34). They reported that the microbial activity stimulated by *Rhizobium* inoculation contributed to the release of several different enzymes which increased their activity in the soil. Similar results were also found in previous results who carried out a field experiment to evaluate the effect of biofertilizers on the biological properties in wheat rhizosphere. Results indicated an increase in dehydrogenase enzymatic activity with the inoculation of *Azotobacter* along with the application of PSB, potassium mobilizing bacteria (KMB) and zinc solubilizing bacteria (ZnSB).

The data from Tables 4 to 7 revealed a strong positive relationship between microbial population and enzymatic activities in soil. Treatments involving the combined inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. (T7) recorded the highest bacterial, fungal and actinomycetes populations, which corresponded with significantly elevated dehydrogenase, acid phosphomonoesterase and alkaline phosphomonoesterase activities at both flowering and harvest stages. This indicates that the proliferation of beneficial microbes enhanced soil enzymatic

Table 5. Effect of inoculation of *Rhizobium* and PSB in combination and alone on the dehydrogenase enzymatic activity in soil at different stages of crop growth

Treatments	**Dehydrogenase enzymatic activity (µg TPF g^{-1} soil hr^{-1})**	
	At 50 % flowering	**At harvesting**
T_1- Control	83.54^c	16.13^d
T_2- 100 % RDF	90.90^{bc}	31.74^b
T_3- *Rhizobium* alone at 10 mL kg^{-1}	83.85^c	23.62^c
T_4- PSB alone at 10 mL kg^{-1}	85.00^c	27.62^{bc}
T_5- *Rhizobium* at 10 mL kg^{-1} + 25 % N+ 100 % PK	102.15^{abc}	31.98^b
T_6- PSB at 10 mL kg^{-1} + 25 % P+ 100 % NK	114.62^{ab}	41.94^a
T_7- *Rhizobium* at 10 mL kg^{-1} + PSBat 10 mL kg^{-1} + 75 % NP+ 100 % K	128.82^a	58.43^a
SeM(±)	8.75	1.98
C D (*P* = 0.05)	26.96	6.09

Values followed by the same small letters do not differ significantly ($p < .05$) between treatments.

Table 6. Effect of inoculation of *Rhizobium* and PSB in combination and alone on the acid phosphomonoesterase activity in soil at different stages of crop growth

Treatments	**Acid phosphomonoesterase activity (mg p NP g^{-1} soil hr^{-1})**	
	At 50 % flowering	**At 50 % flowering**
T_1- Control	0.20^e	0.20^e
T_2- 100 % RDF	0.20^e	0.20^e
T_3- *Rhizobium* alone at 10 mL kg^{-1}	0.21^d	0.21^d
T_4- PSB alone at 10 mL kg^{-1}	0.22^c	0.22^c
T_5- *Rhizobium* at 10 mL kg^{-1} + 25 % N+ 100 % PK	0.24^a	0.24^a
T_6- PSB at 10 mL kg^{-1} + 25 % P+ 100 % NK	0.24^a	0.24^a
T_7- *Rhizobium* at 10 mL kg^{-1} + PSBat 10 mL kg^{-1} + 75 % NP+ 100 % K	0.25^a	0.25^a
SeM(±)	0.01	0.01
C D (*P* = 0.05)	0.03	0.03

Values followed by the same small letters do not differ significantly ($p < .05$) between treatments.

Table 7. Effect of inoculation of *Rhizobium* and PSB in combination and alone on the alkaline phosphomonoesterase activity in soil at different stages of crop growth

Treatments	Alkaline phosphomonoesterase activity (mg p NP g^{-1} soil hr^{-1})	
	At 50 % flowering	At 50 % flowering
T_1- Control	0.20^e	0.20^e
T_2- 100 % RDF	0.32^d	0.32^d
T_3- *Rhizobium* alone at 10 mL kg^{-1}	0.35^c	0.35^c
T_4- PSB alone at 10 mL kg^{-1}	0.40^b	0.40^b
T_5- *Rhizobium* at 10 mL kg^{-1} + 25 % N+ 100 % PK	0.40^b	0.40^b
T_6- PSB at 10 mL kg^{-1} + 25 % P+ 100 % NK	0.47^a	0.47^a
T_7- *Rhizobium* at 10 mL kg^{-1} + PSBat 10 mL kg^{-1} + 75 % NP+ 100 % K	0.51^a	0.51^a
SeM(±)	0.01	0.01
C D (P = 0.05)	0.03	0.03

Values followed by the same small letters do not differ significantly (p <.05) between treatments.

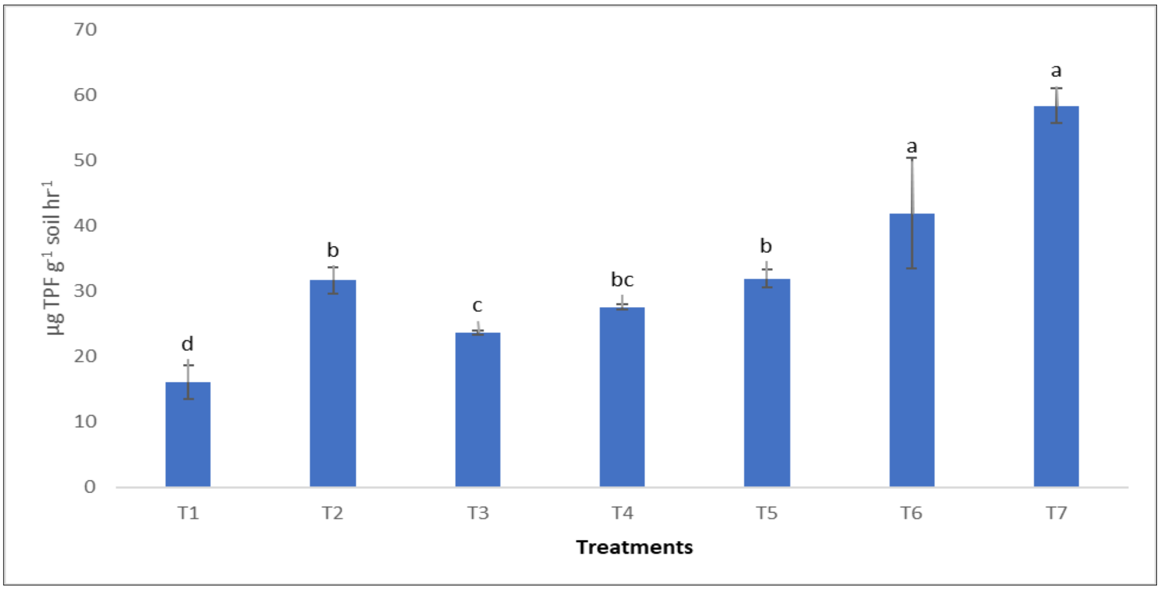

Fig. 4. Effect of inoculation of *Rhizobium* and PSB in combination and alone on the dehydrogenase enzymatic activity in soil at different stages of crop growth.

Values are means and bars represent S D. Different letters indicate significant ($p \leq 0.05$) differences between means.

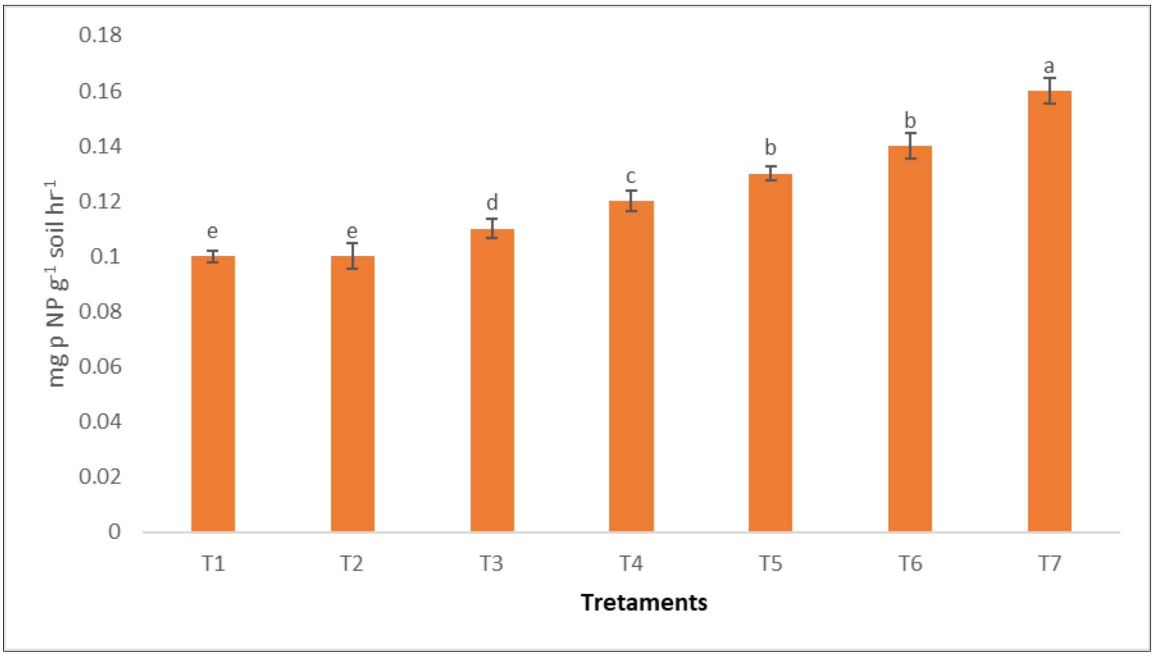

Fig. 5. Effect of inoculation of *Rhizobium* and PSB in combination and alone on the acid phosphomonoesterase activity in soil at different stages of crop growth.

Values are means and bars represent S D. Different letters indicate significant ($p \leq 0.05$) differences between means.

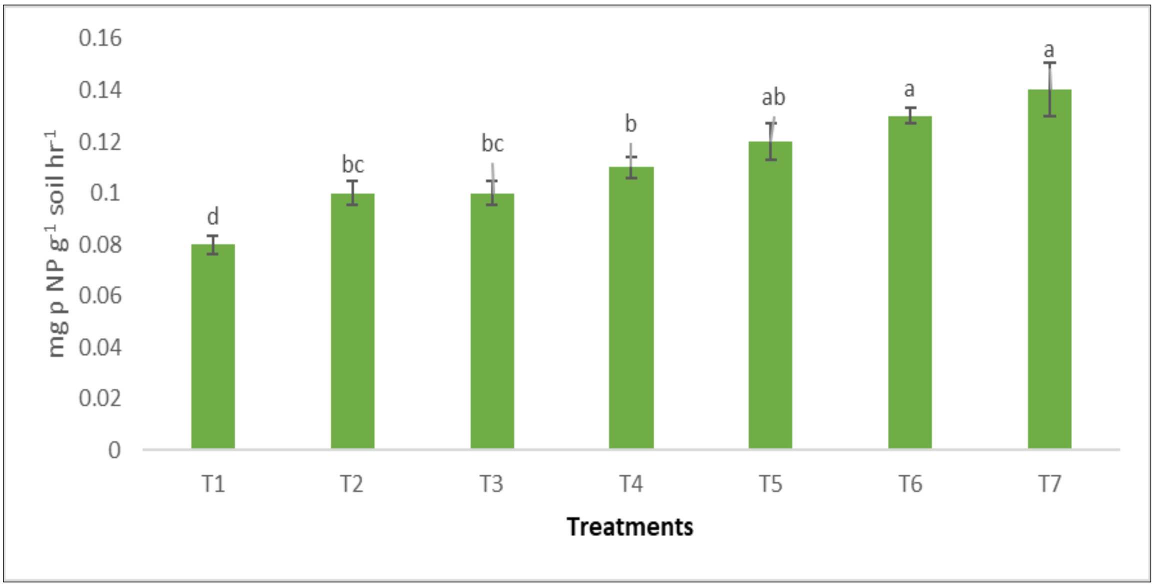

Fig. 6. Effect of inoculation of *Rhizobium* and *Pseudomonas* sp. in combination and alone on the alkaline phosphomonoesterase activity in soil at different stages of crop growth.

Values are means and bars represent S D. Different letters indicate significant ($p \leq 0.05$) differences between means.

activities by promoting organic matter decomposition and nutrient cycling. The results confirm that higher microbial abundance directly contributes to greater enzymatic activity, reflecting improved soil biological health and P transformation efficiency under combined microbial inoculation.

Correlation study

A Pearson correlation study was carried out to assess the relationship between soil P fractions and yield (Table 8). Soluble Pi fraction was found negatively correlated with labile organic P fraction. The Al-Pi was found significantly ($p \leq 0.01$) positively correlated with the entire Pi and Po fractions. Similar trends were observed with Fe_1-Pi fraction and Ca-Pi fraction, respectively. Labile Po fraction was observed significantly positively correlated with moderately labile Po ($p \leq 0.776$). The results are in correspondence with the findings who conducted an experiment and reported that soluble Pi was found to have a negative correlation with the residual P. They also found that Al-Pi had significantly positive correlation with the inorganic and organic P fractions, except residual inorganic P.

Conclusion

The results revealed significant improvement in soil soluble P, P fractions, microbial population and enzymatic activity in P low soil. The co-inoculation of *Rhizobium bangladeshense* and *Pseudomonas* sp. alone or combined with mineral fertilizers improved the P availability and grain yield. The use of *Rhizobium bangladeshense* at 10 mL kg^{-1} seed + *Pseudomonas* sp. at 10 mL kg^{-1} seed + 75 % NP + 100 % K significantly increased

Table 8. Correlation between the analyzed parameters

	Soluble P	Al-P	Fe1-P	Ca-P	Fe2-P	Residual P	Labile-OP	ML-OP	NL-OP	Dehydrogenase	Acid phosphomonoesterase	Alkaline phosphomonoesterase	Grain yield
Soluble P	1.000**												
Al-P	0.603**	1.000**											
Fe1-P	0.575**	0.894**	1.000**										
Ca-P	0.602**	0.882**	0.801**	1.000**									
Fe2-P	0.775**	0.884**	0.874**	0.796**	1.000**								
Residual P	0.594**	0.823**	0.703**	0.853**	0.804**	1.000**							
Labile-OP	0.409NS	0.800**	0.777**	0.673**	0.710**	0.706**	1.000**						
ML-OP	0.668**	0.887**	0.877**	0.905**	0.858**	0.848**	0.776**	1.000**					
NL-OP	0.577**	0.928**	0.862**	0.851**	0.811**	0.771**	0.819**	0.856**	1.000**				
Dehydrogenase	0.466*	0.701**	0.636**	0.628**	0.669**	0.741**	0.742**	0.664**	0.699**	1.000**			
Acid phosphomonoesterase	0.859**	0.752**	0.696**	0.733**	0.774**	0.702**	0.600**	0.813**	0.693**	0.446*	1.000**		
Alkaline phosphomonoesterase	0.875**	0.721**	0.793**	0.726**	0.825**	0.562**	0.567**	0.794**	0.730**	0.420NS	0.870**	1.000**	
Grain yield	0.475*	0.917**	0.853**	0.828**	0.779**	0.792**	0.898**	0.868**	0.950**	0.734**	0.658**	0.648**	1.000**

*= significant at 5 %, ** = significant at 1 % and NS = non-significant.

inorganic, organic P fractions and yield, respectively, over the control and application of 100 % mineral fertilizer. Thus, combined use of microbial inoculants and inorganic chemical fertilizers may be recommended instead of chemical fertilizer application alone to reduce the dependance on chemical fertilizer, to have improvement in soil health for sustainable production of black gram in the low P soil.

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Authors' contributions

AR carried out the conduction of the experiment, soil chemical and microbiological analysis and preparation of manuscript draft. MS carried out the design of experiment and editing of the manuscript draft, AKP carried out the editing of manuscript. AY carried out the interpretation of data. AS carried out the preparation of the manuscript. RK carried out the editing of the manuscript. NS carried out reference editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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