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Plant Growth, Nodulation, Soil Biological Properties, Nutrient Content, Uptake and Yield of Black Gram (*Vigna mungo* L.) as Influenced with Co-Inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. under Low Phosphorus Soil

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ABSTRACT

The study aimed to examine the inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. on the nodulation, growth, enzymatic activity, phosphorus uptake and yield of black gram in P deficient soil. The experimental findings revealed that the combined inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+*Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% NP +100% K yielded the highest nodulation (42), plant height (43 cm $plant^{-1}$), available N (154.96 kg ha^{-1}), available P (16.77 kg ha^{-1}), available K (278.20 kg ha^{-1}). This treatment was also found statistically ($p \leq 0.05$) at par with the application of *Pseudomonas* sp. @ 10 ml kg^{-1} seed+ 75% P+100% NK for root nodulation, plant height, available NPK. As for grain and straw, the nutrient content showed non-significant effects for all the treatments while the nutrient uptake was observed to be highest in the treatment including the combined inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+*Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% NP+100% K. The grain yield (11.83 q ha^{-1}), stover yield (26.70 q ha^{-1}) and biological yield (38.53 q ha^{-1}) was found to be highest in the treatment including combined inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+ *Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% NP+100% K. Hence, these inoculants can be recommended to increase the yield of black gram while mitigating the effect of P deficiency in low P soil.

Keywords Phosphorus, *Pseudomonas, Rhizobium*, Nodulation, Nutrient uptake.

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INTRODUCTION

Black gram (*Vigna mungo* L.) is considered as one of the most important pulse crops that is grown throughout the nation. Black gram, being a summer pulse crop has a short duration for maturity period of just 90 days (Hussain *et al.* 2011). The nutritional composition of black gram comprises of 24% protein, 60% carbohydrates, and 1.3% fat (Molla *et al.* 2024). Despite its importance in nutritional aspects, the yield of black gram is not as much in comparison to other legumes (Siddikee *et al.* 2019). Poor fertility of soil serves as an important factor for the low productivity of black gram (Ghannam *et al*. 2022). The availability of nitrogen (N) and phosphorus (P) ranges between medium to low in Indian soils (Shekhawat *et al.* 2018). The application of P in the soil system leads to a significant increment in the yield of legumes along with the nutritional quality as well (Patel *et al.* 2019). However, the growth and productivity of legume crops are severely restrained if P is low in the soil system, ultimately leading to the reduction in nodule formation, development, and function (Alikhani *et al.* 2006). Despite applications of chemical fertilizers, the growth of black gram constantly seems to show signs of stagnation or low productivity (Jangir *et al.* 2016). This is due to the fact that a significant amount of phosphorus applied through fertilizers can be absorbed by the soil, resulting in it becoming unavailable for plants to utilize (Balemi and Bayissa 2012). Also, the repeated overuse of P fertilizers results in the disturbance of the soil microbial diversity (Huang *et al.* 2016). However, the use of microbial bio-inoculants such as *Rhizobium* and *Pseudomonas* can help in increasing the productivity of black gram as they help in solubilizing the insoluble phosphorus in the soil and make it available for the plant uptake along with the fixation of nitrogen (Peix *et al.* 2001). *Rhizobium* and PSB are also extremely important because they play an important function in N fixation and solubilization of P in the soil (Reddy *et al.* 2024). They are also a cost-effective, sustainable, and renewable source of plant nutrients as compared to chemical fertilizers (Khan *et al.* 2007). *Rhizobium* while possessing the capacity to fix nitrogen, also aid in the root colonization of non-legumes (Chabot *et al.* 1996, Schloter *et al.* 1997) while also stimulating the growth of the plants (Antoun *et al.* 1998, Yanni *et al.* 2001). Meanwhile, *Pseudomonas* sp. produces several growth promoting phytohormones such as IAA, gibberellins and cytokinins which ultimately enhance the root growth and branching for better nutrient and water absorption, thus, increasing the plant growth and yield (Swarnalakshmi *et al.* 2020).

MATERIALS AND METHODS

The field experiment was conducted at Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India during the summer season of the year 2023-2024 to evaluate the inoculation of *Rhizobium* and *Pseudomonas* sp. for growth, nodulation, nutrient availability, uptake of nutrient and yield of black gram under low P soil. The experimental site is placed in the Eastern Plain agro-climatic zone of Uttar Pradesh at 26.7068º N latitude and 82.1336º E longitude with an elevation of 110 meters above the sea level. The optimum temperature during the experimental period was between 23.1℃ to 35.6 ℃. The soil was taxonomically a Typic Ustochrept and silt loam in texture. The soil samples were air dried and passed through a 2 mm IS sieve. Detailed initial characteristics of soil has been mentioned in Table 1. A field experiment was conducted in a Randomized Block Design (RBD) with 7 treatments replicated thrice viz., T_1-Control (no fertilizer), T_2- 100% recommended dose of fertilizers (RDF) i.e., 20:40:20 (N: P_2O_5: K_2O), T_3-inoculation of *Pseudomonas* sp. alone @ 10 ml kg^{-1} seed, T_4-Inoculation of *Rhizobium bengladensis* alone @ 10 ml kg^{-1} seed, T_5- Inoculation of *Rhizobium bengladensis* alone @ 10 ml kg^{-1} seed+25% N+ 100% PK, T_6-Inoculation of *Pseudomonas* sp. alone @ 10 ml kg^{-1} seed+25% P+100% NK, T_7-Co-inoculation *Rhizobium bengladensis* @ 10 ml kg^{-1} seed and *Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% NP + 100% K. K was applied uniformly irrespective of all the treatments from T_2 to T_7. Urea, single super phosphate and muriate of potash was used for nitrogen

Table 1. Initial characteristics of field experimental soil.

Sl. No.	Property	Initial value
1	Soil texture	Silt loam
2	Sand	25.14
3	Silt	48.90
4	Clay	25.96
5	Soil bulk density	1.32
6	Soil particle density	2.60
7	pH	8.20
8	Electrical conductivity (ds m^{-1})	0.345
9	Oxidizable soil organic carbon (OC%)	0.42
10	Available N (kg ha^{-1})	130.58
11	Available P (kg ha^{-1})	10.98
12	Available K (kg ha^{-1})	245.70
13	Bacterial count (10^6 cfu g^{-1} of soil)	13.5
14	Fungal count (10^4 cfu g^{-1} of soil)	7.5
15	Actinomycetes count (10^5 cfu g^{-1} of soil)	10.5
16	*Rhizobium* (10^6 cfu g^{-1} of soil)	13.0
17	PSB (10^6 cfu g^{-1} of soil)	12.5
18	Soil microbial biomass carbon (µg g^{-1} soil)	225.25

Table 2. Characteristics of used bacteria in the study.

Parameters	*Rhizobium bengladensis*	*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.

(N), phosphorus (P) and potassium (K) fertilization, respectively. The inoculation of seeds was done with *Rhizobium bengladensis* and *Pseudomonas* sp. @ 10 ml kg seed with cfu 1.0×10^9 mL^{-1}. The cultures were procured from biofertilizer production unit, Bihar bAgricultural University, Sabour, Bihar, India and the characteristics of inoculants used has been presented in Table 2. All the phosphatic and potassic fertilizers were applied as basal application before sowing while 50% of N was applied as basal application; 25% was top-dressed at the tillering stage; and another 25% was top-dressed at the pod formation stage. The irrigation has applied as per requirement of the crop. The soil samples were collected in poly bags treatment wise after harvest of the crop. After proper mixing the soil was sieved through a 2 mm IS sieve. The texture was determined by the triangular method as prescribed by Lyon *et al.* (1952) and the pH was evaluated by keeping a 1:2.5, soil: water suspension as prescribed by Jackson (1973) whereas the EC of soil samples was analyzed by soil water suspension method (1:2, soil: water) as prescribed by Jackson (1973). The oxidizable organic carbon was measured by wet digestion method as prescribed by Walkley & Black (1934). The available soil N was analyzed by alkaline permanganate method as postulated by Subbaiah & Asija (1956) while available P was determined by method outlined by Olsen *et al*. (1954). Meanwhile, the available K of soil samples was analyzed by treating soil with neutral normal ammonium acetate (NH_4OAc) with using flame photometer as prescribed by Black (1965). To assess the nutrient content and uptake, the samples were collected for chemical analysis of N, P and K content in grain and straw. The N content was determined with Micro Kjeldhal's method (Jackson 1967), K estimation was done by digesting the samples as mentioned by Hanway and Heidel (1952). However, the P content was determined by vandomolybdo phosphoric acid yellow colored method in HNO_3 system as described by Jackson (1974).

Rhizospheric soil samples were obtained by collecting the soil adhering to the roots and root hairs at flowering and at harvesting stage. The 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 (Thom & Raper 1945), Kenknight and Munaier's Medium, Yeast Extract Mannitol Agar medium and Pikovaskaya's Medium (Rao & Sinha 1963) respectively were used. The numbers of colony forming cells were determined in each plot by serial dilution pour plate method (Subba Rao 1982). The activities of soil enzymes: Dehydrogenase activity (Casida *et al.* 1964), acid phosphatase and alkaline phosphatase (Tabatabai & Bremner 1969) were determined. Data were subjected to statistical analysis using statistical package for the social sciences 20.0 (IBM SPSS 20). The effects of the experimental factors studied were determined by one-way analysis of variance (ANOVA). Mean comparisons were carried out by the Duncan ($p\leq0.05$) test. The statistical analysis conducted in this experiment was followed by the standard protocol as IBM Corp (2020).

RESULTS AND DISCUSSION

Plant growth parameters

Statistical analysis of recorded data revealed that co-inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed and *Pseudomonas* sp. @ 10 ml kg^{-1} seed along with the application of 75% NP+ 100% K significantly ($p\leq0.05$) increased the plant height, plant fresh weight and dry weight as compared to the uninoculated control treatment after 40, 50, and 60 days of sowing (Table 3). The maximum height of the plant was obtained at 40, 50 and 60 DAS was recorded in the treatment including the co-inoculation

Table 3. Effect of inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. alone and in combination on plant growth parameters weight of black gram (*Vigna mungo* L.).

Treatments	Plant height (cm plant^{-1})			Plant fresh weight (g plant^{-1})		Plant dry weight (g plant^{-1})	
	At 40 DAS	At 50 DAS	At 60 DAS	At 45 DAS	After harvest	At 45 DAS	After harvest
T_1- Control	16.46^{d}	25.00^{c}	31.31^{b}	29.33^{d}	16.67^{e}	6.40^{c}	4.38^{b}
T_2- 100% RDF	25.43^{bc}	36.07^{ab}	38.00^{a}	31.67^{cd}	17.50^{e}	7.27^{abc}	4.40^{b}
T_3- *Rhizobium* alone @ 10 ml kg^{-1}	23.83^{c}	33.00^{b}	39.80^{a}	31.67^{cd}	21.01^{d}	6.33^{c}	4.53^{b}
T_4- PSB alone @ 10 ml kg^{-1}	24.87^{bc}	36.33^{ab}	41.33^{a}	36.13^{bc}	26.33^{c}	7.00^{bc}	4.74^{b}
T_5- *Rhizobium* @ 10 ml kg^{-1} + 75% N+ 100% PK	25.86^{bc}	35.74^{ab}	41.58^{a}	38.18^{ab}	28.00^{bc}	7.35^{abc}	4.67^{b}
T_6- PSB @ 10 ml kg^{-1} + 75% P +100% NK	28.43^{ab}	37.16^{ab}	42.44^{a}	40.34^{ab}	31.13^{ab}	8.50^{ab}	5.80^{ab}
T_7- *Rhizobium* @ 10 ml kg^{-1} + PSB @ 10 ml kg^{-1} + 75% NP + 100% K	29.56^{a}	38.00^{a}	43.00^{a}	43.00^{a}	34.34^{a}	9.00^{a}	6.73^{a}
SeM (±)	1.10	1.39	1.57	0.56	0.37	0.56	0.48
CD (p=0.05)	3.38	4.29	4.83	1.72	1.14	1.73	1.48

Values followed by the same small letters do not differ significantly ($p < 0.05$) between treatments.

of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed and *Pseudomonas* sp. @ 10 ml kg^{-1} seed along with the application of 75% NP+100% K. Similar results were obtained for the plant dry weight at both 45 DAS and after harvest. However, the highest fresh weight of the plant at 45 DAS and after harvest was obtained in the treatment including the inoculation of *Pseudomonas* sp. @ 10 ml kg^{-1} seed along with the application of 75% P+100% NK. The similar trend was observed for dry weight. This might be due to the more availability of the nitrogen and phosphorus to the plants which improve overall plant growth. The results are aligned with the findings of Ghannam *et al.* (2022), who carried out a field experiment to evaluate the effect of biofertilizers on the growth and yield of black gram and reported that the inoculation of *Rhizobium*+ PSB+ 100% NPK significantly increased plant height over control treatment.

Root nodulation

The data pertaining to nodulation is presented in Table 4. The recorded data revealed that combined inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed +*Pseudomonas* sp. @ 10 ml kg^{-1} seed +75% NP + 100% K significantly ($p \leq 0.05$) increased the number of nodules plant^{-1}, nodule fresh weight and nodule dry weight by 50%, 51.49% and 52%, as compared to uninoculated control and 25.87%, 27.03% and 29.41% as compared to 100% recommended dose

Table 4. Response of inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. alone and in combination on the root nodulation of black gram (*Vigna mungo* L.).

Treatments	Number of root nodules plant^{-1}	Nodule fresh weight (g plant^{-1})	Nodule dry weight (g plant^{-1})
T_1- Control	21.00^{d}	0.81^{e}	0.24^{e}
T_2- 100% RDF	28.33^{c}	1.11^{cd}	0.34^{cd}
T_3- *Rhizobium* alone @ 10 ml kg^{-1}	26.67^{c}	1.03^{de}	0.31^{de}
T_4- PSB alone @ 10 ml kg^{-1}	27.33^{c}	1.06^{de}	0.32^{cde}
T_5- *Rhizobium* @ 10 ml kg^{-1} + 75% N+ 100% PK	32.00^{b}	1.31^{bc}	0.39^{bc}
T_6- PSB @ 10 ml kg^{-1} + 75% P+ 100% NK	37.33^{a}	1.46^{ab}	0.44^{ab}
T_7- *Rhizobium* @ 10 ml kg^{-1}+ PSB@ 10 ml kg^{-1}+ 75% NP+ 100% K	42.00^{a}	1.67^{a}	0.50^{a}
SeM (±)	0.83	0.08	0.02
CD (p=0.05)	2.56	0.24	0.07

Values followed by the same small letters do not differ significantly ($p < 0 .05$) between treatments.

Table 5. Response of individual and combined inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. on the chemical properties of soil and the availability of essential soil nutrients- viz., nitrogen, phosphorus and potassium.

Treatments	Soil pH	Soil EC (dS m^{-1})	Soil OC (% C)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
T_1- Control	8.17^{a}	0.439^{a}	0.45^{a}	137.78^{d}	12.90^{d}	251.73^{e}
T_2- 100% RDF	7.87^{ab}	0.437^{a}	0.45^{a}	143.93^{bc}	14.93^{bc}	262.83^{b}
T_3- *Rhizobium* alone @ 10 ml kg^{-1}	7.63^{bc}	0.376^{a}	0.45^{a}	141.95^{c}	13.57^{d}	254.63^{d}
T_4- PSB alone @ 10 ml kg^{-1}	7.73^{bc}	0.260^{a}	0.46^{a}	142.60^{bc}	14.50^{C}	259.67^{c}
T_5- *Rhizobium* @ 10 ml kg^{-1} + 75% N+ 100% PK	7.57^{bc}	0.328^{a}	0.47^{a}	145.33^{b}	15.23^{b}	265.33^{b}
T_6- PSB @ 10 ml kg^{-1} + 75% P+ 100% NK	7.63^{bc}	0.307^{a}	0.47^{a}	151.31^{a}	16.10^{a}	271.57^{a}
T_7- *Rhizobium* @ 10 ml kg^{-1}+PSB@ 10 ml kg^{-1}+75% NP+ 100% K	7.50^{c}	0.284^{a}	0.47^{a}	154.96^{a}	16.77^{a}	278.20^{a}
SeM (±)	0.11	0.07	0.01	2.17	0.22	0.90
CD (p=0.05)	0.35	NS	NS	6.68	0.69	2.79

Values followed by the same small letters do not differ significantly (p <0.05) between treatments.

of chemical fertilizer. Meanwhile, this treatment was found to be statistically (p≤0.05) at par with the inoculation of *Pseudomonas* sp. @ 10 ml kg^{-1} +75% P +100% NK. The increase in the nodulation might be due to the production of phytohormones and nod factors which trigger the formation of nodules in plant roots. The findings are in conformity with the results of Reddy *et al.* (2024), who found that the combined inoculation of *Rhizobium* sp. and PSB led to the highest nodulation density plant^{-1} in legume crops.

Chemical properties of soil

Soil pH, EC and OC

It was noted that the pH of the soil gradually decreased with the inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. as compared to control (Table 5). However, the statistical analysis of data showed that the soil EC and organic carbon was found to be non-significant among the treatments. The results were in accordance with the findings of Khan *et al.* (2022), who conducted a field experiment to evaluate the efficacy of PSB in enhancing the mungbean P acquisition, N fixation and morphological and yield traits in alkaline calcareous soil.

Available nutrients

The available soil nitrogen, phosphorus and potash recorded significant changes with the application of combined inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+*Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% NP+100% K and the inoculation of *Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% P+100% NK were also found to be statistically (p≤0.05) at par with each other. The available N were recorded to be in the range between 137.78 to 154.96 kg ha^{-1}, 12.90 to 16.77 kg ha^{-1} for available P and 251.73 to 278.20 kg ha^{-1} for available K. The increase in the soil available N may be due to the nitrogen fixation activity by *Rhizobium bengladensis* while the increase in the soil available P may be due to the secretion of organic acids such as gluconic acid, citric acid, lactic acid, by *Pseudomonas* sp. which leads to the solubilization of insoluble phosphate ions into soluble forms. However, the increase in the available K might be due to the mobilization of K due to the acidification and chelation activity by *Pseudomonas* sp. These findings are found to be in correspondence with the findings of Molla *et al.* (2024), who conducted a field experiment to evaluate the effect of seed inoculation of *Rhizobium* and organic nutrient sources at different levels on the growth and yield of black gram.

Microbial population

It was interesting to note that the microbial population decreased after harvest of the crop as compared with the population recorded after 50% flowering stage (Table 6). It might be due to the fact that the microbes die as the loss of root exudates after the harvest of the crop leads to the removal of the primary source of energy for most of the microbes present in the rhizosphere. The statistical analysis of reported data revealed that the co-inoculation of *Rhizobium*

Table 6. Response of inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. in combination and alone on 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 @ 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 @ 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* @ 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 @ 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* @ 10 ml kg^{-1} + PSB@ 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
CD (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 <0.05) between treatments.

bengladensis @ 10 ml kg^{-1} seed+*Pseudomonas* sp. @ 10 ml kg^{-1} seed + 75% NP+100% K resulted in the highest microbial population for all the microbes at both stages. This treatment was found to be statistically (p≤0.05) at par with the inoculation of *Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% P+100% NK while significantly (p≤0.05) increasing as compared with control, application of 100% dose of fertilizer, inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed, inoculation of *Pseudomonas* sp. @ 10 ml kg^{-1} seed and inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+75% N+100% PK. This might be due to the fixation of nitrogen and the activity of root exudate which acts as an energy source for a wide range of microbes. The results are in agreement with the findings of Tilak *et al.* (2006), who stated that the combined inoculation of *Rhizobium* and *Pseudomonas* results in the higher activity of root exudates which significantly enhances the activity of microbes.

Enzymatic activity

It is worth mentioning that the enzymatic activity of all three 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 7). 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 (Zhang *et al.* 2019). Similar findings were found to be reported by Galazka *et al.* (2017), who conducted a field experiment to examine the effects of long-term maize monoculture and crop rotation on the biological activities of soil, especially the soil enzymatic activities and microbial communities. They reported that the enzymatic activity was at peak during 50% flowering stage and gradually declined after the harvest of the crop.

The statistical analysis revealed that the application of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+ *Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% NP+100% K significantly increased dehydrogenase enzymatic, acid phosphomonoesterase and alkaline phosphomonoesterase activities by 29.43%, 34.90%, 34.02%, 20%, 16%, 16.64%, 37.25%, 31.37% and 21.56% over control, 100% application of recommended dose of fertilizers, inoculation of *Rhizobium bengladensis* alone @ 10 ml kg^{-1} seed and inoculation of *Pseudomonas* sp. alone @ 10 ml kg^{-1} seed and found to be

Table 7. Response of the inoculation of *Rhizobium* and PSB in combination and alone on the enzymatic activity in soil.

Treatments	Dehydrogenase enzymatic activity (μg TPF g^{-1} soil hr^{-1})		Acid phosphomonoesterase activity (mg p NP g^{-1} soil hr^{-1})		Alkaline phosphomonoesterase activity (mg p NP g^{-1} soil hr^{-1})	
	At 50% flowering	At harvesting	At 50% flowering	At harvesting	At 50% flowering	At harvesting
T_1- Control	83.54^{c}	16.13^{d}	0.20^{e}	0.10^{e}	0.20^{e}	0.08^{d}
T_2- 100% RDF	90.90^{bc}	31.74^{b}	0.20^{e}	0.10^{e}	0.32^{d}	0.10^{bc}
T_3- *Rhizobium* alone @ 10 ml kg^{-1}	83.85^{c}	23.62^{C}	0.21^{d}	0.11^{d}	0.35^{c}	0.10^{bc}
T_4- PSB alone @ 10 ml kg^{-1}	85.00^{c}	27.62^{bc}	0.22^{c}	0.12^{c}	0.40^{b}	0.11^{b}
T_5- *Rhizobium* @ 10 ml kg^{-1} + 75% N+ 100% PK	102.15^{abc}	31.98^{b}	0.24^{a}	0.13^{b}	0.46^{a}	0.12^{ab}
T_6- PSB @ 10 ml kg^{-1} + 75% P + 100% NK	114.62^{ab}	41.94^{a}	0.24^{a}	0.14^{b}	0.47^{a}	0.13^{a}
T_7- *Rhizobium* @ 10 ml kg^{-1} + PSB@ 10 ml kg^{-1} + 75% NP+ 100% K	128.82^{a}	58.43^{a}	0.25^{a}	0.16^{a}	0.51^{a}	0.14^{a}
SeM (±)	8.75	1.98	0.01	1.98	0.01	0.00
CD (p=0.05)	26.96	6.09	0.03	6.03	0.03	0.01

Values followed by the same small letters do not differ significantly ($p < 0.05$) between treatments.

statistically ($p \leq 0.05$) at par with the application of *Rhizobium bengladensis* @ 10 ml kg^{-1}+75% N+ 100% PK and the application of *Pseudomonas* sp. @ 10 ml kg^{-1} seed + 75% P +100% NK 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 bengladensis* and *Pseudomonas* sp. The results are in align with findings of Siczek and Lipiec (2016), who reported that the microbial activity stimulated by *Rhizobium* inoculation contributed to the release of several different enzymes which increased their activity in the soil.

Nutrient content and their uptake in grain and straw

It was interesting to find that the treatments had non-significant effects for nutrient (N, P, K) content for both grain and Table 8. A significant increment

Table 8. Response of individual and combined inoculation of *Rhizobium bengladensis* and *Pseudomonas* sp. on the nutrient content and their uptake in grain and straw in black gram (*Vigna mungo* L.).

Treatments	N content (%)		N uptake (kg ha^{-1})		P content (%)		P uptake (kg ha^{-1})		K content (%)		K uptake (kg ha^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T_1- Control	3.80^{a}	1.40^{a}	31.93^{d}	29.80^{e}	0.41^{a}	0.23^{a}	3.47^{e}	4.90^{d}	0.90^{a}	1.03^{a}	21.86^{e}	7.59^{e}
T_2- 100% RDF	3.81^{a}	1.40^{a}	36.72^{c}	30.77^{e}	0.42^{a}	0.23^{a}	4.64^{b}	5.42^{c}	0.90^{a}	1.06^{a}	44.91^{b}	9.43^{bc}
T_3- *Rhizobium* alone @ 10 ml kg^{-1}	3.82^{a}	1.44^{a}	35.67^{c}	33.65^{c}	0.42^{a}	0.23^{a}	3.93^{d}	5.05^{d}	0.92^{a}	1.07^{a}	23.23^{d}	8.46^{d}
T_4- PSB alone @ 10 ml kg^{-1}	3.82^{a}	1.44^{a}	37.21^{bc}	31.68^{d}	0.42^{a}	0.23^{a}	4.06^{cd}	5.06^{cd}	0.92^{a}	1.09^{a}	23.54^{d}	9.00^{c}
T_5- *Rhizobium* @ 10 ml kg^{-1} +75% N+100% PK	3.83^{a}	1.46^{a}	39.71^{ab}	36.45^{b}	0.48^{a}	0.24^{a}	4.37^{bc}	5.82^{b}	0.97^{a}	1.97^{a}	27.59^{c}	9.57^{b}
T_6- PSB @ 10 ml kg^{-1}+ 75% P+ 100% NK	3.83^{a}	1.46^{a}	40.34^{a}	38.07^{ab}	0.49^{a}	0.24^{a}	5.06^{a}	6.26^{a}	0.98^{a}	1.99^{a}	51.44^{a}	10.22^{a}
T_7- *Rhizobium* @ 10 ml kg^{-1} + PSB@ 10 ml kg^{-1} + 75% NP + 100% K	3.85^{a}	1.49^{a}	45.30^{a}	38.97^{a}	0.49^{a}	0.24^{a}	5.76^{a}	6.41^{a}	0.99^{b}	1.99^{a}	53.21^{a}	11.58^{a}
SeM (±)	0.03	0.02	0.96	0.58	0.01	0.00	0.13	0.12	0.01	0.01	0.44	0.16
CD (p=0.05)	NS	NS	2.95	1.79	NS	NS	0.39	0.36	NS	NS	1.35	0.49

Values followed by the same small letters do not differ significantly ($p < 0 .05$) between treatments.

Table 9. Response of *Rhizobium bengladensis* and *Pseudomonas* sp. inoculation, alone and in combination on the yield parameters of black gram (*Vigna mungo* L.).

Treatments	Grain yield (q ha^{-1})	Stover yield (q ha^{-1})	Biological yield (q ha^{-1})	Harvest index (%)
T_1- Control	8.40^{c}	21.29^{d}	29.69^{c}	28.30^{c}
T_2- 100% RDF	9.54^{B}	22.58^{c}	32.12^{b}	29.88^{ab}
T_3- *Rhizobium* alone @ 10 ml kg^{-1} of seed	9.37^{b}	21.97^{cd}	31.34^{b}	30.67^{a}
T_4- PSB alone @ 10 ml kg^{-1} of seed	9.73^{b}	22.00^{cd}	31.73^{b}	29.11^{bc}
T_5- *Rhizobium* @ 10 ml kg^{-1} of seed + 75% N+ 100% PK	10.40^{ab}	25.32^{b}	35.72^{a}	28.78^{bc}
T_6- PSB @ 10 ml kg^{-1} of seed + 75% P+ 100% NK	10.53^{ab}	26.07^{ab}	36.60^{a}	30.70^{a}
T_7- *Rhizobium* @ 10 ml kg^{-1} of seed + PSB@ 10 ml kg^{-1} of seed + 75% NP+ 100% K	11.83^{a}	26.70^{a}	38.53^{a}	29.69^{ab}
SeM (±)	0.21	0.28	0.38	0.44
CD (p=0.05)	0.65	0.88	1.16	1.36

Values followed by the same small letters do not differ significantly (p <0.05) between treatments.

of 29.51%, 39.75% and 58.91% for grain N, P and K uptake and a significant increment of 23.53%, 23.55% and 34.45% for straw N, P and K uptake was recorded with co-inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+*Pseudomonas* sp. @ 10 ml kg^{-1} seed + 75% NP +100% K over control, respectively. The findings are in match with the findings obtained by Tewari *et al.* (2022), who had conducted a field experiment to evaluate the effect of different nutrient mobilizers accompanied by the recommended dose of fertilizers on the productivity and nutrient uptake in black gram. They found that the nutrient uptake in grain and straw was found to be significantly higher due to the application of *Rhizobium* while the nutrient content in grain and straw was recorded to have non-significant difference.

Yield and yield parameters

The data presented in Table 9 showed that the co-inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed+*Pseudomonas* sp. @ 10 ml kg^{-1} seed+75% NP+ 100% K recorded significantly higher grain (11.83 q ha^{-1}) and biological yield (38.53 q ha^{-1}) as compared to treatments control, 100% recommended dose of chemical fertilizers, inoculation of *Rhizobium bengladensis* @ 10 ml kg^{-1} seed and inoculation of *Pseudomonas* sp. @ 10 ml kg^{-1} seed, respectively. This treatment also led to an increment of 30.08%, 20.26%, 29.77% and 4.68% was obtained for grain yield, stover yield, biological yield and harvest index, respectively, over control. The outcomes aligned closely with the previous research by Kumar & Singh

Table 10. Correlation between the analyzed parameters.

	pH	OC	EC	Available N	Available P	Straw N uptake	Grain N uptake	Straw P uptake	Grain P uptake	Grain yield
pH	1.000**									
OC	-0.274^{NS}	1.000**								
EC	0.343^{NS}	-0.040^{NS}	1.000**							
Available N	-0.416^{NS}	0.593**	-0.233^{NS}	1.000**						
Available P	-0.550**	0.503*	-0.404^{NS}	0.825**	1.000**					
Straw N uptake	-0.581**	0.515*	-0.457*	0.792**	0.914**	1.000**				
Grain N uptake	-0.519*	0.592**	-0.362^{NS}	0.814**	0.858**	0.837**	1.000**			
Straw P uptake	-0.502*	0.586**	-0.355^{NS}	0.773**	0.876**	0.934**	0.874**	1.000**		
Grain P uptake	-0.443*	0.480*	-0.331^{NS}	0.836**	0.897**	0.842**	0.920**	0.876**	1.000**	
Grain yield	-0.555**	0.536*	-0.362^{NS}	0.799**	0.867**	0.852**	0.986**	0.874**	0.916**	1.000**

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

(2023), who conducted a field experiment to evaluate the growth and yield of chickpea under different levels of bio-fertilizer and P application. It was recorded that the grain and stover yield was maximum under the combined inoculation of *Rhizobium* and PSB along with the application of P fertilizer.

Interrelationship among various parameters

Pearson's correlation study was calculated for determining interrelationship among soil chemical, biological and yield. Correlation coefficient had been presented in Table 10. The pH was found to be negatively correlated with all the study parameters. The organic carbon was found to be significantly correlated with available nitrogen (0.593**), available phosphorus (0.503*), straw nitrogen uptake (0.515*), grain nitrogen uptake (0.592**), straw phosphorus uptake (0.586**), grain phosphorus uptake (0.480*) and grain yield (0.536*), respectively. Available phosphorus content in soil was also found significantly positive correlated with available nitrogen, grain nitrogen uptake (0.858**), straw phosphorus uptake (0.876**), grain phosphorus uptake (0.897**) and negative correlated with soil EC (-0.404). The similar trend had also been observed for available nitrogen in soil.

CONCLUSION

The research has demonstrated that the application of *Rhizobium bengladensis* and *Pseudomonas* sp. along with 75% NP and 100% K increased available NPK, microbial population, plant growth and yield. In summary, this research highlights that the application of microbial inoculant in combination with mineral fertilizer led to 25% reduction in N and P applications in soil without compromising with yield loss. However, there is a need for more research focusing on exploring the more potent symbiotic nitrogen fixing and phosphorus solubilizing bacteria identifications and their evaluation in long term field experiment.

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