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CO-INOCULATION OF PHOSPHATE SOLUBILIZING BACTERIA AND RHIZOBIA FOR IMPROVING GROWTH AND YIELD OF MUNGBEAN (*VIGNA RADIATA* L.)

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ABSTRACT

Four isolates each of *Bacillus* and *Rhizobium* sp were selected and characterized for their P-solubilization and auxin production. All the isolates produced auxin but with different degree of efficacy. The isolates of *Rhizobium* and *Bacillus* having maximum auxin production and P-solubilization were selected and further evaluated for improving growth, nodulation and yield of mungbean at two fertilizer levels (20-25 and 20-50 kg NP ha^{-1}) in a pot experiment. Results revealed that inoculation significantly increased the growth and yield of mungbean as compared to uninoculated control. However, co-inoculation of *R. phaseoli* and *B. megaterium* further enhanced the mungbean growth, nodulation and yield in comparison with controls. Rhizobial inoculation produced 24.0 g pot^{-1} pod yield and 30.20 g pot^{-1} straw yield whereas co-inoculation produced 24.3 g pot^{-1} pod yield and 32.07 g pot^{-1} straw yield at highest fertilizer level. Co-inoculation produced higher root mass (231.3 g), root length (50.54 cm), nodule number (78) and nodular mass (0.216 g) as compared to control. Similarly, co-inoculations also improved the nutrient concentration in mung plant and grains as compared to control. Study suggested that co-inoculation with *R. phaseoli* and *Bacillus* sp. employed assenting effect on yield components and could be a useful approach than their sole application. However, a comprehensive approach to employ the PGPR in legumes should be carried out to explore the hidden potential of PGPR and to promote the quality and yield of crops under field conditions.

Keywords: *Rhizobium*, *Bacillus*, Co-inoculation, Phosphate Solubilizing Bacteria, mung bean

INTRODUCTION

Use of biological alternatives has radically been increased due to the increasing prices of synthetic fertilizers and concurrently threat of agro-chemicals to the environment (Socolow, 1999; Vance, 2001). Microbial inoculants have proved their worth as biological alternatives to compensate agro-chemicals and to sustain environment friendly crop production (Dobbelaere *et al.,* 2003). Various mechanisms involved in plant growth promotion due to these inoculants are N_2-fixation, hormone regulation, improvement in nutrient uptake and enzymatic reactions in plants, phosphate solubilization and stress resistance (Sarwar *et al.,* 1992; Arshad and Frankenberger, 1998). Induction of PGPR in the legume rhizosphere has exerted detrimental effect on the plant health and yields via the above mentioned mechanisms (Khan *et al.,* 1998).

Phosphorus is an integral part of plant body and the second major nutrient limiting plant growth is generally deficient in most of the soils due to its ready fixation (Schachtman *et al.,* 1998). The increasing prices of phosphatic fertilizers have raised an alarming situation for the country like Pakistan possessing an entirely agro-based economy. Therefore, it is very tricky for poor farmers to supplement P fertilizers in the soil to circumvent the P deficiency. Another issue is the reactivity of phosphate anions which are immobilized by forming a complex with Al or Fe in acidic soils (Norrish and Rosser, 1983) or Ca in calcareous soils (Sample *et al.,* 1980). Hence, the amount available to plants is usually a small proportion of the total. Stevenson (1986) reported that about 80% of added P fertilizers precipitated due to metal ion complexes. It has also been guessed that amount of P-fixed if solubilized might be sufficient for the next century (Goldstein *et al.,* 1993).

Several researchers have reported the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, hydroxyapatite, and rock phosphate (Zaidi, 1999; Richardson, 2001) and hence increased the crop yields (Zaidi, 1999; Gull *et al.,* 2004). Phosphate solubilizers besides solubilization of insoluble phosphates, increased nitrogen fixation rate, offered trace elements and produced plant hormones (Gyaneshwar *et al.,* 1998).

The fundamental mechanism for mineral phosphate solubilization is the production of organic acids and chelating oxo acids from sugars (Antoun and Kloepper, 2001; Peix *et al.,* 2001). Production of organic acids results in acidification of the microbial cell and its surroundings. Gluconic acid seems to be the most common acid of mineral phosphate solubilization (Maliha *et al.,* 2004). Nodule forming *Rhizobium* has also been recognized as a P-solubilizer (Halder *et al.,* 1991). *Bacillus* sp. increased grain yield of crops and lowered the P fertilizer requirements (Yuming *et al.,* 2003).

The combined application of phosphate solubilizing bacteria and nodule forming bacteria in legumes stimulated plant growth (Algawadi and Gaur, 1988; Perveen *et al.,* 2002) as compared to their single inoculation. Co-inoculation with N_2-fixing and P-solubilizing microbe was more effective approach for providing balanced plant nutrition (Gupta *et al.,* 1998; Martins *et al.,* 2004). Present study was designed to evaluate the co-inoculation potential of *Bacillus* and *Rhizobium* sp for improving growth, nodulation and yield of mung bean at different P levels.

MATERIALS AND METHODS

Isolation of Rhizobium and P-solubilizer: Mung bean (*Vigna radiata* L.) plants were uprooted from the Soil Bacteriology Section, AARI, Faisalabad and root samples were collected, washed thoroughly with tap water, nodules were separated and placed in Petri dishes. Nodules were surface-sterilized by dipping in 95% ethanol and then rinsed with sterilized distilled water followed by dipping in acidified 0.2% $HgCl_2$ solution for 3-5 minutes and 5-6 times washings with sterilized distilled water (Russell *et al.,* 1982). Surface sterilized nodules were crushed in a minimal volume of sterilized distilled water with the help of sterilized forceps to obtain a suspension. The suspension with the help of an inoculating needle was streaked out on Congo red yeast extract mannitol agar medium (Vincent, 1970). The colonies that don't attain the color of Congo red were picked and re-streaked on plates to obtain pure cultures. The purified rhizobial cultures (*Rhizobium phaseoli*) were stored at 5 ± 1 ^oC on slants and maintained for further screening.

Bacillus was isolated by standard dilution plate technique. The rhizosphere soil of mung bean growing in the permanent layout plot at Soil Bacteriology Section AARI, Faisalabad was used to prepare the dilutions. For the isolation of *Bacillus,* the serial dilutions of rhizosphere soil samples was subjected to heat shock at 80^oC for 30 minutes in an oven as reported by Claus (1964) and then inoculated the selective medium (Nautiyal, 1999). Plates carrying selective medium were incubated at 28 ± 2^oC for seven days. The growth of *Bacillus* was purified and screened out thrice for purification on the selected medium to get a pure culture. Isolates were checked for their solubilization on Pikovskaya's medium (Pikovskaya, 1948). Isolates forming halos on the above mentioned medium were treated as P-solubilizers and maintained to check the extent of solubilization. After preliminary screening following standard methods as outlined in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984) the pure culture was predicted as *Bacillus megaterium*.

Determination of Auxin biosynthesis: Mung bean *rhizobium* and *Bacillus* isolates (four of each) named as (M_1, M_2, M_3, & M_4) and (B_1, B_2, B_3 & B_4) were screened for their auxin biosynthesis potential. Isolates of *Rhizobium* were maintained on the yeast extract mannitol (YMB) for 48-72 hours and *Bacillus* on Pikovskaya's broth culture. Auxin biosynthesis potential was determined as IAA equivalents using Salkowski's reagent as reported by Sarwar *et al.* (1992). *Rhizobium* and *Bacillus* isolates having the highest auxin biosynthesis potential were selected for the experimentation.

Determination of Phosphate Solubilization: The solubilization potential of *Bacillus* isolates (B_1, B_2, B_3 and B_4) were checked on the Pikovskaya's medium (Pikovskaya, 1948). The growth and solubilization diameter were determined after incubation at 28 ± 2 ^oC for seven days on the Pikovskaya's medium. On the bases of diameter of clearing halo zones, solubilization efficiency (SE) and solubilization index (SI) (Gaur, 1990; Nguyen *et al.,* 1992; Vazquez *et al.,* 2000) were determined by using the following formulas.

$$SE = \frac{Solubilization\ diameter}{Growth\ diameter} \times 100$$

Where as solubilization index was determined by the following formula,

$$SI = \frac{colony\ diameter + halozone\ diameter}{colony\ diameter}$$

Auxin biosynthesis potential of mung *Rhizobium* was varied from 8.55-10.83 µg mL^{-1} while *Bacillus* had 2.10-2.99 µg mL^{-1}. Isolates (M_4) and (B_4) having the highest biosynthesis potential and phosphate solubilization (Table 1) were selected for further experimentation.

Inoculum preparation: Inocula of mung *Rhizobium* and *Bacillus* was prepared in YEM and in selective medium, respectively. Both the media was inoculated in 500 mL conical flask containing 150 mL medium and incubated at 28 ± 2^oC under shaking at 100-150 rpm for three days to give an optical density of 0.5 recorded at 535 nm. Peat was sterilized at 121^oC and 15 psi pressure for one hour and inoculated with broth cultures of *R. phaseoli* and *B. megaterium* (100 mL per kg of peat). Peat based inoculum was incubated at 28 ± 2^oC by adding 10% sugar solution to increase the population of respective microbe. Inocula of *Rhizobium* and *Bacillus* @10^8 MPN bacterial cells per gram of peat were applied to mung bean as seed coating.

Pot Experiment: Study was conducted to assess the co-inoculation effect of *Rhizobium* and *Bacillus* sp. on the yield of mung bean in potted soil conditions. The pre-sowing soil samples were collected, air dried, sieved and analyzed for various physico-chemical characteristics. The soil was sandy clay loam having pH 7.4, EC 1.6 dS m^{-1}, soil N 0.031% and available P 10.4 mg kg^{-1} at Soil

Bacteriology Section, AARI, Faisalabad. There were two P fertilizer levels i.e. (25 & 50 kg ha^{-1}) was applied according to the treatments as SSP while uniform dose of N (20 kg ha^{-1}) applied as basal to all the treatments (detail as under) following completely randomized design (CRD).

T_1: 20-25 kg NP ha^{-1}
T_2: 20-50 kg NP ha^{-1}
T_3: T_1+ *Bacillus* inoculation
T_4: T_2+ *Bacillus* inoculation
T_5: T_1+ Rhizobial inoculation
T_6: T_2+ Rhizobial inoculation
T_7: T_1+ Co-inoculation
T_8: T_2+ Co-inoculation

When the crop was at flowering stage, pots were excessively irrigated to uproot plant for recording root parameters like nodule number and mass (three plants per pot), root length and mass. Data regarding pod yield, plant dry matter, N & P-content in plant and grains and post harvest soil N and available P were recorded. Nitrogen was determined according to Kjeldhal method (Bremner and Mulvany, 1982) while phosphorus by modified Olsen method (Olsen and Sommers, 1982). Data were subjected to statistical analysis by following CRD using standard procedures (Steel *et al.,* 1997). The differences among treatment means were checked by applying the Duncan's multiple range tests (Duncan, 1955).

RESULTS

Co-inoculation effect on yield parameters: Inoculation of *Rhizobium* and *Bacillus* considerably enhanced the pod and straw yield (Table 2) while the effect was more pronounced when they are applied in combination as compared to un-inoculated ones. The highest pod yield was produced by co-inoculation (24.32 g pot^{-1}) followed by rhizobial inoculation (24.0 g pot^{-1}) at 50 kg P ha^{-1}. Likewise, co-inoculation produced higher straw yield (32.07 g pot^{-1}) followed by rhizobial inoculation (30.20 g pot^{-1}) as compared to control (22.93 g pot^{-1}). Percent increase in pod yield with co-inoculation was 101 and 57% while with rhizobial inoculation alone 81 and 55% at fertilizer levels 25, 50 kg P ha^{-1}, respectively.

Co-inoculation effect on post harvest soil parameters: Post harvest soil analysis (Table 3) revealed that co-inoculation resulted in higher soil N at both levels as compared to control. The highest soil N was observed with co-inoculation i.e. 0.037 and 0.039% followed by *rhizobium* i.e. 0.036 and 0.037% at 25 and 50 kg P ha^{-1}, respectively. Increase in soil N with co-inoculation was 12.1 and 14.7% followed by rhizobial inoculation alone 9.1 and 8.8% at both levels of P i.e. 25 and 50 kg P ha^{-1}, respectively. The soil N with *Bacillus* inoculation was at par with the un-inoculated ones. Co-inoculation demonstrated maximum available P i.e. 12.53 and 12.80 ppm followed by *Bacillus* i.e. 11.93 and 12.27 ppm at 25 and 50 kg P ha^{-1}, respectively. Increase in available P with co-inoculation was (15.3 and 15.0 %) and with P-solubilizer alone (9.75 and 10.24%) than un-inoculated ones at half and full P doses. Rhizobial inoculation also increased the available P content as compared to control.

Co-inoculation effect on plant and grain NP-content: Data regarding NP content in plant matter and grains were presented in (Table 4 and 5). Rhizobial inoculation produced highest N-content in grains (3.23 and 3.24%) followed by co-inoculation (3.21 and 3.19%) at half and full dose of P fertilizer, respectively. *Bacillus* inoculation enhanced the plant and grain N-content at both fertilizer levels as compared to control. Co-inoculation enhanced the N content in plant matter (1.22 and 1.25%) which was at par with *Bacillus* and rhizobial inoculation alone.

Bacillus inoculation produced highest grain P-content (0.31 and 0.32%) followed by co-inoculation (0.28 and 0.30%) at half and full P level, respectively. The highest NP-content in plant matter was observed with co-inoculation (1.22, 1.25% and 0.16, 0.17) as compared to control. P-content with *Bacillus* inoculation was at par with co-inoculation. The PGPR inoculation alone or combined with rhizobia influenced and the nutrient concentration in plant matter and grains as compared to un-inoculated ones.

Co-inoculation effect on the root parameters: Co-inoculation effect on the root parameters are presented in (Figures 1, 2, 3 and 4). Inoculations either applied separately or interaction of *Bacillus* with rhizobia influenced the root parameters positively. Co-inoculation produced maximum root length and mass i.e. 49.02, 50.54 cm and 175.4, 231.3 g pot^{-1} followed by rhizobial inoculation i.e. 43.18, 48.26 cm and 175.4, 186.6 g pot^{-1} as compared to control at both fertilizer levels, respectively (Figure 1 & 2). Similarly, co-inoculation produced maximum nodule number (56, 78) and mass (0.179, 0.216 g pot^{-1}); followed by rhizobial inoculation alone as compared to control at 25 and 50 kg P ha^{-1}, respectively.

DISCUSSION

Besides nitrogen fixation *Rhizobium* sp. have been well known for hormone production like auxins and P-solubilization that might consider the most credible means in promoting plant growth and yield (Zaidi *et al.,* 2004). Co-inoculation of nodulating forming *rhizobium* with phosphate solubilizing and growth hormone producing microorganisms is a classical approach resulting enhanced legume yield (Garcia *et al.,* 2004; Khan *et al.,* 2006).

Species of *Rhizobium* and *Bacillus* were isolated from nodules of mung bean and the rhizosphere, respectively were characterized for their auxin biosynthesis potential and P-solubilization. All isolates

produced auxin expressed as IAA equivalents (Table 1). Role of microbial production of auxins and in plant growth promotion have been described by number of researchers (Sarwar *et al.,* 1992; Martins *et al.,* 2004; Zahir *et al.,* 2004).

In present study, isolates of *Rhizobium* and *Bacillus* sp. were assessed for the growth promotion of mung bean at different P levels viz. recommended (50 kg ha^{-1}) and half of recommended (25 kg ha^{-1}). Results revealed that significant increases in pod, straw, nodulation, root length and mass were observed with co-inoculation compared to un-inoculated control. Both *Rhizobium* and *Bacillus* inoculation improved the growth and yield of mung bean when they were applied separately. However, the response of mung bean to rhizobial inoculation was more than *Bacillus* inoculation alone. It had also been observed that the bacterial efficiency was fertilizer rate-dependent. These findings are corroborated by the previous researchers who verified the effect of bacterial sp. on the growth and development of various leguminous crops (Garcia *et al.,* 2004; Zaidi *et al.,* 2004).

In this study, inoculations either singly or combined inoculation resulted in increased growth and yield of mung bean. However, co-inoculation effect of *Rhizobium* and *Bacillus* sp. was more pronounced and enhanced the yield components and root parameters as compared to control. Enhancement in nodule number, nodular mass and consequently yield components due to combined inoculation might be the expansion in root length and mass, thus more number of active sites for nodulation by the rhizobial strains. The curling of root hairs and infection thread are the key steps during nodule development that was boosted with co-inoculation due to the production of growth hormones. The root length and mass enhancement owing to the changes in the root system architecture resulted in increased root density, root hairs and surface area due to interaction of microbes with plant roots. This increase in root surface area resulted in better acquisition of nutrients. The increases in root and yield components of crops by inoculation with N_2-fixing and P-solubilizing microbes have also reported by other researcher (Zaidi, 1999; Garcia *et al.,* 2004). Bai *et al.* (2002) demonstrated that introduction of phosphate solubilizer in the rhizosphere of legumes either applied singly or dual inoculation with specific rhizobia enhanced the yield parameters. Similarly, Dashti *et al*. (1998) Yuming *et al*. (2003) reported the synergistic effect of *Bradyrhizobium* with PGPR inoculation on *Glycin ma*x.

In present study, co-inoculation also increased the plant and grain N, P concentrations compared with un-inoculated control. P-solubilizer i.e. *Bacillus* having high (SE and SI) along with *Rhizobium* demonstrated higher N and P contents in plants and grains might be due to increased nutrient concentration in the rhizosphere of plants. Increase in the nutrient concentration in the plants owed to bioavailability of nutrients in the root zone. Microbial release of nutrients enhanced the nutrient concentration in soil and hence more uptake by plants. Co-inoculation enhanced the NP levels in soil due to increase in root hair density, more lateral roots, root surface area / nodulation, thus more nitrogen fixation and phosphate solubilization. Phytohormone producing microbes enhanced the root mass and length thus enhanced the nutrient concentration in plants (Gull *et al.,* 2004).

Table 1. Some important traits of isolates tested during the examination.

Isolates	IAA equivalents (µg mL^{-1})	Solubilization Efficiency (SE)	Solubilization Index (SI)
M_1	10.72	-	-
M_2	8.55	-	-
M_3	9.45	-	-
M_4	10.83	-	-
B_1	2.36	236.4	3.4
B_2	2.78	227.3	3.3
B_3	2.10	208.3	3.1
B_4	2.99	270.0	3.7

Table 2. Co-inoculation effect on pod and straw yield of mung bean.

(Average of 3 repeats)

	Pod Yield (g pot^{-1})		Straw Yield (g pot^{-1})	
	25 kg P ha^{-1}	50 kg P ha^{-1}	25 kg P ha^{-1}	50 kg P ha^{-1}
Control	11.50^{d*}	15.50^{c}	18.57^{e}	22.93^{d}
Bacillus inoculation	15.70^{c}	17.90^{b}	21.80^{d}	25.30^{c}
Rhizobial inoculation	20.80^{b}	24.00^{a}	25.87^{c}	30.20^{b}
Co-inoculation†	23.10^{a}	24.32^{a}	30.97^{ab}	32.07^{a}
LSD	1.92		1.275	

†*Rhizobium* + *Bacillus* inoculation in 1:1 v/v.

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test

Table 3. Co-inoculation effect on post harvest soil N and available P of mung bean.

(Average of 3 repeats)

Treatments	Soil N (%)		Available P (mg kg^{-1})	
	25 kg P ha^{-1}	50 kg P ha^{-1}	25 kg P ha^{-1}	50 kg P ha^{-1}
Control	0.033^{c*}	0.034^{c}	10.87^{f}	11.13^{ef}
Bacillus inoculation	0.033^{c}	0.034^{c}	11.93^{cd}	12.27^{bc}
Rhizobial inoculation	0.036^{b}	0.037^{b}	11.53^{de}	11.87^{cd}
Co-inoculation†	0.037^{b}	0.039^{a}	12.53^{ab}	12.80^{a}
LSD	0.0013		0.42	

†*Rhizobium* + *Bacillus* inoculation in 1:1 v/v.

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test

Table 4. Co-inoculation effect on plant analysis of mung bean.

(Average of 3 repeats)

Treatments	Plant N (%)		Plant P (%)	
	25 kg P ha^{-1}	50 kg P ha^{-1}	25 kg P ha^{-1}	50 kg P ha^{-1}
Control	1.14*	1.18	0.13^{c}	0.14^{bc}
Bacillus inoculation	1.19	1.23	0.16^{ab}	0.17^{a}
Rhizobial inoculation	1.20	1.22	0.13^{c}	0.15^{abc}
Co-inoculation†	1.22	1.25	0.16^{ab}	0.17^{a}
LSD	NS		0.03	

†*Rhizobium* + *Bacillus* inoculation in 1:1 v/v.

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test.

Table 5. Co-inoculation effect on grain analysis of mung bean.

(Average of 3 repeats)

Treatments	Grain N (%)		Grain P (%)	
	25 kg P ha^{-1}	50 kg P ha^{-1}	25 kg P ha^{-1}	50 kg P ha^{-1}
Control	3.11^{d*}	3.13^{cd}	0.23^{c}	0.24^{bc}
Bacillus inoculation	3.18^{bc}	3.20^{ab}	0.31^{a}	0.32^{a}
Rhizobial inoculation	3.23^{ab}	3.24^{a}	0.24^{bc}	0.24^{bc}
Co-inoculation†	3.21^{ab}	3.19^{ab}	0.28^{ab}	0.30^{a}
LSD	0.055		0.042	

†*Rhizobium* + *Bacillus* inoculation in 1:1 v/v.

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test.

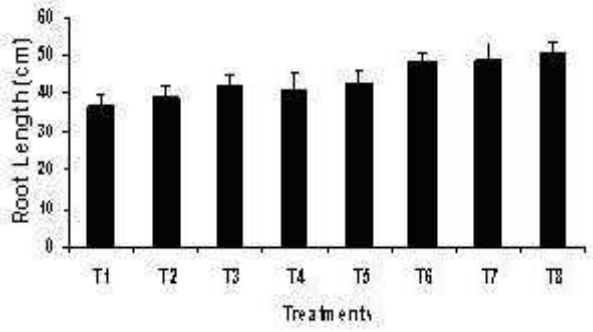

Figure 1. Co-inoculation effect on mungbean root length.

The phosphate solubilizer i.e. *Bacillus* applied positive influences on the mung bean and did not affect the mutualism and not only solubilize the P but also enhance the efficiency of symbionts and acquisition of nutrients.. Enhancing yield with P-solubilizer owed to its capability of growth hormone production and phosphate solubilization. *Rhizobium-Bacillus* association stimulated the mung bean growth and twisted the yield and nutrient concentrations to produce higher levels of yield. This interaction resulted in high nutrient concentration in soil might be due to more exudation, lowered the soil pH due to the production of organic acids and ultimately boosting the yield.

Present study clearly demonstrated that co-inoculation of *R. phaseoli* and *B. megaterium* affected positively the growth and yield of mung bean. Inoculation with either microbe exhibited positive influence on the yield components and nutrient concentration in mung bean and their cumulative effect was more prominent.

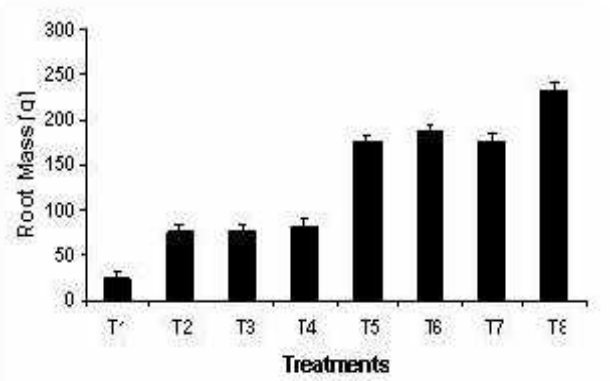

Figure 2. Co-inoculation effect on mungbean root mass.

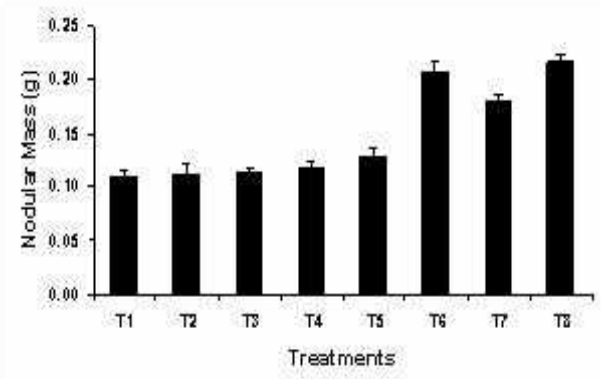

Figure 3. Co-inoculation effect on mungbean nodular mass.

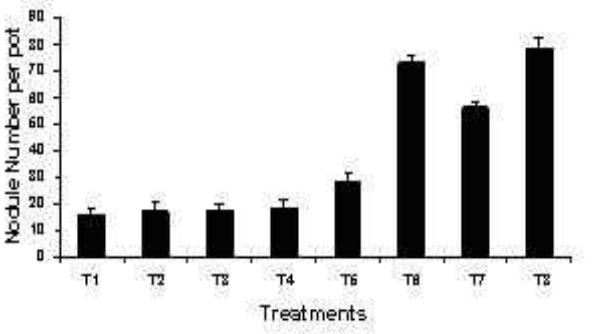

Figure 4. Co-inoculation effect on mungbean nodule number per pot.

REFERENCES

Algawadi, A. R. and A. C. Gaur (1988). Associative effect of *rhizobium* and phosphate solubilizing bacteria on the yield and nutrient uptake of chickpea. Plant Soil 105: 241-246.

Antoun, H. and J. W. Kloepper, (2001). Plant Growth promoting rhizobacteria. Encyclopedia of Genetics. Brenner, S., and Miller, J.F. (Eds in chief) pp. 1477-1480. Academic Press.

Arshad, M. and W. T. Frankenberger. Jr. (1998). Plant growth regulating substances in the rhizosphere: Microbial production and functions. Adv. Agron. 62: 46-51.

Bai, B. P., T. C. Charles, C. Trevor and D. L. Smith. (2002). Co-inoculation dose and root zone temperature for plant growth promoting rhizobacteria on soybean (*Glycin max* L.) growth in soil-less media. Soil. Biol. Biochem. 34: 1953-1957.

Bremner, J. M. and C. S. Mulvaney (1982). Nitrogen Total. p. 595-624. In: A. L. Page (ed.), Methods of soil analysis. Agron. No. 9, Part 2: Chemical and microbiological properties, 2nd ed., Am. Soc. Agron., Madison, WI, USA.

Claus, D. (1964). Anreicherungen and Direktisolierungen aerober sporenbilder Bakterien. In: Anreicherunskultur and Mutantenauslese (ed. H. G. Schlegel), pp. 337-362. Gustav Fischer-Verlag, Stuttgart.

Dashti, N., F. Zhang, R. Hynes and D. L. Smith. (1998). Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean [*Glycin max* (L. Merr.)] under short season conditions. Plant Soil 200: 205-213.

Dobbelaere, S., J. Vanderleyden and Y. Okon (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant Sci. 22: 107-149.

Duncan, D. B. (1955). Multiple Range and Multiple F-Test. Biometrics 11: 1-42.

Garcia, L. J. A., A. Probanza, B. Ramos, J. Barriuso and M. Gutierrez. (2004). Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) and *Sinorhizobium fredii* on biological nitrogen fixation, nodulation and growth of *Glycin max* cv. Osumi. Plant Soil 267: 143-153.

Gaur, A. C. (1990). Phosphate solubilizing microorganisms as biofertilizers, Omega Scientific Publisher, New Delhi, p. 176.

Goldstein, A. H., R. D. Rogers and G. Mead. (1993). Mining by microbe. Biotechnol. 11: 1250–1254.

Gull, F. Y., I. Hafeez, M. Saleem and K. A. Malik. (2004). Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. Aust. J. Exp. Agric. 44: 623-628.

Gupta, A., A. K. Saxena, M. Gopal and K. V. B. R. Tilak. (1998). Effect of plant growth promoting rhizobacteria on competitive ability of introduced *Brayrhizobium* sp. (Vigna) for nodulation. Microbiol. Res. 153: 113-117.

Gyaneshwar, P., N. Kumar and L. J. Parekh. (1998). Effect of buffering on the phosphate solubilizing ability of microorganisms. World J. Microbiol. Biotechnol. 14: 669-673.

Halder, A. K., A.K. Misra and P. K. Chakrabarty. (1991). Solubilization of inorganic phosphates by *Bradyrhizobium*. Indian J. Exp. Biol. 29: 28-31.

Khan, M. S., A. Zaidi and P. A. Wani. (2006). Role of phosphate solubilizing microorganisms in sustainable agriculture-A review. Agron. Sustain. Dev. 26: 1-15.

Khan, M. S., M. Aamil and A. Zaidi. (1998). Moong bean response to inoculation with nitrogen fixing and phosphate solubilizing bacteria. *In:* Deshmukh A.M. (Ed.), Biofertilizers and biopesticides, Technoscience Publications, Jaipur, pp. 40-48.

Krieg, N. R. and J. G. Holt. (1984). Bergey's Manual of Systematic Bacteriology, Vol. 1, pp. 694. Williams and Wilkins, Baltimore, MD, USA.

Maliha, R., K. Samina., A. Najma., A. Sadia and L. Farooq. (2004). Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms under in vitro conditions. Pakistan J. Biol. Sci. 7: 187-196.

Martins, A., O. Kimura, S. R. Goi and J. I. Baldani. (2004). Effect of co-inoculation of plant growth promoting rhizobacteria and rhizobia on development of common bean plants (*Phaseolus vulgaris*, L.). Floresta e Ambiente 11: 33-39.

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Let. 170: 265-270.

Nguyen, C., W. Yan, F. Le Tacon and F. Lapeyrie. (1992). Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) P.D. Orton. Plant Soil 143: 193-199.

Norrish, K. and H. Rosser (1983). Mineral phosphate, in: Soils, an Australian viewpoint. Academic press, Melbourne, CSIRO/London, UK, Australia, pp. 335-361.

Olsen, S. R. and L. E. Sommers (1982). Phosphorus. p. 403-430. In: A. L. Page (ed.). Method of soil analysis, Agron. No. 9, part 2: Chemical and microbiological properties, 2nd ed., Am. Soc. Agron., Madison, WI, USA.

Peix, A., A. A. Rivas-Boyero, P. F. Mateos, C. Rodriguez-Barrueco, E. Martinez-Molina and E. Velazquez (2001). Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium mediterraneum under growth chamber conditions. Soil. Biol. Biochem.33: 103-110.

Perveen, S., M. S. Khan and A. Zaidi. (2002). Effect of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiatus*). Indian J. Agric. Sci. 72: 421-423.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiol. 17: 362-70.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Australian J. Plant Physiol. 28: 897-906.

Russell, A. D., W. B. Hugo and G. A. J. Ayliffo. (1982). Principles and practices of disinfection, preservation and sterilization. Black Wall Scientific, London.

Sample, E. C., R. J. Soper and G. J. Raez. (1980). Reactions of phsopahte fertilizers in soils. In: The Role of phosphate in Agriculture, pp. 263-310. (Eds.) F. E. Khasawneh, E.C. Sample and E. J. Kamprath, Madison, WI: American Society of Agronomy.

Sarwar, M., D. A. Arshad, W. T. Martens and J. R. Frankenberger. (1992). Tryptophan dependent biosynthesis of auxins in soil. Plant Soil 147: 207-215.

Schachtman, D. P., R. J. Reid and S. M. Ayling. (1998). Phosphorus uptake by plants: From soil to cell. Plant Physiol. 116: 447-453.

Socolow, R. H. (1999). Nitrogen management and the future of food: Lessons from the management of energy and carbon. Proc. Nat. Acad. Sci. USA 96: 6001-6008.

Steel, R. G. D., J. H. Torrie and D. A. Dicky. (1997). Principles and Procedures of Statistics- A Biometrical Approach.3rd Edition, McGraw-Hill Book International Co., Singapore. p. 204-227.

Stevenson, F. J. (1986). Cycles of soil carbon, nitrogen, phosphorus, sulphur micronutrients, Wiley, New York.

Vance, C. P. (2001). Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable sources. Plant Physiol. 127: 390-397.

Vazquez, P., G. Holguin, M. E. Puente, A. Lopez-Cortes and Y. Bashan. (2000). Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves growing in a semiarid coastal lagoon. Biol. Fertil. Soils. 30: 460-468.

Vincent, J. M. (1970). A manual for the practical study of root-nodule bacteria. IBP Handbook Number 15, Blackwell, Oxford.

Yuming, B., Z. Xiaomin and D. L. Smith. (2003). Enhanced soybean plant growth resulting from co-inoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci. 43: 1774-1778.

Zahir, Z. A., M. Arshad and W. T. Frankenberger, Jr. (2004). Plant growth promoting rhizobacteria: perspectives and application in agriculture. Adv. Agron. 81: 96-168.

Zaidi, A. (1999). Synergistic interactions of nitrogen fixing microorganisms with phosphate mobilizing microorganisms, Ph.D. Thesis, Aligarh Muslim University, Aligarh.

Zaidi, A., M. S. Khan and M. Aamil. (2004). Bioassociative effect of rhizospheric microorganisms on growth, yield, and nutrient uptake of green gram. J. Plant Nutr. 27: 601-12.