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Full Length Research Paper

Effects of phosphate solubilizing microorganisms and *Rhizobium* sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition

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Effects of Phosphate solubilizing microorganisms (*Glomus intraradices*, *Pseudomonas putida*, *P. alcaligenes*, *P. aeruginosa* (Pa28), *A. awamori*) and *Rhizobium* sp. was observed on the growth, nodulation yield and root-rot disease complex of chickpea under field condition. Inoculation of *Rhizobium* sp. caused a greater increase in growth and yield than *P. putida, P. aeruginosa* or *G. intraradices*. The number of nodules per root system was significantly higher in plants inoculated with *Rhizobium* sp. compared to plants without *Rhizobium* sp. Inoculation of *P. putida* caused highest reduction in galling followed by *P. aeruginosa*, *P. alcaligenes*, *G. intraradices* and *A. awamori* while *Rhizobium* sp. caused almost similar reduction in galling as caused by *P. putida*.

Key words: Chickpea, *Glomus* sp., phosphate solubilizing microorganisms, disease complex.

INTRODUCTION

India is the largest producer and importer of the leguminous crop (Shakya et al., 2008). Amongst the leguminous crops, chickpea (*Cicer arietinum* L.) occupy an important position due to its nutritive values (17-23% protein) in large vegetarian population of the country (Ali and Kumar, 2006). The major chickpea producing states are Andhra Pradesh, Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh contributes 86% of the total production of the country. During last decade the production of chickpea has been declined in the western Uttar Pradesh. The main cause behind this is the root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood and *Macrophomina phaseolina* (Tassi) Goid (Akhtar and Siddiqui, 2007a). The synergistic interaction of these pathogens together causes more loss in term of yield than the sum of their individuals to this important pulse crop (Siddiqui and Husain, 1992; Akhtar and Siddiqui, 2008a, b).

The rhizosphere is the zone of intense microbial activity and provides a front line defense against pathogen attack to root (Weller, 1988). The rhizoplane and surrounding rhizosphere soil are colonized or occupied by a wide range of microorganisms. Of the various microorganisms present, AM (arbuscular mycorrhizal) fungi improves the plant growth, nutrient cycling and protection against biotic and abiotic stresses and soil structure through aggregate formation (Bethlenfalvay and Linderman, 1992; Gianinazzi and Schuepp, 1994; Kapulnik and Douds, 2000; van der Heijden and Sanders, 2002; Jeffries et al., 2003; Barea et al., 2005; Smith and Read, 2008). The AM symbiosis increases the supply of mineral nutrients particularly phosphorus and other minerals like ammonium, zinc and copper. The AM association also improves plant health through increased protection against soil-borne microbial pathogens (Allen, 1996; Gianinazzi et al., 2002; Jefferies et al., 2003; Barea et al., 2005; Akhtar and Siddiqui, 2008a).

Some other microorganisms that are associated with the roots of crop plants and play an important role in mobilization and immobilization of phosphorus are known as phosphate solubilizing microorganisms (PSM). PSM largely include bacteria and fungi viz. some of the

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species of *Bacillus*, *Pseudomonas*, *Penicillium* and *Aspergillus* (Tilak, 1991; Tilak et al., 2005). PSM can solubilize and mineralize P from inorganic and organic pools of total soil P and may be used as inoculants to increase P-availability to plants (Richardson, 2001; Illmer et al., 1995; Whitelaw et al., 1999) and also have the capacity to increase the growth and yield of crop plants (Gupta and Namdeo, 1997; Ozgonen et al., 1999) besides reducing disease severity (Weller, 1988; Siddiqui and Mahmood, 1999). Similarly, presence of rhizobia in the rhizosphere may also protect the host root from damage caused by pathogens (Siddiqui and Mahmood, 1995; Elbardy et al., 2006; Huang and Erickson, 2007).

The consistency in performance of different biocontrol agent have been tested in our previous experiments (Siddiqui and Akhtar, 2007; Akhtar and Siddiqui, 2007a, b; Akhtar and Siddiqui, 2008 a, b). On the basis of the results drawn from the earlier experiments the biocontrol agents which perform best under pot trials were selected for the field trial. The aim of the present study was to investigate the effects of PSM (found best in pot trial in our previous experiments) and *Rhizobium* sp. on the growth, nodulation yield and on the root-rot disease complex of chickpea in field condition and also to find out the best biocontrol agent under field condition.

MATERIALS AND METHODS

Site location and field history

Aligarh is situated at 27˚53’ N latitude, 78˚4’ E longitude and 187.45 m altitude with an area of 3431 sq km. Its climate is sub-tropical, with hot dry summer and cold winters. The winter extends from the middle of October to the end of March. The mean temperature during this season ranges from 23˚C and 12˚C respectively.

The average rainfall is 847.3 mm. More than 85% of the total rainfall occurs during June to September and some 10% in the winter. The winter rainfall is useful for Rabi crops. The relative humidity of the winter season ranges between 56 and 77% with an average of 66.5% that of the summer, between 37 and 49% with an average of 43% and that of the mansoon season, between 63 and 73% with an average 68%.

A field experiment was conducted at University Fort, Aligarh Muslim University. Aligarh generally available for the field research work (soil sandy loam, pH 7.5, porosity 39%, water holding capacity 42%, electrical conductivity 0.67, available N 95.90 mg/kg soil, available P 11.4 mg/kg soil and available K 160.89 mg/kg soil). The site at Fort was selected with a view suitable for chickpea crop.

Maize (*Zea mays*) was harvested September, 2006. The field was prepared by ploughing and removing grasses and plant debris.

Raising and maintenance of plants

Healthy, uniform size seeds of chickpea (*Cicer arietinum* L.) cv. Avarodhi were surface sterilized with 0.1% sodium hypochlorite (NaOCl) and washed 3 times with distilled water. Sowing was done by the usual ‘behind the plough’ method at the rate of 60 kg / ha on October 25, 2006. After germination, the distance between seedlings and rows was maintained at 20 cm and 40 cm respectively. The plots were free from weed, thrice. The plants were irrigated thrice at 40, 80 and 120 days after sowing and were harvested at 150 days.

Nematode Inoculum

Meloidogyne incognita was collected from chickpea field soil identified with the help perineal pattern (Taylor and Sassor, 1978). The pure culture of nematode was multiplied on egg plant (*Solanum melongena* L.) using a single egg mass placed in a glass house at the Department of Botany, A.M.U., Aligarh, India. Egg masses were hand-picked using sterilized forceps and placed in 9 cm diameter sieves of 1 mm pore size, which were previously mounted with cross layered tissue paper and placed in a petri plates containing water just deep enough to contact the egg masses. The sieves were placed in petri dishes with distilled water for hatching and incubated at 27°C. The hatched second stage juveniles (J_2) were collected from the petri plates every 24 h and fresh water was added to the petri plates. The concentration of J_2 of *M. incognita* in the water was adjusted so that each ml contained 200 ± 5 nematodes. 10 ml of this suspension (that is, 2000 freshly hatched J_2) was added to each pot containing a chickpea seedling.

Fungus Inoculum

Macrophomina phaseolina was isolated from infected chickpea root samples collected from Kasimpur, Aligarh (KA1) on potato dextrose agar (PDA). On confirmation its identity, the pure culuture of the fungus was maintained on PDA. Fungal inoculum was prepared by culturing the isolates in Richard’s medium (Riker and Riker, 1936) for 15 days at 25°C. Mycelium was collected on blotting sheets to remove excess water and nutrients. 100 g of mycelium was macerated in 1 l distilled water. 10 ml of this suspension (containing 1 g fungus) was applied to each plant.

Preparation of PSM inoculum

The AM fungus, *Glomus intraradices* Schenck and Smith, was isolated from the soil of chickpea field of Aligarh, India. The species was identified using the synoptic keys of Trappe (1982) and Schenck and Pervez (1990) and the identification was reconfirmed from Curator, INVAM, USA (http://invam.caf.wvu.edu/). For inoculum, the fungus was produced on *Chloris gayana* Kunth (Rhodes grass) grown in sandy loam soil. The population of *G. intraradices* isolate AI03 (Aligarh, India) in the inoculum was assessed by the most probable number method (Porter, 1979). 50 g of inoculum with soil was added around the seed to provide 500 infective propagules of *G. intraradices* per pot (1 g inoculum contains 10 infective propagules). The crude inoculum consisted of soil, extra metrical spores and spore carps, hyphal fragments and infective rhodes grass segments.

Paenibacillus polymyxa (MTCC No. 122), *Pseudomonas putida* (MTCC No. 3604), and *Pseudomonas alcaligenes* (MTCC No. 493) were obtained from Microbial Type of Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India while the *P. aeruginosa* (Pa28) isolated from chickpea soil. These isolates were sub culture on nutrient broth (Hi-Media Laboratories, Mumbai, India) incubated at 37 ± 1°C for 72 h. 1 ml nutrient broth suspension contains about 1.5×10^7 cfu /ml. 10 ml of this suspension was inoculated into each pot around the pea seedling. Another PSM, *Aspergillus awamori* Nakazawa (ITCCF 4680) which was obtained from Indian Agricultural Research Institute, New Delhi and cultured in Richard’s medium at 25 ± 1°C for 15 days. 10 ml (equivalent to 1 g) suspension was inoculated into each pot around the chickpea seedling.

Rhizobium inoculum

100 g commercial culture of *Rhizobium* Jordan (chickpea strain AP)

Table 1. Effect of phosphate solubilizing microroganisms (*G. intraradices, P. putida, P. alcaligenes, P. aeruginosa* (Pa28), *A. awamori*) and *Rhizobium* sp. on the growth of chickpea under field condition.

Treatments		Shoot dry weight (g)	No. of pods/plant	No. of nodules/ root system
Control	C	26.30 ± 0.36 e	26 ± 3.16 g	48 ± 3.16 fg
	Gi	27.91 ± 0.41 d	30 ± 3.74 efg	54 ± 2.74 bcd
	Pp	29.92 ± 0.48 b	34 ± 4.69 e	58 ± 3.74 ab
	Pa	28.61 ± 0.24 c	31 ± 4.36 ef	56 ± 4.00 bc
	A	27.66 ± 0.31 d	29 ± 5.48 fg	53 ± 3.81 cde
	R	30.74 ± 0.15 a	81 ± 5.92 a	62 ± 4.00 a
	Pa28	28.92 ± 0.33 c	32 ± 4.00 ef	56 ± 3.16 bc
M. incognita	C	18.76 ± 0.65 p	9 ± 2.24 jklm	33 ± 3.24 m
	Gi	22.10 ± 0.22 kl	11 ± 2.00 ijkl	40 ± 3.74 j kl
	Pp	24.18 ± 0.48 g	13 ± 2.55 hij	49 ± 2.23 efg
	Pa	22.87 ± 0.45 ij	10 ± 2.54 ijkl	41 ± 2.55 ijk
	A	21.24 ± 0.38 m	12 ± 2.45 hijk	46 ± 3.16 gh
	R	24.88 ± 0.33 f	56 ± 5.48 c	51 ± 2.92 def
	Pa28	23.58 ± 0.40 h	11 ± 3.08 ijkl	46 ± 4.47 gh
M. phaseolina	C	18.88 ± 0.26 p	12 ± 1.87 hijk	36 ± 4.00 lm
	Gi	22.50 ± 0.28 jk	13 ± 2.55 hij	42 ± 3.16 hij
	Pp	24.92 ± 0.48 f	6 ± 1.87 h	51 ± 2.91 def
	Pa	23.34 ± 0.45 hi	12 ± 2.45 hijk	42 ± 3.08 hij
	A	21.67 ± 0.46l m	12 ± 1.87 hijk	48 ± 2.45 fg
	R	25.25 ± 0.30 f	62 ± 4.00 b	53 ± 3.81 cde
	Pa28	24.19 ± 0.52 g	14 ± 2.34 hi	46 ± 3.08 gh
M. incognita* + *M. phaseolina	C	12.55 ± 0.17 r	5 ± 1.41 m	21 ± 2.73 n
	Gi	18.76 ± 0.52 p	8 ± 2.12 klm	33 ± 2.74 m
	Pp	20.54 ± 0.49 n	10 ± 2.55 ijkl	43 ± 3.61 hij
	Pa	19.56 ± 0.17v o	7 ± 2.34l m	37 ± 2.00 klm
	A	18.14 ± 0.19 q	9 ± 2.24 jklm	40 ± 3.16 jkl
	R	21.48 ± 0.18 m	40 ± 5.10 d	45 ± 3.31 ghi
	Pa28	20.10 ± 0.30 n	8 ± 2.12 klm	40 ± 3.74 jkl
L.S.D. p = 0.05		0.47	4	4

C = Control; Gi = G. intraradices; Pp = *P. putida;* Pa = *P. alcaligenes*; A = *A. awamori*; Pa = *P. aeruginosa*; R = *Rhizobium* sp.
*Values within each column followed by same letter are not significantly different (p = 0.05). ± = Standard deviation.

was suspended in 1000 ml distilled water and 10 ml (equivalent to 1 g inoculum) into each pot around chickpea seedling after thinning.

Inoculation technique

For inoculation of *M. incognita*, *M. phaseolina*, PSM (*G. fasciculatum*, *P. putida*, *P. alcaligenes*, *P. aeruginosa* (Pa28), *A. awamori*) and *Rhizobium* sp. and soil around the roots was carefully moved aside without damaging the roots. The inocula of these microorganisms were poured or placed around the roots and the soil was replaced. An equal volume of sterile water was added to control treatments.

Experimental design

Experiment was conducted in randomized block design and the size of each plot was 9 square meter. There were 7 treatments comprising of *G. intraradices*, *P. putida*, *P. alcaligenes*, *A. awamori*, *P. aeruginisa* (isolate 28), *Rhizobium* sp. and control. These 7 treatments were also tested against 4 pathogen combination (7 × 4 = 28 treatments) and each set was replicated 3 times (7 × 4 × 3 = 84 pots) as listed in Table 1.

Observations

The plants were harvested 150 days after inoculation. Data were recorded on shoot dry weight, number of pods, number of nodules, number of galls, grain weight, yield and root-rot index. Each plant was cut with a knife above the base of the root-emergence zone to separate shoot and root. The numbers of galls per root system were counted. For dry-weight determination, shoots were kept in envelops at 60°C for 2-3 days and weight was recorded in g. A root-rot index was determined by scoring on a scale ranging from 0 (no

disease) to 5 (severe root-rot).

Statistical analysis

The entire dataset was analyzed as a single two-factor experiment (pathogens × PSM / *Rhizobium* sp.) as described by Dospekhov (1984). All analyses were carried out using Stat view 5.0 (SAS institute, Cary, NC, USA). Least significant differences (L.S.D.) were calculated at p = 0.05 and Duncan's multiple-range test was used to test significant differences between treatments. Standard deviation was also calculated for the variance. The graph for galling was prepared using the program sigma plot version 9.0 with error bars showing standard deviation.

RESULTS

Treatment of plants without pathogens with *G. intraradices*, *P. putida*, *P. alcaligenes*, *A. awamori*, *P. aeruginosa* (Pa28) and *Rhizobium* caused a significant increase shoot dry weight over uninoculated ones (Table 1). Inoculation of *Rhizobium* to plants without pathogens caused a greater increase in shoot dry weight to that caused by *P. putida*, *P. aeruginosa* or *G. intraradices*. However, increase in shoot dry weight of plants with *P. alcaligenes* was similar to that caused *P. aeruginosa* while *A. awamori* increased shoot dry weight similar to that caused by *G. intraradices* (Table 1).

Inoculation of plants with *M. incognita* and *M. phaseolina* alone and in combination caused a significant reduction in shoot dry weight over uninoculated ones (Table 1). Reduction in shoot dry weight was greater when *M. incognita* and *M. phaseolina* were inoculated together than the inoculation of either of them. Inoculation of *G. intraradices*, *P. putida*, *P. alcaligenes*, A. *awamori*, *P. aeruginosa* and *Rhizobium* sp. significantly increased shoot dry weight of plants with pathogens. Inoculation of *Rhizobium* sp. to plants with pathogens caused a greater increase in shoot dry weight than by *P. putida*, *P. aeruginosa* or *G. intraradices*. However, inoculation of *P. alcaligenes* to plants with pathogens caused almost similar increase in shoot dry weight as by *P. aeruginosa* while *A. awamori* increased shoot dry weight similar to that caused by *G. intraradices* (Table 1).

Inoculation of *M. incognita* / *M. phaseolina* or both significantly reduced the number of pods per plant. Inoculation of *G. intraradices*, *P. putida*, *P. alcaligenes*, *A. awamori*, *P. aeruginosa* and *Rhizobium* sp. significantly increased the number of pods per plant both in pathogens inoculated and uninoculated ones. The number of nodules per root system was significantly higher in plants inoculated with *Rhizobium* sp. compared to uninoculated one (Table 1).

Inoculation of plants without pathogens with *G. intraradices*, *P. putida*, *P. alcaligenes*, *A. awamori*, *P. aeruginosa* and *Rhizobium* sp. significantly increased seed weight (per 100 seeds) and yield over uninoculated ones (Table 2). Inoculation of *Rhizobium* sp. to plants without pathogens caused a greater increase in seed weight and yield than by *P. putida* or *P. aeruginosa* or *G. intraradices*. However, increase in seed weight and yield of plants with *P. alcaligenes* was similar to that caused *P. aeruginosa* while *A. awamori* increased seed weight and yield similar to that caused by *G. intraradices* (Table 2).

Inoculation of plants with *M. incognita* and *M. phaseolina* alone and in combination caused a significant reduction in seed weight and yield over uninoculated controls (Table 2). Inoculation of *G. intraradices*, *P. putida*, *P. alcaligenes*, *A. awamori*, *P. aeruginosa* and *Rhizobium* sp. significantly increased seed weight and yield of plants with pathogens. Inoculation of *Rhizobium* caused a greater increase in seed weight and yield than by *P. putida*, *P. aeruginosa* or *G. intraradices*. However, inoculation of *P. alcaligenes* to plants with pathogens caused almost similar increase in seed weight and yield as caused by *P. aeruginosa* while *A. awamori* increased seed weight and yield similar to that caused by *G. intraradices* (Table 2).

The number of galls per root system was high when *M. incognita* was inoculated alone (Figure 1). In presence of *M. phaseolina* root galling were reduced. Inoculation of *P. putida* caused highest reduction in galling followed by *P. aeruginosa*, *P. alcaligenes*, *G. intraradices* and *A. awamori*. *Rhizobium* sp. caused almost similar reduction in galling as caused by *P. putida* (Figure 1).

Root-rot index was 4 when *M. incognita* and *M. phaseolina* were inoculated together (Figure 2). Index was reduced to 3 when *M. incognita* plus *M. phaseolina* inoculated plants were treated with *G. intraradices* or *A. awamori* while index was found to 2 when *M. incognita* plus *M. phaseolina* inoculated plants were treated singly *with P. putida*, *P. alcaligenes*, *P. aeruginosa* and *Rhizobium* sp. Index was reduced to 1 when *M. phaseolina* inoculated plants was treated singly with *G. intraradices*, *P. putida*, *P. alcaligenes*, *A. awamori*, *Rhizobium* sp. and *P. aeruginosa* (Figure 2).

DISCUSSION

Among the 6 biocontrol agents tested under field condition, *Rhizobium* sp. performed better in the management of root-rot disease complex followed by *P. putida*, Pa28, *P. alcaligenes*, *G. intraradices* and *A. awamori*. Root nodules formed by *Rhizobium* sp. under field condition were larger than the nodules formed in the plants under pot condition (Akhtar and Siddiqui, 2008a). Higher nitrogen fixation by large size nodules may account for reduced development (Barker and Huisingh, 1970). *P. putida* had a greater siderophore production than *P. alcaligenes* (Siddiqui et al., 2007). This may be a reason that *P. putida* was better than *P. alcaligenes* under field condition. Pa28 also showed greater siderophores production among pseudomonads isolates from chickpea fields and performed better that *G. intraradices* and *A. awamori*. Least effectiveness of *G. intraradices* and *A. awamori* may be attributed to their inability to adapt in the fields condition with other soil microflora.

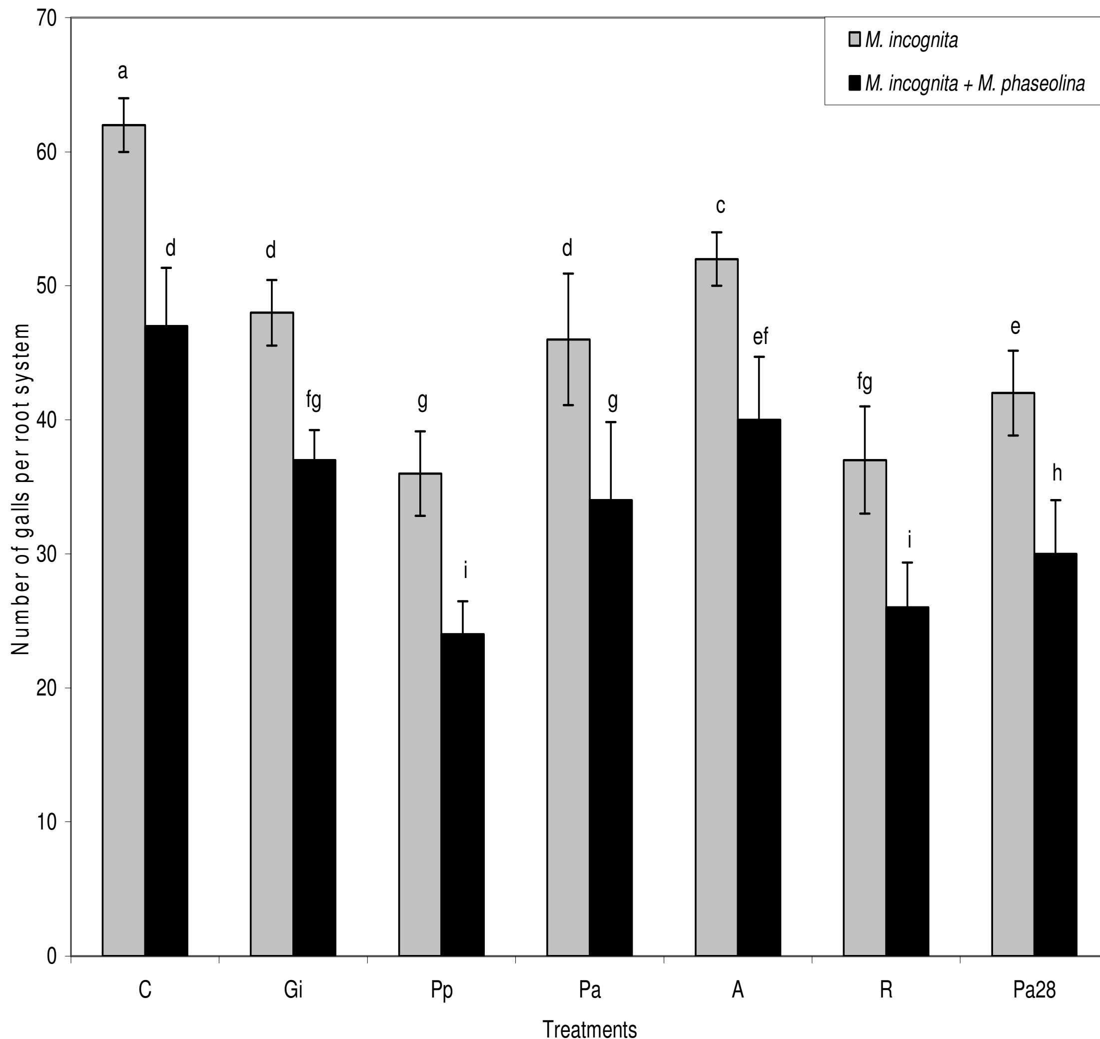

Figure 1. Effect of PSM (*G. intraradices*, *P. putida*, *P. alcaligenes, P. aeruginosa* (Pa28), *A. awamori*) and *Rhizobium* sp. on the galling of chickpea under field condition. C = Control; Gi = *G. intraradices*; Pp = *P. putida*; Pa = *P. alcaligenes*; A = *A. awamori*; Pa = *P. aeruginosa*; R = *Rhizobium* sp.
*Different letters within 1 parameter are significantly different at p = 0.05. Error bars represent Standard deviation.

In this study, *G. intraradices* improved plant growth of nematode-infected plants by reducing nematode multiplication. The root-rot severity of plants inoculated with *M. phaseolina* was also reduced by *G. intraradices* (Akhtar and Siddiqui, 2007b). Bodker et al. (1998) observed a reduction in root-rot of pea caused by *Aphanomyces euteiches*, while Akkopru and Demir (2005) observed about 17% reduction in Fusarium wilt of tomato after inoculation of plants with *G. intraradices*. In addition, the changes in nutrient uptake in the root system, a mycorrhizosphere effect and activation of plant defense mechanisms are thought to be responsible for disease inhibition by AM fungi (Linderman, 1994; Demir and Akkopru, 2005). Moreover, treatment with *Glomus* sp. is also reported to increase phenylalanine and serine in tomato roots (Suresh, 1980), these amino acids have an inhibitory effect on nematodes (Reddy, 1974).

Pseudomonads may also improve plant growth by suppressing parasitic and non-parasitic root pathogens (Oostendrop and Sikora, 1989) through the production of biologically active substances (Gamliel and Katan, 1993) or the conversion of unavailable minerals and organic compounds into forms that are available to plants (Broadbent et al., 1977; Siddiqui and Mahmood, 1999). *Pseudomonas* can synthesize enzymes that can modulate plant hormone levels, may limit the available iron via siderophore production and can also kill the pathogen with antibiotics (Siddiqui, 2006). In addition, induced systemic resistance by *Pseudomonas* is also thought to be a biocontrol mechanism against plant pathogens (Wei et al., 1996). Similarly *Bacillus* isolates have been reported to promote the growth of a wide range of plants

Table 2. Effect of phosphate solubilizing microroganisms (*G. intraradices*, *P. putida*, *P. alcaligenes, P. aeruginosa* (Pa28), *A. awamori*) and *Rhizobium* sp. on the yield and of chickpea under field condition.

Treatment		Grain (100 seeds) weight (g)	Yield (q/ha)
Control	C	14.76 ± 0.43 d	16.04 ±0.26 e
	Gi	15.60 ± 0.14 c	17.02 ±0.33 d
	Pp	16.54 ± 0.26 a	18.25 ± 0.38 b
	Pa	15.92 ± 0.23 b	17.45 ± 0.44 c
	A	15.34 ± 0.17 c	16.87 ± 0.18 d
	R	16.72 ± 0.11 a	18.75 ± 0.26 a
	Pa28	16.20 ± 0.32 b	17.64 ± 0.36 c
M. incognita	C	10.94 ± 0.30 p	11.44 ± 022 p
	Gi	13.06 ± 0.36 k	13.48 ± 0.18 kl
	Pp	14.36 ± 0.26 ef	14.74 ± 0.42 g
	Pa	13.64 ± 0.17 ij	13.95 ± 0.31 ij
	A	12.68 ± 0.13 l	12.95 ± 0.29 m
	R	14.47 ± 0.08 def	15.17 ± 0.24 f
	Pa28	14.04 ± 0.33 gh	14.38 ± 0.30 h
M. phaseolina	C	11.06 ± 0.24 op	11.51 ± 0.42 p
	Gi	13.40 ± 0.24 j	13.72 ± 0.33 jk
	Pp	14.58 ± 0.22 de	15.20 ± 0.19f
	Pa	13.82 ± 0.23 hi	14.23 ± 0.25 hi
	A	12.79 ± 0.19 kl	13.21 ± 0.29 lm
	R	14.60 ± 0.25 de	15.40 ± 0.37 f
	Pa28	14.26 ± 0.08 fg	14.75 ± 0.21 g
M. incognita* + *M. phaseolina	C	9.15 ± 0.32 q	7.65± 0.24 r
	Gi	11.72 ±0.30 n	11.44 ± 0.36 p
	Pp	12.62 ± 0.35 l	12.52 ± 0.28 n
	Pa	12.05 ± 0.31 m	11.93 ± 0.24 o
	A	11.35 ± 0.23 o	11.06 ± 0.21 q
	R	12.85 ± 0.18 kl	13.10 ± 0.44 m
	Pa28	12.15 ± 0.18 m	12.26 ± 0.38 no
L.S.D. p = 0.05		0.31	0.34

C = Control; Gi = *G. intraradices*; Pp = *P. putida*; Pa = *P. alcaligenes*; A = *A. awamori*; Pa = *P. aeruginosa*; R = *Rhizobium* sp.
*Values within each column followed by same letter are not significantly different (p = 0.05).
± **=** Standard deviation

(De Freitas et al., 1997; Kokalis-Burelle et al., 2002). *B. pumilus* induces callose and pectin in close association with phenolic compounds in newly formed wall appositions in pea roots in response to attack by *Fusarium oxysporum* (Benhamou et al., 1996). However, treatment with *B. pumilus*, induced a rapid lignification in cucumber plants in response to ingress of *Colletotrichum orbiculare*, and total peroxidase and superoxide dismutase activities increased more than those in the buffer control (Jetiyanon et al., 1997). These responses may be due to the production of siderophores, antibiotics, wall appositions and defense enzymes, which adversely affect on the pathogens.

G. intraradices, *P. alcaligenes* and *B. pumilus* were used individually and concomitantly to control the root-rot disease complex of chickpea (Akhtar and Siddiqui, 2008b). Phosphate solubilizing bacterial isolate that promote and stimulate colonization by the AM fungus are called mycorrhiza helper bacteria (Barea et al., 1998) and they also stimulate the germination of AM spore and mycelial development (Meyer and Linderman, 1986). The present study demonstrated that PSM can coexist without

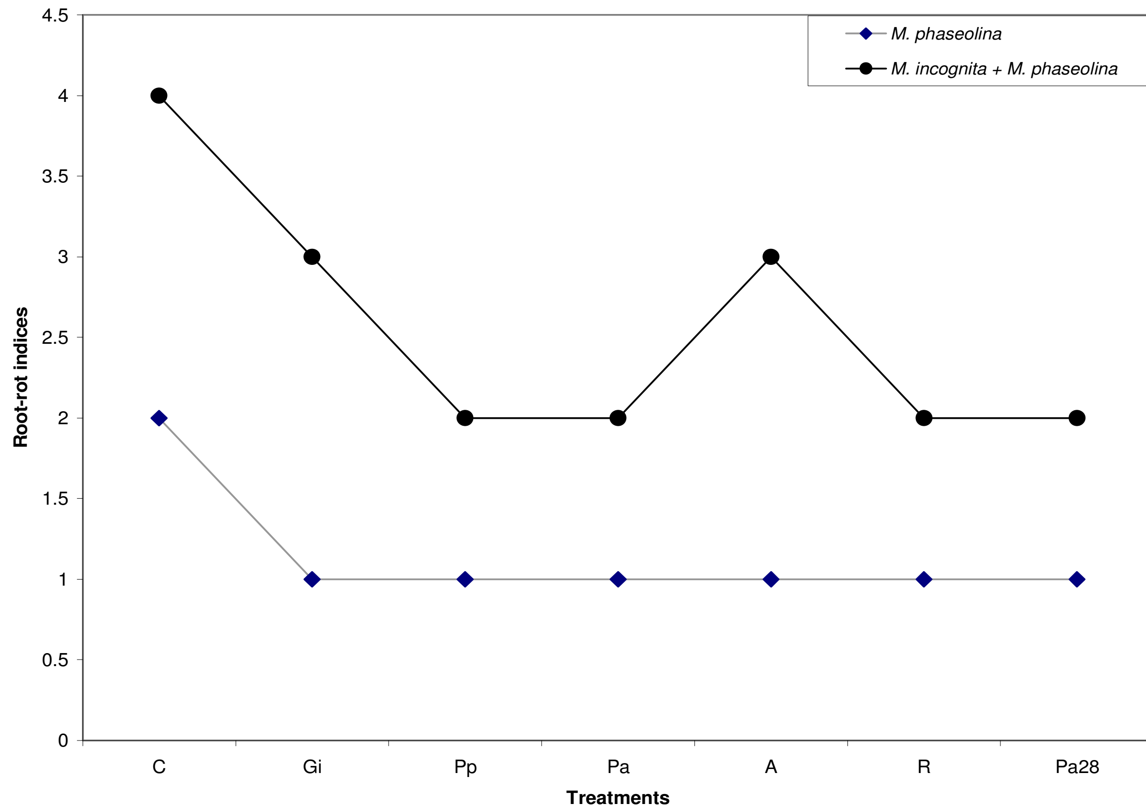

Figure 2. Effect of PSM (*G. intraradices*, *P. putida*, *P. alcaligenes, P. aeruginosa* (Pa28), *A. awamori*) and *Rhizobium* sp. on the on the root-rot indices under field condition.
C = Control; Gi = *G. intraradices*; Pp = *P. putida*; Pa = *P. alcaligenes*; A = *A. awamori*; Pa = *P. aeruginosa*; R = *Rhizobium* sp.

adversely affecting each other. In fact, suitable combinations of these biocontrol agents may be increase plant growth and resistance to pathogens. In future, more detail investigations are needed on the performance of PSM on different hosts of various pathosystems.

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