

BIOCONTROL OF A CHICKPEA ROOT-ROT DISEASE COMPLEX WITH PHOSPHATE-SOLUBILIZING MICROORGANISMS

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SUMMARY

The effects on chickpea (*Cicer arietinum*) of the phosphate-solubilizing microorganisms *Aspergillus awamori*, *Pseudomonas aeruginosa* (isolate Pa28) and *Glomus intraradices* in terms of growth, and content of chlorophyll, nitrogen, phosphorus and potassium and on the root-rot disease complex of chickpea caused by *Meloidogyne incognita* and *Macrophomina phaseolina* were evaluated. Application of these phosphate-solubilizing microorganisms alone and in combination increased plant growth, pod number, and chlorophyll, nitrogen, phosphorus and potassium contents, and reduced galling, nematode multiplication and root-rot index of chickpea. *Pseudomonas aeruginosa* reduced galling and nematode multiplication the most followed by *A. awamori* and *G. intraradices*. Combined inoculation of these microorganisms caused the greatest increase in plant growth and reduced the root-rot index more than individual inoculations. Pathogens adversely effected root colonization by *G. intraradices*. However, root colonization and root nodulation were increased when co-inoculated with *P. aeruginosa* and *A. awamori* whether in the presence or absence of pathogens.

Key words: *Aspergillus*, Biocontrol, Chickpea, *Glomus*, *Macrophomina*, *Meloidogyne*, *Pseudomonas*, root-rot disease complex.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop in India and chief source of dietary protein in the vegetarian diet. This crop is susceptible to the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood and the root rot fungus *Macrophomina phaseolina* (Tassi) Goid. The interaction between *M. incognita* and *M. phaseolina* causes a root-rot disease com-

plex that severely damages this important crop (Siddiqui and Husain 1991; 1992).

Rhizosphere organisms provide an initial barrier against pathogens attacking the root (Weller 1988) and microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents. Phosphate-solubilizing microorganisms have potential for the biocontrol of plant pathogens (Ozgonen *et al.*, 1999) as they change insoluble phosphatic compounds into soluble forms (Duijff *et al.*, 1999; Chin a-Woeng *et al.*, 2000; Ramamoorthy *et al.*, 2002) thus increasing the growth and yield of crop plants (Algawadi and Gaur 1988; Tilak 1991; Gupta and Namdeo 1997). Arbuscular mycorrhizal (AM) fungi also colonize the roots of many crop plants (Smith and Read 1997; Ozgonen *et al.*, 1999) and are of great value in promoting the uptake of phosphorus, minor elements and water (Allen 1996; Ibijbijen *et al.*, 1996; Siddiqui *et al.*, 2001). They also influence the severity of several plant pathogens (Dehne 1982; Siddiqui and Mahmood 1995; Linderman 2000; Barea *et al.*, 2002; Akkopru and Demir 2005). *Glomus intraradices* is a highly infective species on woody and herbaceous plants in a wide range of conditions and greatly enhances plant growth (Duponnois and Plenchette 2003). *Pseudomonas aeruginosa* is also ubiquitous in soil and water and tolerant to a wide variety of physical conditions. It is resistant to high salt concentrations and primarily a nosocomial pathogen. Similarly, inoculations of *Aspergillus awamori* have potential to increase the grain yield of various crops indicating the solubilization of fixed soil phosphorus (Gaur 1985).

This study examined the effects of *Aspergillus awamori*, *Pseudomonas aeruginosa* (isolates Pa28) and *Glomus intraradices* on growth, chlorophyll, nitrogen, phosphorus and potassium contents and the root-rot disease complex of chickpea.

MATERIALS AND METHODS

The root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood and *Macrophomina phaseolina* (Tassi) Goid were the pathogens tested. The phosphate-solubilizing microorganisms *A. awamori*, *P. aerug-*

inosa and *G. intraradices* were applied alone and in combination to chickpea (*Cicer arietinum* cv. Avarodhi). The influence of these treatments on plant growth, number of pods, galling and nematode multiplication, and the root-rot disease complex were assessed in 90-day glasshouse experiments.

Preparation and sterilization of soil mixture. Soil, river sand and organic manure were mixed in ratio of 3:1:1 (v/v) respectively and added to jute bags. Water was poured into each bag to wet the soil before sterilization at 137.9 kPa for 20 minutes. Sterilized soil was allowed to cool to room temperature before using it to fill 15-cm diameter clay pots with 1 kg each.

Growth and maintenance of test plants. Seeds of chickpea cv. Avarodhi were surface sterilized in 0.1% sodium hypochlorite for 2 minutes and then washed three times with distilled water. Five seeds were sown in each pot and later thinned to one seedling per pot. Plants were placed in a glasshouse and watered as needed. Two days after thinning, seedlings received the treatments while uninoculated plants served as a control. The seedlings were inoculated with of *M. incognita*, *M. phaseolina*, *A. awamori*, *P. aeruginosa*, and *G. intraradices*.

Preparation of nematode inoculum. *M. incognita* was collected from chickpea field soil and multiplied on eggplant (*Solanum melongena* L.) using a single egg mass. Egg-masses were hand picked using sterilized forceps and placed in 9-cm diameter sieves of 1 mm pore size which were previously lined with cross-layered tissue paper. The sieves were placed for hatching in Petri dishes with distilled water and incubated at 27°C. Two thousand freshly hatched second stage juveniles (J2) per plant were used as inoculum.

Preparation of fungal inoculum. *M. phaseolina* was isolated from chickpea root and maintained on potato dextrose agar (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. Wet mycelium (100 g) was macerated in 1 l distilled water, and 10 ml of this suspension containing 1 g fungus was inoculated per plant.

Inoculum of phosphate-solubilizing microorganisms. The AM fungus, *G. intraradices* (Schenck and Smith), was produced on *Chloris gayana* (Rhodes grass) grown in sandy loam soil mixed with washed river sand and farm yard manure in ratio of 3:1:1 (v/v) respectively. The population of *G. intraradices* in the inoculum was assessed by the most probable number method (Porter 1979). Fifty grams of inoculum with soil was added around the seed to provide 500 infective propagules of *G. in-*

traradices per pot (1 g inoculum contained ten infective propagules). The crude inoculum consisted of soil, extra-metrical spores and spore carps, hyphal fragments and infective Rhodes grass segments. *Pseudomonas aeruginosa* (isolate Pa28) and *A. awamori* were isolated from chickpea field soil on nutrient agar and PDA respectively. *P. aeruginosa* inoculum was produced from a freshly cultured sub-plate on nutrient broth incubated at 37 ± 2°C for 72 h. Ten ml of the suspension (1.5 × 10⁷ cells/ml) was used as inoculum. *A. awamori* was cultured in Richard's medium for 15 days at 25°C, mycelium was collected on blotting sheets to remove excess of water and nutrients, and 100 g mycelium was macerated in 1 l distilled water. Ten ml (equivalent to 1 g culture) was used as inoculum.

Inoculation techniques. For inoculation of *M. incognita*, *M. phaseolina*, *G. intraradices*, *A. awamori*, and *P. aeruginosa*, soil around the root was carefully removed without damaging the roots. Inoculum suspensions were poured around the roots and the soil was replaced. An equal volume of sterile water was added to control treatments.

Experimental design and measurements. The experiment was carried out in a completely randomized blocked design with four variables: (a) Control (b) *M. incognita* (c) *M. phaseolina* (d) *M. incognita* + *M. phaseolina*. Each set was inoculated with the following eight treatments: (i) Control; (ii) *G. intraradices* (Gl); (iii) *A. awamori* (As); (iv) *P. aeruginosa* (Ps); (v) Gl + As; (vi) Gl + Ps; (vii) As + Ps; (viii) Gl + As + Ps, and the experiment was repeated once.

The plants were harvested 90 days after inoculation. Data were recorded on plant height, dry shoot weight, number of pods, number of nodules, number of galls, percentage root colonization, root-rot index and estimated nematode population. Chlorophyll, nitrogen, phosphorus and potassium content were estimated per 1g of fresh leaf weight. Chlorophyll content of the shoot was estimated by the technique of Arnon (1949) and nitrogen content of the shoot was estimated by the technique of Lindner (1944). Phosphorus and potassium contents were estimated by the methods of Fiske and Subba Row (1925) and flame photometer respectively. A 250 g sub-sample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting method followed by Baermann's funnel extraction to determine nematode population (Southey 1986). A root-rot index was determined by scoring on a scale ranging from 0 (no disease) to 5 (severe root-rot). The proportion of root colonized by *G. intraradices* was determined by a grid intersecting method (Giovannetti and Mosse 1980) after clearing the root with KOH in 0.05 % trypan blue lactophenol.

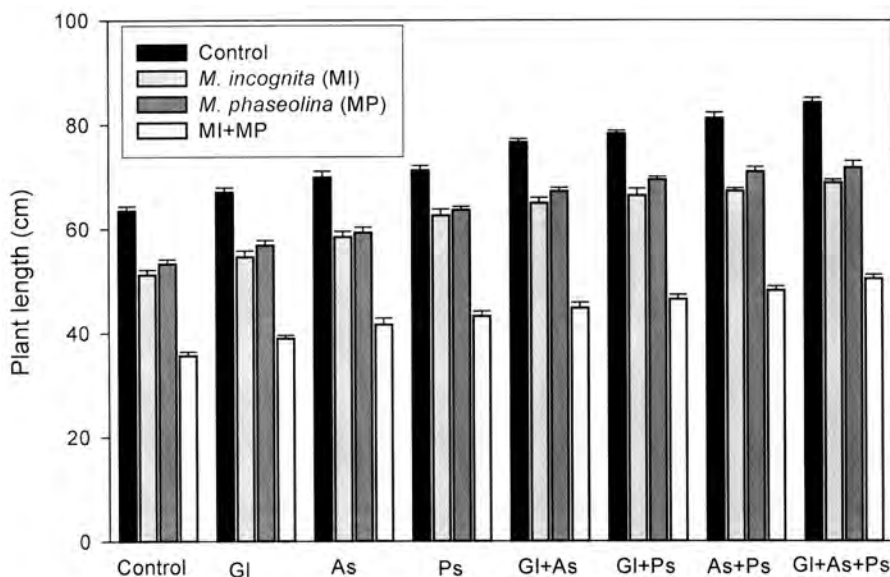


Fig. 1. Effects of *Glomus intraradices* (Gl), *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on height of pathogen-inoculated and uninoculated chickpea.

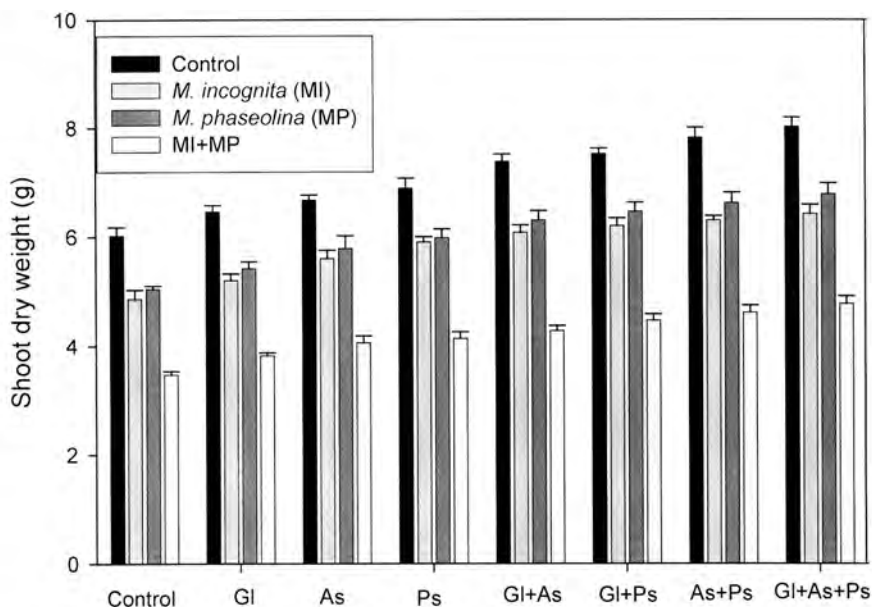


Fig. 2. Effects of *Glomus intraradices* (Gl), *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on the shoot dry weight of pathogen-inoculated and uninoculated chickpea.

Statistical analysis. The data were analyzed statistically and the standard error of each treatment was calculated (Dospekhov 1984). Each parameter studied was graphed using sigma plot 2000.

RESULTS

Inoculation of plants with *A. awamori*, *P. aeruginosa* and *G. intraradices* alone and in combination without the pathogens significantly increased plant growth compared

to uninoculated controls (Fig. 1-2). *Pseudomonas aeruginosa* increased plant growth more than *G. intraradices*.

Increase of plant growth with *A. awamori* or *P. aeruginosa* was similar. Combined inoculation with *G. intraradices* plus *A. awamori* and *P. aeruginosa* increased plant growth more than *G. intraradices* plus *P. aeruginosa* or *G. intraradices* plus *A. awamori* although *A. awamori* with *P. aeruginosa* in the absence of pathogens caused a similar increase in plant growth to that caused by combined inoculations of *G. intraradices* with *A. awamori* and *P. aeruginosa*.

Inoculation with *M. incognita* and *M. phaseolina* alone or in combination significantly reduced plant growth compared to the untreated control. Growth reduction was greater when *M. incognita* and *M. phaseolina* were inoculated together than when plants were inoculated with either of them alone. *Meloidogyne incognita* reduced plant growth to the same extent as *M. phaseolina*.

Inoculation with *G. intraradices*, *A. awamori* and *P. aeruginosa* significantly increased the growth of pathogen-inoculated plants. Inoculation with *P. aeruginosa* increased plant growth of *M. phaseolina*-inoculated plants more than plants inoculated with *G. intraradices* or *A. awamori*.

Combined inoculation with *G. intraradices*, *A. awamori* and *P. aeruginosa* to plants with the pathogens increased plant growth more than by *G. intraradices* with *P. aeruginosa* or *G. intraradices* with *A. awamori*, however, inoculation with *G. intraradices* plus *A. awamori* increased plant growth to the same level as with combined inoculation of *G. intraradices* with *P. aeruginosa* and *A. awamori* (Figg. 1-2).

Inoculation with *G. intraradices*, *A. awamori* and *P. aeruginosa* alone and in combination significantly increased the number of pods per plant in both pathogen-inoculated and uninoculated soil (Fig. 3). The number of pods per plant was reduced when plants were inoculated with *M. incognita*, *M. phaseolina* or both together. Nodulation was very poor on plants inoculated with either pathogen alone or with *G. intraradices*, *A. awamori* and *P. aeruginosa* (Fig. 4). The number of galls per root system and nematode multiplication was reduced in the presence of *M. phaseolina* (Figg. 5-6). Inoculation with *P. aeruginosa* reduced galling and nematode multiplication the most, followed by the *A. awamori* and *G. intraradices* treatments. Combined inoculation with *G. intraradices* and *A. awamori* plus *P. aeruginosa* reduced galling and nematode multiplication the most, followed by *A. awamori* plus *P. aeruginosa* or *G. intraradices* plus *P. aeruginosa* or *G. intraradices* plus *A. awamori* (Figg. 5-6). The pathogens had adverse effects on root colonization by *G. intraradices* (Fig. 7), however, root colonization by the AM fungus was increased when co-inoculated with *P. aeruginosa* and *A. awamori* both in the presence and absence of pathogens (Fig. 7).

Root-rot indices were 3 and 5 when *M. phaseolina* was inoculated alone and together with *M. incognita* (Fig. 8). The index was reduced to 3 when *M. incognita* and *M. phaseolina* inoculated plants were also treated with *A. awamori* or *G. intraradices* or *P. aeruginosa*. The index dropped to 2 when *M. phaseolina* inoculated plants were treated with *A. awamori* or *G. intraradices* or *P. aeruginosa* or when *M. incognita* plus *M. phaseolina* inoculated plants were treated with *G. intraradices* plus *A. awamori* or *G. intraradices* plus *P. aeruginosa*. In other treatments the index was reduced to 1 (Fig. 8).

Inoculation of plants with *A. awamori*, *P. aeruginosa* and *G. intraradices* alone and in combination without the pathogens caused significantly increases in chlorophyll, nitrogen, phosphorus and potassium over uninoculated plants (Figg. 9-12). *P. aeruginosa* inoculated plants without pathogens had more chlorophyll, nitrogen, phosphorus and potassium than plants inoculated with *G. intraradices*. The increase in chlorophyll, nitrogen, phosphorus and potassium in plants inoculated with *A. awamori* were similar to those caused by *P. aeruginosa*. Combined inoculation of plants with *G. intraradices* along with *A. awamori* plus *P. aeruginosa* increased chlorophyll, nitrogen and potassium more than inoculation with *G. intraradices* plus *P. aeruginosa* or *G. intraradices* plus *A. awamori*. *Aspergillus awamori* with *P. aeruginosa* inoculated plants in the absence of pathogens caused a similar increase in chlorophyll, nitrogen, and potassium as that caused by inoculation with *G. intraradices* plus *A. awamori* along with *P. aeruginosa*. Increased phosphorus in *G. intraradices* plus *A. awamori* inoculated plants was similar without pathogens to that with *G. intraradices* plus *A. awamori* and *P. aeruginosa* (Figg. 9-12).

Inoculation of plants with *M. incognita* and *M. phaseolina* alone and in combination significantly reduced chlorophyll, nitrogen, phosphorus and potassium compared with the uninoculated control (Figg. 9-12). The reduction in chlorophyll, nitrogen, phosphorus and potassium contents was greater when *M. incognita* and *M. phaseolina* were inoculated together than with either of them alone. *Meloidogyne incognita* caused a reduction in phosphorus and potassium similar to that caused by *M. phaseolina*. Inoculation of plants with *P. aeruginosa* in the presence of pathogens increased chlorophyll, nitrogen, phosphorus and potassium more than inoculation with *A. awamori* or *G. intraradices*; however, the increased chlorophyll, nitrogen, phosphorus and potassium caused by *A. awamori* alone was similar to that caused by *G. intraradices*. Combined inoculation with *G. intraradices*, *A. awamori* and *P. aeruginosa* increased chlorophyll, nitrogen, phosphorus and potassium more than the increase by *G. intraradices* plus *P. aeruginosa* or *G. intraradices* plus *A. awamori*. However, inoculation with *P. aeruginosa* and *A. awamori* to plants with pathogens caused a similar increase in chlorophyll, nitrogen, phosphorus and potassium to that caused by inoculation with the combination of *G. intraradices*, *A. awamori* and *P. aeruginosa* (Figg. 9-12).

DISCUSSION

Glomus intraradices has the potential to improve plant growth of nematode-infected plants by reducing nematode multiplication (Bagyaraj *et al.*, 1979). The root-rot index of *M. phaseolina* inoculated plants was al-

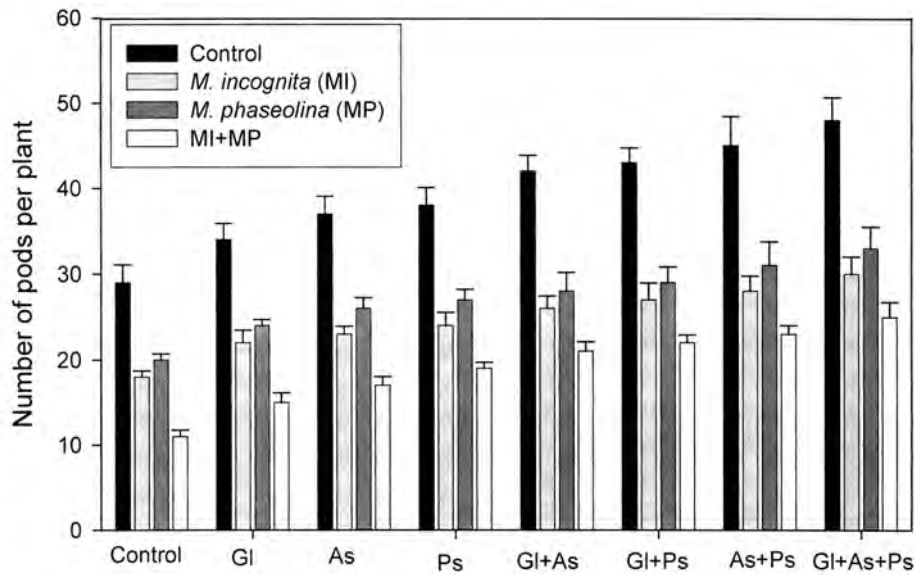


Fig. 3. Effects of *Glomus intraradices* (Gl), *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on number of pods per plant in pathogen-inoculated and uninoculated chickpeas.

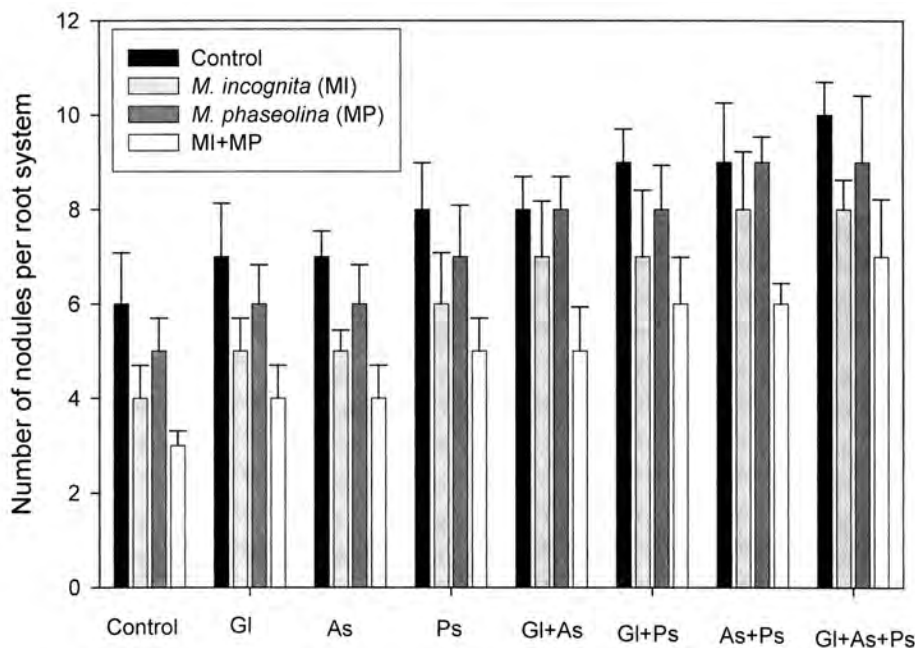


Fig. 4. Effects of *Glomus intraradices* (Gl), *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on number of root nodules on pathogen-inoculated and uninoculated chickpeas.

so reduced by *G. intraradices*. Bodker *et al.* (1998) observed a reduction in root-rot of pea caused by *Aphanomyces euteiches* while Akkopru and Demir (2005) observed reduced Fusarium wilt of tomato by inoculation of plants with *G. intraradices*. Reduced

pathogen damage of mycorrhizal plants may be due to physiological and biochemical changes in the host or to an increase in the flow of nutrients which provides greater mechanical strength (Schonbeck, 1979; Auge 2001). In addition, inoculation with mycorrhizal fungi

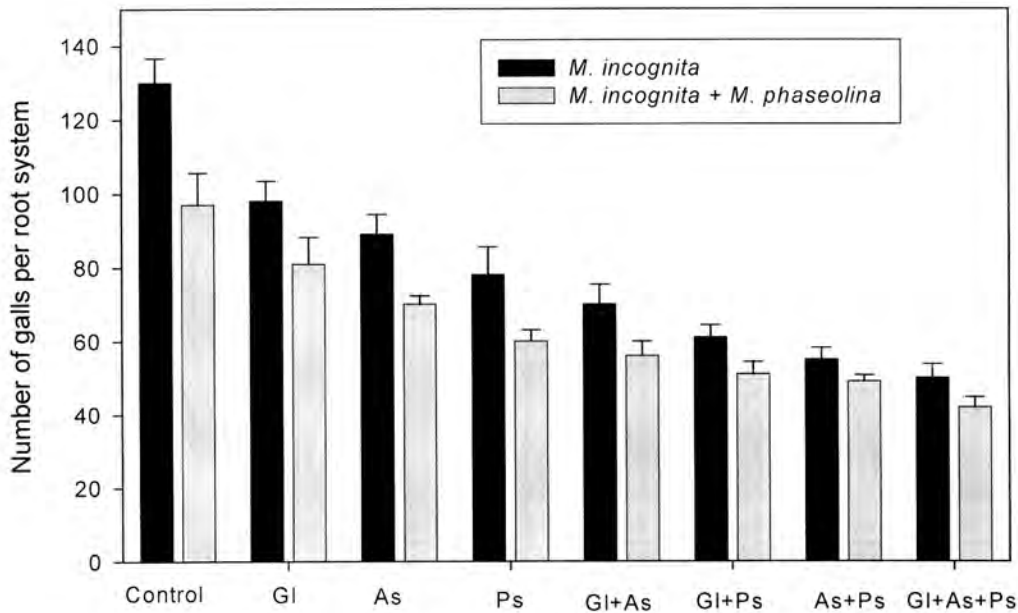


Fig. 5. Effects of *Glomus intraradices* (GI), *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on the number of galls per root system in nematode-inoculated plants.

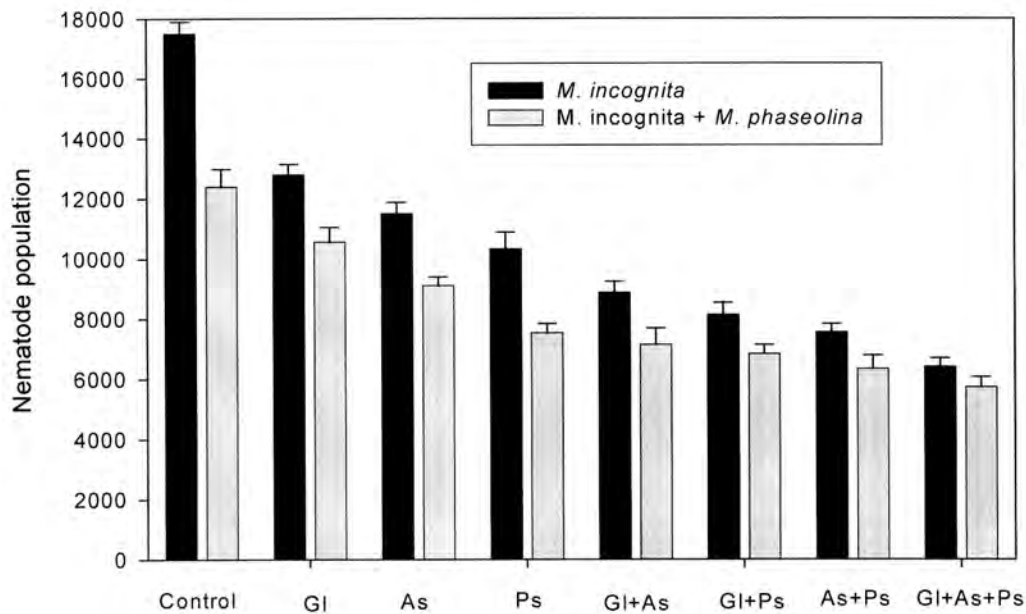


Fig. 6. Effects of *Glomus intraradices* (GI), *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on nematode populations on chickpea.

increases phosphorus enough to offset symptoms caused by the pathogen (Hussey and Roncadori, 1982). Treatment with *Glomus spp.* is also reported to increase phenylalanine and serine in tomato roots (Suresh, 1980) and these amino acids have an inhibitory effect on nematodes (Reddy, 1974).

Phosphate-solubilizing microorganisms improved the

growth of plants possibly through an inhibitory effect on nematode development as reported by Becker *et al.*, (1988); Kloepper *et al.*, (1992); and Haseeb *et al.*, (2005). Pseudomonads may also improve plant growth by suppressing parasitic and non-parasitic root pathogens (Oostendrop and Sikora, 1989), by the production of biologically active substances (Gamiel and

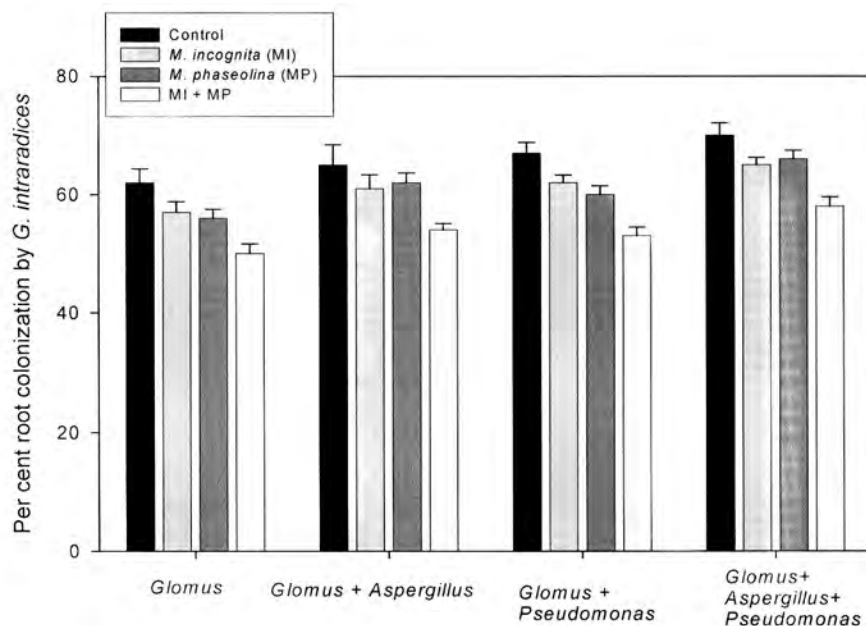


Fig. 7. Effects of, *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on root colonization by *G. intraradices* in pathogen-inoculated and uninoculated soil.

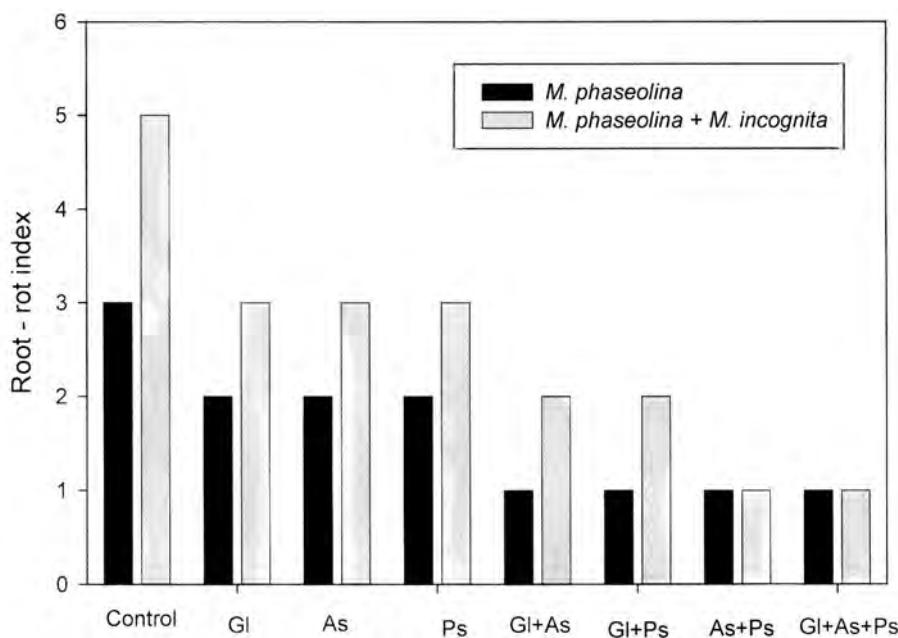


Fig. 8. Effects of *Glomus intraradices* (Gl), *Aspergillus awamori* (As) and *Pseudomonas aeruginosa* (Ps) on chickpea root rot index.

Katan, 1993), or by converting unavailable minerals and organic compounds into forms available to plants (Broadbent *et al.*, 1977, Siddiqui and Mahmood, 1999). In addition, systemic resistance induced by *Pseudomonas* is also considered a mechanism for the biocontrol of plant pathogens (Wei *et al.*, 1996). Inoculation of *A. awamori* produced phenyl ethanol, phenyl acetic acid

and phenoxy acetic acid (Nair and Burke, 1988) which may suppress *Fusarium* spp., *Sclerotium* spp., *Phytophthora* spp. etc. (Palakshappa *et al.*, 1999). Our results show that combined inoculations with phosphate-solubilizing microorganisms increased nodulation and root colonization as well as increasing tissue nitrogen, phosphorus, potassium and chlorophyll compared to indi-

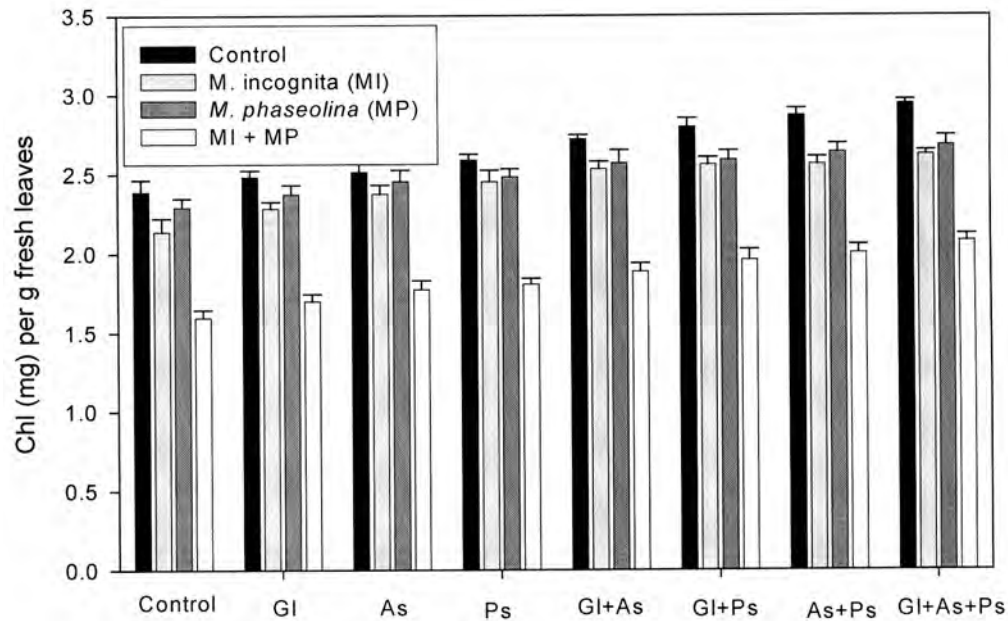


Fig. 9. Effects of *G. intraradices* (GI), *A. awamori* (As) and *P. aeruginosa* (Ps) on total chlorophyll in uninoculated chickpea plants and those inoculated with *M. incognita* and *M. phaseolina*.

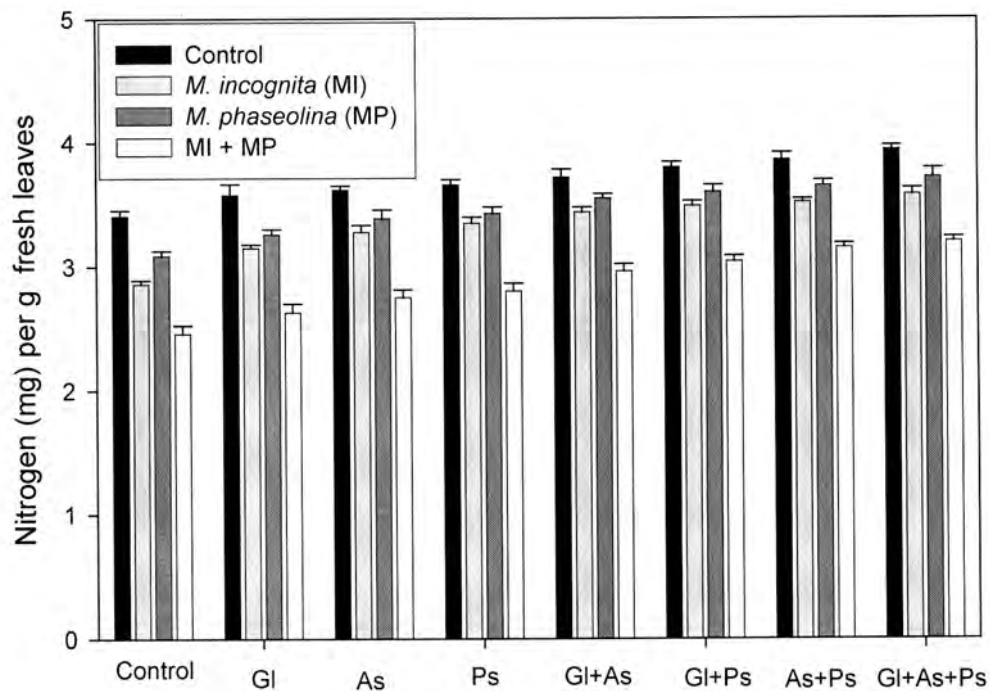


Fig. 10. Effects of *G. intraradices* (GI), *A. awamori* (As) and *P. aeruginosa* (Ps) on the nitrogen content of uninoculated chickpea plants and those inoculated with *M. incognita* and *M. phaseolina*.

vidual inoculation besides reducing the damage caused by pathogens. Inoculation with *G. intraradices*, *A. awamori* and *P. aeruginosa* not only improved the growth, chlorophyll, nitrogen, phosphorus and potassium content of plants but also reduced root-rot.

Despite these positive results, it should be remembered that *P. aeruginosa* is the epitome of an opportunistic pathogen of humans. This bacterium almost never infects uncompromised human tissues but its use as biocontrol agent may involve some health problems.

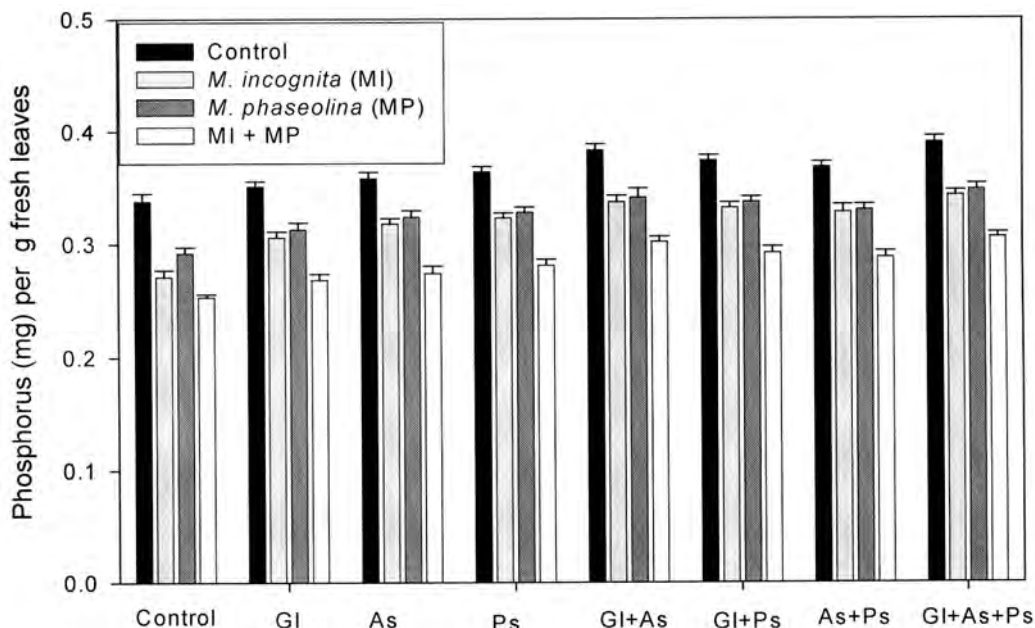


Fig. 11. Effects of *G. intraradices* (GI), *A. awamori* (As) and *P. aeruginosa* (Ps) on the phosphorus.

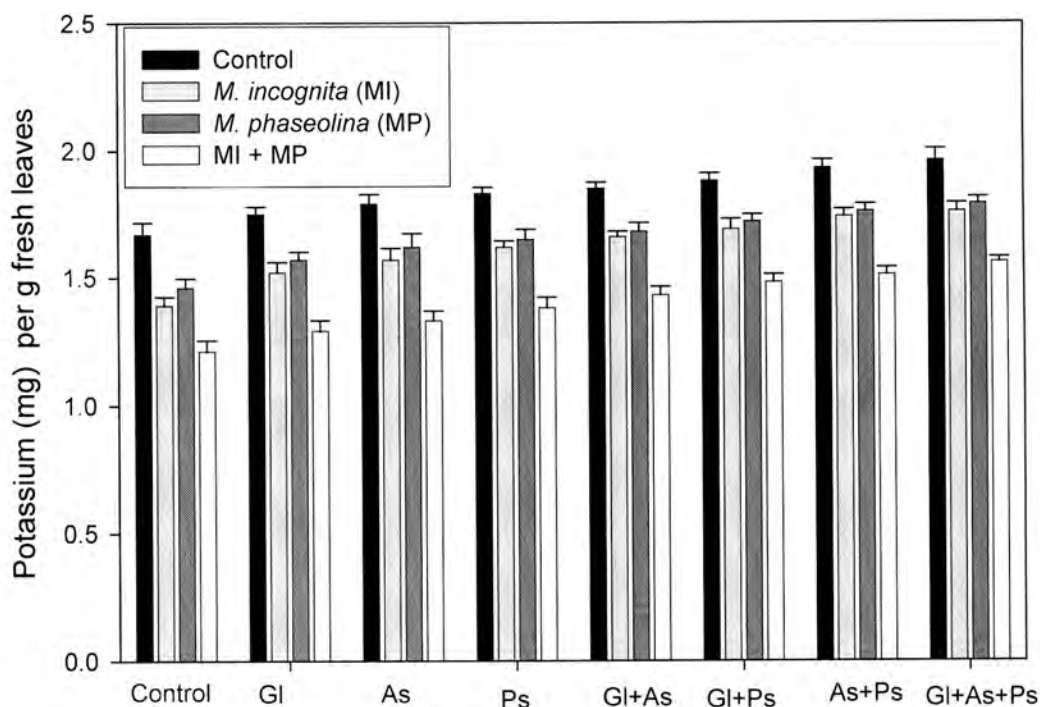


Fig. 12. Effects of *G. intraradices* (GI), *A. awamori* (As) and *P. aeruginosa* (Ps) on the potassium content of uninoculated chickpea plants and those inoculated with *M. incognita* and *M. phaseolina*.

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