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RESEARCH PAPER

Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra

TARIQ MUKHTAR[1], MUHAMMAD ARSHAD HUSSAIN[2] and MUHAMMAD ZAMEER KAYANI[3]

[1] Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
[2] Plant Pathology Section, Regional Agricultural Research Institute, Bahawalpur, Pakistan
[3] Greenbelt Project, Department of Agriculture, Murree Road, Rawalpindi, Pakistan

Summary. The root-knot nematode, *Meloidogyne incognita*, is a sedentary endoparasitic plant pathogen with a very wide host range, which causes annual crop losses amounting to millions of dollars. The small number of available nematicides and restrictions on the use of non-fumigant nematicides due to high toxicity to humans and non-target organisms hinder effective nematode control. A possible alternative to chemical nematicides is the use of biological control agents for the management of this nematode. In the present study, the efficacy of four biocontrol agents was tested against *M. incognita* at different doses. The biocontrol agents *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* were mass produced and mixed with the formalin sterilized soil at the rates of 2×10^3, 4×10^3, 6×10^3, 8×10^3, and 1×10^4 endospores/chlamydospores/cfu per g of soil. Okra seeds (cv. Sabz Pari) were sown in pots of soil amended with the different agents, and 10 d after emergence, the plants were inoculated with 2000 freshly hatched second stage juveniles of *M. incognita*. Data on plant growth parameters and nematode infestations were recorded 7 weeks after inoculation. The antagonists varied significantly in enhancing various growth parameters and reducing nematode infestations in a dose-responsive manner. Both *P. penetrans* and *P. lilacinus* were equally effective and caused maximum reductions in number of galls, egg masses, nematode fecundity and build up as compared with *T. harzianum* and *P. chlamydosporia*. Reductions in these parameters at the concentration of 8×10^3 were statistically similar with those caused at the concentration of 1×10^4 chlamydospores/endospores/cfu. Our results indicate that application of antagonists can suppress galling and reproduction of *M. incognita* resulting in enhancement of plant growth.

Key words: root-knot nematodes, biological control, antagonists, *Abelmoschus esculentus*.

Introduction

The root-knot nematode, *Meloidogyne incognita*, is a sedentary endoparasitic plant pathogen with a broad host range. These nematodes are of considerable economic importance, responsible for low crop yields and annual losses in tropical countries of 22% (Sasser, 1979). These parasites are the most prevalent in the country (Hussain *et al*., 2012; Kayani *et al*., 2012a, 2013) and accounts for growth impairment (Hussain *et al*., 2011a, Irshad *et al*., 2012; Maleita *et al*., 2012). Use of chemicals for nematode control, though very effective, cannot be adopted by the farmers in developing countries due to their high cost. In developed countries, nematicides are undesirable due to associated problems of residual toxicity, environmental pollution and public health hazards (Thomason, 1987). These factors are an impetus for the discovery of alternative methods for nematode control (Tzortzakakis and Petsas, 2003). One alternative to chemical nematicides is the use of biological control agents, either alone or integrated with other pest

Corresponding author: T. Mukhtar
Fax: +92 51 9290771
E-mail: drtmukhtar@uaar.edu.pk

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management strategies (Davies *et al.*, 1991; Hussain *et al*., 2011b; Singh *et al*., 2012).

In biological control, many antagonists have shown efficacy against root-knot nematodes. Among these, the fungus, *Pochonia chlamydosporia*, a parasite of root-knot nematode eggs, is ubiquitous in distribution, and has controlled successfully root-knot nematodes (Kerry, 2001). Another fungus, *Paecilomyces lilacinus* infects eggs and females of *Meloidogyne* spp. and causes death of embryos in 5–7 d. The fungus has given excellent results under varying conditions. Similarly, *Trichoderma harzianum* has been found to be an effective biocontrol agent for the management of root-knot and other nematodes (Casas-Flores and Herrera-Estrella, 2007; Lòpez-Llorca *et al*., 2008; Hallmann *et al*., 2009; Moosavi and Zare, 2012). Furthermore, *Pasteuria penetrans*, an obligate parasite, has been widely investigated for its efficacy against root-knot nematodes throughout the world (Chen *et al*., 1996; Duponnois *et al*., 1999; Hallmann *et al*., 2009). This organism has the potential to be used as a biocontrol agent both by interfering with nematode migration towards host roots and by limiting nematode reproduction (Davies *et al.*, 1991; Oostendorp *et al*., 1991). Information on the comparative study of these antagonists is lacking, so the objective of the present study was to make a comparison of the effectiveness of these antagonists for management of *M. incognita.*

Materials and methods

Nematode inoculum

Meloidogyne incognita, raised from a single egg mass, was used in the experiment. For nematode reproduction, the most susceptible variety of tomato (cv. Money maker) was used as the host plant. Three-week-old tomato plants were transplanted into pots containing 2.5 kg formalin sterilized sandy loam soil. One week after transplantation, the plants were each inoculated with approximately 5,000 freshly hatched second stage juveniles (J2s) of *M. incognita* added to holes in the soil around the stem of each plant. The plants were kept in a green house at 25±2°C and watered as needed.

Sources of antagonists used in the experiment

An isolate of *Pasteuria penetrans* designated as Pp3, derived from *M. incognita* originating from South Africa, and the fungal isolate *Pochonia chlamydosporia* obtained from University of Reading, United Kingdom, were used in the experiment. *Paecilomyces lilacinus* was obtained from the National Nematological Research Centre, University of Karachi, Karachi, Pakistan, and *Trichoderma harzianum* was obtained from the Institute of Agriculture, Punjab University, Lahore, Pakistan.

Mass production of biocontrol agents

Pasteuria penetrans

The isolate of *P. penetrans* was multiplied on *M. incognita* on tomato cv. Money maker in a greenhouse using the procedure described by Stirling and Wachtel (1980). Root powder (100 mg) containing spores was ground in a small amount of water with a pestle and mortar to produce a slurry. This was diluted with tap water and filtered through a 38 μm mesh sieve to remove debris and collected in a beaker. Newly hatched second stage juveniles of *M. incognita* were added to this spore suspension and agitated by bubbling air through it. After 24 h, juveniles were examined with an inverted lens microscope for spore attachment. The majority of juveniles each had six to eight spores attached to their cuticles. The spore-nematode suspension was poured through a 20 μm mesh sieve to separate encumbered J2s from excessive spores unused in the suspension. The spore-encumbered J2s were washed into a 250 mL beaker and the required volume was made up using tap water. These spore encumbered J2s were inoculated around the stems of tomato plants grown in sterilized pots. Pots were kept in a greenhouse at 25±2°C and watered regularly. After 7 weeks, the root systems were carefully removed, gently washed, air dried and each ground in a pestle and mortar. This root powder served as *P. penetrans* inoculum. The spore concentration in powdered roots was determined using a haemocytometer.

Pochonia chlamydosporia

Dried milled barley was washed over a 53 μm mesh sieve and mixed in a 1:1 (v/v) with coarse sand and allowed to partially dry until easily friable. About 150 mL of this culture medium was added to a 250 mL flask, and then autoclaved (30 min, 15 psi), cooled, shaken and inoculated with five plugs (7 mm diam.) of *P. chlamydosporia* on corn meal agar. After 3 weeks incubation at 25°C, the colonized sand/bran medium

was washed through 250 μm, 53 μm and 10 μm mesh sieves with a fine water spray to remove the sand and bran, and the fungal propagules were collected on 10 μm mesh sieve. The deposit was further washed to remove conidia and small hyphal fragments leaving mainly chlamydospores, and was blotted dry to remove extra moisture. Chlamydospores were scraped off and thoroughly mixed at 1:10 (w/w) with fine sand (40-100 mesh) which acted as inert carrier. A 1 g sub-sample of inoculum was shaken in 9 ml of water and the number of chlamydospores per gram of sand was determined using a haemocytometer.

Paecilomyces lilacinus and *Trichoderma harzianum*

For mass production of the inoculum of *P. lilacinus* and *T. harzianum*, chopped wheat grains were immersed in water for about 10–12 h, surface dried using a paper towel, and 250 g was added to each of 500 mL capacity flasks. These were autoclaved at 15 psi for about 50 min. The sterilized wheat grains in flasks were inoculated separately with pure cultures of each of the antagonistic fungi and incubated at 25±1°C for 15 d. The flasks were shaken on alternate days for uniform colonization of the fungus. The number of spores per gram of the grains were counted using haemocytometer after making spore suspensions in distilled water.

Soil used for the experiment

The soil (56% sand; 19% silt; 24% clay; 1% organic matter and pH 7.6) used in the experiment was sterilized with formalin, passed through a 3.5 mm mesh sieve to remove large stones and plant residues and added to pots.

Testing the efficacy of biocontrol agents

To test the comparative biocontrol potential of *P. penetrans*, *P. chlamydosporia*, *P. lilacinus* and *T. harzianum* against *M. incognita* in a pot experiment, the biocontrol agents were each mixed with the formalin sterilized soil at the rates of 2×10^3, 4×10^3, 6×10^3, 8×10^3, or 1×10^4 endospores/chlamydospores/cfu per g of soil. The treated soil was then put in plastic pots (1 kg per pot). Three okra seeds (cv. Sabz Pari) were sown in these pots and after emergence, one healthy seedling was maintained per pot. Ten days after emergence, the plants were inoculated with 2,000 J2s of *M. incognita*. Inoculated plants without antagonists served as experimental controls. The pots were arranged in Completely Randomized Design (CRD) in a greenhouse at 25±2°C, and left for 7 weeks and watered as needed.

Data collection

The plants were carefully removed from the pots. The shoots were excised from the roots. Shoot and root lengths were measured, and root and shoot fresh and dry weights (60°C for 24 h) were determined. The galls and egg masses on each plant root system were counted under a stereo microscope (40× Magnification).

For estimation of total nematode populations, eggs were extracted from the roots of individual plants using the method described by Hussey and Barker (1973). Juveniles were extracted from the soil from each pot using the Tray Method of Whitehead and Hemming (1965). The total number of eggs and nematodes in soil were considered as the total population. The reproductive factor (Rf) was calculated by dividing the final population (Pf) by the initial population (Pi).

Proportional reductions or increases in plant growth parameters and nematode infestations compared to experimental controls were calculated for each treatment as described by Kayani *et al*. (2012b).

Statistical analysis

The experiment was repeated twice. As no significant discrepancies were observed between the corresponding mean values of all the treatments of the repeated experiments, the data of both the experiments were combined before statistical analyses. This gave a total of 10 replications for each treatment. All the data were subjected to analysis of variance using GenStat package 2009, (12^{th} edition) version 12.1.0.3278 (www.vsni.co.uk). Means were compared by Duncan's Multiple Range Test. A significant level of $P\leq0.05$ was used in statistical analyses.

Results

Shoot and root weights

All the antagonists showed significant effects on plant growth parameters. The analyses of variance

showed significant variations in shoot and root fresh weights and shoot dry weight among the different antagonists, concentrations and their interactions.

Maximum increase in shoot weight resulted from application of *P. penetrans,* followed by *P. chlamydosporia*. The increases in shoot weight as a result of application of *P. lilacinus* and *T. harzianum* were statistically similar. Maximum average increase in shoot weight was recorded at a propagule concentration of 1×10^4 spores/ml which was not statistically different from the concentration of 8×10^3. The concentration of 2×10^3 resulted in minimum increase in shoot weights. Maximum individual increase of 19% was recorded at the concentration of 1×10^4 spores of *P. penetrans*. The individual increases in shoot weights at each concentration of the antagonists are given in Table 1. Very similar trends were observed in shoot dry weights (Table 2).

Reduction in root weight is considered as an improvement of root health, because gall formation from nematode infections results in increased root weight. *Pasteuria penetrans* was the most effective as it resulted in the greatest reduction in root weight. All the antagonists differed significantly in reducing root weight. Similarly, concentrations of the antagonists also had significant effects on root weight

Table 1. Mean proportional increases in shoot fresh weights of okra plants after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Increase[a]					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	9 c	11 d	12 de	16 hi	16 hi	13 B
P. lilacinus	7 ab	8 bc	9 bc	14 f	15 fgh	11 A
P. penetrans	9 c	13 ef	16 gh	17 i	19 j	15 C
T. harzianum	6 a	9 bc	9 c	14 fg	14 fg	11 A
Mean[c]	8 A	10 B	11 C	15 D	16 D	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean increase in shoot fresh weight of okra plant after treatment with each antagonist compared to control.
[c] Overall mean increase in shoot fresh weight of okra plant at different concentrations compared to controls.

Table 2. Mean proportional increases in shoot dry weights of okra plants after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Increase[a]					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	5 a	8 bcd	8 cde	11 f	10 f	9 B
P. lilacinus	4 a	7 bc	8 bcd	8 bcd	8 bcd	7 A
P. penetrans	7 b	10 ef	10 ef	13 g	15 h	11 C
T. harzianum	5 a	7 b	8 bcd	9 de	9 cde	7 A
Mean[c]	5 A	8 B	8 B	10 C	10 C	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean increase in shoot dry weight of okra plant after treatment with each antagonist compared to control.
[c] Overall mean increase in shoot dry weight of okra plant at different concentrations compared to controls.

reduction. Maximum reduction was recorded at a propagule concentration of 8×10^3 which did not differ significantly from 1×10^4 concentration. The greatest reduction was seen for *P. penetrans* at 1×10^4 concentration. The individual and average reductions are given in Table 3.

Shoot and root length

The antagonists varied in their ability to enhance shoot and root lengths. Maximum increase in shoot length was recorded from *P. chlamydosporia*. *Paecilomyces lilacinus* and *P. penetrans* both gave similar increases in shoot lengths. Minimum average increase was recorded in the case of *T. harzianum*. Propagule concentrations also affected increase in shoot lengths. Maximum increase was observed at a concentration of 8×10^3. Minimum increases were recorded at the lowest propagule concentrations of antagonists. The average and individual increases in shoot lengths are given in Table 4.

Similarly, root length was also increased by the application of antagonists. Maximum increases were recorded from *P. chlamydosporia* and *P. penetrans* which showed no statistical difference from each other. *Trichoderma harzianum* caused least increase.

Table 3. Mean proportional decreases in root weights of okra plants after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Decrease					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	10 bcd	11 cde	13 fg	13 gh	14 ghi	12 C
P. lilacinus	9 a	9 ab	10 bcd	13 fg	13 fg	11 A
P. penetrans	11 cde	12 ef	14 ghi	14 hi	15 i	13 D
T. harzianum	9 a	10 abc	12 def	14 ghi	13 fg	11 A
Mean[c]	9 A	10 B	12 C	14 D	13 D	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean decrease in root weight of okra plant after treatment with each antagonist compared to control.
[c] Overall mean decrease in root weight of okra plant at different concentrations compared to controls.

Table 4. Mean proportional increases in shoot lengths of okra plants after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Increase[a]					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	5 a	11 cd	13 f	18 gh	17 g	13 C
P. lilacinus	5 a	8 b	12 de	17 gh	17 g	12 B
P. penetrans	4 a	8 b	11 cd	18 h	16 g	12 B
T. harzianum	5 a	9 c	10 c	13 ef	13 ef	10 A
Mean[c]	5 A	9 B	11 C	16 E	16 D	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean increase in shoot length of okra plant after treatment with each antagonist compared to control.
[c] Overall mean increase in shoot length of okra plant at different concentrations compared to controls.

The propagule concentrations also showed variations in improving root lengths. Concentrations of 8×10^3 and 1×10^4 showed no significant differences in increasing root lengths. Similarly, concentrations of 4×10^3 and 6×10^3 were also statistically similar in their efficacy. The average and individual increases in root lengths caused by antagonists at different propagule concentrations are given in Table 5.

Nematode infestation

The analyses of variance of number of galls, egg masses and reproductive factors showed significant variances amongst biocontrol agents and their propagule concentrations.

All the antagonists caused significant reductions in these parameters. *Pasteuria penetrans* and *P. lilacinus* caused maximum reduction in number of galls and proved equally effective. Similarly, *T. harzianum* and *P. chlamydosporia* also showed similar reductions to each other in root galls. Significant variations were also found among concentrations of the antagonists. Maximum reduction was noticed at concentrations of 8×10^3 and 1×10^4. The reduction observed at 1×10^4 concentration was not statistically different from 8×10^3. The average and individual re-

Table 5. Mean proportional increases in root lengths of okra plants after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Increase[a]					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	5 de	7 fghi	7 fghi	8 i	7 ghi	7 C
P. lilacinus	4 bc	4 bcd	4 bcd	6 ef	6 ef	5 B
P. penetrans	5 de	6 fg	6 fgh	7 ghi	8 hi	7 C
T. harzianum	3 a	3 ab	3 ab	5 cde	5 cde	4 A
Mean[c]	4 A	5 B	5 B	6 C	6 C	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean increase in root lengths of okra plant after treatment with each antagonist compared to control.
[c] Overall mean increase in root length of okra plant at different concentrations compared to controls.

Table 6. Mean proportional decreases in number of galls on okra plant roots after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Decrease[a]					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	12 a	21 c	23 cd	32 h	31 gh	24 A
P. lilacinus	14 a	23 d	27 e	36 i	37 i	27 B
P. penetrans	17 b	27 ef	28 ef	33 h	35 i	28 B
T. harzianum	14 ab	23 cd	24 d	29 efg	29 fg	24 A
Mean[c]	14 A	24 B	25 C	32 D	33 D	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean decrease in number of galls on okra plant roots after treatment with each antagonist compared to control.
[c] Overall mean decrease in number of galls on okra plant roots at different concentrations compared to controls.

ductions are given in Table 6. A similar pattern was seen for egg masses. The reductions in egg masses caused by antagonists at different concentrations are given in Table 7.

The antagonists and their concentrations caused significant decline in nematode reproductive factors. Maximum average reduction resulted from *P. penetrans*, followed by *P. lilacinus*. Similarly, the concentration of 1×10^4 caused maximum decline in reproductive factor. The average and individual decreases in nematode reproductive factors are given in Table 8.

Discussion

All tested biocontrol agents proved effective in controlling *M. incognita*. These antagonists significantly increased root and shoot length and weight and caused reductions in the number of galls and egg masses. *Pasteuria* and *P. lilacinus* were equally effective at a concentration of 8×10^3 endospores/cfu per gram of soil in reducing nematode infestations. The improvement in various growth characteristics and reduction in nematode infections can be ascribed to a number of mechanisms. The ability of *P. penetrans* to suppress root-knot nematodes is attributable to (i) reduced root penetration by the spore-encumbered

Table 7. Mean proportional decreases in number of egg masses on okra plant roots after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Decrease[a]					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	11 a	22 c	23 cd	30 ef	30 f	23 A
P. lilacinus	15 b	29 ef	31 f	38 hi	37 ghi	30 C
P. penetrans	15 b	23 cd	35 g	39 ij	41 j	30 C
T. harzianum	16 b	25 d	28 e	36 gh	36 gh	28 B
Mean[c]	14 A	25 B	29 C	36 D	36 D	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean decrease in number of egg masses on okra plant roots after treatment with each antagonist compared to control.
[c] Overall mean decrease in number of egg masses on okra plant roots at different concentrations compared to controls.

Table 8. Mean proportional decreases in reproductive factors of *Meloidogyne incognita* after treatments with different nematode biocontrol agents applied at different propagule concentrations.

Biocontrol agent	% Decrease[a]					Mean[b]
	2×10^3	4×10^3	6×10^3	8×10^3	1×10^4	
P. chlamydosporia	20 a	31 b	35 c	43 ef	43 ef	35 A
P. lilacinus	20 a	41 e	44 f	52 h	52 h	42 C
P. penetrans	38 d	44 ef	48 g	55 i	57 i	48 C
T. harzianum	21 a	37 cd	39 d	45 f	50 h	38 B
Mean[c]	25 A	38 B	41 C	49 D	51 E	

[a] Values are means of ten replicates. Means sharing common letters do not differ significantly from each other ($P<0.05$), Duncan's Multiple Range Test.
[b] Overall mean decrease in reproductive factor of *Meloidogyne incognita* after treatment with each antagonist compared to control.
[c] Overall mean decrease in reproductive factor of *Meloidogyne incognita* at different concentrations compared to controls.

juveniles and/or (ii) failure to form egg masses by the infected females (Davies *et al*., 1991; Chen *et al*., 1996). In the present study maximum reductions in numbers of galls and egg masses were observed after *P. penetrans* treatments (Table 6 and 7), which indicated that there was reduced penetration of juveniles into the roots of okra plants resulting in reduction of these parameters. Many researchers have reported that movement and mobility of juvenile nematodes were reduced, and their capability to locate host roots was affected when juveniles were encumbered with endospores. Reduced motility probably leads to a high mortality of J2s in the soil (Chen *et al*., 1996; Vagelas *et al*., 2012). Since the reproductive systems fail to develop in the infected females of root-knot nematodes, such nematodes do not lay eggs resulting in reduced number of egg masses and reproductive factors (Davies *et al*., 1991). This leads to marked decline in the secondary infection by the second or subsequent generations of juveniles. The maximum reductions in number of egg masses and reproductive factors by the application of *P. penetrans* in the present study (Table 7 and 8) can be ascribed to these mechanisms.

Paecilomyces lilacinus and *P. penetrans* were equally effective in causing reductions in nematode infestations. This fungus appears to be a good root colonizer (Cabanillas *et al*., 1988) and rhizosphere competitor, and has been tested widely for its potential as a biological control agent and shown to suppress nematode population densities and increase plant yields (Noe and Sasser, 1995). The fungus first colonizes the gelatinous matrix of *Meloidogyne* and eventually a mycelial network develops and engulfs the nematode eggs. Penetration of nematode eggs is completed with appressoria or simple hyphae (Jatala, 1986; Holland *et al*., 1999). Both mechanical and enzymatic activities may be involved in the penetration. Morgan-Jones *et al.* (1984) reported that the fungal hyphae penetrated the eggshell of *M. arenaria* through small pores dissolved in the vitelline layer. Following penetration, the fungus grows and proliferates in the eggs in early embryonic development. After depleting all nutrients in the eggs, the mycelium may penetrate and rupture the egg shells and then emerge to infect other eggs in the vicinity. The fungus may also colonize the juveniles within the eggshells, and the 3rd and 4th stages of juveniles on water agar (Holland *et al*., 1999). Culture filtrates of *P. lilacinus* have been shown to be toxic to nematodes (Cayrol *et al*., 1989; Khan and Goswami, 2000). Cuticles of nematodes were ruptured, and the nematodes were killed within a few hours after exposure to the culture filtrates (Cayrol *et al*., 1989). A peptidal antibiotic P-168 has been isolated from culture of *P. lilacinus* and characterized (Isogai *et al*., 1980). This substance has antimicrobial activity against fungi, yeasts, and gram-positive bacteria, and therefore may enable the fungus to compete with soil microorganisms.

Trichoderma harzianum also caused reductions in nematode infestations and was not as effective as *P. penetrans* and *P. lilacinus*. *Trichoderma* is a ubiquitous soil fungus which colonizes root surfaces and root cortices (Sharon *et al*., 2009). Several species of *Trichoderma*, including *T. harzianum, T. viride*, *T. atroviride*, and *T. asperellum*, have provided excellent control of root-knot nematodes in previous studies (Sharon *et al*., 2001, 2007). Application of *Trichoderma* resulted in reduced nematode galling and improved plant growth and tolerance (Spiegel and Chet, 1998). The highly branched conidiophores of *Trichoderma* produce conidia that can attach to different nematode stages. Conidial attachment and parasitism varies among fungal species and strains (Sharon *et al*., 2007). This process was often associated with the formation of fungal coiling and appressorium-like structures. *Trichoderma harzianum* colonizes isolated eggs and J2s of *M. javanica* (Sharon *et al*., 2001). Successful parasitism of the nematode by *Trichoderma* requires mechanisms to facilitate penetration of the nematode cuticles or eggshells. The involvement of lytic enzymes has long been suggested and demonstrated in *Meloidogyne* parasitism (Spiegel *et al*., 2005). Besides direct antagonism, other mechanisms involved in *Meloidogyne* control by *Trichoderma* spp. include production of fungal metabolites and induced resistance (Freitas *et al*., 1995; Goswami *et al*., 2008). In general, *Trichoderma* should be applied before planting to achieve maximum nematode control (Dababat *et al*., 2006) as good establishment of the fungus in plant rhizospheres seems to be important for nematode control.

In our studies *P. chlamydosporia* proved to be the least effective of the organisms tested in reducing nematode infestations, although the fungus resulted in significant improvements in plant growth characteristics which were statistically similar to those obtained with *P. penetrans*. *Pochonia chlamydosporia* (*Verticillium chlamydosporium*), like *P. lilacinus*, can para-

sitize eggs and female stages of the nematode (Morgan-Jones *et al.*, 1982, 1983; Freire and Bridge, 1985; de Leij and Kerry, 1991). *Pochonia chlamydosporia* is primarily regarded as an egg parasite. Observations have shown that during the initial stages of infection, it produces branched mycelial networks that are in close contact with eggshells (Morgan-Jones *et al.*, 1983; Lopez-Llorca and Duncan, 1988; Lopez-Llorca and Claugher, 1990). Penetration of eggshells occurs both from specialized penetration pegs (appressoria) and also from lateral branches of mycelium, and leads to the disintegration of the vitelline layer and dissolution of the chitin and lipid layers of eggshells (Segers *et al.*, 1996; Morton *et al.*, 2004). Enzymes are thought to be important in the infection process, and experiments indicate that a cocktail of proteases and chitinases are necessary to initiate infection. Proteases of nematode parasites have been characterized, including one from *V. suchlasporium* (Lopez-Llorca and Robertson, 1992) and another from *P. chlamydosporia* (Segers *et al.*, 1996). Studies of different isolates of *P. chlamydosporia* have also shown that they produce a range of different subtilisins (Segers *et al.*, 1998). The reduction in efficacy of *P. chlamydosporia* in the present study might be due to the fact that we used an exotic isolate of the fungus which may have not been effective against an indigenous population of *M. incognita*. There are reports that isolates of *V. chlamydosporium*, even those collected from the same soil, differ greatly in virulence against different root-knot populations. Similarly, variations also exist within root-knot populations in reaction to parasitism by *V. chlamydosporium* isolates (Kerry, 2001).

Results from the present comparative study prove that *P. penetrans* and *P. lilacinus* are likely to be the most effective biocontrol agents for reducing nematode infections and improving various plant growth parameters, and are likely to exert sufficient action in soil to suppress the activity of nematodes. These antagonists may be used for the management of root-knot nematodes in okra and other crops in combination with other control strategies (Rahoo *et al.*, 2011; Khan *et al.*, 2012; Mukhtar *et al.*, 2013a, 2013b) in an integrated pest management (IPM) program for important nematode diseases.

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