Turk J Biol
36 (2012) 161-169

doi:10.3906/biy-1008-72

Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves

Mujeebur Rahman KHAN[1], Fayaz Ahmad MOHIDDIN[2], Mohd. Nadeem EJAZ[1], Mohd. Mahmud KHAN[1]
[1]Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh - INDIA
[2]Sher-e-Kashmir Agriculture University of Science and Technology, Srinagar 194 101 - INDIA

Received: 20.08.2010

Abstract: The incorporation of dry neem leaves into the soil significantly enhanced the yield of uninoculated eggplant, but aldicarb treatments at 4 kg a.i./ha proved to be phytotoxic. Inoculation with root-knot nematode *Meloidogyne incognita* (1500 J_2/plant) caused a significant decrease in both the plant growth (12.5%) and the yield (11.9%) of eggplant cv. Pusa Purple Round. Treatments involving neem leaves, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, or aldicarb reduced the suppressive effect of the nematode, leading to a significant increase in the dry matter production and yield of inoculated plants compared to the inoculated control. Neem leaves induced a 19% increase in the weight of fruits/plant of inoculated plants; similar results were obtained using *P. chlamydosporia*, *P. lilacinus*, *T. harzianum*, and aldicarb treatments, with increases over the inoculated control of 11%, 14%, 6%, and 8%, respectively. Declines in galling, egg mass production, and fecundity were found to be greater with aldicarb and lower with the neem leaf treatment. The incorporation of neem leaves into biocontrol treatments increased the efficiency of the treatment and resulted in a 17%, 21%, and 14% increase in the yield with *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* treatments, respectively. Decreases in galling and egg mass production were also greater in the presence of neem leaves than in methods using the biocontrol agents alone. The percentages of infection in adult nematode females and egg masses with *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* applied to plants were considerably greater in the presence of neem leaves (77%-92% and 43%-57%) than in their absence (69%-87% and 33%-47%).

Key words: Biocontrol, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, neem leaves, yield, field trial, nematode disease

Introduction

Eggplant, *Solanum melongena* L., is one of the premier vegetables in India and is widely cultivated. The crop happens to be highly susceptible to infection by root-knot nematodes of the genus *Meloidogyne*, such as *M. incognita* and *M. javanica* (1). A number of good nematicides are available to control the root-knot nematode problem associated with vegetables including eggplant, but their use is expensive and poses hazards to man and the environment (2). Nonchemical means such as cultural and biological methods can be good substitutes for chemicals and provide satisfactory control of root-knots in vegetables and other crops (3). Among numerous organisms that have shown antagonism against root-knot nematodes, *P. chlamydosporia* (4,5), *P. lilacinus* (6,7), and *T. harzianum* (8,9) have been found to be highly suppressive to plant nematodes, especially under greenhouse conditions (3). Under field trials, however, these microorganisms usually do not provide a similar degree of nematode control (10).

Neem, *Azadirachta indica*, is one of the most versatile trees with regard to germicidal activity against an array of microorganisms (11). Its leaves, bark, and seed kernels can be used in nematode management, as can products, such as cakes, that are made of neem. Various compounds such as nimbin, nimbidin, azadirachtin, salannin, thionemon, and meliantriol occur in the seeds, leaves, and bark of neem in high concentrations (12) and are responsible for the tree's antimicrobial and nematicidal activity (13). Powder from the seed kernels and leaves has been found to be suppressive against some nematodes (14).

Biocontrol agents (BCAs) such as *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* generally colonize and establish themselves in soils rich in organic matter (2); in such soils, these agents usually offer improved biocontrol activity (15). Field soils normally contain lower organic matter contents, and it is probably as a result of this deficiency that biocontrol fungi show decreased population and activity against nematodes in the field as compared to pot conditions. Some researchers have shown that soil amendment with neem cake, green manure, or other plant materials may improve the effectiveness of BCAs (16). The incorporation of chopped green leaves of *Crotolaria* spp., *Sesbenia* spp., and *Tagetes* spp. facilitated the colonization of *Trichoderma* spp., *Paecilomyces lilacinus*, and others (14,17). Hence, in the present paper, an attempt was made to use the neem leaves that become available during autumn due to natural senescence in order to control root-knot nematodes in conjunction with biocontrol fungi. To achieve this objective, a field trial was undertaken to determine the effectiveness of *P. chlamydosporia*, *P. lilacinus* (Thom) Samson, and *T. harzianum* (Rifai) singly and in combination with dry neem leaves (*Azadirachta indica* A.Juss.) against root-knot disease of eggplant (*Solanum melongena* L.) caused by *M. incognita* (Kofoid and White) Chitwood. The effects of the treatments were also compared with a known efficacious nematicide, aldicarb (thimet).

Materials and methods

Biocontrol fungi

Cultures of *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* were obtained from the Indian Agricultural Research Institute (IARI) in New Delhi. Mass culture of the fungi was prepared on Richards' liquid medium (RLM) in 250-mL conical flasks. The flasks contained 50 mL of sterilized RLM, and they were inoculated with a loopful of the biocontrol fungi and incubated at 25 ± 2 °C for 10 days in a BOD incubator. The mycelial mat (hyphae and spores) was collected from the flask with the help of a sterilized spatula. The mat was weighed and then blended with a known volume of distilled water in an electric blender for 1 min. The fungal suspension, containing 1.5 g mycelium + spores, was added to soil around the roots of seedlings at the time of transplanting (1.5 g fungus/seedling).

Neem leaves

Leaves were collected from a neem tree and dried under shade for 10 days before being pounded with a wooden stick to make a coarse powder. The powder was applied as a spot application to the soil when the seedling was planted (100 g/seedling).

Treatments and crop culture

About 300 kg of compost was incorporated into a field of 16 × 60 m and watered with drain irrigation. A week later, 42 plots of 2 × 5 m each were demarcated by bunds 0.30 m wide and high. The treatments administered are indicated in Table 1. Each treatment was represented by 3 plots distributed randomly in the field. Aldicarb was applied to the soil at 4 kg a.i./ha in a broadcast manner 2 days before planting. During planting, 3-week-old seedlings of eggplant cv. Pusa Purple Round (PPR), raised in sterilized soil, were planted in 4 rows, with 10 seedlings per row, in each of the 24 plots. Immediately after the transplant, a nematode suspension containing 1500 freshly hatched juveniles of *M. incognita* were added to the soil in the root zone of each seedling of 12 plots and watered with a hose. A week later, the first drain irrigation was given to plots followed by 3 more irrigations at intervals of 4 weeks. The experiment was terminated 4 months after planting. During this period, plants were regularly observed for any disorder and formed flower buds were recorded.

Mature fruits, if there were any, were collected and weighed. Ten plants from each plot were selected randomly for harvest. They were uprooted and the following values were determined: dry weights of shoots and roots, number of flowers and fruits, total weight of fruits/plant, mean fruit weight, number of galls, egg masses/root system, fecundity, soil population, and nematode infection with biocontrol agents.

Infection of females and egg masses with biocontrol agents

Females and egg masses were excised from the inoculated plants grown in biocontrol agent-treated or untreated plots. The excised females and egg masses were surface sterilized with 0.5% sodium hypochloride and inoculated on solidified potato dextrose agar (PDA) in petri plates (10 females/plate) (17). For each treatment, 10 plates were maintained. The plates were incubated at 25 ± 2 °C for 7 days in a BOD incubator. After incubation, plates were examined to determine the percentage of infection of females and egg masses with *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum*.

Fecundity

The number of eggs/egg masses (fecundity) was determined from 100 egg masses excised from the root systems of 10 plants from a treatment. The egg masses were blended in 0.5% sodium hypochlorite solution for 40 s and the resulting suspension was poured over 100- and 500-mesh sieves. The eggs retained on the finer sieve were washed with water and finally transferred to a beaker in 100 mL of water, from which 1 mL was taken to a counting dish and all types of eggs (healthy, fungus-invaded, misshapen) were counted with the aid of a microscope.

Soil population of root-knot nematode juveniles

The population of *M. incognita* was determined at 0, 2, and 4 months using a modified version of Cobb's decanting and sieving method followed by Baermann's funnel technique (18). Soil was collected from the root zone of 10 plants each from 3 plots of a treatment. The soil sample from each plot was mixed and sifted through a coarse sieve, and 1 kg was taken with 10 L of water in a plastic bucket. The soil-water mixture was stirred and then allowed to stand for 1-2 min. The suspension was decanted over a combination of 3 sieves (60-, 200-, and 400-mesh), and the catch from the finest sieve was carefully washed and transferred to a beaker. A small coarse sieve with 2 layers of wet paper towels was kept in a Baermann funnel filled with water. The nematode suspension from the beaker was gently poured onto the sieve and allowed to stand overnight. Because of their wriggling movements, the nematode juveniles migrated through the paper pores into the water and gradually settled down in the bottom of the rubber tubing of the funnel. The nematode suspension recovered from the Baermann funnel was put into a beaker and counted in a dish under a stereomicroscope.

Rhizosphere population of biocontrol fungi

The populations of *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* in the soil were estimated 0, 2, and 4 months after transplanting. The soil collected for the nematode population was used to determine the rhizosphere population of the biocontrol fungi. A 10-g sample of the sieved soil was taken in a sterilized conical flask, to which 100 mL of distilled water was added. The suspension was stirred and poured into a 1000-mL Erlenmeyer flask. The flask containing the suspension was subjected to mechanical shaking for 15 min. Afterwards, 1 mL of the filtrate was added to a sterilized test tube containing 9 mL of distilled water. The procedure was repeated 5 times in order to obtain a dilution of 1:10,000 (10^{-4}). The suspension from the final dilution was pipetted over solidified PDA in petri plates under laminar flow (0.3 mL/plate). Three plates were maintained for each treatment. Inoculated plates were incubated at 25 ± 2 °C for 72 h in a BOD incubator. After incubation, the plates were examined under a colony counter and the colonies of the biocontrol fungi were counted.

Statistical analysis

The mean of 10 plants collected from a plot was calculated and considered as 1 replicate. Hence, there were 3 replicates, which were analyzed by a 2-factor analysis of variance (ANOVA), and the least significance difference (LSD) was calculated at $P \leq 0.05$ (19). Treatments with neem leaves, *P. chlamydosporia*, *P. lilacinus*, *T. harzianum*, and aldicarb were considered as one factor and nematode inoculation as another factor. To identify the significant effect of a treatment, uninoculated-treated

plants were compared with uninoculated-untreated plants (control), and, similarly, inoculated-treated plants were compared with inoculated-untreated plants (inoculated control). The data on galling, egg mass production, and fecundity were processed by single-factor ANOVA.

Results and discussion

Application of control agents did not produce any discernable effect on the dry matter production or yield of the uninoculated eggplants (Table 1); incorporation of neem leaves, however, led to a significant increase in the number and weight of the fruits/plant over the uninoculated control ($P \leq 0.05$). Neem leaves may have improved the organic composition of the soil by providing a more nutritive and porous substrate in the root zone, leading to the promotion of biomass and yield. Aldicarb application caused a somewhat suppressive effect on the plant growth that was not significantly different from the control (Table 1). Inoculation with *M. incognita* resulted in the significant suppression of the dry weight of shoots and roots, and in the number and weight of fruits/plant, when compared to uninoculated plants (Table 1). Eggplant is a highly susceptible host of *M. incognita* and it exhibits significant reductions in plant growth and yield as a result of nematode attack (1,20).

Table 1. Effect of neem leaves, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, and aldicarb on dry matter production and the yield of *Meloidogyne incognita* in infected and uninfected eggplant.

Treatments	Nematode inoculation (Juveniles/plant)	Dry weight (g)		Number/plant		Fruit weight (g)	
		Shoot	Root	Flower	Fruits	Total/plant	Mean fruit
Control	0	60.8	13.1	14.2	13.5	1550	114.8
P. chlamydosporia	0	60.8	12.5	14.2	13.3	1576	114.2
P. lilacinus	0	60.4	12.9	14.8	13.6	1563	114.9
T. harzianum	0	60.5	12.3	14.5	13.1	1561	114.5
Neem leaves (NL)	0	62.4	13.5	15.0	14.4[a]	1712[a]	118.9[a]
Aldicarb	0	58.2	12.6	14.0	13.3	1520	114.3
Control	1500	53.5[a]	11.2[a]	13.5	12.2[a]	1316[a]	102.0[a]
P. chlamydosporia	1500	56.7[a]	11.4	13.7	13.0	1466[a]	104.0
P. lilacinus	1500	57.3[a]	12.3	14.2	13.5	1507[a]	105.3
T. harzianum	1500	55.1	11.1	13.1	12.2	1443[a]	102.9
Neem leaves	1500	56.9[a]	12.2[a]	14.0	13.6	1578[a]	116.1
Aldicarb	1500	58.2	12.0	14.0	12.9	1473[a]	114.2[a]
NL + *P. chlamydosporia*	1500	58.5[a]	11.9	14.1	13.3	1646[a]	123.7[a]
NL + *P. lilacinus*	1500	59.3[a]	12.7	14.6	13.9	1697[a]	122.1[a]
NL + *T. harzianum*	1500	57.1[a]	11.4	13.5	12.5	1673[a]	133.8[a]
NL + aldicarb	1500	58.4	12.2	14.1	13.1	1497[a]	114.3[a]
LSD ($P \leq 0.05$)		6.3	3.1	0.96	0.84	0.91	74.8
F-value							
Treatments (df = 5)		NS	NS	NS	NS	7.2	4.7[b]
Nematode (df = 1)		NS	11.5[b]	NS	10.1[b]	33.5	18.0[b]
Interaction (df = 5)		NS	NS	NS	NS	12.3	17.2[b]

Each value is the mean of 3 replicates; a = significantly different from the respective controls at $P \leq 0.05$; b = significant at $P \leq 0.05$; NS = not significant at $P \leq 0.05$.

The 3 biocontrol agents tested decreased the negative effects of nematodes, leading to a decrease in galling and an enhancement in the growth and yield of the eggplant, but the effect varied according to the treatment and parameter (Table 1). Soil application of *P. chlamydosporia* and *P. lilacinus* significantly improved the dry shoot weight (6%-7%) over the control group. The increase in the dry weight of the root of inoculated plants was significantly greater with *P. lilacinus* (9.3%) and neem leaves (8.9%). The number of flowers and fruits and the mean fruit weight of the inoculated plants remained uninfluenced with the control treatments. The total weight of the fruits/plant was, however, significantly greater with neem leaf treatment (19%), followed by *P. lilacinus* (14.5%), *P. chlamydosporia* (11.4%), *T. harzianum* (9.6%), and aldicarb (11.8%), when compared to the inoculated control (Table 1). According to the ANOVA, the F-value obtained for the nematode inoculation was not significant for the number of flowers and fruits or for the mean fruit weight. Significant F-values for the weight of the fruits/plant were observed for treatments and interactions (Table 1).

Combined treatments of biocontrol fungi and neem leaves induced greater enhancement of the plant growth and yield variable of the eggplant (Table 1). Application of the 3 biocontrol fungi with neem leaves significantly increased the dry weight of the shoot, with the effect being greater with *P. lilacinus* (11%) and lowest with *T. harzianum* (6.7%) over the control group. A significant increase in the dry root weight was recorded with only *P. lilacinus* (13.4%). The mean fruit weight and yield (weight of fruits/plant) were more positively influenced by an integration of the BCAs and neem leaves, resulting in a 20%-31% and 25%-29% increase in the 2 yield variables, respectively. The greatest increase in the mean fruit weight and total yield was recorded with *T. harzianum* and *P. lilacinus*, respectively, in combination with neem leaves. Treatment with aldicarb resulted in a 12% increase in these 2 yield variables of the nematode-infected plants.

Root galls were not observed on plants grown in plots not inoculated with the nematode. However, the plants grown in plots inoculated with 1500 J_2 of *M. incognita* developed more than 100 galls and egg masses on the root system (Table 2). The application of BCAs and neem leaves significantly suppressed the gall formation and egg mass production. The lowest number of galls and egg masses were recorded on the plants treated with aldicarb, 82% and 88% lower than in the control. Notably, a great decrease in galls and egg masses also occurred with *P. lilacinus* (48% and 70% less), *P. chlamydosporia* (44% and 62%), *T. harzianum* (29% and 60%), and neem leaves (32% and 50%), compared to the control group. Joint treatments of BCAs, neem leaves, and *P. lilacinus* (68% and 76%), *P. chlamydosporia* (63% and 73%), and *T. harzianum* (57% and 68%) resulted in a greater decrease in the number of galls and egg masses/root system, respectively, when compared with the control (Table 2).

Table 2. The effect of neem leaves, *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, and aldicarb on the gall formation, egg mass, and egg production of *Meloidogyne incognita* in eggplant and the percentage of infection in mature females and egg masses with the biocontrol fungi.

Nematode inoculation treatment	Galls/root system	Egg masses/ root system	Eggs/ egg mass	Percentage infection**	
				Female	Egg mass
Control	136	129	266	0	0
Neem leaves (NL)	93[a]	64[a]	237[a]		
P. chlamydosporia	76[a]	49[a]	203[a]	81	41
P. lilacinus	71[a]	38[a]	193[a]	87	44
T. harzianum	96[a]	51[a]	213[a]	49	19
Aldicarb	24[a]	16[a]	127[a]		
NL + *P. chlamydosporia*	51[a]	35[a]	193[a]	89	49
NL + *P. lilacinus*	44[a]	30[a]	185[a]	92	57
NL + *T. harzianum*	66[a]	41[a]	196[a]	57	23
NL + aldicarb	22[a]	16[a]	129[a]		
LSD ($P \leq 0.05$)	7.4	23.0	15.6	13.8	3.8
F-value (df = 5)	43.8[b]	11.6[b]	29.2[b]	64.5[b]	31.9[b]

Each value is the mean of 3 replicates; a = significantly different from the control at $P \leq 0.05$; b = significant at $P \leq 0.05$; ** = observations are based on 100 females/egg masses.

Adult females and egg masses excised from the plants treated with *P. chlamydosporia* and *T. harzianum* showed 81% and 41%, 87% and 44%, and 49% and 18% infection with the fungus, respectively, whereas the females from untreated plants did not show infection with the biocontrol fungi (Table 2). In the treatments where the fungi were applied in combination with neem leaves, the percentage of infection in females and egg masses was significantly greater than when the fungi were administered in the absence of neem leaves ($P \leq 0.05$). Microscopic examination revealed that infection by the 3 fungi in the females was initiated through the anus and later spread into the body, destroying its contents. With the exception of *T. harzianum*, the fungus mycelium grew profusely on egg masses and the majority of the eggs showed extensive mycelial growth externally on the shell. In some eggs, the mycelium of *P. chlamydosporia* and *P. lilacinus* invaded the body of the unhatched juveniles.

Among the control agents applied singly, neem leaves produced a 19% increase in yield in inoculated plants compared to other treatments; however, galling, egg mass production, and fecundity were decreased to a lesser extent in this treatment. This indicates that the yield enhancement was partially due to a decline in gall formation (14) and partially due to improved soil fertility (16). *P. lilacinus* (4,5,21) and *P. chlamydosporia* (8,9) are established parasites of nematodes. The BCA may have parasitized the juveniles in soil and suppressed their penetration in roots (5,22), leading to a lower disease intensity (3,23) and improved plant growth (24-26). Both of these fungi invaded the females (4,8,27) and resulted in lower egg mass production and fecundity in *M. incognita* (5). Infection with *P. chlamydosporia* and *P. lilacinus* may greatly suppress the egg formation and laying capacity of a female (3). The degree of nematode control and subsequent yield enhancement was relatively lower with *T. harzianum* than with the other BCAs. *Trichoderma* spp. are basically mycoparasites (28), but in recent years, their suppressive effects against plant nematodes have also been reported (9,29). The presence of neem leaves significantly enhanced colonization and multiplication of the BCA, as evidenced by the higher soil population and the increased percentage of infection in females and egg masses. This resulted in a greater decrease in the galling, egg mass production, fecundity, and soil population and, subsequently, in plant growth and yield enhancement. Biocontrol fungi generally require higher organic contents for multiplication, establishment, and activity against pathogens (15). The addition of neem leaves would have significantly increased the organic matter in the root zone of eggplant, the area where the BCAs were applied, leading to their enhanced activity. Decomposed leaves have been found to support greater sporulation and multiplication of *T. harzianum* and *P. chlamydosporia* (30). Aldicarb, among the best-known nematicides, reduced the disease by 82% but did not provide a corresponding increase in the plant dry weights and yield, apparently due to a phytotoxic effect (31). The soil population of *M. incognita* in control plots increased significantly at 2 (23%) and 4 (14%) months of sampling over the planting populations of 1500 J_2/kg soil (Figure 1). Application of *P. lilacinus* and *P. chlamydosporia* was found to decrease the nematode population by 19% and 9.6% at the 2-month evaluation, but showed a 15% and 43% increase at 4 months in comparison to the planting population, respectively. In the presence of neem leaves, *P. lilacinus*, *P. chlamydosporia*, and *T. harzianum*, the nematode populations decreased by 28%, 22%, and 7.2% over the planting population after 2 months and 19%, 6%, and 2.7% after 4 months, respectively; the corresponding values for aldicarb were 32% and 13%, respectively (Figure 1). When nematode populations were compared with the respective month of control, the decrease in the populations was much greater, being greatest with aldicarb plus neem leaves (45%) and *P. lilacinus* plus neem leaves (62%) at 2 and 4 months, respectively. Other treatments also caused significant decreases in the nematode population over the respective months of control.

The rhizosphere population of the biocontrol agents gradually increased over time, irrespective of nematode inoculation. However, the increase was much greater in the presence of nematodes (Figure 2). In nematode-infested plots, the populations of *P. chlamydosporia*, *P. lilacinus*, and *T. harzianum* increased by 109%-126% (2 months) and 139%-164% (4 months) without neem leaves and 138%-187% (2 months) and 170%-231% (4 months) with neem leaves in comparison to the planting populations

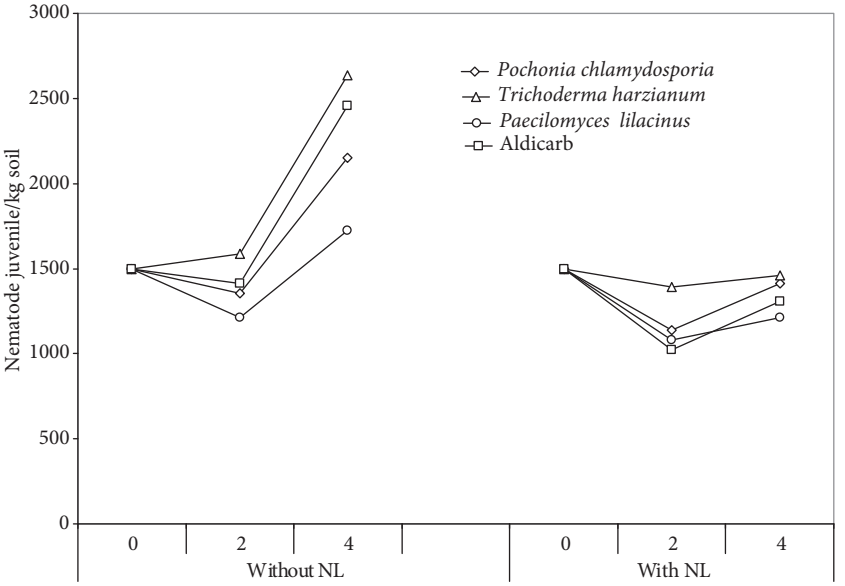

Figure 1. Effect of *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Trichoderma harzianum*, neem leaves, and aldicarb singly and jointly on the soil population of *Meloidogyne incognita*.

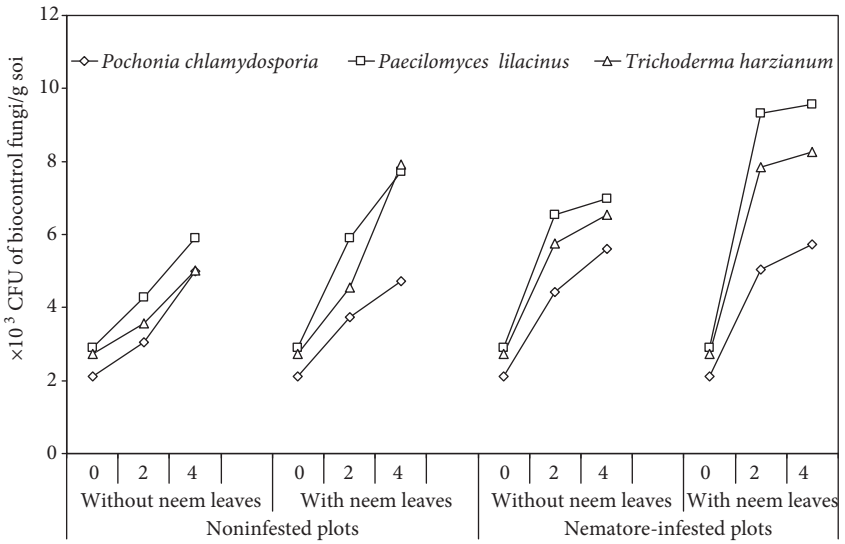

Figure 2. Rhizosphere populations of *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, and *Trichoderma harzianum* in relation to the incorporation of neem leaves and inoculation of *Meloidogyne incognita*.

(Figure 2). The greatest increase in the soil population was recorded for *P. lilacinus* (126%-231%), followed by *T. harzianum* (110%-195%) and *P chlamydosporia* (109%-179%). *P. chlamydosporia* is basically a temperate fungus and shows slower colonization (15). It seems that this is the reason its population was lower than that of the other BCAs. The negative correlation found between the population of BCA and nematode indicates that the BCAs parasitized the nematodes (6,7,9). The significantly greater increase in the population of BCA applied with neem leaves was apparently due to an increase in the soil's organic matter, which is required by the BCAs for colonization and multiplication (2).

The present study has demonstrated that combining *P. lilacinus* with neem leaves can provide

satisfactory control of root-knot disease in eggplant. The integrated treatment not only completely eliminated the suppressive effect of the nematodes but also induced an additional increase of 5%-6% in the yield of eggplant. The treatment is particularly practical in Asia, including India, where neem is a common tree. In autumn, the shed leaves can be collected and applied to fields along with *P. lilacinus* for better root-knot control. The practice can also be used to improve soil fertility.

***Corresponding author**:*
Mujeebur Rahman KHAN
Department of Plant Protectio,n
Faculty of Agricultural Sciences,
Aligarh Muslim University,
Aligarh - INDIA
E-mail: mrkhan777in@yahoo.co.in

References

1. Khan MW, Khan MR, Khan AA et al. Identity of root-knot nematodes on certain vegetables of Aligarh district in northern India. International Nematological Network News 1: 6-7, 1984.
2. Khan MR. Biological Control of Fusarial Wilt and Root-Knot of Legumes. Government of India Publication, Department of Biotechnology, Ministry of Science and Technology. New Delhi, India; 2005.
3. Khan MR. Prospects of microbial control of root-knot nematodes infecting vegetable crops. In: Singh NS. ed. Biotechnology: Plant Health Management. International Book Distributing Co.; 2007: pp. 643-665.
4. Kerry BR. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant parasitic nematodes. Annual Review of Phytopathology 38: 423-432, 2000.
5. Siddiqui IA, Atkins SD, Kerry BR et al. Relationship between saprotrophic growth in soil of different biotypes of *Pochonia chlamydosporia* and the infection of nematode eggs. Annals of Applied Biology 155: 131-14, 2009.
6. Jatala P. Biological control of nematodes. In: Sasser JN and Carter CC. eds. An Advanced Treatise on *Meloidogyne*: Biology and Control. North Carolina State University Graphics; 1985: pp. 303-308.
7. Khan MR, Khan SM, Mohiddin FA et al. Root-knot nematode problem of some winter ornamental plants and its biomanagement. Journal of Nematology 37: 198-206, 2005.
8. Bokhari FAM. Efficacy of some *Trichoderma* species in the control of *Rotylenchulus reniformis* and *Meloidogyne javanica*. Archives of Phytopathology and Plant Protection 42: 361-369, 2009.
9. Siddiqui IA, Shaukat SS. *Trichoderma harzianum* enhances the production of nematicidal compounds *in vitro* and improves biocontrol of *Meloidogyne javanica* by *Pseudomonas fluorescens* in tomato. Letters in Applied Microbiology 38: 169-175, 2004.
10. Khan MR, Mohiddin FA, Khan SM et al. Effect of seed treatment with certain biopesticides on root-knot of chickpea. Nematologia Mediterranea 33: 107-112, 2005.
11. Schmutterer H. The Neem Tree: *Azadirachta indica* A. Juss and Other Meliaceous Plants. VCH Verlagsgesellschaft. Weinheim, Germany; 1995.
12. Kraus W. Biologically active ingredients. In: Schmutterer H. ed. The Neem Tree. VCH Verlagsgesellschaft; 1995: pp. 35-88.
13. Eppler A. Effects on viruses and organisms. In: Schmutterer H. ed. The Neem Tree. VCH Verlagsgesellschaft; 1995: pp. 93-117.
14. Alam MM. Bioactivity against phytonematodes. In: Parmer BS, Randhawa NS, Kumar K. eds. Neem Research and Development. Society of Pesticide Science; 1993: pp. 123-143.
15. Stirling GR. Biological Control of Plant Parasitic Nematodes: Progress, Problems and Prospects. CABI. Wallingford, UK; 1991.
16. Prasad R, Devakumar C, Shivay YS et al. Significance in increasing fertilizer nitrogen efficiency. In: Parmer BS, Randhawa NS, Kumar K. eds. Neem Research and Development. Society of Pesticide Science; 1993: pp. 97-108.
17. Khan MR, Anwer A. Fungal bioinoculants for plant disease management. In: Ahmad I, Ahmad F, Pitchel J. eds. Microbes and Microbial Technology. Springer; 2011: pp. 447-488.
18. Southey JF. Plant Nematology. Her Majesty's Stationery Office. London, UK; 1986.
19. Dospekhov BA. Field Experimentation. Mir Publishers. Moscow; 1984.
20. Sasser JN. Plant Parasitic Nematodes - The Farmer's Hidden Enemy. North Carolina State University Graphics. Raleigh, USA; 1989.
21. Jatala P. Biological control of plant parasitic nematodes. Annual Review of Phytopathology 24: 453-491, 1986.
22. Dunn MT, Sayre RM, Carrel A et al. Colonization of nematode eggs by *Paecilomyces lilacinus* (Thom) Samson as observed with scanning electron microscope. Scanning Electron Microscopy 3: 135-137, 1982.
23. Khan MW, Esfahani MN. Efficacy of *Paecilomyces lilacinus* for controlling *Meloidogyne javanica* on tomato in greenhouse in India. Pakistan Journal of Nematology 8: 95-96, 1990.

24. Khan MN, Verma AC. Biological control of *Meloidogyne incognita* in pointed gourd (*Trichosanthes dioica* Roxb.). Annals Plant Protection Sciences 12: 115-117, 2004.

25. Khan MR, Kaunsar K, Hamid A et al. Effect of certain rhizobacteria and antagonistic fungi on root-nodulation and root-knot nematode disease of green gram. Nematologia Mediterranea 30: 85-89, 2002.

26. Khan MR, Tarannum Z. Effects of field application of various microorganisms on *Meloidogyne incognita* on tomato. Nematologia Mediterranea 27: 233-238, 1999.

27. Morgan-Jones G, Rodriguez-Kabana R. Species of *Verticillium* and *Paecilomyces* as parasite of cyst and root-knot nematodes. Phytopathology 74: 831, 1984.

28. Papavizas GC. *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. Annual Review of Phytopathology 23: 23-54, 1985.

29. Sikora RA. Interrelationship between plant health promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 53: 867-878, 1988.

30. Khan MR, Khan N, Khan SM et al. Evaluation of agricultural materials as substrate for mass culture of fungal biocontrol agents of fusarial wilt and root-knot nematode diseases. Annals of Applied Biology 22: 50-51, 2001.

31. Dhawan SC, Narayanan R, Babu NP et al. Biomanagement of root-knot nematode, *Meloidogyne incognita* on okra by *Paecilomyces lilacinus*. Annals Plant Protection Sciences 12: 356-358, 2004.