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ORIGINAL PAPER

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Interaction of *Glomus mosseae* and *Paecilomyces lilacinus* on *Meloidogyne javanica* of tomato

Abstract The effects of *Glomus mosseae* and *Paecilomyces lilacinus* on *Meloidogyne javanica* of tomato were tested in a greenhouse experiment. Chicken layer manure was used as a carrier substrate for the inoculum of *P. lilacinus*. The following parameters were used: gall index, average number of galls per root system, plant height, shoot and root weights. Inoculation of tomato plants with *G. mosseae* did not markedly increase the growth of infected plants with *M. javanica*. Inoculation of plants with *G. mosseae* and *P. lilacinus* together or separately resulted in similar shoots and plant heights. The highest root development was achieved when mycorrhizal plants were inoculated with *P. lilacinus* to control root-knot nematode. Inoculation of tomato plants with *G. mosseae* suppressed gall index and the average number of galls per root system by 52% and 66%, respectively, compared with seedlings inoculated with *M. javanica* alone. Biological control with both *G. mosseae* and *P. lilacinus* together or separately in the presence of layer manure completely inhibited root infection with *M. javanica*. Mycorrhizal colonization was not affected by the layer manure treatment or by root inoculation with *P. lilacinus*. Addition of layer manure had a beneficial effect on plant growth and reduced *M. javanica* infection.

Key words *Glomus mosseae* · *Paecilomyces lilacinus* *Meloidogyne javanica* · Tomato · Interaction

Introduction

Root-knot nematodes are important plant pathogens affecting crop production throughout the world. In Jordan, *Meloidogyne* spp. are considered a big problem for plants, especially tomato, in irrigated areas (Abu-Gharbieh 1988). Recent problems caused by the intensive use of nematicides have enhanced the development of biocontrol methods for integrated management of plant parasitic nematodes with antagonistic organisms (Cabanillas and Barker 1989).

Predacious and parasitic fungi constitute the largest and most-promising group of nematode antagonists. Parasitic fungi found to be successful bioagents against nematodes include *Paecilomyces lilacinus* (Thom) Samson (Jatala 1986), *Fusarium oxysporum* and *F. solani* (Abu-Laban 1991; Qadri 1989). The use of animal manures as substrates for nematophagous fungi introduction has replaced the traditional methods of delivery, such as infested wheat grains or aqueous spore suspensions (Abu-Laban 1991).

Vesicular-arbuscular mycorrhizal (VAM) fungi and soil-borne pathogens commonly occur together in the roots or rhizosphere of the same plant . However, recent experiments have shown that infection of tomato, white clover, lucerne and grape by VAM fungi decreases the incidence of disease, limits nematode development and activity in plant roots and minimizes growth suppression by root knot nematodes (Cooper and Grandison 1986; Grandison and Cooper 1986; Atilano et al. 1981). Francl and Dropkin (1985) observed that *Glomus fasciculatum* decreased the numbers of first-generation adult females by 26% compared with the nonmycorrhizal control. Furthermore, soybean plants with *G. fasciculatum* and *Heterodera glycines* produced more biomass than did nonmycorrhizal plants with nematodes. Mycorrhizae-induced resistance to pathogens has been linked to better host nutrition and improved phosphorus uptake by VAM plants (Dehne 1982; Hussey and Roncadori 1982). The parasitization of plants by endoparasitic nematodes can be influenced by the establishment of a VA mycorrhiza. The penetration rate of parasitic nematodes can be decreased, their development inside the root may be retarded or the degree of damage caused by the nematode may be lowered (Dehne 1982). Growth responses of tomato plants in the field due to VAM have been reported (Al-Raddad 1987).

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The objective of the present study was to evaluate the combined effects of two biocontrol agents *Glomus mosseae* and *Paecilomyces lilacinus* on the development of root-knot nematodes of tomato.

Materials and methods

Plant materials

Tomato plants (*Lycopersicon esculentum* L. cv. Claudia RAF) were raised from seeds in a soil-sand potting mixture that had been fumigated with methyl bromide at a concentration of 100 g/m^3 for 24 h. Five weeks later, seedlings were transplanted into 20-cm diameter plastic pots (one seedling per pot) containing a mix of sandy loam and sand (2:1). This soil had a pH of 7.1 and a P concentration of 15 ppm.

Inoculum preparation

A mycorrhizal inoculum of pot culture soils was prepared from *Zea mays* stock culture plants colonized with *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe. Stock culture plants were first checked for absence of pathogens. At transplanting, each plant recieved 100 ml of culture soil from the previous mother inoculum containing 500 ± 50 chlamydospores. This inoculum was dispersed through the soil around and below the plant roots. Nonmycorrhizal plants recieved the same quantity of noninoculated soil to ensure the same microflora (Menge and Timmer 1982).

The nematode inoculum of *Meloidogyne javanica* was extracted from infected roots of eggplant (*Solanum melongena*). Five thousand eggs per pot were pipetted 1 cm deep in the soil over the plant roots 6 days after seedlings were transplanted (Abu-Laban 1991; Barker 1985). All treatments were inoculated with *M. javanica* except the control. In layer manure treatments, each pot received 10 g of sterilized manure.

Paecilomyces lilacinus (Thom.) Samson was provided by Dr. Jatala of the International Potato Center, Lima, Peru. Chicken layer manure was used as carrier substrate for *P. lilacinus* introduction into the soil (Abu-Laban 1991). In *P. lilacinus* treatments, each pot was inoculated with 10 g of colonized layer manure containing 4×10^8 spores of *P. lilacinus*.

There were seven treatments: Controls not treated, plants inoculated with *G. mosseae* + *M. javanica* at transplanting, plants inoculated with *M. javanica* alone at transplanting, plants inoculated with *G. mosseae* + *P. lilacinus* and *M. javanica* at transplanting, plants inoculated with *G. mosseae* + *M. javanica* and fertilized with layer manure, plants inoculated with *P. lilacinus* + *M. javanica*, plants inoculated with *M. javanica* and fertilized with layer manure. Each treatment was replicated five times. Pots were maintained in randomized, replicated blocks in a greenhouse during January through March (10-h day, 12–25° C).

Harvesting of plants

Plants were harvested 90 days after transplanting, which potentially allowed for the development of at least two life cycles of nematodes. Fresh and dry weights of shoots were recorded after oven drying and fresh weights of roots after removal of excess water. Plant height was also measured at the end of the experiment. Roots were cut into segments of 1–2 cm, mixed and divided on a fresh weight basis for measurements of mycorrhizal root length and nematode infection.

Evaluation of mycorrhizal colonization and nematode infection

The extent of mycorrhizal infection was assessed microscopically after clearing the root system with 10% KOH, staining in lactophenol and trypan blue and examining 50 root segments per pot (Phillips and Hayman 1970). The amount of infection was expressed as either incidence (percentage of infected segments) or intensity (proportion of cortex containing endomycorrhiza). Mycorrhizal intensity was scored on a scale of 0–10 for each root segment (Biermann and Lindermann 1981).

To assess nematode infection, roots were stained in acid fuchsin in lactophenol (Southey 1970), and galling index was evaluated using the scale of 0–5 (0 = no galling, 1 = 1–10% root galling, 2 = 11–25% root galling, 3 = 26–75% root galling, 4 = 76–90% root galling, 5 = 91–100% root galling) as described by Barker (1985).

Data were subjected to one way analysis of variance. Mean separation was performed with Duncan's multiple range test.

Results

Mycorrhizal inoculation did not markedly improve height, shoot or root growth of tomato plants inoculated with *M. javanica* when compared with control plants (Table 1). Inoculation of nonmycorrhizal plants with nematodes at transplanting reduced root and shoot growth.

Plants inoculated with *G. mosseae* + *P. lilacinus* achieved height and shoot weights similar to those of inoculated plants with each fungus and manure separately. Root development was more extensive when mycorrhizal plants were inoculated with *P. lilacinus* before inoculation with nematodes.

Table 1 Effect of *Glomus mosseae* and *Paecilomyces lilacinus* on height and weight of tomato plants inoculated with *Meloidogyne javanica*. Within columns, values with at least one letter in common are not significantly different at the 5% level

Treatment		Plant height (cm)	Shoot (g/plant)		Root fresh wt. (g/plant)
Fungi	*M. javanica*		Fresh wt.	Dry wt.	
Control	–	32.4 b	9.4 c	1.7 b	11.7 de
G. mosseae	+	40.6 b	13.3 c	1.6 b	11.8 de
No fungi	+	32.0 b	6.7 c	0.9 b	7.8 e
Layer manure	+	68.8 a	26.6 b	5.9 a	15.5 cd
P. lilacinus	+	64.4 a	44.3 a	5.5 a	26.1 b
G. mosseae + layer manure	+	60.6 a	32.5 ab	5.4 a	21.2 bc
G. mosseae + *P. lilacinus*	+	67.0 a	42.3 a	7.2 a	35.3 a

Table 2 Effect of *G. mosseae* and *P. lilacinus* on *M. javanica* infection and mycorrhizal colonization of tomato plants. Within columns, values with at least one letter in common are not significantly different at the 5% level

Treatment		Gall index (scale 1–5)	Average number of galls per root system	Mycorrhizal colonization (%)	
Fungi	*M. javanica*			Incidence	Intensity
Control	–	0 d	0 d	0 b	0 b
G. mosseae	+	1.2 b	22 b	23 a	3 a
No fungi	+	2.5 a	65 a	0 b	0 b
Layer manure	+	0.6 c	10 c	0 b	0 b
P. lilacinus	+	0 d	0 d	0 b	0 b
G. mosseae + layer manure	+	0 d	0 d	24 a	5 a
G. mosseae + *P. lilacinus*	+	0 d	0 d	30 a	5 a

Shoot fresh weight at the end of the experiment was always highest in plants treated with *P. lilacinus* alone or *G. mosseae* + *P. lilacinus*. Shoot dry weights were 0.9, 5.5, 5.4 and 7.2 g for plants inoculated with *M. javanica*, *P. lilacinus*, *G. mosseae* + layer manure and *G. mosseae* + *P. lilacinus*, respectively. Plants treated with layer manure or *P. lilacinus* or *G. mosseae* + layer manure or the two fungi together were significantly larger than untreated control plants. Growth of plants inoculated with *M. javanica* was not significantly different to the control. Similarly, there were no significant differences between plants treated with layer manure + *M. javanica* + *G. mosseae* and plants treated with only layer manure + *M. javanica*, but mycorrhizal plants inoculated with *M. javanica* and fertilized with layer manure grew significantly more than plants treated with only *M. javanica*. Shoot growth of plants inoculated with *M. javanica* and fertilized with layer manure was significantly higher than that of control plants or plants inoculated with *M. javanica* without manure addition. Shoot growth of plants treated with *M. javanica* + *G. mosseae* was significantly less than that of plants with *G. mosseae* + *M. javanica* and layer manure (Table 1).

At the end of the experiment, the galled roots of plants infected by *M. javanica* were greatly decomposed and had very few feeder roots. Root weights of plants treated with *G. mosseae* and *P. lilacinus* together or each fungus separately tended to be greater than those of control plants, especially in the presence of layer manure. Roots of plants treated with *G. mosseae* + *M. javanica* were approximately the same weight as the control and did not significantly differ from the *M. javanica* treatment. Plants treated with *G. mosseae* + *P. lilacinus* + *M. javanica* showed the highest root fresh weight. The roots of layer manure treatments were not significantly different to layer manure combined with *G. mosseae* (Table 1).

The nematode populations were very low at the end of the experiment according to the root galling indices, due to root decomposition in nematode-infected root systems. The galling index was higher in the presence of *G. mosseae* than in the layer manure treatment. Inoculation of tomato roots with *G. mosseae* suppressed the galling index by 50% compared with nonmycorrhizal control seedlings. Both beneficial fungi (*G. mosseae* or *P. lilacinus*) together or separately in the presence of layer manure completely inhibited root galling with *M. javanica*. The same trend was clear for the average number of nematode galls per root system (Table 2).

Root systems inoculated with *G. mosseae* alone or with *G. mosseae* + *P. lilacinus* either in the presence or absence of layer manure showed a similar incidence and intensity of mycorrhizal infection (23–30% incidence and 3–5% intensity). Root colonization with *G. mosseae* was unaffected by nematode inoculation (Table 2).

Discussion

Meloidogyne javanica was found to cause the most severe growth inhibition of tomato plants in a study of four parasitic nematode genera, each represented by one species (Van Gundy and Kirkpatrick 1975). After *M. javanica* invades the vascular cylinder, the root tissues around developing females usually enlarge to form "knots" or "galls". Transport of water and metabolites through the altered roots is disrupted and this may interfere with the movement of metabolites needed by mycorrhizal fungi (Jenkins and Taylor 1967). In our study, mycorrhizal plants grew better than noninoculated controls and tomato plants inoculated with *M. javanica* alone. Several investigators have indicated an inhibition of nematode activity in mycorrhizal plants (Saleh and Sikora 1984; Hussey and Roncadori 1977), whilst others demonstrated no difference between mycorrhizal and nonmycorrhizal plants with respect to nematode reproduction (Roncadori and Hussey 1977). Results presented here show that inoculation of tomato plants by either *G. mosseae* or *P. lilacinus* separately or together in the presence of layer manure increased the host's resistance to *M. javanica*. Seedlings inoculated with *M. javanica* alone showed a reduction in shoot and root fresh weight by 50 and 66% respectively compared to mycorrhizal seedlings inoculated with *M. javanica*. Preinoculation with mycorrhizal fungi significantly reduced root infection with root-knot nematode. This ability of mycorrhizal plants to grow well despite infection by nematodes is generally considered to be the

principal effect of mycorrhizal fungi or the interaction of host plants with parasitic nematodes (Hussey and Roncadori 1982).

Nematode reproduction was also suppressed by inoculation with *G. mosseae* and *P. lilacinus*, with inoculated plants showing low numbers of galls per root system. The plant's resistance to nematode development was increased further when tomato plants received layer manure. Development and reproduction of nematodes is often inhibited in mycorrhizal plants (Sikora 1978), and preinfection of plants with mycorrhizal fungi and *P. lilacinus* may well afford the plant maximum protection from damage by root-knot nematodes. *P. lilacinus* significantly reduced tomato root galling with *M. javanica* by 64% compared with the nematode-alone treatment; galling index, percentage of parasitized egg masses and second stage juveniles were also significantly reduced with layer manure more than with wheat grain in a field experiment (Abu-Laban 1991). The same author reported a significant increase in the yield by *P. lilacinus* formed on layer manure where the manure was used as a carrier substrate for *P. lilacinus*. The low C/N ratio and high N content of this medium led to stimulation of fungal growth and sporulation (Abu-Laban 1991). Organic matter with a low C/N ratio resulted in broad stimulation of soil microflora (Rodriguez-Kabana et al. 1987). In addition, the decomposition of organic matter by microorganisms in soil might result in increased enzymatic activity of amended soil and accumulation of specific end products with nematicide effect. The magnitude of the organic matter activity against the pathogen depends on the nature of the organic amendment or the chemical composition and the species of microorganisms. Rodriguez-Kabana et al. (1987) reported that materials with low C/N ratios and high protein or amine contents might have nematicidal effects. In our study, pots treated with fungi-free layer manure showed less galling that pots treated with *G. mosseae* alone. Similar findings were reported by Abu-Laban (1991), where animal manure reduced the infection by root-knot nematodes. The best sporulation of *P. lilacinus* and nematode inhibition were achieved on layer manure medium which has a higher N content (5.1% N) than wheat grain (2.7% N). Both the fungi antagonists (*G. mosseae* and *P. lilacinus*) and organic amendments were acting synergistically in the biological control of root-knot nematode and could be of great importance in field trials.

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