BIOLOGICAL CONTROL OF *MELOIDOGYNE INCOGNITA* AND *RHIZOCTONIA SOLANI* IN EGGPLANT

W.M.A. El-Nagdi[1] and H. Abd-El-Khair

Plant Pathology Department, National Research Centre, Dokki, Giza, Egypt

Summary. Eggplant (*Solanum melongena*) is an important vegetable crop that is infected by root-knot (*Meloidogyne incognita*) and root-rot (*Rhizoctonia solani*) pathogens in Egypt. *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Trichoderma viride* were tested for managing these two pathogens *in vitro* and in the greenhouse in comparison with the nematicide oxamyl. The efficacy of the commercial product Micronema was assessed in the field. *In vitro*, culture filtrates of *B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride* at 10% concentration caused nematode mortalities of 100, 99, 98 and 96%, respectively, after 72 hours exposure to the filtrates. Also, *T. harzianum* greatly reduced mycelial growth of *R. solani*, followed by *T. viride*, *B. subtilis* and *P. fluorescens*. In the greenhouse, the most effective culture filtrate, applied as a soil drench, was that of *B. subtilis* at 10%, which reduced the number of juveniles in soil, galls and egg masses of *M. incognita* on the roots of eggplant cv. Pusa Purple Long by 91.9, 82 and 82.6%, respectively. Also, cultures filtrates of *T. harzianum* reduced damping-off and root-rot incidence in eggplants, followed by those of *T. viride*, *P. fluorescens* and *B. subtilis*. All bioagent treatments improved plant growth and their effectiveness was similar to that of oxamyl at 0.01% soil weight. In the field, Micronema protected eggplants from attack of *M. incognita* and *R. solani*, thus increasing yield, and affected populations of soil mycoflora differently.

Key words: *Bacillus subtilis*, biological control, *Pseudomonas fluorescens*, root-knot nematode, *Solanum melongena*, *Trichoderma harzianum*, *T. viride*.

Eggplant (*Solanum melongena* L.) is an important vegetable crop in Egypt. In this country, roots of several vegetable plants are liable to be attacked by root knot nematode and root-rot diseases, which are responsible for considerable losses in yield and fruit quality. Plant-parasitic nematodes reduce yield of the world's 40 major food staples and cash crops by an average of 12.3% (Sasser and Freckman, 1987) and the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., is one of the most important nematodes infecting vegetables. Among fungal pathogens, *Rhizoctonia solani* Kühn is the most common in Egyptian soils and causes damping-off and root-rot diseases on a wide range of vegetable plants, including eggplants. Root-rot disease may cause 10-80% yield losses in different vegetable crops (Hadwan and Khara, 1992). Field observations revealed that root-knot and root-rot diseases were common in eggplant cultivations. A preliminary study showed root-rot disease incidence in eggplant of 15.7% and that soil samples were infested with *M. incognita*, thus suggesting that these two pathogens were causing the main problem in eggplant cultivation in Egypt (Hadwan and Khara, 1992).

Biological agents can successfully control both *M. incognita* and *R. solani* (Arya and Saxena, 1999). Some species of the genus *Trichoderma* are effective against soilborne pathogens (Chet, 1990). *Trichoderma harzianum* Rifai was used to control the root-knot nematodes *M. incognita* and *M. javanica* (Treub) Chitw. (Badr, 2001) and other plant parasitic nematodes (Ismail *et al*., 2005). A culture filtrate of *T. viride* Pers. delayed egg lying by *M. javanica* (Badr, 2001). Siddiqui and Mahmood (1995) reported that satisfactory control of *Heterodera cajani* Koshi and *Fusarium udum* Butler was obtained in pigeonpea by different microorganism, such as *Bacillus subtilis* Cohn and *T. harziaum*. *Pseudomonas fluorescens* Migula has potential to control root-infecting nematodes (Hamid *et al*., 2003). *Gliocladium virens* Corda and *T. hamatum* Bonord protected eggplant, pepper and zinnia seedlings from damping-off caused by *R. solani* (Lewis *et al*., 1995a). The incidence of *Rhizoctonia* damping-off of eggplant in soil was reduced (>50%) with as little as 0.2% (w/w) of *G. virens* granules and 1% (w/w) of *T. hamatum* granules. Lewis *et al*. (1995b) studied the effect of *Cladorrhinum foecundissimum* Sacc. *et* March on the survival and saprophytic growth of *R. solani* in soils and on its ability to reduce the incidence of damping-off of sugar beet, eggplant and pepper. They found that the *C. foecundissimum* prevented damping-off of eggplant and pepper, depending on the rate of inoculum used (Lewis and Larkin, 1998). Nemec *et al*. (1996) reported that *T. harzianum*, *B. subtilis*, *G. virens* and *Streptomyces griseovirdi*s Wak. *et* Henrieni reduced crown rot disease (*Phytophthora parasitica* Dastur) of tomato in the field, and *T. harzianum* and *B. subtilis* were the most effective agents. Damping-off of eggplant, pepper, cucumber, cabbage and zinnia caused by *R. solani* was reduced by *T. hamatum* and *G. virens* (Lewis and Lumasden, 2001). *Bacillus subtilis* and *Trichoderma* spp. were isolated from

[1] Corresponding author e-mail: wafaa_elnagdi@yahoo.com

the rhizosphere and applied against damping-off disease caused by *R. solani* on cauliflower and cabbage plants; *in vitro*, *T. viride* exhibited the greatest control of *R. solani* (Loganathan *et al*., 2001).

Because of the above promising results, the aim of our study was to assess the efficacy of the bacteria *B. subtilis* and *P. fluorescens* and the fungi *T. harzianum* and *T. viride* against *M. incognita* and *R. solani* in *in vitro* tests and in eggplant in a greenhouse. Also, the effect of the new commercial product Micronema against these two soil-borne pathogens was investigated under field conditions.

MATERIALS AND METHODS

Bioagents. The bacteria *B. subtilis* and *P. fluorescens* and the fungi *T. harziamum* and *T. viride*, known to show antagonistic effects against the soil-borne pathogens that cause root-knot and root-rot diseases, were tested *in vitro* and in a greenhouse. The commercial product Micronema (containing strains of *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis*) was obtained from the Agricultural Research Center (Giza, Egypt) and tested in the field.

Root-knot nematode. Females and egg-masses of *M. incognita* were isolated from infected eggplant roots collected from the Nobariya region. A single egg mass culture of this nematode was established and reared on eggplant cv. Pusa Purple Long in a greenhouse at 30 ± 5 °C. Adult females were used to identify the nematode species by the morphological characteristics of the female perineal pattern (Taylor and Sasser, 1978).

Root-rot pathogens. *Rhizoctonia solani* and *Fusarium solani* (Mart.) Appel *et* Wollenw. emend. Snyd. *et* Has., were isolated from naturally diseased eggplants, collected from the Nobariya region, on Potato Dextrose Agar (PDA) medium, as described by Dhingra and Sinclair (1985). The fungi in pure culture were identified from cultural and morphological characters according to keys given by Gilman (1957) and Barnett and Hunter (1972).

Pathogenicity test. The objective of this test was to assess which of the two soil-borne fungi commonly occurring in Egypt was more pathogenic to eggplant. The isolates of *R. solani* and *F. solani* were tested separately in a greenhouse. Each fungus was grown on barley grain medium (200 g of grain/500 ml flasks) at 25 °C for 15 days. The soil (sandy loam) used for the test was then infested with each fungus at a rate of 3% by weight and 20-cm diameter pots were filled with 2 kg of the inoculated soils and placed on benches in a greenhouse at 30 ± 5 °C. The soil of the control pots was mixed with the same amount of non-inoculated autoclaved barley medium. There were five pots per treatment arranged according to a completely randomized design. Ten seeds of eggplants were sown in each pot. After one month, the pots were thinned to five plants per pot. Disease incidence for pre-emergence damping-off and root-rot at 30 and 60 days after sowing, respectively, were assessed as follows:

$$\% \text{ Damping off (pre-emergence)} = \frac{\text{No. of non-emerged seeds}}{\text{No. of sown seeds}} \times 100$$

$$\% \text{ Root-rot (post-emergence)} = \frac{\text{No. of plants with root-rot}}{\text{Total no. of emerged plants}} \times 100$$

***In vitro* tests**

Effect on Meloidogyne incognita. For production of the bioagent culture filtrates, the bacteria *B. subtilis* and *P. fluorescens* were grown, separately, in conical flasks (250 ml) containing 100 ml of nutrient glucose (1%) broth (3 g beef extract, 5 g peptone, 10 g glucose, 1000 ml distilled water and pH 7.2) at 30 °C for 48 h. The fungi, *T. harzianum* and *T. viride* were grown, separately, in conical flasks (250 ml) containing 100 ml of Czapek's medium at 30 °C for one week. Three conical flasks were prepared of each bioagent. The filtrates of each bioagent were obtained by filtration and then centrifugation at 3000 rpm (1090 g) for 15 min. The supernatants (growth products) were sterilized by filtration through a sterile 0.45 µm membrane filter (cellulose nitrate, Whatman) (Tahikalange *et al*., 2005). Two concentrations of each bioagent filtrate were prepared. One concentration was prepared by adding 10 ml of each bioagent filtrate to 90 ml sterile distilled water (10% of the original culture filtrate concentration) and designated as S, and the second was prepared by dilution of the S filtrate to S/2 (5% concentration).

Nematode inoculum. Second stage juveniles (J_2) of *M. incognita* were obtained from the culture maintained in the glasshouse on eggplant plants (cv. Pusa Purple Long). Infected eggplant roots bearing large egg masses were incubated in water for three days at 30 ± 5 °C and hatched J_2 were collected and counted. The concentration of the J_2 suspention was adjusted to 300 J_2 per ml. Nine ml of each culture filtrate concentration were added to 1 ml of nematode suspension containing 300 J_2 of *M. incognita* in 50 ml plastic capsules. Nine ml of water only were added to the capsules for the control treatment. Each treatment was replicated five times. The numbers of live and dead nematodes were counted under a light microscope after 24, 48 and 72 h of exposure to the culture filtrate at 25 °C. From these counts, the percentages of nematode mortality were calculated for each treatment. Nematodes were considered alive if they moved or assumed a winding shape, and they were considered dead if they had adopted a straight shape and were immobile. To avoid incorrect classification, the nematodes in each capsule were then transferred to distilled water for 48 h to check whether dead nematodes regained motility or not. The corrected nematode mortality percentages were calculated according to Abbott's Formula (1925)

$$\text{Mortality (\%)} = \frac{m - n}{100\text{-}n} \times 100$$

where *m* and *n* indicate per cent mortality in treatments and control, respectively.

Effect on Rhizoctonia solani. The antifungal activity of the bacteria *B. subtilis* and *P. fluorescens* and of the fungi *T. harizanum* and *T. viride* against *R. solani* was studied in Petri-dishes containing PDA medium, by the dual culture technique. A disc of antagonistic fungi or a streak of antagonistic bacteria were inoculated on one side of the medium and a disc of the pathogenic fungus was placed on the opposite side of the Petri dish containing the medium. As a reference control, *R. solani* was grown separately on PDA in Petri dishes. Three replicated dishes for each treatment were prepared. Inoculated Petri dishes were incubated at 25 °C for 7 days. Inhibition of fungal growth (%) was calculated from the formula:

$$\text{Inhibition of fungal growth \%} = \frac{A\text{-}B}{A} \times 100$$

Where A = diameter of linear mycelial growth in the control, and B = diameter of linear mycelial growth in the treated dish.

Greenhouse experiment

The efficacy of the four bioagents (*B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride*) on *M. incognita* and *R. solani* was assessed in pots of 20 cm diameter (containing 2 kg of sandy loam soil) in a greenhouse.

Effect on Meloidogyne incognita. Eggplant seedlings (40-days-old) were transplanted to plastic pots of 20 cm diameter, containing a sterilized sandy loam soil (1:1 w/w). Each pot was inoculated with 500 freshly hatched second stage juveniles (J_2) of the nematode. Six replicated pots were prepared per treatment. The bioagent filtrates were applied separately, at the same concentrations used *in vitro*, as a soil drench by adding 25 ml of each bioagent filtrate per pot. For comparison, the nematicide oxamyl 24% L was used at the rate 0.01% of the soil weight. Six additional pots inoculated with nematode but non-treated served as controls. All pots were arranged in a completely randomized design in a greenhouse and maintained at 30 ± 5 °C. Three months after inoculation, the plants were carefully uprooted and J_2 in the soil were extracted using a sieving and decanting technique (Barker, 1985). Nematodes were counted under a light microscope. Numbers of galls and egg-masses on the entire root system of each plant were also counted, and the lengths and weights of shoots and weights of roots were recorded.

Effect on Rhizoctonia solani. The suspensions of the bacteria *B. subtilis* and *P. fluorescens* as 3-days-old cultures and the fungi *T. harzianum* and *T. viride* as 7-days-old cultures, adjusted to 3×10^8 colony forming units (cfu)/ml were used (Abd El-Khair and El-Mougy, 2003). Two kg of a sterilized sandy loam soil (1:1 w/w) in plastic pots (20 cm diameter) was infested with *R. solani* and the biocontrol agent suspensions were then added at a rate of 1% (w/w) one week before sowing the eggplant seeds. Pots without the pathogen and others with *R. solani* but not treated were used as controls. There were five replicated pots per treatment, and each pot was sown with ten eggplant seeds. Pots were thinned to two plants per pot after seed germination. Pots were maintained at 30 ± 5 °C in a greenhouse. Percentages of damping-off and root-rot incidence were calculated after 30 and 60 days as mentioned before (Atia *et al.*, 2003).

Field experiment

The efficacy of the commercial product Micronema was evaluated as a soil treatment in a field naturally infested with *M. incognita* (600 J_2/200 g of soil) and *R. solani* at Nobariya. The experiment consisted of eight plots, each of 8 m² (4 m × 2 m), four treated with Micronema and four left untreated and used as a control, according to a completely randomized design. Each plot consisted of five rows, 4 m long and 40 cm wide, with a 40 cm space between planting stations. One eggplant seedling (40-days-old) was planted at each station. Micronema was applied to the soil at the rate of 9 l/Fedden (= 4200 m²) in irrigation water 7, 15, and 30 days after transplanting. All plots were managed using agricultural practices recommended for organic cultivation systems.

Soil and root samples were collected from each plot at 1, 2, and 3 months after transplanting. A soil sample of about 1 kg was collected from the rhizosphere of five randomly selected plants per plot (200 g soil/plant) and a 5 g root sub samples was collected from each plant. Nematode juveniles were extracted from a 200 g soil sub-sample by sieving and decanting. Eggs were collected by treating a sub-sample (5 g) of roots bearing egg masses with a 0.5% solution of sodium hypochlorite for 1 min (Barker, 1985) and then incubating in fresh water at 30 ± 5 °C for egg hatching. Juveniles emerging after three days were collected and counted.

The per cent of root-rot disease incidence on the eggplants was assessed at 1, 2, and 3 months after transplanting on 10 plants per plot at each observation. Disease incidence (%) was estimated on the basis of the number of infected plants with root-rot disease that died or showed root discolouration (roots showing the rot symptom) with reference to the total number of emerged plants per pot, as mentioned before.

The populations of *R. solani* and mycoflora in the rhizosphere soil of treated eggplants were also determined at 1, 2, and 3 months after transplanting from the same soil samples collected for nematode extraction. Populations of fungi were determined as colony forming units (cfu) in 1 ml of soil suspension cultured on Martin's medium (Rose Bengal agar medium), by poured plate methods and a dilution technique. Thus, one gram of soil was suspended in 99 ml sterile water to obtain a 1/100 di-

lution. Then, serial dilutions were prepared up to 10^{-7} (El-Abbasi *et al*., 2003). Three replicated plates were prepared for each dilution per soil sample. The plates were incubated for 7 days at 25 °C. Fungi that grew out were counted and identified from cultural and morphological characters (Barnett and Hunter, 1972). The frequency of occurrence (%) of each isolated fungus was calculated according to the formula:

Frequency of a fungus % = No. of each fungus / Total no. of fungi ×100

Where: - = no fungi, + = ≤ 5 %, ++ = >5 - ≤10%, +++ = >10 - ≤ 20 % and ++++ = >20 % fungi.

To assess eggplant yield, fruits produced by 10 eggplants per plot were weighed at the end of the growing season and yield (kg) expressed as an average per plant.

Statistical analysis

Data were analyzed using Fisher's Least Significant Difference (LSD). The means were compared by LSD at the 0.01 and 0.05 levels of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Pathogencity test

Rhizoctonia solani had the greatest pathogenic effect on eggplant with damping-off and root-rot disease incidences of 32.5 and 37.1%, respectively. *Fusarium solani* caused 11.1 and 10.0% damping-off and root-rot incidence, respectively. The data suggested that *R. solani* plays a major role as the causal agent of root-rot disease in eggplant (Atia *et al*., 2003) and, therefore, it was selected for further studies.

***In vitro* tests**

Effect on Meloidogyne incognita. The culture filtrates of *B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride*, at the concentration S, caused net mortality percentages of J_2 of 100, 99, 98 and 96% after 72 hr exposure, respectively (Table I). At concentration S/2, and the same exposure time, the filtrates caused net mortalities of 93, 92, 92 and 88% with *B. subtilis*, *T. harzianum*, *T. viride* and *P. fluorescens*, respectively. *Bacillus subtilis* caused the greatest nematode mortality, followed by *P. fluorescens*, *T. harzianum* and *T. viride* at concentration S after exposure for 24 and 72 hr. The statistical analysis showed that the effects of filtrate, concentration, exposure time and the interaction filtrate × concentration were highly significant ($P<0.01$), whereas the interactions between filtrate and exposure time, concentration and exposure time and that between these three factors were not significant (Table I).

Effect on Rhizoctonia solani. The fungi were more effective than the bacteria in reducing mycelial growth of *R. solani*. Percentages of mycelial growth reduction were 51.1, 48.2, 19.5, and 19.2% by *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens*, respectively (Table II).

In general, the results of the *in vitro* tests suggest that these bioagents show promise for controlling root-knot and root-rot pathogens. Also, they agree with those of Stephan *et al*. (1996), who found that *T. harzianum* has a dual effect since it provided significant control of root rot and root-knot disease complex on tomato. In other studies, *T. harzianum* and *B. subtilis* gave the best protection to tomato, pepper, cucumber, cabbage and eggplant against crown rot and damping-off diseases (Nemec *et al*., 1996; Lewis and Lumasden, 2001; Loganathan *et al*., 2001).

Greenhouse experiment

Effect on Meloidogyne incognita. At the concentration S, *B. subtilis* reduced significantly the numbers of J_2 in soil, galls and egg masses on the roots by 91.9, 82 and 82.6%, respectively, followed by the concentration S/2 (Table III). At concentration S, *T. harzianum*, *P. fluorescens*, *T. viride* and oxamyl also reduced significantly the numbers of J_2 in soil, by 79.3, 77.9, 75.4 and 67.6%, respectively.

The culture filtrates also increased length and weight of shoots and weight of roots of eggplant, with the largest increase provided by the higher concentration S. Among the bioagents, *P. fluorescens* gave the greatest increase in length of shoots, followed by *T. viride*, *B. subtilis*, oxamyl and *T. harzianum*. The largest significant increase in weight of shoots was achieved by *B. subtilis*, followed by *T. harzianum*, *T. viride*, *P. fluorescens* and oxamyl, and in root weight by *B. subtilis*, followed by *P. fluorescens*, *T. viride*, *T. harzianum* at concentration S and oxamyl (Table III). In general, the effects of the bioagents were comparable with that of oxamyl.

Effect on Rhizoctonia solani. Damping-off incidence was 26.0 , 16.7, 7.5 and 13.3% in pots treated with culture filtrates of *B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride*, respectively, compared to a 35% incidence in the control (Table II). The effects of the treatments on percentage of root-rot incidence were very similar. *Trichoderma harzianum* was more effective than any of the other bioagents in reducing both damping-off and root-rot incidence in eggplants.

Bacillus subtilis provided the best control of the root-knot nematode and *T. harzianum* was the most effective in reducing root-rot in the greenhouse experiment. Windham *et al*. (1989) found that two isolates of *T. harzianum* suppressed *Meloidogyne arenaria* by producing compounds that act directly on the nematodes or make the roots less attractive. The mechanisms that could explain the antagonistic effect of *Trichoderma* spp. include rapid growth, production of volatile or nonvolatile antibiotics, competition with pathogenic species for space or nutrient limiting factors (carbon, nitrogen, iron, etc.), and lysis of the pathogen by the enzymes secreted by *Trichoderma*.

Table I. Mortality (%) of second-stage juveniles of *Meloidogyne incognita in vitro* as affected by culture filtrates of different bioagents.

Bioagent filtrate	Culture filtrate concentration	% Nematode mortality after hours			% Recovery	% Net mortality
		24	48	72		
Bacillus subtilis	S	95	97	100	0.0	100
	S/2	88	92	93	0.0	93
Pseudomonous fluorescens	S	94	96	99	0.0	99
	S/2	82	85	88	0.0	88
Trichoderma harzianum	S	90	93	98	0.0	98
	S/2	85	86	92	0.0	92
Trichoderma viride	S	89	90	96	0.0	96
	S/2	80	88	92	0.0	92
Distilled water	-	3	2	2	0.0	0.0

LSD	Filtrates (F)	Concen. (C)	F × C	Hours (H)	F × H	C × H	F × C × H
5%	1.8	1.3	2.6	1.6	NS	NS	NS
1%	2.8	1.7	3.5	2.1	NS	NS	NS

Each value is the average of five replicates.
S = 10% of the original culture filtrate; S/2 = 5 % of the original culture filtrate.

Table II. Effects of bioagent treatments on mycelial growth reduction of *Rhizoctonia solani in vitro*[1] and damping-off and root-rot incidence on eggplant caused by the fungus in a greenhouse[2] pot test.

Bioagent	Mycelial growth reduction (%)	Disease incidence (%)	
		Root-rot	Damping-off
Bacillus subtilis	19.5	27.3	26.0
Pseudomonas fluorescens	19.2	15.5	16.7
Trichoderma harzianum	51. 1	10.1	7.5
Trichoderma viride	48.2	11.3	13.3
Control	-	38.5	35.0
LSD: 5%	1.5	0.7	0.8
1%	2.2	1.1	1.2

[1] Dual culture technique was used for the *in vitro* test.
[2] Culture suspensions was used for the green-house test.
Control = *Rhizoctonia solani*.

Table III. Effects of culture filtrates from bioagents on second-stage juveniles (J_2) in the soil, numbers of galls and egg masses of *Meloidogyne incognita* and on growth of eggplant in a greenhouse pot test.

Treatment	Filtrate concentration	J_2 /200 g soil	Galls per plant	Egg masses per plant	Shoot length (cm)	Shoot weight (g)	Root weight (g)
Bacillus subtilis	S	143	32	25	41.0	27.7	16.3
	S/2	330	56	49	37.5	19.8	14.4
Pseudomonas fluorescens	S	403	87	61	44.0	22.3	14.3
	S/2	625	142	91	40.5	19.2	13.2
Trichoderma harzianum	S	338	98	44	37.3	24.5	12.7
	S/2	698	140	86	37.0	19.5	11.6
Trichoderma viride	S	450	118	60	43.3	22.9	13.0
	S/2	608	134	70	34.5	22.1	12.4
Oxamyl 24% L	-	391	91	55	39.0	20.1	10.8
Control	-	1826	178	144	29.5	16.9	9.9
LSD: 5%	-	353.7	43.5	26.7	N.S	10.70	2.98
1%	-	476.9	58.6	35.4	N.S	N.S	4.02

Each value is the average of six replicates.

Field experiment

The commercial product Micronema reduced the number of *M. incognita* juveniles in soil and roots. The effect increased with time and was 42.5 and 44.9% after one month, 73.5 and 67.5% after two months, and 90.5 and 90.4% after three months, respectively (Table IV).

Root-rot incidence on the eggplants was 18.2, 11.1 and 3.3% after 1, 2 and 3 months compared to disease incidences of 23.0, 27.2 and 36.3%, respectively, observed in the control, with a reduction of 90.9% three months after planting, compared with the control.

In the soil, the most common genera and species of fungi were *Aspergillus candidus* Link ex. Fr., *Aspergillus chevalieri* (Mangin) Thom. *et* Curch, *Aspergillus niger* (Van Tiegh), *Aspergillus terreus* Thom., *Penicillium* spp. Link, *P. chrysogenum* Thom., *P. citrinum* Thom., *Rhizopus nigricans* Stolonifer, *R. solani* and *F. solani* (Table V). Soil application of Micronema decreased the populations of *R. solani* and *F. solani* after three months, those of all above fungi, except *A. chevalieri*, *Penicillium* spp. and *P. citrinum*, one month after planting, those of all isolated fungi, except *P. citrinum*, two months after planting, and of all fungi, except *A. niger*, *Penicillium* sp. and *P. chrysogenum*, which instead increased, three months after planting. *Aspergillus niger*, *Penicillium* spp. and *P. chrysogenum* may have had antagonistic effects against the root-knot and root-rot pathogens (Abdel-Raham, 1999), as population densities of the nematode in both roots and rhizosphere soil of eggplants were 10 times less: 50 J_2/5 g roots and 70 J_2/200 g soil in treated plants and 520 J_2/5 g root and 740 J_2/200 g soil in the control, respectively. This indicated that the treatment either reduced or delayed the penetration of *M. incognita* into the roots. Also, the

Table IV. Effect of the commercial biological product Micronema on *Meloidogyne incognita* and *Rhizoctonia solani* and yield of eggplant.

Treatment (T)	Months[1] (M)	Numbers of juveniles		Root-rot incidence (%)	Fruit yield per plant (kg)
		J_2 / 200 g soil	J_2 / 5 g root		
Micronema	1	230	160	18.2	
	2	180	120	11.1	
	3	70	50	3.3	7.5
Control	1	400	290	23.0	
	2	680	350	27.2	
	3	740	520	36.3	4.5

LSD:	No. juveniles in soil			No. juveniles in roots			Root-rot incidence		
	T	M	T × M	T	M	T × M	T	M	T × M
5%	8.0	10.1	0.5	9.8	12.3	0.6	13.9	17.4	0.9
1%	11.4	14.3	0.7	14.0	17.5	0.9	19.7	24.8	1.3

[1]After planting

Table V. Effect of the commercial biological product Micronema on the population of soil mycoflora one, two and three months after application under field conditions.

Fungus	Colony forming units/gram of soil after sowing* after months					
	1		2		3	
	Control	Treated	Control	Treated	Control	Treated
Aspergillus candidus	++	++	+	-	+	-
Aspergillus chevalieri	++	+++	++	++	+	-
Aspergillus niger	+++	+	++++	+++	++++	+++
Aspergillus terreus	+++	+	+	+	-	-
Penicillium spp.	++	+++	+++	+	++	+++
Penicillium chrysogenum	+	-	++	+++	+	+++
Penicillium citrinum	++	+++	++	++++	++	-
Rhizopus nigricans	++	+	+	-	++	-
Rhizoctonia solani	+++	++	++	+	++	+
Fusarium solani	++	+	++	+	++	+
Others	++	+++	++++	++++	+++	++++

* - = no fungi, + = ≤ 5 %, ++ = >5 - ≤10%, +++ = >10 - ≤ 20 %, and ++++ = >20 % fungi.

root-rot incidence was reduced 10 times, 3.3% in the treated plants compared to a 36.6% in the control, three months after planting. The efficacy of Micronema could be due to suppression of colonization by *R. solani* in the rhizosphere or by inhibiting the germ tube elongation (Table IV). The statistical analysis showed that the effects of treatment, time and their interaction were significant ($P<0.01$) on nematode populations in soil and roots, but not on root-rot incidence, except for their interaction (Table IV). These results agree with findings by Tylkowska and Szopinska (1998), who reported that *Penicillium* spp. and *Tichoderma* spp. were more effective than chemical treatments [benomyl + thiram (as Benlate T) and metalaxyl (as Apron)] against onion seed-borne fungi. Also, it was observed that *A. niger* gave the highest inhibition of *F. solani* mycelium, followed by *T. viride*, *Gliocladium* sp., *T. harzianum* and *P. citrinum* (Ambikapathy *et al.*, 2002).

The good control of root-knot nematode and root-rot disease improved plant growth, which resulted in an increase of fruit yield of eggplant of 66.7%. Most probably the effect of Micronema on population changes of the total mycoflora in the soil also played an important role in reducing disease incidence and increasing yield, thus making this product promising for use on a large scale under field conditions. However, it is suggested that the efficacy of this product be confirmed under different field conditions of Egypt.

Also, although the efficacy of the fungal and bacterial bioagents observed *in vitro* has been confirmed in the greenhouse, more experiments are required to assess the suitability of the bioagent isolates in the field.

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