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ISOLATION OF FUNGAL ANTAGONISTS AGAINST THE ROOT-KNOT NEMATODE (*Meloidogyne javanica*)

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ABSTRACT

Isolation trials carried out on eggs, larvae, and adult females of root-knot nematodes extracted from root and rhizosphere soil samples resulted in the isolation of several fungal isolates. Screening of these isolates to verify their antagonistic activity against *M. javanica* resulted in the selection of four fungal isolates exhibiting marked antagonistic activity. They were identified as *Myrothecium verrucaria*, *Acremonium* sp., *Fusarium oxysporum* and *Geotrichum citri-aurantii*. Results of *in Situ* experiments revealed that these four antagonistic fungi were able to invade and parasitize eggs of *M. javanica* in the presence of soil extract. However, they had different preferences to attack stages of nematode in water; *Acremonium* sp. was most effective in reducing egg hatching, while, *M. verrucaria was most effective* against larvae. *As far as we are aware, it is the first time that M. verrucaria was isolated from second stage larvae of root-knot nematode in Egypt.* Pot experiments conducted to control root-knot disease *in Vivo* revealed that performance depends on application method, inoculum-density and antagonist. Some treatments achieved levels of protection against root-knot nematodes and of plant growth comparable to those achieved by the nematicide Furadan 10G. *M. verrucaria* seemed to have some promising attributes, which increase its potential as a biocontrol agent In addition to its ability to achieve significant control levels against root-knot nematode, it can be easily mass produced as it grows abundantly on traditional media.
Key words: Antagonistic fungi, Root-knot nematode, Biological control.

INTRODUCTION

Plant-parasitic nematodes cause significant reduction in yield and quality of many crops. Nematode infection affects the plant nutrient absorption and providing infection sites for a wide range of secondary plant pathogens (Warrior, 1997). In Egypt, estimated losses for vegetable crops due to nematode-related disease complexes amounted to some 15 % in 1986, with losses for field crops ranging from 5 to 20 % (Eissa, 1988). Damage caused by root-knot nematodes, *Meloidogyne spp.* constitutes a major constraint to cultivation of tomato especially in sandy soil and reclaimed desert lands in Egypt.

Since the discovery of the first nematicides in the 1940s, and the introduction of the organophosphate and carbamate nematicides in the 1960s, their use has being increased substantially to control nematode in high value crops. However, the finding that DBCP (1,2- dibromo-3-chloropropane) was carcinogenic (Stirling, 1991) and the high mammalian toxicity of the organophosphate and carbamate contributed to the nematicides created a considerable public pressure to limit or even ban the use of nematicides. To avoid the drawbacks of chemical control, a range of other nematode management strategies have to be developed, and biological

control is one of such alternatives. Therefore, the present study aimed to isolate fungal antagonists of root-knot nematodes (*Meloidogyne* spp.) and evaluating their efficiency as potential biological control agents against these nematode in laboratory and greenhouse.

MATERIALS AND METHODS

1- Isolation of fungi pathogenic to nematodes:

Rhizosphere soil and root samples of tomato plants were collected from three different locations suffering from root-knot nematodes in Egypt, i.e. El-Nubaria, Behira Governorate and Balteem, Kafr El-Sheikh Governorate. Samples were kept in polyethylene bags, labeled and sent directly to the laboratory for extraction of different stages of root-knot nematode. Samples were kept at 4 ºC until needed.

Populations of different root-knot nematode stages (eggs, larvae and adult females) were extracted from both root and rhizosphere soil samples. The collected nematode stages were washed twice with sterilized water and observed in sterilized water for three days. Eggs, larvae or females showing signs of fungal proliferation were transferred to water agar medium. After incubation for 24 hours at 28 ºC, a hyphal tip of each fungus growing from an infected larvae, egg or female was transferred onto potato dextrose agar (PDA) medium containing an antibacterial antibiotic (pencillin, in the form of Benzyl Pencillin Sodium) at the rate of 50 μg/ml according to Stirling, (1991). Stock cultures of the isolated fungi were maintained on (PDA) medium.

2- Screening for antagonistic activity in laboratory:

Individual egg-masses of distinct root-knot nematodes were collected from diseased plants by the aid of a special needle. A stock culture of the second stage juveniles were obtained from the collected mature egg-masses after immersion in sterilized water for 7-10 days. The obtained culture was reared on tomato seedlings planted in pots filled with sterilized soil under greenhouse conditions for more than 45 days to obtain the nematode inoculum. The re-extracted nematodes from diseased tomato plants were identified according to Jepson (1987).

The fungal isolates were prepared by growing on plates of PDA at 28 ºC. After an incubation period of 14 days spore suspensions were prepared by washing the culture surface with sterile water. Concentrations of 10^7 propagules/ml were adjusted using sterilized tap water.

a) Influence of isolated fungi on egg hatching of root-knot nematode:

Fungal suspensions were transferred into 25 ml glass vials (5 ml / vial) to which another 5 ml containing 1000 eggs were added. Vials containing eggs only without fungi served as control treatment. Each treatment consists of four replicates. After inoculation for 14 days at 27± 3 ºC, data were recorded for hatching percentages using the research microscope.

b) Antagonism of isolated fungi on eggs of *M. javanica*:

Ability of the isolated fungi to invade and parasitize eggs of *M. javanica* was determined according to the following method (Dr. Fathy Abd El- Aleem, Plant Pathology Department, North Carolina Stat University, Raleigh, North Carolina,USA, personal communication). Fiberglass screens (2 x 1.5 cm. pore size 0.1 mm) were immersed in petroleum ether for 60 min. and then kept in ethyl alcohol until use. A concentrate suspension of *M. javanica* eggs was prepared by centrifugation. Sodium alignate solution was prepared by continuous mixing of sodium alignate and water (20 g/l) for 4 hours at 60 ºC. Egg suspension and sodium alignate were mixed in the ratio 2:1 v/v. Screens were immersed in the mixture for 1 min and then passed between two glass rods to remove the excess of the mixture. The prepared screens were fixed in calcium gluconate solution (25 g/l) for 10 min. Screens coated with calcium gluconate were washed 2-3 times with sterilized water and examined under microscope to assure that each square of screen contains at least 3-5 eggs. Screens were put onto petri dishes each containing 10 ml of sterilized soil extract inoculated with spores of the fungus to be tested. Petri dishes were incubated at 27 ºC for 7 days and then examined under microscope to determine the percentage of infected eggs.

c) Influence of isolated antagonistic fungi on activity of the second stage juveniles (J_2):

In petri dishes (5 cm in diameter) containing 5 ml of the prepared fungal suspensions, 5 ml containing 500 new second stage juveniles were added. Petri dishes containing only the second stage juveniles without fungi served as control treatment. Each treatment was represented by four replicates. The petri dishes were incubated at 27 ± 3 ºC for 7 days. Every day data were recorded for active and inactive (dead) juveniles.

3- Identification of the antagonistic fungi:

Pure cultures of the effective antagonistic fungal isolates were submitted to identification according to Alexopoulos & Mims (1979), Barnett & Hunter (1979) and Von Arx (1974). The identification results were confirmed by Prof. .W.Gams., Central Bureau Voor Schimmelcultures, Baan, The Netherlands.

4- Greenhouse experiments:

Inocula of the antagonistic fungal isolates were prepared by growing on liquid potato dextrose medium for 14 days at 27 ± 3°C. Fungal mats were decanted and blended in a blender, and the concentration was adjusted.
Plastic pots (15 cm in diameter) filled with unsterilized loamy and sandy soil (3:1 v/v) were used in these experiments. Two tomato seedling (var. Castle-rock) aged 30 days were transplanted in each pot. Soil was infested with 5000 second stage larvae and eggs of nematode/pot at planting. Inoculation with the antagonistic fungal isolates was carried out using 3 different densities (10^3,10^5,10^7 propagules/g soil) applied with 4 different methods:

1. Soil inoculation 7 days before planting.
2. Soil inoculation at planting.

3. Dipping of seedlings roots in a suspension containing fungal propagules for 2 hour prior to planting.
4. Soil inoculation 7 days after planting.

Each treatment was replicated 4 times. Pots were arranged in a completely randomized design and kept under greenhouse condition at 27 ± 3 ºC. After three months, plants were uprooted and number of galls, final nematode population in 250 cm^3 soil and fresh and dry weight of shoots and roots were determined. A treatment with the nematicide Furadan 10G was included for comparison. Furadan 10G was broadcasted and incorporated in soil before irrigation 7 days after planting at the rate of 25 kg/fed. as recommended. Check treatment without antagonistic fungi or nematicide was included.

RESULTS AND DISCUSSION

1- Isolation of fungal antagonists and their influence on egg hatching, egg parasitism and larval activity of Meloidogyne javanica:

Isolation trails carried out using abnormal living or dead stages (eggs, larvae, adult females) of root-knot nematode (*Meloidegyne spp*.) resulted in the isolation of several fungal isolates. Screening of the fungal isolates for antagonistic activity (effect on egg hatching and activity of the 2nd larvae stage) indicated that four isolates exhibited antagonistic activity against *Meloidogyne javanica*. These isolates showed characteristics identical to those known for *Myrothecium verrucaria* Ditmar, *Acremonium sp*. Games, *Fusarium oxysporum* Schl. and *Geotrichum citri-aurantii* Link.

Data in Table (1) indicate that the fungal isolates *Acremonium sp., Fusarium oxysporum* and *Geotrichum citri-aurantii s*ignificantly reduced the percentages of egg hatching in compare with the control treatment. The most effective reduction of hatching was obtained by *Acremonium sp*. (65.12 %) followed by *Geotrichum citri-aurantii* (60.36 %), and *Fusarium oxysporum* (40.82%). On the other hand, the least reduction percentage was recorded for *Myrothecium verrucaria* (1.49 %).

Table (1): Effect of antagonistic fungi on percentage of egg hatching of *Meloidogyne javanica.*

Antagonist	% Egg hatching	Reduction %
Myrothecium verrucaria	69.25 d	1.49
Acremonium sp.	24.52 a	65.12
Fusarium oxysporum	41.60 c	40.82
Geotrichum citri-aurantii	27.87 b	60.36
Control (water only)	70.30 d	-----

Means followed by the different letters are significantly different (P=.05) according to Duncan's new multiple range test.

Data in Table (2) indicate that all tested fungi can invade and parasitize eggs of *M. javanica* in the presence of soil extract. *Acremonium sp*. showed the highest percentage of parasitism (50.8%) followed by *M. verrucaria* (44%). *F. oxysporum* and *G. citri-aurantii* showed less similar tendency to invade eggs (37.2% and 38%, respectively), but their effect was not significantly different from that of *M. verrucaria.*

Table (2): Parasitism of the isolated fungi on eggs of *Meloidogyne javanica*:

Antagonist	Infected eggs (%)
Myrothecium verrucaria	44.02 ab
Acremonium sp.	50.77 a
Fusarium oxysporum	37.23 b
Geotrichum citri-aurantii	37.98 b
Control (water only)	6.05 c

Means followed by the different letters are significantly different (P=0.05) according to Duncan's new multiple range test.

Data in Table (3) show that treating with *M. verrucaria* and *Acremonium* sp. significantly reduced the percentages of active juveniles even after one-day incubation. *F. oxysporum* and *G. citri-aurantii* also reduced the percentages of active juveniles but after 5 and 4 days of incubation period, respectively. All antagonistic fungi significantly reduced the percentages of active juveniles after 5 days. Generally, data reveal that effect of the antagonistic fungi increased by increasing the duration of the exposure. After the 6th day exposure, antagonistic effect of *G. citri-aurantii* was hidden because of the natural mortality of juveniles in the control treatment.

Among the tested fungi, *M. verrucaria* showed a relatively high suppressive effect on the larvae. The percentage of inactive second stage juveniles reached 89.59 % after 7 days incubation while the percentage obtained in the control treatment was 13.05 %.

These results are in agreement with frequent reports implicating antagonists as a factor influencing nematodes (Kerry, 1987; Sayre & Walter, 1991; Stirling, 1991 and Sikora, 1992). Several reports dealt with *Acremonium* sp. (Qadri & Saleh, 1990; Al-Hazmi & Abdel- Razik, 1991 and Roberts *et al*., 1992), *Myrothecium* sp. (Jarvis *et al*., 1990 and Warrior, 1997), *F. oxysporum* (Drysdale, 1984 and Chen & Dikson, 1996) and *Geotrichum* spp (Goswami & Rumpenhorst, 1978; Tovar & Medima; 1983) as supressive biocontrol agents against nematodes, and several modes of action were proposed (Tovar & Medima, 1983; Drysdale, 1984; Jarvis *et al*., 1990 and Roberts *et al*., 1992).

Table (3): Effect of antagonistic fungi on activity of the second stage larvae of *Meloidogyne javanica.*

Antagonists	% of inactive second stage juveniles after different incubation periods (days)						
	1	2	3	4	5	6	7
Myrothecium verrucaria	14.0 a	21.8 a	39.9 a	75.9 a	80.1 a	84.1 a	89.6 a
Acremonium sp.	6.4 b	10.7 b	13.1 b	25.5 b	31.0 b	34.0 b	38.6 b
Fusarium oxysporum	4.6 bc	5.5 c	9.2 b	11.3 cd	16.6 c	19.5 c	28.6 b
Geotrichum citriaurantii	3.6 bc	4.8 c	9.0 b	14.8 c	16.3 c	16.5 cd	18.0 c
Control (water only)	3.0 c	5.7 c	6.0 b	7.2 d	7.6 d	10.7 d	13.1 c

Means followed by the different letters are significantly different (P=0.05) according to Duncan's new multiple range test.

2- Evaluation of the isolated antagonists as biocontrol agents against root-knot nematode (*Meloidogyne javanica*).

The four fungal isolates, i.e., *M. verrucaria, Acremonium sp., F. oxysporum* and *G. citri-aurantii,* which exhibited antagonistic activity against *M. javanica In Vitro*, were further tested in pot experiments to evaluate their performance as compared to the nematicide Furadan 10G to control root-knot disease in tomato incited by the nematode. Number of galls on roots, and final number of second stage juveniles in soil as well as plant growth were used as parameters for efficiency.

Data in Tables (4 - 7) show that soil or seedling inoculation with the tested antagonistic fungi significantly reduced the number of galls on root system and the final population of nematode in soil compared with the check treatment. Few exceptions are noticed specially where low inoculum-densities (10^3 propagules / g or ml) or late treating (1 week after planting) were applied. The nematicide Furadan 10G achieved significantly higher protection levels against root-knot disease than most of the tested treatments. However, some antagonistic treatments achieved reduction levels in root galling and/or final nematode population not significantly different from those of the nematicide.

The protection against nematode was reflected in significant improvement in shoot weight of tomato plants with all the efficient fungal treatments as well as with the nematicide Furadan 10G On the other hand, a decrease in the dry weigh of dry root was noticed by application of the antagonists or the nematicide Furadan 10G probably due to their effect in reducing root galling. However, no distinct correlation could be detected between the effectiveness of these agents in reducing root galling and the decrease in dry weight of root.

Table (4): Effect of *Myrothecium verrucaria* on root galling, nematode population in soil and plant growth of tomato plants.

Treatments		No. of galls on root system/plant	Final nematode population (j_2) / 250 cm^3 soil	Dry weight of shoots g/plant	Dry weight of roots g/ plant
Antagonist					
Method of application	Concentration (propagules)				
Soil inoculation 1 week before planting	10^7 / g soil	70.0 b	312.3 a	8.80 fgh	2.43 b-e
	10^5 / g soil	69.0 b	210.0 a	8.64 fgh	2.31 bcd
	10^3 / g soil	79.0 bc	421.0 c	8.14 d-g	2.10 abc
Soil inoculation at planting	10^7 / g soil	77.0 bc	180.0 a	8.75 fgh	1.93 ab
	10^5 / g soil	78.0 bc	256.7 ab	8.05 c-f	1.78 a
	10^3 / g soil	95.7 c	443.3 cd	7.88 cde	1.66 a
Dipping of seedling roots at planting	10^7 / ml	76.7 c	223.3 ab	8.72 fgh	2.44 b-e
	10^5 / ml	77.0 bc	306.7 b	8.60 e-f	2.47 c-f
	10^3 / ml	96.3 c	446.6 cd	7.82 cd	2.86 d-h
Soil inoculation 1 week after planting	10^7 / g soil	101.0 cd	416.7 c	7.61 cd	2.57 c-f
	10^5 / g soil	121.7 de	420.0 c	7.33 bc	2.59 c-f
	10^3 / g soil	132.7 e	506.7 d	6.76 ab	2.64 c-f
Soil treated with Furadan 10 G		44.0 a	300.0 ab	8.73 fgh	2.35 bcd
Control (with nematode only)		140.0 e	626.7 e	6.59 a	2.98 e-f

Means followed by different letters are significantly different (P=.05) according to Duncan's new multiple range test.

Table (5): Effect of *Acremonium sp.* on root galling, nematode population in soil and plant growth of tomato plants.

Treatment		No. of galls on root system / plant	Final nematode population (j_2) / 250 cm^3 soil	Dry weight of shoots g/plant	Dry weight of roots g/ plant
Antagonist					
Method of application	Concentration (propagules)				
Soil inoculation 1 week before planting	10^7 / g soil	42.3 a	298.3 a	8.24 cde	2.26 cde
	10^5 / g soil	44.0 ab	303.3 a	7.94 cd	1.99 abc
	10^3 / g soil	69.3 cde	296.7 bc	7.63 cd	1.74 abc
Soil inoculation at planting	10^7 / g soil	45.7 abc	370.0 b	8.26 cde	1.91 abc
	10^5 / g soil	47.7 a-d	436.7 d	7.42 c	1.91 abc
	10^3 / g soil	67.0 b-e	601.7 efg	6.09 b	1.47 a
Dipping of seedling roots at planting	10^7 / ml	61.7 a-e	431.7 cd	8.50 cde	1.80 abc
	10^5 / ml	68.0 cde	405.0bcd	7.77 cd	2.20 bcd
	10^3 / ml	70.7 de	575.0 ef	5.19 ab	1.98 abc
Soil inoculation 1 week after planting	10^7 / g soil	79.3 ef	570.0 e	5.86 b	1.58 ab
	10^5 / g soil	81.7 ef	613.3 fg	5.53 ab	1.48 a
	10^3 / g soil	9.5 f	606.7 efg	4.80 a	1.46 a
Soil treated with Furadan 10 G		44.0 ab	300.0 a	9.07 e	2.35 c-f
Control (with nematode only)		140.0 g	626.7 g	5.92 b	2.98 f

Means followed by different letters are significantly different (P=.05) according to Duncan's new multiple range test.

Table (6): Effect of *Fusarium oxysporum.* on root galling, nematode population in soil and plant growth of tomato plants.

Treatments		No. of galls on root system/plant	Final nematode population (j_2) / 250 cm^3 soil	Dry weight of shoots g/plant	Dry weight of roots g/ plant
Antagonist					
Method of application	Concentration (propagules)				
Soil inoculation 1 week before planting	10^7 / g soil	101.3 abc	294.7 b	10.18 c	3.21 de
	10^5 / g soil	105.3 b-e	300.0 b	10.05 c	3.13 cde
	10^3 / g soil	116.7 de	347.3 c	8.60 b	3.15cde
Soil inoculation at planting	10^7 / g soil	97.3 ab	303.3 b	10.29 c	2.86 a-d
	10^5 / g soil	110.7 cde	316.7 bc	9.93 c	2.85 a-d
	10^3 / g soil	133.0 f	348.7 c	8.62 b	2.64 abc
Dipping of seedling roots at planting	10^7 / ml	102.7 a-d	294.7 b	10.31 c	3.04 cd
	10^5 / ml	112.7 cde	306.7 bc	9.92 c	2.96 bcd
	10^3 / ml	138.7 f	412.7 d	7.64 a	2.79 a-d
Soil inoculation 1 week after planting	10^7 / g soil	118.7 e	406.7 d	8.70 b	2.47 ab
	10^5 / g soil	136.7 f	424.0 d	8.63 b	2.48 ab
	10^3 / g soil	144.7 f	480.0 e	6.83 a	2.34 a
Soil treated with Furadan 10 G		85.3 a	229.3 a	11.25 d	3.62 efg
Control (with nematode only)		234.0 g	702.7 f	7.27 a	4.31 hi

Means followed by different letters are significantly different (P=.05) according to Duncan's new multiple range test.

Table (7): Effect of *Geotricum citri-aurantii* on root galling, nematode population in soil and plant growth of tomato plants.

Treatment		No. of galls on root system/plant	Final nematode population (j_2) / 250 cm^3 soil	Dry weight of shoots g/plant	Dry weight of roots g/ plant
Antagonist					
Method of application	Concentration (propagules)				
Soil inoculation 1 week before planting	10^7 / g soil	184.7 bc	596.7 bc	9.02 f	2.51 a
	10^5 / g soil	180.0 b	591.3 b	8.64 ef	2.77 a
	10^3 / g soil	190.0 bcd	601.3 bc	8.23 de	2.62 a
Soil inoculation at planting	10^7 / g soil	200.0 cde	594.0 b	8.56 ef	2.76 a
	10^5 / g soil	197.3 cd	601.3 bc	7.21 b	2.57 a
	10^3 / g soil	203.3 de	608.0 bc	6.44 a	2.75 a
Dipping of seedling roots at planting	10^7 / ml	187.3 bcd	588.7 b	8.06 cde	2.57 a
	10^5 / ml	191.3 bcd	296.7 bc	8.17 de	2.63 a
	10^3 / ml	202.7 de	614.0 c	77.46 bc	2.48 a
Soil inoculation 1 week after planting	10^7 / g soil	219.3 fg	636.0 d	8.33 def	2.71 a
	10^5 / g soil	214.0 ef	643.3 d	7.82 bcd	2.70 a
	10^3 / g soil	220.0 fg	571.3 e	8.35 def	2.69 a
Soil treated with Furadan 10 G		85.3 a	229.3 a	11.25 g	3.63 b
Control (with nematode only)		234.0 g	702.7 f	7.27 b	4.31 bcd

Means followed by different letters are significantly different (P=.05) according to Duncan's new multiple range test.

Comparing the effect of methods of application tested on reducing number of galls on root and final number of nematode in soil irrespective of antagonist or inoculum density, data in Table (8) indicate that soil inoculation one week before planting was the most effective method among those tested. It achieved a mean reduction of 46.5% in root –galling and 43.1% in final number of nematode. Meanwhile it resulted in the highest increase in dry weight of shoots (28.1%) and the least decrease in dry weight of roots (30.8%). Early application apparently gives the antagonist the chance to be established in soil and hence exerts its effect. This result is in agreement with those reports by Ali (1990) and Al-Hazmi & Abdul-Razik (1991). Soil inoculation or dipping of seedlings at planting was less effective than soil inoculation one week before planting, but still more effective than soil inoculation one week after planting.

Comparing the effect of inoculum-densities tested on reducing number of galls on root and final nematode number in soil irrespective of antagonist or method of application, Table (8) indicate that higher densities 10^7 and 10^5 propagules /g (ml) were obviously more effective than 10^3 propagules /g (ml). High inoculum densities (10^5 and 10^7 propagules /g (ml) achieved 40.4 – 42.9 % reduction in root galling and 36.9 – 40 % reduction in final nematode number in soil. Meanwhile, soil or seedling inoculation with high densities resulted in higher increase (21.2 – 27.7 %) in dry weight of shoots and less decrease in dry weight of root compared to those attained using lower inoculum density.

Comparing the overall effect of the tested antagonists on reducing number of galls on root and final nematode population in soil irrespective of application method or inoculum density, Table (8) indicate that *Fusarium*

oxsporum was the most effective antagonist among those tested. It achieved a mean reduction of 49.5% in root galling and 49.8 % in final number of nematode in soil. In addition, its application resulted in the highest increase in dry weight of shoots (25.1%). However, *Fusarium oxysporum* is known to be pathogenic to several plants. It is true that some biotypes, like that isolated in our study, can live in reconciliation with plants as stated by Booth (1971). Therefore, the risk of becoming pathogenic to tomato or other plants diminshes its potential as biocontrol agent to be inoculated in soil. Although the effect of *Acremonium sp*. on reduction of root –galling was obvious (54.0%), its effect on reduction of nematode number (25.4%) and increase of dry weight of shoots (17.5%) was less pronounced. In addition, mass production of this antagonist was difficult as it grows very slowly on traditional media, thus reducing its biocontrol potential. *G. citri-aurantii* was also excluded, as it was the least efficient antagonist in reducing number of galls on root and final number of nematode in soil. Als, its application resulted in the least increase in dry weight of shoots (9.6%) and to a pronounced decrease in dry weight of roots (36.5%).

Table (8): Comparison between the effect of methods of application, inoculum densities and the tested antagonists on nematode and plant growth.

Compared treatments		% Decrease (-) or increase (+) of control			
		Nematode		Plant growth	
		Root-galling	Final nematode in 250 cm^3 soil	Dry weigh of shoots	Dry weigh of roots
Method of Application	Soil inoculation 1 week before planting	-46.5	-43.1	+28.1	-30.8
	Soil inoculation at planting	-41.9	-36.7	+20.3	-37.9
	Dipping of seedlings roots at planting	-39.8	-34.8	+20.8	-27.7
	Soil inoculation 1 week after planting	-26.1	-19.7	+9.8	-35.6
Inoculum density	10^7 propagules /g (ml)	-42.9	-40.0	+27.2	-32.4
	10^5 propagules /g (ml)	-40.4	-36.9	+21.2	-31.3
	10^3 propagules /g (ml)	-32.5	-23.8	+7.2	-35.2
Antagonist	*M. verrucaria*	-36.1	-46.2	+22.6	-23.3
	Acremonium sp.	-54.0	-25.4	+17.5	-39.3
	F. oxysporum	-49.5	-49.8	+25.1	-33.9
	G. citri-aurantii	-14.9	-12.9	+9.8	-36.5

M. verrucaria, which *as far as we are aware was isolated in Egypt from the second stage larvae of root-knot nematode i*n the present study, seemed to have some promising attributes increasing its potential use as biological control agent. Although it reduced root-galling moderately, (36.1%) its effect on reduction of final nematode population in soil was pronounced (46.2%). Its application resulted in high increase in dry weight of shoot (22.6%) and to the least decrease in dry weight of root (23.3.%). In addition, mass production of *M. verrucaria* is easy, as it grows abundantly on traditional media. These promising attributes of *M. verrucaria* which increase its biocontrol potential

against root-knot nematode. A field experiment is planned, therefore, to test its performance under field conditions.

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عزل فطريات مضادة لنيماتودا تعقد الجذور *Meloidogyne javanica*

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أدت محاولات العزل التى أجريت على بيض ويرقات وإناث كاملة لنيماتودا تعقد الجذور التى تم استخلاصها من عينات جذور مصابة ومن منطقة التربة المحيطة بها الى عزل العديد من العزلات الفطرية . وقد أسفرت اختبارات التأكد من القدرة التضادية لهذه العزلات ضد نيماتودا *M. javanica* عن أن أربعة عزلات منها تظهر قدرة تضادية واضحة ضد هذه النيماتودا. وقد تم تعريف هذه السلالات بأنها *Geotrichum citri-aurantii* ، *Fusarium oxysporum* ، *Acremonium sp.* ، *Myrothecium verrucaria* .

وقد اتضح من التجارب المعملية أن الفطريات التضادية الأربعة يمكنها المهاجمة واحداث التضاد على بيض نيماتودا تعقد الجذور *M. javanica* إلا أنها تتباين فى تأثيرها على أطوار النيماتودا فى وجود الماء، وقد كان فطر *Acremonium sp.* أشدها تأثيرا على الفقس أما فطر *M.verrucaria* فقد كان أشدها تأثيرا على نشاط اليرقات. وتعد هذه هي المرة الأولى – على قدر علمنا – التي يتم فيها عزل فطر *M.verrucaria* من يرقات الطور الثاني لنيماتودا تعقد الجذور في مصر . وعند تقييم أداء الفطريات التضادية الأربعة فى تجارب الأصص لمعرفة كفاءتها فى مقاومة مرض تعقد الجذور فى الطماطم ، أوضحت النتائج أن كفاءتها تعتمد على طريقة استخدامها وكثافة اللقاح المستخدم كما تعتمد على نوع الفطر . ولقد حققت بعض المعاملات مستويات وقاية ضد نيماتودا تعقد الجذور ومستوى نمو للنبات مساوية لتلك التى حققها المبيد النيماتودى فيوردان 10 ./.، ولقد اتضح أن الفطر *M.* له بعض المواصفات المرغوبة التى تزيد من احتمالات نجاحه كعامل مقاومة ضد مرض تعقد الجذور ، فعلاوة على كفاءته فى تحقيق مستويات مقاومة معنوية ضد المرض فإنه سريع النمو على البيئات المعتادة ويسهل انتاجه على نطاق واسع.