J. Agric. Sci. Mansoura Univ., 34 (11): 10761 - 10770, 2009

USE OF BIOCONTROL FUNGI, *Bacillus thuringiensis* AND ORGANIC SOIL AMENDMENT TO CONTROL ROOT-KNOT NEMATODE (*Meloidogyne incognita*) IN TOMATO AND EGGPLANT.

Ibrahim G. H.[*]; S. M. Al-Rehiayani[**] and M. M. Bellal[*]

[*] **Plant Pathology Research Institute, ARC, Giza, Egypt**

[**] **Plant Production & Protection Dept., College of Agric. and Vet. Medicine, Qassim Univ., Saudi Arabia**

ABSTRACT

Fungal colonization was determined for females and cysts of *Heterodera avenae* on wheat roots or rhizosphere soil, and also determined for eggs and juveniles of *Meloidogyne incognita* on tomato. The common fungi isolated from *H. avenae* were *Fusarium oxysporum, Pacilomyces lilacnus, Verticillium chlamydosporium* and *Rhizoctonia solani.* Also, the common fungi isolated from *M. incognita* were *Aspergillus* spp., *Alternaria alternate, F. oxysporum, P. lilacnus* and *V. chlamydosporium.* The effect of biocontrol fungi which isolated from *H. avenae* or *M. incognita* as well as the antagonistic bacterium *Bacillus thuringiensis* were examined against root-knot nematode infected tomato plants and the results indicated that the highest reduction in galls was observed with *P. lilacnus* (82.92%) followed by *V. chlamydosporium* (77.6%), *B. thuringiensis* (60.91%) and *F. oxysporum*(27.92%) as compared with plants infected with *M. incognita* alone. Also, these biocontrol organisms improved the growth of tomato plants in both shoot and root dry weights. The highest increase in root dry weight percentage was recorded when plants treated with non-pathogenic conidia of *F. oxysporum* (223.69%), followed by *V. chlamydosporium* (200.58%) and *P. lilacnus* (196.53%), while the least increase was recorded with treatment of *B. thuringiensis* (78.03%) as compared with *M. incognita.* Similarly, the effect of organic soil amendment were examined against root-knot nematode in eggplants and the results showed that chicken manure alone gave the highest gall reduction (59.02%), followed by eucalyptus leaves and stems dry powder (38.37%), and the mixture of chicken and eucalyptus (39.33%) Organic soil amendments also improved the plant growth of eggplants. Chicken manure gave the highest increase in shoot dry weight (755.6%) followed by mixture of chicken and eucalyptus (570.19%), while the least increase was recorded with eucalyptus treatment (102.33).

Keywords: Biocontrol agents, *Bacillus thuringiensis*, Fungi, *Meloidogyne incognita*, organic soil amendment, Tomato, Eggplant.

INTRODUCTION

Root-knot nematode (*Meloidogyne* spp.) is one of the most economically important pests causing severs damages to a wide variety of crops (Siddiqui and Shaukat, 2003). Pajovic *et al.*, 2007 mentioned that *Meloidogyne* spp. are considered common pathogens in Montenegro, Yugoslavia, and the most prevalent species was *M. incognita* which isolated from roots of many vegetable crops. Their wide host ranges made them difficult to be controlled by rotation and resistant cultivars have variable value because of the occurrence of virulent species and races mixtures (Robertson and Diez-Rojo, 2009). Existing management procedures could be enhancing by the development of biocontrol strategies consequently, there is a great need to increase the control options for managing root-knot nematodes and biological control has been an active area of research (Heydari *et al.*, 2006). Biological control of the plant parasitic nematodes with certain natural plant products or animal wastes or microbial agents singly or in combination with nematicides has been recorded by several researchers (Zaki and Maqbool, 1998; Riegel and Noe, 2000; and El-Sherif *et al.*, 2007). Nematode antagonists have been observed in a wide range of organisms including fungi, bacteria, viruses, richettseae, protozoan, turbellarians, tardigrades, enchytraeids, mites, insects, and nematodes (Li *et al.*, 2000). Numerous fungi have been isolated from nematodes; Crump (1991) listed 129 species of fungi isolated from root-knot and cyst nematodes. Numerous fungi including various types of fungal antagonists of nematodes have been tested for their efficacy in controlling plant-parasitic nematodes. However, only a few fungi have been commercialized. *Pacilomyces lilacnus, Verticillium chlamydosporium, V. lecanii, Hirsutella rhossilliensis, Fusarium oxysporum* and *F. solani* have been more extensively tested and have shown some potential in control of plant-parasitic nematodes (Chen *et al.*, 2004). *P. lilacnus* is a typical soil-borne fungus that has been reported from numerous parts of the world (Domsch, *et al.*, 1980). The fungus has been isolated from eggs, egg masses, females, and cysts of many plant-parasitic nematodes; eventually a mycelia network develops and engulfs the nematode eggs. Penetration of nematode eggs is completed with an appressorium or simple hyphae. Both mechanical and enzymatic activities may be involved in the penetration. The fungus may also colonize the juveniles within the eggshell (Holland, *et al.*, 1999). Culture filtrates of *P .lilacnus* were toxic to nematodes (Chen, *et al.*, 2000). *V. chlamydosporium* has been found on various nematodes but mainly species of *Heterodera* and *Meloidogyne* (Gams, 1988). The fungus forms branched mycelia network and penetrate eggs by simple branches of hyphae or by formation of appressoria (Lopez-Llorca and Claugher, 1990). *V. chlamydosporium* may produce toxins that inhibit hatching or kill nematode eggs (Caroppo, *et al*,. 1990). *Fusarium* spp. has been isolated from females, cysts, egg masses, and eggs of nematodes. *F. oxysporum* and *F. solani* are the most commonly encountered species (Nigh *et al.*, 1980). Species of *Fusarium* produce a large range of toxins, which are antagonistic to *Streptomyces*, bacteria, fungi, and nematodes (Ciancio *et al.*, 1988). A number of rhizobacteria or plant growth promoting rhizobacteria have been reported to have nematicidal effects. The bacteria produce metabolites, excretory enzymes, and antibiotics that are detrimental to nematodes (Sikora, 1997). A diversified group of bacteria have been reported to be nematicidal. They include genera *Acinebacter, Agrobacterium, Bacillus, Burkholderia, Chromobacterium, Enterobacter, Pseudomonas, Serratia, Stenotrophomonas,* and *Streptomyces* (Chen *et. al*, .2004). *Bacillus* spp. is a large group of bacteria that have shown diversified effects on both free-living and plant-parasitic nematodes. Various strains of *Bacillus thuringiensis* were reported to have nematicidal effects against free-living nematodes, as well as plant-parasitic nematode, *Heterodera glycines* and *Meloidogyne* spp. (Mena *et al.*, 1997). It has been suggested that extra cellular toxins cause the deaths of the nematodes (Carneiro *et al.*, 1998). The addition of green manure crops and soil amendments can effectively control plant-parasitic nematodes. Some of the green manure crops used successfully for nematode management in the United States includes *Brassica napus, Sorghum bicolor, Mucuna deeringiana, Raphanus sativus*, and *Sinapis alba* (Al-Rehiayani and Hafez 1998). Soil amendments comprise a much broader category, usually consisting of various waste materials. Often, the waste is a direct by product of agriculture production such as pressed seed meal or pomade. In other cases it is waste from other sources such as animal manure, crustacean shells, and even human wastes (Chen *et al.*, 2004). Joshi and Patel (1995) reported that application of poultry manure showed improved growth of groundnut crop and reduced nematode population in comparison to controls. Khan and Shaukat (1998) observed that pigeon manure and poultry manure were effective in controlling population of *Helicotylenchus indicus*, *Merlinius brevidens* and *Hoplolaimus seinhorsti* associated with garlic.

The objective of this study were to (1) isolate the fungi from root-knot and cyst nematodes and tested these fungi and other microorganisms to show its ability to control root-knot nematode, *M. incognita* in tomato plants, (2) study the effect of organic soil amendments, i.e. chicken manure and eucalyptus leaves and stems powder to control root-knot nematode, *M. incognita* in eggplants.

MATERIALS AND METHODS

Fungal Isolation and Identification:

Wheat roots infected by *Heterodera avenae* were washed with tap water and put in sterilized water. The young white females that contained zero or few eggs were removed with the aid of a stereomicroscope and transferred to sterilized water. Brown cysts were extracted from the soil and soil debris remaining on the sieve by a modified sugar flotation-centrifugation technique (Chen *et al.*, 1994). Also, tomato roots which showing symptoms of root-knot nematode disease were collected. Eggs of root-knot nematode were extracted by shaking the infected roots in 2 % sodium hypochlorite solution, collected on a 400 mesh sieve. Juveniles were obtained by teasing gall root tissues with the help of a sterilized needle under a stereomicroscope and transferred to sterilized water. Juveniles were washed thoroughly with sterile distilled water (Amer Zareen *et al.*, 2000). The extracted females and cysts of *H. avenae* and the extracted eggs and juveniles of *M. incognita* were plated into water agar plates supplemented with penicillin (100,000 units/l) and streptomycin (0.2 g/l) and incubated at room temperature (25-30 C°) for 3-5 days. As soon as some fungal colonies appeared, hyphal fragment was transferred into potato dextrose agar (PDA) plates and fungi were identified according to Booth (1971), Nelson *et al.* (1988) and Domsch *et al.* (1980).

Source of *Bacillus thuringiensis*:

Bacillus thuringiensis (Bt) was obtained from Ministry of Egyptian Agriculture as a commercial product (Protecto) which is recorded under No. 541, with an active ingredient 9.4%, inert ingredient carrier 90.6% and the recommended dose is 300 g/ Fadden.

Greenhouse evaluation and soil treatments:

(A) The antagonistic effects of the isolated fungi and Bt on the root-knot nematode, *Meloidogyne incognita* were studied in tomato plants. Seeds of tomato Supermarmande cultivar susceptible to root-knot nematode, *M. incognita* were sown in seedbeds filled with autoclaved sandy-loam soil. Tomato seedlings of 21 days-old were transplanted in 15-cm plastic pots diameter filled with steam sterilized sand loam soil (1:1 w/w) and planted with two tomato seedling/pot. Eggs of *M. incognita* were harvested from infected roots using sodium hypochlorite (Hussey and Barker, 1973), suspended in sterilized distilled water and the inoculum was standardized to 5000 eggs per pot. The micro conidia of the isolated fungus growing on PDA plates at 25 C° for 14 days were harvested by flooding the plates with sterilized distilled water. The resulted suspension was strained through cheesecloth and then the inoculum potential was adjusted to $1x10^6$ spore/ml using haemocytometer. Each pot containing 2 plants received 25 ml of the spore suspension. *Bacillus thuringiensis* was added at the recommended dose that was 0.01 g/pot. and treatments were arranged as follow: (1) soil infested with *M. incognita* (5000eggs/pot); (2) soil infested with *V. chlamydosporium* + *M. incognita*; (3) soil infested with *F. oxysporum* + *M. incognita*; (4) soil infested with *P. lilacinus* + *M. incognita* (5) soil infested with *Bacillus thuringiensis* + *M. incognita.* Each treatment was replicated 3 times.

(B) The effect of organic soil amendments on the root-knot nematode infected eggplant was studied. Seeds of eggplant cv. black beauty susceptible to root-knot nematode, *M. incognita* were sown in 15-cm plastic pots diameter. Dry leaves and stems of eucalypts plant (*Eucalyptus citriodora*) and dry chicken manure were used in this study. Treatments were arranged as follow: (1) soil infested with *M. incognita* alone; (2) soil mixed with 5gram eucalypts leaves and stems + *M. incognita*; (3) soil mixed with 5gram chicken manure + *M. incognita*; (4) soil mixed with a mixture of 2.5 gram eucalyptus leaves, stems and 2.5 gram chicken manure + *M. incognita*. Each treatment was replicated 3 times.

The pots in experiments (A) and (B) were randomly arranged in the greenhouse, watered every other day for 40 days. Plant height, dry weight of shoot and root systems were investigated. Root-knot nematode disease was counted as number of galls/ g root fresh weight. The experiments were carried out twice. Data were statistically subjected to analysis of variance (ANOVA) Gomez and Gomez (1984), followed by Duncan's multiple-range test to compare means (Duncan, 1955).

RESULTS AND DISCUSSION

The obtained data of fungal isolation from the females and cysts of *H. avenae* showed the presence of *Fusarium, Pacilomyces, Rhizoctonia,* and *Verticillium* spp. These results are in parallel with other studies, where the species of *Cylindrocarpon, Exophiala, Fusarium, Pacilomyces, Phoma, Taricibium* and *Verticillium* were among the fungi most frequently encountered from females and cysts of *H. avenae* and *H. glycines* (Kerry and

Crump, 1977; Chen *et al.*, 1994).While the isolated fungi from eggs and juveniles of *M. incognita* were *Aspergillus, Alternaria, Cladosporium, Fusarium, Pacilomyces* and *Verticillium* spp. The obtained data are in line with that of Amer-Zareen *et al.*,(2000) who isolated *Aspergillus, Alternaria, Acremonium, Cladosporium, Cephalosporium, Curvularia, Fusarium, Pacilomyces* and *Ulocladium* spp. from eggs and juveniles of *M. incognita*. The identified species used in this study were *F. oxysporum, P. lilacnus,* and *V. chlamydosporium*. Data in Table (1) revealed that the effect of antagonistic fungi and Bt on *M. incognita* infected tomato plants. The maximum reduction in nematode galls was observed with *P. lilacnus* (82.92%) followed by *V. chlamydosporium* (77.6%), *B. thuringiensis* (60.91%) and *F. oxysporum* (27.92%). However, the maximum increase in shoot dry weight was recorded with *V. chlamydosporium* (170.65%) followed by *F. oxysporum* (153.35%), *P. lilacnus* (64.72%) and *B. thuringiensis* (45.87%). The highest increase in root dry weight was obtained with *F. oxysporum* (223.69%), followed by *V. chlamydosporium* (200.58%), *P. lilacnus* (196.53%) and then *B. thuringiensis* (78.03%). These results agreed with several investigators. Amer-zareen *et al.*, (2001) found that *P. lilacnus* reduced gall formation, egg mass production of root-knot nematode on okra plants. Zaki and Maqbool (1998) found that the use of *V. chlamydosporium, P .lilacnus* and *Talaromyces flavus* alone or mixed with carbofuran reduced root-knot nematode on okra plants and increased shoot and root fresh weights. Chen *et al.*, (1996) reported that one isolate of *F. oxysporum* and one isolate of *F. solani* could colonize more than 30% and 20% of the eggs, respectively. Hallmann and Sikora (1994) reported that isolates of *F. oxysporum* reduced nematode root galls on tomato by 52% to 75%, and reported the culture filtrates of *F. oxysporum* killed juveniles of *M. incognita* within 8 hours. El-Sherif *et al.*, (2007) observed that *B. thuringiensis* reduced number of galls, females and egg masses of root-knot nematode on eggplants by 32.2, 36.6, and 37.7%, respectively and increased the shoot and root dry weights of eggplants. Also, Several investigators used fungal filtrates in biological control of plant-parasitic nematodes and they found that the toxic metabolites produced by biocontrol fungi may cause deterioration of giant cells, reduce hatching, and immobilize the second-stage juveniles of root-knot nematode (James *et al.*, 1999). Cayrol, (1989) found that the culture filtrates which produced in liquid media by *Fusarium* spp., *Aspergillus niger* and *P. lilacinus* were active against eggs, larvae and adults of *Meloidogyne* spp. Siddiqui and Husain (1991) used culture filtrate of *F. solani* to control *M. incognita* on chickpea. Hallmann and Sikora (1994) found that tomato roots treated with non-pathogenic mycelium of *F. oxysporum* or its culture filtrate inhibited root penetration with *M. incognita* and gave 50 % control of *M. incognita* in pot experiment. They also found that the culture filtrate has a nematicidal effect *in vitro*, and may be a source for new active substances important for nematode control. Zaki, (1994) showed that, culture filtrate of *P. lilacinus* inhibited egg hatching of *M. javanica in vitro*. Data in Table (2) showed the effect of eucalyptus leaves and chicken manure alone or as a mixture on the root-knot nematode, *M. incognita* infected eggplants. Results indicated that all the tested organic manures caused remarkable increase in the growth parameters of eggplant and reduced number of nematode galls. It is evident that pots received chicken manure either alone or mixed with eucalypts leaves and stems at the half dose of each component improved plant growth parameters than pots received eucalyptus leaves and stems alone, and reduced number of nematode galls. The increase % of total plant height was 98.03% in the mixture of chicken manure and eucalyptus leaves and stems, followed by 83.37% in chicken manure alone, 36.15% in eucalyptus leaves and stems alone. Also, the highest increase percentage in shoot dry weight was obtained with chicken manure alone (755.60%) followed by the mixture of chicken and eucalyptus leaves and stems powder (570.19%), while the lowest percentage was obtained with treatment of eucalyptus leaves and stems powder (102.33%).

In addition, chicken manure alone gave the highest reduction of nematode galls (59.02%). Similar results were obtained by Khan *et al.*, (2001), who found that chicken manure, pigeon manure and sawdust reduced the population of *Meloidogyne* spp. and the highest reduction of nematode population v/as achieved with. chicken manure. Also, they observed that horse and donkey manures reduced numbers of *Helicotylenchus, Meloidogyne* and *Merlinius* spp. associated with garlic crop. However, El-Sherif *et al.*, 2007 found that horse manure improved plant growth response and reduced number of root-knot nematodes on eggplants. Riegel and Noe, (2000) demonstrated that the addition of poultry litter compost to field soil reduced numbers of *M. incognita* and generally increased plant growth of cotton. Everts *et al.*, (2006) observed that the use of castorbean or sorghum sudangrass as cover crops, poultry litter and poultry litter compost reduced nematode populations of *M. incognita* over three years of vegetable crop rotation. Kratochvil *et al.*, (2004) mentioned that sorghum sudangrass effectively reduced numbers of root-knot nematode, *M. incognita* in Maryland. The incorporation of green manures of mustard, clover and sorghum sudangrass successfully improved controling of nematodes in a subsequent potato crop in the northwest United States (Eberlein *et al.*, 1997). Also, McSorley *et al.*, (1994) showed that the use of switchgrass and sorghum sudangrass in rotation systems reduced nematode and increased yields of vegetables in the southeastern United States.

Table 1: Effect of biocontrol fungi and Bt against the root-knot nematode, *M. incognita* (MI) on tomato plants.

Treatment	Shoot dry weight (g)	Increase %	Root dry weight (g)	Increase %	No. of galls/ plant	Reduction %
(MI)	1.029 a	0.0	0.173 a	0.0	256.67 d	0.0
V. chlamydosporium + (MI)	2.785 c	170.65	0.520 c	200.58	57.5 a	77.60
F. oxysporum + (MI)	2.607 c	153.35	0.560 c	223.69	185 c	27.92
P. lilacnus + (MI)	1.695 b	64.72	0.513 c	196.53	43.83 a	82.92
Bt +(MI)	1.501 b	45.87	0.308 b	78.03	100.33 b	60.91

Data are average of 3 replicates.
Means followed by the same letter within the same column are not significantly different according to Duncan's multiple range tests ($p \leq 0.05$).

Table 2: Effect of organic amendments against the root-knot nematode, *M. incognita* (MI) on eggplants.

Treatment	Total plant height (cm)	Increase %	Shoot dry weight (g)	Increase %	No. of galls/ plant	Reduction %
(MI)	51.17 a	0.0	0.473 a	0.0	624 c	0.0
Eeucalyptus leaves and stems (5g/ pot) +(MI)	69.67 b	36.15	0.957 b	102	385 b	38.37
Chicken manure +(MI)	93.83 c	83.37	4.047 d	755	256 a	59.02
Eeucalyptus leaves and stems +chicken manure (2.5g/ pot) +(MI)	101.33 c	98.03	3.17 c	570	379 b	39.33

Data are average of 3 replicates.
Means followed by the same letter within the same column are not significantly different according to Duncan's multiple range tests (p≤ 0.05).

REFERENCES

Al-Rehiayani, S. and S. Hafez 1998. Host status and green manure of selected crops on *Meloidogyne chitwoodi* race 2 and *Pratylenchus neglectus*. Nematropica 28:213-230.

Amer-Zareen, I. A. Siddiqui and M. J. Zaki. 2000. Fungal parasites of root-knot nematodes. Pak. J. Biol. Sci., 3: 478-480.

Amer-Zareen, N. J. Khan, and M. J. Zaki. 2001. Biological control of *Meloidogyne javanica*, root-knot nematodes of okra. Pak. J. Biol. Sci., 4: 990-994.

Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute. New survey, England, pp.132.

Carneiro, R. G., I. de Souza, and L. C. Belarminoand. 1998. Nematicidal activity of *Bacillus* spp. strains on juveniles of *Meloidogyne javanica*. Nematologia Brasileira 22: 12-21.

Caroppo, S., B. Perito, and O. Pelagatti. 1990. *In vitro* evaluation of nematicide activity by several fungi against *Meloidogyne incognita* eggs. Redia 73: 451-462.

Cayrol, J.C. 1989. Nematicidal toxins of fungi. Revne Horticol 293: 53-57.

Chen, S. Y., D. W. Dickson, and D. J. Mitchell.1994. Fungi associated with females and cysts of *Heterodera glycines* in a Florida soybean field. J. of Nematology 26: 296-303.

Chen, S. Y., D. W. Dickson, and D. J. Mitchell.1996. Pathogenicity of fungi to *Heterodera glycines*. Journal of Nematology 28:145-158.

Chen, S. Y., D. W. Dickson, and D. J. Mitchell.2000. Viability of *Heterodera glycines* exposed to fungal filtrates. J. of Nematology 32:190-197.

Chen, Z. X., S. Y., Chen and D. W. Dickson .2004. Nematology advances and perspectives: Nematode management and utilization. CABI Publishing London, UK.

Ciancio, A., A. Logrieco, F. Lamberti, and A. Bottalico. 1988. Nematicidal effects of some *Fusarium* toxins. *Nematologia Mediterrananea* 16: 1370 138.

Crump, D. H. 1991. Fungal species isolated from beet (BCN), cereal (CCN) and potato (PCN) cyst nematodes. Buletin Section Regional Quest Palearctique (SROP) 14: 58-64.

Domsch, K. H., W. Gams, and T. H. Anderson. 1980. Compendium of soil fungi, vols. 1 and 2. London: Academic Press.

Duncan, D.B. (1955). Multiple ranges and multiple F. test. Biometrics, 11: 142.

Eberlein, C. V., Boydston, R., Al-Khatib, K., Davis, J. R., Guttieri, M. J., Santo, G. S., and Pan, W. 1997. Brassica green manure systems for weed, disease, and nematode control in potatoes. Proc. West. Soc. Weed Sci. 50: 24-25.

El-Sherif, A. G., A. R. Refaei, M. E. El-Nagar and H. M. Salem. 2007. Integrated management of *Meloidogyne incognita* infecting eggplant by certain organic amendments, *Bacillus thuringiensis* and oxamyl with reference to N P K and total chlorophyll status. Plant Pathology J. 2: 147-152.

Everts, K. L., Sardanelli, S., Kratochvil, R. J., Armentrout, D. K., and Gallagher, L. E. 2006. Root-knot and root-lesion nematode suppression by cover crops, poultry litter, and poultry litter compost. Plant Dis. 90: 487-492.

Gams, W. 1988. A contribution to the knowledge of nematophagous species of *Verticillium*. Netherlands Journal of Plant Pathology 94: 123-148.

Gomez, Z. A. and A. A. Gomez. 1984. Statistical procedures for agriculture research. New York, U.S.A. pp: 680.

Hallman, J., and R. A. Sikora. 1994. *In vitro* and *in vivo* control of *Meloidogyne incognita* with culture filtrates from nonpathogenic *Fusarium oxysporum* on tomato. J. of Nematology 26:102-(Abstr.).

Heydari, R., E. Pourjam and Mohammadi, G. 2006. Antagonistic effect of some species of *Pleurotus* on the root-knot nematode, *Meloidogyne javanica in vitro*. Plant Pathology J. 5: 173-177.

Holland, R. J., K. L. Williams, K. Alamgir, and A. Khan. 1999. Infection of *Meloidogyne javanica* by *Paecilomyces lilacnus*. Nematology 1: 131-139.

Hussey, R. S. and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Report., 57: 1025- 1028.

James, K. N, Susan, L. F., and David, J. C. 1999. *In vitro* assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode antagonistic fungal compound. J. of Nematology 31: 172-183.

Joshi, P. R. and H. R. Patel. 1995. Organic amendments in management of *Meloidogyne javanica* on groundnut. Indian J. Nematol., 25: 76-78.

Kerry, B. R. and Crump, D. H. 1977. Observations on fungal parasites of females and eggs of the cereal cyst nematode *Heterodera avenae* and other cyst nematodes. Nematologica 23: 193-201.

Khan, A. and S. S. Shaukat. 1998. Effect of some organic amendments on density of nematodes associated with garlic (*Allium sativum* L.) Appl. Ento. Phytopath. 66: 13-19.

Khan, A., S. S. Shaukat, and I. Ahmad. 2001. Effect of organic manures and carbofuran on nematodes associated with garlic (*Allium sativum* L.). Pak. J. Biol. Sci., 4: 319-320.

Kratochvil, R. J., Sardanelli, S., Everts, K. L., and Gallagher, L. E. 2004. Evaluation of crop rotation and other cultural practices for management of root-knot and lesion nematodes. Agron. J. 96: 1419-1426.

Li, T. F., K. Q. Zhang, and X. Z. Liu. 2000. Taxonomy of nematophagous fungi. Beijing: Chinese Scientific and Technological Publication.

Lopez-Llorca, L. V., and D. Claugher. 1990. Appressoria of the nematophagous fungus *Verticillium suchlasorium*. Micron and Microscopica Acta 21: 125-130.

McSorley, R., Dickson, D. W., and Brito, J. A. 1994. Host status of selected tropical rotation crops to four populations of root-knot nematodes. Nematropica 24: 45-53.

Mena, J., E. Pimentel, R. Vazquez and J. D. Mencho. 1997. Results of the use of *Bacillus thuringiensis* in the control of *Radopholus similes* in banana and plantain plantations. Centro Agricola 24: 41-49.

Nigh, E. A., I. J. Thomason, and S. D. Van Gundy. 1980. Identification and distribution of fungal parasites of *Heterodera schachtii* eggs in California. Phytopathology 70: 884-889.

Nelson, P. E., T. A. Toussoun and W. F. Marasas. 1988. *Fusarium* species. An illustrated manual for identification. The State Univ. Press, pp: 193.

Pajovic, I., S. Sirca, B. Geric Stare, G. Urek. 2007. The incidence of root-knot nematodes *Meloidogyne arenaria, M. incognita* and *M. javanica* on vegetables and weeds in Montenegro. Plant Dis. 91: 1514-1518.

Riegel, C. and J. P. Noe. 2000. Chicken litter soil amendment effects on soil-borne microbes and *Meloidogyne incognita* on cotton. Plant Dis. 84: 1275-1281.

Robertson, L. and M. A. Diez-Rojo. 2009. New host races of *Meloidogyne arenaria, M. incognita* and *M. javanica* from horticultural regions of Spain. Plant Dis.93: 180-184.

Siddiqui, L.A. and S. Shaukat 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: Importance of bacterial secondary metabolite, 2, 4-diacetylpholoroglucinol. Soil Biol. Biochem., 35: 1615-1623.

Siddiqui, Z. A. and Husain, S .I. 1991. Control of *Meloidogyne incognita* on chickpea by fungal filtrates. Pakistan J. of Nematology.9: 1310137.

Sikora, R. A. 1997. Biological system management in the rhizosphere an inside-out/outside-in perspective. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universities Gent. 62: 105-112.

Zaki, F. A. 1994. Effect of culture filtrates of *Paecilomyces lilacinus* on *Meloidogyne javanica. Nematologia Mediterranean* 22: 41-43.

Zaki, M. J. and Maqbool, M. A. 1998. Use of biocontrol fungi with carbofuran in the control of root-knot nematodes of okra. Pak. J. Biol. Sci., 1: 27-28.

استخدام الفطريات الحيوية وبكتيريا باسيلس سيرينجينسس والمخلفات العضوية لمقاومة نيماتودا تعقد الجذور ميليدوجينى انكوجنيتا فى نباتات الطماطم والباذنجان

جمال الدين حامد إبراهيم* ، سليمان محمد الرحياني** و مدحت محمود بلال*
* معهد بحوث أمراض النباتات- مركز البحوث الزراعية-الجيزة
** قسم إنتاج النبات ووقايته – كلية الزراعة والطب البيطري – جامعة القصيم- المملكة العربية السعودية

تم عزل وتنقية وتعريف الفطريات المصاحبة لكل من إناث وحويصلات نيماتودا حوصلات الحبوب هيتيرودرا أفينى التي تصيب نباتات القمح وكذلك الفطريات المصاحبة لبيض ويرقات نيماتودا تعقد الجذورميليدوجينى انكوجنيتا التي تصيب نباتات الطماطم . واتضح أن الفطريات المعزولة من نيماتودا حوصلات الحبوب هي فيوزاريم اوكسيسبورم ؛ باسيلوميسس ليلاسينس ؛ فرتيسليم كلاميدوسبوريم و ريزوكتونيا سولانى وكذلك عزلت الفطريات الاسبرجلس ؛ الترناريا الترناتا ؛ فيوزاريم اوكسيسبورم ؛ باسيلوميسس ليلاسينس و فرتيسليم كلاميدوسبوريم من بيض ويرقات نيماتودا تعقد الجذور. أيضا اجري اختبار لمعرفة كفاءة بعض الفطريات المعزولة من النيماتودات السابقة بالإضافة إلى بكتيريا باسيلس سيرينجينسس في خفض أعداد العقد الجذرية لنيماتودا تعقد الجذور في نباتات الطماطم، واتضح أن المعاملة بالفطر باسيلوميسس ليلاسينس أعطت أعلى تأثير حيث أدت المعاملة الى انخفاض أعداد العقد الجذرية النيماتودية بنسبة ٨٢،٩٢% يليها المعاملة بالفطر فرتيسليم كلاميدوسبوريم بنسبة ٧٧،٦% ثم بكتيريا باسيلس سيرينجينسس بنسبة ٦٠،٩١% ثم فطر فيوزاريم اوكسيسبورم بنسبة ٢٧،٩٢% . وقد اتضح أن استخدام هذه الكائنات التضادية ذات التأثير الحيوي أدى الى تحسين بعض الصفات المحصولية لنباتات الطماطم، فقد ادت المعاملة بالفطر فيوزاريم اوكسيسبورم الى زيادة النسبة المئوية للوزن الجاف للجذور بنسبة (٢٢٣،٦٩%) يليها المعاملة بالفطر فرتيسليم كلاميدوسبوريم (٢٠٠،٥٨%) ثم المعاملة بالفطر باسيلوميسس ليلاسينس (١٩٦،٥٣%)، وادت المعاملة بالبكتيريا باسيلس سيرينجينسس الى زيادة الوزن الجاف بنسبة (٧٨،٠٣ %) . وعلى الجانب الآخر اجري اختبار لمعرفة كفاءة بعض المخلفات النباتية والحيوانية على خفض أعداد العقد الجذرية لنيماتودا تعقد الجذور على نباتات الباذنجان، وقد اتضح أن مخلفات الدواجن أعطت اعلي تأثير حيث ادت الى انخفاض فى اعداد العقد النيماتودية بنسبة ٥٩،٠٢% كذلك انخفضت النسبة إلى ٣٨،٣٧% عند استخدام أوراق وسيقان نبات الكافور الجافة كمخلفات نباتية والى ٣٩،٣٣% عند استخدام خليط من مخلفات الدواجن وأوراق وسيقان الكافور معا عند مقارنتهم بالتربة المعدية بنيماتودا ميليدوجينى انكوجنيتا فقط. وقد لوحظ أيضا أن استخدام المخلفات النباتية والحيوانية ادى الى تحسين الصفات المحصولية لنباتات الباذنجان. فقد اتضح ان استخدام مخلفات الدواجن سبب أعلى زيادة فى النسبة المئوية للوزن الجاف للمجموع الخضرى (٧٥٥،٦%) يليها المعاملة بخليط من مخلفات الدواجن واوراق وسيقان الكافور (٥٧٠،١٩%) وادت المعاملة باوراق الكافور فقط الى اقل تأثير (١٠٢،٣٣٥ %).

قام بتحكيم البحث

أ.د / أحمد جمال الشريف	كلية الزراعة – جامعة المنصورة
أ.د / محمد أنور محمد الصعيدى	خارجي