The Journal of Animal & Plant Sciences, 27(1): 2017, Page: 252-258
ISSN: 1018-7081

EFFECTS OF NEMATOPHAGOUS FUNGI ON VIABILITY OF EGGS AND JUVENILES OF *MELOIDOGYNE INCOGNITA*

M. Hussain*, M. Zouhar and P. Ryšánek

Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic
*Corresponding author: manzoor.sahi@gmail.com; hussain@af.czu.cz

ABSTRACT

The present study was carried out to determine the efficacy of six fungal genera on the viability of eggs and second stage juveniles (J2) of *Meloidogyne incognita*. Cultures of all test fungi were prepared on potato dextrose agar (PDA). *Lecanicilliummus carium* was found to be highly effective in infecting eggs (79.6%), reducing egg hatching (6.9%), and causing the mortality of J2(78%), followed by *L. psalliotae* and *Trichodermahamatum,* compared to the controls. In contrast, *Fusariumsolani* demonstrated low infection of nematode eggs and J2. *Aspergillus niger* and *A. nidulans* did not show significant (*P*= 0.05) egg parasitism but these species were highly toxic againstJ2causing high mortality (50%).All the other fungal species showed moderate levels of infection of both eggs and J2. Our study demonstrated that *L. Muscarium* might be a potential biocontrol agent for the management of *M. incognita,* but further studies are needed to test its efficacy in the field.

Keywords: Parasitism, biocontrol agents, *Lecanicilliummus carium*, *Fusariumsolani*, *Aspergillusniger.*

INTRODUCTION

Economic losses due to plant parasitic nematodes infield crops, vegetables and fruit trees are amajor worldwide problem (Sasser and Freckman, 1987; Anwar and McKenry, 2012; Singh andKumar,2015). Among these nematodes, the root knot nematodes (*Meloidogyne* spp.) are considered to becryptic enemies for crops compared to other plant parasitic nematodes that are polyphagous, endoparasites and sedentary vascular root feeders. The growth and harvested yield of crops are affected by many biotic and abiotic factors, but plant parasitic nematodes, particularly *M. incognita* and *M.javanica* could become the most yield-limiting biological factors. The penetration of plant roots by second stage juveniles (J2) of these nematodes leads to a surge in physical and physiological changes, leading to the formation of giant cells and root galls (Sharon *et al.,* 2001). The management of these soil pathogens becomes difficult due to their high reproduction rate,short life cycle,wide host range and endoparasitic nature (Trudgill and Blok, 2001; Manzanilla-Lopez *et al.,*2004).

Among various approaches, biological control has recently drawn the attention of nematologists. It involves predacious and parasitic organisms including fungi, bacteria, protozoa, viruses, nematodes, and mites. Nematophagous fungi are widespread in association with nematodes in the soil. These fungi parasitize nematodes directly using trapping devices such as sticky branches, networks, knobs, constricting and non-constricting rings, or attack them indirectly by producing toxic substances and enzymes (Barron, 1977; Bird and Herd,1995; Mukhtar and Pervaz, 2003; Zouhar *et al.,*2013). Gan *et al*. (2007) has described the process of pathogenesis as a series of mechanisms comprising of attachment, penetration, digestion and propagation of the parasite into the host. Nematophagous fungi must first attach to the host followed by penetration ofthe host for successful infection(TunlidandJansson,1991; Gan*et al.,*2007). The main barriers preventing fungal penetration for successful infection are the nematode cuticle and egg shell. It is assumed that nematophagous fungi have developed mechanisms to break these barrierseither through direct penetrationusing mechanical force or by producing hydrolytic enzymes including chitinases, glucanases, proteases or collagenases (Meyer and Wergin, 1998; Yang *et al.,*2007).

The hazardous effects of chemicals on human health and ecology and the evolution of pathogen strains that are resistant to notorious chemicals have caused such chemicals to undergo the process of restriction (Zuckerman and Esnard, 1994). The carcinogenic effects of these fatal chemicals on human and animal life on earth have forced scientists to determine other ways to control the pathogens. Nematophagous fungi can play an important role in controlling the soil-borne nematodes. In-vitro screening is a vital method to identify potential biocontrol agents among nematophagous fungi. The classification of fungal biocontrol agents is normally based on their mode of action and efficacy in terms of the secretion of nematicidal substances, enzymes and direct parasitism (Nitao *et al.,*1999).The objective of this study was to explore the potential of these fungi on the viability of eggs and J2 of *M. Incognita* under laboratory conditions.

MATERIALS AND METHODS

Nematode culture: Nematode-galled roots of tomato plants from the greenhouse at the Czech University of Life Sciences, Prague, were collected. The egg masses were isolated, and a single egg mass was used to establish a pure nematode population. The eggs were extracted from 45-day old galls on the roots of tomato seedlings by using 0.05%NaOCl (Hussey and Barker,1973).The extracted eggs were gently washed with tap water to remove NaOCl. *Meloidogynein cognita* eggs were identified based on morpho-metrical characteristics (Eisenback, 1985). The extracted eggs were maintained at room temperature (25˚C) to hatch J2. The eggs and J2 were counted and exposed to the test fungi.

Fungus culture: All tested fungi were isolated from egg masses of root knot nematodes collected from twenty vegetable fields of tomato, eggplant, carrot and chili. The egg masses on the galled root systems revealed simultaneous infection by nematodes and fungi, which were in black color. These egg masses were surface-sterilized and then inoculated axenically onto Petri plates containing potato dextrose agar (PDA) amended with streptomycin at 1g/L after surface sterilization with 0.5% NaOCl for 2 min (Singh andMathur,2010). The Petri plates were incubated at 25°C for one week in an environmentally controlled chamber. The fungal colonies were isolated and purified by repetitive culturing, and were later identified and confirmed using the molecular technique polymerase chain reaction (PCR) at theMolecular Plant Pathology Lab, United States Department of Agriculture, Washington, DC, USA. The fungi used for the experiment included *Aspergillusniger, A. nidulans, Fusariumchlamydos porium, F. oxysporum, F. solani, Purpureocillium lilacinus, Pochoniachl amydosporia, Trichoderma harzianum, T. viride, T. hamatum, Lecanicilliummus carium* and *L. psalliotae* (Table 1). The conidia were harvested in deionized water containing 0.03% Tween 80 and filtered through four layers of sterile cheesecloth to remove mycelium (Güçlü *et al.,*2010). The conidia were counted and standardized using a haemocytometer under a compound microscope. The conidial suspension was adjusted to 1×10^3 conidia/ml for each fungal isolate and sprayed onto agar media for the Lab experiments (Güçlü *et al.,* 2010). The viability of the conidia was confirmed before each experiment using methods previously described by Wekesa *et al.*(2005) with some modifications. In all cases the viability was close to 100%.

Effect of fungi on egg parasitism: Each fungal isolate spore suspension at a concentration volume of spore suspension of 1×10^3 conidia/ml was inoculated onto the center of aPetri dish containing PDA. The inoculated plates were incubated at 25˚C for 10 days, and each plate was spread uniformly with 100 *M. incognita* eggs by a pipette. Five replicates for each fungus were used whereas eggs without fungi were maintained as controls for the comparison of infection. The effects of the fungi were noted after 24, 48, and 72 h, and the percent parasitized eggs was measured by staining them with cotton blue and counting them under a stereo-binocular microscope. Eggs with the direct penetration of hyphae or with disintegrated contents were considered to be infected (Khan *et al.,* 2006; Singh and Mathur, 2010). Eggs containing live J2 and that had hatched J2 from eggs were counted as viable.

Effect of fungi on egg hatching: A 1-mL suspension containing 100 nematode eggs was placed onto each sterile Petri plates simultaneously with a 1×10^3 conidia/ml suspension of the test fungi. The plates were incubated at 25˚C for 10 days. Five replicates were used for each treatment. The number of J2 was counted under a stereo-binocular microscope, and the percentage of hatched eggs was determined. The eggs in sterile water and non-inoculated potato dextrose broth (PDB) were used as controls for comparison.

Effect of fungi on juvenile activity and mortality: One mL of each fungal suspension containing 1×10^3 conidia and 1mL of a nematode suspension containing 100 J2 were placed on PDB in sterile Petri plates. Nematode activity was evaluated after 24, 48, and 72 h. The fungal efficacy was based on the percentage of paralyzed nematodes. Rigid, elongated, and bent J2 were considered to be immobilized if they did not react after probed with a fine needle (Cayrol and Pijarowski, 1989; Singh andMathur,2010). A revival test was run by centrifugation for 3 min at 1000rpm, and incubation in sterile water for one day. If the nematodes were found to be inactive after one day, they were considered as dead. Five replicates were used in this study.

Statistical analysis: The experiments were repeated, and the data from the experiments were subjected to ANOVA using the software SPSS 0.1. Duncan's multiple range test was conducted to determine the significance of the treatments.

RESULTS

All the fungi were found to be effective in parasitizing eggs or paralyzing J2 except *A. niger, A. nidulans* and *F. solani*, which were only capable of infecting J2 to various extents. The infection was monitored at 24 h, 48 h, and 72 h intervals and the maximum infection was recorded for*L. muscarium* (79%), followed by *T. hamatum* (68%), *L. psalliotae* (65%), *P. lilacinus* (64%) and *T. harzianum (*62%) at 24 h. *Aspergillusniger*, *A. nidulans* and *F. solani* did not show any parasitic activity towards eggs even at 72 h. All

Table 1. Efficacy of various fungi on viability of eggs and juveniles of root knot nematodes, *Meloidogyne incognita.*

	Percent Egg infection (Mean±SE) at different time intervals			**Percent Egg hatching (Mean±SE)**	**Percent Inactive nematode (Mean±SE) at different time intervals**			**Percent Dead nematode* (Mean±SE)****
Fungi/treatments	24h	48h	72h	10 DAYS	24h	48h	72h	
Aspergillusniger	0±0.00^{f}	0±0.00^{f}	0±0.00^{g}	24.2±0.86^{d}	20.6±0.93^{b}	34.8±1.43^{c}	55.6±1.33^{d}	45±0.71^{d}
A. nidulans	0±0.00^{f}	0±0.00^{f}	1±0.32^{g}	34±2.05^{c}	14±1.00^{de}	25.6±1.08^{e}	58±1.41^{d}	41.4±2.66^{d-f}
Fusariumchlamydosporum	13.2±0.66^{cd}	31±1.14^{cd}	44.2±0.97^{e}	41.4±1.03^{b}	11.8±0.86^{e}	23.4±0.68^{ef}	42.4±1.08^{g}	33.4±0.68^{g}
F. oxysporum	9.6±0.87^{e}	22±0.89^{e}	34.6±1.03^{f}	33.4±0.68^{c}	8.2±0.86^{f}	21±1.14^{fg}	32.4±1.08^{h}	26.4±1.08^{h}
F. solani	0±0.00^{f}	0.4±0.24^{f}	0.6±0.40^{g}	19.6±1.08^{e}	8±1.00^{f}	14±1.34^{h}	21±1.41^{i}	14.4±0.75^{i}
Purpureocillium lilacinus	23±1.41^{a}	40.4±1.40^{ab}	64.4±1.78^{c}	21±1.41^{de}	11.8±0.86^{e}	20.2±1.02^{g}	35±1.58^{h}	30.6±1.36^{g}
Pochoniachl amydosporia	23±1.41^{a}	35.2±1.07^{b-d}	77±1.14^{a}	21.4±1.72^{de}	13.8±1.02^{de}	25.2±0.66^{e}	46.6±1.21^{f}	38.4±1.17^{f}
Trichoder maharzianum	17.2±1.20^{b}	23±0.71^{e}	62.8±1.56^{c}	23.4±1.33^{d}	16.8±1.36^{c}	34±1.00^{c}	51.4±2.48^{e}	42.6±1.72^{de}
T. viride	11.8±0.86^{de}	22±0.89^{e}	51.6±1.50^{d}	18±1.00^{e}	15.8±1.02^{cd}	23.2±0.80^{ef}	48.4±2.04^{ef}	38.8±.91^{ef}
T. hamatum	13.8±0.86^{cd}	35.6±6.48^{bc}	68.6±1.21^{b}	11.8±0.86^{f}	20.2±1.02^{b}	29.8±1.02^{d}	66.2±1.02^{c}	55.8±1.62^{c}
Lecanicillium mu carium	22.6±1.29^{a}	42.6±2.32^{a}	79.6±1.89^{a}	6.8±0.66^{g}	32.4±1.08^{a}	63±1.41^{a}	84.4±2.04^{a}	78±1.41^{a}
L. psalliotae	15.6±1.08^{bc}	29.6±1.08^{d}	65.4±1.21^{bc}	10.2±0.86^{fg}	31±1.14^{a}	44±1.10^{b}	75.8±0.86^{b}	65.6±1.72^{b}
PDB medium (control)	0±0.00^{f}	0±0.00^{f}	0±0.00^{g}	89.8±1.59^{a}	0±0.00^{g}	2.6±0.68^{i}	5.8±0.80^{j}	2.6±0.51^{j}
Distilled water (control)	0±0.00^{f}	0±0.00^{f}	0 ±0.00^{g}	89.8±1.46^{a}	0 ±0.00^{g}	1.8±0.58^{i}	3.6 ±0.60^{j}	1.6±0.51^{j}

* After revival in water; **Means in each column with different letters differ significantly (P<0.05).

the rest of the fungi showed moderate infection of eggs (Table 1).

In terms of the egg hatching rates, all fungi performeddifferently, but four fungi, including *L. muscarium, L. psalliotae, T. viride* and *T. hamatum* were dominant in reducing the rate of egg hatch comparedto the controls (Table 1). All other fungi had moderate effects on emergence of J2 from eggs. The fungi having the least effect on J2 emergence were of *F. chlamydosporum* (41%), *A. nidulans* (34%) and *F. oxysporum* (33%).

Lecanicilliummus carium inactivated or paralyzed 84% of the J2. Six fungi,*A. niger, A. nidulans T. harzianum, T. hamatum, L. muscarium, and L psalliotae* were determined to be better parasites of J2 by inactivating more than 50% of J2 after 72 h. The other fungi, *F. chlamydosporium, F. oxysporum, P. lilacinus, P. chlamydosporia,* and *T. viride* had mild effects on killing or paralyzing J2 (Table 1). The water and PDA controls had similar effects on eggs and J2.

The revival test of J2 showed that *Lecanicillium* spp. and *T. hamatum* did not lose their effectiveness and proved to be potential biocontrol agents against nematodes (Table 1). They killed the highest number of J2 compared (P= 0.05) to the others treatments. The lowest mortality of J2 was occurred when they were exposed to *Fusarium* spp.

DISCUSSION

Parasitic activities against nematodes by different fungi as potential biocontrol agents against nematode has been extensively documented (Cayrol *et al.*,1989; Saifullah, 1996; Zaki, 1999; Nicola *et al.*,2014). This experiment incorporated time intervals of 24h, 48h and 72h. The results showed that J2 infection by fungi increased with exposure time. All fungal isolates showed nematicidal effects of varying degrees on *M. incognita* except *A. niger, A. nidulans,* and *F. solani* which had no effect on egg mortality although they effectively parasitized J2. Our results validate the studies by Singh and Mathur (2010).

The *Lecanicillium* spp., which were fast growing fungi on PDA, were the most effective egg parasites which reducing the level of hatching and increasing the mortality of J2 (Hussain *et al*., 2017a, b, c). The high degree of nematophagous potential of these fungi might be due to the production of toxins (Sugimoto *et al.*,2003). They produce high levels of chitinases, which havea key role in the parasitic mechanisms, allowing fungi to infect host cells containing chitin, for example, egg shells and the cuticle. *Trichoderma*spp., ranked second in effectiveness and behaved very positively in the reduction of egg hatching. *Trichoderma* spp., are believed to have great potential against many pathogens including fungi, bacteria, and nematodes due to the release of lytic enzymes (Elad *et al.*,1982; Limon *et al.*,1998; Sharon *et al.*,2001), production of volatile or non-volatile antibiotics (Barker and Griffin,1995), or nutrient-limiting factors that affect their hosts(Sivan and Chet,1989) and either direct parasitism mechanically or indirectly through the release of lytic enzymes (Chet *et al.*,1997). Such characteristics are useful against soil-borne pathogens such as nematodes and others due to ecosystem factors and their high colonizing capacity, high shelf life and efficiency with respect to the habitat and target (Spiegel and Chet, 1998). Furthermore, the use of *Trichoderma* spp., in combination with other fungi that produce trapping structures could be a promising approach to the management of soil-borne diseases such as nematodes in addition to promoting the growth of plants as *Trichoderma* spp. play a vital role in the growth and vigor of plants (Goswami *et al.*, 2008).

Purpureocillium lilacinus ranked third in terms of causing egg infection, and affecting the egg hatching rate and mortality. This fungus is considered to be a biocontrol agent against various pathogens, especially nematodes and fungi. This fungus is believed to secrete toxic metabolites that have neurotropic actions (Cayrol *et al.*, 1989).

Amat of mycelium was found inside and around eggs and J2, but some of these were lacked mechanical injuries but were found to bedead, which could be due to toxic enzymes secreted by the fungi. The nematicidal effects increased with an increase in exposure time. This in line with previous studies (Hallmann and Sikora, 1996; Meyer *et al.*,2004; Park *et al.*, 2004). The PDB media did not possess any nematicidal activity and did not significantly differ from water (P= 0.05),which contradicts the studies by Nitao *et al*.(1999), who observed more hatching in PDB than in water. This difference might be related to differences in experimental conditions. *Fusarium* spp., were not very effective against egg parasitism but behaved equally wellin terms of the immobility and death of J2. This observation is in line with studies by Ciancio (1995), Vaishnav *et al*.(1985) and Zia *et al*.(2002).The juvenile cuticle is mainly composed of proteins that could be degraded by the proteolytic activities of fungi (Blaxster and Robertson, 1998). Some fungi have spiny structures or other trapping devices that could facilitate the mechanical penetration of eggs as well as J2. Eggs have surface binding components in a gelatinous matrix (gm), which might facilitate fungal spore attachment, germination and penetration (Sharon *et al.*,2002; Sharon *et al.*, 2007; Sharon *et al.*, 2009). The presence of gmon nematode eggs might have contributed to fungal parasitism which needs further study in the future.

In our study, *L. muscarium* was found to be a potential biocontrol agent and might be a useful for commercial purposes. It has two different modes of action, higher parasitism and higher level of toxin

production at a wide range of temperatures (5-30°C) and humidity (Fenice *et al.*,1996; Fenice *et al.,* 1997; Fenice *et al.,* 2012; Kope *et al.,* 2008), which might be advantageous over the fungi. Further investigations must be carried out to characterize the nematicidal compounds produced by this fungus.

Acknowledgements: We are thankful to Czech University of Life Sciences Prague and Ministry of Agriculture for providing funds to conduct the part of this research under project number PROJ201500056 and QJ1310226 respectively.

REFERENCES

Anwar, S.A., and M.V. McKenry (2012). Incidence and population density of plant parasitic nematodes infecting vegetable crops and associated yield losses. Pakistan J. Zool. 44: 327-333.

Barker, R., and G.J. Griffin (1995). Molecular strategies for biological control of fungal plant pathogens. In R. Reuveni (Eds.), Novel approaches to integrated pest management: (pp. 153). Boca Raton, FL, USA: Lewis Publishers Inc.

Barron, G.L., (1977). The nematode destroying fungi, Canadian Biological Publications, Guelph, Ont. pp.140.

Bird, J., and R. P Herd. (1995). *In-vitro* assessment of two species of nematophagous fungi (*Arthrobotrys oligospora* and *Arthrobotrys flagrans*) to control the development of infective cyathostome larvae from naturally infected horses. Vet. Parasitol. 56: 181-187.

Blaxster, M. L., and W. M. Robertson (1998). The Cuticle, In R.N. Perry and D.J. Wright (Eds.),Free living and Plant Parasitic nematodes. (pp. 25-48). CAB. International, Wallingford, UK.

Cayrol, J.C., C. Djian, and L. Pijarowski (1989). Study of the Nematocidal properties of the culture filtrate of the nematophagus fungus *Paecilomyces lilacinus.* Revue de Nematologie, 12: 331-336.

Chet, I., J. Inbar and I. Hadar (1997). Fungal antagonists and mycoparasites. In D.T. Wicklow and B. Söderström (Eds.), The Mycota IV: Environmental and microbial relationships. (pp. 165-184). Springer-Verlag, Berlin.

Ciancio, A., (1995). Observations on the nematicidal properties of some mycotoxin. Fund. Appl. Nematol. 18: 451- 454.

Eisenback, J. D. (1985). Detailed morphology and anatomy of second stage juveniles' males, and females of the genus *Meloidogyne* (root knot nematode). In J.N. Sasserand C.C. Carter (Eds.), An Advanced Treatise on *Meloidogyne*, Vol. 1 Biology and Control, North Carolina State University Graphics, Raleigh.

Elad, Y., I. Chet and Y. Henis. (1982). Degradation of plant pathogenic fungi by *Trichoder maharzianum.* Can. J. Microbiol. 28: 719-25.

Fenice, M., P. Barghini, L. Selbmann and F. Federici (2012). Combined effects of agitation andaeration on the chitinolytic enzymes production by Antarctic fungus *Lecanicillium muscarium* CCFEE 5003. Microb. Cell Fact. 11-12.

Fenice, M., L. Selbmann, R. Di Giambattista, M. Petruccioli and F. Federici (1996). Production of extracellular chitinolytic activities by a strain of the Antarctic entomogenous fungus *Verticilliumcfr. Lecanii"*. In R.A. Muzzarelliand A. Grottammare (Eds.), Chitin Enzymology (pp. 285-292). 2. Atec Edizioni, Italy.

Fenice, M., L. Selbmann, L. Zucconi and S. Onofri (1997). Production of extracellular enzymes by Antarctic fungal strains. Polar Biol. 17: 275-280.

Gan, Z.W., J.K. Yang, N. Tao, L.M. Liang, Q. Mi, J. Li and K.Q. Zhang (2007). Cloning of the gene *Lecanicilliumpsalliotae* chintinase LpChi1 and identification of its potential role in the biocontrol of root knot nematode *Meloidogyne incognita.* Appl. Microbiol. Biotechnol. 76: 1309-1317.

Goswami, J., R.K. Pandey, J.P. TewariandB.K. Goswami(2008). Management of root knot nematode on tomato through application of fungal antagonists, *Acremoniumstrictum* and *Trichodermaharzianum*. J. Environ. Sci. Heal. 43: 237-240.

Güçlü, Ş., K. Ak, C. Eken, H. Akyol, R. Sekban,B. Beytutand R. Yildirim(2010). Pathogenicity of *Lecanicilliummuscarium* against *Ricaniasimulans.* Bulletin Insectolgy. 63: 243-246.

Hallmann, J., and R.A. Sikora(1996). Toxicity of fungal endophyte secondary metabolites to plant parasitic nematodes and soil borne plant pathogenic fungi. Eur. J. Plant Pathol. 102:155-162.

Hussey, R.S., and K.R. Barker (1973). Comparison of methods for collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Rep. 57: 1025-1028.

Hussain, M., M. Zouhar and P. Ryšánek. (2017a). Comparison between biological and chemical management of sugar beet nematode, *Heteroderaschachtii.* Pakistan J. Zool. 49(1): 45-50.

Hussain, M., M. Zouhar and P. Ryšánek. (2017b). Potential of some nematophagous fungi against *Meloidogynehapla* infection in Czech Republic. Pakistan J. Zool. 49(1): 35-43

Hussain, M., M. Zouhar and P. Ryšánek. (2017c). Effect of some nematophagous fungi on reproduction of

a nematode pest, *Heteroderaschachtii*, and growth of sugar beet. Pakistan J. Zool. 49(1): 89-96.

Khan, A., K.L. Williams and H.K. Nevalainen(2006). Control of plant parasitic nematodes by *Paecilomyceslilacinus* and *Monacrocporium lysipagum* in pot trials. Biol. Control. 51: 463-658.

Kope, H.H., R.I. Alfaro and R. Lavallée(2008). Effects of temperature and water activity on *Lecanicillium* spp. conidia germination and growth, and mycosis of *Pissodesstrobe*. Bio Control. 53: 489-500.

Limon, M.C., J.A. Pintor-Toro and T. Benitez (1998). Increased antifungal activity of *Trichodermaharzianum* transform ants that overexpress a 33-kDa chitinase. Phytopathol. 89: 254-261.

Manzanilla-Lopez, R.H., E. Kenneth and J. Bridge (2004). Plant diseases caused by nematodes. In Z.X. Chen., Chen, S.Y. and D. W.Dickson (Eds.), Nematology advances and perspectives II; Nematode management and utilization (pp. 637-716), Cambridge, MA: CABI publishing.

Meyer, S.L.F., and W.P. Wergin(1998). Colonization of soybean cyst nematode females, cysts and gelatinous matrices by the fungus *Verticilliumlecanii*. J. Nematol. 30: 436-450.

Meyer, S.L.F., R.N. Huettel, X.Z. Liu, R.A. Humber, J. Juba and J.K. Nitao(2004). Activity of fungal culture filtrate against soybean cyst nematode and root knot nematode egg hatch and juvenile motility. J. Nematol. 36: 23-32.

Mukhtar, T., and I. Pervaz (2003). *In vitro* evaluation of ovicidal and larvicidal effects of culture filtrate of *Verticilliumchlamydosporium* against *Meloidogynejavanica*. Int. J. Agri. Biol. 4: 576-579.

Nicola, L.,S. Tosi and D. Savini (2014). In vitro evaluation of nematophagous activity of fungal isolates. J. Basic Microbiol. 54: 1-5.

Nitao, J.K., S.L.F. Meyer and D.J. Chitwood(1999). *In vitro* assays of *Meloidogyneincognita* and *Heteroderaglycines* for detection of nematode antagonistic fungal compounds. J. Nematol. 31: 172-183.

Park, J.O., J.R. Hargreaves, E.J. Mcconville, G.R. Stirling, E.L. Ghisalberti and K. Sivasithamparam (2004). Production of leucinostatins and nematicidal activity of Australian isolates of *Paecilomyceslilacinus.* (Thom) Samson. Lett. Appl. Microbiol. 38: 271-275.

Saifullah, (1996). Nematicida land nematostatic effect of cell free culture filtrates of *Verticilliumchlamydosporium*. Goddard *in vitro*. Afro-Asian J. Nematol. 6: 32-5.

Sasser, J. N., and D. W. Freckman (1987). A world perspective on Nematology. In J.A. Veech and D.W. Dickson (Eds.), The role of society. Vistas on nematology (pp. 7-14) SON Inc., Hyattsville, MD.

Sharon, E., M. Bar-Eyal, I. Chet, A. Herrera-Estrella, O. Kleifeldand Y. Spiegel (2001).Biological control of the root knot nematode *Meloidogynejavanica* by *Trichodermaharzianum*. Phytopathol. 91: 687-693.

Sharon, E., I. Chetandand Y. Spiegel (2009). Improved attachment and parasitism of *Trichoderma* on *Meloidogynejavanica in-vitro*. Eur. J. Plant Pathol. 123: 291-299.

Sharon, E., I. Chet, A. Viterbo, M. Bar-Eyal, H. NaganandG.J. Samuels (2007). Parasitism of *Trichoderma* on *Meloidogynejavanica* and role of the gelatinous matrix. Eur. J. Plant Pathol.118: 247-258.

Sharon, E.,Y. Spiegel, R. Solomon and R. Curtis (2002). Characterization of *Meloidogynejavanica* surface coat using antibodies and their effect on nematode behavior. Parasitol. 125: 177-185.

Singh, S., and N. Mathur (2010). In vitro studies of antagonistic fungi against the root knot nematode, *Meloidogyneincognita*. Biocon. Sci. Technol. 20: 275-282.

Singh, R., and U. Kumar (2015). Assessment of nematode distribution and yield losses in vegetable crops of Western Uttar Pradesh in India. Int. J. Sci. Res. 4 (5): 2812-2816.

Sivan, A., and I. Chet (1989). The possible role of competition between *Trichodermaharzianum* and *Fusariumoxysporum* on rhizosphere colonization. Phytopathol. 79: 198-203.

Spiegel, Y., and I. Chet (1998). Evaluation of *Trichoderma* spp. as a biocontrol agent against soil borne fungi and plant parasitic nematodes in Israel. Integr. Pest Manag. Rev. 3: 169-175.

Sugimoto, M., M. Koike, N. Hiyamaand H. Nagao (2003). Genetic, morphological, and virulence characterization of the entomopathogenic fungus *Verticilliumlecanii*. J. Invertbr. Pathol. 82: 176-187.

Trudgill, D.L., and V.C. Blok (2001). Apomictic polyphagous root knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Ann. Rev. Phytopathol. 39: 53-77.

Tunlid, A., and S. Jansson(1991). Proteases and their involvement in the infection and immobilization of nematodes by nematophagous fungus *Arthrobotrysoligospora*. Appl. Environ. Microbiol. 57: 2868-2872

Vaishnav, M.U., H.R. Patel and I.U. Dhruj(1985). Effect of culture filtrates of *Aspergillus* spp. on

Meloidogynearenaria.Ind. J. Nematol. 15: 116-117.

Wekesa, V.W., N.K. Maniania, M. Knapp and H.I. Boga(2005). Pathogenicity of *Beauveriabassiana* and *Metarhiziumanisopliae* to the tobacco spider mite *Tetranychusevansi*. Exp. Appl. Acarol. 36: 41-50.

Yang, J.K.,B.Y. Tian, L.M. LianyangandK.Q. Zhang (2007). Extracellular enzymes and the pathogenesis of nematophagous fungi. Appl. Microbiol.Biotechnol. 75: 21-31.

Zaki, M.J., (1999). Effect of fungal culture filtrates on mortality and hatching of *Meloidogynejavanica*. Pakistan J. Biol. Sci.2: 161-3.

Zia, T., I.A Siddiqui, S.S. Shaukatand S.N. Hasan(2002). Trigonellafoenumgracecum (fenugreek) Mediated Suppression of *Meloidogynejavanica* in Mung Bean. *ActaAgrobotanica*. 55: 121-132.

Zouhar, M., O. Douda, J. Nováková, E. Doudová, J. Mazáková, J. Wenzlová, P. RyšánekandM. Renčo(2013). First report about the trapping activity of *Strophariarugosoannulata*acanthocytesfor Northern Root Knot Nematode. Helminthologia. 2: 127-131.

Zuckerman, B.M., and J. Esnard(1994). Biological control of plant nematodes: current status and hypothesis. Jpn. J. Nematol. 24: 1-13.