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STUDIES ON THE EFFICACY OF SOME FUNGI AND BIOPREPARATIONS FOR CONTROL OF *Meloidogyne incognita*

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Abstract

A glasshouse trial was conducted to ascertain the effects of fungi, plant and bacterial products against *Meloidogyne incognita*. All the tested formulations suppressed nematode multiplication and root galling significantly. The nematicide had the most suppressive effect on gall formation. The smallest galling index, number of galls and nematode population were in soils treated with oxamyl followed by neem-Azal – 0.3%, neem-oil – 0.3%, *Fusarium* spp., *Trichoderma* spp. and Treisr – 480 EC (*Saccharopolyspora spinosa*). The best control was provided by application of the neem products – neem-Azal (0.3%) (*Azadirachta indica* A. Juss.), and neem-oil – 0.3%. They were highly effective in reducing disease incidence. The nematode density was decreased from 32.0% to 60.0%. The percentage of infected females was from 10.3% to 22.3%. The plant growth for the treated plants enhanced as compared with that of inoculated with *M. incognita*.

Key words: *M. incognita,* root - knot nematode, bioagents, reproductive index

The root-knot nematode *Meloidogyne incognita* is serious pest of many cultivated crops around the word. The *Meloidogyne* spp. complex is one of the economically most important pests in Bulgaria (Choleva, 1973; Stoyanov, 1980). The control of root knot nematodes by crop rotation is rather difficult because of their wide host range. The nematicides are expensive and may cause pollution. For several years, there has been considerable interest in the use of soil fungi and bacteria that parasitize the females and eggs of certain nematodes (Sharon et al., 2001; Zuckerman et al., 1993). According Sharon et al. (2006), Randey (2005), Mayer et al. (1990) several species (and isolates) of the genus *Trichoderma* were evaluated as biocontrol agents against *Meloidogyne javanica* and cyst nematode *Heterodera glycines* and exhibited significant biocontrol activity.

Natural parasitism of cyst nematodes by *Fusarium* and *Trichoderma spp.* is well-known (Sharon et al., 2001; Suazez et al., 2004; Nagesh and Reddy, 2004; Randey, 2005; Khan et al., 2005). Endoparasitic fungi which infect nematodes with their conidia have been suggested as possible biocontrol agents, although very little is known about their biology and isolation in pure culture. Investigations on extracts from various plants and neem (*Azadirachta indica* Juss.) products have revealed that some of them are effective against insects and nematodes (Sharma, 2000).

This has encouraged scientists to search for alternative sources of effective and ecofriendly chemicals for nematode control. These bacterial and fungal egg parasites can be integrated for the management of nematodes. Therefore, a glasshouse test was conducted to evaluate the effects of different bioagents for the biological control of a root-knot nematode.

MATERIAL AND METHODS

Pathogens: Isolates of *Fusarium* spp. and *Trichoderma* spp. (local isolates accession number R4-134-BPI and T - 9) were obtained from roots and lower stems of naturally diseased tomato plants. The inoculums of the two fungal pathogens was developed in pure cultures on barley (*Hordeum vulgare* L.) grains, preliminary placed in Petri dishes (200 kernels in each) soaked in distilled water for 24 h, drained and autoclaved at 121°C. Each dish was inoculated individually with a 5 mm-agar disc of the fungus from margins of the actively growing 7 and 10-day-old OA cultures, respectively. Dishes were incubated at 25°C until barley grains were completely colonized (Vatchev, 1995).The fungi were used at rates – 5 g mycelium/pot for *Fusarium* spp. and 2 g mycelium/pot for *Ttichoderma* spp. The bacterial bioagent was added to the soil at the time of planting as 1 ml bacteria suspension (containing around 10^8 spores/ml) of the commercial preparation: Treisr 480 CK – *Saccharopolyspora spinosa.*

The two plant products were used – two comercial formulations of neem (*Azadirachta indica* A. Juss) – neem-Azal (0.3%) and neem-oil (0.3%). The formulations were obtained from the National Service for Plant Protection – Sofia. Sixteen days after transplanting, the soil in the pot was drenched with 20 ml of each of the plant product separately. The inoculums were carefully disposed around the root zone and covered with soil. Oxamyl (Vidate – 10 G) at the rate of 1.5 mg/g soil was applied through irrigation carried out before seedling planting. There were four replicates of each treatment. Non-inoculated plants were used as a control.

Nematode. The population of root-knot nematode *M. incognita* (Kofoid & White) Chitw. was collected from tomato plants and cultured on *L. esculentum* cv. Ideal from a single egg mass under glasshouse conditions. Mature egg masses were hand-picked from the roots and incubated in Baermann trays (Southey, 1986). Hatched juveniles were collected each 24 h and stored at 20 °C temperature. Identity of the nematodes was made by standard technique.

Treatments. In the glasshouse experiment plastic pots 12 cm in diameter with a capacity of 600 g steam sterilized soil were used. They were planted with two-three week – old tomato seedlings *Lycopersicon esculentum* Mill, cv. Ideal, raised in sterilized soil. One week after planting the seedlings were inoculated with 2000 freshly hatched juveniles suspended in 25 ml wither.

The experiments were terminated after 65 days or after completion of the nematodes life-cycle. The plants were uprooted from the pots and roots were washed in running tap water and blotted dry to record the number of galls on the roots and to estimate the number of females parasitized by *Trichoderma* spp., *Fusarium* spp. and *Saccharopolyspora spinosa*. The plant height and root length were recorded after 2 months.

The whole root system was examined visually to count galls. Immediately after harvest, root-knot index (RKI = G.I.) was assessed using a 0-5 scale of Taylor and Sasser (1978), where 0 = no gall on the roots; 1 = 1-2 galls; 2 = 3-10; 3 = 11-30; 4 = 31-100 and 5 = more than 100 galls per root.

The percentage of parasitized eggs was determined by plating a 100 eggs on water agar (WA), incubating for two days at room temperature and counting the proportion of eggs showing fungal growth (i.e. parasitized eggs) under the microscope at × 100 magnification (Kerry and Crump, 1977). The following categories were used: infected egg (those developing a fungal colony or bacterial spores), distorted eggs (those whose contents were not identifiable as juveniles or embryos) and normal eggs (containing embryos or second stage juveniles.

Second-stage juveniles in soil. 250 g of the soil from each pot was washed for extraction of second-stage juveniles by Cobb, s sieving and modified Baerman funnel methods (Southey, 1986). An aliquot of 2 ml was pipette out and the number of juveniles counted under stereozoom binocular microscope. The process was repeated five times and average number of juveniles/ml counted was multiplied by the volume of the suspension.

Table 1. Effect of different treatments on the plant growth of tomato and the density of *Meloidogyne incognita*

Treatment	Length, cm		Egg parasitism, %	Nematode density in soil	Relative reduction, %
	shoot	root			
1.	48.1*	7.2*	-	220.0*	60.0
2.	45.3*	8.0*	-	302.0*	32.0
3.	45.2*	8.0*	19.8	332.2*	28.0
4.	40.0*	7.2*	22.3	312.3*	32.0
5.	40.0*	7.0*	10.3	374.2*	24.0
6.	35.2*	8.7	-	570.2	-
7.	51.5*	10.0	-	-	-
8.	49.2*	7.5*	-	-	90.0
SD	*4.3*	*1.2*		*7.4*	

* - Significant differences from the control (nematode) at P ≤ 0.05.
Legend: 1 - neem-Azal 0.3%; 2 - neem-oil 0.3%; 3 - *Trichoderma* spp.; 4 - *Fusarium* spp.; 5 - Treisr 480 SC; 6 - nematode; 7 - untreated; 8 - oxamyl G.

The data were subjected to factorial analysis of variance and treatments were compared using Duncan`s multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The experimental results on the efficacy of some fungi, bacteria and plant products for the control of *M. incognita* on tomato indicate that the treatments improved plant growth significantly over control (Table 1) with a corresponding reduction in all the population parameters. However, the greatest increase was given oxamyl and neem-Azal – 0.3%. Except for treated with nematode plants, the other treatments significantly increased shoot and root length compared to the untreated uninoculated plants. The untreated control plants had the greatest growth. Infection by *M. incognita* caused a significant decrease in the growing of tomato plants compared to the uninoculated control. The treatments of plants suppressed gall formation and nematode reproduction, thus helping to increase growing of the plants. Similar results were obtained by Kumar and Khanna (2006).

All treatments caused significant reduction in the number of galls compared to the untreated control. Inoculation of plants with nematode alone resulted in the highest gall index (G I = 5.0).When fungus was applied at planting at rates of 2 or 5 g/pot they caused significant reductions in the number of galls of root system (Figure 1). The best control of the gall index showed preparation neem-Azal 0.3% (G.I. = 2.0). Maximum reduction over control was observed in oxamyl treatment – 90.0%, followed by neem-Azal (0.3 – 60.0%) and neem-oil (0.3 – 32.0%).

In all treatments population density of *Meloidogyne incognita* drastically declined compared with control at 90 days after treatments (Table 1). In all plants the number of second stage juveniles of the nematode in the soil were significantly reduced when compared to the inoculated but untreated check. The neem-Azal (0.3%) was the most effective. Also effective, but less so than those were treatments with fungal and bacterial bioagents. The highest reproductive rate of nematode was registered in the infected untreated plants (570.2 nematode/250 g soil). The soil application of the bioagents significantly suppressed the second stage juveniles (J_2) of the nematode (P ≤ 0.05).

The nematicidal principles (azadirachtin, nimbidin, phenolics, aldehydes etc.) of neem have been reported by Khan (1976) and Mojumder (1995). According Hasan (1992) Hasan and Khan (2004) the different neem products suppress root-knot disease in various crops. Our results confirmed conclusions of Akhtar and Mahmood (1993) that also found that neem oil induce plant resistance against plant-parasitic nematodes present in naturally infested soil on tomato.

Our results indicate that application of the commercial insecticide – Treisr 480 EC (*S. spinosa*), bioagents – *Fusarium* spp., *Trichoderma* spp. and plant products neem-Azal and neem-oil reduced the population density of *Meloidogyne incognita* in tomato. Under the conditions of this experiment, in use rates may to reducing nematode level and could be an alterna-

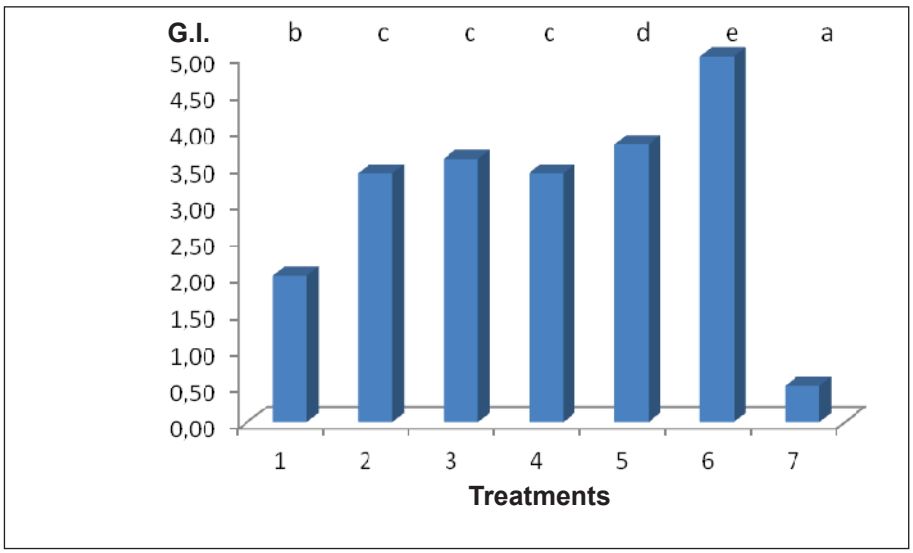

Fig. 1. Gall index (G.I.) for different treatments

Legend: 1 - neem-Azal 0.3%; 2 - neem-oil 0.3%; 3 - *Trichoderma* spp.; 4 - *Fusarium* spp.; 5 - Treisr 480 SC; 6 - nematode; 7 - oxamyl G.
Columns with same letters are not significantly (P ≤ 0.05) different according to Duncan`s Multiple Range Test.

tive control option for the management of root-knot nematode.

The low numbers of J_2 in the soil and galling of tomato roots indicates that these bioagents were effective against the root-knot nematode, thus confirming previous findings of Nagesh and Reddy (2004), Daglish et al. (2008) and Negesh and Reddy (2004).

Therefore, the use of test products shows promise to suppress nematode populations and may provide an alternative to chemicals that is both environmentally safe and economical. However, further evaluation under field conditions is necessary to assess the feasibility of using these components in the integrated nematode management strategy.

CONCLUSIONS

The results obtained from the research of the soil treatments with the biopreparation – Treisr 480 EC and fungi like *Trichoderma* spp. and *Fusarium* spp. neem products as neem-Azal – 0.3% and neem-oil – 0.3% for control of *Meloidogyne incognita* allowed us to make the following conclusions:

All treatments improved the plant growth being greatest with neem-Azal – 0.3%.

The neem-Azal – 0.3% and neem-oil – 0.3% caused significant inhibitory effect on the multiplication and galling of the root-knot nematode. They decreased reproduction rate from 32.0% to 60.0%.

The best reduction in the population density was obtained in the application of oxamyl – 90.0%, following by neem-Azal – 0.3 – 60.0%, neem-oil – 0.3% and *Fusarium* spp. – 32.0%, *Trichoderma* spp. – 28.0%, and Treisr 480 EC – 24.0%.

The percentage of infected females is 22.3%, 19.8% and 10.3% when applied the fungi *Fusarium* spp., *Trichoderma* spp. and bacterial preparation Treisr 480 EC respectively.

REFERENCES

Akhtar, M., I. Mahmood. 1993. Control of plan-parasitic nematodes with neem and some plant oils by bare-root dip treatment. *Nematologica Mediterranea*, v. 21, p. 89-92

Choleva, B. 1973. Root - knot nematodes in greenhouses. *Plant Protection*, v 11, p. 30-35

Daglish, G. J., M. B. Head, P. B. Hughes. 2008. Field evalution of spinosad as a grain protectant for stored wheat in Australia. *Australial Journal of Entomology*, v. 47, p. 70-74

Hasan, A. 1992. Allelopathy in management of root-knot nematodes (p. 413-441). In: Allelopathy – Basic and Applied Aspects (Rizvi, S. J. H. and V. Rizvi, eds). *Chapman & Hall*, London, U.K.

Hasan, A., M. N. Khan. 2004. Interaction of root-knot nematode, vam fungus and neem cake in amended on gladiolus. *Nematologica Mediterranea*, v. *32,* p. 95-99

Khan, A. M. 1976. Control of diseases caused by nematodes by the application of oil-cake manures. Final Tech. Reptr. Grant-No. FG-IN-426, Department of botany, Aligarh Muslim University, Aligarh, India.

Khan, T. A., S. I. Husain. 1991. Effect of Interaction of Meloidogyne incognita and Fusarium solani on the Growth of Papaya and Nematode Reproduction. *Nematologia Mediterranea*, v. 19, p. 195-196

Kumar, S., A. S. Khanna. 2006. Effect of neem-based products on the root-knot nematode, Meloidogyne incognita and growth of tomato. *Nematologica Mediterranea*, v. 34, p. 141-146

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

Mayer, S. L., R. N. Huettel, R. M. Sayr. 1990. Isolation of fungi from Heterodera glycines and in vitro bioassays for their antagonism to eggs. *Journal of Nematology*, v. 22, p. 532-537

Mojumber, V. 1995. Nematoda, Nematodes. p. 129-150. In: The Neem Tree, V. C. H., Wein heim, Germany.

Nagesh, M., P. P. Reddy. 2004. Biochemical changes in Glomus facicilatum colonized roots of Lycopersicon esculentum in presence of Meloidogyne incognita. *Indian Journal of Experimental Biology*, v. 42, p. 721-727

Rao, M. S., P. P. Reddy, M. Nagesh. 1996. Evolution of plant based formulations of Trichoderma harzianum for the management of Meloidogyne incognita on eggplant. *Nematologia Mediterranea*, v. 26, p. 59-62

Randey, R. 2005. Field application of bio-organics in the management of Meloidogyne incognita in Mentha arvensis. *Nematologia Mediterannea*, v. 33, p. 51-54

Saifullah, B., J. Thomas. 1996. Studies on the parasitism of Globodera rostochiensis by Trichoderma harzianum using low temperature scanning electron microscopy. *Afro-Asian Journal of Nematology*, v. 6, p. 117-122

Sharma, G. C. 2000. Efficacy of neem based formulations against the root-knot nematode M. incognita. *Pesticide Research Journal*, v. 12, p. 183-187

Sharon, E., M. Bar-Eyal, I. Chel, A. Herrera-Estrella, O. Kleifeld, J. Spiegel. 2001. Biological control of the root-knot nematode Meloidogyne javanica by Trichoderma harzianum. *Phytopathology*, v. 91, p. 687-693

Sharon, E., I. Chet, A. Viterbo, Y. Spiegel. 2006. Mechanisms in biocontrol of Meloidogyne javanica by Trichoderma. XXVIII International Symposium, 5-9 June, Blagoevgrad, Bulgaria, p. 53-54

Southey, J. F. 1986. Laboratory methods for work with plant and soil nematodes. Her majesty stationary office. London, U.K., p. 202

Steel, R. G., J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York, U.S.A., p. 633

Stoyanov, D. 1980. Control of plant parasitic nematodes. *Zemizdat*, Sofia, p. 193.

Suazez, B., M. Rey., P. Castillo, E. Mont., A. Llobello. 2004. Isolation and characterization of PRA, a trypsilike protease from the biocontrol agent Trichoderma harzianum displaying nematicidal activity. *Applied Microbiology and Biotechnology*, v. 65, p. 43-55

Taylor, A. L., L. N. Sasser. 1978. Biology, Identification and Control of Root-knot nematodes (Meloidogyne species). Nort Carolina State University Graphics, Baleigh. USA, p. 111

Vatchev, T. D. 1995. Studies on Soil-borne Pathogenic Fungi in Greenhouse Tomatoes. − Ph. D. Tesis, Plant Protection Institute, Sofia, p. 192

Изследване ефикасността на някои гъби и биопрепарати за борба срещу галовата нематода *Meloidogyne incognita*

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Резюме

При оранжерийни условия е оценявана ефикасността на гъби, растителни и бактериални препарати срещу галовата нематода *Meloidogyne incognita*. Всички тестирани продукти супресират нематодното размножаване и галообразуване. Най-висок супресивен ефект има нематоцидния препарат. Стойностите на галовият индекс, броят на галите и нематодната плътност са най-ниски в почва, третирана с оксамил, следвана от neem-Azal – 0,3%, neem-oil – 0,3%, *Fusarium* spp., *Trichoderma* spp. и Treisr - 480 EC (*Saccharopolyspora spinosa*). Най-добър биоконтрол се наблюдава при прилагане на неем продуктите – neem-Azal – 0,3% (*Azadirachta indica* A. Juss.) и neem-oil – 0,3%. Те редуцират плътността на нематодата от 32,0 до 60,0%. Инфектираните женски нематоди са от 10,3 до 22,3%. Растежните параметри на третираните растения са по-високи в сравнение с инокулираните с *M. incognita* растения.