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*Full Length Research Paper*

# Biological control of root-knot nematode and *Meloidogyne incognita* in the medicinal plant

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**The medicinally important plant; *Withania somnifera*, is highly susceptible to root knot nematode; *Meloidogyne incognita*. Various nematode antagonistic fungi have been studied for their use as biocontrol agents. In the experiment, the potential of fungi *Trichoderma harzianum*, *Paecilomyces lilacinus* and *Arthrobotrys oligospora* along with natural organic compound (Neem compound mix) to control the nematode; *M. incognita* was evaluated. Also, their potential to control nematodes was compared with that achieved by using the chemical control agent; carbofuran. The fungal agents evaluated significantly controlled nematode population and enhanced plant growth.**

**Key words:** *Withania somnifera*, *Meloidogyne incognita*, biocontrol.

## INTRODUCTION

*Withania somnifera* (local name: Ashwagandha) is a medicinal plant. Its alkaloids and steroidal lactones (withanoids) are used in pharmaceutical industries.

*W. somnifera* is highly susceptible to the root knot nematode; *Meloidogyne incognita.* Infestation results in root galling, stunted growth of the plant and low produc-tivity (Pandey and kalra, 2003). Not only ashwagandha but many other commercially important plants such as betelvine, ginger and tomato suffer severe damage from *M. incognita* infections (Bhatt et al., 2002a, b; Vadhera et al., 1998). Chemical methods have been mostly used to control nematodes. Chemical agents such as halogen-nated aliphatic hydrocarbons (e.g., 1,3-dichloropropene), methyl isothiocynate mixtures, oxamyl, Thionazin and carbofuran are effective in the management of nema-todes but are not ecofriendly and in the course of time may cause serious threat to the ecological balance. In soil these agents increase the probability of mutagenesis in microbes. Chemical pesticides have been tested and evaluated for their ill effects such as reproductive toxicity and carcinogenesis in mammals. High doses of these agents have been proved to be fatal to animals. These facts have been reported under 'Food and Environment Protection Act, 1985, Part III. Control of Pesticide regula-tions 1986' by Pesticide Safety Directorate (Kings Pool, York Y01 7PX) in 1992. Therefore, biological control agents are gaining importance in the field of nematode management. Another importance of these agents is their role as plant growth promoting microorganism (Sharon et al., 2001). *Trichoderma* spp. found in close association with roots contribute as plant growth stimulators (Ousley et al, 1994). Many fungal and bacterial agents have been examined over a period of time for their potential as biocontrol agents. Li et al (2008) evaluated expression of Cry5B protein from *Bacillus thuringiensis* as environment friendly nematicidal proteins. In research performed on fungi, it has been shown that fungi possess appropriate characteristics for biological control of nematodes, for example, fungal enzymes such as chitinases are capable of rupturing nematode egg shells contributing to para-sitism of fungi on nematodes (Gortari and Hours, 2008). Also, mutualistic endophytic fungi such as non- patho-genic strains of *Fusarium oxysporum* and species of *Tri-choderma* have been evaluated for their activity against plant parasitic nematodes (Sikora et al., 2008).

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## MATERIALS AND METHODS

In the experiment, the natural antagonists of nematodes isolated from infected nematodes and healthy plant roots from nematode

**Photograph 1.** The nematode; *Meloidogyne incognita* trapped by nematode trapping fungi *Arthrobotrys oligospora*

infested area were screened for their nematicidal potential against nematode; *M. incognita.*

Fungal lawns were developed on Potato Dextrose Agar medium (PDA) for each of the test fungi. PDA block of one cm diameter containing mycelial growth was transferred to nematode inoculated Water Agar medium. The interaction of the fungi with nematodes was studied microscopically (Photograph 1). Similar screening was performed against nematode eggs.

Isolated strains of *Trichoderma harzianum*, *Paecilomyces lilacinus* and *Arthrobotrys oligospora* were selected for the green house pot experiment on the basis of screening test. Besides potential biocontrol agents, Carbofuran as chemical treatment and Neem (*Azadiracta indica*) compound as natural organic compound treatment were also included in the experiment. Healthy seedlings of *W. somnifera* were planted in pots containing autoclaved soil. The three test fungi grown on Maize Sand medium were separately mixed with the soil of experimental pots, at concentrations of $10^9$ cfu/gm of medium. Carbofuran and Neem compound were also include-ed in the experiment, at 5gm/pot, in separate treatments. After two days, nematodes (approx. 1000/pot) were inoculated in each of the experimental pots.

Four sets of controls were maintained in comparison with the experimental sets. Seedling planted pots with the following combinations were taken as controls:-

Maize Sand medium + Nematode Inoculated
Maize Sand medium + Nematode Uninoculated
No medium + Nematode Inoculated No
medium + Nematode Uninoculated

Control (i) was employed for statistical comparison with other treatments. Pots were watered as required and observations were taken after one month. The observations were subjected to statistical analysis. The experiment was done in triplicates. Statistical analysis was done by employing Completely Randomized Experimental Design. ANOVA analysis was performed with critical difference at 5% level of significance.

## RESULTS AND DISCUSSION

The efficiency of the potential biocontrol agents in the management of root knot nematode was assessed from the reduction in root galling expressed in terms of Root Knot Index (RKI). The efficiency of *T. harzianum* was found to be comparable to that of carbofuran (RKI=2), followed by *P. lilacinus*, *A. oligospora* and neem compound. Besides reducing nematode infestation, the biocontrol agents also enhanced the growth of the plant (Table 1). It is evident from the table that most of the treatments showed significant results (at p= 0.05) for shoot /root fresh and dry weights, as well as, shoot and root length of the plant; *W. somnifera*. *P. lilacinus* treatment resulted in highest fresh weight measurement for both plant shoot and root followed by *T. harzianum* treatment. *P. lilacinus* and *T. harzianum* were similarly found to show good results for dry weight and length of shoot and root. *A. oligospora* showed significant results except for root length. Neem compound treatment also resulted in improved plant growth. Carbofuran did not show significant results for shoot dry weight and shoot length but observations for shoot and root fresh weight, root dry weight and root length were comparable to that of neem compound.

**Table 1.** Measurement of plant growth parameters and Root Knot Index (RKI) in *Withania somnifera* after application of treatments against nematode; *Meloidogyne incognita*

| | Treatments | Fresh Weight (gm) | | Dry Weight (gm) | | Length (cm) | | RKI |
|---|---|---|---|---|---|---|---|---|
| | | Shoot | Root | Shoot | Root | Shoot | Root | |
| 1. | Trichoderma harzianum | 38.66 | 8.50 | 10.60 | 2.40 | 30.50 | 18.07 | 2.0 |
| 2. | Paecilomyces lilacinus | 46.00 | 10.60 | 10.63 | 3.17 | 36.33 | 17.37 | 2.33 |
| 3. | Arthrobotrys oligospora | 34.73 | 6.57 | 8.70 | 1.93 | 32.67 | 9.47* | 2.66 |
| 4. | Neem compound | 33.66 | 7.47 | 8.00 | 1.87 | 26.87 | 12.33 | 2.66 |
| 5. | Carbofuran | 26.00 | 8.40 | 3.97* | 2.2 | 17.33* | 15.23 | 2.0 |
| | **Controls** | | | | | | | |
| i | Medium + NI | 12.33 | 3.17 | 2.90 | 0.77 | 12.30 | 6.47 | 4.0 |
| ii | Medium + NU | 23.66 | 6.77 | 5.13 | 1.83 | 26.37 | 13.27 | -- |
| iii | No medium + NI | 10.66 | 2.77 | 1.67 | 0.57 | 12.10 | 7.60 | 3.66 |
| iv | No medium + NU | 18.50 | 4.93 | 4.90 | 1.33 | 14.83 | 10.53 | -- |
| | C.D. (p=0.05) | 9.14 | 1.87 | 1.65 | 0.80 | 9.10 | 3.51 | 0.66 |

*non-significant, C.D.= Critical Difference, NI=Nematode Inoculated, NU=Nematode Uninoculated observations subjected to ANOVA statistical analysis.

Biocontrol agents improve the health of plants and thus contribute to overall productivity. These agents are also self propagating under favourable conditions, and therefore, may remain in the soil for a long period.

Although chemical agents like carbofuran are efficient in controlling nematodes (Adegbite and Agbaje, 2007), their persistence may pose ecological problems (Li et al., 2008). Therefore, biocontrol is suggested to be a safer solution. Various fungal antagonists of nematodes have shown promising results. These mainly include endoparasitic fungi, parasites of nematode egg and nematode trapping fungi.

The fungi, *P. lilacinus* , is an egg parasitic fungi which infects by direct hyphal penetration. The hyphae branch and grow across the egg shell (Khan et al., 2006). It has been suggested that its parasitism is associated with the enzyme serine protease which is nematicidal in activity. It acts by degrading egg shell and prevents hatching (Zareen et al., 2001). *P. lilacinus* is one of the potential biocontrol agents which can also colonize organic matter in soil and develop in the rhizosphere of plants. Another fungi, *T. harzianum* parasitizes eggs and larvae of *M. incognita*. The hyphae penetrate the eggs and larval cuticle by dissolving the chitin layer through enzymatic activity. They proliferate within the organism and produce toxic metabolites (Dos Santos et al., 1992). Thus, the enzymes produced by *Trichoderma* spp. such as chitinases, glucanases and proteases seem to play an important role in parasitism (Haran et al., 1996). *Trichoderma* has not only been proved to parasitize nematodes and inactivate pathogen enzymes but also help in tolerance to stress conditions by enhanced root development. It participates in solubilization of inorganic nutrients. Thus, *Trichoderma* colonized roots require lesser supply of manmade nitrogen fertilizers (Harman, 2000). Another important group of antagonists of nematodes is the nematode trapping fungi. This group can trap nematodes nonspecifically. These fungi release chemo-attractants which bring nematodes close to the fungal mycelia where they are immobilized in special trapping organs such as sticky pads or constricting/ non constricting rings. One of such fungi is *A. oligospora* , in which, researchers have identified two pathogenicity factors- a carbohydrate binding protein(lectin) and an extracellular serine protease (Ahman et al., 2002). Proteases have been found to be involved in immobilization of nematodes captured by *A. oligospora.* The adhesion of fungal structures, penetration and immobilization of nematodes take not more than one hour as suggested by Tunlid and Jansson (1991).

Although the biocontrol agents seem to work well under laboratory conditions , their effect may decrease under field conditions due to dilution by water or interaction with the biotic and abiotic components of the surrounding environment.

Besides the natural antagonists of nematodes, naturally occurring organic compounds such as neem compound may also be effectively used (Pandey and kalra, 2003). The active principle of neem such as nimbidin and thionimone were reported to be highly active against nematodes (Fatema and Ahmad, 2005). Other active principles such as Azadirachtin, Salannin and Meliantriol are also found to be effective. These compounds act by various mechanisms like blocking molting of larvae, disrupting mating and sexual communication of nematodes, reducing the motility of gut and by inhibiting the formation of chitin (Ramasamy, 2008).


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