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# Effect of Different Fungal Filterates on Egg Masses of *Meloidogyne Incognita* under Laboratory Condition

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**Abstract:**

*Six antagonistic fungi were isolated from naturally infested soil with nematodes, purified, identified and grown on PDA. The grown isolates were sterile-filtered to remove fungal biomass and the filtrates were placed into well plates to test their effects on the hatching of Meloidogyne incognita egg-masses. M. incognita egg-masses hatch ranged from 85.0% when using Arthrobotrys superba to 98.3 when using Trichoderma harzianum compared with untreated control.*

**Key words**: Antagonistic fungi: *M. incognita,* fungal filterates

## Introduction

Plant parasitic nematodes account worldwide for an average estimated by 10- 20% yearly loss of agricultural products, including food and industrial crops (Lamberti, 1981). Their control was achieved in the past through crop rotation. The world increasing demand for food and the structural changes of

rural societies and production techniques, requiring continuous cropping systems over thousands of feddans, provided the best conditions for nematode uncontrolled multiplication and spread. Plant parasitic nematodes can be moved by water, wind, animals and phytoparasitic species, including vectors of plant viruses as were even found in irrigation waters and canals (Roccuzzo and Ciancio, 1991). Further studies revealed that a wide diversity of microbial antagonists to nematodes exist, including, actinomycetes, bacteria, acquatic fungi and endoparasitic Hyphomycetes (Kerry, 1990).

The present experiment was conducted to screen some antagonistic soil microorganisms from the agro-ecosystem and evaluate their influences as bio-control agents in controlling *M.incognita* to reduce the use of hazardous nematicides.

## Materials and Methods

### Nemotode stock culture:

*Meloidogyne incognita* (Kafoid and White) stock culture was initiated from well identified single egg-masses which were collected from galled tomato roots. The fresh egg- masses were then propagated on tomato seedlings (cv. Heem Sana) cultivated in sterilized soil in pots. The pure culture of *M. incognita* was then maintained in greenhouse.

Six antagonistic fungi were isolated in PDA from naturally infested soil with nematodes, purified and identified by Faculty of Plant Pathology, SHIATS, Allahabad, Deemed to be University. The identified microorganisms were as follow: *Arthrobotrys oligospora, A. superba, Dactylella brochopaga, Paecilomyces lilacinus, Trichoderma harzianum and T. viridi.*

### Fungal culture filtrates:

The six antagonistic fungi were grown on Czapek's Dox Broth in 150 ml conical flasks. The flasks were incubated at 25 C° under complete darkness conditions (Abd-El-Moity and Shatla,

1981). After 11 days, each culture was blended for 3 minutes and the mixture was filtrated by filter paper (Wattman-1), the filtrate was centrifuged for 15 minutes at 3000 RPM to separate the fungal spores. Filtrates were then sterilized by using test tube.

### Effect of fungal filterates on *M. incognita* egg-masses in the laboratory

Five ml of three concentrations (100%, 50% and 25%) of each filterate were used to five hand pecked egg- masses of *M. incognita* in petri-dishes (5-cm-dim.). The control treatment was 5 ml. distilled water containing the same egg- masses number. Each treatment was replicated three times. The nematode egg hatching inhibition (%) was recorded after 3 days of incubation at room temperature.

## Results

### Effect of different fungal filterates on *Meloidogyne incognita* egg- masses under *in vitro* conditions:-

Percentage hatching inhibitions of M. incognita treated with six fungal filtrates are presented in Table (1). It is apparent that the inhibition of nematode hatching was affected by concentrations of fungal filtrate. In general, the percent inhibition increased as the fungal filtrate concentration increased. After 3 days, percent inhibition of the high concentration was 88.3, 85.7, 90.7, 92.7, 98.3 and 97.7%, occurred by *A. oligospora, A. superba, Dactylella brochopaga, Paecilomyces lilacinus, T. harzianum and T. viridi* filterates respectively.

**Table (1): Percentage of hatching inhibition of *Meloidogyne incognita* egg-masses treated with thirteen fungal filtrates after 3 days.**

| Concentration / Treatments (fungal filtrate) | 100 % | 50 % | 25 % |
|---|---|---|---|
| | Egg hatching inhibition (%) | | |
| *Arthrobotrys oligospora* | 88.3 | 73.3 | 45.7 |
| *Arthrobotrys superba* | 85.7 | 69.3 | 42.7 |
| *Dactyleria brochopaga* | 93.0 | 76.7 | 54.3 |
| *Paecilomyces lilacinus* | 90.3 | 74.3 | 53.7 |
| *Trichoderma harzianum* | 98.0 | 86.3 | 60.3 |
| *Trichoderma viridi* | 90.7 | 68.3 | 42.3 |
| *control* | 0 | 0 | 0 |

L.S.D = 5% 1%
2.96 3.96

$$\% \text{ inhibition} = 100 - \frac{\text{Hatching in control} - \text{hatching in treatment}}{\text{Hatching in control}}$$

The lowest concentration had the lowest effect on nemaode egg hatching inhibiting. The most effective fungus was *T. Harzianum* followed by *Dactylella brochopaga, Paecilomyces lilacinus, Trichoderma viridi, Arthrobotrys oligospora* and *A. superba.*

## Discussion

A wide range of activities was recorded from the filtrates of the tested fungal isolates against egg-masses. They may have nematode species- specific effect for some filtrates on nematodes as several isolates produced filtrates that strongly inhibited or stimulated egg hatching (Gourd *et al*., 1994; Mayer *et al*., 1999). Some scientists have reported secretion of nematicidal metabolites in culture of *Arthrobotrys* spp. also daily observation showed that number of killed nematode was increased from 4th day and seen up to 8th day (Eefje, 1994; Sandeepa and Kanitkar, 2003). Regarding to *Trichoderma* and

*Dactyleria* species, Ghisalberti, (2002) reported that they are ubiquitous in the environment, especially in soil, most of their species are non- phytopathogenic, and, have the ability to express high cellulase activity and has been commercially exploited. Chitinolytic enzymes produced by these fungi are thought to be responsible for the degradation of cell walls, they also had ability to produce a wide range of secondary metabolites with diverse biological actions including gliotoxin and gliovirin and this is the end step after the penetration of germ tubes into the nematode body and subsequent death and degradation of the nematodes (Zhang *et al.*, 2008). Olubunmi and Rajani, (2004) found that the tests of *Trichoderma* against *Meloidogyne* spp. prevented nematode egg hatching and also resulted in 100% mortality of nematode juveniles *in vitro*. *Trichoderma harzianum* was able to penetrate nematode egg-mass matrix and significantly decreased nematode egg hatching level because direct parasitism of eggs through the increase in extracellular chitinase activity, which would be indicator of eggs infection capability (Sahebani and Hadavi, 2008).

In conclusion, 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. However, the results of using the above mentioned bio-control agents may be considered in the integrated control of *M. incognita* and may be other plant parasitic nematodes.

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