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# Tropical soil microflora of spice-based cropping systems as potential antagonists of root-knot nematodes

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**Abstract**

Suppression of plant parasitic nematodes with nematode predators, parasites or antagonists is an eco-friendly approach than the toxic chemicals. In a study, soil borne fungi from the rhizosphere of major spice crops were collected from diverse cropping systems prevailing in three southern states of India. A series of in vitro studies were conducted using 73 freshly collected fungal isolates and 76 isolates obtained from other sources. Out of this 67 isolates were not parasitic on females of root-knot nematodes whereas 115 isolates, though colonized the egg masses, did not show any signs of parasitism on nematode eggs. Fifty-nine isolates showed 50–90% inhibition in egg hatch. *Pochonia chlamydospora*, *Verticillium lecanii*, *Paecilomyces lilacinus*, and few isolates of *Trichoderma* spp. showed >25% parasitism on root-knot nematode eggs. The most promising isolates in this study were one isolate each of *Aspergillus* (F.45), *Fusarium* (F.47), and *Penicillium* (F.59); three each isolates of *Trichoderma* (F.3, F.52, and F.60) and *Pochonia* (F.30 and Vc.3) *Verticillium* (Vl); and two isolates of fungi that could not be identified (F.28 and F.62). Parasitism by *Aspergillus tamarii*, *Aspergillus ustus*, *Drechslera* sp., *Humicola* sp., and *Scopulariopsis* sp. on root-knot nematode eggs or females, reported in the present study, are new reports.


*Keywords:* Biological control; Bioassay; Hatching suppression; Microflora; *Meloidogyne incognita*; Parasitism; Plant parasitic nematodes

## 1. Introduction

Plant parasitic nematodes are important pathogens on most food, horticultural and fiber crops and without appropriate control will cause loss of yield and quality. Approximate yield losses due to plant parasitic nematodes have been estimated to be $ 100 billion worldwide each year (Sasser and Freckman, 1987). Among the various pests and diseases of spices, the damage caused by plant parasitic nematodes is quite serious. Nematode management is difficult and complicated and at present chemical control is followed in many crops to maintain their populations below economic threshold levels. Suppression of plant parasitic nematodes with nematode predators, parasites or disease agents is a desirable alternative to chemicals. The tropical soils are rich in biodiversity of beneficial microbes and the biocontrol potential of the resident microbial fauna and flora is under exploited.

Though hundreds of organisms, which parasitize or prey on nematodes, are reported fungal antagonists are predominantly used for the biological suppression of nematodes. Several reviews have been published exclusively on the fungal antagonists of nematodes (Barron, 1977; Gray, 1987, 1988; Jansson and Nordbring-Hertz, 1988; Kerry, 1984; Morgan-Jones and Rodriguez-Kabana, 1988). In general biological control of nematodes is yet to gain momentum in India and the isolated efforts in this direction still need consolidation. In view of this a study has been undertaken to collect and screen the soil-borne fungi from plantations and gardens of different ecosystems in South India for their bioefficacy in suppressing root-knot nematodes.

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## 2. Materials and methods

### 2.1. Sampling

Soil and root samples were collected from the rhizosphere of major spice crops such as black pepper (*Piper nigrum* L.), cardamom (*Elettaria cardamomum* Maton), and ginger (*Zingiber officinale* Rosc.) from Kerala (108), Karnataka (44), and Tamil Nadu (16) (Table 1). Fields from a wide range of agro-ecological conditions were chosen to obtain a wide range of nematode antagonists that are adapted to diverse climatic conditions. The top 3–5 cm of soil and litter layer were removed and about 250 $cm^3$ of soil and 10 g of feeder roots were collected up to a depth of 30 cm. The samples were collected in polythene bags, sealed, and taken to the laboratory for isolation of various antagonists.

### 2.2. Isolation and culturing

Fungi from the rhizosphere soil were isolated by the standard dilution plate method (Waksman, 1922). Fungi were isolated directly from root-knot nematode egg masses or individual eggs or females. For this, roots were washed in tap water and the egg masses were collected after staining with Phloxin B (Holbrook et al., 1983), rinsed in tap water followed by sterile distilled water (SDW). They were then transferred to 2% water agar (WA) plates. After 2 days, egg masses showing fungal growth were aseptically transferred to potato-dextrose agar (PDA) containing streptomycin sulphate (50 $\mu g\ L^{-1}$).

For isolating fungi that parasitize root-knot nematode females, root galls were selected from fresh roots, rinsed in tap water and SDW. Intact females with egg masses were extracted by carefully tearing the galls using sterile forceps and blades. The females were washed in SDW and were placed on a sterile cover slip (24 $mm^2$) kept in a petri plate (9 cm diameter) containing 2% WA. The females were crushed on the cover slips and incubated at 28 °C for 4 days (Crump, 1987). The fungi growing out were transferred to PDA. Similarly a suspension of nematode eggs was prepared by dispersing the egg masses of root-knot nematodes in SDW (Hussey and Barker, 1973). One milliliter of the suspension containing about 100 eggs was poured into a petri plate containing 2% WA and was incubated at 28 °C for 4 days. The fungi growing from the eggs were aseptically transferred to new WA plates and again incubated for 4 days (Godoy et al., 1983).

Single fungal colonies isolated from nematode eggs, females or soil were transferred and cultured on PDA medium in 9 cm diameter petri dishes for 10 days at 28 °C. The purified fungal isolates were then maintained on PDA slants for further studies.

Table 1
Sampling sites and number of samples collected from major spice crops in three states of South India

| Area of sampling | No. of samples collected | | | |
|---|---|---|---|---|
| | Black pepper | Cardamom | Ginger | Total |
| *Kerala* | | | | |
| Idukki | 8 | — | 8 | 16 |
| Kannur | 3 | — | 7 | 10 |
| Kasaragod | — | — | 6 | 6 |
| Kozhikode | 21 | — | 21 | 42 |
| Wyanad | 9 | — | 25 | 34 |
| *Karnataka* | | | | |
| Dakshina Kannada | 5 | — | — | 5 |
| Kodagu | 20 | 9 | — | 29 |
| Uttara Kannada | 4 | 6 | — | 10 |
| *Tamil Nadu* | | | | |
| Nilgiris | 6 | — | — | 6 |
| Valparai | 10 | — | — | 10 |
| Total | 86 | 15 | 67 | 168 |

### 2.3. In vitro bioassays

In vitro bioassays are rapid, space-efficient, and repeatable and hence were adopted in the current investigation as a preliminary step to identify the potential biocontrol agents (Merriman and Russell, 1990). As bioassays using free-living nematodes like *Caenorhabditis elegans* that are very sensitive to test organisms can be misleading (Anke et al., 1995), eggs and J2 of root-knot nematodes were chosen as test organisms. Culture medium also is reported to influence the percentage of eggs parasitized (Kim et al., 1998; Nitao et al., 1999) and therefore initial laboratory screening was done on simple water-agar plates. The test fungi were evaluated for their ability to parasitize various life stages of the nematode viz. eggs, juveniles, females, and egg masses. Simultaneously, to detect the involvement of any toxic metabolites on nematode inhibition, their effect on egg hatching was also studied.

#### 2.3.1. Nematode parasitization

A 4 mm diameter plug of each fungal isolate, grown on PDA, was transferred colony-side-up to the centre of a 80 × 15 mm petri plate containing 2% WA and was incubated at 28 °C until the whole agar surface was covered with the fungal mycelium. A suspension (0.5 ml) containing c.100 eggs or c.100 juveniles (J2) was then spread over each fungal covered WA surface. For studying their parasitization on female nematodes 5 each surface-sterilized females were placed on such WA plates that had been inoculated with the respective fungal isolate. Control plates had received the same quantity of eggs, juveniles or females, but a non-colonized PDA plug. The plates were arranged in a randomized design with three replications and were incubated in dark for 2 weeks at 28 °C. After the incubation period, a

few eggs or juveniles or females were retrieved randomly and stained with a drop of 0.05% cotton blue in lactophenol and were examined under a research microscope at 100× magnification for recording the parasitization by the fungus.

#### 2.3.2. Effect on hatching

Three freshly extracted root-knot nematode egg masses were surface sterilized and placed on sterile WA plates that had been inoculated with the test fungus, as described above. Control plates had no fungus. After 14 days, the egg masses were crushed in a droplet of 0.01% NaOCl solution to dissolve the gelatinous matrix. The number of eggs and juveniles present in each egg mass was counted at 100× magnification. These eggs and juveniles were suspended in 1 ml of SDW and the suspension was poured on a facial tissue paper nested on a 2 cm diameter sieve. This sieve was placed in a water-filled petri dish (40 × 15 mm) and the juveniles were allowed to hatch and migrate into the water. They were incubated at 28 °C for 48 h and the number of juveniles hatched was determined using the formula

$$\% \text{ hatch} = (J_f \times 100)/(E_i + J_i),$$

where $J_i$ is the number of J2 observed initially, $E_i$ is the number of intact eggs observed initially, and $J_f$ is the number of J2 hatched.

### 2.4. Test organisms

The fungi used in this study included 73 freshly collected isolates and 76 isolates obtained from other sources. All these isolates were obtained from nematode eggs/egg masses, females or nematode-suppressive soils and consisted of four isolates of *Pochonia* sp., 8 isolates of *Paecilomyces* spp., 88 isolates of *Trichoderma* spp., and 48 isolates of various other fungi. An isolate of root-knot nematode (*Meloidogyne incognita*), originally collected from black pepper, was used in all bioassay studies.

## 3. Results

### 3.1. Sampling, isolation, and identification of fungi

Samples were taken from the rhizosphere of black pepper (41 nos.) and ginger (67 nos.) in Kerala, whereas from Karnataka they were collected from black pepper (29 nos.) and cardamom (15 nos.) rhizosphere (Table 1). Sampling was confined to black pepper rhizosphere (16 nos.) only in Tamil Nadu. Diverse cropping systems were chosen to obtain a wide range of nematode antagonists that are adapted to various climatic conditions.

The rhizosphere samples were collected from various locations yielded a large number of fungi. However, those isolates with distinct features and characteristics in the colony morphology were only selected and maintained for further studies. Out of the 73 fungi obtained (Table 2), 61 isolates were saprophytes, some of which were previously reported as facultative parasites of nematodes. Majority of them (17 isolates) belonged to the genus *Trichoderma*, whereas 7 isolates were of *Aspergillus* spp., 6 each of *Paecilomyces* spp. and *Pencillium* spp., 5 isolates of *Fusarium* spp., and 2 isolates of *Pochonia* spp. Taxonomic identity of 22 isolates could not be established as there was no sporulation.

Table 2
Soil-borne fungi isolated from the samples collected from the rhizosphere of three spice crops cultivated in various parts of South India

| Antagonist | Black pepper | Cardamom | Ginger | Total |
|---|---|---|---|---|
| *Aspergillus* sp. | 1 | — | — | 1 |
| *A. fumigatus* | 1 | — | 1 | 2 |
| *A. nidulans* | — | — | 1 | 1 |
| *A. restrictus* | — | — | 1 | 1 |
| *A. tamarii* | — | — | 1 | 1 |
| *A.ustus* | — | — | 1 | 1 |
| *Aurobasidium* sp. | 1 | — | — | 1 |
| *Cephalosporium* sp. | 1 | — | — | 1 |
| *Drechslera* sp. | 1 | — | 1 | 2 |
| *Fusarium* sp. | — | — | 4 | 4 |
| *F. oxysporum* | — | — | 1 | 1 |
| *Humicola* sp. | — | — | 2 | 2 |
| *Paecilomyces* sp. | 1 | — | 1 | 2 |
| *P. carneus* | — | — | 1 | 1 |
| *P. lilacinus* | — | 1 | 2 | 3 |
| *Penicillium* sp. | 1 | — | 2 | 3 |
| *P. citrinum* | — | — | 1 | 1 |
| *P. fumiculosm* | — | — | 1 | 1 |
| *P. digitatum* | — | — | 1 | 1 |
| *P. chlamydospora* | 1 | — | — | 1 |
| *Scopulariopsis* sp. | — | — | 1 | 1 |
| *Scolecobasidium* sp. | — | — | 1 | 1 |
| *Trichoderma* sp. | 7 | — | 3 | 10 |
| *T. harzianum* | — | 3 | 1 | 4 |
| *T. virens* | — | 1 | — | 1 |
| *T. viride* | — | 1 | 1 | 2 |
| *V. lecanii* | — | 1 | — | 1 |
| Unidentified | 17 | — | 5 | 22 |
| Total | 32 | 7 | 34 | 73 |

### 3.2. Nematode antagonism

The pathogenicity of fungi to eggs of root-knot nematode, *M. incognita* on water agar varied among species and isolates of various fungi (Figs. 1 and 2). Sixty-seven out of the 110 isolates screened were not parasitic on females of root-knot nematodes. Significant parasitism on adult females was observed only in three isolates viz. *Paecilomyces lilacinus* (Pl. 1), *Trichoderma harzianum* (C.22), and *Verticillium lecanii* (Vl).

Out of the 149 fungal isolates screened, 115 isolates colonized the gelatinous matrix of root-knot nematode

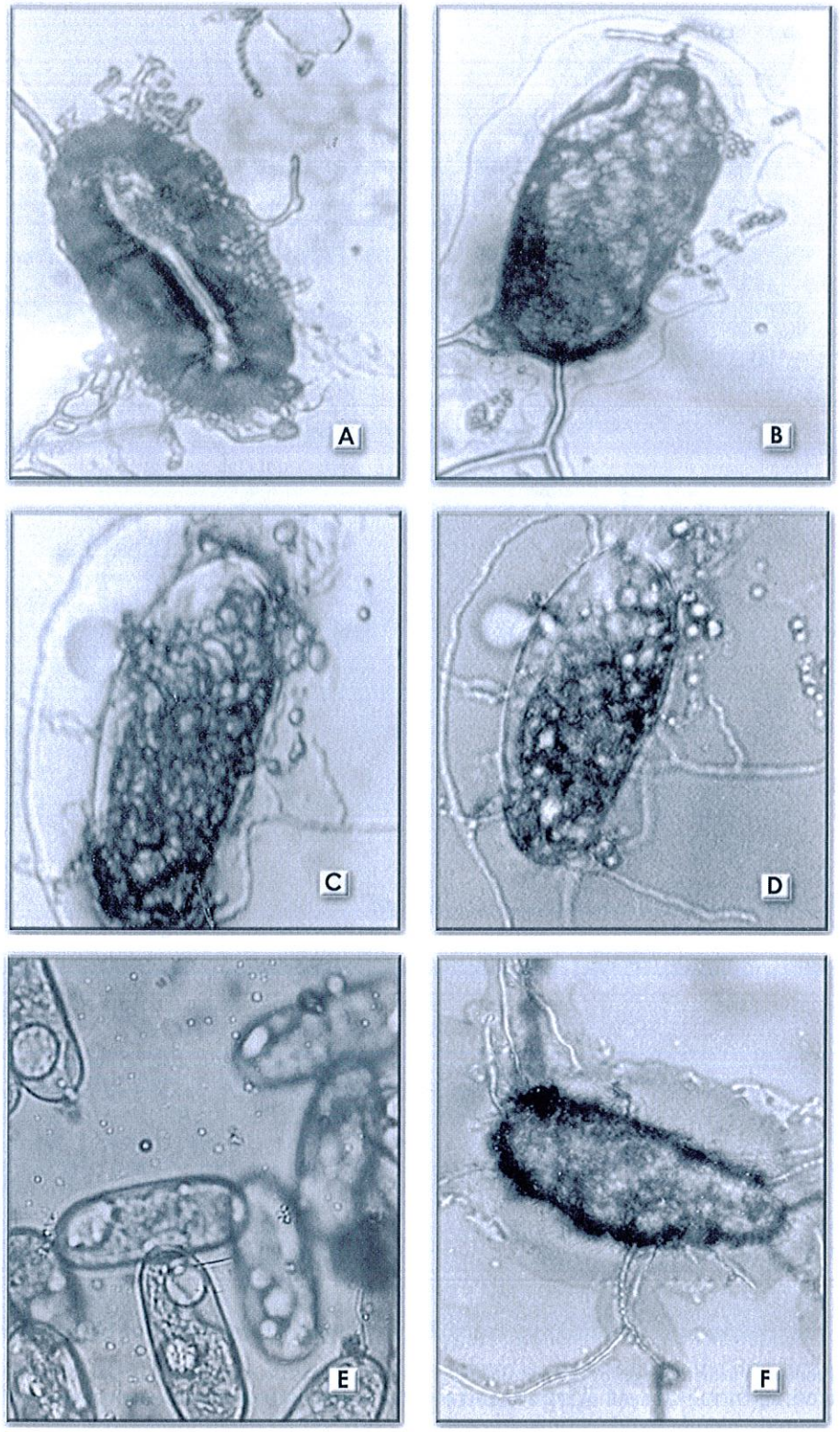


Fig. 1. Parasitization of root-knot nematode eggs by egg parasitic fungi: (A) *Trichoderma virens*, Gv. 21; (B) *Trichoderma* sp., Pat. 5; (C) *Trichoderma virens*, Gv. 13; (D) *Trichoderma virens*, Gv. 13; (E) *Penicillium digitatum*, Is. 23; (F) *Trichoderma harzianum*, C.22.

egg masses (77.18%). Majority of them (73.8%), though colonized the egg masses, did not parasitize eggs. Only four isolates showed >25% egg parasitism and they were two each isolates of *Pochonia chlamydospora* (F.30 and Vc.3) and *P. lilacinus* (Pl.1 and Pl.2) (Fig. 2). Another 33 isolates exhibited 10–25% egg parasitism. These in-

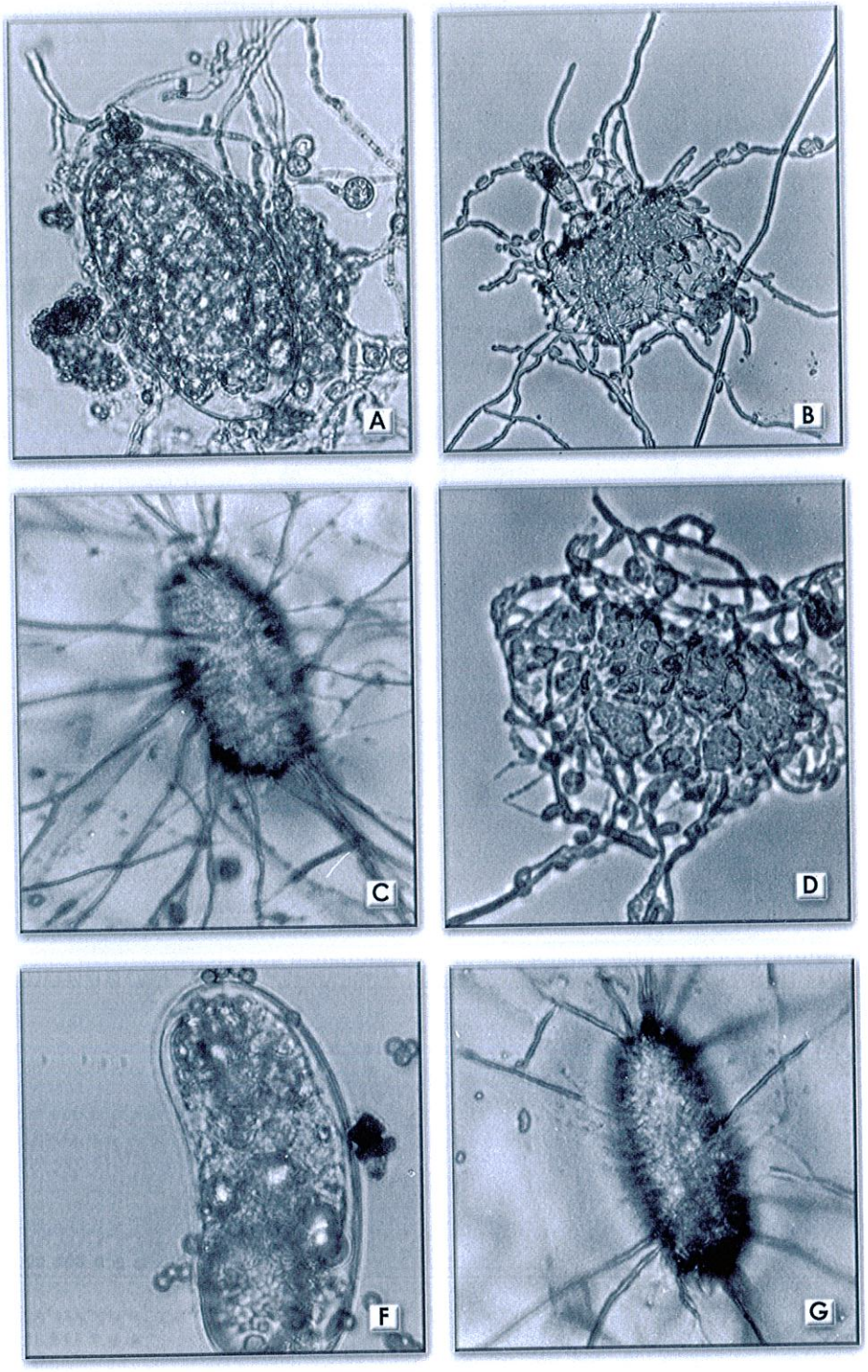


Fig. 2. Parasitization of root-knot nematode eggs by egg parasitic fungi (cont'd): (A) *Fusarium oxysporum*, Is. 11; (B) *Scolecobasidium* sp., Is. 15; (C) *Pochonia chlamydospora*, Vc. 3; (D) *Scopulariopsis* sp., Is. 14; (E) *Aspergillus tamarii*, Is. 2; (F) *Paecilomyces lilacinus*, Pl. 2.

cluded fungi belonging to the genera *Aspergillus* (F.6, F.34, F.37, F.40, F.45, and F.46), *Fusarium* (F.41 and F.47), *Trichoderma* (F.3, F.5, F.52, F.60, F.71, Gv.21, Th.12, Th.22a, Th.29/11, Th.32, Th.44, Th.45, Thm.7b and Thm.22b), *Drechslera* (F.32), *Humicola* (F.43), *Scopulariopsis* (F.50), *Verticillium* (Vl), and four unidentified strains of fungi (F.2, F.15, F.21, and F.62). Most of these isolates were parasitic on root-knot nematode females too. But none of the 149 isolates of fungi was parasitic on the juveniles.

In the bioassay for suppression of hatching, except two isolates (F.48 and F.57), all the fungi screened

Table 3
Comparison of different nematode suppression mechanisms of promising fungal antagonists displayed in the in vitro bioassays

| Fungal antagonist | Isolate No. | Parasitism on | | Hatching suppression[b] (%) |
|---|---|---|---|---|
| | | Eggs[a] (%) | Females[a] (%) | |
| *A. restrictus* | F.45 | 20.82 | 0.00 | 91.8 |
| *F. oxysporum* | F.47 | 21.22 | 0.00 | 95.71 |
| *P. lilacinus* | Pl.1 | 42.32 | 28.12 | 78.12 |
| | Pl.2 | 36.12 | 24.22 | 72.06 |
| *P. digitatum* | F.59 | 0.00 | 0.00 | 100.00 |
| *P. chlamydospora* | F.30 | 35.36 | 18.36 | 95.45 |
| | Vc.3 | 26.42 | 19.48 | 91.24 |
| *Trichoderma* sp. | F.52 | 14.66 | 10.21 | 97.2 |
| | F.60 | 16.38 | 0.00 | 93.56 |
| *T. harzianum* | C.22 | 24.26 | 32.82 | 88.89 |
| *T. viride* | F.3 | 10.26 | 15.38 | 91.03 |
| *V. lecanii* | Vl | 24.36 | 26.48 | 91.89 |
| Unidentified | F.28 | 0.00 | 12.36 | 90.64 |
| | F.62 | 19.36 | 12.48 | 95.45 |

Values are mean of three replications. Percentage data transformed to arc sine for analysis and converted to original means. Table compiles results of different screening experiments carried out separately. Hence, no direct comparisons among isolates were attempted. However, results allow classification of each isolate based on its ability to suppress nematodes.

[a] Proportion of eggs/females parasitized by the candidate fungus.

[b] Hatching suppression = $(1 - T/C) \times 100$, where $T$ is the mean of J2 hatching in the treatment and $C$ is the mean of J2 hatching in the control.

showed various degrees of adverse effect on the egg hatch process. But only 11 isolates had shown >90% suppression in hatching. Fifty-nine isolates showed 50–90% inhibition in egg hatch. The most promising isolates in this study were one isolate each from *Aspergillus* (F.45), *Fusarium* (F.47), and *Penicillium* (F.59); three isolates of *Trichoderma* (F.3, F.52 and F.60), two isolates of *Pochonia* (F.30 and Vc.3), and an isolate of *Verticillium* (Vl); and two isolates of fungi that were not identified (F.28 and F.62). Considerable variability was observed among isolates with regard to inhibition of egg hatch (0–100%), parasitism on eggs or females (0–42.32 and 0–32.82%, respectively).

The results clearly indicated that the promising fungal antagonists have different modes of action (Table 3). A few of them like *P. lilacinus*, *Trichoderma* spp., and *Pochonia* spp., possessed multiple modes of action. *P. chlamydospora*, *V. lecanii*, *P. lilacinus*, and few isolates of *Trichoderma* spp. invaded the root-knot nematode eggs (Figs. 1 and 2). Infected eggs were defined as those which were full of fungal hyphae and the embryo or juvenile had been destroyed. Immature eggs in early embryonic development stage were more susceptible to these fungi. Those eggs containing ready to hatch second stage juveniles were seldom parasitized. Hyphae of fungi like *Trichoderma virens* (Figs. 1C and D), *F. oxysporum* (Fig. 2A), *Scolecobasidium* sp. (Fig. 2B), *P. chlamydospora* (Fig. 2C), *Scopulariopsis* sp. (Fig. 2D), and *P. lilacinus* (Fig. 2F) ramified over the egg surface and sometimes formed an extensive network. In some cases grooves were observed on the egg surface (Fig. 1A). Eggs with networks of hyphae had a wrinkled and shrunken appearance (Figs. 1F, 2B, and D). In many cases conidiating hyphae were also seen outside the egg surface (Figs. 1C and D, 2A and D). Infected eggs were pale, yellowish-brown and in most cases, no trace of juveniles could be detected. They were stained easily due to the increased permeability of the eggshell. The embryonic development was arrested by the fungal invasion. Fungal mycelium radiated profusely from eggs in the advanced stage of infection. In some cases high vacuolation was also observed within the infected eggs (Fig. 1E).

## 4. Discussion

Majority of the fungi collected in the random survey belonged to the group of opportunistic fungi, which are predominantly saprophytes. Earlier studies in spice agro ecosystems showed the prevalence of *Trichoderma*, *Aspergillus*, *Pencillium*, *Paecilomyces*, and *Fusarium* in the rhizosphere soil of spices like black pepper and cardamom (Sankaran, 1981). This was mainly due to the fact that the isolation was done mainly from egg masses and rhizosphere soil and not from single eggs. As nematodes are more abundant in rhizosphere than in the bulk soil, their obligate parasites will also probably be more numerous in the rhizosphere. That is why rhizosphere is considered as the first line of defense for roots against attack by soil-borne pathogens (Weller, 1988). Recent studies have shown that microbial population was more in egg masses of root-knot nematodes than in the rhizosphere (Kok et al., 2001). Fungi like *A. niger*, *F. oxysporum*, and *P. lilacinus* were frequently isolated from egg masses of root-knot nematodes by other workers too (Goswami et al, 1998). Some fungi, such as *P. chlamydospora*, are largely confined to the rhizosphere.

Though 73.8% of the fungi screened colonized egg masses, only very few isolates showed considerable degree of egg parasitism. Isolates of many of these fungi differed significantly in their ability to parasitize the eggs of different nematode species as reported by earlier workers (Kerry, 1981; Khan and Goswami, 2000; Sosnowska et al., 2001; Stirling and Mankau, 1979). Generally, eggs in early developmental stages have been found to be more susceptible to fungal infection (Irving and Kerry, 1986; Lopez-Llorca and Duncan, 1991). No adverse effects have been observed in eggs containing juveniles, i.e., in advanced stage of embryonic development. Since eggs of mixed developmental stages were used in this study this fact could not be confirmed. Holland et al. (1999) proved that eggs of all stages, including those containing unhatched juveniles, were infected by an isolate of *P. lilacinus*. Mere presence of fungi in females or egg masses may not necessarily mean that the fungus is parasitic as shown in females and eggs of *M. incognita* on black pepper in which the presence of *Phytophthora palmivora* and *Nectria haemococca* was observed (Freire, 1982). Grooves and shrinkage observed on some egg surfaces have probably been formed by enzyme action. Enzymes could thus break down the egg shell by attacking the protein layer itself, proteins that cross link the chitin layer, the egg shell lipids or the chitin, to enable a narrow infection peg to push through.

Fungi like *Trichoderma*, *Fusarium*, and *Aspergillus* are not regular candidates for biological control of nematodes. However, many of these saprophytic fungi are reported as occasional parasites of nematodes in literature indicating that they have an important role to play in the natural suppression of root-knot nematodes affecting spice crops. In literature there are only very few reports on the nematicidal activity of fungi like *Drechslera* sp. (Charles et al., 2000), *Fusarium* spp. (Pocasangre et al., 2000; Zareen et al., 2001), *Aspergillus* spp. (Ayoub et al., 2000; Goswami et al., 2001; Siddiqui et al., 2001), and *Trichoderma* spp. (Saifullah and Thomas, 1996; Santos et al., 1992; Sharon et al., 2001; Spiegel and Chet, 1998; Windham et al., 1989). Potential isolates of fungi belonging to all the above groups were obtained in this study too. *Humicola* sp., *Scopulariopsis* sp., and *Scolecobasidium* sp. have been reported as parasites of cyst nematodes (Kuczynska, 1997; Sosnowska and Banaszak, 1998). Parasitism by *Aspergillus tamarii*, *A. ustus*, *Drechslera* sp., *Humicola* sp., and *Scopulariopsis* sp. on root-knot nematode eggs or females, reported in the present study, are new reports.

In contrast, majority of the fungal isolates (98.7%) inhibited egg hatch at varying levels indicating the involvement of mechanisms other than parasitism. The presence of fungi like *Aspergillus* spp., *Fusarium* spp., *Trichoderma* spp., and *Pochonia* spp. in the vicinity of nematode eggs inhibited the egg hatching suggesting an exogenous effect. The enzymatic disintegration of vitelline and chitin layers might have increased the permeability of eggshell and enhanced the mycelial penetration leading to total disintegration of the egg contents.

The variability in nematicidal action can be explained as differences in fungal strains, culture media, and pH (Cayrol et al., 1989; Kim et al., 1998; Meyer et al., 2000). Variations of toxin production have been observed among strains within a species (Hallmann and Sikora, 1996).

In conclusion, the study has brought out the potential of a number of soil-borne fungi which if judiciously deployed can remarkably suppress plant parasitic nematodes. It also highlights the rich biodiversity present in the tropics, which has to be conserved to sustain the biological equilibrium of the rhizosphere. Further studies are needed to understand the exact chemistry of nematode–fungus interaction, their rhizosphere competence, and amenability to mass multiplication for commercially exploiting them as nematode biocontrol agents.

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## References

Anke, H., Stadler, M., Mayer, A., Sterner, O., 1995. Secondary metabolites with nematicidal and antimicrobial activity from nematophagous fungi and Ascomycetes. Can. J. Bot. 73, 932–939.

Ayoub, R., Zareen, A., Zaki, M.J., Khan, S., 2000. Female parasitism of *Aspergillus* species and their effect on root penetration by root knot nematode *Meloidogyne javanica*. Pakistan J. Phytopathol. 12, 115–117.

Barron, G.L., 1977. The Nematode Destroying Fungi. Canadian Biological Publications, Guelph, Ontario.

Cayrol, J.C., Djian, C., Pijarowski, L., 1989. Study of the nematocidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. Rev. Nematol. 12, 331–336.

Charles, J.S.K., Vijayalakshmi, A.V., Chitra, R., Varma, A.S., 2000. A new report on the biological suppression of cyst nematode, *Heterodera oryzicola* (Rao and Jayaprakash, 1978) infecting banana (*Musa* AAB cv. Nendran) in Kerala, India. J. Trop. Agric. 38, 102–103.

Crump, D.H., 1987. A method for assessing the natural control of cyst nematode populations. Nematologica 33, 232–243.

Freire, F.C.O., 1982. Interactions of Fungi and Nematodes of Black Pepper (*Piper nigrum* L.). Ph.D. Thesis, University of London, London, UK.

Godoy, G., Rodriguez-Kabana, R., Morgan-Jones, G., 1983. Fungal parasites of *Meloidogyne arenaria* eggs in an Alabama soil. A mycological survey and greenhouse studies. Nematropica 13, 201–213.

Goswami, B.K., Mittal, A., Singh, S., 2001. Conducive role of *Fusarium solani* and suppressive role of different fungi associated with root-knot nematode infecting brinjal around Yamuna river belt in Delhi. Indian Phytopathol. 54, 385–386.

Goswami, B.K., Uma Rao, Singh, S., 1998. Association of some deuteromycetous fungi with the egg masses of *Meloidogyne incognita* infecting vegetables. Ann. Agri. Res. 19, 149–152.

Gray, N.F., 1987. Nematophagous fungi with particular reference to their ecology. Biol. Rev. 62, 245–304.

Gray, N.F., 1988. Fungi attacking vermiform nematodes. In: Poinar, G.O., Jansson, H.B. (Eds.), Diseases of Nematodes, vol. II. CRC Press, Boca Raton, pp. 3–38.

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

Holbrook, C.C., Knauft, D.A., Dickson, D.W., 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Dis. 57, 957–958.

Holland, R.J., Williams, K.L., Khan, A., 1999. Infection of *Meloidogyne javanica* by *Paecilomyces lilacinus*. Nematology 1, 131–139.

Hussey, R.S., Barker, K.R., 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp. including a new technique. Plant Dis. Rep. 57, 1025–1028.

Irving, F., Kerry, B.R., 1986. Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium* Goddard. II. Factors affecting parasitism of cyst nematode eggs. Nematologica 32, 474–485.

Jansson, H.B., Nordbring-Hertz, B., 1988. Infection events in the fungus–nematode system. In: Poinar, G.O., Jansson, H.B. (Eds.), Diseases of Nematodes, vol. II. CRC Press, Boca Raton, pp. 59–72.

Kerry, B.R., 1981. Fungal parasites: a weapon against cyst nematodes. Plant Dis. 65, 390–393.

Kerry, B.R., 1984. Nematophagous fungi and the regulation of nematode populations in soil. Helminthol. Abstr. Ser. B 53, 1–14.

Khan, M.R., Goswami, B.K., 2000. In vitro evaluation of Indian isolates of *Paecilomyces lilacinus* against *Meloidogyne incognita* eggs. Indian J. Nematol. 30, 96–97.

Kim, D.G., Riggs, R.D., Correll, J.C., 1998. Isolation, characterization and distribution of a biocontrol fungus from cysts of *Heterodera glycines*. Phytopathology 88, 465–471.

Kok, C.J., Papert, A., Hok-a-hin, C.H., 2001. Microflora of *Meloidogyne* egg mass: species composition, population density and effect on the biocontrol agent *Verticillium chlamydosporium* (Goddard). Nematology 3, 729–734.

Kuczynska, J., 1997. The influence of soil mycoflora on the reduction of population of the golden nematode (*Globodera rostochiensis* Wo.). Prog. Plant Prot. 37, 120–122.

Lopez-Llorca, L.V., Duncan, G.H., 1991. Effects of fungal parasites on cereal cyst nematodes (*Heterodera avenae* Woll.) from naturally infested soil—a scanning electron microscopy study. Can. J. Microbiol. 37, 218–225.

Merriman, P., Russell, K., 1990. Screening strategies for biological control. In: Hornby, D. (Ed.), Biological Control of Soil-borne Plant Pathogens. CAB International, Oxon, UK, pp. 427–435.

Meyer, S.L.F., Massoud, S.I., Chitwood, D.J., Roberts, D.P., 2000. Evaluation of *Trichoderma virens* and *Burkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita*. Nematology 2, 871–879.

Morgan-Jones, G., Rodriguez-Kabana, R., 1988. Fungi colonizing cysts and eggs. In: Poinar, G.O., Jansson, H.B. (Eds.), Diseases of Nematodes, vol. II. CRC Press, Boca Raton, pp. 39–58.

Nitao, J.K., Meyer, S.L.F., Chitwood, D.J., 1999. In-vitro assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. J. Nematol. 31, 172–183.

Pocasangre, L., Sikora, R.A., Vilich, V., Schuster, R.P., 2000. Survey of banana endophytic fungi from central America and screening for biological control of the burrowing nematode (*Radopholus similis*). Infomusa 9, 3–5.

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

Sankaran, K.V., 1981. Studies on the Rhizosphere Mycoflora of Black Pepper (*Piper nigrum* L.) Ph.D. Thesis, University of Calicut, Calicut, India.

Santos, M.A., dos Ferraz, S., Muchovej, J.J., 1992. Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* Race 3. Nematropica 22, 183–192.

Sasser, J.N., Freckman, D.W., 1987. World perspective on nematology: the role of the Society. In: Veech, J.A., Dickson, D.W. (Eds.), Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists. Society of Nematologists, Hyattsville, MD, USA, pp. 7–14.

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

Siddiqui, I.A., Ali, N.I., Zaki, M.J., Shaukat, S.S., 2001. Evaluation of *Aspergillus* species for the biocontrol of *Meloidogyne javanica* in mungbean. Nematol. Medit. 29, 115–121.

Sosnowska, D., Banaszak, H., 1998. Occurrence of parasitic fungi on population of sugar beet nematode (*Heterodera schachtii* Schmidt) in Torun region. Prog. Plant Prot. 38, 457–460.

Sosnowska, D., Mauchline, T.H., Bourne, J.M., Kerry, B.R., 2001. Screening of Polish, other European and tropical isolates of *Verticillium chlamydosporium* to assess their potential as biological control agents of root-knot nematodes. J. Plant Prot. Res. 41, 77–83.

Spiegel, Y., Chet, I., 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.

Stirling, G.R., Mankau, R., 1979. Mode of parasitism of *Meloidogyne* and other nematode eggs by *Dactylella oviparasitica*. J. Nematol. 11, 282–288.

Waksman, S.A., 1922. A method of counting the number of fungi in soil. J. Bacteriol. 7, 339–341.

Weller, D.M., 1988. Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26, 379–407.

Windham, G.L., Windham, M.T., Williams, W.P., 1989. Effects of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. Plant Dis. 73, 493–494.