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Evaluation of fungal bioagents for management of root-knot nematodes in ginger and turmeric fields

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Abstract

Ginger (*Zingiber officinale Rosc.*) and turmeric (*Curcuma longa* L.) are important herbaceous spices cultivated extensively in different states of India. Being vegetatively propagated, they are challenged with several pests and diseases including plant parasitic nematodes. Root-knot nematode problem in these crops is currently managed with nematicides, cover crops and organic amendments. Realizing the scope of biological control in these crops, a series of experiments were conducted at Indian Institute of Spices Research, Calicut, Kerala to screen and evaluate various fungal bioagents for control of root knot nematodes (*Meloidogyne incognita*) infesting ginger and turmeric under field conditions. Ten antagonistic fungi were evaluated in different field experiments conducted in root-knot infested ginger and turmeric fields at two locations. The most promising isolates that suppressed root knot nematodes were Aspergillus nidulans (Is.10), *Fusarium oxysporum* (Is.11), *Paecilomyces lilacinus* (Is.36), Trichoderma viride (Is. 25), *Verticillium lecanii* (Is.35) and *Pochonia chlamydosporia* (Is.32). From these, three fungi viz. *F. oxysporum*, *T. viride* and *P. chlamydosporia* were further tested in ginger fields using two delivery systems, soil bed application and seed rhizome dipping generally applicable in dry shed treatment. The final results showed that *P. chlamydosporia* significantly suppressed root knot nematodes in ginger and gave the maximum yield irrespective of the mode of application.

Introduction

Ginger (*Zingiber officinale Rosc.*) and turmeric (*Curcuma longa* L.) are important herbaceous tropical plants belonging to Zingiberaceae family. They are cultivated widely in India, Jamaica, Sierra Leone, Nigeria, Malaysia, Southern China and Japan. They require warm and humid climate and thrive well from sea level to an altitude of 1500 m above MSL having a well distributed rainfall (150 to 300cm). Major areas under ginger cultivation in India are located in Orissa, Kerala, Karnataka, West Bengal, Himachal Pradesh and North Eastern states. Andhra Pradesh, Orissa and Tamil Nadu are the predominant turmeric growing states. Both ginger and turmeric are used in food processing, medicine and has wider applications in indigenous medicines.

Though several plant parasitic nematodes

have been recorded from ginger and turmeric, the most important nematode pests are root knot and burrowing nematodes (Guo et al., 2004; Kaur *et al*., 1989; Luqman *et al*., 1998; Routaray *et al*., 1987; Sheela *et al*., 1995; Vadhera *et al*., 1998). Stunting, chlorosis, poor tillering and necrosis of leaves are the common symptoms of nematode infestation in these plants. The affected plants mature and dry faster than healthy ones, resulting in a poor crop stand. Root knot nematodes cause galling and rotting of roots and underground rhizomes. Infested rhizomes have brown, water-soaked areas in the outer tissues leading to crop loss.

Integrated management of the plant parasitic nematodes infesting these crops has been successful with combined application of carbofuran and neem oil cake (Makhnotra et al., 1997; Mohanty *et al*., 1992; Mohanty et al., 1995; Ray *et al*., 1995; Sheela *et al*., 1995). Mulching, crop rotation, intercropping and organic amendments have been recommended as alternatives to the use of toxic nematicides (Das, 1999; Sharma and Bajaj, 1998; Stirling, 1989; Stirling and Nikulin, 1998). Considering the annual nature of these crops and the high organic status of the soil in which they are cultivated, biological control is an ideal option for managing plant parasitic nematodes infesting ginger and turmeric. However, except for a study on mycorrhiza there are no previous reports on biological management of nematodes in these two Zingiberaceous spices (Sampat *et al*., 2003). The present study is outcome of a series of experiments conducted to screen and evaluate various fungal bioagents against root knot nematode (*Meloidogyne incognita*) infesting ginger and turmeric under field conditions.

Materials and methods

Test organisms

The selection of the fungal biocontrol agents for the field evaluation was on the basis of the above microplot evaluation and *in vitro* studies conducted earlier (Eapen *et al*., 2005). *P. chlamydosporia* (Is. 31 & 32), *Trichoderma* spp. (Is. 25 & 56), *Penicillium* sp. (Is. 4), *Verticillium lecanii* (Is. 35), *P. lilacinus* (Is. 36), non pathogenic *F. oxysporum* (Is.11), *Scopulariopsis* sp. (Is. 14) and *A. nidulans* (Is.10) were selected and evaluated in various experiments conducted as part of this study. All the organisms were multiplied on steam sterilized sorghum as per the standard procedures.

Screening in microplots

Thirteen promising fungal biocontrol agents, selected based on *in vitro* screening, were screened in a pilot trial conducted in 2000 in microplots (1m x 1 m) using turmeric (variety Prabha) as the test plant. The test fungi were two isolates of *P. chlamydosporia* (Is. 31 and Is.57), one isolate each of *P. lilacinus* (Is. 36), *P. carneus* (Is. 27), *Fusarium* sp. (Is. 13), F. oxysporum (Is. 11), *A. nidulans* (Is. 10), *Scopulariopsis* sp. (Is. 14), *Scolecobasidium* sp. (Is. 15) and *Trichoderma* sp. (Is. 56); and three unidentified fungi (Is. 12, 52 and 54). There were three controls viz. absolute control, carrier material sorghum and rice and each treatment was replicated thrice. The fungi were multiplied on sorghum or rice grains and applied @ 75 g/plot at the time of sowing. The spore count in the biocontrol inoculum varied from 107 - 1010 /g carrier substance. After one month each microplot was inoculated with 1000 juveniles of *M. incognita*. Root and soil samples were drawn after three months and population of root-knot nematodes and total fungi were estimated using standard procedures.

Field Evaluation

Field evaluation of the promising fungal biocontrol agents was conducted at two locations, one at IISR farm, Chelavoor (only in 2001), and the other at IISR Experimental Farm, Peruvannamuzhi consecutively for three years (2001-2003). In all the experimental fields either turmeric or ginger was grown previously and were naturally infested with root knot nematode. The experiments were conducted in randomized block design with six treatments, both for ginger and turmeric at Peruvannamuzhi and

seven treatments in ginger experiment at Chelavoor. The bioagents used in the ginger field trial at Chelavoor were two isolates of *Trichoderma* spp. (Is. 25 & 56), one isolate each of *Pencillium* sp. (Is. 4), V. lecanii (Is. 35) and *P. chlamydosporia* (Is. 31). *P. chlamydosporia* was tried as a seed treatment as well as soil bed application in this trial. In the second year five new isolates viz. *P. lilacinus* (Is. 36), *F. oxysporum* (Is. 11), *Scopulariopsis* sp. (Is. 14), A. nidulans (Is. 10) and *P. chlamydosporia* (Is. 32) were evaluated at Peruvannamuzhi on both ginger and turmeric. The biocontrol agents, multiplied on sorghum, were incorporated in the soil (@ 50 g/bed of 3 x 1 m) at the time of sowing. There were four replications at both locations. The test varieties were 'Himachal' (ginger) and 'Prathibha' (turmeric).

In the final field trial conducted during 2003-04, three promising fungi viz. *P. chlamydosporia* (Is. 32), *T. viride* (Is. 25) and *F. oxysporum* (Is. 11) were once again evaluated in ginger fields by employing two types of delivery methods. In the first method, the fungi were multiplied on sorhum grains and incorporated into ginger beds at the time of sowing. In the second method, fungal spores were mixed with starch solution and the seed rhizomes were dipped in this suspension for 15 min, dried under shade and planted in beds.

Plant growth parameters like height of the plants, number of tillers per plant were recorded in all the above experiments. Nematode populations in the roots were estimated using standard procedures described earlier. The data were analyzed using ANOVA and the means were separated using Duncan's Multiple Range Test.

Results and discussion

Evaluation in microplots

The number of nematodes in turmeric roots and soil varied widely in beds treated with different fungal bioagents. *A. nidulans* (Is.10) totally suppressed root knot nematodes followed by *Scopulariopsis* sp. (Is. 14) and *P. chlamydosporia* (Is. 57). *Fusarium* spp. (Is. 11 & Is. 13) and another isolate of P. *chlamydosporia* (Is. 31) were comparatively less efficient in suppressing these nematodes. Out of these, *P. chlamydosporia* is a known egg parasite of nematodes and is widely used as a biocontrol agent of nematodes. However, *Aspergillus* spp. and *Fusarium* spp. infesting different stages of root knot nematodes have been encountered rarely in field samples (Goswami *et al.*, 1998; Amer-Zareen *et al.*, 2000; Eapen *et al.*, 2005).

Field trial

None of the treatments resulted in significant change in the number of tillers of turmeric plants where as the height of the plant increased significantly in all the treatments with biocontrol agents (Table 1). All the fungal biocontrol agents evaluated were effective in suppressing root knot nematode population. However, significant suppression of root knot nematodes could be achieved only by the application of *F. oxysporum* (100%) and *P. lilacinus* (97.4%). No adverse effect was noticed on turmeric plants treated with F. oxysporum confirming the nonpathogenic nature of this isolate. Nonpathogenic isolates of *F. oxysporum* are now widely used as nematode biocontrol agents in different crops (Mennan *et al.*, 2005; Athman, 2006). Though all the biocontrol agents significantly increased the height of turmeric plants compared to control, there was no significant difference among the treatments in the number of tillers produced. Significant increase in yield (40.9%) could be observed only in plants treated with *F. oxysporum*.

In ginger, the first experiment was conducted at IISR Farm, Chelavoor in which all the inoculated beneficial fungi improved the growth (Table 1). Significant reduction in root-knot nematodes was observed with three fungi viz. *T. viride* (Is. 25), *V. lecanii* and *P. chlamydosporia* (soil application). As in the case of turmeric, in the second trial conducted at Exp. Farm, Peruvannamuzhi too, there was no significant difference among the treatments in the number of tillers of ginger

plants (Table 1). Significant increase in the height of the plant was achieved with the application of *P. lilacinus*, *Scopulariopsis* sp. and *A. nidulans*. But except *Scopulariopsis* sp., all the other fungal isolates significantly suppressed the nematode population.

In the final field trial, where the three best isolates viz. *P. chlamydosporia*, *T. viride* and *F. oxysporum* were evaluated by adopting two delivery methods, the best performance was noticed with *P. chlamydosporia* (Table 2). *P. chlamydosporia* significantly reduced the root-knot nematode population over control which was non-significant. Both *T. viride* and *F. oxysporum* failed to suppress the nematode population. There was no significant difference between the two methods of inoculum delivery, though the soil incorporation biocontrol inocula was slightly superior to seed rhizome dipping. The nematophagous fungi with a saprophytic phase in their life cycles are much affected by conditions in the rhizosphere and the biological control they mediate is density

Table 1. Field evaluation of fungal biocontrol agents for the suppression of root knot nematodes infesting ginger and turmeric (Meaof four replications)

a. Ginger - IISR Farm, Chelavoor

Treatment	No. of tillers	Height of plant (cm)	Nematode population (per g root)			
			Eggs	Juveniles	Females	Total
Tri. (Is. 25)	7.47 a	50.17 a	0.00 b	0.00 b	0.00 a	0.00 b
Pen. (Is. 4)	7.98 a	48.01 a	10.06 ab	6.00 ab	0.46 a	16.52 ab
V l (Is. 35)	7.70 a	49.71 a	0.46 b	0.00 b	0.00 a	0.46 b
Tri. (Is. 56)	7.13 a	48.89 a	4.24 ab	4.75 ab	0.46 a	9.45 ab
P c (Is. 31) -Soil	7.44 a	47.94 a	0.67 b	0.00 b	0.00 a	0.67 b
P c (Is. 31) - Seed	8.94 a	47.70 a	2.97 ab	3.02 ab	1.32 a	7.31 ab
Control	6.56 a	46.83 a	51.48 a	24.64 a	0.00 a	76.12 a

b. Turmeric - Peruvannamuzhi Farm

Treatment	No. of tillers	Height of plant (cm)	Nematode population (per g root)			
			Eggs	Juveniles	Females	Total
P l (Is. 36)	2.24 a	129.13 c	5.54 b	0.00 b	0.46 b	6.00 b
F o (Is. 11)	2.14 a	134.43 a	0.00 b	0.00 b	0.00 b	0.00 b
Sco. (Is. 14)	2.06 a	133.04 b	8.20 ab	1.45 b	1.60 ab	11.25 ab
Asp. (Is. 10)	2.06 a	134.49 a	10.02 ab	6.07 ab	0.00 b	16.09 ab
P c (Is. 32)	2.35 a	134.64 a	3.23 b	8.09 ab	0.00 b	11.32 ab
Control	2.38 a	118.35 d	158.59 a	61.66 a	6.93 a	227.18 a

c. Ginger - Peruvannamuzhi Farm

Treatment	No. of tillers	Height of plant (cm)	Nematode population (per g root)			
			Eggs	Juveniles	Females	Total
	7.47 a	50.17 a	0.00 b	0.00 b	0.00 a	0.00 b
P l (Is. 36)	6.01 a	73.42 b	0.46 b	1.33 c	0.00 a	1.79 b
F o (Is. 11)	6.07 a	70.45 c	3.03 b	2.13 bc	0.00 a	5.16 b
Sco. (Is. 14)	6.84 a	75.76 a	10.11 ab	26.93 ab	0.46 a	37.5 ab
Asp. (Is. 10)	6.06 a	74.37 ab	7.33 ab	2.13 bc	0.46 a	9.92 b
P c (Is. 32)	6.15 a	70.19 c	3.58 b	1.14 c	0.00 a	3.72 b
Control	5.94 a	71.57c	66.92 a	126.93 a	0.46 a	194.31 a

Pc - *P. chlamydosporia*, Tri. - *Trichoderma* sp., Pen. - *Pencillium* sp., Vl - *Verticillium lecanii*,

Pl - *Paecilomyces lilacinus*, Sco.- *Scopulariopsis* sp., Asp. - *Aspergillus* sp., Fo - *Fusarium oxysporum*

Table 2. Evaluation of fungal bioagents in a ginger field trial at Peruvannamuzhi (Mean of four replications)

Treatment	Height (cm)			Tillers			Yield (kg - 3x1 m bed)			Nematodes/g root		
	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
Control	76.29a	73.66a	74.98a	9.93a	8.85a	9.39a	5.83a	4.46b	5.15bc	13.49b	35.31b	24.40b
P. chlamydosporia	76.83a	71.16a	73.99a	9.96a	8.69a	9.32a	5.90b	5.29b	5.83c	2.88a	1.01a	1.95a
T. harzianum	79.43a	75.45a	74.75a	8.55a	8.95a	8.75a	3.69a	4.27a	3.98a	7.68ab	6.26ab	6.97ab
F. oxysporum	74.38a	72.60a	73.49a	9.30a	9.43a	9.36a	4.46b	5.04b	4.75ab	37.55ab	9.79ab	23.67b
Mean	75.39	73.22	-	9.43	8.98	-	4.97	4.77	-	15.40	13.09	-

1 - Incorporation in soil; 2 - Seed rhizome coating

dependent (Kerry, 2000). *P. clamydosporia* can provide sustainable nematode control in an intensive cropping system like ginger (Kerry and Evans, 1996).

The study has clearly proved the potential of different antagonistic fungi in managing plant parasitic nematodes of ginger and turmeric under an organic cultivation especially the P. chlamydospora. Such an approach may take care of the soil-borne fungal pathogens of these crops as indicated by others (Balakrishnan *et al.*, 1997; Meena and Mathur, 2003; Sharma, 1998). Further it was also proved that none of the fungal isolates used had any adverse effect on the test plants and can be exploited as potential biocontrol agents.

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