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Pathogenicity of selected antagonistic soil fungi on *Meloidogyne incognita* (Kofoid & White) eggs and egg masses under *in vitro* and *in vivo* conditions

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ABSTRACT: **Four indigenous antagonistic fungi belonging to 3 species, *Gliocladium virens* Miller Giddens & Foster, *Paecilomyces lilacinus* (Thom) Samson, *Trichoderma harzianum* Rifai and *T. viride* Pers. Ex S. F. Gray evaluated for their pathogenicity to eggs and egg masses of *Meloidogyne incognita, in vitro* through the Petri-dish assay and *in vivo* under glasshouse conditions were found pathogenic. Under both the conditions, maximum egg mass and egg parasitization was observed to be by *P. lilacinus* followed by *T. viride, G. virens* and *T. harzianum*. There was a general decline in parasitization by these fungi, under *in vivo* conditions compared to that under *in vitro* conditions. Fungal colonization in galled roots was higher than in healthy roots. Comparatively *T. viride* and *G. virens* recorded higher root colonization closely followed by *P. lilacinus*. Among the four fungi, *P. lilacinus* recorded consistently higher pathogenicity against root-knot nematodes both *in vitro* and *in vivo* conditions, indicating that *P. lilacinus* was more efficient against root-knot nematodes compared to other species.**

KEY WORDS: Antagonistic fungi, *Meloidogyne incognita*, pathogenicity

Several soil-borne fungi were found to be effectively parasitizing not only fungal pathogens (Chet, 1987; Elad *et al.*, 1986; Mukhopadhyay, 1994), but also insect pests and plant parasitic nematodes (Stirling, 1991; Vyas *et al.*, 1995), thus playing a beneficial role in agricultural pest management. Promotion of such antagonistic biotic factors forms an integral part of sustainable nematode management. Earlier studies demonstrated that *Gliocladium virens* and *Trichoderma* spp. caused antagonism to *Heterodera glycines* by producing toxic substances which not only inhibited egg hatching but also caused nematode mortality *in vitro* (Meyer *et al.*, 1990). In the present study, the above local isolates of *G. virens, Paecilomyces lilacinus, Trichoderma harzianum* and *T. viride* were studied *in vitro* for their pathogenicity to eggs and egg masses of *M. incognita* through the Petri-dish assay and *in vivo*, under glasshouse conditions. The study was carried out in order to compare the pathogenic efficacy of these antagonistic fungi *in vitro* and *in vivo*, and identify the fungus, which exhibited consistently high pathogenicity.

MATERIALS AND METHODS

Studies were conducted in Indian Institute of Horticultural Research, Bangalore, during 1997-99. Pure cultures of local strains of *Gliocladium*

virens Miller Giddens & Foster, *Paecilomyces lilacinus* (Thom.) Samson, *Trichoderma harzianum* Rifai and *T. viride* Pers. Ex S. F. Gray were maintained on potato dextrose agar medium. Fresh and healthy egg masses of *Meloidogyne incognita* were collected from infested roots of tomato; surface sterilised with NaOCl (1.0%) for 5 seconds and washed thoroughly with sterile distilled water. The fungi were tested against *M. incognita* egg masses by Petri- dish bioassay technique (Mc Innis and Jaffe, 1989). Spores of respective fungi were sprayed on water agar plates (2%) at the rate of 12 x 10^6 spores/plate followed by the placement of 10 egg masses/plate. In control plates, only sterile distilled water was used. Treatments were replicated 5 times using completely randomized design. The plates were incubated at 27 ± 1°C and fungal proliferation and colonization of egg masses were recorded 48h interval for 10 days. Parasitization of egg masses and eggs were studied under inverted compound microscope. Data on number of egg masses parasitized, number of eggs parasitized/egg mass were recorded.

Further, to confirm fungal parasitization of eggs and egg masses, tomato plants were raised in plastic pots (10cm diam) containing autoclaved sand. Ten-day old plants were inoculated with freshly hatched juveniles of *M. incognita* at 500/ pot, followed by incorporation of 10ml of aqueous spore suspension (2-4 x 10^7 spores/ml) of each fungus. Thirty days later, the plants were removed from the pots; roots were cleared of sand and washed thoroughly with sterile distilled water. Ten egg masses from treated plants were collected, surface sterilized and placed in Petri-plates containing respective fungal selective medium (Davet, 1979; Elad and Chet, 1983; Mitchell *et al.*, 1987). The Petri-plates were incubated at 27 ± 1°C for 2 weeks. Glasshouse experiment was repeated subsequently.

The proportion of eggs infected with the fungi was estimated by taking 10 egg masses at random, washed several times in sterile water and exposed to NaOCl (0.1%) for 2-3 seconds and transferred to 5ml sterile water. One ml of this stock was then plated individually on Petri-plates containing thin layer of selective media for *P. lilacinus* and *Trichoderma* spp. The plates after incubation at 27 ± 1°C for 36h were observed under microscope for recording the number of infected eggs. The percentage of infected egg masses and eggs were arrived at accordingly.

Tomato roots from each treated pot were collected, cleared of soil, washed thoroughly with sterile water and cut into small pieces (approx. 1cm). Ten root bits were surface sterilized with NaOCl (0.1%) for 1 min and placed in Petri- plates containing semi-selective media for respective fungi. Similarly, one gram of soil from each pot was collected, suspended in 9 ml of sterile water and serially diluted. One ml of each sample was placed in Petri-plates containing semi-selective media to record the populations of the fungi in soil. These Petri-plates were incubated at 27 ± 1°C for 4-6 days to observe number of root bits colonized with the antagonistic fungi and populations of fungi in soil. Further, the re-isolated fungi were stained and microscopically examined for their morphology.

RESULTS AND DISCUSSION

In vitro and *in vivo* studies showed that all the 4 fungi under study parasitized the egg masses and eggs. Under *in vitro* conditions, maximum egg mass and egg parasitization (92.5 and 78.0%, respectively) were observed to be by *P. lilacinus* followed by *T. viride, G. virens* and *T. harzianum*. Similar trend was also observed under *in vivo* conditions. There was a general decline in parasitization by the fungi under study, under *in vivo* conditions compared to that under *in vitro* conditions, possibly due to congenial conditions under *in vitro* conditions compared to that under *in vivo* conditions. Highest per cent reduction in parasitization of egg massses and eggs by the fungi *in vivo* over *in vitro* (>40%) was observed in case of *T. harzianum* and *T. viride*, while it was comparatively lower in case of *P. lilacinus*, followed by *G. virens* (<40%). Between the two *Trichoderma* species, *T. viride* consistently exhibited higher parasitization of egg masses and

eggs both under *in vitro* and *in vivo* conditions over that of *T. harzianum*. The decline in parasitization between *in vitro* and *in vivo* conditions is attributed to rapid rate of growth and multiplication of fungi, and monoxenic conditions serve as medium for growth of the soil-borne saprophytic and other micro-organisms. Besides, galled roots contain higher number of root-knot nematode egg masses, which are easily parasitized by these antagonistic fungi. These fungi

Table 1. Pathogenicity of 4 antagonistic fungi against eggs and egg masses of *M. incognita* and colonization in tomato roots

Treatment	Fungal parasitization (%) (Mean±SD)				Fungal colonization (%) (Mean ± SD)	
	In vitro conditions		*In vivo* conditions			
	Egg masses	Eggs	Egg masses	Eggs	Roots in toto	Galled root only
Control	0	0	0	0	0	0
T. harzianum	67.5±5.50	48.5±3.00	35.0±5.25 (48.14)*	28.5±2.45 (41.23)	56.0±1.50	68.0±3.50
G. virens	69.5±2.75	5.5±3.50	46.5±2.25 (33.09)	34.5±1.50 (37.83)	74.0±2.00	79.0±2.00
T. viride	75.0±4.75	66.5±1.25	45.0±3.75 (40.00)	37.5±3.30 (43.60)	72.0±3.50	79.0±4.00
P. lilacinus	92.5±3.50	78.0±1.60	65.0±4.50	49.5±3.80	68.0±2.50	77.0±2.50
CD (P=0.05)	2.11	4.36	4.54	3.77	5.11	6.35

* Values in parentheses indicate per cent reduction in parasitization *in vivo* over in two conditions.

under *in vitro* conditions. Our earlier studies showed that *T. viride* sporulated in 21days while *T. harzianum* sporulated in 28 days on paddy grains. The number of spores per unit weight of grains were also higher in *T. viride* (Anon., 1997).

Further, fungal colonization on roots was higher in case of *T. viride* followed by that of *P. lilacinus* and *T. harzianum*, while *G. virens* colonization was on par to that of *T. viride* (Table 1). Fungal colonization in galled roots was higher compared to that in healthy roots. Colonization in galled roots was higher in case of *T. viride* and *G. virens* (74 and 72%, respectively), closely followed by *P. lilacinus* (68%). Galled root tissues generally (propagules) were successfully re-isolated from the infected/parasitized egg masses on respective selective media, and from soil.

Bio-agents such as *G. virens, P. lilacinus, Trichoderma* spp., are known to produce hydrolytic enzymes including, chitinases, ß-1,3-glucanases, proteases and volatile and non-volatile antibiotics (Lumsden and Locke, 1989; Smith *et al.*, 1990). Chitin, an insoluble linear ß-1,4-linked polymer of N-acetyl glucosamine (GlcNAc), is one of the most abundant polysaccharides in nature (Deshpande, 1986). It was conclusively demonstrated that the eggshell is the structure in nematodes that contains chitin (Bird and Self,

1995), although chitin was reported to be detected from gelatinous matrix of egg mass of the root-knot nematode (Spiegel and Cohn, 1985). Literature cited attributed mycoparasitism by these bio - agents, to these hydrolytic enzymes, which degraded the fungal cell walls and ensured fungal growth. Similarly, in the present study, the root knot-nematode egg and egg mass parasitization by the antagonistic fungi could be related to the hydrolytic enzymes such as, chitinases, endochitinases, proteases, etc. The difference in parasitization levels could be due to differences in production of hydrolytic enzymes by individual species and strains.

The literature cited suggest that these enzymes aided degradation of chitin and related proteins in the egg shells/walls, thus aiding the antagonistic fungi to establish on the contents of the ruptured eggs and egg masses. Since the chitin is a protective polysaccharide in nematode eggs, the chitinases are essential for the fungi to parasitize and have pathogenic effect. Earlier experiments showed that the culture filtrates of *P. lilacinus, Trichoderma* spp. and other fungi not only inhibited egg hatching but also caused mortality to *M. incognita* juveniles at different concentrations (Goswami and UmaRao, 1997).

The present study demonstrated that among the four antagonistic fungi studied, *P. lilacinus* recorded consistently higher pathogenicity to the eggs and egg masses of root-knot nematodes both *in vitro* and *in vivo* conditions, indicating that *P. lilacinus* was more efficient against root-knot nematodes compared to other species. Further, only about 60 per cent of the parasitization under *in vitro* conditions can be expected under *in vivo* conditions. Studies on the pathogenicity through the production of hydrolytic enzymes by these fungi need to be carried out to further understand the mechanisms of nematode suppression by these fungi.

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