Recent Advances in Plantation Crops Research, 2000, pp. 382-386.

TRICHODERMA SPP. FOR THE MANAGEMENT OF ROOT KNOT NEMATODES AND RHIZOME ROT DISEASE IN CARDAMOM NURSERIES

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Trichoderma spp. isolated from root and soil samples of cardamom plantations situated in Kerala and Karnataka were tested under *in vitro* conditions for their interaction with different life stages of *Meloidogyne incognita*. *T. harzianum*, *T. viride* and *T. virens* were found to inhibit nematode development, though no direct parasitization was observed. *T. harzianum* caused maximum suppression of nematodes, especially in native, non-sterile soil under green house conditions. All the isolates promoted the growth of cardamom seedlings, whether or not the plants were infested with root knot nematodes. A mixture of *Trichoderma* isolates when applied in two 'sick' cardamom nurseries reduced the incidence of rhizome rot disease and root knot nematode population significantly.

INTRODUCTION

The spice crop, cardamom (*Elettaria cardamomum* Maton) is being propagated vegetatively through suckers but seedlings are the main source for large scale planting. Root knot nematodes (*Meloidogyne incognita)* and damping off/rhizome rot disease caused by *Pythium vexans* de Bary and *Rhizoctonia solani* Kuhn. take a heavy toll of seedlings. Root knot nematodes invariably in association with *Pythium-Rhizoctonia* complex caused greater damage compared to nematodes alone (Ali and Venugopal, 1992). Environmental and health hazards associated with the recommended agrochemicals have prompted the search for ecofriendly crop protection measures using biological control agents. Various isolates of *Trichoderma* and *Gliocladium* spp. suppressed rhizome rot of ginger (Usman *et al.*, 1996) and cardamom capsule rot (Susheela Bhai *et al.*, 1993). Preliminary studies carried out with biocontrol agents at Indian Institute of Spices Research have shown their efficacy in suppression of *P. vexans* and *R. solani*. Present study was executed to evaluate the potentiality of local isolates *of Trichoderma* spp. against root knot nematodes under laboratory conditions. Further, promising isolates were evaluated in a green house and in 'sick' cardamom nurseries to assess their suppressive effect on *M. incognita* and rhizome rot.

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MATERIALS AND METHODS

Isolates of *Trichoderma aureoviride* Rifai, *T. hamatum* (Bon.) Bain., *T. harzianum* Rifai., *T. koningii* Oud, *T. longibrachiatum* Rifai, *T. polysporum* (Link ex Pers.), *T. pseudokoningii* Rifai, *T. viride* Pers. ex S.F. Gray and *T. virens*

Miller *et al.* were isolated from soil and roots collected from various cardamom nurseries and plantations in Kerala and Karnataka. Stock cultures were maintained on potato dextrose agar medium. *M. incognita* obtained from infested cardamom plants were multiplied on tomato seedlings (susceptible local variety) grown in sterile soil. Egg masses of *M. incognita* were hand picked from tomato roots and surface sterilized (Hussey and Barker, 1973). In order to get second stage juveniles (J2) egg masses were incubated in sterile distilled water for 24 h.

To study the interaction between *Trichoderma* spp. and *M. incognita*, three egg masses/plate, 500 eggs/plate and 100 juveniles per plate were added separately to petri dishes containing two per cent water agar. Two mm discs of three day old *Trichoderma* colonies were placed near the vicinity of egg masses and at the centre for eggs and juveniles. Controls consisted of plates with egg masses, eggs or juveniles alone. Plates were incubated at 28°C under dark condition. There were 11 treatments and each treatment replicated thrice. Ten days after incubation, egg masses, eggs and juveniles were sampled at random from respective treatments, stained with 0.05 per cent cotton blue in lactophenol and observed for fungal parasitization. One egg mass from each treatment was transferred to tap water and maintained for 24 h to record the hatching rate. Culture filtrate of *Trichoderma* isolates were tested against *M. incognita* parasitisation using cavity blocks containing 10 juveniles of *M. incognita* in 0.5 ml distilled water. Each treatment was replicated thrice with pure distilled water as control. Toxicity was estimated after 24 h according to the mean percentage of paralysed nematodes.

Malabar type cardamom seedlings of uniform size and age raised in sterile soil were transplanted to plastic pots containing (four numbers/pot) 10 l sterile/non sterile soil. Rhizosphere was inoculated with different *Trichoderma* isolates (2.9 - 3.6 x 10^7 c.f.u./g) raised in decomposed coffee husk. One week after biocontrol agents' inoculation, aqueous suspensions of *M. incognita* eggs and juveniles (1000 No. per pot) were incorporated. Treatment details are presented in Table 3. Each treatment was replicated four times in a completely randomised block design. After six months, plants were uprooted gently, washed and weighed individually. Roots were sampled and stained appropriately for counting the egg masses/ nematode population as per standard procedures (Byrd *et al.*, 1983). Ten egg masses were removed at random to check fungal colonization. Fungi were reisolated from soil and root samples and compared with the test isolates.

Three isolates of *T. harzianum* and one *T. viride* isolate were multiplied on sterilized neem cake and decomposed coffee husk (1:1 w/w) mixture. Fifteen day old biocontrol inocula were mixed well with nursery soils (5 x 1 m bed) thrice; once before sowing (50 g/m^2) followed by second inoculation at rhizome formation (75 g/m^2) and finally at tillering stage (100 g/m^2). Nursery beds received neem cake/coffee husk mixture without any biocontrol agent served as control. Beds were prepared in two sick cardamom nurseries where there was a high incidence of root knot nematodes and rhizome rot during previous years. Cardamom seeds of a high yielding selection (Clone 37) were sown at the rate of 1000 seeds/ bed. Each treatment replicated eight times in a complete randomised block design. Germination, growth of seedlings and incidence of rhizome rot/ damping off were monitored periodically. After nine months, 20 seedlings were selected at random from each bed and their total biomass/ seedling and number of nematodes/g root were recorded. Data were subjected to analysis of variance.

RESULTS AND DISCUSSION

Trichoderma isolates colonised root knot nematode egg masses but none of them parasitized either eggs or juveniles (Table 1). Embryonic development was inhibited in the

Table 1. Interaction of *Trichoderma* spp. with *Meloidogyne incognita* egg masses under *in vitro* conditions

Trichoderma species	*M. incognita* egg masses Colonization of matrix	Egg parasitization	Hatching rate
T. aureoviride	+	-	79.7 abcd
T. hamatum	+	-	95.0 abc
T. hamatum I	+	-	*30.3 e*
T. hamatum II	+	-	49.0 de
T. koningii	+	-	75.0 abcd
T. longibrachiatum	+	-	84.3 abcd
T. polysporum	+	-	104.7 a
T. pseudokoningii	+	-	97.67 ab
T. viride	+	-	58.0 cde
T. virens	+	-	62.3 bcde
Control	-	-	102.7 a

Mean number of juveniles hatched per egg mass. Figures followed by same alphabet in the column are statistically not significant

presence of the biocontrol agents as evident from distortions and deformities in nematode eggs. Santos and others (1992) have reported more than 50 per cent parasitization of *M. incognita* eggs by *T. harzianum* in *in vitro*. Fungal isolates showed considerable variability in their biocontrol ability on nematode development. Since *Trichoderma* is an opportunistic and ectoparasitic fungi sustained contact with the nematode eggs or juveniles is essential for penetration of the fungi. This was not very much ensured in agar plates and might be a reason for absence of parasitization. It is also reported that only eggs in the early stages of embryonic development are vulnerable to fungal parasitism (Goswami and Uma, 1995). This also might have contributed to the absence of fungal development in eggs. Eggshell wall degrading enzymes may be responsible for disrupting nematode development inside the eggs when challenged by these fungi. Cell walls of the target pathogens are weakened by chitinolytic enzymes thus helping the uptake of diffusible toxic metabolites released by the fungi (Goldman *et al.*, 1995). Complete mortality of *M. incognita* larvae in culture filtrates of *Trichoderma* and related species did support this (Djian *et al.*, 1991; Sharma and Saxena, 1992).

Among the five isolates tested, *T. harzianum* I and II found efficient in suppressing nematode populations than any other isolates and it was more prominent in native soil than in sterilised soil (Table 2). *T. harzianum* reduced the nematode population by 60.5 to 86.8 per cent in sterile soil and by 50.4 to 82.1 per cent in non sterile soil. This suggests that the natural complement of soil microflora available in native soil supported the initial establishment and colonisation of the soil by the biocontrol agents. Alternately, indigenous nematode antagonists present in the native soil might have acted synergistically with the introduced biocontrol agents. Reduction in nematode population in control plants also substantiates this statement.

Table 2. Interaction of *Trichoderma* isolates with *Meloidogyne incognita* infesting cardamom seedlings under green house conditions

Treatment	No of egg masses		No fo nematodes per g root	
	Sterile soil	Non sterile soil	Sterile soil	Non sterile soil
Trichoderma sp.	10.45 abcd	4.18 a	285.42 abc	163.44 ab
T. viride	16.45 abc	7.85 a	671.98 a	104.93 ab
T. harzianum I	7.20 bcd	8.52 a	86.70 bcd	118.58 ab
T. harzianum II	9.99 abcd	4.98 a	259.62 abc	42.66 b
T. virens	15.70 abc	9.35 a	449.82 ab	318.15 a
Together	19.02 ab	6.13 a	372. 25 ab	54.98 ab
M. incognita alone	23.45 a	13.54 a	656.66 a	238.88 ab

Mean values followed by same alphabet in a column are statistically not significant

Table 3. Effect of *M. incognita*, *Trichoderma* and *Gliocladium* isolates on growth of cardamom seedlings under greenhouse conditions

Treatment	Total biomass (fr.wt g/seedling) Sterile soil	Non sterile soil	Difference
Trichoderma sp.	23.01 abc	22.58 bcde	0.43
T. viride	21.08 bcdef	24.62 abc	3.54
T. harzianum I	22.12 abcde	19.04 defgh	3.08
T. harzianum II	25.00 ab	26.75 ab	1.75
T. virens	22.86 abc	21.46 cdefg	1.40
Together	19.20 cdefg	29.12 a	9.92*
Trichoderma sp.+RKN	15.17 gh	17.02 fgh	1.85
T. viride + RKN	17.37 defg	18.25 defgh	0.88
T. harzianum I + RKN	17.92 cdefg	15.21 hi	2.71
T. harzianum II + RKN	16.50 fg	22.85 bcd	6.35*
T. virens + RKN	18.96 cdefg	15.08 hi	3.88
Together +RKN	17.08 efg	20.54 cdefgh	3.46
M. incognita (RKN)	15.33 gh	11.33 i	4.00
Control	19.16 cdefg	20.29 cdefgh	1.05

Mean value followed by the same alphabet in a column do not differ at five per cent probability

* indicates significant difference between means in a row

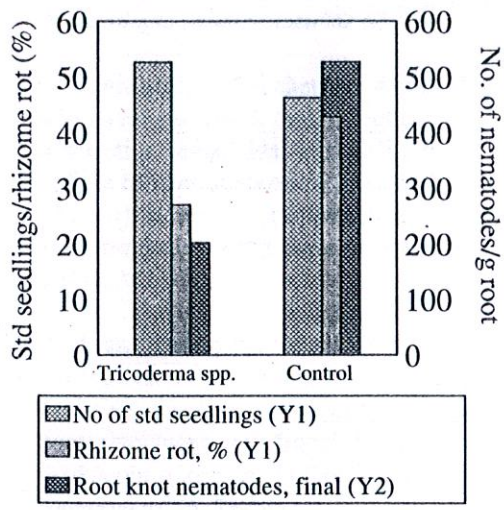

Fig.1. Effect of Trichoderma spp. on rhizome rot and root knot nematodes in sick nurseries

Biocontrol agents in spite of nematode infestation (Table 3) promoted growth of cardamom seedlings. This could be due to the diffusible metabolites released by the fungi might be acting as growth promoting substances. Increased growth response was more evident for *T. harzianum* II both in sterile and non sterile soil. But significant growth promotion in the nematode infested plants was observed in native soil only. Here again, maximum growth response was observed with *T. harizanum* II followed by combined application and *T. viride*. Reisolation studies revealed that *T. harzianum* was the dominating species in combined application. Chitinolytic enzymes of *T. harzianum* are reported to be active against a broad range of pathogens and exhibit the highest degree of synergy with other enzymes or with other control agents (Goldman *et al.*, 1994).

Trichoderma spp. controlled the root knot nematode multiplication rate and enhanced the growth of cardamom seedlings under field conditions (Fig.1). As a result, the number of quality seedlings was more in *Trichoderma* treated plots and the incidence of rhizome rot was reduced to 26.44 per cent. Suppression of soil-borne pathogens by *Trichoderma* spp. is well established in several crops and the reported mechanisms involved are competition, mycoparasitism and production of antibiotics (Mukhopadhyay, 1987). In a pilot study, Eapen and Venugopal (1995) reported the efficacy of

Trichoderma spp. in controlling root knot nematodes and diseases like rhizome rot/damping off, especially in solarised soil. In the present study, *T. harzianum* II was identified as the best biocontrol agent followed by *T. harzianum*, *T. viride* and *T. virens* which suppressed the populations of root knot nematodes and other soil borne fungal pathogens of cardamom.

ACKNOWLEDGEMENTS

The authors are thankful to the Spices Board officials for granting the permission to carry out the field trials at their nursery at Biligeri, Coorg District, Karnataka.

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