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Use of *Trichoderma* Species in the Control of *Meloidogyne Javanica*, Root Knot Nematode in Okra and Mungbean

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Abstract: The efficacy of *Trichoderma viride, T. harzianum, T. hamatum, T. koningii* and *T. pseudokoningii* was tested for the control of *Meloidogyne javanica*, root knot nematode in okra and mungbean. Culture filtrates of *Trichoderma* spp., significantly reduced egg hatching and showed nematicidal activity by killing second stage juveniles of *M. javanica*. Soil application with conidial suspension of *T. harzianum* significantly reduced nematode population densities and root knot development in okra and mungbean. Apart from suppressing root knot nematode, *T. harzianum* also elevated plant height and fresh shoot weight of both okra and munhbean.

Key words: *Trichoderma spp.*, culture filtrate, *Meloidogyne javanica*, okra, mungbean

Introduction

Fungi and plant parasitic nematodes being common inhabitants of crop rhizosphere show synergetic relation (Inagaki and Powell, 1969; Jorgenson, 1970). Since they occur together in the rhizpsphere, the toxic metabolites naturally produced by microorganisms may be responsible for keeping low level of nematode populations. Filtrates of nematophagous or entomophagous fungi have been found to be active against free living nematodes (Alam *et al.*, 1973; Cayrol *et al.*, 1989; Ali, 1990).

Among the fungal antagonists, various species of *Trichoderma* have shown promising results in the control of soilborne plant pathogens (Elad *et al.*, 1983). Use of *Trichoderma* species in the biological control of soilborne root-infecting fungi like *Sclerotium rolfsii* Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn and *Fusarium* spp., has also been reported (Chet *et al.*, 1981; Elad *et al.*, 1971; Bell *et al.*, 1982; Wells *et al.*, 1972; Ghaffar, 1992). Beside control of root-infecting fungi, *T. harzianum* has also been found to antagonize plant-parasitic nematodes (Saifullah, 1996a; Dos Santos *et al.*, 1992). It shows good potential for the control of *Globodera rostochiensis, G. pallida* and *M.* javanica (Saifullah, 1996b; Saifullah and Thomas, 1996). Experiments were therefore carried out to examine the ability of *Trichoderma* spp., to control *M. javanica*, root-knot nematode in okra and mungbean.

Materials and Methods

Cultures of *Trichoderma viridi*, (Karachi University Culture Collection (KUCC-656), *T. harzianum* (KUCC-195), *T. koningii* (KUCC-427), *T. pseudokoningii* (KUCC-93) and *T. hamatum* (KUCC-29) were multiplied on potato dextrose agar (PDA) medium and were grown in Erlenmyer flasks containing Czapek's Dox liquid medium. The pH of the medium was adjusted to 6.7 before autoclaving. A5-mm diam., disc from a 5-day old culture of the fungus was inoculated in each flask. The flasks were kept at 28±2 °C in an incubator and 2 weeks later the liquid was passed through Whatman No.1 filter paper several times to remove any spores. The solution obtained was stored in refrigerator before use. One ml of the spore free culture filtrate of *Tricoderma* spp., was transferred to watch glasses separately in which two hand picked medium sized egg masses were placed. Egg masses kept in 2 ml Czapek's Dox liquid medium without the fungus served as control. After 48 h, the number of hatched juveniles were counted under a stereo microscope. The egg masses from culture filtrate were then transferred to sterile distilled water and their hatching in water was recorded to ascertain whether the egg masses kept in the filtrate had been permanently or temporarily inactivated. The emergence of juveniles was again recorded after 48 h.

To study the effects of *Trichoderma* spp., on mortality of *M. javanica* larvae, one ml of the culture was transferred into watch glasses. One ml juvenile suspension was added to each watch glass (at 30-45 juveniles/watch glass). Juveniles kept in 2 ml Czapek's Dox liquid medium without the fungus served as control. Number of dead juveniles were counted after 24 and 48 h intervals. The nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al.*, 1989).

Sandy loam soil (pH 8.1) was filled in 8-cm diam., plastic pots (350 g/pot). The soil was excavated to a depth of 3 cm and a conidial suspension of *Trichoderma* spp., viz., *T. viride* (cfu 3.6 x 10^7 ml^{-1}); *T. harzianum* (cfu 2.8x 0^7 ml^{-1}); *T. koningii* (cfu 1.8x 0^7 ml^{-1}) and *T. pseudokoningii* (cfu 3.6x10^7 ml^{-1}) were drenched separately in each pot @ 25ml/pot. After treatment, 8 seeds of okra and mungbean were sown in each pot. After germination only four seedlings were maintained in each pot. There were three replicates of each treatment and pots were randomized on the green house bench of Soil-borne Diseases Research Laboratory, Department of Botany, University of Karachi. The soil in each pot was kept at 50% W.H.C. After one week of the seedling emergence, roots in each pot were inoculated with 2000 freshly hatched, second stage juveniles of *M. javanica*. Plants were uprooted 45 days after the addition of nematode and plant height, root length and fresh weight of shoot and root were recorded. Number of galls induced by *M. javanica* and nematode populations in soil and roots were recorded. The data were subjected to analysis of variance (ANOVA). Treatments means were compared using Duncan's multiple range test (Sokal and Rolf, 1995).

Results and Discussion

Trichoderma species showed significant ($p<0.05$) inhibition in egg hatching and caused larval death of *M. javanica*. Maximum inhibition in egg hatching (44%) was recorded after treatment with culture filtrate of *T. viride* followed by *T. harzianum* which resulted in 40% inhibition in egg hatching as compared to untreated controls Table 1. Likewise culture filtrates of *T. harzianum* at 48 h caused 41% mortality of *M. javanica* compared to *T. viride* which produced 31% juveniles death (Table 2).

Soil drenches with conidial suspension of *Trichoderma* spp.,

Table 1: Effects of culture filtrates of *Trichoderma* spp., on egg hatching of Meloidogyne javanica.

Treatments	Number of juveniles hatched		Total no. of eggs hatched	Inhibition % over control
	Culture filtrate	Distilled water*		
Czapek's dox broth	122a	94a	216	-
Trichoderma koningii	121a	67bc	188	12.9
T. harzianum	83ab	47c	130	39.8
T. hamatum	93ab	82ab	175	18.9
T. pseudokoningii	92ab	82ab	174	19.4
T. viride 65b	55c	120	44.4	

* After a 48 hour exposure period in culture filtrates, egg masses were transferred to distilled water. Data represent mean of three replicates; Means followed by the same letters in each column are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test.

Table 2. Effects of culture filtrates of *Trichoderma* spp., on mortality of *Meloidogyne javanica*.

Treatments	Exposure time (hours)	
	24	48
Czapek's dox broth	0b	0c
Trichoderma koningii	17a	30ab
T. harzianum	19a	41a
T. hamatum	8ab	18abc
T. pseudokoningii	3b	16bc
T. viride	21a	31ab

Data represent mean of three replicates; Means followed by the same letters in each column are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test.

significantly reduced root knot formation due to *M. javanica* ($p<0.01$) and root knot nematode population in soil ($p<0.001$) and root ($p<0.05$). Soil application with *T. harzianum* resulted in the greatest reduction in nematode populations in soil (27% and 37%) and root (36% and 42%) and subsequent root knot disease severity (46% and 38%) in okra and mungbean respectively, as compared to controls (Table 3). Similarly *T. harzianum* significantly ($p<0.05$) increased plant height (51%) and >47%) and fresh weight of shoots (71% and 50%) of okra and mungbean respectively, in comparison with controls. The longer roots compared with untreated controls in both okra and mungbean were produced in treatments where *T. hamatum* was used whereas fresh weights of root did not differ significantly from those of controls in any of the treatments (Table 4).

Trichoderma spp., significantly reduced egg hatching, caused mortality of *M. javanica* juveniles and when incorporated in soils as drench significantly suppressed nematode populations in soil and root and subsequently reduced root knot development in both okra and mungbean. The production f antibiotics (Denis and Webster, 1971) and extra cellular lytic enzymes (Elad *et al.*, 1982) by *Trichoderma* spp., are known to be involved in the antagonism. There are reports where use of *T. harzianum* significantly suppressed root knot disease in maize (Windhum *et al.*, 1989). *T. harzianum* has also been found as an egg parasite of *M. incognita* race-3 killing 53% of eggs *in vitro* (Dos Santos *et al.*, 1992). Khan *et al.* (1977) reported a significant control of *M. incognita* and *Fusarium solani* disease complex in papaya with *T. harzianum*. Similarly *Trichoderma* spp. used individually or in combination

Table 3: Effects of *Trichoderma* spp., on the development of root knot infection and nematode population in soil and root of okra and mungbean.

Treatments	Galls per root system		Root knot nematode population			
			Soil (250 g)		Juveniles/g root	
	Okra	Mung	Okra	Mung	Okra	Mung
Control	45	55	2150	2410	95	125
Trichoderma viride	30	42	1620	1800	76	76
T. koningii	30	42	1930	1910	64	112
T. hamatum	25	40	1590	1750	69	91
T. harzianum	24	34	1580	1530	61	72
T. pseudokoningii	35	47	2050	2230	72	91
LSD ($p=0.05$) Treatments	7		362		22	
Host	4		209		13	

Table 4: Effects of *Trichoderma* spp., on growth of okra and mungbean plants.

Treatments	Plant height		Shoot weight		Root length		Root weight	
	Okra	Mung	Okra	Mung	Okra	Mung	Okra	Mung
Control	21.3	16.4	0.7	0.5	7.4	9.3	0.6	0.8
Trichoderma viride	26.4	21.9	0.8	0.7	8.4	10.4	0.6	0.5
T. koningii	24.6	21.0	0.8	0.6	8.3	10.4	0.6	0.6
T. hamatum	27.7	22.5	1.0	0.6	9.0	13.8	0.8	0.7
T. harzianum	31.5	24.7	1.2	1.0	8.7	13.2	0.6	0.6
T. pseudokoningii	25.2	20.0	0.8	0.5	8.2	13.5	0.5	0.8
LSD ($p=0.05$)	3.9	2.3	0.4	0.1	2.4	3.1	0.3	0.3

with *Pseudomonas aeruginosa*, a pant growth promoting rhizobacterium, significantly controlled root rot-root knot disease complex in chili (Siddiqui *et al.*, 1999). Further investigation is needed for the isolation and characterization of nematicidal compound (s) produced by *Trichoderma* spp.

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