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Effect of Culture Filtrate of Fungi in the Control of *Meloidogyne javanica*, Root Knot Nematodes on Okra and Broad Bean

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Abstract

Fungal cell free filtrates were used in two different doses, enhanced plant growth and root knot nematodes infection was reduced where high doses of filtrate (100% concentration) were applied, in all test fungal filtrates. Culture filtrates of *Paecilomyces lilacinus* and *Verticillium chlamyosporium* at 100 percent concentration showed significant reduction in *Meloidogyne javanica* root knot infection on okra and broad bean as compared to *Trichoderma harzianum*, *T. koningii*, *T. viride*, *Aspergillus restrictus* and *Aspergillus sp.*, which found less effective.

Introduction

Chemical nematicides are generally used for the control of plant parasitic nematodes (Gowen, 1992). Many of the chemicals have proved to be carcinogens, build up residues in food plants and infiltrate into ground water, which has led to the total ban in developed countries (Zuckerman & Ensard, 1994). Biological control is considered as an alternate method to chemical pest management. Several microorganisms produce toxic substances in culture media, which effect nematode activity (Ali, 1989; Sakhuja *et al.*, 1978).

Experiments were therefore carried out to study the efficacy of fungal cell free culture filtrates of some microbial antagonists in the control of *Meloidogyne javanica* (Treub) Chitwood root knot nematode on okra (*Abelmoschus esculentus* Moench.) and broad bean (*Phaseolus vulgaris* L.) under green house conditions.

Material and Methods

Czapek's Dox liquid medium in 250 ml Erlmyer flasks (100 ml/flask) were inoculated with 5 mm diam., discs of test fungi viz., *Paecilomyces lilacinus* (Thom.) Samson, *Verticillium chlamyosporium* Goddard, *Trichoderma harzianum* Rifai, *T. koningii* Oudem, *T. viride* Pers. ex Gray, *Aspergillus restrictus* G.Sm, and *Aspergillus sp.* species, incubated at 28°C. After three weeks growth, the culture was filtered through No. 1 Whatman filter paper (atleast twice) and the filtrates were designated as 100 percent concentration (standard dilution 'S') which was diluted 50 percent by addition of sterilized distilled water. Sandy loam soil, pH 8.1 was obtained from experimental fields of Botany Department, University of Karachi was transferred in 8 cm diam., plastic pots, 300 g soil/ pot. Five seeds of each test crop viz., okra and broad bean were sown in each pot. Each set of pots was treated with 25 ml of culture filtrates. Distilled water served as control. After two weeks growth three plants were kept in each pot. *Meloidogyne javanica* root knot nematode eggs obtained using the method of Hussey & Barker (1973), were inoculated into three holes around the base of each plant @ 2000 eggs & 1/2 pot. Treatments were replicated three times and randomized on green house bench. Plants were uprooted after 45 days of nematode inoculation. Roots were gently washed to remove adhering soil, fresh weights and length

of shoots and roots were measured. Galls were counted under binocular microscope and RKI determined using 0-5 scale of Taylor and Sasser (1978). The roots were stained with lactophenol acid fuchsin (Franklin, 1949) and nematode population in roots counted under stereoscope microscope. Population of nematode in soil was determined using Baermann modified funnel technique (Schindler, 1961). Data were statistically analyzed according to Gomez & Gomez (1984).

Results

Okra: Cell free culture filtrates of fungi significantly suppressed nematodes infection on okra plants. Nematode reproduction, gall formation, egg mass production, nematode population in soil and nematode invasion was significantly decreased compared to control. Maximum reduction in gall formation, egg mass production, nematode population in soil and invasion in roots were recorded in treatments where 100 percent conc. of *P. lilacinus* was used as compared to 50 percent conc. of the filtrate which did not show significant reduction (Table 2). Cell free culture filtrate of *V. chlamyosporium* (100% conc.) found less effective as compared to *P. lilacinus* (100% conc.). *Trichoderma harzianum* (100% conc.) showed lethal effect against root knot nematodes whereas *T. koningii*, *T. viride*, *A. restrictus*, *Aspergillus sp.* also showed less degree of control.

Culture filtrates, of fungi also showed an increase in plant height, shoot weight and root length as compared to untreated plants (Table 1.). Significant ($p < 0.05$) results were observed where *P. lilacinus* (100% conc.), *T. harzianum* (100% conc.) were used as compared to control.

Broad Bean: Where culture filtrates of *P. lilacinus* (100% conc.) and *V. chlamyosporium* (100% conc.) were used respectively 74.07 percent and 70.21 percent reduction in soil population of nematode was obtained compared to control (Table 4.). Culture filtrate of other fungi found less effective in controlling root knot infection on broad bean. *P. lilacinus* (100% conc.) was found more effective against root-knot nematodes by limiting their soil population density and invasion in broad bean roots.

There was significant ($p < 0.05$) effect on plant growth where *T. harzianum* culture filtrate (100%) was applied, an

Table 1. Effect of cultural filtrate of fungal antagonists on growth of okra (*Abelmoschus esculentus* Moench.)

Treatments	Plant length (cms)	Shoot weight (g)	Root length (cms)	Root weight (g)
Sterilized Distilled Water	30.22	2.87	6.43	3.05
<i>Paecilomyces lilacinus</i> (50%)	36.36	3.04	10.47	0.80
<i>P. lilacinus</i> (100%)	38.83	3.20	12.11	0.68
<i>Verticillium chlamydosporium</i> (50%)	36.36	2.74	9.86	0.88
<i>V. chlamydosporium</i> (100%)	37.70	3.62	11.33	0.83
<i>Trichoderma harzianum</i> (50%)	39.99	2.91	10.08	1.23
<i>T. harzianum</i> (100%)	41.58	3.41	12.36	0.70
<i>T. koningii</i> (50%)	38.25	2.77	9.75	1.00
<i>T. koningii</i> (100%)	40.33	3.56	15.08	0.80
<i>T. viride</i> (50%)	35.83	2.98	9.83	0.84
<i>T. viride</i> (100%)	37.55	3.91	11.83	0.75
<i>Aspergillus restrictus</i> (50%)	32.99	2.99	10.16	0.86
<i>A. restrictus</i> (100%)	36.47	3.10	12.33	0.73
<i>Aspergillus species</i> (50%)	34.00	2.89	10.21	0.95
<i>spergillus species</i> (100%)	36.08	3.13	10.32	0.88
SED (p 0.05)	2.11	0.57	1.44	0.28

Table 2: Effect of cultr filtrates of fungal antagonists on the development of root knot nematodes *Meloidogyne javanica* on okra plants.

Treatments	Galls / root system	RKI (0-5)	Egg masses/ root sys.,	Population			Rf = Pfl
				/300cc soil	root pop./pot	Total	
Sterilized Distilled Water	86.44	4.71	120.99	2035.80	2692.09	4727.89	2.36
<i>Paecilomyces lilacinus</i> (50%)	47.77	3.25	45.88	1095.99	1153.24	2249.23	1.12
<i>P. lilacinus</i> (100%)	30.22	3.10	32.33	631.99	699.72	1331.71	0.66
<i>Verticillium chlamydosporium</i> (50%)	49.44	3.60	44.66	1152.00	690.89	2112.89	1.05
<i>V. chlamydosporium</i> (100%)	35.5	3.12	34.22	715.99	593.02	1309.01	0.65
<i>Trichoderma harzianum</i> (50%)	50.22	3.58	47.55	1215.99	1449.40	2665.39	1.33
<i>T. harzianum</i> (100%)	47.99	3.37	43.33	864.00	1306.60	2170.60	1.08
<i>T. koningii</i> (50%)	58.55	3.68	51.21	1296.00	1582.20	2878.20	1.43
<i>T. koningii</i> (100%)	50.58	3.45	46.88	1020.00	1240.47	2260.47	1.33
<i>T. viride</i> (50%)	50.21	3.40	47.11	1548.00	1634.88	3182.88	1.59
<i>T. viride</i> (100%)	45.94	3.38	40.99	1047.96	1304.88	2352.84	1.17
<i>Aspergillus restrictus</i> (50%)	61.88	3.88	53.11	1229.19	1684.51	2913.70	1.45
<i>A. restrictus</i> (100%)	49.11	3.42	44.50	1095.96	1595.74	2691.70	1.34
<i>Aspergillus species</i> (50%)	62.33	3.86	58.44	1584.00	1786.72	3370.72	1.68
<i>Aspergillus species</i> (100%)	40.44	3.55	39.66	1070.70	1308.88	2379.58	1.18
SED (p 0.05)	5.01	0.18	6.72	727.44	291.42	-	-

Table 3: Effect of cultural filtrate of fungal antagonists on growth of broad bean (*Phaseolus vulgaris* L.)

Treatments	Plant length (cms)	Shoot weight (g)	Root length (cms)	Root weight (g)
Sterilized Distilled Water	55.09	5.97	9.11	2.88
<i>Paecilomyces lilacinus</i> (50%)	62.00	7.73	11.2	1.35
<i>P. lilacinus</i> (100%)	81.88	8.76	12.83	1.25
<i>Verticillium chlamydosporium</i> (50%)	63.83	7.36	11.05	1.38
<i>V. chlamydosporium</i> (100%)	65.16	8.26	11.77	1.27
<i>Trichoderma harzianum</i> (50%)	78.16	8.83	12.33	1.77
<i>T. harzianum</i> (100%)	83.16	9.89	16.66	1.37
<i>T. koningii</i> (50%)	73.33	7.13	10.60	1.79
<i>T. koningii</i> (100%)	78.05	8.05	12.88	1.22
<i>T. viride</i> (50%)	63.44	6.47	9.33	1.79
<i>T. viride</i> (100%)	69.33	7.49	12.16	1.16
<i>Aspergillus restrictus</i> (50%)	64.16	5.18	10.05	1.72
<i>A. restrictus</i> (100%)	70.33	7.45	10.16	1.18
<i>Aspergillus species</i> (50%)	61.00	6.47	6.50	1.85
<i>Aspergillus species</i> (100%)	68.00	7.12	9.33	1.50
SED (p 0.05)	5.88	1.29	2.36	0.29

Table 4: Effect of culture filtrates of fungal antagonists on the development of root knot nematodes *Meloidogyne javanica* on broad bean

Treatments	Galls / root system	RKI (0-5)	Egg masses/ root sys.,	Population		Rf = Pf/Pi	
				/300cc soil	root pop./pot		
sterilized Distilled Water	44.66	3.83	33.22	1773.00	1407.36	3180.36	1.59
<i>Penicillium lilacinus</i> (50%)	25.44	3.16	22.19	759.60	460.24	1219.84	0.60
<i>Penicillium lilacinus</i> (100%)	19.75	3.00	15.53	459.60	359.85	819.45	0.40
<i>Verticillium chlamyosporium</i> (50%)	27.44	3.27	27.22	948.00	480.85	1428.85	0.71
<i>Verticillium chlamyosporium</i> (100%)	21.66	3.10	18.05	528.00	333.45	861.45	0.43
<i>Trichoderma harzianum</i> (50%)	24.66	3.16	20.94	1052.40	673.65	1726.05	0.86
<i>Trichoderma harzianum</i> (100%)	21.25	3.10	20.36	583.99	491.81	1075.8	0.53
<i>Trichoderma koningii</i> (50%)	30.55	3.28	31.12	1110.0	734.81	1844.81	0.92
<i>Trichoderma koningii</i> (100%)	27.33	3.20	28.38	696.00	461.33	1157.33	0.57
<i>Trichoderma viride</i> (50%)	26.38	3.19	26.05	1074.00	675.83	1749.83	0.87
<i>Trichoderma viride</i> (100%)	25.01	3.11	24.27	787.92	421.44	1209.36	0.60
<i>Aspergillus restrictus</i> (50%)	30.33	3.30	27.11	1161.60	723.18	1884.78	0.94
<i>Aspergillus restrictus</i> (100%)	23.33	3.16	26.44	1027.20	458.75	1485.95	0.74
<i>Aspergillus species</i> (50%)	32.27	3.55	30.04	1092.00	801.84	1893.84	0.94
<i>Aspergillus species</i> (100%)	28.25	3.37	27.33	942.00	574.19	1519.19	0.75
ED (p 0.05)	4.6	0.2	3.93	60.55	145.85		

Increased in plant height, shoot weight and root length was observed (Table 3). Cultural filtrate of *P. lilacinus* (100%) found slightly less effective to enhance broad bean growth. All the plant growth parameters viz., plant height, shoot weight, root length and weight was influenced by cultural filtrate of *P. lilacinus* (100%). Cultural filtrates of all the remaining test fungi showed less degree of growth enhancement.

Discussion

The results of present studies showed reduction in the root knot index and nematode population in root and soil and an increase in plant growth parameters in the treatments where okra and broad bean plants were grown in soil drenched with culture filtrate of *P. lilacinus* (100% conc.). *P. lilacinus* has been reported as an effective biological control agent of root knot nematodes (Jatala, 1986). The activity of the toxic metabolites of *P. lilacinus* suggests a neurotropic mechanism (Cayrol *et al.*, 1989). Serine protease from *P. lilacinus* liquid culture plays an important role in the penetration of the fungus through egg shell of nematodes as incubation of purified protease with nematode eggs significantly influenced their development (Bonants *et al.*, 1995). The reduction in root knot index observed in *P. lilacinus* treatment may be due to less invasion of the root knot juveniles.

V. chlamyosporium has also been reported to parasitize mature females of cyst nematode eggs and female of root knot nematodes (Lopez-Llorca and Duncan, 1991; Zaki, 1993). *V. chlamyosporium* produces a number of lytic enzymes which help in pathogenesis (Webb *et al.*, 1972). *In vitro*, culture filtrate of *P. lilacinus* has shown its toxicity against root knot nematodes by hampering their hatching and increasing mortality rate with increase in exposure of time (Zaki, 1999). *V. chlamyosporium* has shown good control of root-knot nematode infestation on okra (Leij *et al.*, 1992). It has been reported that species

of *Verticillium* produce compounds like Verticillin which may be responsible for suppression of root knot nematodes both *in vitro* and *in vivo*.

Very little attention has been given on the interaction between *Trichoderma* spp. and nematodes (Windham *et al.*, 1989). Dos Santos *et al.*, (1992) reported *T. harzianum* as an egg parasites of *Meloidogyne incognita* race-3, which was found to kill 53 percent of eggs *in vitro*. In the present studies *T. harzianum*, *T. koningii* and *T. viride* showed enhancing effect on test crops and proved to be beneficial agents in hindering the nematode infection.

In the present study, culture filtrates of *A. restrictus* and *Aspergillus* sp. suppressed root knot infestation, which may be due to the metabolites produced in culture filtrate. There are reports that *Aspergillus* species may cause killing effect against nematodes (Mankau, 1969., Desai *et al.*, 1972, Khan *et al.*, 1984).

The presence of toxic metabolites in fungal culture filtrate has been reported to reduce root knot infection and enhance plant growth (Alam *et al.*, 1973; Sakhuja *et al.*, 1978; Khan *et al.*, 1984; Mani & Sethi, 1984; Ali, 1990; Zaki, 1999). Use of hyperparasitic fungi as cell free culture filtrate could provide better plant health, yield and protection against phytonematodes in environmentally acceptable way.

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