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Effect of Fungal Culture Filtrates on Mortality and Hatching of *Meloidogyne javanica*

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Abstract

Mortality of *Meloidogyne javanica* larvae was directly proportional to the concentration of culture filtrates of all tested fungi viz., *Verticillium chlamydosporium*, *Paecilomyces lilacinus* and *Talaromyces flavus* and the duration of exposure. Maximum mortality of larvae was observed in higher concentration of *P. lilacinus* (82%) followed by *V. chlamydosporium* (66%). Similarly different dilutions of culture filtrates significantly inhibited hatching of *M. javanica* eggs. Larval emergence was however, inversely proportional to filtrate concentrations. Significant lower hatching was observed with *P. lilacinus* 17% followed by *V. chlamydosporium* (15%) in 'S' concentrations.

Introduction

Various biocontrol agents viz., *Verticillium chlamydosporium* and *Paecilomyces lilacinus* which are parasites of eggs of root knot and cyst nematodes (Jatala *et al.*, 1979; Zaki and Maqbool, 1993) have also been found to effectively control root knot nematodes on crop plants (Zaki and Maqbool, 1991, 1996). *Talaromyces flavus*, a biocontrol agent of root infecting fungi (Fahima and Henis, 1990; Ehteshamul-Haque *et al.*, 1994) has been reported to reduce root knot infestation on okra plants (Zaki and Maqbool, 1998). Fungi and phytoparasitic nematodes being a common inhabitant of crop rhizosphere showing synergistic, antagonistic or antibiotic relationship (Jorgenson, 1970). Since they occur together in the rhizosphere, the toxic metabolite naturally produced by microorganisms may be responsible for keeping low level of nematode population. Comparatively little work has been done about the effect of toxic fungal metabolites on nematodes. It was therefore, considered useful to study the effect of fungal culture filtrates of *V. chlamydosporium*, *P. lilacinus* and *T. flavus* on hatching and mortality of root knot nematode larvae.

Materials and Methods

Pure cultures of *V. chlamydosporium*, *P. lilacinus* and *T. flavus* were obtained from the Culture Collection of the Department of Botany, University of Karachi. Culture filtrates were prepared by growing these fungi in Potato Dextrose Broth in 250 mls flasks, 100 mls in each, for 15 days at 28°C. Mycelial mats were removed and the media were filtered through Whatman No. 1 filter paper. This solution was designated as standard (S). Further dilutions (25, 50 and 75%) were prepared by adding requisite amount of sterilized distilled water. Two mls of each solution and 0.1 ml of distilled water containing about 50-60 second stage larvae of *M. javanica* were poured in each cavity block. Nematodes kept in distilled water served as control. Each treatment was replicated thrice. Number of dead nematodes (unmoved) in each cavity block were

counted after 24, 48 and 72 hours.

Freshly collected equal sized egg masses were transferred to each cavity block (one egg mass per cavity block) containing 4 mls of different dilutions of culture filtrates. Egg masses placed in distilled water served as control. There were three replicates for each treatment. Number of hatched larvae in each treatment was counted after 24, 48 and 72 hours. Means egg hatch and larval mortality were calculated and data was statistically analysed.

Results and Discussion

The larval mortality percentage differed with species and concentrations of culture filtrates. Mortality of *M. javanica* larvae was directly proportional to the concentrations of filtrates and the duration of exposure (Table 1). Culture filtrates of *P. lilacinus* and *V. chlamydosporium* were more effective in causing death of larvae as compared to filtrate of *T. flavus* and control. Maximum mortality of larvae was observed in higher concentration of *P. lilacinus* (82%) followed by *V. chlamydosporium* (66%).

Similarly different dilutions of culture filtrates significantly inhibited hatching of *M. javanica* eggs. Larval emergence was, however, inversely proportional to filtrate concentrations. Significant lower hatching was observed with *P. lilacinus* (7%) followed by *V. chlamydosporium* (15%) in "S" concentrations (Table 2).

There are reports that culture filtrates of several soil borne fungi are known to exhibit nematicidal action beside inhibiting larval emergence of plant parasitic nematodes (Alam *et al.*, 1973; Cayrol *et al.*, 1989; Ali, 1990). *Helminthosporium nodulosum*, *Trichoderma lignorum*, *Curvularia tuberculata*, *Penicillium corylophilum* and *A. niger* killed the nematode and inhibited the egg hatch of *M. incognita* and *Hoplolaimus indicus* (Alam *et al.*, 1973). Culture filtrates of *Aspergillus* spp., were found to have some nematicidal properties (Mankau, 1969; Khan *et al.*, 1984). Culture filtrates of *Fusarium oxysporum* and *F. solani* exhibited high degree of nematicidal effect on

Zaki: Nematicidal effect, culture filtrates, root-knot nematode, biocontrol agents

M. incognita (Mani and Sethi, 1984). *Arthrobotrys oligospora* and *Dactylaria brachopage* inhibited hatching of *M. incognita* (Walia and Swarup, 1985).

Table 1: Effect of fungal culture filtrates on mortality percentage of second stage larvae of *Meloidogyne javanica*

Treatments (%)	Mortality %age (hr. of exposure)		
	24	48	72
Control			
PD Broth S	Oa	Oa	2h
100	Oa	Oa	2h
75	Oa	Oa	Oh
50	Oa	Oa	Oh
25	Oa	Oa	Oh
<i>V. chlamydosporium</i> S			
100	40d	56d	66b
75	20c	30c	38d
50	2b	4b	18e
25	Oa	Oa	Oh
<i>P. lilacinus</i> S			
100	40d	56d	82a
75	24c	30c	54c
50	4b	6b	10f
25	Oa	Oa	2h
<i>T. flavus</i> S			
100	2b	4b	10f
75	Oa	2b	6g
50	Oa	Oa	Oh
25	Oa	Oa	Oh

Means followed by the same letters in each column are not significantly different at 5% level according to Duncan's multiple range test

Table 2: Effect of fungal culture filtrates on hatching of *Meloidogyne javanica* eggs

Treatments (%)	No. of eggs hatched in hours of exposure		
	24	48	72
Control	38b	75a	117a
PD Broth S			
100	35c	65c	100bc
75	32d	69b	101bc
50	39ab	57de	98c
25	42a	71b	105b
<i>V. chlamydosporium</i> S			
100	2g	5kd	15i
75	4g	15i	20i
50	10f	35g	50g
25	40ab	60d	80e
<i>P. lilacinus</i> S			
100	1g	3k	7j
75	2g	10j	18i
50	10f	25h	40h
25	25c	55e	The
<i>T. flavus</i> S			
100	8f	12ij	18i
75	25e	35g	40h
50	39d	40f	60f
25	40ab	60d	90d

Means followed by the same letters in each column are not significantly different at 5% level according to Duncan's multiple range test

In the present studies the culture filtrates of all tested fung inhibited egg hatch and mortality of *M. javanica* to a varying degree. The difference in response to the nematode may due to differences in the nature of toxic metabolites due to differences in the nature of toxic metabolites *Verticillium* and *Talaro myces* are known to produce toxins and antibiotic like *paecilotoxin*, *verticillin* A, B and C, *vermiculin*, *vermicillin*, *talaron* and vermiotatin (Minato *et al.*, 1973; Fuska *et al.*, 1979; Mikami *et al.*, 1989). Our results strongly indicate the production of toxins by *P. lilacinus* and *V. chlamydosporium* in growth media whip have adverse effect on the hatching of eggs and mortality of second stage larvae of *M. javanica*. There is need to characterize the nematicidal principle present in the culture filtrates of these fungi.

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