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In Vitro Evaluation of Ovicidal and Larvicidal Effects of Culture Filtrate of *Verticillium chlamydosporium* Against *Meloidogyne javanica*

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ABSTRACT

Effects of culture filtrate of *Verticillium chlamydosporium* were evaluated on mortality of larvae and hatching of eggs of *Meloidogyne javanica*. Mortality of *M. javanica* larvae was found to be directly proportional to concentrations of culture filtrate of *V. chlamydosporium* and duration of exposure. Maximum mortality of larvae (65.5%) was observed in 100% concentration of culture filtrate after 72 h followed by 80% concentration which caused 53.25% mortality. Similarly different dilutions of culture filtrate significantly inhibited hatching *of M. javanica* eggs. Larval emergence was, however, inversely proportional to filtrate concentrations. Significant lower hatching was observed in 100% concentration.

Key Words: Culture filtrate; *Verticillium chlamydosporium;* Mortality; Hatching; *Meloidogyne javanica*

INTRODUCTION

Controlling any soil inhabitant pathogen in a crop is difficult and root-knot disease caused by *Meloidogyne* spp. is no exception. Nematicides may play a major role in crop protection and production threatened by diseases when other practical and economical measures are lacking. In recent years the ban on certain nematicides due to health hazards and environmental concerns has prompted the search for alternatives to manage nematode damage.

Of various approaches that could be adopted for the control of nematodes, biological control is receiving increased interest among nematologists. Biological control includes the use of predaceous or parasitic organisms such as fungi, bacteria, protozoa, viruses, nematodes and mites etc. Nematode destroying fungi are widespread in soil and consistently associated with the bio-control of nematodes in nature. These fungi kill and destroy nematodes by prey catching devices such as sticky branches, networks, knobs, constricting and non-constricting rings; by producing toxic substances and adhesive spores and by colonizing nematode reproductive structures.

VerticilIium chlamydosporium is a wide spread fungus that proliferates in the rhizosphere and parasitizes females and eggs of cyst and root-knot nematodes. A lot of literature is available on *V.chlamydosporium* which suggests that the fungus has potential as a biocontrol agent for cyst and root-knot nematodes (Kerry *et al*., 1984; De Leij & Kerry, 1991; Mousa *et al*., 1995). Since fungi and nematodes occur concomitantly in the rhizosphere, the toxic metabolites naturally produced by these fungi may be responsible for keeping low levels of nematode population. Adverse effects of such substances present in the culture filtrates of several soil borne fungi on hatching and mortality of root-knot nematodes have been studied by many workers (Desai *et al*., 1972; Cayrol *et al.*, 1989; Saifullah, 1996). The present study was, however, conducted to evaluate the ovicidal and larvicidal effects of culture filtrate of *V. chlamydosporium* against *M. javanica*.

MATERIALS AND METHODS

Culture filtrates were obtained by growing *V. chlamydosporium* on Potato Dextrose broth. The medium (100 mL in 250 mL flasks) was autoclaved at 15 psi for 15 minutes. Each flask was then inoculated with four scoops of 5 mm diameter of the fungus from an actively growing culture of the fungus on corn meal agar under sterilized conditions and incubated at 25^0C for 15 days. Some flasks were inoculated with the scoops of the medium only. At the end of incubation period, the cultures were passed through Whatman filter paper No. 1 to remove the mycelial mats. Filtrates thus obtained were designated as 100% concentration. Further concentrations (20, 40, 60, and 80%) were made by adding requisite amount of sterilized distilled water. The filtrates were stored at 4^0C until use.

The population of *Meloidogyne javanica* used in the experiment was already maintained on the roots of tomato plants in the glasshouse of the Department of Plant Pathology, University of Agriculture, Faisalabad and mass cultured on tomato cv. Money maker. Eggs were isolated and nematodes were extracted (Hussey & Barker, 1973).

To investigate the effect of different concentrations of the culture filtrate of *V. chlamydosporium* on larval hatching of *M. javanica*, 5 mL of culture filtrate from each concentration was pipetted into 5-cm-dia. Petri dishes.

Three uniformed-sized egg masses of *M. javanica* were hand picked and placed into each dish. Egg masses placed in distilled water served as control. Each treatment was replicated five times. All dishes were maintained at 25^0C. Data on egg hatching were recorded after 24, 48, and 72 h. Fresh fungal filtrate was replaced after each data. The same procedure was repeated using potato dextrose broth in order to compare the effect of culture filtrate with that of the medium.

Similarly, to study the effect of different concentrations of the culture filtrate on larval mortality, 5 ml of the culture filtrate from each concentration was poured into each Petri dish and about 80 freshly hatched second-stage juveniles of *M.javanica* in 0.2 mL distilled water were added to each Petri dish. Juveniles added in distilled water served as control. Each treatment was replicated five times. Number of dead (unmoved) larvae in each Petri dish were counted after 24, 48 and 72 h and their percentages were calculated. In order to compare the effect of culture filtrate with that of the medium, the same procedure was followed using the medium. All the data collected were analyzed statistically by using Analysis of Variance and Regression Analysis (Muhammad, 1991).

RESULTS

Effect of culture filtrates on egg hatching of *M. javanica.* The analysis of variance regarding effect of culture filtrates on hatching of *M.javanica* juveniles showed significant interaction ($P < 0.01$) between filtrates and concentrations (F X C), filtrates and time interval (F X T), concentrations and time(C X T) and among filtrates, their concentrations and time (F X C X T).

Data presented in Table I showed that mean number of juveniles hatched in medium filtrate was significantly higher than those hatched in the culture filtrate. As mean hatching observed in different concentrations of the medium filtrate was found to be statistically similar to that observed in distilled water (0% concentration), it was concluded that medium itself did not show ovistatic or ovicidal property.

Larval emergence was found to be inversely proportional to filtrate concentration and time interval. Different dilutions of the culture filtrate significantly inhibited hatching of *M. javanica* eggs. Maximum inhibition in hatching was observed in 100% and 80% concentrations of the culture filtrate followed by 60%, which, however, was statistically not different from the highest concentrations. Significantly lesser juveniles emerged after 72 h as compared to 48 and 24 h interval.

With an increase in the concentration of culture filtrate there was a significant decrease in hatching. These relationships are shown by regression equations given below.

24 h $Y= 2.20 – 0.0057 X \quad R^2 = 0.83$
48 h $Y= 1.60 - 0.0092 X \quad R^2 = 0.79$
72 h $Y= 1.33 – 0.0115 X \quad R^2 = 0.81$

Effect of culture filtrates on larval mortality of *M. javanica.* The analysis of variance regarding effect of culture filtrate on percent mortality of *M. javanica* juveniles showed significant interaction ($P< 0.01$) between filtrates and concentrations (F X C), filtrates and time interval (F X T), concentrations and time (C X T) and among filtrates, their concentrations and time (F X C X T).

It is apparent from the perusal of the data presented in Table-II that mean percent mortality in culture filtrate of the fungus differed significantly from that in the medium filtrate and it is concluded that medium itself had no nematostatic or nematicidal effect as the mean mortality observed in different concentrations of the medium filtrate was statistically similar to the mortality observed in distilled water (0% concentration).

Percent juvenile mortality in the culture filtrates of the fungus was found to be directly proportional to the concentrations of the culture filtrate and the duration of exposure. Maximum mortality (50.92%) was observed in 100% concentration of the fungal filtrate whereas it was minimum in 20% concentration which was statistically similar to that in 0% concentration (distilled water).

The mean percent mortality in fungal filtrate after 24 hours was 13.25% which increased significantly to 20.42% after 48 hours and finally reached to 28.42% after 72 h.

Mean individual mortality (65.5%) was observed in 100% concentration of culture filtrate after 72 h followed by 80% concentration which caused 53.25% mortality. Mean percent mortalities in medium filtrate and culture filtrate at different concentrations and durations are given in Table II.

A progressive increase in the concentrations of the culture filtrate resulted in increase in the percent mortality of juveniles. The relationships between the concentrations and percent mortality at different time intervals are given by regression equations

24 h $Y= - 5.43 + 0.374 X \quad R^2= 0.91$
48 h $Y= - 7.15 + 0.551 X \quad R^2= 0.92$
72 h $Y= - 5.76 + 0.684 X \quad R^2= 0.95$

DISCUSSION

Adverse effects of culture filtrates of several fungi on hatching and mortality of root-knot nematodes have been reported by many workers (Cayrol *et al.*, 1989; Saifullah, 1996 & Zaki, 1999).

Nematicidal action of culture filtrates of *V.chlamydosporium* against *M.javanica* may be attributed to the production of certain enzymes (Webb *et al.*, 1972; Segers *et al.*, 1994) and toxins like Verticillin A, B and C (Minato *et al.*, 1973) which help in weakening and dissolving the barriers of its hosts. Species of *Aspergillus, Penicillium, Talaromyces, Curvularia* and *Aternaria* are known to produce toxins and antibiotics like aflatoxin, penicillin, vermiculin, vermicillin, talaron, vermistatin, viridin, fusaric acid, rhizopin, lilacinin, leucinostatin, P-168 and phytoalternarin (Nafe-Roth, 1972; Arai *et al.*, 1973; Das

Table I. Effect of culture filtrate of *V. chlamydosporium* on hatching of *M. javanica eggs*

Filtrates	Concentration (%)	Hatching after			Mean
		24 hours	48 hours	72 hours	
	0	218.8 a	60.6 e	43.6 fgh	107.7 A
Medium	20	209.4 ab	57.0 e	37.0 h	101.1 A
Filtrate	40	205.0 b	58.8 e	40.8 gh	101.5 A
(No fungus)	60	212.2 ab	57.4 e	40.8 gh	103.5 A
	80	207.2 b	56.4 e	39.4 h	101.0 A
	100	213.6 ab	55.0 e	37.6 h	102.1 A
		211.03 A	57.53 C	39.87 D	102.81 A
	0	218.8 a	60.6 e	43.6 fgh	107.70 A
	20	99.8 c	20.8 i	6.4 jk	42.33 B
Culture	40	77.4 d	15.2 ij	5.0 jk	32.53 C
Filtrate	60	61.4 e	9.6 ijk	5.2 jk	25.40 CD
	80	54.8 ef	4.6 jk	2.6 k	20.67 D
	100	51.8 efg	8.6 jk	1.8 k	20.73 D
		94.00 B	19.90 E	10.77 F	41.56 B

LSD values at 0.05 for Filtrates (F) = 13.35; Concentrations (C) = 5.45; Time (T) = 3.29; F X C = 7.71 F X T = 4.65; C X T = 8.04; F X C X T = 11.39

Table II. Effect of culture filtrate of *V. chlamydosporium* on mortality of *M. javanica*

Filtrates	Concentration (%)	% Mortality			Mean
		24 hours	48 hours	72 hours	
	0	0.00 n	0.25 mn	2.25 ijkl	0.83 E
Medium	20	0.00 n	0.50 lmn	3.50 hi	1.33 E
Filtrate	40	0.00 n	0.00 n	1.75 ijklmn	0.58 E
(No fungus)	60	0.50 lmn	0.75 lmn	1.25 jklmn	0.83 E
	80	0.50 lmn	1.00 klmn	1.50 jklmn	1.00 E
	100	0.50 lmn	1.00 klmn	2.00 ijklmn	1.17 E
		0.25 E	0.58 E	2.04 D	0.96 B
	0	0.00 n	0.25 mn	2.25 ijkl	0.83 E
	20	1.50 jklmn	2.75 hijk	4.25 h	2.83 E
Culture	40	3.00 hij	4.25 h	15.00 g	7.42 D
Filtrate	60	13.75 g	24.50 f	30.25 e	22.83 C
	80	25.50 f	39.25 c	53.25 b	39.33 B
	100	35.75 d	51.50 b	65.50 a	50.92 A
		13.25 C	20.42 B	28.42 A	20.69 A

LSD values at 0.05 for Filtrates (F) = 4.58; Concentrations (C) = 2.24; Time (T) = 0.55; F X C = 3.17; F X T = 0.78; C X T = 1.36; F X C X T = 1.92

& Pal, 1974; Wheeler, 1975; Fuska *et al*., 1979; Isogai *et al*., 1981; Agarwal & Bisen, 1984; Ghewande *et at*., 1984; Mikamy *et al*., 1989). There are some fungi which produce appreciable amount of oxalic acid in the culture medium (Franke & de Boer, 1961) which killed root-knot nematode larvae (Desai *et al*., 1972).

These toxic chemicals may affect the phytonematodes in different ways. Cayrol *et al.* (1989) suggested that the toxic metabolites have a neurotropic action upon the nervous receptors of the nematodes. Saifullah (1996) found that culture filtrate of *V. chlamydosporium* caused 100% mortality of potato cyst nematode males and *Panagrellus redivivus* and a strong contraction of the nematode muscles causing the spicules to be pushed out. Nematodes ruptured in the middle probably because of the digestive enzymes, the fungus released into the medium. The enzymes of the fungus were not able to make the cuticles of nematodes permeable. The toxic substances might, therefore, have entered through the natural pores.

A number of nematophagous fungi are known to have proteolytic and chitinolytic activities which cause alteration in eggs' cuticular structure, changes in egg shell permeability or cause perforations in the cuticle which allows seepage of toxic metabolites into the eggs and cause physiological disorders (Webb *et al*., 1972; Jatala *et al*., 1985; Lopez-Llorca, 1990). These factors may have important role in the inhibition of egg hatch. *V. chlamydosporium* produces a subtilisin, which breaks down the outer membrane of the egg shell and exposes the chitin layer of root-knot nematode eggs (Segers *et al*., 1994). Lopez-Llorca and Robertson (1992) reported the secretion of a serine protease designated P-32, produced by *V. chlamydosporium* during infection of nematode eggs. Similar enzyme was also produced by nematode trapping fungus, *Arthrobotrys oligospora* (Tunlid *et al*., 1994). It has also been reported that 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).

It is suggested that the study should be extended to characterize the substances produced in the culture filtrate of *V. chlamydosporium*.

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