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EFFECT OF CULTURE FILTRATES OF *PAECILOMYCES LILACINUS* ON *MELOIDOGYNE JAVANICA*

by
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Summary. Laboratory experiments showed that fungal filtrates of *Paecilomyces lilacinus* inhibited the hatching of *Meloidogyne javanica* at all the concentrations used when exposed for 72 hours, which, however, resumed partially in water. Irreversible inhibition ranged from 47 to 62 per cent. The fungal filtrates immobilized second-stage juveniles, more, in higher concentrations. Activity of filtrates obtained from the buffered medium was stronger than that from unbuffered medium. However, activity of J2 resumed in all the cases after 24 h exposure.

Jatala (1986) and Zaki (1988) recorded some abnormalities in the shape and size of nematode eggs exposed to or parasitized by *Paecilomyces lilacinus* (Thom) Samson implying that the fungus produces some substances antagonistic to the nematode egg. The present tests were conducted to ascertain the effects of *P. lilacinus* fungal filtrates on the hatching of *Meloidogyne javanica* (Treub) Chitw. and on second-stage juveniles.

Materials and methods

A loopful fungal spore suspension of *P. lilacinus* was inoculated into 250 ml Erlenmeyer flasks containing sterilized Czapek Dox medium. The flasks were incubated for two weeks at 30±1 °C. The entire contents were passed through Whatman filter paper No. 1 twice and filtrates thus obtained were stored at 4 °C in a refrigerator until used. Concentration of fungal filtrates, viz., 0, 20, 40, 60, 80 and 100 per cent were made with demineralized-sterile water (DSW). Ten ml of each concentration were pipetted into 5 cm-diam. Petri plates containing 15 egg masses each of *M. javanica*. Each treatment was replicated three times and incubated at 30±1 °C in B.O.D. incubator. DSW only served as control.

Observations on egg hatch were recorded after 24, 48 and 72 hours. Fresh fungal filtrate was replaced after each observation. At 72 h egg masses were transferred to DSW after 2-3 washings and again incubated at 30±1 °C. The number of second stage juveniles (J2) hatched in DSW was recorded 72 h later, i.e. 144 h after the start of the experiment to determine the resumption of hatching.

Two types of culture media for growth of the fungus were used. The first set consisted of buffered Czapek Dox medium (CDM) with varying pH amended with either tris or citrate buffer. The second set consisted of unbuffered CDM with varying pH amended with either NaoH or HCl. The treatments were:

	Treatment	Initial pH 7 days after inoculation	Final pH
T_1	Medium buffered with tris	9.0	7.9
T_2	do	8.0	7.6
T_3	do	7.0	6.9
T_4	Medium buffered with citrate	6.0	6.8
T_5	do	5.0	5.7
T_6	Medium unbuffered amended with NaoH	9.0	6.8
T_7	do	8.0	6.7
T_8	Medium unbuffered amended either with HCl or NaoH	7.0	6.6
T_9	Medium unbuffered amended with HCl	6.0	6.5
T_{10}	do	5.0	6.5

The sterilized media thus prepared were inoculated with the spore suspension of *P. lilacinus* and fungal filtrates obtained by the method referred to in the effect on hatching. Initial pH of media and final pH of the fungal filtrates were recorded. Concentration of the fungal filtrates were prepared in 5 cm-diam. Petri plates containing 1 ml of J_2 suspension (approximately 100 J_2). DSW was added

to provide 10 ml of 0, 20, 40, 60 and 80 per cent concentrations of fungal filtrates. In 100 per cent concentration no DSW was used and the J_2 were hand-picked. Each treatment was replicated three times and zero concentration served as control. Observations on the nematicidal/nematostatic effects were recorded after 24 hours. Immobility of the J_2 was taken as an indication of nematoxicity. About ten immobilized J_2 from each plate were transferred to DSW after observation to record their revival.

The data obtained on per cent immobility of J_2 were analysed as per C.R.D. factorial set design.

Results and discussion

Data presented in Table I show that egg hatch was significantly reduced after 24 h at all concentrations of fungal filtrates tested. Further reduction in the egg hatch was observed after 48 h and a similar effect was recorded after 72 h when only 10-34 J_2 hatched in the filtrate. Analysis of cumulative numbers of J_2 hatched revealed that the inhibition in hatching at all concentrations was significantly different from the control, suggesting that the fungal filtrate, even at the lowest concentration (20%), was effective in reducing the egg hatch (65%).

When the egg masses from the filtrates were transferred to water, hatching increased and was similar to control. Differences among the treatments were not statistically significant. Data on total hatching indicated that there was a significant reduction in total hatch by exposing eggs to various concentrations of filtrates, inspite of hatching being resumed after transferring them to water. Computing irreversible per cent inhibition over control, the highest (62%) was observed in 100% concentration and the lowest (47%) in 40% concentration.

The fungus has a strong proteolytic and chitinolytic activity with the help of which metabolites seep into the egg and cause physiological disorders. The fungus produces antibiotics like leucinostatin and lilacinin (Arai *et al.*, 1973). These factors may have important role in the inhibition of egg hatch.

Per cent J_2 immobilized are presented in Table II. The data indicate that immobility increased with the increased concentrations of fungal filtrates, the maximum being in 100 per cent. The fungal filtrates obtained from buffered medium proved to be more potent in terms of per cent immobility at all the concentrations than unbuffered medium. The fungus had the tendency to neutralize the initial pH of the medium both in acidic or alkaline medium buffered or unbuffered. Obviously, fungus had to produce higher amounts of pH neutralizing metabolites in buffered system which eventually increased the activity of the fil-

TABLE I - *Effect of fungal filtrates of* Paecilomyces lilacinus *on hatching in* Meloidogyne javanica

Concentration (%)	No. of juveniles hatched after 24 h	48 h	72 h	Cumulative hatch	Per cent inhibition	Hatching resumed in water	Total hatch	Irreversible inhibition compared to check
O (Check)	1045[a] (32.2)	307[a] (17.5)	203[a] (14.1)	1555[a] (39.3)	—	657 (25.6)	2211[a] (46.9)	—
20	496[b] (22.0)	37[c] (5.9)	10[b] (3.1)	543[b] (23.1)	65.1	550 (22.0)	1092[b] (32.6)	50.6
40	415[bc] (20.2)	104[b] (9.8)	35[b] (5.7)	554[b] (23.2)	64.4	617 (24.8)	1170[b] (34.1)	47.1
60	320[bc] (17.5)	41[bc] (6.2)	25[b] (4.6)	386[b] (19.2)	75.1	541 (23.0)	927[b] (30.0)	58.1
80	266[bc] (16.0)	28[c] (4.8)	10[b] (3.1)	304[b] (17.1)	80.4	646 (25.4)	950[b] (30.7)	57.0
100	260[c] (15.9)	48[bc] (6.7)	14[b] (3.8)	323[b] (17.8)	79.2	516 (22.3)	839[b] (28.6)	62.0
C.D. 5%	6.2	3.8	2.9	6.8	–	N.S.	8.8	—

Figures in parentheses are $\sqrt{n}$ transformed values; figures in a column followed by the same letter do not differ significantly.

TABLE II - *Effect of culture filtrates of* P. lilacinus *on second-stage juveniles of* M. javanica.

Conc (%)	Per cent second-stage juveniles immobilized in fungal filtrate										Mean
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	
0	0	0	0	0	0	0	0	0	0	0	0^a
	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)	(1.8)
20	26.2	36.6	9.6	24.1	10.6	1.7	2.7	3.7	18.0	1.5	13.5^b
	(30.6)	(37.3)	(17.6)	(29.2)	(18.8)	(7.7)	(9.6)	(11.3)	(25.1)	(7.1)	(19.4)
40	77.1	97.5	25.4	63.3	48.6	42.1	25.7	18.7	20.5	26.7	44.6^c
	(61.8)	(81.4)	(30.4)	(52.8)	(44.3)	(40.5)	(30.6)	(25.7)	(26.9)	(31.1)	(42.5)
60	78.3	100.0	70.4	100.0	49.6	36.1	32.7	34.00	35.7	19.4	55.6^d
	(62.4)	(90.0)	(57.5)	(90.0)	(44.8)	(37.0)	(34.7)	(35.7)	(36.8)	(26.1)	(51.5)
80	64.9	100.0	91.7	100.0	57.4	49.6	49.5	60.0	54.1	64.3	69.1^e
	(53.8)	(90.0)	(75.3)	(90.0)	(49.3)	(44.8)	(44.8)	(51.0)	(47.5)	(53.4)	(59.9)
100	91.0	100.0	97.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.8^f
	(72.9)	(90.0)	(84.6)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(85.1)
Mean	56.2^C	72.3^A	49.1^D	64.6^B	44.4^E	38.2^{FG}	35.1^G	32.8^H	38.0^F	35.3^G	
	(47.2)	(65.1)	(44.5)	(58.9)	(41.5)	(36.9)	(35.2)	(31.5)	(38.0)	(34.9)	

C.D. 5% Fungal filtrates (T) = 2.4. Concentration (C) = 1.86. T x C = 5.87

Figures in parentheses are n + 0.1 angular transformed values; figures in column or row followed by the same letter do not differ significantly; legends of T8 given in Materials and methods.

trates against the nematode in the buffered medium. The highest immobility (72%) was observed in fungal filtrates from the medium with initial pH 8, buffered with tris and the lowest (33%) in unbuffered medium at pH 7.

However, initial or final pH gradient of the filtrates did not follow a definite trend. The immobilized J_2 from each Petri plate were transferred to water at the end of the experiment. There was 100% revival in all the treatments demonstrating that the effect on J_2 was nematostatic but not nematicidal.

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