Nematol. medit. (1983), *11*: 119-123.

Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh - 202001, India

INHIBITORY EFFECT OF CULTURE FILTRATES OF SOME RHIZOSPHERE FUNGI OF TOMATO AS INFLUENCED BY OILCAKES ON THE MORTALITY AND LARVAL HATCH OF *MELOIDOGYNE INCOGNITA*

by
S. P. SINGH, VEENA PANT, A. M. KHAN and S. K. SAXENA

It has been suggested that as a result of organic amendments, changes occur in the rhizosphere mycoflora which adversely affect the nematode populations (Singh and Sitaramaiah, 1970; Khan *et al.*, 1973). Similarly, tomato seeds coated with oilcakes when sown in the field bring about changes in mycoflora of the rhizosphere (Singh *et al.*, 1980). It is likely that metabolic products of some of these fungi might adversely influence nematode activity. Hence it was considered interesting to study the effect of culture filtrates of some of the rhizosphere fungi on the hatching and mortality of larvae of the root knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw.

Materials and Methods

It is generally recognized that *Alternaria humicola* Oudemans, *Aspergillus niger* van Tieghem, *Curvularia lunata* (Walker) Boedijn, *Sclerotium rolfsii* Saccardo, *Trichoderma lignorum* (Tode) Harz and *Trichoderma viride* Pers. have antimicrobial properties. These fungi also found in the rhizosphere of tomato cv. Marglobe raised from seeds coated with oilcakes of castor (*Ricinus communis* L.), mustard (*Brassica campestris* L.) and neem (*Azadirachta indica* Juss.) were, therefore, selected for testing. Filtrates were obtained from the cultures grown on Czapek's liquid medium in 250 ml Erlenmeyer flasks

for 15 days these were designated as standards (S). Dilutions viz., S/2, S/10, S/100 and S/1000 were prepared with sterilized distilled water. About 100 freshly hatched larvae of *M. incognita* were transferred to 10 ml of test solutions in 4 cm petri dishes. After 12, 24 and 48 hours of incubation, the number of immobilized larvae was counted and these were then transferred to water to ascertain their mortality (if they did not regain mobility within 12 hours then were considered dead).

To study filtrate activity on larval hatch of *M. incognita*, 5 average sized eggmasses were transferred to petri dishes containing 10 ml of test concentrations of the culture filtrates or distilled water control and the larval hatch counted after 5 days. All the treatments were replicated five times.

Analysis of filtrates for free phenols, o-dihydroxyphenols and free aminoacids was established by using the technique of Biehn *et al.* (1968) with Folin Ciocalteau reagent (Bray and Thorpe, 1954) for total free phenols and that of Johnson and Schaal (1952) with Arnows reagent for o-dihydorxyphenols; total free aminoacids were estimated by using modified ninhydrin reagent (Moore and Stein, 1954).

Results and Discussion

The results presented in Fig. 1 clearly indicate that the culture filtrates of all the fungi tested were toxic to larvae of *M. incognita* to a varying degree. The mortality of larvae was directly correlated with the concentration of culture filtrates and the period of exposure. The culture filtrates of all the fungi were least effective at S/1000 and S/100 concentrations at 12 hours of exposure. The highest kill of larvae was observed after 48 hours in S/2 concentration and after 12 hours in S concentration. Similarly, inhibition of hatching increased with the increase in the concentration of the culture filtrates (Table I). These results are in agreement with those of Walker *et. al.*, (1965), Mankau (1969) and Shukla and Swarup (1971).

Of six fungi tested, the culture filtrate of *T. viride* had higher amounts of total free phenols and o-dihydroxyphenols followed by *T. lignorum, S. rolfsii, C. lunata, A. humicola* and *A. niger*. On the other hand, the culture filtrate of *A. humicola* had higher amount of aminoacids (Table II). The higher mortality of nematode larvae in

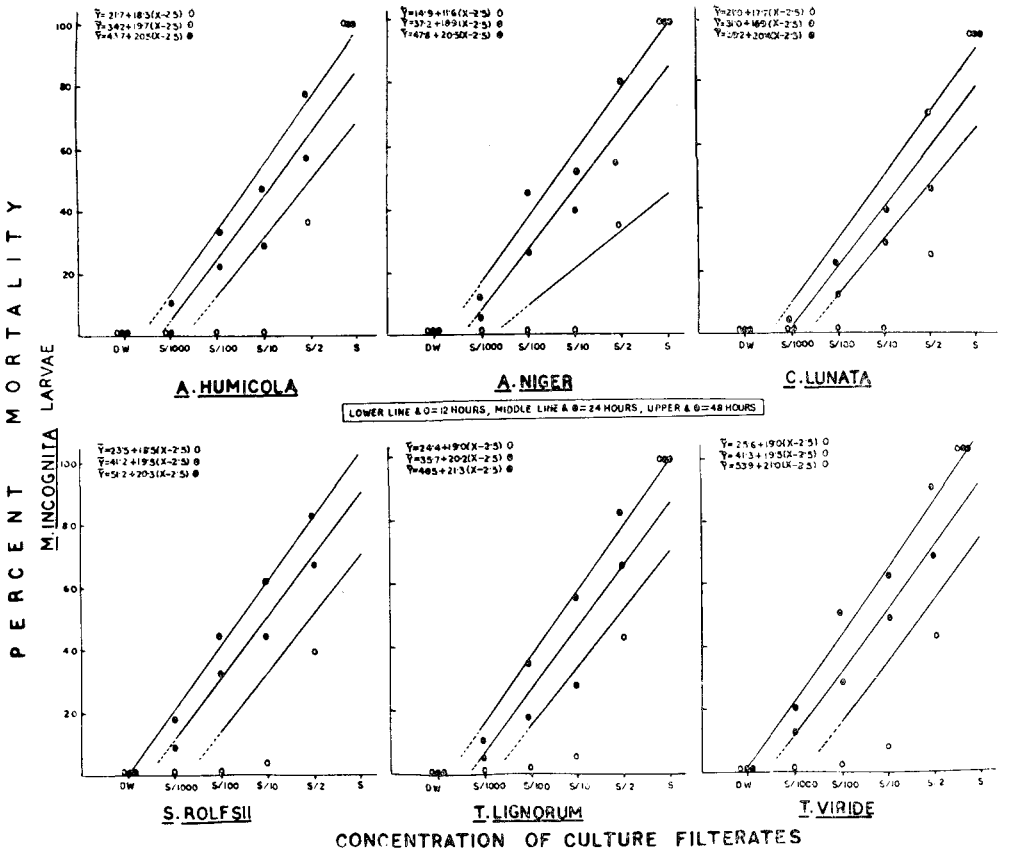

Fig. 1 - Regression lines of mortality of *Meloidogyne incognita* in different dilutions of the culture filtrates of various fungi after different exposure periods.

culture filtrate of *T. viride* is correlated with high amounts of phenolics.

Mortality of larvae attributes the presence of certain nematicidal compounds in the culture filtrates of fungi. Phenols and aminoacids have also been reported to inhibit the hatching and to induce the mortality of nematodes (Prasad and Webster, 1967; Sitaramaiah and Singh, 1974; Hassan, 1977 and Setty *et al.*, 1977). The presence of these compounds in soil may be one of the factors responsible for suppression of nematodes. The release of such compounds may be

Table I - *Effect of culture filtrate of six fungi on hatching of larvae of* M. incognita.

Fungi	Cumulative larval hatch (after 5 days)					
	DW	S	S/2	S/10	S/100	S/1000
Alternaria humicola	1610	20	150	600	1180	1580
Aspergillus niger	1610	15	160	610	1220	1600
Curvularia lunata	1610	15	140	510	1200	1600
Sclerotium rolfsii	1610	20	130	520	1200	1570
Trichoderma lignorum	1610	15	130	500	1060	1550
T. viride	1610	10	110	460	950	1450

N. B.: each value is an average of 5 replicates; DW = Distilled water; S = Culture filtrate obtained after growing the fungus on Czapek's medium for 15 days.

Table II - *Amount of total free phenols, o-dihydroxyphenols and aminoacid contents in culture filtrate of six fungi.*

Fungi	Total free phenols mg/1 ml	o-dihydroxy-phenols mg/1 ml	Total aminoacids mg/1 ml
Alternaria humicola	0.27	0.023	0.51
Aspergillus niger	0.25	0.019	0.35
Curvularia lunata	0.41	0.040	0.45
Sclerotium rolfsii	0.67	0.029	0.37
Trichoderma lignorum	0.81	0.048	0.41
T. viride	0.87	0.057	0.30
L.S.D. (at 5% level)	0.0304	0.0030	0.381
L.S.D. (at 1% level)	0.0424	0.0042	0.533

N. B.: each value is an average of 3 replicates.

from the decomposing oilcakes or due to increased microbial activity like antagonism and the break down products of fungi in the soil ecosystem.

The award of C.S.I.R. Senior Fellowship to the first two authors (S.P.S. and V.P.) is gratefully acknowledged.

SUMMARY

The effect of culture filtrates of *Alternaria humicola, Aspergillus niger, Curvularia lunata, Sclerotium rolfsii, Trichoderma lignorum* and *T. viride* obtained from rhizosphere of tomato cv. Marglobe raised from seeds coated with oilcakes, on the mortality and larval hatch of *Meloidogyne incognita* has been studied. The culture filtrates of all the fungi tested proved to be nematoxic and inhibited the hatching of larvae of *M. incognita*. Highest mortality occurred in the culture filtrate of *T. viride* presumably because of the presence of higher amount of phenols.

LITERATURE CITED

BIEHN W. L., KUC J. and WILLIAMS E. B., 1968 - Accumulation of phenols in resistant plants, fungi interactions. *Phitopathology, 58*: 1255-1260.

BRAY H. G. and THORPE W. V., 1954 - Analysis of phenolic compounds of interest in metabolism. *Meth. Biochem. Anal., 1*: 27-52.

HASSAN N., 1977 - Effect of oilcake, sawdust and certain chemical compounds on the development of root-knot on egg plant and tomato. Ph. D. Thesis. Aligarh Muslim University, Aligarh (India).

JOHNSON G. an. SCHAAL L. R., 1952 - Relation of chlorogenic acid to the scab resistance in potatoes. *Science, 115*: 627-629.

KHAN M. W., KHAN A. M. and SAXENA S. K., 1973 - Influence of certain oilcake amendments on nematodes and fungi in tomato fields. *Acta Bot. Indica, 1*: 49-54.

MANKAU R., 1969 - Nematicidal activity of *Aspergillus niger* culture filtrates. *Phytopathology, 59*: 1170.

MOORE H. and STEIN W. H., 1954 - Modified ninhydrin reagent for spectrophotometric determination of aminoacids. *J. Biol. Chem., 24*: 904-913.

PRASAD S. K. and WEBSTER J. M., 1967 - The effect of aminoacids antimetabolites on 4 nematode species and their host plants. *Nematologica, 13*: 318-320.

SETTY K. G. H., KRISHNAPPA K. and PRASAD S. K., 1977 - Effect of DL-phenyl alanine on root-knot nematode, *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 on tomato. *Current Res., 6*: 135-136.

SHUKLA V. N. and SWARUP G., 1971 - Studies on root-knot of vegetables VI. Effect of *Sclerotium rolfsii* filtrate on *Meloidogyne incognita. Indian J. Nematol., 1*: 52-58.

SINGH R. S. and SITARAMAIAH K., 1970 - Control of plant parasitic nematodes with organic soil amendments. *Pans., 16*: 287-297.

SINGH S. P., AHMAD M., KHAN A. M. and SAXENA S. K., 1980 - Effect of seed treatments with certain oilcakes and nematicides on the growth of tomato cv. Marglobe and population of rhizosphere nematodes and fungi. *Nematol. medit., 8*: 193-198.

SITARAMAIAH K. and SINGH R. S., 1974 - The possible effects on *Meloidogyne javanica* of phenolic compounds produced in amended soil (Abstr.). *J. Nematol., 6*: 152.

WALKER J. T., SPECHT C. H. and BEKKER J., 1965 - Nematicidal activity to *Pratylenchus penetrans* by culture fluids from actinomycetes and bacteria. *Can. J. Microbiol., 12*: 347-351.

Accepted for publication on 14 March 1983.