SOME CHARACTERISTICS OF CULTURE FILTRATE OF *FUSARIUM SOLANI* TOXIC TO *MELOIDOGYNE INCOGNITA*[1]

A. Mani and C. L. Sethi

Respectively, Citrus Improvement Project, Andhra Pradesh Agricultural University, Tirupati-517 502, India; and Division of Nematology, Indian Agricultural Research Institute, New Delhi-110 012, India.

Accepted:
21.V.1984
Aceptado:

ABSTRACT

Mani, A., and C. L. Sethi, 1984. Some characteristics of culture filtrate of *Fusarium solani* toxic to *Meloidogyne incognita*. Nematropica 14:121-129.

The toxic principle in the culture filtrate (CF) of *Fusarium solani* (Mart.) Sacc. is thermostable and also independent of the pH of CF. The inhibitory effect of CF was due to fungal toxin and unused residues of sugar and salts present in CF. Among the sugar and salts tested, potassium chloride and ferrous sulphate showed maximum inhibitory effect after 48 hr of exposure. The fungal toxin was water soluble.

Additional key words: culture filtrate, toxin.

RESUMEN

Mani, A., y C. L. Sethi. 1984. Algunas características de los filtrados de cultivos de *Fusarium solani* tóxicos al *Meloidogyne incognita*. Nematropica 14:121-129.

El principio tóxico de los filtrados de cultivos de *Fusarium solani* (Mart.) Sacc. es termoestable e independiente de su pH. El efecto inhibitorio de dichos filtrados es debido a la toxina fungosa y a los residuos no usados de azúcares y sales presentes en los mismos. De los azúcares y sales probados, cloruro de potasio y sulfato ferroso mostraron el máximo efecto inhibitorio después de 48 horas de exposición. La toxina fungosa es soluble en agua.

Palabras claves adicionales: filtrados de cultivos, toxina fungosa.

INTRODUCTION

The culture filtrates (CFs) of *Fusarium oxysporum* Matuo and Sato and *Fusarium solani* (Mart.) Sacc., have been found to have adverse effects on hatch and mobility of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, which were attributed to factors like low pH and toxins produced by the fungal pathogens during their initial growth period (5, 6). Since earlier studies suggested that CF of *F. solani* was more potent than that of *F. oxysporum* f. sp. *ciceri* in inhibiting nematode

activities (5), CF of *F. solani* alone was selected for the present investigations. However, before starting the isolation and identification of toxin(s) from CF, a series of experiments were carried out to study the properties of CF and confirm the production of toxin(s) by *F. solani*. Results of these tests are reported below.

MATERIALS AND METHODS

The CF was obtained by growing *F. solani* on Czapek's liquid medium (4) for 15 days at 28 ± 2 C in an incubator. The filtrate thus collected was passed through Whatman® No. 42 filter paper to remove mycelial fragments. The pH of the CF was found to be 5.8 and it was neutralized with the addition of 0.45 ml of 2N NaOH to 120 ml of CF to obtain a pH level of 7.1. The CF was tested against second-stage juveniles of *M. incognita* in 5-cm-diam Petri plates. The CF was diluted to 10, 20, 40, and 80% concentrations and tested for toxicity (evaluated in terms of nematode mobility) at 4, 8, 24, and 48 hr intervals. Four CFs were tested in this manner: (i) unneutralized CF boiled for 5 min; (ii) unneutralized CF autoclaved for 25 min at a pressure of 1.05 kg/cm^2; (iii) neutralized CF; (iv) fresh, unboiled, unautoclaved, and unneutralized CF which served as a control.

The CF and autoclaved Czapek's liquid medium were dialyzed separately for 3 hr against tap water by changing water every 45 min. The dialyzed solutions were collected and tested for toxicity along with the sterilized but undialyzed Czapek's liquid medium at concentrations of 20 and 40%. A sugar and 5 salt solutions, the components of Czapek's liquid medium, were prepared individually at the same concentration as in the medium and tested for toxicity to find out their individual effects.

The CF was added to 15-cm-diam Petri dishes and left for 18 hr in a freezer, after which the frozen sample was dried under vacuum at −40 C. The residue in the Petri dishes was dissolved in 5 ml of distilled water and different concentrations were prepared and tested for toxicity.

All data were subjected to analysis of variance following standard statistical procedures (8). Differences between means were evaluated for significance following a modified Duncan's multiple range test (8).

RESULTS AND DISCUSSION

A steady decrease in juvenile mobility was observed with increase in concentration and period of exposure to untreated CF. Mobility was zero at concentrations of 40% and above after 48 hr of exposure (Table 1). The results of this experiment are in agreement with the earlier findings (2, 3, 7) regarding the effects of concentration and period of exposure

Table 1. Effect of untreated (control) and boiled culture filtrates of *Fusarium solani* on mobility of second-stage juveniles of *Meloidogyne incognita*.[z]

Concentration (%)	Untreated (control) % mobility at different time intervals (hrs)					Boiled % mobility at different time intervals (hrs)				
	4	8	24	48	Mean	4	8	24	48	Mean
Check (Water)	100 a	99 a	99 a	98 a	99 a	99 a	99 a	98 a	98 a	99 a
10	94 b	92 b	89 b	83 b	89 b	98 a	98 a	73 b	70 b	85 b
20	92 bc	89 b	87 b	14 c	70 c	98 a	94 b	64 c	10 c	67 c
40	89 c	88 b	85 b	0 d	66 d	97 a	90 c	38 d	3 d	57 d
80	83 d	78 c	66 c	0 d	57 e	96 a	87 cd	18 e	0 e	50 e
100	78 d	73 c	65 c	0 d	54 e	92 b	82 d	3 f	0 e	44 f
Mean	89	87	82	33		97	92	49	30	

[z]Data in columns followed by the same letter and data in rows underscored by the same line are not significantly different (P=0.05) according to Duncan's multiple range test; each value is the mean of three replications.

Table 2. Effect of autoclaved and neutralized culture filtrates of *Fusarium solani* on mobility of second-stage juveniles of *Meloidogyne incognita*.[z]

Concentration (%)	Autoclaved					Neutralized				
	% mobility at different time intervals (hrs)					% mobility at different time intervals (hrs)				
	4	8	24	48	Mean	4	8	24	48	Mean
Check (Water)	100 a	99 a	99 a	98 a	99 a	99 a	99 a	99 a	98 a	99 a
10	98 ab	94 b	77 b	55 b	81 b	98 a	96 a	40 b	29 b	66 b
20	96 b	85 c	59 c	19 c	65 c	96 ab	92 ab	25 c	11 c	56 c
40	90 c	78 c	43 d	1 d	53 d	93 b	89 bc	16 c	0 d	50 cd
80	82 d	66 d	12 e	0 d	40 e	83 c	82 cd	12 cd	0 d	44 d
100	78 d	60 d	6 e	0 d	36 e	80 c	76 d	8 d	0 d	41 d
Mean	91	80	49	29		91	89	33	23	

[z]Data in columns followed by the same letter and data in rows underscored by the same line are not significantly different (P=0.05) according to Duncan's multiple range test; each value is the mean of three replications.

Table 3. Effect of dialyzed culture filtrate and sterilized Czapek's liquid medium on mobility of second-stage juveniles of *Meloidogyne incognita*.[z]

Concentration (%)	Dialyzed culture filtrate % mobility at different time intervals (hrs) 4	8	24	48	Mean	Sterilized Czapek's liquid medium % mobility at different time intervals (hrs) 4	8	24	Mean
Check (Water)	100 a	98 a	99 a	99 a	99 a	100 a	100 a	99 a	99 a
20	98 ab	91 b	78 b	72 b	85 b	96 b	95 b	80 b	90 b
40	90 b	86 b	82 b	48 c	77 b	96 b	93 b	72 c	87 b
Mean	96	92	86	73		97	96	84	

[z]Data in columns followed by the same letter and data in rows underscored by the same line are not significantly different (P = 0.05) according to Duncan's multiple range test; each value is the mean of three replications.

to CF on the mobility of *M. incognita*. The toxic principle(s) present in CF responsible for larval immobility was not affected by either boiling or autoclaving (Tables 1 and 2). In fact, the effect of boiled and autoclaved CFs was expressed even earlier than the unboiled/unautoclaved CFs, as 24-hr exposure caused significant reduction in larval mobility. The test carried out with neutralized CF indicated that acidic pH was not responsible for toxicity (Table 2), contrary to the reports that, besides toxins, low pH was one of the possible factors responsible for the inhibitory effect of CF of fungi (1, 2, 3). In the present study, the neutralized CF showed the same effect observed without neutralization. The above results indicate the toxic principle of CF was thermostable and also independent of pH.

Observations on the effect of dialyzed CF revealed that immobility

Table 4. Effect of individual chemicals of Czapek's medium on mobility of second-stage juveniles of *Meloidogyne incognita*.[z]

Chemicals	Concentration (%)	% mobility at different time intervals (hrs) 4	8	24	48	Mean
Check (Water)		100 a	100 a	99 a	99 a	99 a
Sucrose	3.00	96 b	94 b	90 d	88 c	92 c
Sodium nitrate	0.20	99 a	98 a	95 c	94 b	96 b
Dipotassium hydrogen phosphate	0.10	99 a	98 a	95 bc	95 b	97 ab
Magnesium sulfate	0.05	100 a	99 a	98 ab	97 ab	99 ab
Potassium chloride	0.05	95 b	84 c	82 e	80 d	85 d
Ferrous sulfate	0.001	89 c	86 c	83 e	81 d	85 d
Mean		97	94	92	91	

[z]Data in columns followed by the same letter and data in rows underscored by the same line are not significantly different (P = 0.05) according to Duncan's multiple range test; each value is the mean of three replications.

rate at the 40% concentration was reduced to half compared to undialyzed CF at that concentration (Table 3), which indicated that the activity of CF was due to toxin(s) as well as unused residues of sugar and salts present in CF. Sterilized Czapek's liquid medium also affected the mobility of larvae, but to a lesser extent than control CF (Table 3). Larvae continued to be mobile even at 40% concentration after 48 hr of exposure to sterilized and dialyzed Czapek's liquid medium. It suggested that the dialyzed medium was non-toxic to larvae as a result of removal of sugar and salts during the process of dialyzation. The above results suggest that sugar and salts present in liquid medium and a toxic substance produced by *F. solani* in culture medium are responsible for the inhibition of nematode activity.

Among the sugar and salts tested, 0.05% potassium chloride and 0.001% ferrous sulfate caused 20 and 19% larval immobility, respectively after 48 hr, whereas 0.05% magnesium sulfate exhibited only 3.0% toxicity. The toxicity of chemicals tested were, in descending order: ferrous sulfate, potassium chloride, sucrose, sodium nitrate, dipostassium hydrogen phosphate, magnesium sulfate, water (Table 4).

Toxicity tests carried out with the residue obtained from freeze-dried samples of CF revealed that larval mobility rate was decreased with in-

Table 5. Effect of freeze-dried residue of *Fusarium solani* culture filtrate on mobility of second-stage juveniles of *Meloidogyne incognita*.[z]

Concentration (%)	% mobility at different time intervals (hrs)				Mean
	4	8	24	48	
Check (Water)	100 a	100 a	99 a	99 a	99 a
0.2	100 a	100 a	99 a	96 b	99 a
0.4	98 b	98 b	97 b	89 c	95 b
0.6	97 bc	95 c	93 c	87 cd	93 bc
0.8	97 bc	95 c	91 c	83 d	91 c
1.0	96 bc	94 c	90 c	76 e	89 c
2.0	95 c	92 c	90 c	69 f	86 cd
Mean	97	96	94	85	

[z]Data in columns followed by the same letter and data in rows underscored by the same line are not significantly different (P = 0.05) according to Duncan's multiple range test; each value is the mean of three replications.

crease in concentration of residue and length of exposure period (Table 5). The toxic principle is apparently water-soluble.

LITERATURE CITED

1. AHMED, A., and A. M. KHAN. 1964. Factors influencing larval hatching in the root-knot nematodes, *Meloidogyne incognita* (Kofoid and White, 1919), Chitwood, 1949 and *M. javanica* (Treub, 1885), Chitwood, 1949. I. Effect of temperature and hydrogen-ion concentration. Indian Phytopath. 17(2):98-101.
2. ALAM, M. M., M. WAJID KHAN, and S. K. SAXENA. 1973. Inhibitory effect of culture filtrates of some rhizosphere fungi of okra on the mortality and larval hatch of certain plant parasitic nematodes. Indian J. Nematol. 3:94-98.
3. AZAM, M. F., A. M. KHAN, and S. K. SAXENA. 1979. Effect of culture filtrates of certain fungi on hatch and mortality of larvae of root-knot nematode. Acta Botanica Indica 7(2):122-125.
4. LEVINE, M., and H. W. SCHOENLEIN. 1930. A compilation of culture media for the cultivation of micro organisms. Baltimore, 969 pp.
5. MANI, A. 1983. Studies on interaction of root-knot nematode, *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium solani* on chickpea. Ph.D. Thesis. Indian Agricultural Research Institute, New Delhi, India.
6. SAKHUJA, P. K., I. SINGH, and S. K. SHARMA. 1978. Effect of some fungal filtrates on the hatching of *Meloidogyne incognita*. Indian Phytopath. 31(3):376-377.
7. SHUKLA, V. N., and G. SWARUP. 1971. Studies on root-knot of vegetables. VI. Effect of *Sclerotium rolfsii* filtrate on *Meloidogyne incognita*. Indian J. Nematol. 1:52-58.
8. STEEL, R. G. D., and J. D. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York, 481 pp.

ACKNOWLEDGEMENTS

The authors thank Dr. Gopal Swarup and Dr. D. R. Das Gupta, Division of Nematology, and Dr. S. L. Mehta, Nuclear Research Laboratory, I.A.R.I., New Delhi, for their comments and suggestions. Special thanks are due to Dr. Ivan J. Thomason, Department of Nematology,

University of California, Riverside, for his suggestions during the preparation of the manuscript.

Received for publication:
10.I.1984
Recibido para publicar:

---

[1]Portion of a thesis submitted to P.G. School, I.A.R.I., New Delhi by senior author for the award of Ph.D. degree in Nematology.