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Observations on the nematicidal properties of some mycotoxins

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Summary – Secondary metabolites produced by species of *Fusarium* and other soil fungi were tested against some plant parasitic nematodes. Assays were conducted *in vitro* using toxin concentrations of 0.02, 0.2, 2, 20, and 200 ppm in 4 % methanol. T2-toxin, moniliformin, verrucarin A and cytochalasin B significantly reduced the viability of *Meloidogyne javanica* juveniles at 2 ppm or higher concentrations after 72 hours exposure at 22.5 °C. Enniatin B showed a significant increase in *M. javanica* mortality at 20 ppm. T2-toxin also showed a nematicidal effect on *M. hapla* and *Pratylenchus neglectus* at 0.2 and 2 ppm, respectively. When tested in soil against *M. javanica* parasitizing tomato plants, T2-toxin and moniliformin did not affect nematode density. In the treated pots a larger number of galls per g of root was also observed. Data suggest that mycotoxins can interact with nematodes through antagonistic or synergistic effects related to the concentration levels present in the plant rhizosphere.

Résumé – ***Observations sur les propriétés nématicides de certaines mycotoxines*** – Les métabolites secondaires produits par certaines espèces de *Fusarium* et d'autres champignons du sol ont été testés contre des nématodes parasites des plantes. Les essais ont été conduits *in vitro* avec des concentrations de 0.02, 0.2, 2, 20, et 200 ppm en solution aqueuse à 4 % de méthanol. La toxine T2, la moniliformine, la verrucarine et la cytochalasine B ont significativement réduit la viabilité des juvéniles de *Meloidogyne javanica* à partir de 2 ppm après 72 heures d'exposition à 22.5 °C. L'enniatine B a montré une augmentation significative de la mortalité des larves de *M. javanica* à 20 ppm. La toxine T2 possède également un effet nematicide sur *M. hapla* et *Pratylenchus neglectus* à 0.2 et 2 ppm, respectivement. Testées dans le sol contre *M. javanica* sur tomate, la toxine T2 et la moniliformine n'ont pas réduit la densité des nématodes. Dans les pots traités, une plus grande quantité de galles par g de racine a été observée. Les données suggèrent que les mycotoxines peuvent interagir avec les nématodes par effets synergiques ou antagonistes, en relation avec les concentrations présentes dans la rhizosphère des plantes.

Key words : toxins, nematodes, *Fusarium*, synergism, toxicity.

The biological response of invertebrates to secondary metabolites produced by fungi is of interest because of the possible effects occurring during plant parasitism and their potential exploitation as pesticides of natural origin (Belloncik & Parent, 1976; Wright *et al.*, 1982; Gillespie & Claydon, 1989; Peterson *et al.*, 1990).

Filtrates of nematophagous or entomophagous fungi have been found to be active against free-living nematodes (Krizkova *et al.*, 1976; Giuma *et al.*, 1973). Filtrates produced by non-specialized soil fungi were also reported as toxic or lethal to different plant parasitic species (Alam *et al.*, 1973; Mani & Sethi, 1984; Cayrol *et al.*, 1989). *Fusarium* spp. filtrates were toxic to free-living and plant parasitic nematodes *in vitro*. In these studies, however, no information was given on the filtrate components or on the presence of specific *Fusarium* mycotinx released by the isolates tested (Krizkova *et al.*, 1979; Alcantara & Azevedo, 1980; Mani & Sethi, 1984).Little information is also known on the nematotoxic activity of fungal metabolites of known composition and origin (Ciancio *et al.*, 1984).

The occurrence and activity of mycotoxins can be responsible for the different synergistic or antagonistic effects observed among fungi and nematodes (Powell, 1971; Palmer & Mac Donald, 1974; Nordmeyer & Sikora, 1983; Mai & Abawi, 1987). In this note we report on the effects of mycotoxins produced by soil fungi on some species of plant parasitic nematodes.

Materials and methods

Juveniles of a *Meloidogyne javanica* population originating from southern Italy and maintained on tomato (*Lycopersicon esculentum* Mill.) cv. Roma were obtained from eggs hatching on water agar with 0.01 % penicillin or from egg masses placed on nylon microsieves in watch glasses with sterile water. Juveniles of *M. hapla* were obtained as described from egg masses dissected from parasitized roots of *Actinidia deliciosa* (A. Chev.) Liang & Ferguson. Adults and juveniles *Pratylenchus neglectus* were obtained from naturally infested wheat (*Triticum durum* Desf.) plants collected at Castellana (Bari, Italy). The nematodes were extracted from the soil by sieving, using 450 and 120 μ sieves, hand picked and washed twice in sterile distilled water.

Lyophilized powders of commercially available mycotoxins (T2-toxin, moniliformin, cytochalasin B, ver-

rucarin A, enniatin b) were used for *in vitro* tests. Standard solutions were prepare at concentrations of 5×10^3, 5×10^2, 50, 5 and 0.5 ppm in pure methanol. Solutions and lyophilized powders were stored prior to use at 4 ± 2 °C.

The mycotoxins tested *in vitro* are produced by species frequently isolated from soil and causal agents of several plant diseases. Moniliformin is produced by *Fusarium moniliforme* var. *subglutinans, F. graminearum, F. fusarioides, F. chlamydosporium, F. avenaceum* and *F. oxysporum.* Cytochalasin B is produced by *Ascochita* sp., Phoma sp., *Helminthosporium dematioideum* and *Hormiscium* sp. Verrucarin A is produced by *Myrothecium verrucaria* and *M. roridum.* Finally, several species and isolates of *Fusarium* produce Enniatin B or T2-toxin (Cole & Cox, 1981).

Juveniles of *M. javanica* were suspended in sterile distilled water and poured for testing in multi-well tissue culture plastic plates (Sterilin Ltd, U.K.) to obtain a final volume of 480 µl distilled water. After nematode introduction, 20 µl of each toxin standard solution was poured in the micro-wells by means of a sterile microcapillary tube (Drummond Sc. Co., USA) to provide a final volume of 500 µl and final toxin concentration of 200, 20, 2, 0.2 and 0.02 ppm. Four replications were used with approximately ten to seventeen juveniles per replication. In a second test, T2-toxin was tested against juveniles of *M. hapla* and adults and juveniles of *P. neglectus.* The toxin was dissolved as previously described. Four replications were used with approximately ten to twelve nematodes for each species and replication. For all tests, controls included nematodes in 4 % methanol water solution. Nematode viability was assessed by the movements and contractions induced by pH change when a drop of 4 % lactic acid in water was placed in each micro-well. The plates were checked after 72 hours incubation at 22.5 ± 2 °C.

The effect of mycotoxins on *M. javanica* development in soil was tested using 25 ppm T2-toxin or 25 ppm moniliformin in a 2 % aqueous methanol solution per each replication. Tomato plants (cv. Roma) were transplanted in 18 mm diam. plastic pots with sterile sandy soil and sealed at the bottom with parafilm. The plants were inoculated four days after transplanting with 3500 juveniles previously multiplied on tomato and hatched in water with gentle air bubbling from approximately 30 egg masses. The juveniles were previously checked under a stereomicroscope to assess their viability and density. For each treatment and replication 10 ml of the T2 toxin solution of 6 ml of the moniliformin solution were poured into soil. The plants were treated four days after the nematode inoculum, in order to study the mycotoxins' effects on nematode reproduction and plant damage. The treatments were repeated at 4, 6, 12 and 25-day intervals. A total amount of 1 mg of T2-toxin or 0.6 mg of moniliformin was introduced for each pot. Untreated plants with nematodes were used as controls. The plants were placed in greenhouse at 20-24 °C. The nematodes were extracted from soil by sieving five weeks after the last treatment and were counted with a Hawksley counting chamber. The eggs were extracted from the roots with the hypochloride method (Hussey & Barker, 1973). Five replications were used.

Results

All the toxins tested, except enniatin B, reduced significantly the rate of *M. javanica* juveniles' survival *in vitro* at 2 ppm or higher concentrations (Table 1). At 200 ppm juveniles mortalities were 5.8, 5.6, 4.6 and 4.8 fold higher than control for T2-toxin, moniliformin, verrucarin A and cytochalasin B, respectively. Enniatin showed a significant increase in nematodes mortality at 20 ppm concentration, with a mortality 3.2 fold higher than control at 200 ppm (Table 1). Similarly, T2-toxin showed a nematicidal effect on *Meloidogyne hapla* and *Pratylenchus neglectus* significant at 0.2 and 2 ppm, respectively (Table 2).

In the greenhouse test, T2-toxin and moniliformin did not significantly reduce the nematode density, but higher root weights and juvenile densities and fewer eggs/g roots were observed. A larger number of galls/g root was observed for both toxins, significant at $P < 0.05$ (Table 3).

Discussion

The nematicidal activity showed *in vitro* by the mycotoxins tested suggests a possible ecological role in the plant rhizosphere. Moniliformin and T2-toxin nematicidal activity *in vitro,* however, was not observed in soil where *M. javanica* densities were higher than in the untreated control. This effect can be related to the antibiotic, fungistatic, or phytotoxic activities of the toxins

Table 1. In vitro *nematicidal activity of mycotoxins on juveniles of* Meloidogyne javanica.

Treatments (ppm)	Nematodes mortality (%)				
	E	M	CB	VA	T2 *
200	47.7 *a*	83.6 *a*	99.0 *a*	94.5 *a*	84.1 *a*
20	43.3 *a*	37.9 *b*	53.7 *b*	89.2 *a*	80.0 *a*
2	15.5 *b*	46.2 *bc*	44.9 *b*	58.2 *b*	80.9 *a*
0.2	13.2 *b*	9.3 *cd*	19.5 *c*	7.0 *c*	48.2 *b*
0.02	25.1 *b*	5.5 *d*	13.2 *c*	6.0 *c*	43.2 *b*
Control	14.8 *b*	14.8 *d*	20.4 *c*	20.4 *c*	14.3 *c*

* E = Enniatin, M = Moniliformin, CB = Cytochalasin B, VA = Verrucarin, T2 = T2 toxin. Means flanked in columns by the same letter are not statistically different according to Duncan's Multiple Range test ($P < 0.01$).

Table 2. *Activity of T2-toxin on* Meloidogyne hapla *and* Pratylenchus neglectus.

Treatments (ppm)	Nematodes mortality (%) and stages	
	M. hapla (juv.)	*P. neglectus* (juv. & ad.)
200	83.9 *a*	87.6 *a*
20	72.5 *a*	82.5 *a*
2	70.0 *a*	61.0 *b*
0.2	38.8 *b*	43.6 *c*
0.02	19.0 *c*	39.1 *c*
Control	7.5 *c*	30.5 *c*

Means flanked in columns by the same letter are not statistically different according to Duncan's Multiple Range test ($P < 0.01$).

Table 3. *Effect of T2-toxin and moniliformin treatments on* Meloidogyne javanica *parasitizing tomato in posts.*

Treatment	Juveniles/ 100 cc soil	10 eggs/g root	Galls/g root	Plant dry weight (g)
T2-toxin	3606 ± 1978	252 ± 59	207 ± 63 *	1.1 ± 0.3
Moniliformin	3486 ± 192	233 ± 71	192 ± 45 *	1.3 ± 0.2
Control	2473 ± 404	261 ± 46	123 ± 48	0.9 ± 0.2

Means flanked in columns by asterisk are significantly different from control according to Student's test ($P < 0.05$).

tested (Burmeister & Hesseltine, 1970; Wright *et al.,* 1982; Schappert & Khachatourian, 1983). At the concentrations used these effects balanced or hid any nematicidal activity.

The two main T2-toxins producing *Fusarium* species, *F. sporothrichioides* and *F. acuminatum,* are soil-borne fungi (Booth, 1971). These species have a worldwide distribution and may occur in crop residues and in decaying plant matter where they may affect nematodes as well as other animals (Davis *et al.,* 1982). Although the *in vitro* tests suggest that T2-toxin and other mycotoxins can act as possible limiting factors for different nematode species in nature, significant nematicidal effects may occur only when nematodes are exposed at high concentrations for long periods. The use of the mycotoxins tested as natural pesticides cannot therefore be recommended, also because of several technical (storage and handling safety, soil and water contamination, hazards to animals and man) and economic considerations.

Finally, even if a low cost and safe formulation could be found for the mycotoxins tested, the high concentration thresholds required for nematicidal activity (2ppm) appear difficult to maintain in natural conditions. Those mycotoxins may be removed from soil through several mechanisms, i.e. a rhizosphere oxidative environment, absorption to organic matter, water solubilization or decomposition by soil microflora (Ueno & Ishi, 1985).

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