Toxicity of Leaf and Stem Extracts to *Tylenchorhynchus dubius*

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Abstract: Plant extracts, made by grinding 2 g of fresh tissue in 5 ml of water, were toxic to *Tylenchorhynchus dubius* and *Hoplolaimus* spp. Such extracts from leaves and stems of bean (*Phaseolus vulgaris* L.) and leaves of tobacco (*Nicotiana tabacum* L.) were most toxic; those from leaves of corn (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill.) and rhododendron (*Rhododendron catawbiense* L.) were less toxic; and extracts of bean roots were nontoxic. Nematode movement slowed markedly within 1 hr in tobacco leaf extract, and within 4 hr in bean leaf extract; both extracts completely inactivated or killed 95% of the nematodes in 24 hr. Heating leaf extract 10 min at 80 C eliminated toxicity. Absorption of fusicoccin, a phytotoxin produced by *Fusicoccum amygdali* Del., increased the toxicity of tomato leaf extracts, whereas water extracts of acetone-extracted powder preparations of leaves were about 15-fold more toxic than water extracts of fresh tissue. Addition of homogenized leaves of bean, tobacco and tomato to soil significantly reduced nematode populations within 3 days.

Some plant roots are toxic to nematodes (7, 8, 10). Although residues of decomposing rye (*Secale cereale* L.) and timothy (*Phleum pratense* L.) have nematicidal properties (9, 11), the authors are not aware of any report that leaf extracts are toxic to nematodes. In this study, we report nematicidal activity in extracts of leaves and stems of bean, the leaves of tobacco, and to a lesser extent in leaves of corn, tomato and rhododendron. The effect of fusicoccin [a fungal toxin produced by the actively growing mycelium of *Fusicoccum amygdali* Del. (2)] on the nematicidal activity of leaf extracts of tomato and bean plants that had absorbed the toxin, was also investigated. Finally, we added aqueous homogenates of leaf material to soil to determine whether its nematicidal activity was retained in the soil.

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MATERIALS AND METHODS

Plant extracts were obtained by grinding 2 g of fresh whole leaves of corn (*Zea mays* L. 'Pa602A'), beans (*Phaseolus vulgaris* L. 'Pinto'), tobacco (*Nicotiana tabacum* L. 'Bel. W3'), tomato (*Lycopersicon esculentum* Mill. 'Bonny Best') and rhododendron (*Rhododendron catawbiense* L.), in 5 ml of water with a mortar and pestle and filtering each sample through cheesecloth to remove debris. Corn and bean plants were 2 weeks old and tobacco and tomato plants were 25-cm tall. The plants had not been treated with nematicides, fungicides or insecticides before extraction. Two ml of each extract was mixed with 2 ml of a nematode suspension containing 60-80 *Tylenchorhynchus dubius* and 15-25 *Hoplolaimus* spp. A control, containing 2 ml of water plus an equal volume of the nematode suspension, also was prepared. Nematodes were obtained from untreated Windsor fine sandy loam around roots of bluegrass (*Poa pratensis* L.) and native bentgrass (*Agrostis stolonifera*

L.) by decantation, sieving (44-μ screen) and sugar flotation (5). Nematodes in plant extracts were kept at room temperature (23 C) and the active nematodes were counted after 48 hr. In this and successive experiments, treatments were replicated four times.

In another experiment, leaf extracts from bean and tobacco were diluted with water 2-, 6-, 20- and 60-fold. Each ml of the undiluted extracts from leaves of bean and tobacco contained 33 mg and 25 mg of dry matter, respectively. A 2-ml sample of the undiluted extracts and of each dilution was mixed with an equal volume of nematode suspension. The numbers of active and inactive nematodes were counted after 1, 4, 7, 24 and 48 hr. This experiment was repeated twice.

The effect of heat on the toxicity of leaf extracts to nematodes was tested by heating bean and tobacco leaf extracts in a water bath at 80 C for 10 min and then cooling them in cold water. Two-ml samples of unheated and heated extracts were mixed separately with equal volumes of the nematode suspension and the number of active nematodes counted after 24 hr.

Acetone powders (a residue of acetone-extracted homogenized tissues) were made by grinding 30 g of bean or tobacco leaves in 300 ml of cold acetone followed by vacuum filtration. The residue was washed further with 200 ml of acetone, dried and stored at −20 C until used. The acetone was discarded. Thirty grams of fresh tissue yielded 2.7 g of acetone powder. To prepare a mixture for test, 80 mg of acetone powder was soaked in 20 ml of water for 2 min, centrifuged for 10 min and the supernatant retained. Bean and tobacco acetone powders contained 14 mg and 6.5 mg of dry matter/ml, respectively. These extracts were diluted 2-, 6-, 12-, 20- and 60-fold. Two-ml samples of undiluted extracts and of each extract dilution were mixed with equal volumes of nematode suspension and active and inactive nematodes were counted after 24 and 48 hr.

To determine whether fusicoccin would increase the toxicity of plant extracts, tomato and bean plants grown in sand culture were washed free of sand and placed in a greenhouse with their roots in a solution of 5×10^{-6} M fusicoccin or in water. After 24 hours, the leaves of plants in fusicoccin had wilted. Leaf samples from three plants were removed after 8, 24 and 48 hr, frozen and the leaf extracts were made as previously described except that the desiccated leaves of the plants treated with fusicoccin were prepared in 6.8 ml of water to replace the water lost by desiccation of the tissue. A two-ml volume of each extract was mixed with an equal volume of the nematode suspension and the number of active nematodes counted after 24 hr.

Finally, 5-, 10- and 20-g samples of leaf tissue from bean, tobacco and tomato plants were homogenized separately for 10 min in 100 ml of water; then each was mixed with 2 kg of soil and shaken for 5 min. A control was also prepared by mixing 100 ml of water with 2 kg of soil and shaking it for 5 min. After 3, 8 and 21 days, a 100-g sample from each soil mixture was suspended in water, allowed to settle for 3 min and decanted through a 44-μ screen. Nematodes in the material retained by this screen were washed onto a cellulose tissue (6) and the tissue was left in contact with water overnight. By morning, the active nematodes had moved into the water and could be counted.

TABLE 1. Mean percent inactive *Tylenchorhynchus dubius* and *Hoplolaimus* spp. after 48 hours in plant extracts.

Extract source	Mean % inactive	
	T. dubius	*Hoplolaimus* spp.
Control (no extract)	9	12
Bean leaves	100	100
Bean leaves - heated[a]	30	32
Bean stems	100	100
Bean roots	33	25
Tobacco leaves	100	100
Tobacco leaves - heated[a]	36	38
Tomato leaves	31	22
Corn leaves	49	52
Rhododendron leaves	55	38

[a]Extracts heated to 80 C for 10 min and then cooled before nematode suspension was added.

RESULTS

After 48 hr, all *T. dubius* and *Hoplolaimus* spp. in bean and tobacco leaf extracts and bean stem extracts were inactive, 49% and 55% in leaf extracts of corn and rhododendron (respectively) were inactive and 31% in tomato leaf extract were inactive (Table 1). Bean and tobacco leaf extracts heated at 80 C for 10 min were much less toxic (Table 1).

TABLE 2. Mean percent inactive *Tylenchorhynchus dubius* after 1 to 48 hours in various dilutions of water extracts of tobacco or bean leaves. The equivalent concentration of extract (dry weight) per ml of suspension for each dilution is given.

Extract	Dilution	Equivalent extract concn. (mg/ml)	Mean % inactive *T. dubius* after 1 hr	4 hr	7 hr	24 hr	48 hr	24 hr	48 hr
			Test A					Test B	
Bean	0	16.5	4	61	75	96	95	100	100
	2	8.2	10	27	66	86	84	63	66
	6	2.8	5	18	27	49	31	47	34
	12	1.4	11	7	13	24	14	27	21
	20	0.8	4	0	4	14	12	19	15
	60	0.3						18	24
Tobacco	0	12.5	44	77	100	96	100	100	100
	2	6.2	49	76	85	94	92	99	80
	6	2.1	33	39	68	85	60	72[a]	67[a]
	12	1.0	6	5	12	13	48	11	19
	20	0.6	1	2	15	4	21	7	5
	60	0.2						3	3
None (ck)	0	0	4	2	15	21	25	17	20

[a]Active nematodes very sluggish.

The mobility of *T. dubius* and *Hoplolaimus* sp. nematodes slowed visibly within 1 hr in undiluted tobacco leaf extracts and within 4 hr in undiluted bean leaf extracts (Table 2). Since reactions of *Hoplolaimus* spp. and *T. dubius* were very similar, only data on *T. dubius* is presented. Although mobility was reduced after 4 hr in undiluted extract of both plant species, the nematodes were not in the characteristic arc-shape assumed when dead. After 7 hr, all of the *T. dubius* were immobilized in the tobacco extract and 75% were immobilized in the bean extract. Dilution of the bean and tobacco leaf extracts revealed that the tobacco extract was more toxic, since the ED_{50} (mean effective dose) of tobacco leaf extract after 24 hr was 1.8 mg/ml, whereas that of bean leaf extract was 3.8 mg/ml.

The extracts of the acetone powders, prepared from bean and tobacco leaves, were toxic to *T. dubius* (Table 3). The dilution series data revealed that extracts from the acetone powder of tobacco leaves were more toxic than those from bean leaves and that extracts of the acetone powders were considerably more toxic than water extracts from fresh leaf tissue. The ED_{50} for the extracts of acetone powders from tobacco and bean were 0.1 and 0.3 mg/ml, respectively, compared to 1.8 and 3.8 mg/ml of water extracts. Thus, the activity of the toxic fraction was increased approximately 15-fold by acetone extraction.

Tomato leaf extracts from plants that absorbed fusicoccin for 8 hr were not more toxic to *T. dubius* than those from plants which had absorbed water only, but extracts from leaves of plants which absorbed fusicoccin for 24 and 48 hr were more toxic (Table 4). Leaves were wilted by fusicoccin after 24 hr, and were unlikely to absorb any more solution; therefore extracts from leaves sampled after treatment with fusicoccin for 48 hr were no more toxic than those sampled after 24 hr. Nematodes suspended in 10^{-4} M fusicoccin for 48 hr showed no loss of activity.

TABLE 3. Mean percent inactive *Tylenchorhynchus dubius* after 24 and 48 hours in various dilutions of extracts and acetone powders of bean and tobacco leaves. The equivalent concentration of extract (dry weight) per ml of suspension for each dilution is given.

Extract	Dilution	Equivalent extract concn. (mg/ml)	% inactive *T. dubius* after 24 hr	48 hr
Bean	0	7.00	100	100
	2	3.50	89	100
	6	1.17	89	100
	12	0.58	91	90
	20	0.35	70	70
	60	0.12	20	20
Tobacco	0	3.25	100	100
	2	1.62	98	97
	6	0.54	97	98
	12	0.27	95	94
	20	0.16	75	70
	60	0.05	48	40
None	0	0	17	20

TABLE 4. Mean percent inactive *Tylenchorhynchus dubius* after 24 hr in tomato leaf extracts. The plants had been allowed to absorb either 5×10^{-6} M fusicoccin or water through the roots for 8, 24, or 48 hr before sampling.

Hours of absorption	% inactive *T. dubius*	
	Fusicoccin	Water
8	3	30
24	85	40
48	90	18

TABLE 5. Mean percent active *Tylenchorhynchus dubius* in soil 3 days after mixing various amounts of homogenized leaves of bean, tobacco or tomato with the soil. The number of active *T. dubius* separated from soil containing no plant extract is taken as 100%.

Homogenized leaves	Fresh weight (g) of leaves/kg of soil	% active *T. dubius*
None	0	100
Bean	2.5	67
	5.0	40
	10.0	16
Tobacco	2.5	58
	5.0	30
	10.0	29
Tomato	2.5	44
	5.0	36
	10.0	29

Bean, tobacco and tomato leaf extracts added to the soil immobilized many *T. dubius* at all concentrations after 3 days (Table 5). Even at 2.5 g of homogenized leaves per kg of soil, the number of active nematodes was reduced 33-56% compared to that in the control soil. Exposure longer than 3 days did not significantly decrease nematode activity more than at 3 days; therefore, those data are not presented in Table 5.

DISCUSSION

The toxicity of bean and tobacco leaves and stems to *T. dubius* and *Hoplolaimus* spp. has been demonstrated. Extracts from leaves of tomato, corn and rhododendron were less toxic to the nematodes than were those from bean and tobacco. However, the toxicity of the tomato was increased significantly by absorption of fusicoccin. Fusicoccin is known to alter the permeability of cell membranes (3, 12) and to cause electrolyte leakage (1). This study suggests that it also releases into the water extracts nematode-toxic material that otherwise would be discarded with the residues from the water extracts.

The activity of the plant extracts was remarkably rapid. Indeed, nematode movement was visibly slowed after 1 hr in the tobacco extracts and many were inactive after 4 hr. The fact that sluggishness was observed first, followed by cessation of movement, suggests that the toxin in the leaf extract may be toxic to nerves or muscles. Furthermore, the rapid response of the nematodes to the extracts, plus the fact that the acetone powders were also toxic, identifies the toxic fraction as a plant product and rules out the possibility that the toxicity arose from bacteria or a bacterial toxin produced in the extracts. Further work is required to identify the nematicidal material in the leaves.

The ability of the leaf extracts to inactivate nematodes was retained when these were added to the soil. It is well known that certain organic compounds (viz: mycelial residues, cellulose, chitin, soybean meal) mixed with soil will kill nematodes (4), and that plant residues (when allowed to decompose for 10 to 15 days) also act as nematicides (9, 11). Our laboratory studies with the plant extracts indicated that the nematicidal component was a plant product and not a breakdown product; therefore, in the soil its toxic action should be quickly apparent. Indeed, the ability of the homogenized leaves to kill nematodes in the soil within 72 hr and the lack of any further death of nematodes after this time suggests that leaf decomposition need not occur before toxicity is apparent. Finally, the fact that leaves retained their nematicidal properties after mixture with the soil is of practical importance. The possibility of using plant leaves for biological control of nematodes clearly warrants further investigation.

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