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DIOSCOREA FLORIBUNDA, A POTENTIAL SOURCE OF NEMATICIDES OF PLANT ORIGIN

by
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Summary. The nematicidal potential of aqueous extracts of tubers of medicinal yam, *Dioscorea floribunda*, against *Meloidogyne incognita* was evaluated *in vitro* and *in vivo*. Egg hatching was inhibited by 17.5 to 77.6% at 0.5 to 20% concentrations, respectively. Concentrations of the aqueous extracts at 20,10 and 5% killed 100% juveniles within 2.5, 3 and 4 hr, respectively. Soil amendments with chopped tuber significantly suppressed the root-knot development and greatly improved plant growth of tomato as compared with the inoculated control. The plant material possesses strong nematicidal properties, which can be successfully used as pre-plant nursery treatments.

Aqueous extracts and decomposition products of various indigenous medicinal plants have shown antihelminthic properties against plant parasitic nematodes (D'Addabbo, 1995). In the present study an attempt has been made to investigate the nematicidal properties of tubers of an indigenous medicinal yam, *Dioscorea floribunda* Mart. *et* Gal.

Materials and methods

Five concentrations (w/v) of aqueous extracts of fresh tubers of *D. floribunda* were prepared from finely chopped material in the quantities 20, 10, 5, 1 and 0.5 g with each aliquot comminuted in an electric blender in 100 ml distilled water for five minutes. The extracts were then passed through a two layered silk cloth and centrifuged at 3000 rpm for 10 minutes. The supernatants were separated and filtered by filter paper which were used as stock solutions.

The stock culture of *Meloidogyne incognita* (Kofoid *et* White) Chitw. was cultured on brinjal (*Solanum melongena* L.) growing in sterilized soil in clay pots.

To assess ovicidal action, three egg masses of equal size containing more or less the same number of eggs were placed in a glass cavity block (4x4 cm) and 2 ml aqueous plant extract at each concentration were added; distilled water served as control. Each treatment was replicated three times. The egg masses were incubated in each concentration for three days and then transferred to distilled water. The number of juveniles (J_2) freshly hatched from the egg masses were recorded up to 17 days (three days in the extracts and 14 days in distilled water) until hatching ceased. The experiment was conducted in the laboratory at 27 ± 2 °C.

To assess larvicidal action, 50 freshly hatched J_2 of *M. incognita* were placed in a single drop of distilled water in a watch glass immediately followed by the addition of a 2 ml solution of each concentration. The control was distilled water only. The treatments were replicated three times. The juvenile mortality was determined at half hour intervals for six hours and

calculated as the cumulative value. Mortality was checked by transferring the immobile nematode into distilled water for two hours following the treatment to differentiate between those not moving and killed. The results were statistically analysed.

In a further experiment, clay pots (18 cm dia.) were filled with 2 kg of solarised (for 25 days) and steam sterilized soil which comprised sandy-clay soil and cowdung (1:1). Freshly chopped tubers of *D. floribunda* were incorporated into the soil at 25, 50, 75 and 100 g per pot. The pots were watered regularly to ensure thorough decomposition of the organic additives. There were three replications for each treatment and untreated pots served as an inoculated control. After 15 days, one month old tomato (*Lycopersicon esculentum* Mill.) seedlings cv. Pusa-Ruby were transplanted singly into each pot and ten days later the pots were inoculated with 3000 freshly hatched J_2 of *M. incognita.*

Sixty days after inoculation, the plants were depotted, the roots washed gently and blotted to remove excess water. Fresh plant growth parameters such as shoot and root length, shoot and root weight were determined. The number of root galls per plant were counted and 20 individual galls from each plant were measured at random to determine their mean size. At the termination of the experiment soil nematode populations were assayed from 200 cm^3 soil aliquots by a modified Baermann funnel method. Reproduction factor (R) was calculated by dividing the final population of the nematode with the initial population (P_f/P_i). The experiment was conducted outdoors under ambient atmospheric mean temperature of 16 ± 4 °C.

Results

Immersion in 20% concentration of the tuber extract completely suppressed hatching and this effect continued for a further two days when the egg masses were transferred to distilled water (Table I). Total hatch in distilled water was more than four times that in the 20% concentration of the tuber extract, indicating a moderate nematicidal effect. The average hatch of juveniles ranged from 0 to 78.7 at 20 to 0.5% concentration of tuber extract compared to 127 nematodes hatched in control up to three days. Initially hatching was significantly suppressed by higher concentrations of the tuber extract (10 and 20%) but at a later period (7th day) it was marginally improved. On the other hand, in the lower concentrations of the treatments (0.5, 1 and 5%) the inhibitory effect started gradually and the number of juvenile hatched was very low at the later periods (15th and 17th days) of hatching. The mean juvenile hatch ranged from 70 to 259 per egg mass up to 17 days until hatching stopped as compared to 314 J_2 in the control. In all concentrations of the treatments, the juvenile hatching was suppressed and the percentage inhibition ranged from 77.6 to 17.5 compared to control. About 50% hatching inhibition occurred at 5% concentration of the *D. floribunda* tuber extracts.

A total mortality of juveniles was recorded after 2.5, 3, and 4 hr of treatment at the concentration of 20, 10 and 5% respectively, of the tuber extract, whereas 8.3% juvenile mortality was obtained with 1 and 0.5% concentration after four hours. But in the lower concentration of the treatment, the per cent mortality increased very slowly and 16.7 and 13.3% juvenile were killed by 1 and 0.5% concentration up to six hour of the treatment (Table II). Percent mortality increased with the increase in concentration and period of exposure of the tuber extract. There was no significant difference between 1 and 0.5% concentration on juvenile mortality. There was no mortality in the control during the experiment.

M. incognita multiplied freely on tomato plants in inoculated untreated control conditions (Table III). All the treatments with chopped tuber of *D. floribunda* as organic amendment significantly suppressed the devel-

TABLE I - *The effect of different concentrations of tuber extracts of* Dioscorea floribunda *on the hatching of egg masses of* Meloidogyne incognita.

Treatment (% Conc.)	Mean number of juveniles hatched (days)*									Total hatch	Mean hatch per egg mass	Percent inhibition
	3	4	5	7	9	11	13	15	17			
20	0.0 (1.0)	0.0 (1.0)	0.0 (1.0)	5.3 (2.5)	12.7 (3.6)	52.3 (7.3)	97.3 (9.9)	36.0 (6.1)	7.7 (2.9)	211.3	70.4	77.6
10	2.3 (1.8)	0.0 (1.0)	5.0 (2.4)	66.7 (8.2)	91.7 (9.6)	107.7 (10.4)	123.3 (11.1)	17.3 (4.2)	2.0 (1.6)	416.0	138.7	55.8
5	6.0 (2.6)	3.7 (2.1)	27.7 (5.3)	71.0 (8.5)	104.3 (10.2)	166.3 (12.9)	97.7 (9.8)	8.3 (3.0)	0.7 (1.2)	485.7	161.9	48.5
1	17.7 (4.2)	11.7 (3.5)	44.7 (6.7)	126.7 (11.2)	198.0 (14.1)	192.7 (13.9)	90.3 (9.4)	8.0 (2.9)	0.0 (1.0)	689.7	229.9	26.8
0.5	78.7 (8.8)	70.3 (8.4)	83.7 (9.2)	192.0 (13.9)	254.3 (15.9)	77.0 (8.7)	20.7 (4.6)	1.0 (1.4)	0.0 (1.0)	777.7	259.2	17.5
Control	127.0 (11.2)	60.7 (7.8)	63.7 (8.0)	149.3 (12.3)	286.7 (17.0)	171.3 (13.1)	56.7 (7.6)	21.7 (4.7)	5.3 (2.5)	942.3	314.1	–
SEm	0.5	0.2	0.2	0.4	0.3	0.5	0.6	0.2	0.1			
F value	37.4	119.5	133.9	58.4	126.9	16.6	7.7	26.0	15.5			
CD at 5%	1.6	0.6	0.6	1.3	0.9	1.6	1.9	0.6	0.3			
CD at 1%	2.2	0.9	0.9	1.8	1.3	2.2	2.7	0.9	0.4			

($\sqrt{X+1}$ transformed values); * egg masses immersed in extract for 3 days and then transfered to distilled water; control in distilled water throughout.

opment of root-galls, size of galls and the nematode population. There was a linear increase in the growth parameters (shoot and root length, shoot and root weight) of the treated plants with a parallel increase in the concentration of the treatments. Moreover, significant differences were observed in the rate of nematode multiplication which gradually declined with increasing concentration of the soil treatment. The multiplication rate was less than one in all treatments. The results of soil amendments with chopped tuber of *D. floribunda* promoted the growth of tomato plants and was also a potential inhibitor of the rate of multiplication (P_f/P_i ratio) of *M. incognita.*

Conclusions

The investigation indicates that aqueous extracts of the tuber of medicinal yam, *D. floribunda*, are nematicidal. It also showed strong ovicidal and larvicidal action against *M. incognita.* The soil amendments with the chopped tuber significantly reduced root-knot development in tomato plants and increased plant growth. Reduction in size and number of root-galls observed may be due to the fact that the females and the egg masses produced in tuber tissues of *D. floribunda* become surrounded by lignified cells preventing migration of the hatched juveniles into adjacent tissues and cause their death (Jatala and Bridge, 1990). Although working with different plant sources, the nature of hatching and mortality obtained by Desai *et al.* (1973) and Goswami and Vijoylaksmi (1986) were similar but the action of plant extracts used in this experiment were much faster.

The decomposition products of *D. floribunda* are also a potential hatching inhibitor and caused juvenile mortality and can be successfully used in root-knot nematode management in the seed-beds and breeding nurseries and also

TABLE II - *Effect of* D. floribunda *tuber extract on juveniles of* M. incognita.

Treatment (% Conc.)	Mean per cent mortality at 0.5 hour intervals									
	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
20	10.0	75.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	(18.4)	(60.2)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)
10	3.3	13.3	68.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	(8.6)	(20.8)	(55.9)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)
5	0.0	0.0	5.0	18.3	63.3	100.0	100.0	100.0	100.0	100.0
	(0.0)	(0.0)	(12.9)	(25.0)	(53.1)	(90.0)	(90.0)	(90.0)	(90.0)	(90.0)
1	0.0	3.3	3.3	6.7	6.7	8.3	10.0	11.7	13.3	16.7
	(0.0)	(8.6)	(8.6)	(12.3)	(12.3)	(16.6)	(18.4)	(19.9)	(21.3)	(23.9)
0.5	0.0	3.3	5.0	6.7	8.3	8.3	10.0	11.7	11.7	13.3
	(0.0)	(8.6)	(12.9)	(14.7)	(16.6)	(16.6)	(18.0)	(19.9)	(19.9)	(21.3)
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
SEm	1.2	2.2	1.6	2.3	2.9	0.8	0.8	0.6	0.7	0.9
F value	18.9	53.5	254.2	160.1	93.4	1546.0	1363.0	2303.9	2095.1	1009.5
CD ad 5%	3.8	6.9	5.0	7.2	9.1	2.5	2.5	1.9	2.2	2.8
CD ad 1%	5.4	9.9	7.2	10.3	13.0	3.6	3.6	2.7	3.1	4.0

(Angular transformed values).

TABLE III - *Effect of soil amendments with the chopped tuber of* D. floribunda *on* M. incognita *reproduction and growth of tomato.*

Treatment g/Pot	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Rootweight (g)	Final population/2 kg soil	Pf/Pi ratio	Number of galls per root system	Mean size of galls (mm)
25	32.5	16.2	13.3	7.7	1230	0.7	53	2.4
	(5.8)	(4.1)	(3.7)	(2.9)	(34.7)	(1.2)	(7.2)	(1.8)
50	36.6	18.5	14.2	8.7	1890	0.7	44	2.5
	(6.1)	(4.4)	(3.9)	(3.1)	(43.4)	(1.3)	(6.6)	(1.9)
75	36.1	17.3	17.0	10.8	2110	0.6	38	2.2
	(6.1)	(4.3)	(4.2)	(3.4)	(45.9)	(1.3)	(6.2)	(1.8)
100	42.2	20.5	17.5	11.0	2048	0.4	30	1.8
	(6.6)	(4.6)	(4.3)	(3.5)	(45.2)	(1.3)	(5.6)	(1.7)
Control	28.8	14.5	12.0	3.5	5630	1.9	282	3.9
	(5.4)	(3.9)	(3.6)	(2.1)	(74.5)	(1.7)	(16.7)	(2.2)
SEm	0.1	0.1	0.2	0.1	2.5	0.0	0.5	0.0
F value	9.4	5.0	2.2 (N.S)	16.8	36.6	30.6	72.4	21.3
CD at 5%	0.3	0.3	0.6	0.3	7.5	0.0	1.5	0.0
CD at 1%	0.4	0.4	0.8	0.4	10.3	0.0	2.1	0.0

($\sqrt{X+1}$ transformed values)

as a source of green-manure in vegetables for enhanced growth in tomato plants. The present work is the first demonstration of nematicidal activity in *Dioscorea* extract and this might be attributed to the presence of endogenous steroids in the plant material used.

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