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TOXICITY OF HARMAL, *RHAZYA STRICTA*, TO *MELOIDOGYNE INCOGNITA* AND *PRATYLENCHUS JORDANENSIS*

by

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Summary. Aqueous extracts of leaf, root and rhizosphere soil of harmal, *Rhazya stricta*, were tested against *Meloidogyne incognita* and *Pratylenchus jordanensis* under controlled conditions. Leaf extract exhibited ovistatic effect at 12.5 and 25 per cent and ovicidal effect at 50-100 per cent concentrations against *M. incognita*. Sixty-two per cent reduction in egg hatch was recorded at 100% concentration of leaf extract. Leaf and root extracts exhibited higher rate of toxicity against second-stage juveniles of *M. incognita* and all stages of *P. jordanensis* than soil extract. The rate of nematode mortality increased markedly with increase in concentration of leaf and root extracts from 50 to 100 per cent. Application of chopped leaves of harmal at 2.75 T/ha to an alfalfa field reduced the populations of *P. jordanensis* in soil and roots.

Harmal, *Rhazya stricta* Decne, commonly occurs throughout north and central Oman (Ghazanfar, 1992) as well as in other west Asian countries like Iraq, Pakistan, Saudi Arabia and United Arab Emirates. Extracts of harmal are often used in indigenous herbal medicine and more than 30 alkaloids have been identified from the extracts of leaves, roots and fruits (Rahman, *et al.*, 1989). These have been reported to possess anti-cancer activity (Rahman and Malik, 1987; Rahman and Zaman, 1988), antimicrobial activity (Mariee *et al.*, 1988) and insect repellant properties (Parveen and Malik, 1986). The nematicidal properties of *R. stricta* have not been explored so far and therefore a study was carried out to test aqueous extracts of the plant against the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. and the lesion nematode, *Pratylenchus jordanensis* Hashim which are important plant parasitic nematodes in Oman.

Materials and methods

Fresh leaves and roots of harmal were collected, washed and cut into small pieces. Standard extracts were prepared by blending 50 g of chopped leaves or roots in 200 ml of distilled water for 4-5 min and passing the suspension through double layer of muslin cloth. The extracts were further cleared by passing through Whatman no. 1 filter paper. Also, about 500 g soil was collected from the rhizosphere of harmal plants and passed through 1 mm sieve to remove stones. A 200 g portion of the soil sample was stirred for 60 minutes in 200 ml of distilled water and then filtered through Whatman No. 1 filter paper. The standard extracts were diluted to different concentrations and 5 ml of each were placed in 5 cm diameter Petri dishes and tested against nematodes.

Five uniform-size egg masses of *M. incognita*, hand-picked from infected roots of eggplant

(*Solanum melongena* L.), were placed in each Petri dish containing the aqueous leaf or root extracts of harmal. Tap water served as control and each treatment was replicated three times. All the Petri dishes were maintained at 25+1 °C in an incubator. Observation on egg hatch was recorded at three day intervals up to fifteen days after which the egg masses from all the treatments were transferred to tap water. Egg hatch in water was recorded for another fifteen days.

The effect of aqueous extracts of leaf, root and rhizosphere soil were tested against second-stage juveniles (J_2) at various concentrations. One hundred freshly hatched J_2 were added to each Petri dish containing 5 ml extract. Tap water served as control and each treatment was replicated three times. The Petri dishes were incubated at 25+1 °C and observation on juvenile mortality was recorded after 48 hrs of exposure.

Fibrous roots of alfalfa (*Medicago sativa* L.), heavily infected with *P. jordanensis*, were collected and washed free of soil. The roots were cut into small pieces and comminuted with 50 ml water in a Waring blender at low speed for 30 seconds. The root suspension was incubated in a Baermann funnel, containing water with 0.2% hydrogen peroxide, for 24 hrs and the nematodes were collected in a few ml of water using a 38 µm sieve. Two drops of nematode suspension, containing about 100 active nematodes, were added to each Petri dish containing 5 ml of leaf, root or soil extract. Tap water served as control. There were three replications for each concentration and all the Petri dishes were incubated at 25+1 °C. Observations on nematode mortality were recorded after 48 hrs.

An experiment was carried out in an alfalfa field, infested with *P. jordanensis*, at Rumais in Batinah region, from December, 1995 to August, 1996. There were three treatments *viz.*, leaves of harmal applied at 2.75 T/ha, oxamyl at 3 kg a.i./ha and an untreated control. Each treatment was replicated six times and each replication consisted of a plot of 5.7x1.8 m size. The treatments were applied twice (December and May) after cutting the alfalfa crop. Fresh leaves and tender stems of harmal were chopped into small pieces and applied uniformly in the plot. The leaves were covered by sprinkling dry soil obtained from an adjoining fallow field. Oxamyl granules were applied after mixing with an equal quantity of fine sand and mixed into the top soil using a garden tool. The plots were irrigated immediately and subsequently at three day intervals. Soil and root samples were collected from all replications before and after application of treatments. Nematodes were extracted from a composite soil sample of 250 cm^3 by sieving and modified Baermann funnel technique. One g root sample was comminuted in a Waring blender at low speed for 45 seconds and the suspension was incubated in a Baermann funnel for 48 hrs. Nematodes were collected using a 38 µm sieve and counted in one ml aliquot under a stereo binocular microscope. All data were analyzed following standard statistical procedures.

Results and discussion

The results indicated that leaf extract of harmal significantly affected egg hatch of *M. incognita* to varying degrees (Table I). Although there was an ovistatic effect at 12.5 and 25% concentrations, egg hatch continued in water after fifteen days of exposure in leaf extract. There was no difference in total hatch at low concentrations of 12.5 and 25 per cent when compared with the control. However, the extract exhibited a strong ovicidal effect at concentrations greater than 50 per cent (Table I).

The rate of mortality of *M. incognita* and *P. jordanensis* increased progressively with concentration of extracts of leaf and root (Table II). In general, there was a high rate of mortality at concentrations of 50-100% with a higher rate of mortality in root extracts than in leaf extracts.

TABLE I - *Effect of aqueous leaf extract of* Rhazya stricta *on egg hatch of* Meloidogyne incognita.

Concentration (%)	Egg hatch in leaf extract (15 days)	Egg hatch in water (15 days)	Total hatch	Per cent reduction
0 (water)	1221	12	1233 A	–
12.5	1026	366	1312 A	-6.4
25	498	764	1262 A	-2.4
50	106	646	752 B	39.0
75	20	676	696 B	43.6
100	7	462	469 C	62.0

Means followed by the same letter are not significantly different at P≤0.01.

TABLE II - *Toxicity of aqueous extracts of leaf, root and rhizosphere soil of* R. stricta *to* M. incognita *and* P. jordanensis.

Concentration (%)	% mortality in extracts					
	M. incognita			*P. jordanensis*		
	Leaf	Root	Soil	Leaf	Root	Soil
12.5	5.7 D	11.0 C	5.3 B	50.5 C	22.8 C	11.2 C
25	10.4 D	20.0 B	9.2 AB	56.5 C	28.5 C	16.7 C
50	29.2 C	92.8 A	10.1 AB	77.2 B	79.3 B	25.1 B
75	52.6 B	95.0 A	12.6 A	90.3 A	92.4 A	27.9 B
100	70.1 A	95.0 A	14.0 A	93.5 A	92.4 A	35.3 A

Means followed by the same letter are not significantly different at P≤0.01.

The extract of rhizosphere soil exhibited only a weak nematicidal effect on *M. incognita* and *P. jordanensis* even at the highest concentration of 100 per cent.

The results of the field experiment, although not statistically significant, indicated that nematode populations decreased both in soil (72.1%) and roots (80.6%) nine months after the first application of harmal and there was no such reduction in oxamyl treatment when compared with control. Root populations of *P. jordanensis* were relatively high only from May to September when air and soil temperatures were high (Mani and Al Hinai, 1997). Nematode populations in soil and roots were high during May and August both in control and oxamyl and considerably low in the harmal treatment indicating the efficacy of harmal in preventing penetration/multiplication of the nematode. The efficacy of harmal could probably be enhanced by applying it at a higher rate than that used in the present study.

The results obtained are in agreement with earlier reports which indicated that leaf extracts of many plant species inhibited egg hatch and killed J_2 of *M. incognita* due to the presence of toxic compounds (Jain and Hasan, 1984; Mani and Chitra, 1989; Venkata Rao *et al.*, 1986). More than thirty alkaloids were identified from leaves, roots and fruits of *R. stricta* (Rahman and Malik, 1987; Rahman and Zaman, 1988; Rahman *et al.*, 1987 and 1989). Hence, the toxicity of leaf and root extracts of *R. stricta* to *M. incognita* and *P. jordanensis* could be due to the presence of few or many alkaloids. The toxic effect of rhizosphere soil extract of harmal in-

dicated that the roots of harmal exuded some compounds which were toxic to nematodes. Thus, the present findings established the *in vitro* toxicity of various parts of *R. stricta* to *M. incognita* and *P. jordanensis* and its efficacy in reducing the population of *P. jordanensis* in alfalfa field.

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