Screening of some Leguminosae seeds for nematicidal activity

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ABSTRACT

Root-knot nematodes, Meloidogyne spp., are the major nematode pests of economic crops worldwide. Use of conventional nematicides has been one of the most effective methods to reduce their populations. However, the ban on many effective nematicides in recent past has necessitated the search for alternative sources of effective and ecofriendly chemicals for nematode control. The nematicidal potential of fifteen Leguminosae seeds (wild and cultivated) was assessed, in vitro, against juveniles of Meloidogyne incognita (Kofoid & White) Chitwood. Each standard aqueous seed extract, 25g/100ml (w/v), was evaluated at dilutions, 1:10, 1:20, and 1:40 at 27±1°C for 24 hours. The majority of seed extracts caused high mortality rates, especially in 1:10 concentration. Trigonella foenum graceum, Sesbania sesban, Albizzia lebbak, Cassia fistula and Pongamia glabra were the most efficacious (above 90% mortality). S. sesban and P. glabra were significantly effective even in the lowest concentration (1:40).

Key words: *root-knot nematodes, Meloidogyne incognita, seed extracts, plant extracts, botanicals, bioactivity*

1 INTRODUCTION

A good deal of research efforts in nematode management is directed toward root-knot nematodes, *Meloidogyne* spp., the most damaging of plant parasitic nematodes. *M. incognita* is the most widely distributed of root-knot nematodes and has a wide range of hosts.

The environmental hazards associated with the chemical control using conventional pesticides are well documented now and being increasingly highlighted. The use of many hazardous chemical nematicides has been banned. Search for alternative methods for application in pest control is an active area of research at present. The plant based pesticidal chemicals (botanicals) have found favour, in recent times, as an alternative to pesticides. Some of these botanicals are already being exploited commercially in insect control (Agnihotri *et al*. 1999).

Exploration of nematicidal potential of botanicals and their application is on increase. Different plant parts are being tested to identify the sources of nematicidal substances. However seeds have received only limited attention so far. Khurma and coworkers (Khurma and Kumari, 1996; Khurma and Singh, 1997; Khurma and Chaudhary, 1999) reported the strong nematicidal activity of seeds, particularly of some Leguminosae seeds tested, in different evaluation studies. This encouraged the undertaking of the present investigation on nematotoxic evaluations of Leguminosae seeds on juveniles of *M. incognita*. The objective was to confirm the activity of some seed extracts and to screen some of the seeds of this family not evaluated before for their nematicidal properties.

2 MATERIALS AND METHODS

2.1 SEED EXTRACTS

Aqueous seed extracts of fifteen wild and cultivated Leguminosae plant were evaluated, *in vitro*, for their nematicidal and nematostatic effects. Each standard extract was prepared from 25g seeds in 100ml of water. The mature seeds were washed thoroughly. The dried seeds were then powdered in an electric grinder and 25g of powdered seeds was soaked in 100ml of distilled water for 24 hours. The solution was then centrifuged at 4000rpm for 10 minutes and then filtered through Whatman filter paper no.1. This standard solution, taken as 100% seed extract, was used for making dilutions, viz., 1:10, 1:20, and 1:40 by addition of distilled water.

2.2 EXPERIMENTAL NEMATODE

Second stage juveniles (J_2) of *Meloidogyne incognita* (Kofoid & White) Chitwood were used for toxicity evaluations. *M. incognita* population from infected Brinjal roots (from Guru Nanak Dev University campus, Amritsar) was maintained on tomato plants in pot cultures. The juveniles were obtained by hatching of egg masses in distilled water at 27±1°C.

2.3 EXPERIMENTS

Juveniles (J_2) were treated with each concentration at 27±1°C in a BOD incubator. Juveniles in distilled water served as control. For each treatment, 1 ml suspension containing approximately 100 (80-100) juveniles (3-7 days old) was added to 10 ml of test concentration, or distilled water in a 3cm diameter petri dish. Each treatment was replicated four times. Dead and live juveniles were counted after 24 hours. The immobilised juveniles in a typical dead posture were manipulated with a fine quill pick to confirm the mortality. Some of the treated, dead juveniles were transferred to distilled water to check for revival. Mean percentage mortality and LC_{50} values were calculated for comparison of activity (Table 1) of different seed extracts.

3 RESULTS AND DISCUSSION

For most of the seeds strong nematicidal activity was demonstrated in the highest concentration (1:10). Five seeds, namely, *Trigonella foenum graceum, Sesbania sesban, Albizzia lebbak, Cassia fistula* and *Pongamia glabra*, induced very high J_2 mortality (90-97%) in this concentration. LC_{50} values for these seeds indicate that relatively high dilutions of standard extract can cause effective mortality rates (Table 1). Above 70% juvenile mortality was recorded in 1:20 concentration of *T. f. graceum* and *S. sesban* seed extracts, while in 1:40, fairly significant activity was shown only by *P. glabra* and *S.*

sesban (LC_{50} values 1:47 & 1:39, respectively). High activity (85% or above mortality) in 1:10 concentration was also obtained for *Sesbania aculeata, Medicago falcata, Cassia didymbotrys, Cassia occidentalis* and *Acacia acuminata*. For *Acacia auriculiformis* LC_{50} value equals that of *C. fistula* (1:27) though J_2 mortality realised in highest concentration was 78%.

Seeds have been largely unexplored in nematode control though some common oilseed cakes have been tested frequently. Khurma and coworkers, based on screening of seeds of many different plants in separate studies (1996, 1997, and 1999), observed that the seeds in general have a strong nematicidal potential. Khurma and Singh in 1997 concluded that majority of seeds tested, had strong nematicidal properties and Leguminosae seeds had a higher potential. The efficacy of seed extracts was tested on mortality of juveniles as well as hatching of *M. incognita* and *M. javanica*. Nine Leguminosae seeds tested were reportedly quite promising even in low concentrations. *S. sesban*, and three *Cassia* spp. tested were also strongly inhibitory to egg hatch. The seed extract of *S. sesban* was found to be highly effective, exhibiting its strong toxic properties within 8 hours, in an earlier study too (Khurma and Kumari, 1996). *S. sesban* and *C. fistula* seed extracts were observed to be highly nematicidal by Khurma and Chaudhary, 1999, even in low concentrations. In the same study the seed extracts were found to be more effective than extracts of other parts of the respective plants. Results obtained in the present study on *C. fistula, C. occidentalis, S. sesban* and *P. glabra* substantiate the earlier findings on their activity. Seven new seeds of family Leguminosae evaluated for nematicidal properties for the first time in this study also support the previous observations on the activity of seeds of Leguminosae plants.

Table 1. *In vitro* activity of some seed extracts (Family Leguminosae) against *Meloidogyne incognita* J_2 (second stage juveniles) mortality in decreasing order in 1:10, at 27±1°C, after 24 hours

PLANT	DILUTIONS				LC_{50}
	1:10	1:20	1:40	Control	
Trigonella foenum graceum	97	74	38	0	1:33
Sesbania sesban	96	73	49	0	1:39
Albizzia lebbak	94	67	35	1	1:31
Cassia fistula	92	56	37	1	1:27
Pongamia glabra	90	68	56	0	1:47
Sesbania aculeata	89	51	31	0	1:21
Medicago falcata	89	47	32	0	1:19
Cassia didymbotrys	87	32	19	0	1:16
Cassia occidentalis	86	35	25	0	1:17
Acacia acuminata	85	48	19	2	1:19
Acacia auriculiformis	78	62	25	0	1:27
Phaseolus vulgaris	50	9	2	1	-
Pisum sativum	47	13	2	0	-
Jaccaranda mimosifolia	7	2	0	0	-
Tamarindus indica	0	0	0	0	-

Phaseolus lunatus (Leguminosae), and *P. glabra* were reported to be toxic or inhibitory to root-knot nematodes (juveniles, and egg hatch) by Husain and Masood, 1975, and Shahda *et al*. 1998. Aqueous extracts of *C. occidentalis* were shown to be more toxic than their ethyl acetate extracts (Sarosh *et al*. 1989). Apparently the substances toxic to nematodes are water soluble and active in aqueous extracts.

The results obtained in the present study are in agreement with the previous observations on the strong nematicidal potential of the Leguminosae seeds. It is suggested that more seeds and other parts of plants belonging to this family, especially of wild variety, should be screened to identify the sources of nematicidal substances. The seeds with demonstrated high activity should be subjected to further investigation for possible application in nematode management.

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