Nematol. medit. (1997), 25: 49-54

Department of Zoology, Guru Nanak Dev University, Amritsar 143005, India

NEMATICIDAL POTENTIAL OF SEED EXTRACTS: *IN VITRO* EFFECTS ON JUVENILE MORTALITY AND EGG HATCH OF *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA*

by
UMA R. KHURMA and ARCHANA SINGH

Summary. Seed extracts of *Acacia eburnea*, *Azadirachta indica*, *Cassia* sp., *Parkinsonia aculeata*, *Sesbania sesban* and *Poinciana regia* had the highest nematicidal potential against *Meloidogyne incognita* juveniles while maximum potential against *M. javanica* was shown by *A. eburnea*, *Cassia* sp., *Melia azedarach*, *S. sesban* and *Tribulus terrestris*, as indicated by strong activity in all concentrations. Similarly, *Calotropis procera* and *S. sesban* were the best in preventing egg hatch of *M. incognita*, and *C. procera*, *S. sesban* and *Chenopodium album* were the most effective against hatch of *M. javanica* eggs. *S. sesban* was outstanding in killing juveniles and preventing hatching of both the species.

Nematicidal properties of seed extracts have received less attention than those of other plant parts and seed cakes of some common oil-yielding plants (Prot and Kornprobst, 1983; Devakumar *et al.*, 1985; Lee, 1990). This study was undertaken after the encouraging results obtained for some seed extracts, against root knot nematodes, in an earlier study in this laboratory.

Materials and methods

The screening of thirty locally available seeds was carried out, *in vitro*, in 1993 for their nematicidal activity. Seeds of thirty (Table I) and twenty-seven (Table II) species, respectively, were tested against *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw. juveniles (J_2) and twenty-eight (Tables III and IV) for inhibitory effects on egg hatch of both species.

Seeds were ground in a grinder and immersed in 100 ml of distilled water for 24 hours, centrifuged at 4,000 rpm for 10 minutes and then filtered. Treatments were 1:10, 1:20 and 1:40 concentrations. Distilled water was used as the control.

Nematode populations were maintained on brinjal and okra in pot cultures. For mortality studies, 80-100 J_2/ml suspension were exposed to 10 ml of each test solution in 3 cm diameter Petri-dishes with three replicates of each treatment. The dishes were kept at 27 ± 1 °C in a B.O.D. incubator for 24 hours. Dead and live juveniles were then counted. Mortality was ascertained by transferring some of the treated, apparently dead juveniles to distilled water and examining them after 5-6 hours for any revival. Mortality was expressed as mean per cent.

For evaluating the effects on hatching, mature egg masses were separated from fresh roots. Four egg masses were placed in 10 ml of

TABLE I - In vitro *screening of seed extracts for nematicidal activity against* Meloidogyne incognita *second stage juveniles (J_2).*

Plant species	Family	Per cent J_2 mortality, at 27 ± 1 °C, after 24 hrs			
		Dilutions			
		1:10	1:20	1:40	Control
Acacia eburnea Willd.	Leguminosae	100	98	91	0
Azadirachta indica A. Juss.	Meliaceae	100	95	89	0
Cassia sp.	Leguminosae	100	93	86	0
Parkinsonia aculeata L.	Leguminosae	98	92	84	0
Sesbania sesban (L.) Merr.	Leguminosae	98	91	81	0
Eclipta alba (L.) Hassk.	Compositae	98	84	41	0
Acacia sp.	Leguminosae	98	78	61	1
Poinciana regia Boj	Leguminosae	96	94	80	0
Pongamia glabra Vent. Jard. Malm.	Leguminosae	96	85	65	0
Cassia fistula L.	Leguminosae	96	72	44	1
Melia azedarach L.	Meliaceae	96	70	55	0
Calotropis procera R. Br.	Asclepiadaceae	95	80	62	0
Terminalia bellerica Roxb.	Combretaceae	92	82	49	0
Withania somnifera (L.) Dunal	Solanaceae	88	68	13	0
Terminalia arjuna (Roxb. ex DC) Wt. *et* Arn. Prodr.	Combretaceae	87	69	57	0
Cassia occidentalis L.	Leguminosae	86	66	11	2
Parthenium hysterophorus L.	Compositae	86	62	13	0
Amaranthus viridis L.	Amaranthaceae	85	42	11	0
Chenopodium album L.	Chenopodiaceae	83	55	34	0
Nicotiana plumbaginifolia Viv. Elench.	Solanaceae	68	31	9	0
Croton bonplandianum Baill.	Euphorbiaceae	62	43	17	0
Tribulus terrestris L.	Zygophyllaceae	54	32	12	0
Euphorbia hirta L.	Euphorbiaceae	43	18	6	0
Malvastrum coromandelianum[1] (L.) Garcke	Malvaceae	29	15	10	0
Cannabis sativa L.	Cannabaceae	14	9	4	0
Mimusops elengi L.	Sapotaceae	11	2	0	0
Lantana indica L.	Verbenaceae	4	4	0	0
Callistemon lanceolatus DC. Prodr.	Myrtaceae	0	0	0	0
Lagerstroemia speciosa (L.) Pers.	Lythraceae	0	0	0	0
Lagerstroemia thorelii Gagnep.	Lythraceae	0	0	0	0

[1] With nematostatic effect.

each dilution in 3 cm diameter Petri-dishes. Distilled water served as the control. Each treatment was replicated three times. The dishes were kept at 27 ± 1 °C and emerged juveniles were counted after 48 hours exposure. Mortality of emerged juveniles was also noted. Results were expressed as mean per cent hatch and mean per cent mortality.

Results

Nineteen seed extracts were highly toxic (83-100%) to *M. incognita* in the highest concentration (1:10) (Table I) and most of these also showed very good activity at 1:20 dilution. Mortality generally was much less at 1:40 concentration. However, *Acacia eburnea*, *Azadirachta indica*, *Cassia* sp., *Parkinsonia aculeata*, *Sesbania sesban* and *Poinciana regia* were highly effective (80-100%) in all dilutions.

Against *M. javanica*, seventeen seeds were highly active (at 1:10) and five were ineffective at all dilutions (Table II). *A. eburnea*, *Cassia* sp., *Melia azedarach*, *S. sesban* and *Tribulus terrestris* killed more than 80% of juveniles at all dilutions, while the same level of mortality was obtained with *Acacia* sp., *Calotropis procera*, *P. regia*, *Terminalia arjuna* and *T. bellerica* up to 1:20 dilution.

Callistemon lanceolatus, *Lagerstroemia speciosa* and *L. thorelli* had no adverse effect on ei-

TABLE II - In vitro *screening of seed extracts for nematicidal activity against* M. javanica *second stage juveniles* (J_2).

Plant species	Per cent J_2 mortality, at 27 ± 1 °C, after 24 hrs			
	Dilutions			
	1:10	1:20	1:40	Control
Acacia eburnea	100	100	91	0
Cassia sp.	100	96	87	0
Sesbania sesban	100	88	71	0
Ponciana regia	100	81	66	0
Acacia sp.	100	70	61	0
Tribulus terrestris	98	93	79	0
Melia azedarach	98	92	83	0
Terminalia arjuna	98	80	64	0
Azadirachta indica	96	59	19	0
Calotropis procera	95	85	62	0
Cassia fistula	93	61	42	0
Terminalia bellerica	92	85	67	0
Cassia occidentalis	90	51	11	0
Chenopodium album	89	52	22	0
Withania somnifera	83	61	20	0
Parthenium hysterophorus	80	60	32	0
Eclipta alba	72	51	28	0
Euphorbia hirta	67	31	1	0
Lantana indica	64	51	21	0
Croton bonplandianum	62	41	12	0
Cannabis sativa	50	31	12	0
Malvastrum coromandelianum[1]	13	6	1	0
Mimusops elengi	12	4	1	0
Nicotiana plumbaginifolia	9	2	0	0
Callistemon lanceolatus	0	0	0	0
Lagerstroemia speciosa	0	0	0	0
Lagerstroemia thorelii	0	0	0	0

[1] With nematostatic effect.

TABLE III - In vitro *screening of seed extracts for their inhibitory effects on egg hatch and juvenile (J_2) mortality of* M. incognita.

Plant species	Per cent egg hatch, at 27 ± 1 °C, after 48 hrs							
	Dilutions							
	1:10		1:20		1:40		Control	
	% Egg[1] hatch	% J_2 mortality	% Egg hatch	% J_2 mortality	% Egg hatch	% J_2 mortality	Actual egg hatch no.	% J_2 mortality
Calotropis procera	1	100	9	85	50	14	87	0
Sesbania sesban	2	100	16	74	80	71	73	0
Cassia occidentalis	5	51	46	49	80	0	41	0
Chenopodium album	10	100	42	58	75	0	28	0
Terminalia bellerica	13	100	48	66	81	0	79	0
Cassia fistula	21	100	55	79	100	36	47	0
Withania somnifera	21	100	59	50	92	0	57	0
Cannabis sativa	23	74	42	53	94	0	105	0
Cassia sp.	25	100	53	50	97	0	100	0
Melia azedarach	28	100	30	74	53	0	56	0
Azadirachta indica	28	100	64	64	88	40	102	0
Terminalia arjuna	31	67	39	52	96	0	91	0
Parthenium hysterophorus	34	82	68	47	88	0	94	0
Tribulus terrestris	36	33	70	29	92	0	82	0
Euphorbia hirta	42	40	57	31	98	0	56	0
Acacia sp.	43	97	76	80	96	2	104	0
Pongamia glabra	48	89	62	55	88	0	94	0
Acacia eburnea	53	100	73	45	96	0	104	0
Croton bonplandianum	53	96	73	36	100	0	60	0
Amaranthus viridis	57	71	59	58	83	0	64	0
Poinciana regia	65	81	90	35	100	12	72	0
Malvastrum coromandelianum[2]	76	16	89	6	92	0	74	0
Mimusops elengi	83	8	86	1	98	0	83	0
Parkinsonia aculeata	88	50	90	28	91	0	62	0
Lantana indica	88	6	94	2	96	0	89	0
Lagerstroemia thorelii	91	0	93	0	100	0	100	0
Lagerstroemia speciosa	92	0	98	0	100	0	56	0
Callistemon lanceolatus	97	0	100	0	96	0	39	0

[1] Percentage egg hatch in relation to actual hatch in control.
[2] With nematostatic effect.

ther *M. incognita* or *M. javanica*, but *Malvastrum coromandelianum* had a nematostatic effect as indicated by the revival of juveniles after being kept in water for a few hours.

Maximum inhibition of egg hatch occurred with the lowest (1:10) dilution of seed extracts. Only *C. procera* (9%), followed by *S. sesban* (16%) and *M. azedarach* (30%) suppressed the

juvenile emergence of *M. incognita* significantly in 1:20 dilution. *S. sesban* was highly toxic to emerged juveniles, causing mortality at all dilutions.

The greatest inhibitory effect on egg hatch of *M. javanica* was shown by *C. procera, S. sesban, T. arjuna* and *Chenopodium album* at 1:10 dilution. *C. procera* effectively suppressed egg

TABLE IV - In vitro *screening of seed extracts for their inhibitory effects on egg hatch and juvenile (J_2) mortality of* M. javanica.

Plant species	Per cent egg hatch, at 27 ± 1 °C, after 48 hrs							
	Dilutions							
	1:10		1:20		1:40		Control	
	% Egg[1] hatch	% J_2 mortality	% Egg hatch	% J_2 mortality	% Egg hatch	% J_2 mortality	Actual egg hatch no.	% J_2 mortality
Calotropis procera	3	100	24	63	31	32	94	0
Sesbania sesban	3	100	19	85	75	50	56	0
Terminalia arjuna	8	100	48	47	100	0	94	0
Chenopodium album	9	63	28	42	78	0	53	0
Cassia occidentalis	12	100	43	48	97	0	41	0
Terminalia bellerica	12	82	34	12	85	0	101	0
Melia azedarach	13	100	46	37	83	0	73	0
Cassia sp.	14	100	66	46	91	0	104	0
Cannabis sativa	15	81	41	47	88	0	106	0
Withania somnifera	18	100	54	36	95	0	44	0
Euphorbia hirta	22	58	42	49	81	0	77	0
Azadirachta indica	25	100	60	63	92	0	105	0
Cassia fistula	34	100	56	68	85	57	65	0
Parthenium hysterophorous	35	70	71	24	84	0	101	0
Tribulus terrestris	37	39	67	26	95	0	87	0
Acacia eburnea	48	100	68	57	88	33	76	0
Acacia sp.	49	88	69	74	99	0	102	0
Poinciana regia	62	68	75	32	86	16	66	0
Parkinsonia aculeata	63	40	76	32	79	0	69	0
Pongamia glabra	68	49	83	41	98	0	67	0
Croton bonplandianum	69	100	80	50	91	0	36	0
Malvastrum coromandelianum[2]	76	14	76	3	96	0	76	0
Mimusops elengi	79	8	87	2	95	0	105	0
Amaranthus viridis	83	57	92	23	93	0	81	0
Lantana indica	86	8	89	1	98	0	75	0
Callistemon lanceolatus	89	0	91	0	95	0	46	0
Lagerstroemia thorelii	94	0	96	0	100	0	82	0
Lagerstroemia speciosa	96	0	96	0	97	0	94	0

[1] Percentage egg hatch in relation to actual hatch in control.
[2] With nematostatic effect.

hatch at all dilutions (Table IV). *Cassia fistula* and *S. sesban* were most effective against emerged juveniles.

Discussion

Seed extracts of *Acacia eburnea*, *Cassia* sp. and *S. sesban* showed the greatest nematicidal effect at the three concentrations tested against both *M. incognita* and *M. javanica*. Nematicidal specificity of some seeds (*A. indica*, *M. azedarach*, *T. terrestris* and *Eclipta alba*) as indicated by difference in responses of two nematode species needs more trials to be established conclusively.

Inhibitory potential of the extracts does not necessarily correspond with their ability to induce juvenile mortality. Some seed extracts causing high juvenile mortalities did not always inhibit egg hatch effectively, e.g., *A. eburnea*. Besides, the same degree of toxicity was not obtained for mortality of emerging juveniles as compared to when J_2 were exposed directly. The difference could be that continuous hatching of juveniles does not expose all of them to equal or sufficient exposure time. Against *M. incognita*, eleven seeds and against *M. javanica*, twelve seeds allowed less than 30% egg hatch (at 1:10). In general, the extracts were less effective against egg hatch as compared to mortality of emerged juveniles. *C. procera* and *S. sesban* were the most inhibitory for both species. All seeds that were highly effective in suppressing egg hatch, also caused 100% or nearly 100% juvenile mortality, but as the juvenile emergence was low, very few juveniles were actually exposed. Where egg hatch inhibition was not significant, the juvenile mortality rates were important, e.g., *A. eburnea* did not inhibit egg hatch in both species but effectively killed the emerged juveniles, thus indicating the different nature of nematicidal activity. For *M. javanica*, *Croton bonplandianum* and *Acacia* sp. also showed similar results. Interestingly, *Cannabis sativa* seeds inhibited egg hatch for both species but were much less effective in causing juvenile mortality.

Callistemon lanceolatus, *L. speciosa* and *L. thorelii* were ineffective in suppressing hatch or in causing mortality. The results clearly indicate the superior potential of seeds of some families, prominently, Leguminosae, Meliaceae and Asclepiadaceae, to inhibit hatch or increase juvenile mortality.

The inhibition of egg hatch and juvenile mortality of *M. incognita* by *M. azedarach* seed extracts was also reported by Lee (1990). High nematotoxic potential of seeds of *S. sesban*, *M. azedarach* and *C. procera* is in conformity with earlier observations in this laboratory, wherein also the seed extracts of *M. azedarach* and *Ricinus communis* L. were found to be more toxic as compared to leaves. Higher potential of seeds of *Leucaena leucocephala* in comparison to other plant parts was also indicated by Jain and Hasan (1984).

In view of the present findings, there is need for extensive screening of seed extracts from many plant species. The immediate application of seed extracts may be as spot, nursery or root-dip treatments, but comprehensive testing is needed for field applications.

Literature cited

DEVAKUMAR C., GOSWAMI B. K. and MUKERJEE S. K., 1985. Nematicidal principles from neem (*Azadirachta indica A. Juss*). Part I. Screening of neem kernel fractions against *Meloidogyne incognita*. *Indian J. Nematol.*, *15*: 121-124.

JAIN R. K. and HASAN N., 1984. Toxicity of koo-babool (*Leucaena leucocephala* L.) extracts to *Meloidogyne incognita* and *Helicotylenchus dihystera*. *Indian J. Nematol.*, *14*: 179-207.

LEE M. J., 1990. The effect of extracts of *Melia azedarach* on *Meloidogyne incognita*. *Quart. J. Chinese Forest.*, *20*: 1-7.

PROT J. C. and KORNPROBST J. M., 1983. Effects of *Azadirachta indica*, *Hannoa undulata* and *Hannoa klaineana* seed extracts on the ability of *Meloidogyne javanica* juveniles to penetrate tomato roots. *Revue Nematol.*, *6*: 330-332.

Accepted for publication on 3 January 1997.