JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2008. Vol. **2**(2), p. 443-446

Effect of Crude Alcoholic Fractions of Different Plant Extracts on Hatching and Larval Mortality of *Meloidogyne incognita*

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(Received: 20 April 2008; accepted: 08 June 2008)

Experiments were conducted *in vitro* to assess the nematotoxic effect of crude alcohol soluble fractions of *Pongamia pinnata, Lantana camara*, *Euphorbia pulcherrima, Thevetia peruviana, Martynia annua* and *Opuntia* on root-knot nematode *Meloidogyne incognita*. Plant extracts were assayed for ovicidal and larvicidal effect. Leaf extracts of *Pongamia pinnata, Lantana camara*, and *Euphorbia pulcherrima* were found to be most effective where as leaf extract of *Thevetia peruviana* and *Martynia annua* as well as cladode extract of *Opuntia* did not show appreciable results.

Key words: Alcoholic fractions, Ovicidal, Larvicidal, Nematotoxic, and *Meloidogyne incognita*.

Root-knot nematode (*Meloidogyne* spp.) is one of the most important plant parasitic nematode. These organisms are found worldwide and attack thousands of different plant species. *M. incognita* damages plants by devitalizing root tips and stopping their growth. Formation of swelling or knots in the roots reduces the availability of nutrients, disrupts the vascular system and interferes with translocation of water and minerals from soil (Singh and Sitaramiah 1994). Various organic additives of plant origin including oil-seed cakes, leaves and other plant parts are being effectively used for management of nematodes (Iram & Siddique, 2005; Patel *et al*. 2004). Use of plants as herbal biocontrol agents is preferred over the most nematicidal chemicals, as they are safe, cheap and easily available. Hence, in the present study, crude alcoholic fractions of six different plants i.e. *Pongamia pinnata, Lantana camara*, *Euphorbia pulcherrima, Thevetia peruviana, Martynia annua* and *Opuntia* were screened to assess the *in vitro* ovicidal and larvicidal effect on egg hatching and larval mortality of *Meloidogyne incognita.*

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MATERIAL AND METHODS

Plant extract preparation

Healthy leaves of *P. pinnata, L. camera*, *E. pulcherrima, T. peruviana, M. annua* and cladode of *Opuntia* were collected from Botanical garden, College of Science, Udaipur (Raj.). The collected plant parts were washed with sterile

distilled water. 250 gm of clean fresh plant material were ground with 250 ml of absolute alcohol. The mixture was allowed to stand for 48 h at room temperature and was subsequently filtered through filter paper. The solvent was completely evaporated from the extract in hot air oven at 30°-40°C till it become a semisolid material. A stock solution of 10 mg / ml in 1% Tween 80 was made and from this further dilutions such as 1mg/ml, 2 mg/ml, 4 mg/ml and 8 mg/ml were prepared by adding required amount of sterile distilled water.

Ovicidal and Larvicidal effect of plant extract on *M. incognita*

Hatching assay was done according to the method suggested by Sharma and Patel (2001). *M. incognita* egg masses of roughly similar sizes were collected from infected tomato roots and washed with sterile distilled water. The egg masses were kept in glass cavity blocks (1 egg mass /cavity block) containing 3 ml of alcohol extract of respective concentrations. A distilled water control was maintained simultaneously. Three replicates of each set were maintained. Number of juveniles hatched after 24, 48, and 72 h were counted. Mortality assay of plant extracts on root-knot juveniles was done according to the method given by Saravanapriya and Sivakumar (2004). Freshly hatched second stage juveniles of *M. incognita* were transferred to different cavity blocks (100 juveniles / cavity block) containing respective concentrations of plant extract (3 ml / cavity block). Juveniles put in distilled water were treated as control. Three replicates of each experiment were maintained. Percent larval mortality rate was counted at the intervals of 24, 48 and 72 h. Larvae that did not respond to touch by a fine needle were counted as dead.

RESULTS AND DISCUSSION

The results of ovicidal and larvicidal are presented in Table 1and 2. Maximum egg hatching and minimum larval mortality was observed in control i.e. distilled water. Among all six plant extracts *P. pinnata, L. camera*, and *E. pulcherrima* extracts proved most effective *T. peruviana* leaf extract was found to be slightly inhibitory where as *M. annua* and *Opuntia*

Table 1. Effect of crude alcoholic fractions of plant extracts on egg hatching of *M. incognita*

Plant species	Time duration in hours	% Larval mortality at different concentrations*					
		Control	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml	10 mg/ml
P. pinnata	*24*	*20*	*5.9*	*5.1*	*3.4*	*2.5*	*1.6*
	48	28	12.3	10.1	7.8	5.2	3.2
	72	45	16.1	12.1	9.1	7.2	3.7
L. camara	24	20	11.6	10.5	9.3	6.3	4.3
	48	28	18.3	17.8	14.3	11.2	10.1
	72	45	23.0	22.6	18.6	16.3	13.2
E. pulcherrima	24	20	17.9	17.3	15.4	12.0	10.0
	48	28	26.9	25.5	23.3	20.1	18.0
	72	45	44.4	43.0	42.3	38.8	33.6
T. peruviana	24	20	18.6	18.0	16.8	15.0	12.7
	48	28	23.6	21.0	19.0	18.6	15.0
	72	45	41.0	37.6	35.0	32.3	28.3
M. annua	24	20	18.9	16.1	15.0	13.8	11.2
	48	28	25.0	22.6	20.1	17.8	17.1
	72	45	41.0	38.8	38.0	36.6	34.0
Opuntia	*24*	*20*	*19.1*	*18.6*	*18.1*	*16.6*	*14.2*
	48	28	25.0	22.0	21.3	19.6	17.0
	72	45	42.3	40.0	39.6	37.0	34.6

* Average of three replicates

extracts were least effective. 10 mg /ml concentration of alcohol soluble fractions of leaves of *P. pinnata, L. camera* and *E. pulcherrima* showed significant inhibition of egg hatching and maximum larval mortality. Results indicate that rate of mortality and inhibitions of hatching were directly proportional to exposure period and extract concentration.

Nematicidal activity of plant extract has been reported by several workers (Singh *et al.,* 2001; Singh and Dabur, 2004; Saravanapriya and Sivakumar, 2004). The present investigation is in conformity with the finding of Chandravadana *et al*., (1996) who screened 21 plant extracts obtained from 12 edible plants species against root-knot nematode. Kalaiarasan *et al*., (2007) proved that the water soluble fractions of *Jatropha* deoiled cake reduced the rate of hatching and increased juvenile mortality. Inhibition of hatching and increase juvenile mortality of *M. incognita* by methanol extract of five plants haves been reported by Saravanapriya and Sivakumar (2005).

Plants contain several secondary metabolites, which are responsible for their antimicrobial activity (Mcgaw and Eloff, 2005; Baswa *et al.,* 2001; Vaijayantimala *et al.,* 2001).

Table 2. Effect of crude alcoholic fractions of plant extracts on second stage juveniles of *M. incognita*

Plant species	Time duration in hours	% Larval mortality at different concentrations*					
		Control	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml	10 mg/ml
P.pinnata	*24*	*00*	*28.2*	*30.9*	*36.1*	*57.6*	*70.1*
	48	00	30.6	38.6	49.2	72.5	78.1
	72	00	37.2	50.4	68.4	80.2	96.6
L. camera	24	00	15.0	24.2	28.1	38.6	50.3
	48	00	26.1	30.5	40.0	53.3	68.2
	72	00	34.0	36.9	52.1	60.0	80.2
E. pulcherrima	24	00	20.1	27.0	33.1	46.2	58.3
	48	00	30.0	34.2	45.2	57.6	72.5
	72	00	36.1	37.0	57.6	79.6	85.6
T. peruviana	24	00	00.0	10.0	14.2	26.3	30.0
	48	00	5.0	15.0	21.0	32.2	42.0
	72	00	18.0	20.0	27.0	43.2	50.0
M. annua	24	00	00.0	00.0	5.0	16.1	22.1
	48	00	2.1	4.0	9.0	21.3	30.6
	72	00	8.0	12.2	20.1	38.2	40.3
Opuntia	*24*	*00*	*00.0*	*00.0*	*3.0*	*18.0*	*24.2*
	48	00	00.0	10.0	15.0	22.2	30.0
	72	00	4.2	13.0	24.2	32.6	38.6

* Average of three replicates

Most of these secondary metabolite like alkaloids, carbohydrates, tannins, volatile oils, flavonoids, saponin, and steroids are soluble in organic solvents (Harborne, 1984). Thus it can be suggested that inhibition of hatching and juvenile mortality may be due to the presence of these secondary metabolites in the crude alcohol extracts of the test plants.

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