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NEMATICIDAL ACTIVITY OF SELECTED FLORA OF PAKISTAN

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Abstract

Nematicidal activity of selected medicinal plants has been carried out to evaluate their potential toxicity against juveniles of the root-knot nematode *Meloidogyne* spp and *Cephalobus litoralis*. *In vitro* results showed that ethanolic extract of these plants caused appreciable mortality of second stage juveniles of *M. javanica* and *M. incognita* as well as *Cephalobus litoralis*. The concentrations used @ 2% and 1% were found more effective and produced significant results as compared to 0.5%, and 0.25%. The mortality rate increased with increasing exposure time for most of the extracts.

Introduction

Plant parasitic nematodes are the main pathogens on most fiber crops, horticultural, food and vegetable crops and without adequate control cause loss of yield and quality. Approximate yield losses due to plant parasitic nematodes have been estimated to be $ 100 billion worldwide each year (Sasser & Freckman, 1987). Root-knot nematodes (*Meloidogyne* species) infect almost all types of plants and may cause considerable damage (Adekunle & Akinlua**,** 2007). Root knot nematode larvae infect plant root causing the development of root knot galls that drains the plant's photosynthetic and nutrient (Eisenback & Triantaphyllous, 1991).

These nematodes may be controlled by cultural practices, chemical nematicides and the use of resistant cultivars. Although chemical nematicides hold major promise in the nematode control system but the high costs, non-availability at the time of need and the hazards they pose as environmental pollutants discourage most potential users (Elbadri *et al.*, 2008). This has stimulated research on alternative nematode management practices for plant parasitic nematodes. Different plant parts have been tested to identify the sources of nematicidal substances. More attention is being given to natural nematicides from plant extracts as these are more environment friendly. Several plants have more environmentally and toxicologically safe selective and efficacious nematicidal potential (Dawar *et al.*, 2008). In this context as part of our continuous studies on exploring the hidden potential of indigenous flora of Pakistan (Zia-Ul-Haq *et al.*, 2007 a, b; 2008, 2009), we have screened the ethanolic extracts of selected plants to develop biological control methods that are effective against the *Meloidogyne* root-knot nematode species and *Cephalobus litoralis*.

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Materials and Methods

Preparation of crude extract: The plant material of *Ferula assafoetida* resin, *Grewia asiatica* seed and leaves, *Ipomoea hederacea* seeds, *Lepidium sativum* seeds, *Nigella sativa* seeds and *Terminalia chebula* fruits were extracted with 96% EtOH at room temperature. The ethanolic extract were filtered and evaporated under vacuum to obtain a thick gummy mass. All these extracts were tested for nematicidal activity against *Meloidogyne javanica*, *Meloidogyne incognita* and *Cephalobus litoralis*.

Nematicidal activity

Culture preparation: Fresh egg masses of *Meloidogyne javanica* and *Meloidogyne incognita*, collected from stock culture maintained on tomato (*Lycopersicon esculentum*) root tissues were kept in water for egg hatching. The eggs suspension were poured on a cotton-wool filter paper and incubated at 28±2°C to obtain freshly hatched juveniles (J2). Juveniles collected within 48 h were used (Nazli *et al.,* 2008). Culture of *Cephalobus litoralis* was prepared by using a single egg. Green peas (*Pisum sativum*) were mashed in small Petri dishes. A single egg was carefully picked and placed beside pea meal paste .Nematode egg hatched within 72 hours and after 10 days, large number of nematodes in various stages of life cycle was obtained. These were used for screening crude extracts (Qamar *et al.,* 1989).

Mortality test: Crude extracts were dissolved in water (passed through Whatman filter paper No.1) to make dilutions of 2%, 1%, 0.5%, and 0.25 %. Experiments were performed under laboratory conditions at 28±2°C. Glass tubes 15 cm long and 8 cm were taken for bioassay. Three ml were taken from all dilutions in each tube. The required amount of nematode suspension (100 freshly hatched second stage juveniles/3ml suspension) were poured in to tubes to each of which equal amount of plant extract had already been poured). Distilled water with nematode larvae was taken as control. The dead nematodes were observed under stereoscopic binocular microscope after 24 and 48 hours and percentage mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al.*, 1989).

Results and Discussion

The damage caused by plant parasitic nematodes, especially by root-knot nematodes, represents one of the major obstacles for the production of an adequate food supply (Carter & Sasser, 1982). Medicinal plants play an important role for the management of damage caused by nematodes. The use of costly synthetic nematicides and long-term side effects of these synthetic compounds have assumed alarming range. Effective, safe and cheap medicinal agents from plants may appear as potential alternatives for controlling damage caused by nematodes. As Pakistan has wealth of medicinal flora due to its varied climate, so screening of this indigenous wealth is necessary for full exploitation of these neglected indigenous resources. This study has been designed to evaluate the nematicidal potential of some commonly used medicinal flora of Pakistan.

Table 1.Effect of Ethanolic extracts on mortality % of *Meloidogyne incognita* at different time intervals.

Plants	% Mortality observed against different concentration								
	24 hour				48 hour				Control
	2	1	0. 5	0.25	2	1	0. 5	0.25	
Ferula assafoetida	60	50	32	15	80	75	47	19	2
Grewia asiatica	42	30	17	10	65	52	26	15	1
Ipomoea hederacea	45	28	17	12	60	50	22	14	2
Lepidium sativum	60	35	20	5	78	55	35	18	2
Nigella sativa	20	13	12	4	48	24	19	7	1
Terminalia chebula	30	20	13	4	55	38	19	12	1

Table 2.Effect of Ethanolic extracts on mortality % of *Meloidogyne javanica* at different time intervals.

Plants	% Mortality observed against different concentration								
	24 hour				48 hour				Control
	2	1	0. 5	0.25	2	1	0. 5	0.25	
Ferula assafoetida	67	53	34	17	85	79	50	25	1
Grewia asiatica	45	36	19	9	69	50	25	12	2
Ipomoea hederacea	55	30	22	14	65	55	27	19	2
Lepidium sativum	65	39	24	8	88	52	36	16	2
Nigella sativa	25	17	13	6	52	26	17	9	1
Terminalia chebula	35	24	14	9	60	40	22	10	1

Table 3. Effect of Ethanolic extracts on mortality % of *Cephelobus litoralis* at different time intervals.

Plants	% Mortality observed against different concentration								
	24 hour				48 hour				Control
	2	1	0. 5	0.25	2	1	0. 5	0.25	
Ferula assafoetida	42	30	21	11	64	57	32	16	2
Grewia asiatica	28	20	14	6	55	42	22	10	1
Ipomoea hederacea	30	21	15	7	53	40	18	10	1
Lepidium sativum	45	22	12	4	58	40	17	10	2
Nigella sativa	18	10	6	3	40	15	11	5	1
Terminalia chebula	22	17	11	3	50	38	15	8	1

Of the various plants tested for nematicidal activity against larvae of *Meloidogyne incognita* and *Meloidogyne javanica* and *Cephelobus litoralis*, *Ferula assafoetida* appeared to be the most active, as it caused 42-85 % mortality of the nematode larvae after exposure to its extract. *Nigella sativa* and *Terminalia chebula* was found to be least active in its nematicidal activity, as these caused only 18-52 % and 22-60% mortality after the exposure. *Ipomoea hederacea* and *Lepidium sativum* exhibited moderate activity. Despite differences among investigated plants, all plants indicated time and concentration dependent activity. The activity was higher at high concentrations and increased with time. In conclusion, our study proved that many herbal extracts can be

used for the bio-control of plant parasitic nematodes and this method of control can minimize the risks and hazards of toxic synthetic nematicides. The plants like *Ferula assafoetida* which demonstrated high activity should be subjected to further investigation for possible application in nematode management. However for development of bio-nematicides on commercial scale the identification of active compounds responsible for this nematicidal activity should be carried out. Research in this field will open avenues of future exploitation of indigenous resources and their commercialization in modern era.

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