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Full Length Research Paper

Nematicidal potential of selected flora of Pakistan

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Nematicidal activity of selected parts of medicinal plants including *Acacia leucophloea* (bark), *Sphaeranthus indicus* (flowers), *Amaranthus spinuosus* (whole plant), *Semecarpus anacardium* (fruit), *Capparis deciduas* (root), *Saussurea lappa* (rhizome) and *Albizia lebbeck* (seed, flower and bark) has been carried out to evaluate their potential toxicity against second stage juveniles of nematode *Cephalobus litoralis* and *Helicotylenchus indicus*. *In vitro* results showed that methanolic extract of these plants caused appreciable mortality of second stage juveniles of *C. litoralis* and *H. indicus*. Concentrations used were 2 and 1% and was found more effective and produced significant results as compared to 0.5%. The mortality rate increased with increasing exposure time and was highest after 72 h.

Key word: *Acacia leucophloea*, *Helicotylenchus indicus*, pesticide.

INTRODUCTION

Various biotic factors like fungi, bacterial, viruses, nematodes are major hindrance in achieving the expected goal of production of food and vegetable crops in Pakistan. Yield losses in Pakistan caused by nematodes are relative higher because various seasons available in Pakistan provide a conducive environment for flourishing of range of nematode species which have a high reproductive percentage owing to their short life cycle, high temperature, dearth of winter chilling and lengthy growing periods. Man has used nematicides of natural origin discovered by chance observations since long ago to combat pests that cause losses of food, fiber and cash crops. With passage of time demand of nematicides increased and so synthetic nematicides were invented to slash down threats of food crisis.

These synthetic pesticides fulfilled the lacuna of natural pesticides but raised environmental concerns. Residues of the chemicals used in nematicides especially vegetables may accumulate in food chain and when reach human body may even cause death. Further these are very costly, not timely available and potential dangerous impacts on ecology have amplified interest in natural nematicides again. These plant based nematicides are a nature gifted solution to environmental problems caused by synthetic nematicides.

Many plant species are known to be highly resistant to plant parasitic nematodes, plant pathogens, and insect pests. Well-documented examples of these species include marigolds (*Tagets* spp.), rattlebox (*Crotalaria spectabilis*), chrysanthemums (*Chrysanthemum* spp.), garlic (*Allium sativum*), cinnamon (*Cinnamomum verum*) and neem (*Azardiracta indica*) (Duke, 1990; Lee et al., 2001; Satti et al., 2003; Park et al., 2005; Satti and Naser, 2006; Kong et al., 2007).

These nematicides are believed to be non-persistent under field conditions as these are readily transformed by oxygen, light and microorganism into less contaminated products, therefore no residues are expected on ecosystem or product. Plant kingdom is a consecration

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and boon from nature to humans living on this planet. Pakistan is considered a treasure house of many exotic medicinal plant species many of which are unique in extent, composition and endemism. Pakistan, with its mega-biodiversity having varietal emporium of flora may provide a safe, cost effective and indigenous alternative against synthetic nematicides. In this context as part of our continuous studies on exploring the hidden potential of indigenous flora of Pakistan (Ahmad et al., 2010; Zia-Ul-Haq et al., 2007; 2008 a, b; 2009 a, b; 2010, 2011 a, b, c; Nisar et al., 2010 a, b, c, 2011) we have screened the ethanolic extracts of selected plants their potential toxicity against the *Cephalobus litoralis* and *Helicotylenchus indicus.*

MATERIALS AND METHODS

Preparation of crude extract

Plant parts of *Acacia leucophloea* (bark), *Sphaeranthus indicus* (flowers), *Amaranthus spinuosus* (whole plant), ***S****emecarpus anacardium* (fruit), *Capparis deciduas* (root), *Saussurea lappa* (rhizome) and *Albizia lebbeck* (seed, flower and bark) were extracted with methanol: water (85:15) at room temperature. The methanolic extract were filtered and evaporated under vacuum to obtain a thick gummy mass. All these extracts were tested for nematicidal activity.

Nematicidal activity

Culture preparation

Culture of *C. litoralis* was prepared by using a single egg. Green peas (*Pisum sativum*) were mashed in small petri dishes. A single female was picked and placed beside pea meal paste. Female layed eggs within 12 h and then nematode eggs hatched within 72 h 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). For isolation of *H. indicus* nematodes, 500 mg soil samples (Depth 15 to 25 cm) collected from paddy (*Oryza sativa* L.) fields. Soil samples were processed by Cobb sieving (Cobb, 1918) and modified Baermann funnel method (Baermann, 1917). Soil sample was put in a large bucket containing water and the mixture was vigorously stirred into a suspension, which was allowed to settle for about 2 min. The heavy soil particles sank to the bottom but nematodes remained suspended in the water. The remaining suspension was slowly poured over a coarse sieve (60 mesh aperture), which was continuously tapped by hand to avoid blocking. The deposit on the sieve was washed with a gentle jet of water into a beaker.

This water suspension, containing eel shaped nematode, was passed through 200 and 300 mesh sieves. The nematodes thus recovered were mixed and water was decanted after allowing sufficient time for the nematodes to settle down. Then nematode suspension was poured over a piece of tissue paper attached to a perforated plastic sheet placed in a funnel fitted with a rubber tube and clamped at the lower end. The water contained in the funnel barely touched the bottom of the tissue paper. Care was taken not to allow the debris to float off the edges of the tissue paper. After 24 h the nematodes wriggled out into the clear water in the funnel and settled at the bottom, and then 100 ml of water containing the nematodes was drawn into a beaker. The nematode suspension was allowed to settle for 2 h or more, the excess supernatant water was poured off, and the remaining concentrated content was transferred into a cavity block for examination under the stereomicroscope and nematodes picked (Naqvi et al., 1992).

Mortality test

Crude extracts were dissolved in water (passed through whatman filter paper No.1) to make dilutions of 2, 1 and 0.5%. Experiments were performed under laboratory conditions at 28±2℃. G lass 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/3 ml 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 to 48 and 72 h 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

Wide variation in agrogeoclimatological conditions of Pakistan offers the broadest array of flora harbored by forests, deserts, mountains and rivers. This rich floral biodiversity of Pakistan is an impressive pool of ‘natural pharmacy’ from which indigenous communities select ingredients for the treatment, management and control of various diseases. Much potential of this green wealth is however still untapped. 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 (Zia-Ul-Haq et al., 2010). The highest mortality against *C. litoralis* was shown by *A. lebbeck* (bark) followed by *C. deciduas* (root) and *A. lebbeck* (flower). As shown in Table 1. *A. leucophloea* (bark) and *A. spinuosus* (whole plant) showed moderate activity. *S. anacardium* (fruit) and *S. indicus* (flowers) showed same activity at 2% after 24 h while *S. lappa* (rhizome) and *A.* lebbeck (seed) showed low activity.

However still this activity was higher than control. The highest mortality against *H. indicus* as shown in Table 2 was noted for *A. lebbeck* (bark) followed by *A. lebbeck* (flower) and *C. deciduas* (root). *A. leucophloea* (bark) and *S. indicus* (flowers) showed same activity at 2% after 24 h. *S. lappa* (rhizome) and *A. spinuosus* (whole plant) showed moderate activity. *S. anacardium* (fruit) and *A.* lebbeck (seed) showed low activity although this activity was higher than control. Our study proved that many herbal extracts can be used for the bio-control of plant Parasitic nematodes and this method of control can

Table 1. Nematicidal activity against *C. litoralis.*

Plant	Mortality observed against different concentration (%)									Control
	24 h			48 h			72 h			
	2	1	0.5	2	1	0.5	2	1	0.5	
A. lebbeck (seed)	10	3	2	15	4	3	17	4	4	2
A. lebbeck (bark)	80	60	50	92	75	55	95	80	62	1
A. lebbeck (flower)	52	30	18	63	48	28	65	55	33	2
C. deciduas (root)	55	28	16	65	36	21	68	40	23	1
A. leucophloea (bark)	42	23	10	55	28	21	58	35	23	2
S. indicus (flowers)	50	39	16	70	62	30	72	68	43	1
A. spinuosus (whole plant)	40	12	0	58	20	7	61	38	18	1
S. anacardium (fruit)	50	33	15	63	43	18	70	48	22	1
S. lappa (rhizome)	30	21	4	52	28	11	53	30	11	1

Table 2. Nematicidal activity against *H. indicus.*

Plant	Mortality observed against different concentration (%)									Control
	24 h			48 h			72 h			
	2	1	0.5	2	1	0.5	2	1	0.5	
A. lebbeck (seed)	18	12	5	20	16	8	22	17	10	1
A. lebbeck (bark)	90	65	55	96	82	58	98	88	62	1
A. lebbeck (flower)	62	37	20	78	50	31	80	61	40	2
C. deciduas (root)	60	26	20	72	40	25	75	48	27	2
A. leucophloea (bark)	40	22	5	58	42	20	60	45	20	2
S. indicus (flowers)	40	30	13	45	42	22	58	45	27	1
A. spinuosus (whole plant)	38	10	5	60	40	19	63	41	21	2
S. anacardium (fruit)	28	16	6	60	55	17	67	56	20	1
S. lappa (rhizome)	44	25	8	58	38	21	58	39	23	2

minimize the risks and hazards of toxic synthetic nematicides. The plants like *A. lebbeck* and *C. deciduas* 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 (Zia-Ul-Haq et al., 2010).

Conclusion

From this study, we concluded that medicinal plants provide a wide support for nematicidal activity and can be used as a possible basis in agriculture as pesticide. Inclusion of plant botanicals into soil alone or with biocontrol agents has been recommended as a substitute, safe and effective control method for management of plant parasitic nematodes. However it is recommended that before commercial use of plant botanicals as biopesticides, they must be monitored against other nematode species also.

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