NEMATICIDAL ACTIVITY OF BARK OF SOME TREE SPECIES AGAINST ROOT KNOT NEMATODE *MELOIDOGYNE JAVANICA* (Treub) Chitwood

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Abstract

Root-knot nematodes (*Meloidogyne* species) are the most abundant and destructive nematodes around tropical areas of world. Barks of 10 indigenous tree species namely *Azadirachta indica, Tamarindus indica, Delbergia sissoo,* E*ucalyptus* sp., *Aegle marmelos, Guaiacum officinale, Thespesia populnea, Pithocellobium dulce, Prosopis juliflora and Samanea saman* were assessed against root-knot nematodes *Meloidogyne javanica* (Treub) Chitwood under laboratory and greenhouse conditions. Aqueous extract of bark of *Eucalyptus* sp. revealed greatest inhibition of hatching of root-knot nematodes juveniles. Similarly, highest mortality (98.4%) of juveniles was observed in *Eucalyptus* sp. treatment. Soil amendments with barks of different trees greatly enhanced Seed germination, Shoot and root heights and weights in okra. *A. indica* bark provided maximum growth enhancement in okra plants. Root-knot nematode parasitism in okra decreased with the bark treatments. However, the bark of *A. indica* was the most effective against root-knot nematode parasitism in okra.

Introduction

Root-knot nematodes (*Meloidogyne* species) are the most abundant and destructive nematodes around tropical areas of world. *Meloidogyne* species are mainly parasitizing number of cultivated plant species belonging to Families Cucurbitaceae and Solanaceae (Taba *et al*., 2008). Root-knot nematodes parasitization of plants causes yellowing their leaves and reduces their growth and yield. However, the characteristic symptoms are the formation of galls on roots due to the development of giant cells. World-wide crop losses exceed US$125 billion annually (Chitwood, 2003). In Pakistan, root-knot disease causes more severe losses due to favorable environmental conditions and availability of a wide host range. Further, root-knot nematodes with other biotic and abiotic stresses create more complex infection. These factors along with high reproductive rate of the nematodes make it difficult to manage root-knot parasitism. Nematicidal chemicals were effectively used to reduce losses due to nematodes parasitism in past, however, due to harmful effects on environment and other organisms including humans it became necessary to identify safe and more effective methods to reduce nematode parasitism (Abad *et al.,* 2008).

Plant products from 57 families have been reported to contain nematicidal activity (Sukul, 1992). Use of different plant products against nematode parasitism exhibit many benefits compared to other methods like easy availability and applicability, economical, increasing soil fertility and no hazardous affects on environment (Kumar *et al*., 2005).

Soil amendments with plant products including bark of trees was reported as a successful strategy to reduce soil borne pathogens to cultivated plant species (Harkin and Rowe, 1971). Barks of some trees have also been reported to reduce the nematodes parasitism (Dawar *et al.,* 2007; Parveen and Bhat, 2011).

The objective of this work was to evaluate the effects of bark extract of some local trees on *M. javanica in-vitro* and determine effects of such organic amendment (using bark extracts) on growth of host plant – Okra in the present case.

Materials and Methods

Collection of the plant Material: Barks of 10 different trees namely *Azadirachta indica* A. Juss, *Aegle marmelos* (L.) Corrêa, *Dalbergria sisso* Roxb. ex DC., *Eucalyptus* sp, *Guaiacum officinale* L., *Pithocellobium dulce* (Roxb.) Benth., *Prosopis juliflora* (Sw) DC., *Samanea saman* (Jacq.) Merr., *Tamarindus indica* L., *Thespesia populnea* (L.) Sol. ex Corrêa were collected from the University of Karachi campus. The barks were cleaned by the help of brush to remove soil particles and other debris. The bark powder was stored in tightly closed brown glass bottles for further studies.

Preparation of aqueous extracts: Ten gram bark powder of each plant was soaked in 100ml of sterilized distilled water for 24 hours. The solution was filtered with Whatman filter paper to obtain stock extract. Different dilutions (25% and 50%) were made by adding sterilized distilled water.

Eggs hatching test: Eggs of root-knot nematodes (*Meloidogyne javanica*) were extracted using 1% Na (OCl) from roots of egg plant (*Solanum melongeana* L.) (Hussey and Baker, 1973). One ml Egg suspension (100 eggs approx.) was transferred to glass cavity blocks (2.5 cm diameter, capacity 4 ml) and treated with 0, 25 and 50% of stock of bark extract. Each treatment was replicated three times. The numbers of hatched juveniles were counted under stereoscope binocular microscope (4X). Numbers of hatched second stage juveniles were counted at 24, 48 and 72 h of exposure.

Juvenile mortality test: Egg masses of *M. javanica* were picked from the infected roots of egg plants. The egg masses were kept in distilled water and incubated at room temperature to obtain J2 (juveniles). The freshly hatched juveniles were used to test the effect of bark extract. Aqueous bark extract was diluted in appropriate amount of distilled water to make 25 and 50% of the stock. Juvenile suspension in sterilized distilled water was considered as control. Each treatment was replicated three times. Number of dead juveniles was observed after 24, 48 and 72 hours. Nematodes were considered dead when they did not move while touched with a needle (Caryol *et al*., 1989).

Green house experiment: Sandy loam soil was collected from the experimental plot of Karachi University. Dry powder of different plant barks mixed with soil @ 1 and 2% w/w, transferred into 8 cm diameter plastic pots. Pots were randomized on a screen house bench. The pots were watered regularly for 10 day. Then 5 seeds of okra were sown in each pot. After germination two seedlings were kept in each pot. When plant achieved two leaves stage plants were inoculated second stage Juveniles (J2) of *M. javanica* @ 2000 J2 per pot. The plants were uprooted after 45 days of nematode inoculation.

Analysis of data: Data were subjected to either Analysis of variance (ANOVA) or Factorial analysis of variance (FANOVA) depending upon experimental design (Gomez and Gomez, 1984). The follow up of FANOVA included Least Significant Difference (LSD).

Results

Screening of bark extracts on root knot nematodes *in-vitro:* The aqueous bark extracts of different plants reduced eggs hatching of *M. javanica* to a varying degree (Table 1). Aqueous extracts of bark of *Eucalyptus* spp, *Guaiacum officinale, Samanea saman* and *Azadirachta indica* significantly inhibited egg hatching of *M. incognita*. However, the lowest hatching (4.6%) was observed when *Eucalyptus* sp. was used @ 50% concentration of the stock extract followed by 50% concentration of *G. officinale* (12.12%) after 72h of incubation), *S. saman* (15.4%) in 50% extract and *A. indica* (16.7%) after 72 hours of exposure. The hatching was more inhibited in 50% extract as compared to the lower concentration of 25% of the stock extract. As compared to other plants egg hatching was higher in *Tamarindus indica* extract (45.3 and 72.4% in 50 and 25% extract.

All the bark extracts showed potential nematicidal effects and caused higher mortality of juveniles of *M. javanica to* varying degree. Exposure time plays an important role in the mortality because there was an increase in mortality of juveniles as the exposure period increased ($p<0.001$). Aqueous extract caused significant mortality compared to control. Aqueous extract of *Eucalyptus* sp and *P. juliflora* caused more than 98% mortality of juveniles after 72 h of incubation. Bark of *Samanea saman* caused 93.5 % juvenile mortality. Mortality in control was 19.8% only (Table 1).

Effect of bark powder as organic amendment on plant growth and nematode infection of Okra: Germination of Okra seeds was significantly increased in bark amended soils as compared to control ($p<0.01$). Soil treated with 1% or 2% (w/w) bark powder enhanced the plant growth. The untreated control plants were shorter in height, had lower root length and were lighter in weight. The results clearly indicated that plants grown on the amended soil better growth than the plants grown on un-amended soil ($p<0.001$)(Table 2).

Interestingly, the bark powder applied @ 1 and 2 % more or less equally suppressed the number of knots on the roots of okra. Maximum reduction in the number of galls was observed when *Eucalyptus* sp bark powder was used @ 1 or 2% w/w. The control plants exhibited the highest number of galls on the roots. Growth improvement along with reduction in root knot was also observed when soil amended with *Prosopis juliflora* and *Samanea saman.* Both showed almost comparable growth enhancement however, the number of galls was much reduced in case of bark of *S. saman*. The experimental results revealed increase in shoot length, root length, shoot weight and significant decrease in development of root galls (Table 2).

Table 1. Effect of aqueous bark extracts of different plants on egg hatching and mortality of J2 of root knot nematodes.

S. No	Treatments	Hatching (%)			Mortality (%)		
		24 H	48 H	72 H	24 H	48 H	72 H
1	Control	18.8	29.9	61.6	9.4	15.5	19.8
2	B1 (25%)	9.4	14.1	17.9	25.9	33.3	68.6
3	B1 (50%)	3	9.1	16.7	36.1	47.4	63.2
4	B2 (25%)	22.9	43.3	72.4	15.1	27.2	30.2
5	B2 (50%)	17	37.5	45.3	16.1	29.8	36.7
6	B3 (25%)	36.2	52.8	62	13.9	23.3	39.3
7	B3 (50%)	14.2	20.8	30.6	14.1	20.5	30.8
8	B4 (25%)	4.2	7.4	11.6	91.2	96.2	96.3
9	B4 (50%)	0.6	1.5	4.60	91.1	96.5	98.3
10	B5 (25%)	21.8	38.3	51.3	12.7	22.6	34.7
11	B5 (50%)	10.7	25.8	35.3	10.9	25	42.7
12	B6 (25%)	11.1	14.1	18.6	11	22.8	41.3
13	B6 (50%)	5.9	11.1	12.2	10.7	18.7	28.4
14	B7 (25%)	19.9	28.2	41	17.2	28	30.8
15	B7 (50%)	11	14.1	19.2	90.3	91.4	92.5
16	B8 (25%)	11.2	23.2	46	81.5	86.8	88.2
17	B8 (50%)	11.8	16.3	34.2	79	80.5	85.4
18	B9 (25%)	10.4	24.9	41	87.5	90	96.3
19	B9 (50%)	3.1	5.9	20	94.8	96.8	98.4
20	B10 (25%)	8.1	15.8	25.7	79.8	87.6	90.6
21	B10 (50%)	4.6	6.2	15.4	80.2	88.6	93.5
$LSD_{0.05}$		Treatments = 6.66; Time = 0.75; Concentration = 15.606			Treatments = 7.29882; Time = 13.965; Concentration =17.109		

B1=*Azadirachta indica*, B2= *Tamarindus indica*, B3=Dalbergia sissoo, B4=*Eucalyptus* sp., B5=*Aegle marmelos*, B6=*Guaiacum officinale*, B7=*Thespesia populnea* B8=*Pithocellobium dulce*, B9=*Prosopis juliflora*, B10=*Samanea saman*

Discussion

The initial screening of bark extracts showed potential nematicidal activity against *M. javanica*. There was maximum reduction of hatching of *M. javanica* when eggs were exposed to *Eucalyptus* sp, *A. indica, P. juliflora* and *S. saman*. Complete inhibition of hatching of Juveniles was obtained in case of *Eucalyptus* sp after 24 hours. Dawar *et al,* (2007) also reported that aqueous and ethanol extracts of different parts (leaves, stem, bark and fruit) of *Eucalyptus* sp. reduced the hatching of eggs. Similarly, the water soluble extracts of leaf fruit and bark of neem were reported to possess potential nematicidal properties (Parmar, 1987). The present data indicated that plant bark extracts were highly toxic to larvae and resulted in death of *M. javanica* juveniles. Mortality increased with the exposure time. Aqueous extracts of *Eucalyptus* sp, *P. juliflora, S. saman A. indica* showed greater mortality of juveniles. *Eucalyptus* species contain some essential oils which are known to be toxic against bacteria, fungi, insects, weeds and nematodes (Batish *et al.,* 2008). Complex compounds of *A. indica* like triterpenes or limonoids are reported as active factors responsible for bioactivity of neem against nematodes and insects (Alam 1993; Kraus 1995). *S. saman* contain alkaloids, glycosides, terpenes, etc. (Wiesner *et al.,* 1968; Nigum *et al.,* 1971). The hexane and methanol extracts of *S. saman* exhibited antibacterial, antifungal and insecticidal activity (Azhar *et al.,* 2009).

Table 2. Effects of organic amendments with barks powder of plant species on plant growth of okra.

S.No	Treatments	Germination (%)	Shoot Length (cm)	Root Length (cm)	Fresh shoot Weight (g)	Fresh root Weight (g)	Root knot Index
1	Control	66.6	7.5	13.1	0.87	0.4	4
2	B1 (1%)	53.3	21.3	25.3	2.32	0.55	2
3	B1 (2%)	73.3	15.8	27.3	1.18	0.47	2
4	B2 (1%)	80	17.2	16.8	1.03	0.27	3
5	B2 (2%)	46.6	17.2	16.9	1.48	0.65	2
6	B3 (1%)	66.6	15.5	18.5	1.86	0.48	2
7	B3 (2%)	80	18.4	23.8	1.36	0.57	3
8	B4 (1%)	86.6	14.6	15.1	0.83	0.21	1
9	B4 (2%)	80	16	14.6	0.48	0.31	1
10	B5 (1%)	46.6	19.1	20.7	0.83	0.71	2
11	B5 (2%)	53.3	13.8	20.1	1.24	0.52	2
12	B6 (1%)	60	16.1	20.6	1.0	0.39	2
13	B6 (2%)	93.3	17.7	19.7	1.2	0.52	2
14	B7 (1%)	53.3	13.1	16.0	1.5	0.47	2
15	B7 (2%)	60	14.4	18.4	1.1	0.49	2
16	B8 (1%)	46.6	16.8	16.8	1.0	0.49	3
17	B8 (2%)	66.6	17.9	16.5	1.2	0.49	3
18	B9 (1%)	80	18.5	20.4	1.5	0.61	2
19	B9 (2%)	66.6	17.3	16.8	1.1	1.38	3
20	B10 (1%)	80	15.9	16.1	1.5	0.44	1
21	B10 (2%)	93.3	18.6	18.0	1.8	0.57	1
$LSD_{0.05}$ Treatment =		13.71	1.95	3.69	0.35	0.174	3.59
Concentration =		32.14	4.58	8.66	0.81	0.407	8.44

B1=*Azadirachta indica*, B2 = *Tamarindus indica*, B3 =*Dalbergia sissoo*, B4=*Eucalyptus* sp. B5=*Aegle marmelos*, B6 = *Guaiacum officinale*, B7 =*Thespesia populnea*, B8 =*Pithocellobium dulce*, B9 =*Prosopis juliflora*, B10 =*Samanea saman*

Application of the soil amendment caused significant reduction in the nematode parasitism in roots of okra. Addition of bark powder to soil also enhanced growth of okra plants. The shoot length, root length and shoot weight were significantly increased when grown in soil amended with *A. indica* @ 2% w/w. Similarly Alam, (1993) reported that soil amendment with Neem was the most useful means for nematode management. Neem constituents such as nimbin, solanin, thionemone, azadirachtin and a range of flavonoids have nematicidal action (Thakur *et al.*, 1981). Parveen and Bhat (2011) reported nematicidal activities of neem bark powder in pot experiment against *Rotylenchulus reniformis* and *Meliodogynae incognita* on castor. Jabri *et al.* (1991) reported reduced hatching of *M. incognita* and nematicidal potential of bark extracts of bottle brush. Bark of *Thespesia populnea* also showed nematicidal potential. Leaves of *T. populnea* have been reported to exhibit nematotoxic activities against *M. incognita* (Shekshavali and Hugar, 2002). Increase in growth and decreased in nematodes parasitism was recorded in plants amended with *Eucalyptus* spp. *Eucalyptus* might be highly toxic to juveniles of nematode. Dawar *et al,* (2007) reported similar observation. Leaves of *Eucalyptus microtheca* are reported to control plant parasitic nematodes (Elbadri *et al.,* 2008). The bark of *A. indica, Eucalyptus* sp., *P. juliflora and S. saman* contain high amount of potential nematicidal compounds. In pot experiment bark of these plants reduced root-knot nematode parasitism and increased growth of okra. Further studies are recommended to find out active compounds responsible for nematicidal activity.

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