Turk J Biol
31 (2007) 225-230

Use of *Avicennia marina* (Forsk.) Vierh in the Control of Root Knot Nematode *Meloidogyne javanica* (Treub) Chitwood on Okra and Mash Bean

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Received: 23.03.2007

Abstract: Aqueous and ethanol extracts of *Avicennia marina* (Forsk.) Vierh. plant parts, namely leaves, stem and pneumatophore, were tested for their nematicidal activity against root knot nematode *Meloidogyne javanica* (Treub) Chitwood. Results showed that *A. marina* exerted more lethal effect in mortality of juveniles compared to hatching of juveniles. Stem showed more nematicidal effect compared to leaves and pneumatophore in aqueous extract, whereas pneumatophore showed more nematicidal activity in ethanol extract. Soil amendment at the rates of 0.1, 1 and 5% w/w was carried out with dried powder of leaves, stem and pneumatophore of *A. marina* for the control of root knot nematode on mash bean and okra plants. The results pertaining to seed germination percentage, shoot length, root length, shoot weight and root weight showed improvement with maximum inhibition in root knot indices in both mash bean and okra when *A. marina* plant parts, namely leaves, stem and pneumatophore powder, were used at 5% w/w. Maximum root and shoot weight were observed in mash bean when *A. marina* plant parts powder was used at 5% w/w. All plant parts of *A. marina* powder were effective in the control of root knot nematodes.

Key Words: *Avicennia marina*, biological control, soil amendment, *Meloidogyne javanica*, mash bean, okra

Avicennia marina (Forsk.) Vierh'in Bamya ve "Mash Bean"de Kök Uru Nematodu Olan *Meloidogyne javanica* (Treub) Chitwood'un Kontrolünde Kullanımı

Özet: *Avicennia marina* (Forsk.)'nın yaprak, gövde ve hava kök kısımlarının su ve etanol özütleri, kök uru nematodu *Meloidogyne javanica* (Treub) Chitwood üzerine nematisid etkisi bakımından incelenmiştir. Sonuçlar *A. marina*' nın, genç bireylerin ölümü üzerine, kuluçkadan çıkan genç bireylerle karşılaştırıldığında, daha ölümcül bir etkisinin olduğunu göstermiştir. Gövdenin suda özütü, yapraklar ve hava kökü ile karşılaştırıldığında daha fazla nematisidal etki gösterirken hava kökünün etanol özütü daha fazla nematisidal etki göstermiştir. 0,1, 1 ve %5 w/w oranlarında toprak ıslahı, kök uru nematodu üzerine etkisini belirlemek için *A. marina*' nın yaprak, gövde ve hava kökü kuru tozları ile sağlanmıştır. Tohum çimlenme yüzdesi, gövde uzunluğu, kök uzunluğu, gövde ağırlığı ve kök ağırlığı ile ilgili sonuçlar *A. marina*'nın yaprak, gövde ve hava kökü bitki kısımlarının kuru tozlarının %5 w/w uygulandığı bamya ve "mash bean" kök uru indislerinin maksimum inhibisyonu ile gelişme göstermiştir. En fazla kök ve gövde ağırlığı *A. marina* bitki kısımlarının kuru tozlarının %5 w/w uygulandığında "mash bean" de gözlenmiştir. *A. marina*'nı tüm bitki kısımları kök uru nematodlarının kontrolünde etkilidir.

Anahtar Sözcükler: *Avicennia marina*, biyolojik kontrol, toprak ıslahı, *Meloidogyne javanica*, "mashbean", bamya

Introduction

Mangroves are widespread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coast lines. The Indus delta mangrove area occupies about 250,000 ha (1) and therefore it ranks as the fifth or sixth largest single mangrove area in the world (2). A small (20 ha) mangrove forest also occurs near the western border of Pakistan, along the Makran coast (3). Nematodes are often referred to as "hidden enemies" among plant pathogens causing serious losses to crop plants. Root knot nematode *Meloidogyne* spp are widely found in many parts of the world and are known to attack a wide variety of crops (4). Of a total 70 *Meloidogyne* spp identified so far (5), only 4 species, namely *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood and *M. hapla* (Chitwood), are of major economic importance. *M. javanica* root knot nematode is the most abundant and damaging nematode in Pakistan, infecting about 102 plant species (6). The various species of

Meloidogyne induce major morphological and physiological changes within roots by attacking nearly every crop sown, resulting in reduced yields and poor-quality products (7). Damage caused by root-knot nematode is much higher in tropical and subtropical countries (8). In Pakistan, *Meloidogyne* root knot nematodes are recognized as important parasites of vegetable crops, where more than 102 plants have been found infested with root knot nematode from different cultivated zones of the country (9,10).

Although the application of chemical nematocides has been found as an effective measure for the control of nematode, due to the high toxic residual effect of these chemicals (11), there is a need to develop alternative nematode control strategies (12). Organic amendments are generally used for the improvement of crop plants and for increasing agricultural productivity. Addition of organic matter amendments (organic wastes and plant residues) to field soils suppresses a variety of soil-borne diseases (13). Various organic amendments have a suppressive effect on plant parasitic nematodes (14,15). Use of *A. marina* (Forsk.) Vierh as an organic amendment showed promising results in the control of soil-borne root-infecting fungi like *Macrophomina phaseolina, Fusarium solani*, *Rhizoctonia solani* and root knot nematodes in tomato (16). Mangroves have been reported to contain compounds like tannins, alkaloids and polyphenols (17), which have antimicrobial activity (18-20). *A. marina* releases some compounds toxic to nematodes like phenols, tannins, azadirachtin, and ricinine (21,22) or they are derived from the decomposition process in the soil like ammonia, nitrate, and hydrogen sulfide (23). The aim of the present study was to use *A. marina* as a biocontrol agent in the suppression of root knot nematode and to increase the productivity of mash bean and okra.

Materials and Methods

Leaves, stem and pneumatophore of *A. marina* (Forsk.) Vierh were collected from coastal areas of Karachi, dried under shade and ground in an electric grinder. Dried powder was used as organic amendment at the rates of 0.1, 1 and 5% w/w. For the aqueous extraction of plant material, 200g powdered mangrove plant parts were soaked in 500 ml distilled water for 24 h, filtered with Whatman filter paper and stored at 6 ºC, which gives 100% extract. Half quantity of 100% extract was dissolved in distilled water, which gives 50% extract. Likewise, 200g powdered plant parts of mangroves were soaked in 500 ml ethanol for two weeks, filtered twice, and filtrate was concentrated under a rotary vacuum evaporator. An appropriate amount of extract was dissolved in ethanol to make 1000, 500 and 250 ppm concentrations.

In vitro experiments

Nematicidal activity of *A. marina* was tested using a modified method of Meyer et al. (24) in which 50, 100% (in aqueous) and 250, 500, 1000 ppm (in ethanol) concentrations of plant parts were prepared. Eggs of *M. javanica* were obtained using the method of Hussey and Barker (25). Egg suspension prepared in distilled water and 2 ml suspension containing 20-40 eggs were poured in each cavity block with or without aqueous and ethanol extract and kept at room temperature (34-38˚C). Cavity blocks without extract served as control. There were three replicates of each of the three treatments. The numbers of juveniles were counted at 24-, 48- and 72-h intervals.

For mortality test, freshly hatched second stage juveniles of *M. javanica* were suspended in sterile distilled water and 2 ml of this suspension containing 15-20 larvae/ml was placed in each cavity block. Cavity blocks without extract served as control. There were three replicates of each treatment. The number of juveniles that were killed at the 24-, 48- and 72-h intervals was recorded using a stereoscope.

In vivo experiments

Roots of eggplant (*Solanum melongena* L.) infested with *M. javanica* root knot nematode were collected from the experimental plot of the Department of Botany, University of Karachi. Roots infected with root knot nematodes showing characteristic galls were cut and placed in a wide-mouth bottle in which sodium hypochloride (1% w/w) solution was added and the bottle tightly closed. The bottle was shaken vigorously by hand for 3 mins. The contents were poured onto a 100 mesh screen, fitted over a 400 mesh screen, washed under running water for 1 min and the residue collected on a 400 mesh screen transferred into a 250 ml beaker. Number of eggs/juveniles per ml of suspension was

determined in counting chamber (25). Soil was obtained from the experimental plots of the Department of Botany, University of Karachi. *A. marina* plant parts, viz. leaves, stem and pneumatophore, were mixed with sandy loam soil (sand, silt, clay; 70, 19, 11%, respectively) of pH 8.1 to give a concentration of 1% w/w and transferred into 8 cm diameter plastic pots at 300 g/pot. The soil was watered daily for the decomposition of the organic substrate. After 1 week of amendment, 5 seeds of mash bean and okra were sown in each pot. Freshly hatched second stage juveniles obtained from brinjal (*Solanum melongena* L.) were inoculated near the roots of 1-week-old seedlings. A set without nematode inoculation was also kept. Pots without amendment served as control. There were three replicates of each treatment and pots were kept randomized on the screen house bench of the Department of Botany, University of Karachi. The experiment was terminated after 45 days of nematode inoculation. Plant growth parameters in terms of plant height, shoot weight, root length and fresh weight of roots were recorded. Root knot galling was recorded on a 0-5 scale (8). Data were analyzed and subjected to analysis of variance (ANOVA) including Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT) (26).

Results

In vitro

A. marina plant parts, viz. leaves, stem and pneumatophore, showed nematicidal effect, reduced the hatching of eggs of *M. javanica* to varying degree. Aqueous extract of leaves used at 100% showed maximum reduction (65%) in hatching of eggs ($P < 0.01$), whereas ethanol extract of stem used at 1000 ppm showed maximum reduction (87%) in egg hatching as compared to control ($P < 0.001$) (Table 1). Of the different plant parts used, leaves showed more nematicidal effect in aqueous and ethanol extracts as compared to pneumatophore and leaves. Killing of second stage juveniles of *M. javanica* increased with the time interval. Aqueous extract of stem at 100% ($P < 0.01$) exerted maximum lethal effects (235%), whereas ethanol extract of pneumatophore at 1000 ppm (916%) showed highest mortality percentage of juveniles as compared to control ($P < 0.001$) (Table 1). Ethanol extract showed more significant results as compared to aqueous extract.

In vivo effect:

An experiment was carried out to study the efficacy of *A. marina* plant parts powder, namely leaves, stem and pneumatophore, for the control of root knot nematodes on mash bean and okra. All plant parts used at 5% showed an increase in plant growth as compared to control. Germination of seeds showed significant increases when *A. marina* plant parts were used at 5% w/w in mash bean and okra (Table 2). Maximum shoot and root weight were observed in mash bean when *A. marina* dried leaves, stem and pneumatophore powder were used at 5% w/w ($P < 0.001$). Shoot length, shoot weight, root length and root weight of okra plant were significantly increased ($P < 0.001$) (Table 2). The present results showed that *A. marina* plant parts, namely leaves, stem and pneumatophore, were more effective in the control of *M. javanica* infection in mash bean and okra used. Maximum inhibition in root knots of okra and mash bean was recorded when *A. marina* plant parts were used at 5% w/w followed by 1% w/w ($P < 0.001$). Powder of all plant parts of *A. marina* was equally effective in control of *M. javanica* (Table 2).

Discussion

In the present study, ethanol extract used at 1000 ppm ($P < 0.001$) showed more nematicidal effect as compared to aqueous extract ($P < 0.01$). In both extracts, hatching of eggs decreased and mortality of juveniles increased as the concentration of extract increased with increase in exposure time. Similar results were obtained by Siddiqui et al. (27) by using ethyl acetate and hexane fraction at different concentrations against *M. javanica*. In the present study, aqueous and ethanol extracts of *A. marina* plant parts, namely leaves, stem and pneumatophore, caused significant mortality of *M. javanica* and caused maximum lethal effect at 100% ($P < 0.05$) and 1000 ppm treatment ($P < 0.001$). Mehdi et al. (28) observed the same results by using aqueous, methanolic and chloroform extracts of *A. marina*, which caused significant mortality of *M. javanica* juveniles. Of the different parts, stem showed more lethal effect as compared to leaves and pneumatophore in aqueous extract, whereas in ethanol extract, pneumatophore showed more satisfactory results. The addition of organic materials to soil infested with plant pathogens has been clearly demonstrated as a satisfactory

Table 1. Effect of Avicennia marina aqueous and ethanol extracts on hatching and mortality of Meloidogyne javanica at different time intervals.

Aqueous extract								
	50%				100%			
Treatments	Time (hrs)							
	Population at 0 day	24	48	72	Population at 0 day	24	48	72
	Hatching %							
Control	24	21	21	33 a	21	19	29	43 a
Leaves	23	13	17	26 ab (21.22)	20	10	15	15 b (65.11)
Stem	24	8	13	17 ab (48.49)	22	9	14	18 b (58.13)
Pneumatophore	26	8	12	12 b (63.64)	19	16	21	16 b (62.81)
LSD0.05 =				6.482				3.051
	Mortality %							
Control	23	0	9	13 a	21	5	10	14 a
Leaves	26	4	12	27 b (107.6)	20	20	25	25 b (78.5)
Stem	27	11	11	19 c (46.15)	17	18	24	47 c (235.7)
Pneumatophore	24	4	13	29 b (123.0)	18	17	22	33 d (135.7)
LSD0.05 =				2.424				2.684

Ethanol extract												
	250 ppm				500 ppm				1000 ppm			
Treatments	Time (hrs)											
	Population at 0 day	24	48	72	Population at 0 day	24	48	72	Population at 0 day	24	48	72
	Hatching %											
Control	40	13	63	70 a	40	13	63	70 a	40	13	63	70 a
Leaves	41	2	7	10 b (85.72)	38	3	5	5 b (92.86)	40	0	5	5 b (92.8)
Stem	37	5	8	11 b (84.29)	41	2	2	7 b (90)	33	0	3	9 b (87.14)
Pneumatophore	44	5	7	9 b (87.15)	38	5	5	11 b (84.2)	42	0	2	7 b (90)
LSD0.05 =				2.684				2.921				3.033
	Mortality %											
Control	32	0	3	6 a	32	0	3	6 a	32	0	3	6 a
Leaves	33	6	9	15 b (150)	30	13	17	37 b (516.6)	37	18	35	56 b (833.3)
Stem	33	9	12	18 b (200)	30	10	17	27 c (350)	34	15	24	38 c (533.3)
Pneumatophore	32	6	9	16 b (166.6)	34	12	18	38 b (533.3)	31	19	35	61 b (916.6)
LSD0.05 =				1.853				3.033				5.611

Mean followed by same letters in each column are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.
Parenthesis shows reduction % or increase % as compared to control.

control method particularly against the root knot nematode (29). *A. marina* plant parts, viz. leaves, stem and pneumatophore, used as a biological control for the suppression of root knot nematodes showed that germination of seed, root weight, shoot weight, root length, and shoot length increase in both mash bean ($P < 0.001$) and okra ($P < 0.001$) when soil was amended with *A. marina* at 5% w/w. Maximum inhibition of knots was obtained on okra plant at 5% w/w followed by 1% w/w ($P < 0.001$). Powder of all plant parts of *A. marina* was equally suitable for the control of *M. javanica*. Similarly, Mehdi et al. (28) reported that *A. marina* and *R. mucronata* with or without *Pseudomonas aeruginosa* significantly reduced the root knot infection in tomato.

Table 2. Effect of soil amendment with Avicennia marina plant parts on plant growth and root knot infestation of mash bean and okra plants.

	MASH BEAN						OKRA					
Treatments	Shoot Germ. %	Shoot length (cm)	Root weight (g)	Root length (cm)	weight (g)	RKI	Shoot Germ. %	Shoot length (cm)	Root weight (g)	Root length (cm)	weight (g)	RKI
Control	62.5 a	19.04 a	0.38 a	4.18 a	0.04 a	4 a	50 a	16.74 a	0.47 a	4.75 a	0.02 a	4 a
0.1% leaves	66.66 ab	18.30 ab	0.42 ab	3.71 ab	0.04 b	2 ab	50 a	18.15 b	0.46 ab	4.82 ab	0.04 a	4 ab
1% leaves	70.83 ab	18.77 ab	0.66 abc	3. 50 abc	0.06 bc	1 ab	54.16 ab	19.66 b	0.42 abc	6.06 bc	0.05 ab	2 ab
5% leaves	91.66 bc	21.97 ab	0.96 abc	5.72 abc	0.09 bcd	0 bc	75 ab	21.5 b	0.78 bcd	7.44 bcd	0.11 bc	0 bc
0.1% stem	62.5 bc	18.07 ab	0.46 abc	3.23 abc	0.03 bcd	3 bc	29.16 abc	17.65 b	0.48 bcd	4.28 bcd	0.03 bc	3 c
1% stem	70.83 c	18.09 ab	0.63 abc	2.52 abcd	0.05 bcd	2 cd	62.5 bcd	19.34 b	0.63 cd	5.74 bcd	0.05 bc	2 c
5% stem	91.66 c	21.16 ab	0.89 abc	4.87 bcd	0.11 bcd	0 de	70.83 bcd	23.56 b	0.85 cd	6.96 cd	0.09 c	0 c
0.1% pneumatophore	54.16 c	18.87 b	0.55 abc	3.83 bcd	0.03 cd	3 ef	37.5 cd	17.42 b	0.48 d	5.19 cd	0.03 c	3 d
1% pneumatophore	62.5 c	19.33 b	0.556 bc	4.48 cd	0.06 cd	3 ef	37.5 cd	21.11 b	0.64 d	5.51 d	0.05 c	2 d
5% pneumatophore	100 c	21.01 b	0.84 c	5.28 d	0.08 d	0 f	75 d	21.37 b	1.04 d	9.09 d	0.08 c	0 d
LSD0.05 =	22.33	2.93	0.33	1.739	0.05	0.851	20.67	2.37	0.27	1.94	0.03	0.74

Germ.: Germination. RKI: Root knot index.

Mean followed by same letters in each column are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

Conclusion

Mangroves have been reported to contain compounds like tannins, alkaloids and polyphenols, which have antimicrobial activity. *A. marina* releases some compounds toxic to nematodes like phenols, tannins, azadirachtin, and ricinine or they are derived from the decomposition process in the soil like ammonia, nitrate, and hydrogen sulfide. It is hypothesized that amendment of soil with dry powder of different parts of mangrove releases nematicidal compounds after decomposition insert, which may reduce activity and damage cause by the nematode to test plants and ultimately improve plant growth and increase crop productivity.

Acknowledgements

We are grateful to HEC for providing financial support under grant no. 20-236/R&D/03/321 in order to carry out this research work.

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