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Effects of *Rhizophora mucronata* (Mangrove) in the Control of Root-knot Nematode and Root-infecting Fungi of Tomato

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Abstract: *Rhizophora mucronata* (mangrove) used alone or in combination with *Paecilomyces lilacinus* significantly suppressed root infection caused by *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* the root-infecting fungi and *Meloidogyne javanica*, the root-knot nematode in tomato. An *R. mucronata-P. lilacinus* treatment also improved plant growth. Organic amendments and *P. lilacinus* used together showed better results in the control of root-rot and root-knot disease complex as compared to their separate use.

Key words: *Rhizophora mucronata, Paecilomyces lilacinus*, organic amendment, root-infecting fungi, root-knot nematode

Introduction

Toxicity to beneficial fauna and flora of the soil, development of resistance in parasitic nematodes and environmental degradation often results from their continuous and injudicious use (Akhtar, 1991). Organic soil-amendments have been found to effectively suppress the noxious nematodes to varying extant depending upon the type of organic matter, nematode, host species and the prevailing ecological conditions (Alam, 1990; Siddiqui *et al*., 1998). Significant results have been achieved in controlling a large number of nematode species with neem products such as neem cake and leaves. Soil amendment with neem cake gave better results than leaves and it has been found to show similar results as standard nematicides (Alam, 1993). Similarly, *Avicennia marina* (mangrove) has also been reported to suppress root-infecting fungi like *Macrophomina phaseolina, Fusarium solani, Rhizoctonia solani* and the root-knot nematode (*Meloidogyne javanica*) in tomato (Mehdi *et al*., 1999). Experiments were therefore carried out to examine the effects of *Rhizophora mucronata* (mangrove) in the control of soil-borne root-infecting fungi and root-knot nematode on tomato (*Lycopersicon esculentum* Mill.).

Materials and Methods

One kg of air dried mangrove (*Rhizophora mucronata*) leaves were percolated in ethanol and disintigrated in a homogenizer. After two weeks, the extract was filtered through cotton wool and the filtrate concentrated in a Rorary Vacuum Evaporator (EYLA) under reduced pressure at 37°C. *In vitro* experiments were conducted in the soil-borne Disease Research Laboratory, Department of Botany, University of Karachi. To determine the effects of extract of *R. mucronata* on egg hatching of *M. javanica*, two ml of the different concentrations of crude extract prepared in ethanol were transferred in cavity blocks and allowed to evaporate the organic solvent. After 48 hours, 2 egg masses in distilled water were transferred in each cavity block. Number of juveniles hatched were counted using a low power microscope. After a 48 hour hatching period in extract, the egg masses were transferred to distilled water to see whether the egg masses kept in extract had been permanently or temporarily inhibited. Juveniles emergence was recorded for further 48 hours in distilled water. To determine the effects of extract of *R. mucronata* on *M. javanica* larvae, 2 ml of the different concentrations of extract was transferred in cavity blocks and allowed to dry for 48 hours. Nematode suspension containing 15-20 larvae/ml prepared in distilled water was transferred in each cavity block at 2 ml/cavity block. Nematicidal activity was recorded for 5 days. For the assessment of the activity of mangrove against soil-borne root-infecting fungi, a 5 mm-diam., disc of Whatman No.1 filter paper was soaked in extract (10 mg/ml) and placed on one side of the Petri dishes containing Czapek's Dox agar medium. On other side of the Petri dish, a 5 mm-diam., disc of actively growing test fungus was inoculated. After one week incubation at 30-35°C, zone of inhibition (if any) was recorded. Soil used for the experiment was a sandy-loam, had a natural population of 3-8 sclerotia of *Macrophomina phaseolina* as estimated by wet sieving and dilution technique (Sheikh and Ghaffar, 1975); 6.5% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 2840 cfu g^{-1} of soil of *Fusarium solani* as found by soil dilution technique (Nash and Snyder, 1962). Chopped mangrove leaves were mixed with soil to give the concentration of 0.5 and 1.0% w/w and transferred into 8 cm-diameter plastic pots at 350 g/pot. The soil was watered daily to allow for the decomposition of the organic substrate. After three weeks, upper soil surface was removed to a depth of 3 cm and conidial suspension of *Paecilomyces lilacinus* prepared in 25 ml distilled water containing 2.4×10^8 cfu ml^{-1} was drenched in each pot. Soil drenched with sterile distilled water used as control. After soil treatment, surface was covered and 3 week-old tomato seedlings raised in sterile soil was planted in each pot at 3 seedlings/pot. In another similar set of pots, after one week of the seedling establishment, 2000 eggs/J2 of *M. javanica* were inoculated in the root zone by making three holes around tomato roots. Treatments were replicated three times and pots were kept in a Randomized Block Design fashion. The soil was kept at 50% W.H.C. The experiment was terminated 45 days after the addition of nematode and plant growth parameters were recorded. Number of galls produced on the entire root system were counted under a low power microscope. To determine the incidence of root-infecting fungi, 5-mm-long root pieces after surface disinfestation with 1% $Ca(OCl)_2$ were plated onto PDA plates at 5 pieces/plate. After one week incubation, root infection caused by root-infecting fungi were recorded. All the data were analysed using one way or two way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD) according to (Gomez and Gomez, 1984).

Results

Egg hatching test: Ethanolic extract of *R. mucronata* showed a reduction in egg hatching activity of *M. javanica*. Activity of the extract increased with the increase in extract concentration. Maximum inhibition in *M. javanica* egg hatching was achieved where extract at 10 mg/ml was used (Table 1).

Table 1: Effect of *R. mucronata* on *M. javanica* egg hatching

Treatment	Egg masses in extract Exposure time (Hours)			Total	Egg masses in D.W. Exposure time (Hours)			Total	Total eggs hatched In extract + D.W	% age reduction over control
	24	78	72		24	48	72			
Control	100	51.74	32.00	185.75	40.50	22.00	48.25	110.75	294.50	
R. mucronata 100,000 ppm	38.00	18.50	43.50	100.00	61.25	38.25	49.75	148.75	248.75	-15.53
R. mucronata 10,000 ppm	60.00	12.50	59.00	131.50	130.75	7.50	12.00	150.25	281.75	-4.32
R. mucronata 1000 ppm	81.25	6.00	46.50	133.75	148.00	5.00	13.00	166.00	299.75	-1.78

Table 2: Effect of *R. mucronata* on root infecting fungi

Test fungus	Zone of inhibition (mm)
Macrophomina phaseolina	5
Fusarium solani	9
Fusarium oxysporum	*
Rhizoctonia solani	*

Table 3: Effect of *R.mucronata* and *Paecilomyces lilacinus* on the development of root-knot and root-rot infection of tomato

Treatment	Galls/ Root system	*M. phaseolina* Infection%	*F. solani*	*R. solani*
Control	46.1	66.6	88.8	77.7
R. mucronata 0.5% (A)	37.5	55.5	77.7	66.6
R. mucronata 1.0% (B)	30.0	55.5	72.2	61.1
P. lilacinus	33.5	49.9	66.6	66.6
P. lisacinus + A	28.1	38.3	61.1	49.9
P. lilacinus + B	23.1	33.3	49.9	38.8
LSD<	3.9	(Treatment = 17.6, Fungi = 24.9)		

Table 4: Effect of *R. mucronata* on growth of tomato in nematode infested soil

Treatment	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
Control	13.2	0.7	5.1	1.0
R. mucronata 0.5% (A)	15.3	1.0	7.3	0.9
R. mucronata 1.0% (B)	17.9	1.3	7.5	0.4
P. lilacinus	16.9	1.3	6.9	0.6
P.lilacinus + A	17.7	1.5	8.9	0.3
P.lilacinus + B	17.9	1.6	10.1	0.7
LSD < 0.05	1.37	0.07	1.04	0.09

Table 5: Effect of *R. muncronata* in the control of root infecting fungi on tomato fungi on tomato in soil not infected with nematode

Treatment	*M. phaseolina*	*F. solani*	*R. solani*
		Infection %	
Control	22.0	77.7	44.4
*R. mucronata*0.5% (A)	22.0	55.5	33.3
*R. mucronata*0.1% (B)	33.3	38.6	22.0
P. lilacinus	33.3	38.6	33.3
P. lilacinus + A	11.1	22.2	22.2
P. lilacinus + B	00.0	33.3	11.1
LSD < 0.05	Treatment = 26.0	Pathogen = 18.38	

Table 6: Effect of *R. murconata* on growth of tomato in nematode infested soil

Treatments	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weighyt (g)
Control	17.4	1.0	5.8	0.5
R. mucronata 0.5% (A)	18.8	1.4	8.2	0.7
R. mucronata 0.1% (B)	17.9	1.5	9.9	0.7
P. lilacinus	22.5	1.7	6.4	0.7
P. lilacinus + A	23.9	1.7	8.4	0.8
P. lilacinus + B	21.1	1.4	8.8	0.7

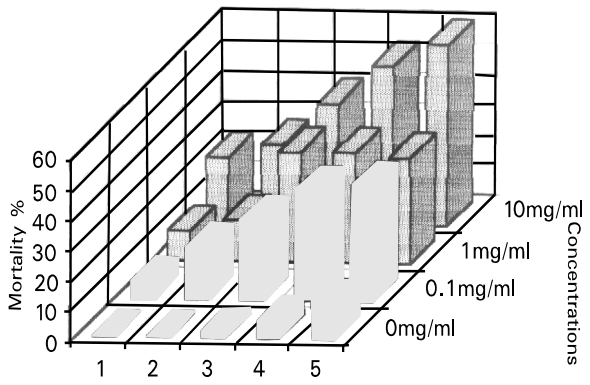

Fig. 1: Effect of *Rhizophora mucronata* on mortatity on *Meloidogyne Javanica* Larvae

Ethanolic extract of leaves of *R. mucronata* exerted lethal effects on *M. javanica* second stage juveniles. An increase in extract concentration and exposure time increased juveniles death. Extract concentration at 10 mg/ml resulted in the maximum larval mortality whereas concentration below this level was found ineffective in the suppression of root-knot nematode (Fig. 1). A zone of 9 and 5 mm respectively were produced against *F. solani* and *M. phaseolina* whereas extract was found ineffective against *R. solani* and *F. oxysporum* (Table 2). Use of *R. mucronata* alone or in combination with *P. lilacinus* showed promising results in the suppression of *M. javanica*, the root-knot nematode infection in tomato. Maximum inhibition in galling rate was achieved where *R. mucronata* at 1.0% w/w was used with *P. lilacinus* (Table 3). Maximum suppression (50% as compared to control) in *M. phaseolina* infection was recorded in the treatment where *R. mucronata* at 1.0% w/w was used with *P. lilacinus* followed by in the treatment where *R. mucronata* at 0.5% w/w was used with *P. lilacinus*. Maximum inhibition in *F. solani* infection was observed in the treatment where *R. mucronata* at 1.0% w/w was used with *P. lilacinus*. Similarly, maximum inhibition in *R. solani* infection was found in the treatment where *R. mucronata* at 0.5% w/w was used with *P. lilacinus* (Table 3). Maximum plant height was found in the treatment where *R. mucronata* at 1.0% w/w was used alone followed by in the treatment where *R. mucronata* was used at 1.0% w/w with *P. lilacinus*.

The greatest fresh weight of shoot was recorded in the treatment where *R. mucronata* at 1.0% w/w was used with *P. lilacinus* followed by in the treatment where *R. mucronata* at 0.5% w/w used with *P. lilacinus*. Maximum root length was produced in the treatment where *R. mucronata* at 0.5% w/w and *P. lilacinus* were used together whereas highest fresh weight of root was produced in untreated control plants (Table 4). In soil not artificially infested with root-knot nematode, a complete suppression in *M. phaseolina* infection was observed in the treatment where *R. mucronata* at 1.0% w/w was used with *P. lilacinus*. Similarly, *R. mucronata*

at 0.5% w/w in combination with *P. lilacinus* reduced *M. phaseolina* infection by more than 50%. *R. mucronata* at 0.5 or 1.0 % w/w used with *P. lilacinus* suppressed *F. solani* infection by more than 50%. *R. mucronata* at 1.0% w/w used alone or *R. mucronata* at both the dosage levels mixed with *P. lilacinus* resulted in more than 50% inhibition in *R. solani* infection (Table 5). *R. mucronata* at 0.5% w/w mixed with *P. lilacinus* resulted in the maximum plant height. *P. lilacinus* used alone produced maximum fresh weight of shoot. *R. mucronata* at 1.0% used individually produced greatest root length whereas *R. mucronata* at 0.5% w/w used with *P. lilacinus* showed the highest fresh weight of root (Table 6).

Discussion

Use of organic wastes, which are available in the developing countries, for pest control provides a new channel for their save disposal. In the present study, use of *Rhizophora mucronata* (mangrove) as soil organic amendment significantly controlled root-infecting fungi and root-knot nematode in tomato. There does not appeared to be any report on the use of *R. mucronata* as soil organic amendment for the control of plant pathogens. Ethanolic extract of *Avicennia marina* (mangrove) was found to reduced egg hatching, produced nematode mortality and inhibit radial growth of root-infecting fungi *In vitro*, when incorporated in soil as organic amendment significantly suppressed root-rot and root-knot diseases in tomato (Mehdi *et al.,* 1999) have reported the presence of compounds like tannins, alkaloids and polyphenols in mangrove which play an important role in the suppression of deleterious microorganisms (Jamale and Joshi, 1998; Nishiyama *et al*., 1978; Ross *et al.,* 1980). It is interesting to note that *R. mucronata* used with *P. lilacinus* showed better results in the suppression of root-rot and root-knot diseases as compared to their separate use. Beside direct effects of organic amendments to the pathogen, organic matter to soil stimulates the microbial activity of bacteria, fungi, algae and other microorganisms (Sayre, 1980; Rodriguez-Kabana *et al*., 1987; Siddiqui *et al.,* 1999a). Increased microbial activity in amended soil enhance enzymatic activities (Rodriguez-Kabana *et al.*, 1983) and accumulation of decomposition end products and microbial metabolites are deleterious to plant parasitic nematodes (Rodriguez-Kabana *et al.,* 1965). Reddy *et al.* (1991), have reported that the use of castor and neem cake with *P. lilacinus* was more effective in increasing plant growth and in reducing the population of *Tylenchulus semipenetrans* both in soil and roots. *P. lilacinus*, an egg parasite of the root-knot nematode (Jatala, 1985) have been found to reduce root-rot and root-knot infection in mungbean and mashbean and nematode population densities in soil (Siddiqui *et al.,* 1999b). The results of the present study would therefore suggest that *R. mucronata* with a compatible biocontrol agent has great potential in the suppression of root diseases caused by fungi and plant-parasitic nematodes which may result in better plnt growth.

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