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Effect of *Avicennia Marina* and *Paecilomyces lilacinus* on Root Rot - Root Knot Diseases of Tomato

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bstract

tential of *Avicennia marina* (mangrove) with *Paecilomyces lilacinus* for the control of root infecting fungi viz., *acrophomina phaseolina, Fusarium solani, Rhizoctonia solani* and *Meloidogyne javanica* root knot nematode was evaluated *vitro* and under green house conditions. Soil amendment with *A. marina* alone or in combination with *P. lilacinus* gnificantly controlled root rot-root knot diseases in tomato with enhancement in plant growth. Organic amendment hanced biocontrol efficacy of *P. lilacinus* in the control of root pathogens.

troduction

ant diseases produce serious losses to crop plants and lversely affect the agricultural economy of a country. Of e disease producing organisms, the soilborne root fecting fungi and root knot nematode produce root rot and ot knot disease complex resulting in death of plants. rganic amendments are generally used for the nprovement of crop plants and increasing agricultural oductivity. The addition of arganic materials to soil nfested with root infecting fungi and root knot nematode as been clearly demonstrated as a satisfactory control ethod against phytoparasitic nematodes (Siddiqui *et al.*, 998) and root infecting fungi (Ehteshamul-Haque *et al.*, 998), particulary in developing countries because of the heapness and easy availabilty of materials. The Northern art of the Indus delta, which includes the Korangi Phitti reeks, is under the control of Port Qasim authority. This is an area of about 64,000 hacter or just over one tenth of the intertidal area of the Indus delta (600,000 hacter). It contains 10,500 ha., of dense mangrove, 4645 ha., of medium cover mangroves and 3690 ha., of sparse mangrove. The rest consists of tidal-creeks, mud flats and sand. The area contains some of the densist mangrove cover in the Indus delta. The predominent species of mangrove in this area is *Avicennia marina* (Mehdi, 1999). There are reports where use of *Paecilomyces lilacinus* (Thom) Samson, an egg parasite of *Meloidogyne javanica* oot knot nematode (Jatala, 1985) showed significant esults in the control of root knot - wilt disease complex of omato (Stephen *et al.*, 1996). There does not appear any eport on the use of mangrove in the control of plant arasitic nematodes, experiments were therefore carried out the use of mangrove as organic amendment on the fficacy of *P. lilacinus* in the control of root infecting fungi nd root knot nematode in tomato (*Lycopersicon sculentum* Mill.).

laterials and Methods

reparation of ethanolic extract of leaves of *A. marina*: resh leaves of *A. marina* were soaked in ethanol and ointigrated in a homogenizer. After storing for 2 week, hanolic extract was filtered and residue resuspended and ired with ethanol. The ethanolic extract was dried in a tary vacuum evaporator under reduced pressure at room temperature (37°C).

***In vitro* nematicidal activity of ethanolic extract of *A. marina*:** Two ml of different concentrations of crude extract (0.1, 1.0 and 10.0mg/ml) was transferred in watch glass and allow to dry. After 48 hours, two ml suspension of freshly hatched second stage juveniles (15-20 juveniles/ml) of *M. javanica* obtained from brinjal (*Solanum melongena* L.) roots were placed in watch glass. There were three replicates of each treatment and watch glasses were kept randomized. Nematicidal activity (Larval mortality) was observed at 24, 48, 72, 96 and 120 hours interval.

***In vitro* egg hatching test:** Two ml of different concentrations of *A. marina* crude extract (0.1, 1.0 and 10 mg/ml) was transferred in watch glass and allowed to dry. After 48 hours 2 medium size egg masses of *M. javanica* were placed in watch glasses in 2ml distilled water. Egg masses placed in sterile distilled water served as control. There were three replicates of each treatment and watch glasses were incubated at room temperature (25-30°C). The counts of juveniles was done after every 24 hours. After 72 hours of incubation period, the egg masses after thorough washing in running tap water were transferred in 2ml sterile distilled water to see whether the egg masses kept in the extract had been permanently or temporarily inactivated. The emergence of juveniles were again recorded for further 72 hours.

***In vitro* testing against root infecting fungi:** Ethanolic extract (10mg/ml) of *A. marina* was impregnated on a 5mm disc of Whatman no. 1 filter paper @ 20µl/disc and placed 5mm inside of the edge of petri dishes containing Czapak Dox Agar medium pH 7.2. Disc inoculated with ethanol served as control, was placed apart from the disc containing ethanolic extract. A 5mm disc of the test fungus was inoculated at the center of the plate. There were three replicates of each treatment and plates were incubated at room temperature (25-30°C). Distance covered by fungus and zone of inhibition (if any) was measured daily.

Green house experiment: Sandyloam soil obtained from the experimental field of the Department of Botany, University of karachi was used. The soil had a natural infestation of 3-

Table 1: Effect of *Avicennia marina* on egg hatching of *Meloidogyne javanica*

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 contro
	24	48	72		24	48	72			
Control	100.0	68.7	41.2	210	36.0	22.0	11.0	69.0	279.0	
A. marina 0.1mg/ml	49.5	59.5	35.0	144	83.0	12.0	20.0	115.0	259.0	-7.16
A. marina 1.0 mg/ml	80.0	78.5	19.5	178	56.0	17.0	8.0	81.0	259.0	-7.16
A. marina 10 mg/ml	110.0	64.2	20.7	195	54.0	11.0	7.0	72.0	267.0	-4.30

* Distilled water

Table 2 Effect of *Avicennia marina* on root infecting fungi *in vitro*

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

* No inhibition

8 sclerotia g^{-1} of soil of *M. phaseolina* as estimated by wet sieving and dilution technique (Sheikh and Ghaffar, 1975), 6.5 percent colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 2840 cfu g^{-1} of soil of mixed population of *Fusarium* spp., as found by soil dilution technique (Nash and Snyder, 1965). Air dried leaves of *A. marina* were chopped in an electric grinder and mixed with soil @ 0.5 and 1.0 percent w/w. Amended soil was transferred in 8cm diam., plastic pots @ 350 g/pot. Soil was watered daily for the decomposition of the organic substrate. After one month of soil amendment, three week old tomato seedlings raised in sterilized soil was planted in each pot. In another similar set before seedling transplantation soil was drenched with conidial suspension of *P. lilacinus* (cfu 2.5x108 ml^{-1}). Soil without organic amendment and/or biocontrol agent served as control. There were three replicates of each treatment and pots were randomized on a screen house bench where soil was kept at 50 percent W.H.C. (Keen and Raczkowski, 1921). In another similar experiment, after one week of the seedling transplantation 2000 eggs/J_2 of *M. javanica* were inoculated near the root zone in each pot.

Experiment was terminated after 45 days of nematode inoculation and plant growth parameters such as plant height, root length, fresh weight of shoot and root were recorded. Infection of root knot nematode was estimated using 0-5 sclae of Taylor and Sasser (1978). To determine the infection by fungi, roots from each plant after thorough washing in running tap water cut into 1cm long pieces and after surface sterilization in 1 percent Ca $(OCl)_2$ five pieces were plated onto PDA medium supplimented with penicillin (100,000 units/L.) and streptomycin (0.2g/L.). Plates were incubated at 28°C and after one week incidence of root infecting fungi were determined. Data were analysed and subjected to factorial ANOVA (FANOVA) followed by Least Significant Differences (LSD) according to Gomez and Gomez (1984).

Results

Effects of ethanolic extract of *A. marina* on mortality of *M. javanica* larvae: Ethanolic extract of *A. marina* showed a significant mortality in *M. javanica* second stage juveniles. An increase in extract concentration and exposure time increased mortality in juveniles. Maximum mortality (22.6 %) in *M. javanica* larvae was found where high concentration (10mg/ml) of ethanolic extract of *A. ma* was used Fig.1.

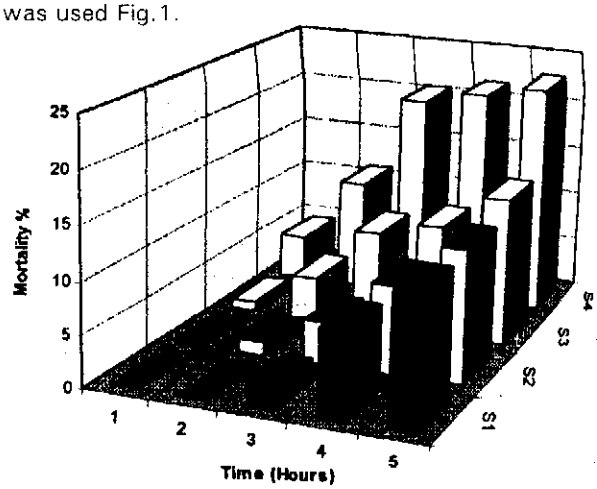

Fig. 1: Effect of *Avicennia marina* on mortality of *Meloidogyne javanica* larvae 1 = 24 hour, 2 = hour, 4 = 96, 5 = 120 hour S1 = Control, S2 = mg/ml, S4 = 10 mg/ml

Effects of ethanolic extract of *A. marina* on egg hatchi *M. javanica*: There was less emergence of juveniles the egg masses of *M. javanica* kept in (10mg/ml), ex an inhibitory effect on the hatching of egg masses. A end of 72 hours hatching period in ethanolic extract *marina*, egg masses were transferred to sterile di water. More juveniles emerged from egg masses transf from ethanolic extract but the total number were le compared to untreated control (Table 1).

Effects of ethanolic extract of *A. marina* on root inf fungi: Ethanolic extract of *A. marina* (10mg/ml) produ zone of inhibition of 5 and 9mm against *M. phaseolin* Fusarium solani whereas radial growth of *F. oxysporu R. solani* was not inhibited by *A. marina* crude e (Table 2).

Green house experiments:

Effects of *A. marina* and *P. lilacinus* on growth of to soil artificially infested with *M. javanica* roo nematode: Maximum plant height and fresh weight o was recorded in treatment where *A. marina* @ 1.0 p w/w was used with *P. lilacnius*. maximum root leng recorded in treatment where *A. marina* @ 0.5 perce was used with *P. lilacinus* whereas greatest fresh w root was found in control plants (Table 3).

e 3: Effect of *Avicennia marina* and *Paecilomyces lilacinus* on growth of tomato in soil artificially infested with *Meloidogyne javanica* root knot nematode

tments	Plant height (cm)	Snoot weight (gm)	Root length (cm)	Root weight (gm)
trol	13.40	0.70	6.76	1.00
cennia marina 0.5% w/w (A)	15.10	1.05	7.10	0.84
cennia marina 1.0 % w/w (B)	17.20	1.30	7.66	0.56
cilomyces lilacinus	19.20	1.13	6.55	0.64
lacinus + A	21.00	1.64	8.66	0.78
lacinus + B	23.30	1.75	7.50	0.34
p<0.05	0.64	0.20	0.81	0.10

le 4: Effect of *Avicennia marina* and *Paecilomyces lilacinus* on the development of root rot- root knot disease complex of tomato in soil artificially infested with *Meloidogyne javanica* root knot nematode

atment	Galls/ plant	RKI 0-5 scale	Infection % M.p.*	F.s.*	R.s.*
trol	47.00	4.00	33.33	88.88	44.44
cennia marina 0.5% w/w (A)	40.00	3.77	33.33	77.77	33.33
cennia marina 1.0 % w/w (B)	35.00	3.77	33.33	77.77	0.00
cilomyces lilacinus	30.00	3.33	11.11	66.66	33.33
lacinus + A	26.00	3.00	0.00	77.77	16.66
lacinus + B	24.00	3.00	0.00	72.20	16.66
p<0.05	7.47	0.36	Treatment = 20.43,	Fungi = 14.44	

M.p. = *Macrophomina phaseolina*, F.s. = *Fusarium solani*, R.s. = *Rhizoctonia solani*

ble 5: Effect of *Avicennia marina* and *Paecilomyces lilacinus* on growth of tomato in soil not artificially infested with *Meloidogyne javanica* root knot nematode

atments	Plant height (cm)	Shoot weight (gm)	Root length (cm)	Root weight (gm)
ntrol	17.30	1.11	7.90	0.65
cennia marina 0.5% w/w (A)	18.10	1.40	8.10	0.67
cennia marina 1.0 % w/w (B)	20.20	1.58	9.44	0.75
cilomyces lilacinus	18.00	1.71	9.99	0.78
lacinus + A	23.00	1.76	11.22	0.81
lacinus + B	24.20	1.81	10.26	0.95
p<0.05	1.15	0.11	1.10	0.04

ble 6: Effect of *Avicennia marina* and *Paecilomyces lilacinus* on the development of root rot disease of tomato in soil not artificially infested with *Meloidogyne javanica* root knot nematode

atment	Infection % *M. phaseolina*	*F. solani*	*R. solani*
ntrol	44.44	77.77	43.33
icennia marina 0.5% w/w (A)	38.80	72.20	38.80
icennia marina 1.0 % w/w (B)	0.00	66.66	33.33
cilomyces lilacinus	33.33	49.96	27.70
lacinus + A	27.76	61.10	33.33
lacinus + B	0.00	55.55	22.22
p<0.05	Treatment = 25.87,	Fungi = 18.29	

ects of *A. marina* and *P. lilacinus* on the development of ot knot infection in soil artificially infested with *M. anica* root knot nematode: Use of *A. marina* with *P. cinus* showed significant control of root knot infection in mato. *A. marina* used as organic amendment enhanced control efficacy of *P. lilacinus* in the suppression of root ot infection. Maximum reduction in gall formation by *M. anica* was observed where *A. marina* @ 1.0 percent w/w as used with *P. lilacinus* followed by where *A. marina* @ 5% w/w mixed with *P. lilacinus* (Table 4).

Effects of *A. marina* and *P. lilacinus* on root rot infection in soil infested with *M. javanica* root knot nematode: *A. marina* at both dosages mixed with *P. lilacinus* showed complete suppression in *M. phaseolina* infection. Similarly *P. lilacinus* used alone showed more than 75 percent reduction in *M. phaseolina* infection. A significant ($p<0.05$) suppression in *F. solani* infection was recorded in the treatment where *P. lilacinus* was used separately. *A marina* @ 1.0 percent w/w used alone provided complete protection to roots from infection caused by *R. solani*.

Similarly use of *A. marina* at both the dosages with *P. lilacinus* showed more than 50 percent reduction in *R. solani* infection (Table 4).

Impact of *A. marina* and *P. lilacinus* on growth of tomato in soil not artificially infested with *M. javanica* root knot nematode: Maximum plant height and fresh weight of shoot and root were observed in the treatment where *A. marina* @ 1.0 percent w/w was used with *P. lilacinus*. Greatest root length was found in the treatment where *A. marina* @0.5 percent w/w was used with *P. lilacinus* (Table 5).

Effects of *A. marina* and *P. lilacinus* on root rot infection in soil not artificially infested with *M. javanica* root knot nematode: *A. marina* @ 1.0 percent w/w used alone or mixed with *P. lilacinus* showed complete inhibition of *M. phaseolina* infection. *P. lilacinus* used alone showed significant control of *F. solani* infection. *R. solani* infection was effectively suppressed by the addition *of A. marina* and / or *P. lilacinus* but their effects were statistically non-significant (Table 6).

Discussion

Non pesticide control is being regarded favourable in agriculture as environmental awareness increases. The adddition of organic materials to soil infested with plant pathogens has been clearly demonstrated as a satisfactory control methods particularly against root knot nematode (Ehteshamul-Haque *et al.*, 1995). Amendments may also provide a favourable substrate of soil microfauna and microflora (Linford, 1937) which can include direct predators (micro-arthopods) or pests (fungi, bacteria) of nematodes, or which suppress soil nematode population indirectly through the production of enzymes (Rodriguez-Kabana, 1986; Galper *et al.*, 1990) or toxic metabolites such as antibiotics of bacteria origin.

In the present study use of A. marina as organic amendment showed promising results in the control of soilborne root infecting fungi like *M. phaseolina, F. solani, R. solani* and *M. javanica* root knot nematode in tomato. mangrove have been reported to contain copmounds like tannins, alkaloids, polyphenols (Combs and Anderson, 1949) which have antimicrobial activity (Jamale and Joshi, 1978; Nishiyama, 1978; Ross *et al.*, 1980). Use of *P. lilacinus* either used alone or in combination with *A. marina* showed significant results in the suppression of root rot and root knot diseases. There are reports where use of *P. lilacinus* an egg parasite of *Meloidogyne javanica* root knot nematode (Jatala, 1985) showed promising results in the control of root knot-wilt disease complex on tomato (Stephen *et al.*, 1996). Similarly, use of *P. lilacinus* on brinjal and mungbean (Zaki and maqbool, 1992) on groundnut (Patel *et al.*, 1995) significantly controlled plant parasitic nematodes.

Ethanolic extract of *A. marina* inhibited radial growth of *M. phaseolina* producing zone of inhibition and showed nematicidal activity against *M. javanica* root knot nematode. Presumably crude extract of *A. marina* contain compounds which have inhibitory effects on root infecting fungi and root knot nematode.

It is interesting to note that in this study, ethanolic extract of *A. marina* was not found to inhibit radial growth of *oxysporum* and *R. solani in vitro* but showed significa control when used in pot experiments. This may presumab be due to the fact that compounds released *in vitro* we quantitatively insufficient to control root pathogens. It also hypothesized that in soil organic amendment with *marina* might not have direct effect on pathogens but m have stimulate other soil microorganisms which presumab inhibited root pathogens.

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