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Avicennia marina (mangrove) soil amendment changes the fungal community in the rhizosphere and root tissue of mungbean and contributes to control of root-knot nematodes

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Summary. The effect of soil amendment with *Avicennia marina* (mangrove) on mungbean growth and mungbean infestation with *Meloidogyne javanica* was determined in greenhouse pot experiments. Galling and final nematode population densities were reduced by all soil amendments with mangrove. To better understand whether nematode suppression by *A. marina* was caused directly by the release of nematicidal factor(s) into the soil, or was due indirectly to changes in the fungal community, the diversity of the rhizosphere populations of culturable fungi was assessed before organic amendment (day 0), after decomposition but before seed sowing (day 15) and at harvest (day 73). Thirteen out of 20 fungal species were isolated from both *A. marina*-amended and unamended soils, the most frequent genera being *Alternaria, Aspergillus, Fusarium, Penicillium, Trichoderma, Mucor, Myrothecium* and *Rhizoctonia*. The other seven were found only in amended soils. At different times in the course of the experiment amended and unamended soils differed significantly in the fungi isolated from the rhizosphere and/or in the concentrations of *A. marina*. *Trichoderma viride* was isolated only from surface-sterilized mungbean roots grown in amended soils, whereas *Chaetomium* sp. was isolated only from unamended soils.

Key words: fungal diversity, *Meloidogyne javanica*, nematicidal compounds, organic amendment, soil-borne fungi.

Introduction

Organic soil amendments are commonly used in agricultural systems to recycle nutrients and energy as well as to improve soil conditions for plant growth (Hadar *et al.,* 1992; Muchovej and Pacovsky, 1997). Some organic amendments suppress soil-borne plant pathogens and/or the diseases they cause, and several also control plant-parasitic nematodes (Rodríguez-Kábana, 1986; Ali *et al.,* 2001). Since organic amendments require high rates of application to be effective against plant-parasitic nematodes, these organic materials must be cheap and of local origin if they are to be exploited practically.

The northernmost part of the Indus delta, which includes the Korangi Phitti creeks, is an area of about 64,000 ha or just over one-tenth of the intertidal area of the entire Indus delta (600,000 ha). It contains 10,500 ha of dense mangrove forest, 4645 ha of medium-density mangroves and a 3690 ha of sparse mangroves (Mehdi, 1999). The predominant species of mangrove in this area is *Avicennia marina*. Mangrove contains compounds like tannins, alkaloids, polyphenols (Comb and Anderson, 1949) and also compounds with antibiotic activity

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(Jamale and Joshi, 1978; Nishiyama *et al*., 1978; Ross *et al*., 1980). Methanolic extract of the leaves of *A. marina* caused substantial mortality *in vitro* of the root-knot nematode *Meloidogyne javanica* and inhibited radial growth of *Macrophomina phaseolina*, a charcoal rot fungus. Powdered leaves of this plant incorporated into the soil significantly reduced root infection caused by soil-borne fungi and root-knot nematode in tomato (Mehdi *et al*., 1999). Mortality of *M. javanica* juveniles by mangrove, observed *in vitro*, was due to nematicidal compounds of a polar nature. Soil amendment with *A. marina* and *Rhizophora mucronata* (mangrove), under greenhouse conditions, markedly reduced root-knot development due to *M. javanica* with enhanced growth of seedlings (Mehdi *et al*., 2001).

In general, soil amendment with toxic plants suppresses plant pathogens directly by releasing toxic substances like phenols, and indirectly by enhancing soil micro-organisms that inhibit phytopathogens, and also plant-parasitic nematodes (Ali *et al*., 2001; Shaukat *et al*., 2001; Shaukat and Siddiqui, 2001a, 2001b). The aim of this study was to determine whether *A. marina*-mediated suppression of *M. javanica* was related to particular changes in the structure and composition of fungal communities in the rhizosphere and in the roots of mungbean [*Vigna radiata* (L.) Wilczek] roots.

Materials and methods

Unsterilized sandy loam soil (pH 8.1; moisture holding capacity 38%) obtained from a field near the Department of Botany, University of Karachi was mixed with powdered leaves of *A. marina* at concentrations of 0.5 or 1.0% w:w or left unamended. The amended soils were placed in 8-cm-diam. plastic pots at 350 g/pot and watered daily to facilitate the decomposition of the plant material. Three weeks after amendment, eight mungbean seeds were sown in each pot, and following germination four seedlings were retained in each pot. One week after seedling emergence, the soil in each pot was inoculated with 2000 juveniles of *M. javanica*. Juveniles were less than one-week-old and were obtained from infected brinjal roots using the Baerman funnel technique. Each treatment and control had eight replicates and pots were arranged in randomized complete block design. The total fungal count was measured at the start of the experiment (day 0); at day 15, which was after decomposition but before the seed sowing date, day 21; and at the time of harvest (day 73). Four replications of each treatment were used to evaluate the fungal populations.

The experiment was terminated 45 days after nematode addition, and at that time plant growth parameters (plant height, f wt of shoot and root) and the galls produced in the root system were counted. Final population densities of *M. javanica* in the soil and roots, and total fungal counts from the rhizosphere of mungbean were determined as described by Shaukat and Siddiqui (2001b). Data were subjected to one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test and Duncan's multiple range test. Several diversity indices including general species diversity (H'), variance of general diversity (Var H'), equitability component (J) and its variance (Var J), and species richness ($\hat{S}$) were computed as described in detail elsewhere (Shaukat and Siddiqui, 2001b).

Results and discussion

Effect of *A. marina* on population density and infectivity of *M. javanica* and on the growth of mungbean

Soil amendment with *A. marina* at both 0.5 and 1% significantly ($P<0.05$) reduced nematode population densities in the soil and roots, and lowered root-knot development in mungbean as compared with the controls (Table 1). *A. marina* at 1% caused 24, 41 and 38% reductions in nematode populations in the soil and root and in root-knot infection respectively, compared with the controls. Soil amendment at this concentration also increased plant height by 21% and fresh weight of shoot by 72% over the controls. However, root weight in the amended soils was slightly lower. Several mechanisms may be involved, including release of compounds toxic to nematodes, changes in microbial communities enhancing nematode suppressiveness and stimulation of biological control organisms (Hallmann *et al*., 1999; Ali *et al*., 2001; Shaukat and Siddiqui 2001a, 2001b).

Effect of *A. marina* on fungal composition and species numbers in the rhizosphere

Isolation of fungi from the rhizosphere of mungbean yielded a broad fungal spectrum dominated by rather widespread genera and species frequently

found in agricultural soils and in the rhizosphere and roots of crop plants. A total of 20 culturable fungal species including a Mycelia sterilia species, were recovered from the mungbean rhizosphere (Table 2); they belong to 12 different genera. In general, compared with the controls, the number of culturable fungi in the rhizosphere increased in *A. marina*-amended soils. The greatest number of fungal species and colony counts were isolated from 1% *A. marina*-amended soil at harvest time, 73 days after amendment. *Paecilomyces varioti* was isolated exclusively from *A. marina*-amended soils (at both dosages), *Penicillium notatum* from unamended and 1% *A. marina* amended soils (Table

Table 1. Effect of soil amended with powdered leaves of *Avicennia marina* on *Meloidogyne javanica* populations in the soil and in the roots of mungbean, and its effect on the growth of mungbean.

Treatment	Galls/ root system	*M. javanica* population in		Plant height (cm)	Shoot weight (g)	Root weight (g)
		250 g soil	1 g root			
Control	85	3750	148	15.5	1.8	1.1
A. marina 0.5%	67	3130	104	17.9	2.8	1.0
A. marina 1%	53	2870	88	18.8	3.1	0.9
$LSD_{0.05}$	12.4	559	26	1.4	0.8	0.2

Table 2. Effect of soil amended with powdered leaves of *Avicennia marina* at concentrations of 0, 0.5 and 1% on soil fungal community structure expressed as $\log_{10}$ (x+1) and recorded at 0 (start of the experiment), 15 (6 days before seed sowing) and 73 (time of harvest) days.

Fungal species	Sampling time (days)								
	Unamended			0.5% *A. marina*			1% *A. marina*		
	0	15	73	0	15	73	0	15	73
Aspergillus sp.	0.34	0	0	0	0.44	0	0.34	0	0.44
A. candidus	0	0	0.34	0	0.34	0	0	0.44	0.34
A. flavus	0.44	0.53	0.34	0.34	0.49	0.84	0.34	0	0.56
A. niger	1.41	2.15	1.53	0.84	2.74	2.69	0.99	1.38	2.76
Alternaria alternata	0.34	0.44	0	0.34	0.34	0.44	0.56	0.44	0.56
Chaetomium globosum	0	0.44	0.34	0	0	0.34	0	0	0.34
Cladosporium herbarium	0.34	0.34	0	0.34	0	0	0	0.44	0
Drechslera halodes	0	0	0.34	0	0.34	0.34	0	0	0.44
Fusarium equiseti	0	0.34	0	0	0	0.44	0	0	0
F. oxysporum	0.49	0.34	0.44	0.49	0.56	0.56	0.34	0.56	0.84
F. solani	0.84	0.99	1.59	0.56	1.06	1.38	0.56	1.38	2.45
Mucor sp.	0.56	0.99	1.39	0.49	1.38	1.41	0.44	1.06	1.84
Myrothecium sp.	0	0.34	0	0.34	0	0	0	0	0
Paecilomyces varioti	0	0	0	0	0.34	0	0	0.44	0.34
Penicillium sp.	0.34	0.44	0	0.34	0.34	0.44	0.44	0.34	0.49
P. crysogenum	0	0	0.34	0	0	0.34	0	0.34	0.34
P. notatum	0.44	0	0.34	0	0	0	0.34	0	0.34
Rhizoctonia solani	0.44	0.34	0.34	0.34	0.34	0	0.44	0	0
Trichoderma viride	0	0.34	0	0	0.44	0.56	0	0.56	0.44
Mycelia sterilia	0.49	0.56	0.34	0.44	0.34	0.56	0.44	0.44	0.34
Total species (No.)	12	14	12	11	14	13	11	12	16
Total genera (No.)	8	11	8	9	9	9	7	9	10

2). By contrast, Shaukat and Siddiqui (2001b) observed that soil amended with *Lantana camara* was associated with several fungal species such as *Acremonium* sp., *Aspergillus fumigatus, Drechslera halodes, Fusarium culmorum, Penicillium notatum* and *Trichoderma viride* that did not occur in unamended soils. In the current study, *Fusarium equiseti* and *Myrothecium* sp. were recovered exclusively from unamended and 0.5% *A. marina* amended soils. However, in a previous study, all fungi in unamended soils were also recorded from *L. camara*-amended soils (Shaukat and Siddiqui, 2001b).

Interestingly, species of *Aspergillus*, *Mucor* and *Trichoderma viride* were isolated in relatively large numbers from the amended soils. Enhanced populations of these fungi in the rhizosphere following soil amendment with *A. marina* could be of significant advantage. *Fusarium, Penicillium* and *Trichoderma* are decomposers of celluloses and hemicelluloses (Domsch *et al.,* 1980). *Fusarium* and *Trichoderma* protect plants against pathogenic fungi through competition, parasitism, antagonism, and/or induced resistance (Alabouvette and Steinberg, 1995; Chet *et al.,* 1997; Fuchs *et al.,* 1997). Non-pathogenic *Fusarium* (Amer-Zareen *et al.,* 2001), *Trichoderma* (Siddiqui *et al.,* 2001b) and *Aspergillus* (Siddiqui *et al.,* 2001a), also suppress root-knot nematode populations and their infectivity.

Effect of *A. marina* on the diversity, equitability and species richness of the fungal communities

In general, fungal species diversity (*H'*) and equitability (*J'*) decreased with time, while species richness (*S*) increased slightly (Table 3). In *A. marina*-amended soil fungal diversity and equitability declined markedly compared to the non-amended soil, but species richness was slightly higher. This agrees well with a previous study in which diversity and equitability decreased but species richness increased in *L. camara*-amended soils compared with the controls (Shaukat and Siddiqui, 2001b). In the present study, the greater dominance of *Aspergillus niger* in amended soils, particularly at day 15 and 73, explains the lower diversity and equitability in these soils.

Effect of *A. marina* on endophytic colonization by fungi

Six of the culturable fungi isolated from the rhizosphere (*Alternaria alternata, Chaetomium* sp., *Fusarium solani, Penicillium* sp., *Rhizoctonia solani* and *Trichoderma viride*), colonized the root tissues (Table 4). Among non-pathogenic fungi *T. viride* was the predominant species isolated from mungbean roots grown in *A. marina*-amended soil, whereas *Chaetomium* sp. predominated in non-amended soil. These results suggest that endophytic fungi are predominantly recruited from the rhizosphere, from where they presumably enter the roots through wounds and natural openings. Lytic enzymes produced by these fungi may also contribute to enhance penetration and colonization. Endophytes colonize the same root tissues as sedentary plant-parasitic nematodes; this association of endophytic fungi with nematodes throughout the nematode life cycle makes these fungi excellent candidates for biocontrol strategies. This remains true even though some of these fungi cause hypersensitive reactions in plants.

Table 3. General diversity (*H'*), equitability (*J'*) and species richness (*S*) of the fungal communities in *Avicennia marina*-amended soils (0, 0.5 and 1%) at various sampling dates (0, 15, 73 days). Var (*H'*) = variance of *H'*; Var (*J'*) = variance of *J'*.

Diversity	Sampling time (days)								
	Unamended			0.5% *A. marina*			1% *A. marina*		
	0	15	73	0	15	73	0	15	73
H'	1.777	0.940	1.540	2.258	0.439	0.603	2.166	1.839	1.016
Var (*H'*)	0.034	0.012	0.010	0.023	0.002	0.003	0.028	0.015	0.0011
J'	0.715	0.356	0.619	0.942	0.166	0.235	0.903	0.740	0.366
Var (*J'*)	0.005	0.001	0.002	0.004	0.0004	0.0004	0.005	0.002	0.0001
_	12	14	12	11	14	13	11	12	16

Table 4. Percent colonization of mungbean roots growing in soils amended with different *A. marina* amendments (0.5 and 1%) and in unamended soil by fungi detected in the plant rhizosphere.

Fungal species	Percent colonization		
	0% *A. marina* unamended	0.5% *A. marina*	1% *A. marina*
Alternaria alternata	4	6	0
Chaetomium globosum	4	0	0
Fusarium solani	27	18	21
Penicillium sp.	2	0	5
Rhizoctonia solani	31	22	25
Trichoderma viride	0	8	11
$LSD_{0.05}$	9	7	7

Species of *Fusarium* and *Rhizoctonia* are common inhabitants of most agricultural fields in Pakistan and are considered very serious pathogens causing severe losses in economically important crops including mungbean. In the present study, fungi differed significantly ($P<0.05$) in their colonization pattern. *F. solani* and *R. solani* colonized mungbean roots grown in both amended and unamended soils, but their colonization was lower in the amended soils; *F. oxysporum* did not colonize mungbean roots in any of the soils. The release of phytoalexins by the plants in response to colonization by *Fusarium* spp., and *R. solani* may have contributed to these reductions in fungal penetration and colonization. Soils also harbour a variety of micro-organisms, including saprotrophic bacteria, that in plants induce systemic resistance to pathogenic fungi and nematodes.

Conclusion

The use of organic amendments is a very promising disease control strategy in Pakistan for mungbean and other high-value crops. However, further research is needed in order to evaluate the impact of such amendments on pathogens.

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