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Nematicidal, Alleopathic and Antifungal Potential of *Launaea procumbens*

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Abstract: Powdered shoot extract of ***Launaea procumbens***, a tropical ruderal and agrestal weed, inhibited egg hatch and caused mortality of ***Meloidogyne javanica*** juveniles ***in vitro***. However, ethanol extract of ***L. procumbens*** did not inhibit radial growth of root-infecting fungi including ***Macrophomina phaseolina***, ***Fusarium solani*** and ***Rhizoctonia solani in vitro***. Soil amendment with powdered shoot of ***L. procumbens*** markedly reduced root-knot infection caused by ***M. javanica*** in mungbean. Population densities of ***M. javanica*** were significantly lower in soil amended with 5.0% ***L. procumbens*** while a 2.5% amendment did not produce significant reduction in the nematode populations in soil. Whereas low dosage (2.5%) of ***L. procumbens*** significantly enhanced plant growth, high dosage (5%) reduced fresh shoot and root weights of mungbean indicating allelopathic effect. Soil amendment with ***L. procumbens*** resulted in marked changes in fungal community structure and composition. Fungi like ***Fusarium semitectum*** and a sterile fungus (red pigmented) were exclusively isolated from ***L. procumbens*** amended soils. On the other hand, all the fungal species isolated from ***L. procumbens*** amended soils were also present in unamended soils. Soil amendments with ***L. procumbens*** also altered fungal community structure in the root tissues of mungbean. Both general diversity and equitability of fungal community at 2.5% ***L. procumbens*** increased appreciably over the controls but at 5% dosage substantially decreased compared to controls, substantially though species richness declined at both the dosages. Dominance concentration followed an opposite trend to that of general diversity.

Key words: Organic amendment, root-knot nematode, root-infecting fungi, allelopathy, species diversity

Introduction

The soil-borne root-knot nematode and root-infecting fungi play a major role in the development of root-rot disease complexes on many important field and horticultural crops that often results in reduced growth and even death of plants. The problem of such pathogens is more serious in Pakistan than in most developing countries. Firstly, because of tropical climatic conditions, fungi and nematode proliferate rapidly throughout the year. Secondly, sandy soils particularly those which are irrigated have conditions conducive to the growth and survival of the pathogens. There exists unquestionable potential for managing plant diseases incited by soil-

borne phytopathogens and increasing crop productivity with the application of botanical toxicants to the soil. The interest in the use of organic amendment for the management of plant-parasitic nematodes has recently been intensified because of the phasing out of the chemical pesticides from the market of the developed countries in the year 2005. It is likely that the farmers in the developing countries will consume large quantities of such chemical pesticides. Therefore, effective alternative methods to combat soil-borne root-infecting fungi and root-knot nematode are urgently needed.

Allelopathy is a plant-plant or plant-microorganism biochemical interaction (Rice, 1981). Many weeds interfere with the crop plants through production of chemical substances (allelochemicals) that inhibit their growth and development. The allelochemicals produced by plants are varied including phenolic acids, terpenes, terpenoids, glycosides, alkaloids and flavonoids (Whittaker and Feeny, 1971; Mandava, 1985; Blum, 1996). Besides, a number of secondary metabolites of plants are toxic to nematodes and fungi. ***Tegetes*** spp., produce α-terthienyl whereas ***Crotalaria*** spp. produce mono-crotaline, both of which have nematicidal qualities (Fassuliotis and Skucas, 1969; Gommers and Bakker, 1988). Similarly, some toxic compounds synthesized by ***Lantana camara*** cause substantial mortality of ***Meloidogyne javanica*** juveniles ***in vitro*** (Ali ***et al.***, 2001). In another study, Shaukat and Siddiqui (2001) showed that ***L. camara*** also possesses antifungal agents, which inhibit radial growth of root-infecting fungi including ***Macrophomina phaseolina***, ***Fusarium solani*** and ***Rhizoctonia solani in vitro***. It was demonstrated that when powdered shoot material was added to the soil, ability to cause root-infection by such fungi was greatly abated. Shaukat ***et al.*** (2001) demonstrated ***in vitro*** nematicidal activity of the powdered shoot extract of ***Argemone mexicana*** that is known to produce a number of phenolic acids including salicylic acid.

Launaea procumbens, a tropical ruderal and agrestal weed, grows abundantly in waste grounds, vacant lots, lawns and abandoned and cultivated fields in Southern Sind. Shaukat ***et al.*** (2003) showed that shoot extract of ***L. procumbens*** caused seed germination and early seedling growth of four test species including mustard, bulrush millet, corn and spinach. In the same study, these authors showed that ***L. procumbens*** produces compounds like salicylic acid, vanillic acid, syringic acid, 2-methyl-resorcinol and gallic acid (Shaukat ***et al.***, 2003) which is likely to be responsible for the inhibition of plant growth. Whereas allelopathic potential of ***L. procumbens*** is known, nematicidal and antifungal activities of this weed have remained largely unexplored.

The aim of the present investigation was to examine I) the nematicidal, allelopathic and antifungal activity, if any, of ***L. procumbens in vitro***, ii) to test the nematicidal and allelopathic potential of the weed in pot cultures under glasshouse conditions and iii) to investigate the fungal community structure of the rhizosphere, in particular, community diversity as influenced by the weed residues.

Materials and Methods

Plant material and preparation of shoot extract

L. procumbens was collected from a waste ground in Malir, Karachi and its shoot material was air dried and powdered in an electric grinder. The powdered shoot material (50 g) was

soaked in 500 ml sterile distilled water and left for 72 h at room temperature. The extract was filtered through two layers of Whatman No.1 filter paper and kept at 6°C prior to use. This was called stock solution and from it 50% stock solution was obtained using sterile distilled water.

Nematode and fungal inoculum

Meloidogyne javanica (Treub) Chitwood was obtained from pure cultures maintained on roots of eggplants (***Solanum melongena*** L.). The entire root system was dipped in water and soil was removed gently without detaching egg sacs. Eggs were extracted by vigorous shaking of infested roots in a 1% sodium hypochlorite solution for 3 min. The resulting suspension was then passed through a range of different mesh sieves. The eggs collected on a fine sieve (38 µm) were washed in tap water to remove all traces of sodium hypochlorite before use. Hatched juveniles of ***M. javanica*** were obtained by placing the eggs in sterile distilled water for 3-5 days at 28°C. The inoculum was used for laboratory and glasshouse tests.

Three root-infecting fungi were tested. ***Macrophomina phaseolina*** was isolated from infected bean (***Phaseolus vulgaris*** L.) roots of plants growing at Ghulamullah, 62 km east of Karachi while ***Rhizoctonia solani*** and ***Fusarium solani*** respectively, were isolated from infected tomato and brinjal roots obtained from Mirpur Sakro, 69 km east of Karachi. The fungi were routinely cultivated on potato dextrose agar (PDA) plates supplemented with appropriate quantities of penicillin and streptomycin sulphate.

Effect of shoot extract on egg hatch of *M. javanica*

To study the effects of the weed extract on egg hatching of ***M. javanica***, two medium sized egg masses with 2 ml of the aqueous shoot extract of the weed (***L. procumbens***) were transferred into a 1 cm diameter cavity glass slide. The egg masses placed in sterile distilled water served as controls. Each treatment was replicated three times and the cavity glass slides were randomized on laboratory bench. The numbers of hatched juveniles were counted after 48 h. The egg masses were then transferred into cavity glass slides containing 2 ml sterile distilled water to ascertain whether the egg masses kept in the culture filtrate had been temporarily or permanently inactivated. The juveniles were counted again after a further 48 h period. The experiment was performed three times.

Effect of shoot extract on mortality of *M. javanica* juveniles

To study the effects of aqueous extract of the weed on mortality of ***M. javanica***, two ml of each filtrate were poured in a glass cavity slide and about 30-40 second stage juveniles of ***M. javanica*** placed in each glass slide. Juveniles kept in freshly prepared liquid medium served as controls. Treatments were replicated three times and dead nematodes in each cavity slide were counted after 24 and 48 h. The nematodes were considered to be dead when they did not move on probing with a fine needle. The experiment was conducted twice.

Preparation of ethanolic extract and its antifungal activity

Fresh shoots (50 g) of ***L. procumberns*** were soaked in ethanol (100 ml) and disintegrated in a homogenizer. After storing for 2 weeks, ethanolic extract was filtered through 2 layers of

Whatman No.1 filter paper. The ethanolic extract was dried in a rotary vacuum evaporator under reduced pressure at room temperature (30°C). The gummy substance so obtained was weighed and dissolved in ethanol. To determine the antifungal activity, the ethanolic extract (10 mg ml^{-1}) of ***L. procumbens*** was impregnated on a 5 mm diam disc of Whatman No. 1 filter paper at 10 µl $disc^{-1}$ and placed 5 mm inside of the 9-cm-diam. Petri plates containing Czapek's Dox Agar (CDA) medium, pH 7.2. Disc dipped in ethanol served as control and was placed apart from the disc containing ethanolic extract of ***L. procumbens***. A 5-mm-diam. disc of the test fungus was placed at the center of the Petri plate. There were four replicates for each test fungus and plates were incubated at room temperature (30°C). Distance covered by the fungus and zone of inhibition (if any) was measured after 7 days for ***M. phaseolina*** and ***R. solani*** and 10 days for ***Fusarium solani*** as this fungus is slow growing.

Nematicidal, antifungal and allelopathic responses of *Launea procumbens* in mungbean

The soil (sandy loam; pH 7.8; moisture holding capacity 38%) was obtained from Crop Disease Research Institute, Karachi University campus. The soil was naturally infested with 6 species of plant-parasitic nematodes and some free-living nematodes. The air-dried soil was passed through a 2 mm mesh sieve to discard non-soil particles. The experiment consisted of 2 treatments: non-amended soil planted with mungbean and ***L. procumbens***-amended soil planted with mungbean The experiment was set up as a randomized complete block design with 4 replications. The soil was thoroughly mixed with ***L. procumbens*** at 2.5 or 5.0% w/w and filled in 8-cm-diam. plastic pots at 400 g pot^{-1}. The pots with soil were placed in a glasshouse (18-26°C) and kept moist for 2 weeks. Subsequently, eight surface sterilized mungbean seeds were sown in each pot and following germination only four seedlings were retained pot^{-1}. One week after emergence, 2000 freshly hatched juveniles of ***Meloidogyne javanica*** were added by making 4 holes in the soil around the seedlings in the pots with mungbean. The soil in each pot was sprinkled with 200 ml of sterile distilled water each day. The experiment was terminated 45 days after nematode inoculation and plant height, fresh weight of shoot and root, number of galls induced by ***M. javanica*** and nematode populations in the soil were estimated (see below).

Isolation and identification of fungi from rhizosphere

At harvest, one mungbean plant was randomly chosen from each replicate pot to study the rhizosphere microfungi. The roots were excised and weighed after the excess soil had been shaken-off. The roots were then shaken vigorously in a test tube containing sterile distilled water, blotted dry and reweighed. A serial dilution of the soil suspensions was prepared and was tested for the enumeration of fungi. A 0.5 ml aliquot from 10^2 and 10^3 dilutions was plated onto CDA medium, supplemented with penicillin (100,000 units l^{-1}) and streptomycin sulphate (0.2 g l^{-1}) to avoid bacterial contamination. After incubation at 28°C the plates were examined for total fungal counts. Most isolates were obtained after a few days of incubation, but plates were checked over several weeks to allow isolation of slow-growing fungi. Developing fungal colonies were sub-cultured into pure isolates and identified by their microscopic morphology using standard mycological literature (Booth 1971; Domsch ***et al.***, 1980; Thom and Rapper, 1945).

Isolation of fungi from roots

The roots of all plants (including the one which was tested for the isolation of rhizosphere fungi) were cut into small segments (5 mm) and after surface sterilization in 1% $Ca(OCl)_2$ for 3 min, 5 such segments were plated onto potato dextrose agar (PDA) plates supplemented with penicillin (100,000 units l^{-1}) and streptomycin sulphate (0.2 g l^{-1}). The plates were incubated at 28°C for one week and emerging fungi from each root segment were identified. Colonization percentage was determined by using the following formula:

Colonization (%) = (no. of root pieces colonized by a fungus/total no. of root pieces) × 100

Root-knot development and nematode soil populations

The numbers of galls induced by ***M. javanica*** on the entire root system were counted with the aid of a hand lens. For nematode counts, 100 cm^3 soil aliquots were incubated for 72 h using a modified Baerman funnel technique (Rodríguez-Kábana and Pope, 1981).

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) followed by the least significant differences test (LSD) or Duncan's multiple range test using STATISTICA software (1995, ver. 5.0; StatSoft Inc., Tulsa, Oklahoma, U.S.A.). Fungal rhizosphere populations were transformed to $\log_{10}(x+1)$ before the analysis.

Diversity measurement

Species diversity is an important parameter of natural or organized community and several diversity indices have been proposed (Magurran, 1988). Diversity indices represent a useful means to quantify community diversity and have been instrumental in revealing the impact of biocontrol inoculants on resident population assemblages (Natsch ***et al.***, 1997). Several diversity indices were employed to compare treatment effects. Various diversity measures estimate different aspect of community structure. The general species diversity of the fungal communities was measured by the generally accepted Shannon-Wiener information theory function:

$$H' = -\sum_{i=1}^{s} p_i \log p_i$$

where H' is the general species diversity and p_i the proportion of total number of cfu for fungi or counts for nematodes, N belonging to the its species (Shannon and Weaver, 1963). The variance of general diversity var (H') was calculated in accordance with Magurran (1988), as follows: $Var(H') = \sum pi (\log p_i)^2 - (\sum p_i \log p_i)^2/N + (S-1)/2N^2$.

Dominance concentration (complement of diversity) was measured by using Simpson's index (Southwood and Henderson, 2000) as: $D = \sum \{[n_i(n_i-1)]/[N(N-1)]\}$ in which n_i = number of cfu for fungi or counts for nematodes. The general diversity incorporates two components of diversity: species richness, which expresses the number of species (S) as a function (ratio) of the total number of individuals (N) and equitability that measures the evenness of allotment of individuals among the species (Magurran, 1988). The equitability component of diversity and its variance

were measured in accordance with Pielou (1975): $J' = H'/H'_{max}$. The equitability index J' is the ratio between observed diversity (H') and maximal diversity (H'_{max}). Variance of equitability was estimated as: $Var (J') = Var (H')/(\log S)^2$. Species richness was calculated in accordance with Menhinick (1964) as $d = S/\sqrt{N}$, where S equals the number of species and N the total number of individuals (colony counts).

Results

Effect of shoot extract on egg hatch of *M. javanica*

Shoot extract of ***Launaea procumbens*** significantly inhibited ***in vitro*** egg hatching of ***Meloidogyne javanica*** ($P<0.01$) compared to the controls (Table 1). The inhibitory effect was not circumvented even after transfer of eggs from shoot extract to distilled water.

Effect of shoot extract on mortality of *M. javanica* juveniles

At both the time periods (24 and 48 h) shoot extract of *L.* ***procumbens*** caused significantly ($P<0.001$) greater mortality of ***Meloidogyne javanica*** compared to water controls (Table 2). The nematicidal effect was substantially more pronounced at 48 h compared to that at 24 h.

Effect of *L. procumbens* extract against root-infecting fungi

When the effect of shoot extract of *L.* ***procumbens*** was tested against three different root-infecting fungi ***Rhizoctonia solani***, ***Fusarium solani*** and ***Macrophomina phaseolina*** it was observed that the fungal growth in Petri plates extended and even overgrew the disc impregnated with *L.* ***procumbens*** extract (data not presented).

Effect of *L. procumbens* on the development of root-knot infection and the growth of mungbean

Soil amendment with *L.* ***procumbens*** at both the dosages markedly ($P<0.05$) reduced galling intensity due to ***M. javanica*** compared to the controls (Table 3). No significant difference between two dosages of ***L. procumbens*** was observed with respect to galling. Population densities of ***M. javanica*** were significantly ($P<0.05$) lower in soil amended with 5.0% ***L. procumbens*** while a 2.5% amendment failed to reduce nematode populations in soil. When compared to the controls, soil application of ***L. procumbens*** resulted in a significant ($P<0.05$) increase in plant height at 2.5% but not at 5.0% ***L. procumbens***. Shoot weight was significantly ($P<0.05$) enhanced at 2.5% while a significant ($P<0.05$) reduction occurred in shoot and root weights at 5% ***L. procumbens***.

Effects of *L. procumbens* on fungi in the rhizosphere

A total of 16 microfungal species comprising of 10 genera were isolated from the rhizosphere of mungbean amended with or without *L.* ***procumbens*** (Table 4). The numbers of fungal species were significantly higher in soil amended with ***L. procumbens*** compared with unamended soil. Similarly, colony counts of the fungi were markedly higher in ***L. procumbens*** amended soils. When two dosages of ***L. procumbens*** were compared, cfu of the fungi including

Table 1: Effects of *Launea procumbens* on egg hatching of *Meloidogyne javanica*

Weed species	Number of eggs hatched Extract	 Distilled water[a]	Total no. of eggs hatched	Inhibition % over control
Control	123	156	257	-
L. procumbens extract	78	113	191	25.68
$LSD_{0.05}$	33	37	-	-

[a]After a 48 h hatching period in culture filtrate, the egg masses were transferred to sterile distilled water

Table 2: Effects of aqueous extract of *Launaea procumbens* shoot extract on mortality of *Meloidogyne javanica*

Treatment	Mortality % Exposure time (h) 24	 48
Control	3	7
L. procumbens extract	29	46
$LSD_{0.05}$	9	12

Table 3: Effects of *Launaea procumbens* on root-knot development due to *Meloidogyne jacanica* and growth of mungbean plants

Treatment	Galls g^{-1} root	*M. javanica* 250 g^{-1} soil	Plant height (cm)	Shoot weight (g)	Root weight (g)
Control	89a	1680a	19.0a	2.7a	1.8a
L. procumbens (2.5%)	68b	1585a	23.4b	3.6b	2.0a
L. procumbens (5%)	55b	1395b	20.2a	1.7c	1.3b
$LSD_{0.05}$	16	133	2.0	0.8	0.4

Means with the same letter are not significantly different at $P<0.05$

Table 4: Effect of soil amendment with or without shoot powder of *Launaea procumbens* on soil fungal community structure expressed as $\log_{10}$ (x+1) in mungbean

Fungus	[(log cfu g^{-1} dry soil)+1] Control	 *L. procumbens* (2.5%)	 *L. procumbens* (5%)
Acremonium butyri	0.75	0.75	1.50
A. flavus	0	0	0.75
A. niger	0.82	1.50	3.36
A. quadrilinatus	0.90	0.75	1.61
Aspergillus sp.	0.75	0.90	0.82
Cephalosporium sp.	0	0.75	0
Cladosporium herbarum	0.82	1.57	1.57
Fusariumj oxysporum	0.75	0.82	0.75
F. semitectum	0	1.61	0.75
F. solani	0.86	0.90	1.50
Macrophomina phaseolina	1.50	1.57	2.25
Mycelia sterilia (White)	0.75	0.82	0.75
Mycelia sterilia (Red)	0	2.32	1.57
Penicillium brefeldianum	2.25	1.61	2.32
Rhizoctonia solani	1.73	1.50	0.90
Rhizopus stolonifer	2.32	2.57	1.57
Total species	12	15	15
Total genera	8	10	9

Table 5: General diversity H', equitability (J'), species richness (d) and dominance (D) of the fungal communities affected by soil amendment with or without *Launaea procumbens* in mungbean rhizosphere. Var (H')= variance of H'; Var (J') = variance of J'

Diversity	[(log cfu g^{-1} fresh root wt.)+1]		
Indices	Control	*L. procumbens* (2.5%)	*L. procumbens* (5%)
H'	1.565	1.783	0.951
Var (H')	0.002	0.002	0.0007
J'	0.629	0.658	0.351
Var (J')	0.0004	0.0002	0.0001
d	0.525	0.516	0.277
D	0.289	0.265	0.628

Table 6: Percent colonization of the fungi isolated from the mungbean roots growing in soils amended with *L. procumbens*

	Colonization (%)		
Fungus	Control	*L. procumbens* (2.5%)	*L. procumbens* (5%)
Aspergillus sp.	13	32	27
Fusarium solani	43	57	48
Mycelia sterilia	0	13	5
Macrophomina phaseolina	13	7	7
Rhizoctonia solani	48	23	27
Total species	4	5	5
$LSD_{0.05}$	11	14	19

Acremonium butyric*, *Aspergillus flavus*, *A. niger A. quadrilinatus*, *Macrophomina phaseolina and ***Penicillium brefeldianum*** were markedly higher in soil amended with 2.5% ***L. procumbens***. Colony numbers for other fungi were higher in soil amended with 5.0% ***L. procumbens***. ***Fusarium semitectum*** and a sterile fungus (red pigmented) were exclusively isolated from ***L. procumbens*** amended soils. None of the fungi were specifically isolated from unamended soils and all fungi isolated from ***L. prcumbens*** amended soils were also recorded from unamended soils

Effects of *L. procumbens* on the diversity of rhizosphere fungi

Both general diversity and equitability of fungal community at 2.5% ***L. procumbens*** increased appreciably over the controls but at 5% dosage decreased substantially compared to controls (Table 5). On the other hand, species richness declined at both the dosages. Dominance concentration as measured by Simpson's index followed an opposite trend to that of general diversity.

Effects of *L. procumbens* on root colonization by fungi

The fungi isolated from the roots of mungbean included ***Aspergillus*** sp., ***Fusarium solani***, Mycelia sterilia, ***Macrophomina phaseolina*** and ***Rhizoctonia solani*** (Table 6). ***Aspergillus*** sp. and ***Fusarium solani*** exhibited greater colonization percentage of roots in amended soils (2.5 and 5% ***L. procumbens***) compared to the controls, whilst Mycelia sterilia occurred only in the amended soils. On the other hand, ***Macrophomina phaseolina*** and ***Rhizoctonia solani*** colonized mungbean roots to a lesser extent in the amended soils compared to non-amended controls.

Discussion

Soil amendment with ***Launaea procumbens*** caused significant reduction of ***M. javanica*** population densities in soil, nematode penetration and subsequent root-knot infection in mungbean. Soil amendments with ***L. procumbens*** also resulted in marked changes in fungal communities both in the rhizosphere and inner root tissues. Understanding how ***L. procumbens*** amendment controls root-knot infection and what soil factors regulate activity is critical for reducing the application rates needed and improving the efficacy of the organic amendment. Several mechanisms of disease suppression can be involved including compounds toxic to nematodes (Ali ***et al.***, 2001; Shaukat and Siddiqui, 2001a) changes in microbial communities suppressive to nematode (Hallmann ***et al.***, 1999; Shaukat and Siddiqui, 2001b) and stimulation of the activity of biological control organisms (Siddiqui ***et al.***, 1999). Observed reduction in nematode population densities in the soil and root suggests that toxic compound in the organic amendment generated following incorporation, could be involved. Disease reduction due to toxic compounds is the easiest mechanism to assess, providing one can determine the viability of a pathogen in the soil.

Since most potential fungicides are highly toxic to human beings and produce environmental hazards, application of botanical toxicants in the soil provides an effective alternative means to control fungal pathogens. However, the majority of soil fungi are nonpathogenic and a large number of these may even be beneficial to plants and/or contribute positively to ecosystem functioning. Indeed, nonpathogenic saprotrophic microfungi perform key ecological role in the soil ecosystem through decomposition of organic matter, nutrient cycling, natural control of plant pathogens and a myriad of other functions (Cooke and Rayner, 1984; Curl and Truelove, 1986; Dix and Webster, 1995). In this context, it is surprising that saprotrophic rhizosphere fungi have been largely neglected asnon-target, beneficial resident microorganisms potentially affected by specific organic amendment, especially when the latter produce antifungal and/or nematicidal metabolites with a relatively broad range of action.

It is interesting to note that species of ***Aspergillus*** and ***Rhizopus stolonifer*** were isolated relatively in large numbers from the amended soils. Enhanced populations of these fungi in the rhizosphere following soil amendment with ***L. procumbens*** could be of significant advantage. ***Penicillium*** and ***Fusarium*** are well documented as decomposers of celluloses and hemicelluloses(Domsch ***et al.***, 1980). The ability of certain strains of saprotrophic ***Fusarium solani*** strains to protect plants against pathogenic fungi through competition, parasitism, antagonism and/or induced resistance is well known (Alabouvette and Steinberg , 1995; Chet ***et al.***, 1997; Fuchs ***et al.***, 1997; Amer-Zareen ***et al.***, 2001). Species of ***Aspergillus*** (Siddiqui ***et al.***, 2001) and non-pathogenic ***Fusarium*** (Amer-Zareen ***et al.***, 2001) are also known to suppress root-knot nematode populations and their infectivity.

Species of ***Fusarium*** and ***Rhizoctonia*** are common inhabitants of most agricultural fields in Pakistan and are considered as the most devastating pathogens causing severe losses in economically important crops including mungbean. In the present study, populations of ***F. oxysporum*** and ***F. solani*** in the rhizosphere markedly increased with time and that amended soils supported larger populations of both the fungi. In contrast, populations of ***R. solani*** were

relatively higher in non-amended soils. Interestingly, inner root colonization by ***F. solani*** and ***R. solani*** reduced in amended soils compared with those of non-amended soils while ***F. oxysporum*** did not colonize mungbean roots in any of the soils. It is possible that the release of phytoalexins in response to colonization by ***Fusarium*** spp. and ***Rhizoctonia solani*** could have been a contributing mechanism in reducing fungal penetration and colonization. Furthermore, soil harbors a variety of microorganisms including saprophytic bacteria that are known to induce systemic resistance in plants against pathogenic fungi and nematodes.

Isolation of fungi from the rhizosphere of mungbean yielded a broad fungal spectrum dominated by genera and species rather widespread and frequently found in agricultural soils, rhizospheres and roots of crop plants. This fungal spectrum overlaps the one obtained by Hong (1969) and Girlanda ***et al.*** (2001) who found that rhizosphere fungi protect cucumber seedlings against damping-off caused by ***Fusarium oxysporum***f. sp. ***cucumerinum***. No oomycetes (***Pythium*** and ***Phytophthora***) were isolated in this study, despite the fact that they can grow on the laboratory medium used. This is in accordance with the fact that disease pressure is usually low in the experimental field of the Department of Botany, University of Karachi. Furthermore, cool climate favours the growth and survival of these fungi in the soil, which during mungbean cultivation season was not available. It is also possible that oomycetes were present at population levels too low to be detected, or perhaps they were not competitive enough on the culture plates.

While changes in the soil fungal community following ***L. procumbens*** amendment were anticipated, interestingly, the endophytic fungal community of mungbean was also considerably influenced quantitatively. Five fungal species were recorded as endophytes and only one was specifically present in the amendments. However, quantitative differences in controls and amendments were amply prominent. These results suggest that endophytic fungi are predominantly recruited from the rhizosphere where they presumably use wounds and natural opening to enter the roots. Lytic enzymes produced by these fungi might also contribute to more efficient penetration and colonization. Endophytes colonize the same root tissues as sedentary plant-parasitic nematodes therefore, this association of endophytic fungi with nematodes throughout the nematode life cycles makes these fungi excellent candidates for biocontrol strategies. Nevertheless some of these fungi may cause hypersensitive reactions in plants.

In the present study, whereas soil amendment with powdered shoot of ***L. procumbens*** at low dosages (2.5% w/w) enhanced shoot growth of mungbean, a high concentration (5% w/w) invariably reduced shoot and root growth of mungbean plants. The inhibitory effect of ***L. procumbens*** is presumably the result of the presence of phenolic compounds including salicylic acid, vanillic acid, syringic acid, 2-methyl resorcinol, gallic acid and two unknowns in the shoot of ***L. procumbens*** (Shaukat ***et al.***, 2003) due to which it expresses its allelopathic activity. The toxic effects of the phenolic compounds on seed germination and plant growth have been previously reported (Blum, 1996; Inderjit, 1999; Burhan and Shaukat, 2000). However, the presence of allelopathic secondary metabolites other than phenolic compounds in ***L. procumbens*** cannot be ruled out. The development of microbial populations in response to

high dosages of ***L. procumbens*** powdered shoot could constitute another factor that impaired plant growth.

Organic by-products are a source of energy and nutrients and when applied to soil change its biological, physical and chemical properties. Potential benefits from use of these soil amendments include (1) suppression of disease (2) improved soil physical characteristics (3) increased soil microbial diversity/population size (4) delivery of biological control agents or plant growth-promoting rhizobacteria (5) use for so called waste by-products. Potential risks include contamination of the environment and detrimental effects on plant growth and the growth of beneficial microorganisms such as plant growth-promoting rhizobacteria and mycorrhizal fungi. We must ensure that agricultural soils do not become a refuse centre for organic trash. If there is to be a role for amendments as a viable disease control strategy, we need to determine where and how amendments affect diseases, manipulate amendments and their dosages in the soil for maximum efficacy and provide farmers access to skilled technical assistance. Presently in the developing countries like Pakistan, organic amendments are not widely used to control plant diseases because of the lack of available information of the impact of such toxicants on pathogens. We believe that use of organic amendments will become a viable disease control strategy in Pakistan for mungbean and other high value crops. In other areas, the potential for use of amendments is even greater where the cultivation of higher value crops, options for double and triple cropping and the occurrence of soils of extreme alkalinity or acidity exist.

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