http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (10): 1240-1243, 2001

Biological and Chemical Control of Soil-borne Fungi and Effect of These on Growth of Mungbean

S. Shahid Shaukat, [1]Imran Ali Siddiqui, [2]Nasima Imam Ali and [2]M. J. Zaki
National Nematological Research Center, University of Karachi, Karachi-75270, Pakistan
[1]P.A.F. Intermediate College, Korangi-Creek, Karachi-75190, Pakistan
[2]Department of Botany, University of Karachi, Karachi-75270, Pakistan

Abstract: Efficacy of soil amendment with *Lantana camara* and various concentrations of three phenolics (caffeic acid, *p*-hydroxybenzoic acid and *(p*-coumaric acid) were tested against the soil-borne root-infecting fungi (*Fusarium solani* and *Rhizoctonia solani*) in unsterilized sandy-loam soil. The potential impact of *L. camara* amendment on the rhizosphere population of *Pseudomonas aeruginosa* and consequent biocontrol potential was also evaluated. Powdered *L. camara* and its aqueous extract caused substantial suppression of *F. solani* and *R. solani* infection in mungbean roots. At high concentration of *L. camara* (1% w/w), population of *P. aeruginosa* in the rhizosphere declined but not to a degree that could reduce biological control and growth promoting potential of the bacterium. *L. camara* and *P. aeruginosa* used together caused greater suppression of the root-infecting fungi as compared to their individual application. *P. aeruginosa* mixed with *L. camara* also resulted in enhanced plant growth. Soil application of caffeic acid at the rate of 10-μg/g soil caused complete inhibition in germination of mungbean. With an increase in phenol concentration, plant growth was progressively reduced and root infection caused by *F. solani* and *R. solani* was suppressed. Caffeic acid at 5-μg/g soil caused greater suppression of *F. solani* whereas *p*-hydroxybenzoic acid at 10 μg/g resulted in the maximum inhibition of *R. solani*.

Key words: *Lantana camara, Fusarium solani, Rhizoctonia solani,* allelopathy

Introduction

Chemical control agents are either environmentally unsafe or too expensive. Plant resistance is often non-existent and crop rotation mostly uneconomical. Addition of organic materials to soil has been demonstrated as a satisfactory control method against plant pathogens including fungi and plant-parasitic nematodes. There are many reports that organic amendments of the soil enhance the activity of biocontrol agents in the suppression of plant pathogens (Cook, 1977; Sitaramaiah, 1990).

Lantana camara, a native of Tropical America is widely naturalized in many tropical and sub-tropical regions. Various parts of the plant are used in folklore and indigenous systems of medicine for the treatment of cuts, ulcers, swellings, eczema, malaria and tumors (Kirtikar and Basu, 1981). Although literature is available on the allelopathic potential of *L. camara* (Casado, 1995; Rajbansi and Inubushi, 1997), little attention has been paid to the fact that it may also be toxic to soilborne root-infecting fungi. Recently, Begum *et al.* (2000) isolated four different compounds (lantanoside, linoroside, camarinic acid and lantanone) from the aerial parts of *L. camara*, of which the first three showed nematicidal activity against *Meloidogyne incognita*, root-knot nematode. Similarly, in our previous studies, soil amendment with *L. camara* markedly suppressed *M. javanica* in mungbean (Ali *et al.*, 2001). *L. camara* is also known to contain phenolic allelochemicals (Narwal, 1994).

Allelopathy appears to be a promising and natural component of sustainable agriculture since the benefits of crop rotation and cover cropping are already well established with regard to maintaining and improving soil quality (Halbrendt, 1996). Potential allelopathic compounds identified in living and decomposing tissue of small grain-cover crops include phenolic acids (Liebl and Worsham, 1983; Barnes *et al.*, 1986; Blum *et al.*, 1991), hydroxamic acids (Nair *et al.*, 1990; Gagliardo and Chilton, 1992), other organic acids (Chou and Patrick, 1976, Lynch, 1977) and volatile substances (Buttery *et al.*, 1985; Bradow, 1991). Among these, phenolic acids have been the most frequently identified as phytotoxins.

The objectives of this study were : 1) to evaluate the potential of *L. camara* alone or in combination with *Pseudomonas aeruginosa* in the control of root-infecting fungi—— *Rhizoctonia solani* and *Fusarium solani* in mungbean, and 2) to find the possible impact of various concentrations of three phenolic acids including *p*-hydoxybenzoic acid, *p*-coumaric acid and caffeic acid on same two root-infecting fungi and the growth of mungbean.

Materials and Methods

Leaves of *L. camara* (L.) collected from shrubs grown at Karachi University Campus, were air-dried and powdered. The soil used for the experiment was obtained from the experimental field of the Department of Botany, University of Karachi. It (sandy-loam, pH-8.1, moisture holding capacity 40%) was passed through 2-mm sieve to discard non-soil particles. The soil was naturally infested with 3000-cfu g^{-1} soil of mixed population of *Fusarium* spp., *(F. oxysporum* and *F. solani*) as estimated by soil dilution technique (Nash and Snyder, 1962) and 5-7% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955). In addition, the soil was also infested with 100-150 larvae/250 g soil of a mixed population of *Helicotylenchus brassicae, Rotylenchulus reniformis* and *Tylenchorhynchus brassicae*. The soil was mixed with *L. camara* to make 0.5, 1, 3 or 5% concentrations and put into 8-cm-diam. plastic pots (350 g/pot). The soil was watered daily and after 21 days, a 25-ml aqueous cell suspension of *Pseudomonas aeruginosa* (Schroeter) Migula strain-78 containing 3.1 x 10^8 cfu/ml was drenched in each pot. Pots without *L. camara* and *P. aeruginosa* served as controls. Eight mungbean *Vigna radiata* (L.) Wilczek seeds were sown in each pot and after germination four seedlings were retained in each pot. Each treatment had four replicates and pots were kept in a randomized complete block design. Concentrations of 3% and 5% were markedly injurious to plants, and were excluded from the experiments. The experiments were terminated after 52 days of growth and

plant height and fresh weight of shoots were recorded. To determine the incidence of root-infecting fungi, 5-mm-long root pieces from each plant were surface sterilized with 1% $Ca(OCl)_2$ and plated onto PDA plates containing penicillin (100, 000 units/L.) and streptomycin sulphate (0.2 g/L.). After incubation for one week at 28°C, the incidence of root-infecting fungi was recorded as follows:

Infection % = (No. of plants infected by the fungus / Total no. of plants) x 100

To establish whether decomposition was necessary for the release of toxic principles or the aqueous extract was responsible for the suppression of the two fungi, in another experiment soil was drenched with 12.5 or 25 ml extract (200g /500 ml water = stock solution). The bacterium was added simultaneously. The rest of the procedure was the same as outlined above.

Populations of *P. aeruginosa* in rhizosphere were estimated following the modified method of Pillay and Nowak (1997). One g root samples with adhering soil were placed in 250 ml flasks containing 10 ml of 0.1M $MgSO_4$ solution (pH 6.5) plus 0.02% Tween-20 and shaken vigorously for 15 min. Ten-fold serial dilutions of the suspension were prepared and 50 μL aliquots from the appropriate dilutions were plated onto KB medium. The plates were incubated at room temperature (28 °C) for 48h and the number of cfu was recorded. The plants grown in soil not treated with *P. aeruginosa* were also checked for the presence of contaminants. No growth of *P. aeruginosa* was detected in non-bacterized soil.

In a previous report, leaf extract of *L. camara* contained 14 phenolic compounds viz., protocatechuic acid (1.5mM), gentisic acid (2.9mM), *p*-hydroxybenzoic acid (5.6mM), vanillic acid (0.9mM), caffiec acid (1.4mM), syringic acid (11.3mM), *p*-coumaric acid (26.4mM), m-coumaric acid (3.3mM), ferulic acid (7.0mM), salicylic acid (2.0mM), vanillin (2.1mM), and methyl coumarin (2.8mM). The phytotoxicity of its leaf extracts was due to complex interactions between these phenolic compounds (Narwal, 1994). In another experiment, potential impact of *p*-coumaric acid, *p*-hydroxybenzoic acid and caffeic acid was tested in the suppression of root-infecting fungi and its role on growth of mungbean. Eight mungbean seeds were sown in 8 cm-diam., plastic pots containing 350 g sandy-loam soil. Before planting, the soil was drenched with 20 ml of the phenolic compounds (*p*-coumaric acid, *p*-hydroxybenzoic acid or caffeic acid) to give concentrations of 2.5, 5 and 10 μg/g soil. Soil drenched with 20 ml sterile distilled water served as the control. Treatments were replicated four times and arranged in a randomized complete block design. After one week, the percentage of seed germination was recorded. Since no growth of mungbean seedlings was detected in caffeic acid at 10 μg/g, therefore, this concentration was excluded from the experiment. The experiment was terminated 45 days after seedling emergence and growth-parameters (plant height and fresh weight of shoot) and incidence of root-infecting fungi were recorded as described earlier.

The data were subjected to analysis of variance (ANOVA) followed by least significant difference (LSD) in accordance with Sokal and Rohlf (1995). The data were transformed to $\log_{10} x+1$, where necessary.

Results

Effect of decomposed leaves of *L. camara* on root-infecting fungi: Soil amendement with *L. acamara* alone or mixed with *P. aeruginosa* significantly ($p<0.001$) suppressed two soil-borne fungi including *F. solani* and *R. solani* (Table 1). With an increase in *L. camara* concentration, root-rot infection was gradually suppressed. *P. aeruginosa* used without the organic amendment was effective ($p<0.001$) in the suppression of *R. solani* but not that of *F. solani*. *P. aeruginosa* used with 1% *L. camara* inhibited *F. solani* upto 56% while when mixed with 0.5% *L. camara* it suppressed *R. solani* infection by more than 83%. *P. aeruginosa* used with *L. camara* at both the dosages (i.e., 0.5% and 1% w/w) enhanced plant height and fresh weight of shoots of mungbean. *P. aeruginosa* used with *L. camara* at 1% w/w gave maximum (>32% increase over the controls) plant height and fresh weight of shoot (>63% increase compared with the controls). Although there was no significant impact of the organic amendment on the population of *P. aeruginosa*, the bacterial population slightly increased in the presence of *L. camara* applied at 0.5% w/w while, at 1% *L. camara*, the bacterial population slightly decreased.

Effect of aqueous extract of *L. camara* on root-infecting fungi: Aqueous extract of *L. camara* at 25 ml (>38%) used alone or

Table 1: Effects of powdered leaves of *Lantana camara* and *Pseudomonas aeruginosa* on *Fusarium solani* and *Rhizoctonia solani*, growth of mungbean and population of *P. aeruginosa* ($\log_{10}$ cfu x+1) in the rhizosphere.

Treatments	Infection %		Plant Height (cm)	Shoot weight (g)	Bacterial rhizosphere population
	Fusarium solani	*Rhizoctonia solani*			
Control	100	78	14.9	1.9	-
L. camara 0.5%	75	75	18.3	2.1	-
L. camara 1.0%	75	56	17.5	2.4	-
P. aeruginosa	81	44	18.9	2.5	5.02
L. camara 0.5 % + *P. aeruginosa*	56	13	19.8	2.5	5.12
L. camara 1.0 % + *P. aeruginosa*	44	25	19.7	3.1	4.96
$LSD_{0.05}$	26.2	19.7	2.1	0.5	0.12

Table 2: Effect of aqueous extract of *Lantana camara* (Ss = stock solution) and *P. aeruginosa* on *Fusarium solani* and *Rhizoctonia solani*, growth of mungbean and population of *P. aeruginosa* ($\log_{10}$ cfu x+1) in the rhizosphere.

Treatments	Infection %		Plant Height (cm)	Shoot weight (g)	Bacterial rhizosphere population
	Fusarium solani	*Rhizoctonia solani*			
Control	91	63	15.3	1.6	-
Lantana camara 12.5 ml Ss	81	75	17.8	1.8	-
Lantana camara 25 ml Ss	56	63	19.3	2.1	-
Pseudomonas aeruginosa	75	38	18.3	1.8	4.93
L. camara 12.5 ml Ss + *P. aeruginosa*	63	56	20.1	2.2	5.01
L. camara 25 ml Ss + *P. aeruginosa*	44	25	20.3	2.1	4.96
$LSD_{0.05}$	18.2	16.8	2.4	0.5	0.18

Table 3: Effect of phenolic compounds on *Fusarium solani* and *Rhizoctonia solani* and growth of mungbean.

Treatments	Infection %		Plant Height (cm)	Shoot weight (g)	Bacterial rhizosphere population
	Fusarium solani	*Rhizoctonia solani*			
Control	91	75	91	19.5	0.49
p-hydroxybenzoic acid 2.5 µg/g	75	81	91	21.9	0.68
p-hydroxybenzoic acid 5 µg/g	75	56	75	18.7	0.59
p-hydoxybenzoic acid 10 µg/g	56	38	75	13.5	0.38
p-coumaric acid 2.5 µg/g	75	78	81	19.8	0.55
p-coumaric acid 5 µg/g	81	56	91	16.2	0.49
p-coumaric acid 10 µg/g	38	56	75	12.8	0.39
Caffeic acid 2.5 µg/g	63	63	75	17.5	0.52
Caffeic acid 5 µg/g	25	44	53	14.3	0.41
$LSD_{0.05}$	16.3	19.8	17	2.8	0.14

its 12.5 ml (>30%) or 25 ml (>51%) in combination with *P. aeruginosa* significantly ($p<0.05$) suppressed *F. solani* infection over the controls (Table 2). Similarly, *P. aeruginosa* used alone (>39%) or applied in combination with 25 ml extract of *L. camara* (>60%) caused significant ($p<0.05$) inhibition of *R. solani* infection over the controls. *L. camara* at 25 ml concentration mixed with *P. aeruginosa* gave maximum plant height whereas *L. camara* at 12.5 ml concentration in conjunction with *P. aeruginosa* produced the highest fresh weight of shoot. Addition of the aqueous extract at both the dosages increased population of *P. aeruginosa* in rhizosphere.

Effect of phenolic acids on root-infecting fungi: The three phenolics significantly ($p<0.05$) suppressed *F. solani* and *R. solani* infection in mungbean (Table 3). In general, caffeic acid was more effective in the suppression of root-infecting fungi compared with *p*-hydroxybenzoic acid or *p*-coumaric acid. Caffeic acid at 5 µg/g soil resulting in the greatest suppression (>72%) of *F. solani* infection whereas *p*-hydroxybenzoic acid at 10 µg/g soil caused maximum suppression (>49%) of *R. solani* infection over the controls. High concentration of the phenolics significantly inhibited seed germination and plant height and fresh weight of shoot. However, *p*-hydroxybenzoic acid 2.5 µg/g resulted in an increased plant height and fresh weight of shoot and root.

Discussion

Present study as well as our previous report (Ali *et al.*, 2001) clearly suggested that *L. camara* has the potential to cause suppressiveness against soilborne plant pathogens including root-infecting fungi and plant-parasitic nematodes. The observed suppression of the two root-infecting fungi could either be direct or indirect. The direct impact could be related with *L. camara*-mediated changes in plant physiology resulting in the inhibition of the phytopathogens. Moreover, accumulation of the toxic substances including phenolic acids and other volatile substances in the crop rhizosphere might have prevented root colonization of the two fungi. Lafontaine and Benhamou (1996) have reviewed chitin-induced physiological changes in plants. They reported that amendment with chitosan, a derivative of crab shell chitin, caused protection of tomato against the pathogen *F. oxysporum* f.sp. *radicis-lycopersici* through induction of physiological and structural changes in the host plant (Benhamou *et al.*, 1994). Indirectly, soil amendment with *L. camara* might have resulted in the changed population structure of the rhizosphere microbial community especially, those with chitinolytic activity, thus inhibiting soilborne pathogens. Further study should be directed towards shift in microbial community in the crop rhizosphere in response to the addition of the added botanical toxicants including *L. camara*. *P. aeruginosa* mixed with *L. camara* caused greater suppression of the two root-infecting fungi and enhanced plant growth as compared to their individual applications. Plant beneficial effects provided by *P. aeruginosa* are widely reported, emphasizing the enormous potential of this species in plant-microbe interactions (Siddiqui *et al.*, 1999; Siddiqui *et al.*, 2000; Siddiqui and Ehteshamul-Haque, 2000). Whereas *P. aeruginosa* showed compatibility with *L. camara*, its population was slightly affected by the organic amendments but not to an extent, where it could reduce biocontrol and growth promoting potentials of the bacterium.

This strongly suggests that the physiology of a plant growing in *L. camara*-amended soil is different from one grown in the absence of *L. camara*. Hence, it may be possible to use organic amendments to manipulate the soil microflora and to induce the desired changes in the endophytic microflora. The results described here come from the single crop. It is not unusual to obtain better control of the soil pathogen in the second crop following *L. camara* amendment than in the first crop and this might be due to more bacteria, actinomycetes and mycoparasitic-fungi in the amended soil.

In present study, phenols exerted an inhibitory effect on soilborne root-infecting fungi thus preventing their colonization in mungbean. The role of these phenolics on fungi could only be speculative. Addition of the phenolic acids might have resulted in increased microbial population specially those having chitinolytic activity, inhibiting root-infecting fungi. Furthermore, greater release of the toxic compounds in response to the application of phenolic acids caused suppressiveness against fungi.

The results described here clearly demonstrate that phenolics have the potential to suppress soil-borne root-infecting fungi though their high concentrations are phytotoxic. In this study efforts were directed towards exploring the impact of single phenolic acids against root-infecting fungi. However, under field conditions a variety of phenolic acids as well as other toxic and non-toxic organic compounds occur that interact, possibly in some additive manner, with the crops and the associated microorganisms. Moreover, this study concentrates on the influence of phenolic acids on two soilborne root-infecting fungi, the examination of the effects on beneficial soil microorganisms was not attempted.

References

Ali, N.I., I.A. Siddiqui, M.J., Zaki and S.S. Shaukat, 2001. Nematicidal potential of *Lantana camara* against *Meloidogyne javanica* in mungbean. Nematol. Medit., 29: 99-102.

Barnes, J.P., A.R. Putnam and A. Burke, 1986. Allelopathic activity of rye (*Secale cereale* L.). In: The science of allelopathy. pp: 271-286. (A.R. Putnum and Tang, C.S. eds.). John Wiley, New York.

Begum, S., A. Wahab, B.S. Siddiqui and F. Qamar, 2000. Nematicidal constituents of the aerial parts of *Lantana camara*. J. Nat. Prod., 63: 765-767.

Benhamou, N., P.J. Lafontaine and M. Nicole, 1994. Induction of systemic resistance to Fusarium crown and root rot in tomato plants by seed treatment with chitosan. Phytopathol., 84: 1432-1444.

Blum, U., T.R. Wentworth, K. Klein, A.D. Worsham, L.D. King, T.M., Grieg and S.W. Lyu, 1991. Phenolic acid content of soils from wheat no till, wheat-conventional till, and fallow-conventional till soybean cropping systems. J. Chem. Ecol., 17: 1045-1068.

Bradow, J.M., 1991. Relationships between chemical structure and inhibitory activity of C6 through C9 volatiles emitted by plant residues. J. Chem. Ecol., 17: 2193-2212.

Buttery, R.G., C.J., Xu and L.C. Ling, 1985. Volatile components of wheat leaves (stems): Possible insect attractants. J. Agric. Food Chem., 33: 115-117.

Casado, C., 1995. Allelopathic effects of *Lantana camara* (Verbenaceae) on morning glory (*Ipomea tricolor*). Rhodora, 97: 264-274.

Chou, C.H. and Z.A. Patrick, 1976. Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. J. Chem. Ecol., 2: 369-387.

Cook, R.J., 1977. Management of the associated microbiota. pp. 145-160. In: Plant Disease. Vol. 1. (J.G. Horsfall and E.B. Cowling eds). Academic Press, New York.

Gagliardo, R.W. and W.S. Chilton, 1992. Soil transformation of 2(3H)-benzoxazolone of rye into phytotoxic 2-amino-3-H-phenoxazin-3-one. J. Chem. Ecol., 18: 1683-1691.

Halbrendt, J.M., 1996. Allelopathy in the management of plant-parasitic nematodes. J. Nematol., 28: 8-14.

Kirtikar, K.R. and B.D. Basu, 1981. *Indian Medicinal Plants*. S.N. Basu (ed.). Panni Office, Bhuwaneswari Asrama, Bahadurganj, Allahbad, India, pp: 984.

Lafontaine, P.J. and N. Benhamou, 1996. Chitosan treatment: an emerging strategy for enhancing resistance of greenhouse tomato plants to infection by *Fusarium oxysporum* f.sp. radicis-lycopersici. Biocont. Sci & Technol., 6: 111-124.

Liebl, R.A. and A.D. Worsham, 1983. Inhibition of morning glory (*Ipomoea lacunosa* L.) and certain other weed species by phytotoxic components of wheat (*Triticum aestivum* L.) straw. J. Chem. Ecol., 9: 1027-1043.

Lynch, J.M., 1977. Phytotoxicity of acetic acid produced in anaerobic decomposition of wheat straw. J. Appl. Bacteriol., 42: 81-87.

Nair, G.N., C.J. Whitenack and A.R. Putnam, 1990. 2,2′-oxo-1,1′-azobenzene a microbially transformed allelochemical from 2,3-benzoxazolinone: I. J. Chem. Ecol., 16: 353-364.

Narwal, S.S., 1994. Allelopathy in Crop Production. Scientific Publishers, India, pp: 105.

Nash, S.M. and W.C. Snyder, 1962. Quantitative estimation by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathol., 52: 567-572.

Pillay V.K. and J. Nowak, 1997. Inoculum density, temperature and genotype effect on *In vitro* growth promotion and endophytic colonization of tomato (*Lycopersicon esculentum* Mill.) seedlings inoculated with a *Pseudomonas* bacterium. Can. J. Microbiol., 43: 354-361.

Rajbansi, S.S. and K. Inubushi, 1997. Chemical and biochemical changes during laboratory scale composting of allelopathic plant leaves (*Eupatorium adenophorum* and *Lantana camara*) Weed Sci., 26: 66-71.

Siddiqui, I.A. and S. Ehteshamul-Haque, 2000. Use of *Pseudomonas aeruginosa* for the control of root rot-root knot disease complex in tomato. Nematol. Medit., 28, 189-192.

Siddiqui, I.A., S.A. Qureshi, V. Sultana, S. Ehteshamul-Haque and A. Ghaffar, 2000. Biological control of root rot-root knot disease complex of tomato. Plant & Soil, 227: 163-169.

Siddiqui, I.A., S. Ehtshamul-Haque and A. Ghaffar, 1999. Root dip treatment with *Pseudomonas aeruginosa* and *Trichoderma* spp., in the control of root rot-root knot disease complex in chili (*Capsicum annum* L.). Pak. J. Nematol., 17: 67-75.

Sitaramaiah, K., 1990. Mechanism of reduction of plant parasitic nematodes in soil amended with organic materials. pp. 263-295. In: Progress in Plant Nematology. (S.K. Saxena, M.W. Khan, A. Rashid and R.M. Khan eds). CBS Publishers and Distributors, New Delhi.

Sokal, R.R. and E.J. Rohlf, 1995. Biometry 3rd ed. Freeman, San Francisco.

Wilhelm, S., 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. Phytopathol., 45: 180-181.