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ENHANCEMENT OF BIOCONTROL POTENTIAL OF *PSEUDOMONAS AERUGINOSA* AND *PAECILOMYCES LILACINUS* AGAINST ROOT ROT OF MUNGBEAN BY A MEDICINAL PLANT *LAUNAEA NUDICAULIS* L.

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Abstract

Pseudomonas aeruginosa, a plant growth promoting rhizobacterium and *Paecilomyces lilacinus*, an egg parasite of root knot and cyst nematodes inhibited the growth of *Macrophomina phaseolina, Fusarium solani* and *F. oxysporum* in dual culture plate assay. Application of *P. aeruginosa* with a medicinal plant *Launaea nudicaulis* @ 0.5% as soil amendment resulted in maximum reduction in *M. phaseolina* infection on mungbean roots in screenhouse experiments. *Launaea nudicaulis* @ 0.5 and 1% w/w also significantly suppressed infection by *M. phaseolina.* Use of *P. aeruginosa* alone or with *L. nudicaulis* @ 0.1% resulted in complete control of *Rhizoctonia solani* infection. Significant control of *F. solani* was also achieved by the application of *P. aeruginosa* and *P. lilacinus* alone and by the combined use of *P. aeruginosa* and *P. lilacinus* with different dosages of *L. nudicaulis.* Greater plant height resulted when *P. lilacinus* was used alone followed by *P. aeruginosa* used with *L. nudicaulis* @ 0.1%. Significant increase in fresh shoot weight resulted when *P. lilacinus* and *P. aeruginosa* were used alone or *P. aeruginosa* used with *L. nudicaulis* @ 0.1%

Introduction

There is an increasing awareness that pesticides and fertilizers cause damage to the environment and effect human health (Perkins & Patterson, 1997). As a consequence, there is a trend toward finding ways to minimize the use of fungicides (Maas & Galletta, 1997). The use of biocontrol agents (Elad & Shtienberg, 1996) and alternative treatments (e.g., cultural practices, cover crops, organic amendments) are perceived to be less harmful than conventional fungicides and may be an alternative in controlling plant diseases (Cutler & Hill, 1994). With the growing interest in the introduction of biocontrol microbes into the rhizosphere, it becomes of particular importance to characterize the effective biocontrol agents under different conditions. The biocontrol agents are frequently found to be ineffective because of microbial competition or adverse environmental conditions (Lazarovits & Nowak, 1997) and combinations of more than one method provide more protection than either component alone (Ehteshamul-Haque *et al*., 1995; Izhar *et al*., 1995).

Management and manipulation of natural communities of antagonistic microorganisms through organic amendments have received less attention, in spite of the fact that these strategies have resulted in highly effective forms of biological control (Hoitink & Boehm, 1999). Soil amendments have the potential to provide disease control through a variety of mechanisms, including chemical, such a producing antimicrobial compounds during decomposition (Brown & Morra, 1997, Tenuta & Lazarovits, 2002) and biological (Chen *et al*., 1988, Mazzola, 2004).

Plants with therapeutic effects have received the attention of scientists as an alternate method of disease control which would also protect our environment from the use of hazardous chemicals. *Launaea nudicaulis* L., a common medicinal plant of Pakistan

(Baquar, 1989) has shown anti-fungal and antibacterial activity (Rashid *et al*., 2000). It is a common weed of field crops with allelopathic affect, but its effect on root infecting organism has yet not been investigated. The present report describes the effect of soil amendment with *L. nudicaulis* on the efficacy of biocontrol agent *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* in reducing the infection by root-infecting fungi on mungbean.

Materials and Methods

Biocontrol agents and antifungal activity: *Paecilomyces lilacinus* was isolated from rhizosphere soil of *Luffa aegyptica* collected from an agricultural field of Hub, Baluchistan, using soil dilution technique. *Pseudomonas aeruginosa* was isolated from rhizosphere soil of mungbean collected from a farmer's field at Malir, Karachi on S1 medium (Gould *et al*., 1985).

Pseudomonas aeruginosa was streaked on one side of the Petri dish containing Czapek's Dox Agar, pH. 7.2. On the other side of the Petri dish a 5mm diam., disc of root-infecting fungi viz., *M. phaseolina, R. solani, F. oxysporum, F. solani* was inoculated (Drepeau *et al.*, 1973). The dishes were incubated at 28ºC and radial growth of test organisms and zone of inhibitions measured daily upto 10 days. A 5 mm disc of actively growing culture of *P. lilacinus* was inoculated and remaining test was identical as for *P. aeruginosa*.

Screenhouse experiment: Dry powder of *L. nudicaulis* was mixed in sandy loam soil, pH 8.0, @ 0.1, 0.5 and 1.0% w/w. The soil was naturally infested with 3-7 sclerotia of *Macrophomina phaseolina* g^{-1} of soil as determined by wet sieving and dilution plating (Sheikh & Ghaffar 1975), 2-6% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu g^{-1} of soil of a mixed population of *Fusarium oxysporum* and *F. solani* as determined by soil dilution (Nash & Snyder, 1962). Amended soil was transferred to 8 cm diam plastic pots each containing 300 g soil. Pots were kept at 50% W.H.C by watering daily (Keen & Raczkowiski, 1921). After three weeks of tissue decomposition, aqueous suspensions of *P. aeruginosa* (3.8×10^8 cfu/mL) grown on KB agar and *P. lilacinus* (1.1×10^8 cfu/mL) grown on potato dextrose agar were drenched onto amended and non-amended soil in pots at 25 ml per pot. Aqueous suspension (100 ppm) of a fungicide, benomyl was also drenched at 25 ml per pot. Six seeds of mungbean *(Vigna radiata* (L.) Wilczek) was sown in each pot. After germination 4 seedlings were kept per pot and excess were removed and discarded. Pots without amendment/antagonists or fungicides served as controls. Each treatment was replicated 4 times and pots were placed in a screen house in a randomized complete block design.

Determination of fungal infection and growth parameter: To assess the efficacy of *P. aeruginosa* and *P. lilacinus* in suppression of root disease, plants were uprooted after 6 weeks of growth. To determine the incidence of fungi, roots were washed with running tap water then surface disinfested with 1% $Ca(OCl)_2$ and 1 cm long root pieces from tap roots, (5 from each plant) were plated onto potato dextrose agar plates amended into penicillin (100,000 units/litre) and streptomycin (0.2 g/litre). After incubation for 5days at 28ºC, the incidence of root infecting fungi was recorded. Infection percentage for each pathogen was calculated using the formula:

$$\text{Infection \% of a pathogen} = \frac{\text{Number of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

Table 1. Growth inhibition of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* by *Pseudomonas aeruginosa* and *Paecilomyces lilacinus*

Bacterium/Fungus	Host	*M. phaseolina*	*R. solani*	*F. solani*	*F. oxysporum*
		Zone of inhibition (mm)			
P. aeruginosa	*Vigna radiata*	2	*	6	10
P. lilacinus	*Luffa aegyptica*	7	A	9	2

A = Colonies inhibited by each other
* = No inhibition

Table 2. Effects of *Launaea nudicaulis, Pseudomonas aeruginosa* and *Paecilomyces lilacinus* on the development of root rot disease of mungbean caused by *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani.*

Treatments	Infection %		
	M. phaseolina	*R. solani*	*F. solani*
Control	75.0	43.7	50.0
L. nudicaulis at 0.1% w/w	81.2	43.7	18.7
L. nudicaulis at 0.5% w/w	43.7	37.5	12.5
L. nudicaulis at 1.0%	37.5	25.0	0.0
P. aeruginosa	56.2	0.0	12.5
P. lilacinus	93.7	50.0	12.5
Benomyl	56.2	6.2	6.2
L. nudicaulis at 0.1% w/w + *P. aeruginosa*	81.2	0.0	25.0
L. nudicaulis at 0.1% w/w + *P. lilacinus*	68.7	25.0	0.0
L. nudicaulis at 0.1% w/w + Benomyl	56.2	25.0	25.0
L. nudicaulis at 0.5% w/w + *P. aeruginosa*	25.0	18.7	25.0
L. nudicaulis at 0.5% w/w + *P. lilacinus*	37.5	12.5	6.2
L. nudicaulis at 0.5% w/w + Benomyl	81.2	62.5	0.0
L. nudicaulis at 1.0% w/w + *P. aeruginosa*	37.5	43.7	25.0
L. nudicaulis at 1.0% w/w + *P. lilacinus*	56.2	81.2	0.0
L. nudicaulis at 1.0% w/w + Benomyl	62.5	56.2	12.5

$LSD_{0.05}$; Treatments = 25.7[1]; Pathogens = 9.9[2]

[1]Mean values for treatments in columns showing differences greater than the LSD value are significantly different at $p<0.05$

[2]Mean values for pathogens in rows showing differences greater than the LSD value are significantly different at $p<0.05$

Plant growth parameters, such as plant height and fresh weight of shoot, were also recorded. The experiment was conducted twice.

Statistical analysis: Data were analysed and subjected to analysis of variance and means were separated using Fisher's least significant difference (LSD) according to Gomez & Gomez (1984).

Results

***In vitro* growth inhibition of root-infecting fungi:** *Pseudomonas aeruginosa* inhibited the radial growth of *M. phaseolina, F. solani* and *F. oxysporum* by producing a zone of inhibition of 2, 6 and 10 mm respectively. *P. lilacinus* also showed a inhibitory effect on these root infecting fungi and produced 7, 9 and 2 mm zone of inhibition respectively against *M. phaseolina, F. solani* and *F. oxysporum* (Table 1).

Table 3. Effects of *Launaea nudicaulis, Pseudomonas aeruginosa* and *Paecilomyces lilacinus* on the growth of mungbean.

Treatments	Plant height (cm)	Fresh shoot weight (g)
Control	9.3	3.2
L. nudicaulis at 0.1% w/w	9.1	3.5
L. nudicaulis at 0.5% w/w	9.5	3.9
L. nudicaulis at 1.0%	9.8	2.9
P. aeruginosa	12.4*	4.7*
P. lilacinus	10.5	4.9*
Benomyl	11.4	3.8
L. nudicaulis at 0.1% w/w + *P. aeruginosa*	12.1*	4.8*
L. nudicaulis at 0.1% w/w + *P. lilacinus*	9.7	3.5
L. nudicaulis at 0.1% w/w + Benomyl	9.7	4.5
L. nudicaulis at 0.5% w/w + *P. aeruginosa*	11.5	3.7
L. nudicaulis at 0.5% w/w + *P. lilacinus*	10.6	3.5
L. nudicaulis at 0.5% w/w + Benomyl	9.9	3.5
L. nudicaulis at 1.0% w/w + *P. aeruginosa*	10.9	3.5
L. nudicaulis at 1.0% w/w + *P. lilacinus*	8.7	4.7*
L. nudicaulis at 1.0% w/w + Benomyl	11.8	4.0
$LSD_{0.05}$	2.5[1]	1.5[2]

[1] Mean values for treatments in columns showing differences greater than the LSD value are significantly different at $p<0.05$
* = Significant at $p<0.05$

Screenhouse experiment: Application of *L. nudicaulis* @ 0.5 and 1% w/w, *P. aeruginosa* alone and *L. nudicaulis* @ 0.5% with *P. aeruginosa* and *P. lilacinus* significantly ($p<0.05$) suppressed infection by *Macrophomina phaseolina*. Maximum suppression of *M.phaseolina* was achieved where *L. nudicaulis* @ 0.5% used with *P. aeruginosa* (Table 2). Complete control of *R. solani* was found by the application of *P. aeruginosa* alone or with *L. nudicaulis* @ 0.1%. Use of the fungicide, benomyl and microbial antagonist *P. lilacinus* used with *L. nudicaulis* also significantly ($p<0.05$) inhibited *R. solani* (Table 2).

Significant ($p<0.05$) control of *F. solani* was achieved by the application of *P. aeruginosa*, *P. lilacinus* or benomyl alone and by the combined use of *P. aeruginosa*, *P. lilacinus* or benomyl with different dosages of *L. nudicaulis.* The addition of *L. nudicaulis* @ 1%, *P. lilacinus* used with *L. nudicaulis* @ 0.1 & 1% and benomyl with *L. nudicaulis* @ 0.5% resulted in complete control of *F. solani* infection (Table 2).

Greater plant height resulted when *P. lilacinus* was used alone followed by *P. aeruginosa* used with *L. nudicaulis* @ 0.1% (Table 3). Significant increased in fresh shoot weight resulted when *P. lilacinus* and *P. aeruginosa* were used alone or *P. aeruginosa* used with *L. nudicaulis* @ 0.1% and *P. lilacinus* also used with *L. nudicaulis* @ 1% (Table 3).

Discussion

Control of root-infecting fungi using antagonistic plants and phytochemicals offers an alternate strategy to the prevalent use of synthetic pesticides. A wide variety of plant materials have been found effective against plant parasitic fungi associated with crop plants (Ehteshamul-Haque *et al*., 1995; 1996). In the present study application of *L. nudicaulis* significantly suppressed the infection by *M. phaseolina* and *F. solani* on mungbean. There is report that latex of *L. nudicaulis* is used in constipation, leaves to relieve fever in children, treatments of itches, cuts, ulcer and eczema (Rashid *et al.*,

2000). The plant also showed hypoglycaemic effect on rats (Shabana *et al.*, 1990). Similarly antibacterial and antifungal activity of *L. nudicaulis* has also been reported (Rashid *et al.*, (2000).

In this study application of *L. nudicaulis* with the PGPR *P. aeruginosa* and microbial antagonist *P. lilacinus*, significantly suppressed infection by *M. phaseolina, R. solani* and *F. solani*. PGPR have been reported to improve plant growth either through direct stimulation or by suppression of pathogens (Weller, 1988; Weller *et al*., 2002). Of the various rhizosphere bacteria, fluorescent Pseudomonads are aggressive colonizers of the rhizosphere of various crop plants and have broad spectrum antagonistic activity against plant pathogens (Parveen *et al.*, 1998; Weller *et al.*, 2002; Raajimakers & Weller 1998). Species of *Pseudomonas* are also reported to induce systemic resistance in plants against invading pathogens (Zhou & Paulitz 1994; De Meyer *et al.*, 1999). Similarly *P. lilacinus* an egg parasite of root knot nematode (Jatala, 1986) has also been reported to suppress the infection by root infecting fungi (Ehteshamul-Haque *et al.*, 1995).

In this study application of *P. aeruginosa* and *P. lilacinus* with *L. nudicaulis* significantly increased their suppressive potential respectively against *M. phaseolina* and *F. solani* and *R. solani*. The delivery of microbial antagonists with urban and agricultural wastes as mulches were found to be very effective in suppressing root pathogens of avocado and citrus (Casale *et al*., 1995). Similarly addition of some composts to soil increased the population of PGPR in the tomato rhizosphere exhibiting antagonism towards *Fusarium oxysporum* f.sp. *radicis-lycopersici, Pyrenocheta lycopersici, Pythium ultimum* and *Rhizoctonia solani* (Alvarez *et al*., 1995). Application of PGPR or *P. lilacinus* in combination with common medicinal weed *L. nudicaulis* holds promise for the control of root infecting fungi of mungbean.

References

Alvarez, M.A., S. deGauge and H. Antoun. 1995. Effect of compost on rhizosphere microflora of tomato and on the incidence of plant growth promoting rhizobacteria. *Applied and Environmental Microbiology*, 61: 194-199.

Baquar, S.R. 1989. *Medicinal and Poisonous Plants of Pakistan.* Printas, Karachi, Pakistan. pp. 506.

Brown, P.D. and M.J. Morra. 1997. Control of soil-borne plant pests using glucosinolate containing plants. *Advances in Agronomy*, 61: 167-231.

Casale, W.L., V. Minassian, J.A. Menge, C.J. Lovatt, E. Pond, E. Jhonson and F. Guillement. 1995. Urban and agricultural wastes for use as mulches on avocado and citrus and delivery of microbial agents. *Journal of Horticultural Science,* 70: 315-332.

Chen,W., H.A.J. Hoitink, A.F. Schmitthenner and O.H. Tuovinen. 1988. The role of microbial activity in suppression of damping-off caused by *Pythium ultimum. Phytopathology,* 78: 314-322.

Cutler, H.G. and R.A. Hill. 1994. Natural fungicides and their delivery systems as alternate to synthetics. In: *Biological Control of Postharvest Diseases–Theory and Practice.* (Eds.): C.L. Wilson and W. Wisniewski CRC Press, Florida. pp. 135-142.

De Meyer, G., K. Capiau, K. Audenaert, A. Buchala, J.P. Metraux and M. Hofte. 1999. Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. *Molecular Plant Microbe Interaction,* 2: 450-458.

Drapeau, R., J.A. Fortin and C. Gagnon. 1973. Antifungal activity of *Rhizobium. Canadian Journal of Microbiology*, 51: 681-682.

Ehteshamul-Haque, S., M. Abid and A. Ghaffar. 1995. Efficacy of *Bradyrhizobium* sp., and *Paecilomyces lilacinus* with oil cakes in the control of root rot of mungbean. *Tropical Science,* 35: 294-299.

Ehteshamul-Haque, S., M. Abid, V. Sultana, J. Ara and A. Ghaffar. 1996. Use of organic amendments on the efficacy of biocontrol agents in the control of root rot and root knot disease complex of okra. *Nematologica Mediteranea,* 24: 13-16.

Elad, Y. and D. Shtienberg. 1996. *Trichoderma harzianum* T39 (Trichodex) integrated with fungicides for the control of grey mould of strawberry, vegetable, greenhouse crops and grapes. In: *Advances in Biological Control of Plant Diseases*. (Eds.): T. Wenhua, R.J. Cook and R. Rovira China Agricultural University Press, Beijing, China. pp. 310-319.

Gomez, K.A. and A.A. Gomez. 1984. *Statistical Procedures for Agricultural Research.* 2nd. ed., Wiley, New York, pp. 680.

Gould, W.D., C. Hagedorn, T.R. Bardinelli and R.M. Zablotswicz. 1985. New selective media for enumeration and recovery of fluorescent *Pseudomonas* from various habitats. *Applied and Environmental Microbiology,* 49: 28-32.

Hoitink, H.A.J. and M.J. Boehm. 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual Review of Phytopathology,* 37: 427-446.

Izhar, I., S. Ehteshamul-Haque, M. Javeed and A. Ghaffar. 1995. Efficacy of *Pseudomonas aeruginosa* and *Bradyrhizobium* sp., in the control of root rot disease in chickpea. *Pakistan Journal of Botany,* 27: 451-455.

Jatala, P. 1986. Biological control of plant parasitic nematodes. *Annual Review of Phytopathology,* 24: 453-489.

Keen, B.A. and H. Raczkowski. 1921. The relation between clay content and certain physical properties of soil. *Journal of Agricultural Science,* 11: 441-449.

Lazarovits, G. and J. Nowak. 1997. Rhizobacteria for improvement of plant growth and establishment. *Horticultural Science,* 32: 188-192.

Mass, J.L. and G.J. Galletta. 1997. Recent progress in strawberry disease research. *Acta Horticulturae,* 439: 769-780.

Mazzola, M. 2004. Assessment and management of soil microbial community structure for disease suppression. *Annual Review of Phytopathology,* 42: 35-59.

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

Parveen, S., S. Ehteshamul-Haque and A. Ghaffar. 1998. Efficacy of *Pseudomonas aeruginosa* and Paecilomyces *lilacinus* in the control of root rot-root knot disease complex of some vegetables. *Nematologica*. *Mediteranea,* 26: 209-212.

Perkins, J.H. and B.R. Patterson. 1997. Pests, pesticides and the environment: a historical perspective on the prospects for pesticide reduction. In: *Techniques for Reducing Pesticide Use*. (Ed.): D. Pimental. John Wiley & Sons, Chichester. pp. 13-33.

Raaijmakers, J.M. and D.M. Weller. 1998. Natural plant protection by 2,4-diacetylphloroglucinol producing *Pseudomonas* spp., in take-all decline soils. *Molecular Plant Microbe Interaction.* 11: 144-152.

Rashid, S., M. Ashraf, S. Bibi and R. Anjum. 2000. Antibacterial and antifungal activities of *Launaea nudicaulis* Roxb., and *Launaea resedifolia* Linn. *Pakistan Journal of Biological Science,* 3: 630-632.

Shabana, M.M., Y.W. Mirhom, A.A. Gnenah, E.A. Aboutabl and H.R. Amer. 1990. Study into wild Egyptian plants of potential medicinal activity. Ninth communication: hypohlycaemic activity of some selected plants in normal fasting and alloxanised rats. *Archiv fur Experimentelle Veterinarmedizin (Leipzig),* 44: 389-394.

Sheikh, A.H. and A. Ghaffar. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pakistan Journal of Botany,* 7: 13-17.

Tenuta, M. and G. Lazarovits. 2002. Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahlia. Phytopathology,* 92: 255-264.

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology,* 26: 379-407.

Weller, D.M., J.M. Raaijimakers, B.B.M. Gardener and L.S. Thomashow. 2002. Microbial population responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology,* 40: 309-348.

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

Zhou, T. and T.C. Paulitz. 1994. Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp., on cucumber. *Journal of Phytopathology,* 142: 51-63

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