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Control of the root-rot and root-knot disease complex by *Pseudomonas aeruginosa*: impact of bacterial rhizosphere colonization

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Summary

The potential of 3 *Pseudomonas aeruginosa* strains as biocontrol agents of root-infecting fungi *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* and the root-knot nematode *Meloidogyne javanica* was tested on chili and uridbean under greenhouse conditions. All the three strains significantly reduced nematode populations in soil, invasion, multiplication and gall formation due to *M. javanica.* Root infection by fungi was also effectively suppressed following *P. aeruginosa* application. Bacterial antagonists exhibited better biocontrol and growth promoting activity in 15-day-old plants than did those harvested at 30 or 45 days. Population of the bacterium in the rhizosphere declined rapidly after 15 days of nematode inoculation. Strain Pa-5 showed maximum nodulation in 15-day-old samplings while strain Pa-7 showed highest number of nodules in 30 and 45-day-old uridbean plants.

Keywords: *Pseudomonas aeruginosa,* root-knot nematode, root-infecting fungi, rhizosphere colonization.

INTRODUCTION

Chili (*Capsicum annum* L.) and uridbean (*Vigna mungo* (L.) Hepper) are the important vegetable crops in Pakistan. Both the crops are highly susceptible to root-infecting fungi *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* and the rot-knot nematode, *Meloidogyne javanica* which are serious constraints for successful cultivation of the crops (G h a f f a r, 1995).

Since soil applied pesticides are costly and produce environmental hazards, several fungi and bacteria have received considerable attention in the control of root-infecting fungi and root-knot nematode (J a t a l a, 1995; R o d r i g u e z-K a b a n a et al., 1984; I z h a r et al., 1995; O o s t e n d o r p and S i k o r a, 1989). Of the bacterial antagonists, *Pseudomonas aeruginosa* (Schroeter) Migula is known to colonize a wide range of crop plants (P i l l a y and N o w a k, 1997), and has shown promising results in the control of root-infecting fungi and plant-parasitic nematodes (S i d d i q u i and E h t e s h a m u l-H a q u e, 2000 a,b). Besides having biocontrol potential, the bacterium is also known to promote plant growth via the production of certain phytohormones (H u s s a i n and V a n c u r a, 1970; B r o w n, 1972). An experiment was carried out to examine the potential of 3 strains of *P. aeruginosa* as biocontrol agents of root-infecting fungi and root-knot nematode in chili and uridbean.

MATERIAL AND METHODS

The soil used for the experiment was a sandy-loam (Sand: Silt: Clay, 70: 19: 11%), pH 8.1 with moisture holding capacity of 36% and had a natural population of 2-9 sclerotia g^{-1} of soil of *M. phaseolina* as assessed by a wet sieving and dilution technique (S h e i k h and G h a f f a r, 1975); 6.8% colonization of *R. solani* on sorghum seed used as baits (W i l h e l m, 1955) and 3500 cfu g^{-1} of soil of mixed population of *Fusarium* spp., as determined by soil dilution technique (N a s h and S n y d e r, 1962). Five-day-old bacterial cultures maintained on King's B medium were scrapped from the surface with a glass rod after adding 10 ml sterilized distilled water. Four, 3-week-old chili seedlings raised in sterilized soil or four uridbean seeds were planted in 8-cm-diam. plastic pots at 350 g/pot. Before seedling transplantation or seed sowing, soil in each pot was drenched with the cell suspension of *P. aeruginosa* strain Pa-5 (cfu 1.5 x 10^8), Pa-7 (cfu 1.7 x 10^8) and IE-6 (1.5 x 10^8) prepared in 25 ml sterile distilled water. The soil in each pot was kept at 50% of maximum water holding capacity. After one week of seedling transplantation or seed sowing, roots in each pot were inoculated with 2000 eggs of *M. javanica* (Treub) Chitwood obtained from infested aubergine (*Solanum melongena* L.) roots. Treatments and controls were replicated 12 times and pots were kept in randomized block design. Plants were harvested 15, 30 and 45 days after nematode inoculation (4 pots at each harvest). At each harvest, plant height, root length and fresh weight of shoot and root were recorded. Number of galls and egg mass were counted using a stereomicroscope. To determine the nematode population, soils from replicates in each treatment were

pooled and 250-cc soil was used for the extraction of nematode using modified Baermann funnel technique. To determine the nematode invasion, 1g root from each plant was boiled, stained in 0.25% acid fuchsin-lactic acid, cooled in glycerol : water, 1:1 plus a few drops of lactic acid and macerated for 45 seconds in an electric grinder. Nematodes in 25 ml of sample (five replicates) were counted and the population size calculated.

To determine the incidence of fungi, tap root from each plant after washing in running tap water was cut into 1-cm-long pieces and surface disinfected with 1% $Ca(OCl)_2$ for 3 minutes. Sterilized root pieces were then plated onto PDA plates at 5 pieces per plate. PDA medium was supplemented with penicillin (100,000 units/L) to avoid the bacterial contamination.

To determine the bacterial population in the rhizosphere, method used by P i l l a y and N o w a k (1997) was modified where 1g root from each replicate with adhering soil was placed in a 250-ml Erlenmeyer flask containing 10 ml of 0.1M $MgSO_4$ solution (pH 6.5) + 0.02% Tween 20 and shaking vigorously for 15 min. Ten-fold serial dilutions of the suspensions were prepared and 50 µml aliquots from the appropriate dilutions were plated on to King's B medium. Plates were incubated for 48 h at room temperature (25-30°C) and number of cfu recorded. Roots grown in non-bacterized soil were also checked for the presence of contaminants (*P. aeruginosa*). Data were analysed and subjected to factorial analysis of variance (FANOVA) followed by least significant difference (LSD) according to G o m e z and G o m e z (1984). Data of bacterial rhizosphere populations were transformed to $\log_{10}(x+1)$.

RESULTS

Effects of *Pseudomonas aeruginosa* on plant growth and *Bradyrhizobium*-nodulation: *P.aeruginosa* significantly increased biomass of foliage in both chili and uridbean plants. Maximum plant height, root length and fresh weight of shoot and root in both chili and uridbean were produced in plants where strain Pa-7 of *P. aeruginosa* was used at 15 days harvest. At 30-day-harvest, maximum plant height, shoot weight, root length and fresh weight of root of chili were obtained by strain Pa-7. At 45 day sampling, strain IE-6 showed maximum plant height and root length of chili seedlings while strain Pa-7 produced maximum plant height and root length in uridbean. Strain Pa-7 in chili and strain IE-6 in uridbean yielded highest fresh weight of shoot. In both the crops, greatest fresh weights of root were recorded in control plants (Table 1-2). In uridbean, maximum numbers of nodules were produced following treatment with strain Pa-5 in 15-day-old plants whereas strain Pa-7 showed highest nodulation in 30 and 45-day-old samplings (Table 3).

Impact of *Pseudomonas aeruginosa* on root fungi: *P. aeruginosa* significantly suppressed root infection by fungi on chili and uridbean. More than 75% suppression of *M. phaseolina* infection was observed in 15-day-old chili and uridbean plants where strain IE-6 was used while strain Pa-5 was effective at second harvest of uridbean.

In 45-day-old uridbean plants, all the isolates of *P.aeruginosa* gave more than 50% suppression of *M. phaseolina* infection. Strain Pa-5 and IE-6 in 15 and 30-day-old uridbean plants and strain IE-6 in 30-day-old chili plants caused more than 50% reduction in *F. solani* infection. A complete suppression of *R. solani* was recorded in 15-day-old uridbean plants where strain IE-6 was used. In 15-day-old chili plants more than 75% inhibition of *R. solani* infection was given by all the isolates used while strain Pa-7 caused more than 50% reduction in 15-day-old uridbean plants. In 30-day-old uridbean plants more than 50% suppression of *F. solani* infection was observed after treatment with strain Pa-7. Likewise, strain Pa-7 and IE-6 gave more than 75% suppression and strain Pa-5 exhibited more than 50% suppression of *R. solani* infection in 30-day-old chili plants. Strain Pa-7 in 45-day-old chili plants and strain IE-6 in uridbean produced >50% suppression in *R. solani* infection whereas strain Pa-7 resulted in more than 50% reduction in 45-day-old uridbean plants (Table 4).

Effect of *Pseudomonas aeruginosa* on *M. javanica* root-knot nematode: Root-knot infection caused by *M. javanica* increased with the passage of time, with more severe infection in uridbean than in chili. Use of *P. aeruginosa* strains significantly ($p<0.05$) suppressed nematode penetration and consequent gall formation due to *M. javanica* in both the crops. Bacterial antagonists showed better biocontrol effects in 15-day-old plants than plants harvested after 30 or 45 days. Maximum reduction in gall formation and nematode population density was observed in treatment where *P. aeruginosa* strain IE-6 was used in both chili and uridbean at all the time intervals (Table 5).

Effect on bacterial colonization in the rhizosphere: Bacterial population in 15-day-old plants was comparatively higher in the uridbean rhizosphere (3.5×10^7 to 1.4×10^8 cfu g^{-1}) as compared to chili (1.1×10^7 to 1.0×10^8 cfu g^{-1}). A drastic decline in bacterial population was observed in 30-day-old chili plants (1.9×10^4 to 1.1×10^6 cfu g^{-1}) and in uridbean (9.0×10^4 to 1.4×10^6 cfu g^{-1}) which increased slightly in 45-day-old sampling (5.7×10^5 to 1.4×10^6 cfu g^{-1}) in chili and (9.0×10^5 to 1.2×10^7 cfu g^{-1}) in uridbean (Table 6).

DISCUSSION AND CONCLUSIONS

There seems to be a need for careful selection of effective isolates in the development of biological control agents. Species- and even strain-specific differences are known for bacteria with respect to their preferred niche in the rhizosphere environment (J a g n o w et al., 1991). All the three isolates of *P. aeruginosa* used in this study showed effective biocontrol and growth promoting effects. Even though all the isolates survived successfully in the rhizosphere throughout the experiment, strain IE-6 was found more rhizospheric competent. A good biocontrol agent should have the quality to be able to persist for a longer period of time and still retain its effectiveness (E h t e s h a m u l-H a q u e, 1994). Reduction in nematode population and invasion was related with the extensive root colonization of the bacterial antagonists.

Use of *P. aeruginosa* significantly reduced nematode population, invasion, gall formation and egg mass production. Colonization of roots by rhizosphere bacteria have been reported to reduce nematode invasion (Siddiqui and Ehteshamul-Haque, 2000 a,b). In the present study, *P. aeruginosa* isolates also suppressed root-colonization by soilborne fungi like *M. phaseolina, F. solani* and *R. solani* in both chili and uridbean. Siderophore activity, antibiotic production, toxic development and induced resistance are the major mechanisms reported against pathogenic fungi (Weller, 1988). *P. aeruginosa* has been reported to reduce growth of *R. solani*, *Sclerotium rolfsii, M. phaseolina, F. solani* and *Verticillium dahliae* (Podile et al., 1988; Izhar et al., 1995; Sharma and Nowak 1998).

It is interesting to note that bacteria exhibited better biocontrol and growth-promoting effects in 15-day-old seedlings compared to 30 and 45-day-old plants. Presumably, metabolites released by *P. aeruginosa* were initially toxic to inoculated nematode that made nematodes less attractive to penetrate their host. By the time of later harvests the nematode might have overcome bacterial toxicity resulting in greater penetration. Alternatively, decreased population levels of *P. aeruginosa* in the rhizosphere could have resulted in increased nematode invasion.

In the present study, pathogens in their pathogencity and *P. aeruginosa* strains in their biocontrol potential against different pathogens on each of the two hosts showed considerable variability. Curl (1982) has reported a variable efficacy of a biocontrol agent against a pathogen on different host plants grown in similar soil conditions indicating that differences in root exudates of different plants could have a stimulatory or inhibitory effect on either the pathogen or the antagonists. Development of tolerance or ability of a pathogen to overcome the effect of inhibitory metabolites would also affect the efficacy of biocontrol agents.

Biological control agents are unlikely to have the widespread effectiveness of most chemical treatments. The efficacy of biocontrol agents is likely to be affected by the level of nematode infestation, root colonization by fungi, host plant, and other biotic and abiotic factors. The significance of these factors need to be elucidated so that application rates and methods can be developed to deliver sufficient inoculum to obtain effective pathogen control in a range of conditions (de Leij and Kerry, 1991). In essence, for a successful application of a biocontrol agent, compatibility between host, antagonists and pathogen is essential. Selection or development of strains of biological control agents effective against more than one pathogen on more than one host plant would obviously be extremely advantageous.

REFERENCES

Brown M.E. 1972. Plant growth promoting substances produced by microorganisms of soil and rhizosphere. J. Appl. Bacteriol., 35: 443-445.

Curl E.A. 1982. The rhizosphere: relation to pathogen behavior and root disease. Plant Diseases, 66: 624-630.

de Leij F.A.A.M., Kerry B.R. 1991. The nematophagous fungus, *Verticillium chlamydosporium* Goddard, as a potential biological control agent for *Meloidogyne arenaria* (Neal) Chitwood. Revue de Nematologie, 14: 157-164.

Ehteshamul-Haque S. 1994. Use of rhizobia in the control of root rot fungi of crop plants. Ph.D. thesis, Department of Botany, University of Karachi.

Ghaffar A. 1995. Biological control of root rot-root knot disease complex of vegetables. Final Research Report. Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Hussain A., Vancura V. 1970. Formation of biologically active substances by rhizosphere bacteria and their effect on plant growth. Folia Microbiol., (Prague), 15: 468-478.

Izhar I., Ehteshamul-Haque S., Javed M., Ghaffar A. 1995. Efficacy of *Pseudomonas aeruginosa* and *Bradyrhizobium* sp., in the control of root rot disease of chickpea. Pak. J. Bot., 27: 451-455.

Jagnow G., Hoflich G., Hoffmann K.H. 1991. Inoculation of non-symbiotic rhizosphere bacteria: Possibilities of increasing and stabilizing yields. Angew. Bot., 65: 97-126.

Jatala P. 1985. Biological control of nematodes. In: An advanced Treatise on *Meloidogyne*. Vol. 1. Biology and Control. pp. 303-309. Taylor, J.N. and Carter C.C. (eds.). North Carolina State University, USAID, Raleigh, NC, USA.

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

Oostendorp M., Sikora R.A. 1989. Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. Revue de nematologie, 12: 77-83.

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

Podile A.R., Kumar B.S.D., Dube H.C. 1988. Antibiosis of rhizobacteria against some plant pathogens. Indian J. Microbiol., 28: 108-111.

Rodriguez-Kabana R., Morgan-Jones G., Goodoy, Gintis B.D. 1984. Effectiveness of species of *Gliocladium, Paecilomyces* and *Verticillium* for the control of *Meloidogyne arenaria* in field soils. Nematropica, 14: 155-170.

Sharma V.K., Nowak J. 1998. Enhancement of *Verticillium* wilt resistance in tomato transplant by *in vitro* co-culture of seedlings with a plant growth promoting rhizobacterium (*Pseudomonas* sp. strain PsJN). Can. J. Microbiol., 44: 528-536.

Sheikh A.H., Ghaffar A. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. Pak. J. Bot., 7: 13-17.

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

Siddiqui I.A., Ehteshamul-Haque S. 2000b. Effect of *Verticillium chlamydosporium* and *Pseudomonas aeruginosa* in the control of *Meloidogyne javanica* on tomato. Nematol. medit., 28: 193-196.

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

Użycie *Pseudomonas aeruginosa* do zwalczania chorób korzeni pomidora: wpływ różnych zagęszczeń *Meloidogyne javanica* i inokulum *Rhizoctonia solani*

Streszczenie

Badania przeprowadzono w szklarni, a dotyczyły one określenia *Pseudomonas aeruginosa* szczep IE-6 w biologicznym zwalczaniu *Melogyne javanicum, Rhizoctonia solani, Macrophomina phaseolina, Fusarium oxysporum*

i *F. solani*. W doświadczeniu uwzgledniono cztery kombinacje zagęszczenia *M. javanicum* tj. 0; 250; 500; 1000 i 2000 jaj /doniczkę oraz *R. solani* w trzech poziomach inokulum - 0; 1 i 3 ml zawiesiny grzyba/kg gleby. Biologiczne zwalczanie chorób korzeni powodowanych przez *R. solani* i *M. javanicum* przeprowadzono na roślinach pomidora z użyciem gleby sterylizowanej i nie sterylizowanej. Porażenie korzeni było silniejsze wraz ze wzrostem koncentracji grzyba i szkodnika. Efekt *P. aeruginosa* w biologicznym zwalczaniu okazał się najlepszy przy małym poziomie populacji *M. javanicum* i *R. solani*, aniżeli przy dużym zagęszczeniu szkodnika i patogena. Szkodliwość chorób korzeni była większa w sterylnej glebie, aniżeli w niesterylnej. Duże zagęszczenie populacji *R. solani* (3 ml/kg gleby) oraz *M. javanicum* (2000 jaj/doniczkę) w glebie wysterylnej przyczyniło się do całkowitego obumierania roślin. Podczas gdy niektóre rośliny przetrwały w glebie niewysterylizowanej. Na podstawie uzyskanych wyników można stwierdzić, że istnieje korelacja pomiędzy gęstością populacji *M. javanicum* a kolonizacją korzeni przez *R. solani*. Porażenie korzeni przez inne trzy grzyby jak *Macrophomina phaseolina*, *Fusarium oxysporum* i *F. solani* było niższe w obecności *P. aeruginosa* w niewysterylizowanej glebie. Ponadto bakteria ta wzmacnia rozwój roślin w obu typach gleby.

Table 1.

Effect of different strains of *P. aeruginosa* on growth of chili.

Treatment	Plant height (cm)			Shoot weight (g)			Root length (cm)			Root weight (cm)		
	Harvest time (days)											
	15	30	45	15	30	45	15	30	45	15	30	45
Control	7.2	10.9	13.8	0.2	0.4	0.6	5.3	9.0	9.6	0.16	0.30	0.55
P. aeruginosa (Pa-5)	8.7	12.6	16.8	0.3	0.7	0.7	5.6	11.8	10.0	0.16	0.40	0.33
P. aeruginosa (Pa-7)	9.0	12.6	16.0	0.4	0.4	0.8	7.2	11.4	10.4	0.20	0.28	0.53
P. aeruginosa (IE-6)	8.7	11.7	16.8	0.2	0.5	0.7	6.4	9.7	10.6	0.16	0.27	0.35
SED $p<0.05$	0.7	1.7	1.2	0.04	0.20	0.19	0.92	1.16	1.54	0.04	0.08	0.08

Table 2.

Effect of different strains of *P. aeruginosa* on growth of uridbean.

Treatment	Plant height (cm)			Shoot weight (g)			Root length (cm)			Root weight (cm)		
	Harvest time (days)											
	15	30	45	15	30	45	15	30	45	15	30	45
Control	16.6	19.6	21.1	1.2	2.0	3.2	10.6	15.3	16.2	0.31	0.53	0.64
P. aeruginosa (Pa-5)	17.6	22.5	32.3	1.3	2.5	3.1	11.6	16.5	18.0	0.52	0.52	0.54
P. aeruginosa (Pa-7)	19.4	22.0	24.4	1.3	3.0	3.3	12.4	18.0	18.9	0.45	0.60	0.53
P. aeruginosa (IE-6)	18.6	19.7	23.3	1.3	2.4	3.3	13.0	15.0	19.6	0.33	0.45	0.59
SED $p<0.05$	0.69	0.77	0.39	0.11	0.38	0.31	1.80	2.31	1.40	0.06	0.11	0.05

Table 3.

Effect of different strains of *P. aeruginosa* on *Bradyrhizobium*-nodules formation in uridbean.

Treatment	Number of nodules Harvest time (days)		
	15	30	45
Control	7	7	9
P.aeruginosa (Pa-5)	10	12	13
P.aeruginosa (Pa-7)	9	14	15
P.aeruginosa (IE-6)	10	12	14
SED p<0.05	0.9	1.1	1.7

Table 4.

Effect of different strains of *P. aeruginosa* in the control of root-infecting fungi on chili and uridbean.

Treatment	Infection%					
	Chili			Uridbean		
	Harvest time (days)					
	15	30	45	15	30	45
	Macrophomina phaseolina					
Control	25	41	50	25	44	75
P. aeruginosa (Pa-5)	25	41	41	25	19	31
P. aeruginosa (Pa-7)	16	25	33	19	31	25
P. aeruginosa (IE-6)	8	25	33	13	25	31
SED p<0.05	18	24	22	18	17	13
	Fusarium solani					
Control	25	66	75	31	44	44
P. aeruginosa (Pa-5)	17	41	50	13	19	35
P. aeruginosa (Pa-7)	25	41	50	19	25	25
P. aeruginosa (IE-6)	17	25	41	13	19	31
SED p<0.05	12	21	25	14	13	16
	Rhizoctonia solani					
Control	25	33	25	13	19	25
P. aeruginosa (Pa-5)	8	17	17	13	13	19
P. aeruginosa (Pa-7)	8	8	8	6	6	13
P. aeruginosa (IE-6)	8	8	25	0	13	6
SED p<0.05	13	16	17	7	14	15

Table 5.

Effect of different strains of *P. aeruginosa* in the control of root-infecting fungi on chili and uridbean.

Treatment	Chili			Uridbean		
	Harvest time (days)					
	15	30	45	15	30	45
	Galls per root system					
Control	5	17	30	6	17	48
P. aeruginosa (Pa-5)	3	14	23	3	14	31
P. aeruginosa (Pa-7)	2	16	23	2	14	27
P. aeruginosa (IE-6)	1	14	19	1	11	24
SED p<0.05	2	2	2	0.7	1	3
	Egg mass per root system					
Control	–	–	20	–	–	31
P. aeruginosa (Pa-5)	–	–	12	–	–	21
P. aeruginosa (Pa-7)	–	–	11	–	–	17
P. aeruginosa (IE-6)	–	–	7	–	–	12
SED p<0.05	–	–	1.8	–	–	2.7
	Nematode soil population (250-cc soil)					
Control	710	1030	2170	990	1310	3150
P. aeruginosa (Pa-5)	530	890	1980	920	1190	2780
P. aeruginosa (Pa-7)	410	930	1860	880	1260	2840
P. aeruginosa (IE-6)	500	750	1800	840	1150	2550
SED p<0.05	125	157	254	127	214	336
	Nematode soil population (one-g root)					
Control	7	27	96	13	52	113
P. aeruginosa (Pa-5)	4	23	36	10	42	68
P. aeruginosa (Pa-7)	3	15	57	10	55	62
P. aeruginosa (IE-6)	3	11	28	5	37	51
SED p<0.05	1	5	20	3	18	13

Table 6.

Bacterial rhizosphere colonization in chili and uridbean.

Treatment	Colonization [(log cfu g^{-1} fresh weight of root)+1]					
	Chili			Uridbean		
	Harvest time (days)					
	15	30	45	15	30	45
Control	0.00	0.00	0.00	0.00	0.00	0.00
P. aeruginosa (Pa-5)	7.37	5.81	5.95	8.04	5.27	6.87
P. aeruginosa (Pa-7)	7.76	5.92	6.04	8.05	4.15	6.43
P. aeruginosa (IE-6)	7.75	6.01	6.08	8.08	6.03	6.95
SED $p<0.05$	0.15	0.11	0.05	0.09	0.22	0.15