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Combination of *Pseudomonas aeruginosa* and *Pochonia chlamydosporia* for Control of Root-Infecting Fungi in Tomato

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With 2 figures

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Abstract

A plant growth-promoting rhizobacterium, *Pseudomonas aeruginosa* strain IE-6, and a fungal antagonist, *Pochonia chlamydosporia*, were tested for their ability to inhibit mycelial growth of root-infecting fungi under laboratory conditions including *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani*. Biocontrol effectiveness of the bacterium and the fungus alone or in combination was also determined for the control of root-infecting fungi under field conditions. In a dual-culture plate assay, the colonies of *P. chlamydosporia* and *P. aeruginosa* met each other and no further growth of either organism occurred. Against *M. phaseolina*, *F. solani* and *R. solani*, an ethyl acetate extract of the culture filtrates of *P. aeruginosa* inhibited fungal growth greater than the hexane extract, but against *F. oxysporum* the hexane extract caused greater inhibition of fungal growth. By contrast, against *M. phaseolina*, *F. oxysporum* and *F. solani*, the hexane extract of *P. chlamydosporia* was more effective in the inhibition of fungal growth than the ethyl acetate fraction. Ethyl acetate extracts of *P. aeruginosa* at 1.0 mg/ml not only inhibited the radial colony growth of *R. solani* but also lysed the fungal mycelium. *P. aeruginosa* produced siderophores and hydrogen cyanide under laboratory conditions. Field experiments conducted in 1997 and repeated in 1998 revealed that *Pochonia chlamydosporia* and *P. aeruginosa* significantly suppressed the root-infecting fungi *M. phaseolina*, *F. oxysporum*, *F. solani* and *R. solani* and that the combination of the two caused greater inhibition of the fungal pathogens than either alone. Application of *P. chlamydosporia* and *P. aeruginosa* as a soil drench also resulted in enhanced growth of tomato plants.

Introduction

Public concern about chemical pesticides has fostered an interest in the application of bacteria for the biological control of pathogenic fungi affecting agricultural crops (Raupach et al., 1996). It seems inevitable that fewer pesticides will be used in the future and that greater reliance will be placed on biological technologies including the use of microorganisms as antagonists (Backman et al., 1997). In evaluating the research on biological control in the last decade, it is clear that most biocontrol agents, including strains of antibiotic-producing *Pseudomonas* spp., are still too variable in their performance to be successfully used commonly in agriculture and horticulture. It is likely that most cases of naturally occurring biological control result from mixtures of antagonists, rather than from high populations of a single antagonist. Thus, a mixture of antagonists that render the soil disease suppressive can be identified and exploited for control of soilborne plant pathogens (Lemanceau and Alabouvette, 1991; Schippers, 1992). Consequently, application of a mixture of introduced biocontrol agents would more closely mimic the natural situation and might broaden the spectrum of biocontrol activity, enhance the efficacy and reliability of control (Fry et al., 1992), and allow a combination of various mechanisms of action against the target pathogens without the need for genetic engineering (Janisiewicz, 1996).

Populations of competitive bacteria and fungi may provide some degree of spatial separation within the rhizosphere, whereas a combination of compatible bacteria and fungi may provide better control of seed-borne and root-rot pathogens than either used alone (Chao et al., 1986). Of the microbial antagonists, fluorescent bacteria of the genus *Pseudomonas* e.g. *Pseudomonas aeruginosa* (Schroeter) Migula that

colonize roots of a wide range of crop plants, are reported to suppress root-infecting fungi and root-knot nematodes (Siddiqui et al., 2001). *Pochonia chlamydosporia* (*Verticillium chlamydosporium*) Goddard, a widespread fungus that parasitizes females and eggs of cyst and the root-knot nematodes (Kerry, 1975; Freire and Bridge, 1985), effectively controlled *Meloidogyne* spp. (de Leij and Kerry, 1991). In a previous study, *P. aeruginosa* and *P. chlamydosporia* applied together afforded better biocontrol and promoted growth of tomato plants compared with either antagonist alone or the untreated control (Siddiqui and Ehteshamul-Haque, 2000).

In selecting potential candidates among *Pseudomonas* isolates for *in situ* biological control of plant-pathogenic fungi, several criteria should be met by the bacteria. These include: (i) ecological fitness, including rhizosphere competence, to maintain an effective population size *in situ*; (ii) rapid root colonization to antagonize fast-growing pathogens (e.g. *Rhizoctonia solani* and *Pythium ultimum*); (iii) stable production of antifungal agents under variable growth conditions to sustain antagonism during root development (Nielsen et al., 1998). Unfortunately, discrepancies exist between the antagonistic effect under *in vitro* conditions and the corresponding *in situ* efficacy (Reddy et al., 1993; Elsherif and Grossmann, 1994), although some studies have demonstrated that production of specific antibiotics *in vitro* is indeed correlated with their production and antagonism *in situ* (Thomashow et al., 1990; Howie and Suslow, 1991; Keel et al., 1992).

Our specific objectives were: (i) to determine the *in vitro* compatibility of *P. aeruginosa* strain IE-6 and *P. chlamydosporia*; (ii) to determine the solubility of potential antifungal compounds in various solvents; (iii) to test the potential of *P. aeruginosa* in combination with *P. chlamydosporia* for enhancing consistency and level of biocontrol against four soil-borne root-infecting fungi (*Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani*), and to evaluate the efficacy of mixtures of biocontrol agents under field conditions.

Materials and Methods

Microorganisms and culture conditions

Pseudomonas aeruginosa strain IE-6S$^+$ is a spontaneous streptomycin-resistant derivative of the strain IE-6 that was originally isolated from the rhizosphere of sunflower grown in a sandy-loam soil of a field in Darsanochanoo, Pakistan (Siddiqui, 2002). The bacterium was routinely cultivated in liquid King's medium B (KMB; King et al., 1954) amended with 100 mg/ml of streptomycin on a rotary shaker (100 rpm) for 48 h at 24°C. The bacterial cells were harvested by centrifugation (2800 × ***g***, 20 min), washed with and resuspended in sterile $MgSO_4$ (0.1 M). *P. chlamydosporia* (KUCC-839) was originally isolated from an infected *Meloidogyne javanica* female and maintained on corn meal agar (CMA) medium for 4 weeks at 20°C.

In vitro experiments

In vitro compatibility of P. aeruginosa *and* P. chlamydosporia. To observe *in vitro* compatibility between *P. aeruginosa* and *P. chlamydosporia*, the dual-culture plate method was employed (Siddiqui et al., 2001). *P. aeruginosa* strain IE-6 was streaked on one side of a Petri dish (9-cm diameter) containing Czapek's dox agar (CDA) at pH 7.2. The other side of the Petri plate was inoculated with a 5-mm disc of the fungus. The plates were incubated at 28°C for 1 week and the zone of inhibition (if any) was measured after 1 week.

Solubility of the active antifungal principle(s). Pochonia chlamydosporia (KUCC-839) was grown in 250-ml Erlenmeyer flasks containing 100 ml Czapek's dox broth for 7 days in the dark at 24°C. The broth was filtered through two folds of Whatman no. 1 filter paper and the filtrate collected in a beaker. *P. aeruginosa* strain IE-6 was also grown in Erlenmeyer flasks containing King's B liquid medium (King et al., 1954) at 30°C in the dark. After 48 h, the bacterial culture was centrifuged twice at 2800 × ***g*** for 20 min to obtain a cell-free culture filtrate. Culture filtrates of *P. chlamydosporia* and *P. aeruginosa* were extracted with ethyl acetate or hexane (1 : 2) and concentrated on a rotary vacuum evaporator (Eyela, Pikakikai Co. Ltd., Tokyo, Japan) under reduced pressure at 37°C. Dilutions of 1.0, 0.1 and 0.01 mg/ml of the extracts were prepared in their respective solvents and impregnated separately on 5-mm-diameter disc of Whatman no. 1 filter paper. The treated discs were dried aseptically under a laminar flow hood and then placed (in a clockwise manner according to the concentration) on Petri plates containing CDA medium. A 5-mm-diameter. disc of the test fungus was placed at the centre of the Petri plate. Ethyl acetate- or hexane-treated discs served as controls. Each treatment and control experiment was replicated three times. After incubation for 1 week at 28°C, the zone of inhibition (if any) was measured.

Detection of siderophores and HCN production from P. aeruginosa. Production of siderophores was determined by the method of Schwyn and Neilands (1987), using the chrome azurol S (CAS) reagent (Fluka Chemicals, Buchs, Switzerland). *P. aeruginosa* was grown on CAS agar plates supplemented with 2% glucose and 0.5% L-glutamic acid (neutralized). The presence of orange haloes was recorded up to 7 days after incubation. Detection of HCN production was performed by the method of Bakker and Schippers (1987). Production of cyanide was determined by a colour shift from yellow to orange in the filter paper.

Field experiment

The experiment was carried out in 2 × 1-m microplots on an experimental field of the Department of Botany, University of Karachi in November 1997. *P. aeruginosa* strain IE-6 was multiplied on King's B medium at room temperature, whereas *P. chlamydosporia* was

grown on CMA medium for 4 weeks at 20°C. After removing the soil to a depth of 12 cm, a suspension of microbial antagonists in sterile distilled water (SDW) was drenched at 300 ml/m furrow. Suspensions of *P. aeruginosa* contained 3.2×10^8 cfu/ml and of *P. chlamydosporia* 2.5×10^8 cfu/ml. In other plots, soil was drenched with a mixture of both *P. aeruginosa* and *P. chlamydosporia* (150 + 150 ml/m furrow). Soil drenched with SDW served as the control. From each plot, two plants were harvested 30 days after seedling establishment while the remaining four plants were harvested after 60 and 90 days. Plant growth parameters in terms of plant height and fresh weight of shoot were recorded. To determine the root infection caused by fungi, the fresh root samples were washed in running tap water. After surface disinfection with 1% $Ca(OCl)_2$, 1-cm-long root pieces from tap root were transferred onto potato dextrose agar (PDA) containing benzyl penicillin (0.1 g/l) and streptomycin sulphate (0.2 g/l). The plates were incubated for 5 days at 28°C to confirm infection and colonization of roots by soil-borne root infecting fungi. Infection percentage of the root-infecting fungi was calculated in accordance with Siddiqui et al. (2001) as follows:

$$\text{Infection (\%)} = (\text{number of plants infected by a fungus/total number of plants}) \times 100$$

The experiment was repeated in October, 1998 with the same experimental design.

Statistical analyses

Data were subjected either to one-way analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) depending upon the experimental design using STATISTICA (version 5.0 software; Tulsa, Oklahoma, USA). Following ANOVA and FANOVA, least significant difference (LSD) test was used to compare the treatment means (Sokal and Rohlf, 1995).

Results

In vitro experiments

Compatibility between P. chlamydosporia *and* P. aeruginosa. In dual culture, colonies of *P. aeruginosa* and *P. chlamydosporia* met each other and neither organism grew further; no inhibition zone was produced between bacterium and the fungal colonies (data not presented).

Solubility of the active antifungal principle(s). Ethyl acetate and hexane fractions of the biological control agents inhibited the radial growth of the four root-infecting fungi (Table 1). Against *M. phaseolina*, *F. solani* and *R. solani*, the ethyl acetate extract of *P. aeruginosa* was more effective in the inhibition of mycelial growth than the hexane extract, while against *F. oxysporum*, the hexane extract inhibited mycelial growth greater than ethyl acetate. By contrast, against *M. phaseolina*, *F. oxysporum* and *F. solani*, the hexane extract of *P. chlamydosporia* was more effective than the ethyl acetate fraction. Extracts of *V. chlamydopsorium* failed to inhibit radial growth of *R. solani*. Hexane extracts of *P. chlamydosporia* at 1 mg/ml produced a zone of inhibition of 4, 2 and 6 mm respectively, against *M. phaseolina*, *F. oxysporum*, and *F. solani*. Ethyl acetate fractions of *P. aeruginosa* at 1 mg/ml produced a zone of 7, 2, and 5 mm respectively, against *M. phaseolina*, *F. oxysporum*, and *F. solani* while, its hexane extracts at the same concentration produced a zone of inhibition of 3, 3, and 2 mm respectively, against *M. phaseolina*, *F. oxysporum* and *R. solani*. Ethyl acetate extract of *P. aeruginosa* at 1 mg/ml also lysed the mycelium of *F. solani*.

Production of siderophores and hydrogen cyanide by P. aeruginosa. *P. aeruginosa* produced an orange diffuse halo surrounding the colonies indicating the production of siderophores. The isolate also produced

Table 1
Antifungal activity of the various concentrations of ethyl acetate and hexane fractions of *Pochonia chlamydosporia* and *Pseudomonas aeruginosa*

Organism	Solvent	Concentration (mg/ml)	Zone of inhibition (mm)/type of reaction			
			M. phaseolina	*F. oxysporum*	*F. solani*	*R. solani*
P. chlamydosporia	Ethyl acetate	0	a	a	a	a
		0.01	a	a	a	a
		0.1	a	a	a	a
		1.0	b	4 ± 0.4	3 ± 0.2	a
	Hexane	0	a	a	a	a
		0.01	a	a	a	a
		0.1	b	a	2 ± 0.2	a
		1.0	4	4 ± 0.5	6 ± 0.4	a
P. aeruginosa	Ethyl acetate	0	a	a	a	a
		0.01	b	a	2 ± 0.3	a
		0.1	4 ± 0.3	b	3 ± 0.4	a
		1.0	7 ± 0.4	2 ± 0.2	5 ± 0.3[c]	4 ± 0. 3
	Hexane	0	a	a	a	a
		0.01	a	a	a	a
		0.1	b	a	a	a
		1.0	3 ± 0.4	5 ± 0.5	b	a

[a]no inhibition; [b]colony of the test fungus met with the disc and no further growth of the fungus was observed; [c]after inhibition, the mycelium of the test fungus lysed; ±, standard error.

orange-red pigmentation on the filter paper which indicates the production of HCN.

Field trial

1 *Effect on root-infecting fungi*

November 1997. *P. aeruginosa* used alone or in combination with *P. chlamydosporia* significantly ($P < 0.01$) reduced *M. phaseolina* infection in tomato roots. Harvest time, and interaction of treatment and harvest time showed non-significant effects. *P. chlamydosporia* used separately, completely inhibited *M. phaseolina* infection in 30-day-old sample while, in 60-day-old sample, *P. chlamydosporia* and *P. aeruginosa* used individually or in combination resulted in complete suppression of *M. phaseolina* infection. In 90-day-old sample, *P. aeruginosa* used alone or in combination with *P. chlamydosporia* caused >66% inhibition of *M. phaseolina* infection over the untreated controls (Fig. 1).

Pseudomonas aeruginosa and *P. chlamydosporia* used alone or in combination failed to suppress *F. oxysporum* infection. *F. oxysporum* infection significantly ($P < 0.01$) increased with time. In 30-day-old samples, *P. aeruginosa* used alone or in combination with *P. chlamydosporia* resulted in complete suppression of *F. oxysporum*. In plants harvested after 60 days, *P. aeruginosa* used individually or in combination with *P. chlamydosporia* showed more than 50% control of *F. oxysporum* infection compared with the untreated controls. In 90-day-old sample, only *P. aeruginosa* used alone caused more than 50% suppression of *F. oxysporum* infection compared to the controls. In 30- and 90-day-old sample, a complete suppression of *F. solani* infection was found in the treatment in

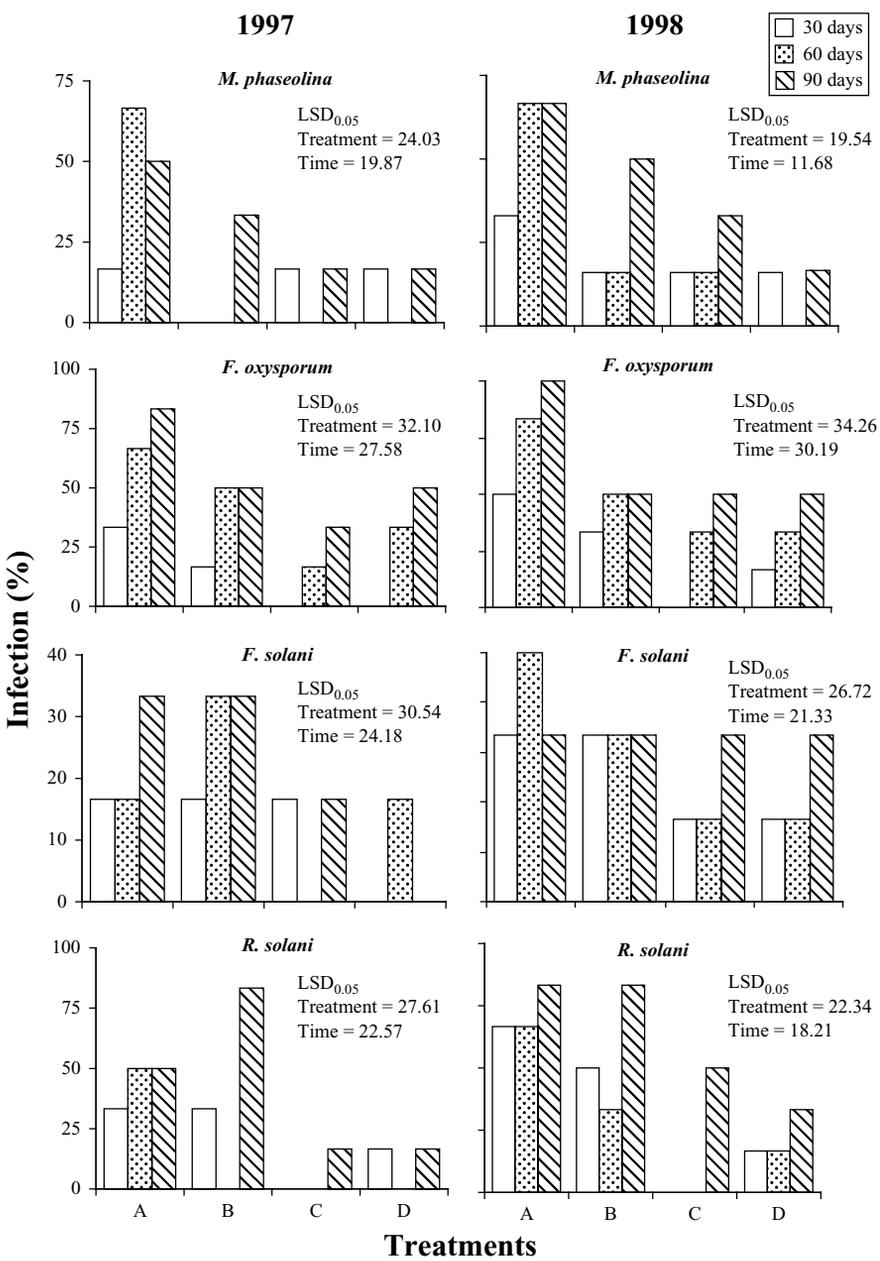

Fig. 1 Effects of soil drench with *Pseudomonas aeruginosa* and *Verticillium chlamydosporium* on infections of *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* in tomato under field conditions. (A) control; (B) *Pochonia chlamydosporia*; (C) *Pseudomonas aeruginosa*; D = B + C

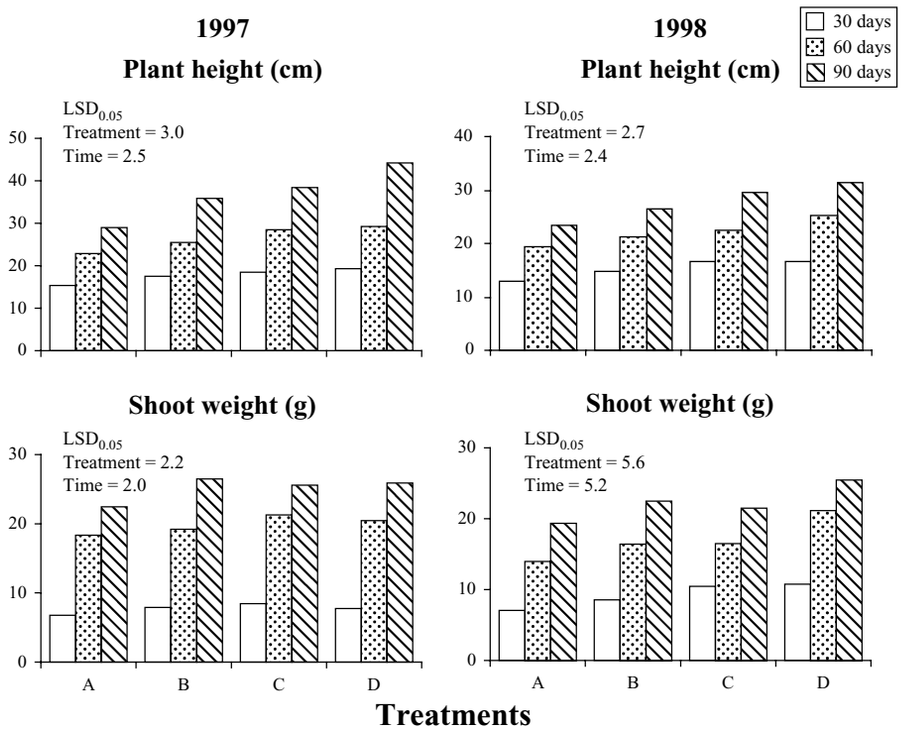

Fig. 2 Effects of soil drench with *Pseudomonas aeruginosa* and *Verticillium chlamydosporium* on tomato under field conditions. (A) control; (B) *Pochonia chlamydosporia*; (C) *Pseudomonas aeruginosa*; D = B + C

which *P. aeruginosa* was used together with *P. chlamydosporia*. In 60-day-old samplings, *P. aeruginosa* used alone caused complete inhibition of *F. solani* infection (Fig. 1).

Rhizoctonia solani infection was significantly ($P < 0.001$) inhibited after soil drench with both the biological control agents. Harvest time also differed ($P < 0.001$) significantly as *R. solani* infection increased with time. In 60-day-old samples, *P. aeruginosa* when used singly or in combination with *P. chlamydosporia* showed complete inhibition of *R. solani* infection. In 90-day-old samples, *P. aeruginosa* used alone or in combination with *P. chlamydosporia* showed more than 66% inhibition of *R. solani* infection (Fig. 1).

October 1998. *P. aeruginosa* used alone or mixed with the fungal antagonist significantly suppressed *F. oxysporum* ($P < 0.05$) and *R. solani* ($P < 0.001$) infection in tomato with no significant effects on *M. phaseolina* and *F. solani*. However, in 30-day-old sample, *P. aeruginosa* and the fungal antagonist used individually or in combination produced 50% inhibition of *M. phaseolina* infection. In 60-day-old plants, *P. aeruginosa* and *P. chlamydosporia* used individually or in conjunction gave more than 50% inhibition of *M. phaseolina* infection. In plants harvested after 90 days, *P. aeruginosa* used alone or in association with *P. chlamydosporia*, caused >50% suppression of *M. phaseolina* infection. In 30-day-old samplings, *P. aeruginosa* used alone showed complete control of *F. oxysporum* infection whereas *P. aeruginosa* used with *P. chlamydosporia* showed >50% reduction of *F. oxysporum* infection. Similarly, in 60- and 90-day-old plants, *P. aeruginosa* used individually or in conjunction with *P. chlamydosporia* produced >50% reduction of *F. oxysporum* infection compared with the controls. *P. aeruginosa* mixed with *P. chlamydosporia* showed more than 50% inhibition of *F. solani* infection compared with controls. Biological control agents were found ineffective in the suppression of *F. solani* infection in 90-day-old samplings. *P. chlamydosporia* used alone showed complete control of *R. solani* infection whereas *P. aeruginosa* used with *P. chlamydosporia* showed more than 50% inhibition of *R. solani* infection in both 30- and 60-day-old samplings. Similarly, *P. aeruginosa* used with the fungal antagonist showed >50% reduction in *R. solani* infection in 90-day-old plants as compared to untreated controls (Fig. 1).

2 *Effect on plant growth*

In both years (1997 and 1998) at each harvest, *P. chlamydosporia* and *P. aeruginosa* used together produced greater plant height ($P < 0.001$) compared to the application of either antagonist alone (Fig. 2). However, the two organisms applied either alone or in combination increased fresh weight of shoots ($P < 0.001$) only in 1997 (Fig. 2).

Discussion

The results indicate that the ethyl acetate extract of *P. aeruginosa* culture filtrate was more effective than the hexane extract in the inhibition of mycelia of *M. phaseolina*, *F. solani* and *R. solani* while its hexane extract was more effective than ethyl acetate against *F. oxysporum*. This suggests that the active principle(s) responsible for the inhibition of *M. phaseolina*, *F. solani* and *R. solani* are polar in nature while compounds of non-polar nature were largely responsible for the

inhibition of *F. oxysporum*. Production of secondary metabolites (e.g. antibiotics, Fe-chelating siderophores, and cyanide), is most often associated with fungal suppression by fluorescent pseudomonads. In addition, cellulolytic activity and chitinolytic activity have occasionally been reported among fluorescent pseudomonads (Nielsen et al., 1998) and microfungi (Schirmböck et al., 1994). In a previous study, six strains of four *Pseudomonas* spp. including *Pseudomonas aeruginosa*, *P. cepacia*, *P. putida* and *P. fluorescens* inhibited radial growth of *R. solani*, a rice sheath blight pathogen (Rosales et al., 1995). In the same study, the antifungal compound pyrrolnitrin was isolated from two *P. cepacia* strains, while 2,4-diacetylphloroglucinol was isolated from *P. putida*. Phenazine-1-carboxylic acid and pyocyanine were produced by two strains of *P. aeruginosa* while several unidentified compounds were produced by a strain of *P. fluorescens*. Of these antifungal compounds, the former two are specifically soluble in ethyl acetate. Based on the results obtained by Rosales et al. (1995), it is speculated that *P. aeruginosa* strain IE-6 used in this study also produces similar compounds inhibitory to *M. phaseolina*, *F. solani* and *R. solani*. In the present study, hexane extract of *P. chlamydosporia* was more effective than ethyl acetate fraction against *M. phaseolina*, *F. oxysporum* and *F. solani* indicative of non-polar nature of the active principle(s). Further studies are required to identify the potential metabolites produced by *P. aeruginosa* and *P. chlamydosporia* that are involved in the inhibition of fungal growth.

The varying degrees of inhibition zones produced against different root-infecting fungi by ethyl acetate extracts of *P. aeruginosa* and *P. chlamydosporia* are indicative of the differences in active compounds both qualitatively and quantitatively. In our previous study, 32 strains of *P. aeruginosa* isolated from the rhizosphere and rhizoplane of different crop plants produced inhibition zones of varying degrees against *M. phaseolina*, *F. solani* and *R. solani* (Siddiqui et al. 2001). Similarly, Rodriguez and Pfender (1997) observed that strain Pf-5 of *P. fluorescens* and its mutants produced inhibition zones of varying degrees against *Pyrenophora tritici-repentis, Sclerotinia homeocarpa, Drechslera poae*. These authors suggested that complexity of nutritional effects on antibiotic production, the possibility that a given fungus may be sensitive to more than one antibiotic, and the possibility that additional unknown antibiotics may be produced by these bacteria makes it difficult to assign antagonistic effects to specific antibiotics.

Pseudomonas aeruginosa and *P. chlamydosporia*, which were used in combination for the first time, induced remarkable disease protection against four soil-borne root-infecting fungi under field conditions. When applied individually and the combination of the two exhibited a general trend towards significant disease suppression and greater consistency with regard to pathogen control. Previous studies on using combinations of biological control agents for plant disease control have included mixtures of fungi (Paulitz et al., 1990; Datnoff et al., 1993, 1995; Budge et al., 1995), mixtures of bacteria (Johnson et al., 1993; Waechter-Kristensen et al. 1994; Stockwell et al. 1996; Wei et al. 1996; de Boer et al., 1997; Siddiqui and Shaukat, 2002) and mixtures of fungi and bacteria (Janisiewicz, 1988, 1996; Park et al., 1988; Duffy and Weller, 1995; Duffy et al., 1996; Leeman et al., 1996; Hassan et al., 1997; Siddiqui et al., 2000; Siddiqui and Ehteshamul-Haque, 2000). Most of these reports on using mixtures of biocontrol agents showed that combining antagonists resulted in improved biocontrol. However, there also are reports of combinations of biological control agents that do not result in improved suppression of disease compared with the separate antagonists (Hubbard et al., 1983; Sneh et al., 1984; Dandurand and Knudsen, 1993). Incompatibility of the coinoculants can arise because biocontrol agents may also inhibit each other as well as the target pathogen or pathogens (Leeman et al., 1996). Thus, an important prerequisite for successful development of strain mixtures appears to be the compatibility of the coinoculated microorganisms (Raupach and Kloepper, 1998).

Although both organisms inhibited colonial growth of the root-infecting fungi and infection of tomato roots under field conditions, further studies are required to identify the metabolites and to determine whether they are produced in the rhizosphere. Under natural conditions, factors like temperature, moisture, sunlight etc. are not constant but tend to fluctuate. These factors are known to influence the production of antibiotics by bacteria both qualitatively and quantitatively. If biological control through antibiotics is to be effective under field conditions, antibiotics must be produced in concentrations adequate for fungal inhibition and be active against the target pathogens during periods favourable for plant infection. Thus, parameters affecting antibiotic production need to be more fully understood (Rodriguez and Pfender 1997). As the combination of *P. aeruginosa* and *P. chlamydosporia* caused greater suppression of fungal pathogens and promoted plant growth compared with their individual application, it is not certain whether compounds produced by *P. aeruginosa* act singly or synergistically with the mycotoxins produced by *P. chlamydosporia* to elicit suppressive responses against root-infecting fungi. Further studies are required to investigate this.

Our results show that *P. aeruginosa* and *P. chlamydopsoria* release antifungal compounds *in vitro* that have the potential to suppress soil-borne root-infecting fungi, and demonstrate that production of antifungal compounds by the microorganisms may be one possible explanation of disease suppressiveness and tomato growth enhancement. However, it does not eliminate the possible involvement of other mechanisms of disease suppression, such as resource competition among microorganisms and production of phytoalexins in response to pathogen attack. In natural systems it is difficult to separate these mechanisms of disease suppression from toxin production. The results presented

here clearly indicate that mixtures of *P. chlamydosporia* and *P. aeruginosa* can provide enhanced disease protection and improve the consistency of biological control. Therefore, it is essential to investigate microbial interactions that enhance or detract from biocontrol to understand and predict the performance of mixtures of specific biocontrol agents.

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