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Efficacy of *Pseudomonas aeruginosa* and other biocontrol agents in the control of root rot infection in cotton

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Abstract

Seed dressing with *Pseudomonas aeruginosa, Paecilomyces lilacinus* and *Trichoderma koningii* significantly ($p<0.05$) reduced infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* on cotton roots in pot experiments and in field. Combined use of *P.aeruginosa* strain CMG63 with *T. koningii* produced greater plant height and fresh weight of shoot in field as compared to CMG52 which showed better results in pot experiments.

Key words: *Pseudomonas aeruginosa*, Biocontrol agents, Cotton root rot.

INTRODUCTION

Soilborne root infecting fungi attack roots of plant, limit nutrient uptake by plant and produce root rot disease complex resulting in the death of plants. Of the disease causing organisms, *M. phaseolina* is widely distributed in tropical and subtropical countries of the world is known to cause seedling blight, charcoal rot, root rot, stem rot, pod rot on more than 500 species of plants with 64 hosts recorded from Pakistan (Dhingra & Sinclair, 1978; Sinclair, 1982; Ghaffar, 1988; Ehteshamul-Haque and Ghaffar, 1994). Of the species of *Fusarium*, *F. oxysporum* and *F. solani* are very common in agricultural fields of Pakistan and cause root rot, stem rot and wilt diseases on a wide range of plants (Booth, 1971; Ehteshamul-Haque and Ghaffar, 1994). Similarly *R. solani* which is known to produce seed rot, damping off of seedlings, wilt and root rot on over 2000 species of plants (Parmeter, 1970) has been reported from atleast 68 hosts in Pakistan (Ghaffar, 1988; Ehteshamul-Haque and Ghaffar, 1994).

Many species of *Pseudomonas* promote plant growth and also reduce the population of deleterious rhizospheric fungi and bacteria (Schroth, Hancock, 1981; Weller, 1988). Among the fluorescent *Pseudomonas* some strains of *P. aeruginosa* were found to enhance the growth of many crops like soybean, sunflower, wheat, mashbean and chickpea (Ehteshamul-Haque, 1996) and also inhibited the growth of root rot pathogens viz., *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp., (Shamim et al., 1994). Application of these bacteria significantly reduced root rot disease in chickpea (Izhar et al., 1995). Duffy and Weller (1992), reported better control of take-all disease of wheat where *Trichoderma koningii* was used with *Pseudomonas fluorescens* than their separate use. Combined use of *Bradyrhizobium japonicum* with *Trichoderma* spp., showed better control of root infecting fungi in soybean than their separate use (Ehteshamul-Haque and Ghaffar, 1995). In the present study some strains of *Pseudomonas aeruginosa* were used with or without *Paecilomyces lilacinus* and *T. koningii* in the control of root rot disease of cotton caused by *M. phaseolina, R. solani* and *Fusarium* spp.

MATERIALS AND METHODS

Five day old cultures of *Pseudomonas aeruginosa* strain CMG63, CMG52 and CMG58 grown on Nutrient agar medium whereas *T. koningii* and *P. lilacinus* on Potato Dextrose Agar were used. A cell suspension of CMG63 @ $6x10^8$ cfu ml^{-1}, CMG52 @ $2.5x10^8$ cfu ml^{-1}, CMG58 @ $4.2x10^8$ cfu ml^{-1}, *P. lilacinus* @$1.8x10^8$ cfu ml^{-1} and *T. koningii* @ $8x10^8$ cfu ml^{-1} was prepared in 1% gum arabic. Delinted seeds of cotton (*Gossypium arboreum*) were dipped in cell suspension of *P. aeruginosa* strains CMG63, CMG52 and CMG58 used alone or mixed with *P. lilacinus* and *T. koningii*. After treatment with microbial suspension seeds were sown in 8 cm diam., plastic pots, containing 250 g soil @ 8 seeds per pot. There were 4 replicates of each treatment. The pots were randomized on a screen house bench at the M.A.H. Qadri Biological Research Centre, University of Karachi and kept at 50% Water Holding Capacity (Keen, Raczkowski, 1921). In a separate set seeds were sown in rows of 2x1 meter microplots at the Department of Botany, University of Karachi in randomized complete block design. There were 3 replicates of each treatments. The soil had a natural infestation of 3-10 sclerotia of *M. phaseolina* g^{-1} of soil as found by wet sieving and dilution technique (Sheikh, Ghaffar, 1975), 8% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3500 cfu g^{-1} of soil of a mixed population of *F. oxysporum* and *F. solani* as assessed by soil dilution technique (Nash, Snyder, 1962).

Plants were uprooted after 6 weeks growth and after washing with running tap water, 5 one cm long root pieces from each plant were cut, surface sterilized with 1% $Ca(OCl)_2$ for 3 minutes and transferred onto PDA plates containing penicillin (100000 units/litre) and streptomycin (0.2 gm/litre). After incubation for 5 days at 28°C, the incidence of root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., were recorded using the formula:

$$\text{Infection \%} = \frac{\text{Total no. of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

Fresh weight and length of shoots were also recorded. Data were analysed and subjected to Factorial ANOVA (FANOVA) followed by least significant differences (LSD) according to Gomez and Gomez (1984).

RESULTS

Pot experiment

P. aeruginosa strains CMG63, CMG52 and CMG58, *P. lilacinus* and *T. koningii* significantly ($p<0.05$) reduced infection of *M. phaseolina* when used alone or where *P. aeruginosa* was used with *P. lilacinus* and *T. koningii* (Table 1). Complete control of *R. solani* was observed where *P. aeruginosa* strains CMG63, CMG58 and *P. lilacinus* were used alone or CMG63 was used with *P. lilacinus*. Use of strain CMG52 and *T. koningii* also significantly ($p<0.05$) reduced *R. solani* infection. Complete control of *F. solani* infection was obtained where CMG58 was used alone or where CMG58 or CMG52 were used with *T. koningii*. CMG63 and *P. lilacinus* also significantly ($p<0.05$) controlled infection of *F. solani* on cotton roots (Table 1). Greater plant height was observed where *T. koningii* was used with CMG52. Combined use of *T. koningii* with CMG58 resulted in maximum fresh weight of shoot (Table 1).

Field experiment

More than 50% control of *M. phaseolina* infection was observed where *P. aeruginosa* strain CMG63, CMG58, *P. lilacinus* were used alone or where *P. lilacinus* was used with CMG63, CMG58 or CMG52 and *T. koningii* used with CMG63 and CMG52 (Table 1). Complete control of *R. solani* infection was observed where CMG63 was used alone or where *P. lilacinus* was used with CMG63 or CMG52 and *T. koningii* used with CMG63 or CMG58. Complete control of *F. solani* infection was found in treatments where *P. lilacinus* was used alone or with *P. aeruginosa* strains (Table 1). Maximum plant height was observed where CMG63 was used alone or with *T. koningii*. Greater fresh weight of shoot was produced by CMG52 with *T. koningii* followed by *P. lilacinus* used with CMG63 or CMG58 (Table 1).

DISCUSSION

In the presented study, strains of *P. aeruginosa* gave significant ($p<0.05$) results in controlling the infection of *M. phaseolina, R. solani* and *F. solani* on cotton. Among the fluorescent *Pseudomonas*, *P. fluorescens* is known to reduce infection of many

Table 1 – Tabela 1

Effect of different strains of *Pseudomonas aeruginosa, Paecilomyces lilacinus* and *Trichoderma koningii* in the control of root infecting fungi and growth of cotton plant.

No.	Treatments	*M.phaseolina*	*R. solani*	*F. solani*	Plant height (cm)	Fresh weight (g)
POTS EXPERIMENT						
1.	Control	47	64	39	10.1	1.4
2.	*P.aeruginosa* (CMG63)	21	0	12	9.6	1.2
3.	*P.aeruginosa* (CMG52)	23	12	14	9.8	1.5
4.	*P.aeruginosa* (CMG58)	33	0	0	12.4	2.4
5.	*P.lilacinus* (PL)	16	0	6	10.6	1.3
6.	*T.koningii* (TK)	14	14	37	10.1	1.2
7.	PL+63	21	0	27	9.4	1.5
8.	PL+52	21	6	25	10.6	1.3
9.	PL+58	23	21	6	9.4	1.0
10.	TK+63	12	8	14	11.6	2.0
11.	TK+52	14	14	0	12.5	1.7
12.	TK+58	14	12	0	11.4	2.2
		$LSD_{0.05}$ (Treatments) = 14.3 (Pathogens) = 7.1			2.3	0.61
FIELD EXPERIMENT						
1.	Control	58	11	19	19.1	2.1
2.	*P.aeruginosa* (CMG63)	8	0	8	25.5	2.6
3.	*P.aeruginosa* (CMG52)	41	41	11	23.1	2.5
4.	*P.aeruginosa* (CMG58)	6	11	8	24.4	1.7
5.	*P.lilacinus* (PL)	19	19	0	24.4	1.7
6.	*T.koningii* (TK)	50	16	8	24.1	2.2
7.	PL+63	25	0	0	24.9	3.1
8.	PL+52	19	0	0	23.5	2.7
9.	PL+58	19	27	0	25.6	3.1
10.	TK+63	11	0	11	21.6	1.5
11.	TK+52	25	19	19	23.3	3.3
12.	TK+58	33	0	8	23.4	2.7
		$LSD_{0.05}$ (Treatments) = 15.4 (Pathogens) = 7.7			3.9	0.92

pathogens including *F. oxysporum* in banana (Sivamani, Gnanamanickam, 1988) and *Verticillium dahliae* on potato (Leben *et al.*, 1987). *P. aeruginosa* has also been reported to reduce growth of *R. solani, Sclerotium rolfsii* and *F. solani* (Podile et al., 1988). It is interesting to note that different strains of *P. aeruginosa* showed variability against different pathogens. Presumably like rhizobia (Chao, 1990), the antagonistic ability of *P. aeruginosa* also varies with strains. Since use of some strain of *P. aeruginosa* with *P. lilacinus* or *T. koningii* showed better control of *F. solani* than either used alone. A better control of *Gaeumannomyces graminis* var. *tritici* was observed by *T. koningii* when used with fluorescent *Pseudomonas* (Duffy, Weller, 1992). Use of rhizobia with *Trichoderma* spp., have given better results in the control of root rot pathogens on fenugreek as compared to their separate use

(Ehteshamul-Haque, Ghaffar, 1992). Combined use of *T. koningii* with *P. aeruginosa* also showed an increase in plant height and better fresh weight of shoot as compared to their separate use. In the rhizosphere, population of bacteria and fungi may avoid competition by colonizing different niches and or by some degree of spatial separation within the rhizosphere. Combination of compatible bacteria and fungi may therefore provide better control of seed and root rot pathogens than either used alone (Chao et al., 1986).

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Skuteczność *Pseudomonas aeruginosa* i innych preparatów biologicznych w kontroli zgnilizny korzeni bawełny

Streszczenie

Zastosowanie *Pseudomonas aeruginosa*, *Paecilomyces Lilaciunus* i *Trichoderma koningii* znacznie ($p<0.05$) zmniejszyło występowanie zakażenia korzeni bawełny bakteriami *Macrophomina phaseolina*, *Rhizoctonia solani* i *Fusarium solani*, zarówno w eksperymentach polowych jak i doniczkowych. Wspólne zastosowanie *P. aeruginosa*, szczep CMG63 i *T. koningii* w eksperymentacyh polowych sprawiło większy przyrost roślin i świeżej masy pędów, zaś zastosowanie szczepu CMG52 dało lepsze rezultaty w eksperymentach doniczkowych.

Pojęcia kluczowe: *Pseudomonas aeruginosa*, preparaty biologiczne, zgnilizna korzeni bawełny.