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EFFICACY OF *PSEUDOMONAS AERUGINOSA* AND *BRADYRHIZOBIUM* SP., IN THE CONTROL OF ROOT ROT DISEASE IN CHICKPEA

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Abstract

Use of growth promoting becterium *Pseudomonas aeruginosa*, strain Pa_6 and Pa_{12} significantly ($p < 0.05$) reduced the infection of *Macrophomina phaseolina* and *Rhizoctonia solani* on chickpea. *P.aeruginosa* strain Pa_{12} was found effective against *F.oxysporum* and Pa_6 against *F.solani*. Combined use of *Bradyrhizobium* sp., (TAL 480) and *P.aeruginosa* showed complete control of *R.solani* and *F.oxysporum* infection. Use of bradyrhizobia with *P.aeruginosa* also showed better control of *F.solani* infection than either used alone. Greater number of nodules per plant were produced where bradyrhizobia was used with strains of *P. aeruginosa* as compared to bradyrhizobia used alone.

Introduction

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents since the rhizosphere provides front line defence for roots against attack by pathogens (Weller, 1988). Of these, the fluorescent *Pseudomonas* were found effective biocontrol agents for the control of soilborne plant diseases (Bashan *et al.*, 1993). Many species of *Pseudomonas* promote plant growth and also reduce the population of deleterious rhizospheric fungi and bacteria when used as seed or root inoculation (Schroth & Hancock, 1981). There are reports where *P. fluorescens* and *P.putida* improved the growth of potatoes (Burr *et al.*, 1978), sugarbeet (Suslow & Schroth, 1982) and radish (Kloepper & Schroth, 1978). Some strains of *P.aeruginosa* were found to inhibit the growth of root rot pathogens viz., *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium oxysporum in vitro* (Shameem *et al.*, 1993). In recent past rhizobia, the root nodule bacteria was also found as a good biocontrol agent against root rot pathogens (Tu, 1978; Ehteshamul - Haque & Ghaffar, 1993). Experiments were therefore carried out to study the effect of *P.aeruginosa* with or without rhizobia in the control of root rot disease of chickpea caused by *M.phaseolina, R.solani* and *Fusarium* spp.

Materials and Methods

Seeds of chickpea (*Cicer arietinum*) after treatment with 5 day old culture of *Bradyrhizobium* sp., (cfu $3x10^9$) using 1% gum arabic as sticker were sown in 8 cm diam., plastic pots each containing 250 gm soil. After sowing the seeds, inoculum of *P.aeruginosa* strains, Pa_5 (cfu $1.3x10^8$), Pa_6 (cfu $3.1x10^8$), Pa_3 (cfu $2.3x10^8$), and Pa_{12} (cfu $5.5x10^8$) were drenched in each pot @ 25 ml /pot. A set of pots inoculated with

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different strains of *P.aeurignosa* and bradyrhizobia were also kept for comparision. Pots without bacterial inoculum served as control. The soil had a natural infestation of 3-10 sclerotia of *M. phaseolina* g^{-1} of soil as found by wet seiving 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). There were 4 replicates of each treatment and 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% WHC (Keen & Raczkowski, 1921).

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. Data were analysed and subjected to Factorial ANOVA (FANOVA) followed by least significant differences (LSD) according to Gomez & Gomez (1984).

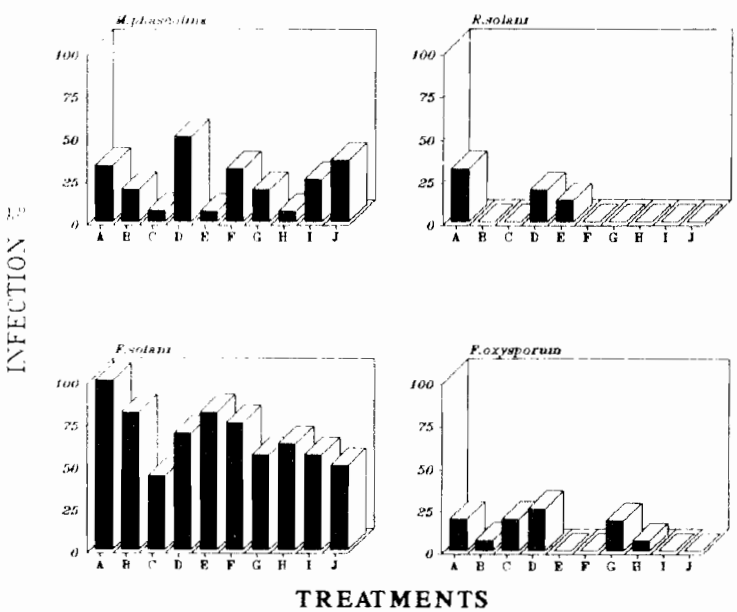

Fig.1. Effect of strains *Pseudomonas aeruginosa* (Pa) and *Bradyrhizobium* sp., in the control of root rot disease of chickpea.

A = Control, B = Pa_5, C = Pa_6, D = Pa_3, E = Pa_{12},
F = *Bradyrhizobium* sp., G = bradyrhizobia + Pa_5,
H = bradyrhizobia + Pa_6, I = bradyrhizobia + Pa_3,
J = bradyrhizobia + Pa_{12}

$LSD_{0.05}$ (Treatments) = 14.9

$LSD_{0.05}$ (Pathogens) = 8.6

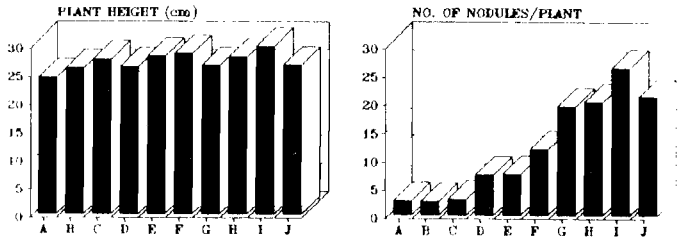

Fig.2 Effect of strains *Pseudomonas aeruginosa* (Pa) and *Bradyrhizobium* sp., on plant height and root nodulation in chickpea.

A = Control, B = Pa_5, C = Pa_6, D = Pa_3, E = Pa_{12},
F = *Bradyrhizobium* sp., G = bradyrhizobia + Pa_5,
H = bradyrhizobia + Pa_6, I = bradyrhizobia + Pa_3,
J = bradyrhizobia + Pa_{12}
$LSD_{0.05}$ (Plant height) = 3.8
$LSD_{0.05}$ (Nodulation) = 10.8

Results

Use of *P.aeruginosa* strains Pa_6 and Pa_{12} alone or strain Pa_6 with bradyrhizobia significantly ($p<0.05$) controlled *M.phaseolina* infection in chickpea (Fig.1). Complete control of *R.solani* infection was observed in treatments where Pa_5, Pa_6 and bradyrhizobia were used alone or where *Bradyrhizobium* sp., was mixed with *Pseudomonas* strains Pa_3, Pa_5, Pa_6 or Pa_{12}. Significant ($p<0.05$) control of *F.solani* infection was produced where Pa_6 was used alone or where *Bradyrhizobium* was used with Pa_5, Pa_3 or Pa_{12}. Similarly complete control of *F.oxysporum* was found where Pa_{12} or bradyrhizobia were used alone or where bradyrhizobia was used with Pa_3 or Pa_{12} (Fig.1). Greater plant height and number of nodules were produced where *Bradyrhizobium* was used with Pa_3 (Fig.2).

Discussion

In the present study, strains of *P.aeruginosa* showed significant ($p<0.05$) results in controlling the infection of *M.phaseolina, R.solani* and *Fusarium* spp., on chickpea. Among the fluorescent *Pseudomonas*, *P. fluorescens* is known to reduce infection of many 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. *Bradyrhizobium* sp., also showed good control of *F.solani* and *F.oxysporum*. Some strains of rhizobia have shown promising results in the control of root rot pathogens (Ehteshamul - Haque & Ghaffar, 1993). In

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