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USE OF *VERTICILLIUM CHLAMYDOSPORIUM* IN THE BIOLOGICAL CONTROL OF ROOT-ROT DISEASE OF CHICKPEA

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Abstract

Verticillium chlamydosporium isolated from eggs of *Meloidogyne incognita*, root knot nematode, inhibited the growth of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F.oxysporum in vitro*. In a field experiment *V.chlamydosporium* was found more or equally effective than *Paecilomyces lilacinus, Talaromyces flavus* and *Bradyrhizobium japonicum* in controlling the infection of *M.phaseolina, R.solani, F.oxysporum* and *F.solani* in chickpea. Combined use of *V.chlamydosporium* and *B. japonicum* showed better control of *F.oxysporum* than their separate use. Combined use of *B.japonicum* and *T.flavus* produced greater plant height and fresh weight of shoot in chickpea.

Introduction

Whereas pesticides produce environmental hazards, use of biocontrol agents in the control of plant diseases has given promising results. It is desirable that a single biocontrol agent should have the potential to control more than one pathogen. Of the various biocontrol agents, *Verticillium chlamydosporium* found in cyst and soil in many parts of the world (Rodriguez-kabana *et al.*, 1984.) has been identified as an egg parasite of root knot nematode in Pakistan (Zaki & Maqbool, 1993a). The fungus was found to inhibit the radial growth of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani in vitro*. Experiments were therefore carried out to see the effect of *V.chlamydosporium* in the control of root rot disease of ckickpea. The efficacy of *V.chlamydosporium* was also compared with other biocontrol agents viz., *Paecilomyces lilacinus*, an egg parasite of root knot nematode (Jatala, 1985) and *Talaromyces flavus* (Fahima & Henis, 1990) with or without *Bradyrhizobium japonicum*.

Materials and Methods

Pure culture of *V. chlamydosporium* isolated from eggs of root-knot nematode (Zaki & Maqbool, 1993) and cultures of root infecting fungi viz., *R. solani, M. phaseolina, F. solani* and *F. oxysporum* isolated from roots of infected chickpea plants, were obtained from Karachi University Mycological Culture Collection, Department of

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Table 1. Inhibition of growth of root infecting fungi by *Verticillium chlamydosporium* in dual culture plates.

Pathogens	Zone of inhibitions mm
Rhizoctonia solani	2
Macrophomina phaseolina	8
Fusarium solani	17
F. oxysporum	20

Botany, University of Karachi were used. Five mm diam., disc of actively growing culture of *V. chlamydosporium* was placed on Czapek's Dox Agar in 90 mm diam., Petri dishes approximately 65 mm apart from the test organisms. Each fungus was also inoculated separately as control. There were three replicates of each treatment. The Petri dishes were incubated at $25 \pm 1^{o}C$ and growth was observed daily.

In another set culture of *Bradyrhizobium japonicum* (TAL 102) obtained from Nitrogen Fixation in Tropical Legumes, Hawaii (NifTAL), *P. lilacinus*, *V. chlamydosporium* and *T. flavus* from KUMH culture collection, University of Karachi, Karachi, Pakistan, were used. The inoculum of fungi were multiplied on sterilized rice grains while rhizobia was multiplied on wheat bran used as substrate. One g of infested wheat bran inoculum contained 10^{9} cfu of *B. japonicum*. Inoculum of *V. chlamydosporium*, *P. lilacinus* and *T. flavus* multiplied on rice grain were mixed with sterilized rice grain which gave an equal population of $0.1x10^{8}$ cfu g^{-1} for each fungal antagonist. Experiments were carried out in 2x1 meter microplots at the Department of Botany, University of Karachi in randomized complete block design with 3 replicates in December 1993. The soil had a natural infestation of 5-11 sclerotia of *M. phaseolina* g^{-1} of soil as found by using 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). Biocontrol agents were applied in soil in rows @ 130 g/1 meter row when used alone and @ 65 g/1 meter row when fungal inoculum was used with rhizobial inoculum (@ 65 g / 1 meter row), to give a final inoculum of 130 g / row. After inoculation of soil with biocontrol agents, 20 seeds of chickpea cv., CM-68 were sown in 1 meter rows. In a comparable set soil without inoculum or where sterilized rice grain or wheat bran were used served as control.

Plants were uprooted after 40 days of growth. Five 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 $28^{o}C$ incidence of root infecting fungi viz., *M. phaseolina*, *R. solani*, *F. solani* and *F.oxysporum* were recorded. Data were analysed and subjected to Factorial ANOVA (FANOVA) followed by least significant differences (LSD) according to Gomez & Gomez (1984).

Results

In dual culture plate assays *V. chlamydosporium* was found to inhibit radial growth of *R. solani, M. phaseolina, F. solani* and *F. oxysporum*, respectively, producing zones of inhibition of 2, 8, 17 and 20 mm (Table 1).

More than 50% control of *M.phaseolina* infection was produced where *B.japonicum, V.chlamydosporium, P.lilacinus* were used alone or where *B.japonicum* was mixed with *P.lilacinus* and *T.flavus*. Similarly more than 50% control in *R.solani* infection was observed where *B.japonicum, V.chlamydosporium* were used alone or where *B.japonicum* was used with *V.chlamydosporium* or *T.flavus*. Infection of *F.solani* reduced by more than 50% only in the treatment where *B.japonicum* was mixed with *T.flavus*. Similarly more than 50% control of *F.oxysporum* infection was produced in the treatments where *B.japonicum, V.chlamydosporium, P.lilacinus* or *T.flavus* were used alone or where fungal antagonists were separately mixed with *B.japonicum*. *T.flavus* alone did not produce more than 50 % reduction in *M.phaseolina* and *F.solani* infection (Fig. 1).

Greater fresh weight of shoots was produced in plants treated with mixed inoculum of *B. japonicum* and *T. flavus* followed by *B. japonicum* used alone. Highest plant height was observed in treatments where *T. flavus* was mixed with *B. japonicum* followed by *P.lilacinus* used alone (Fig. 2).

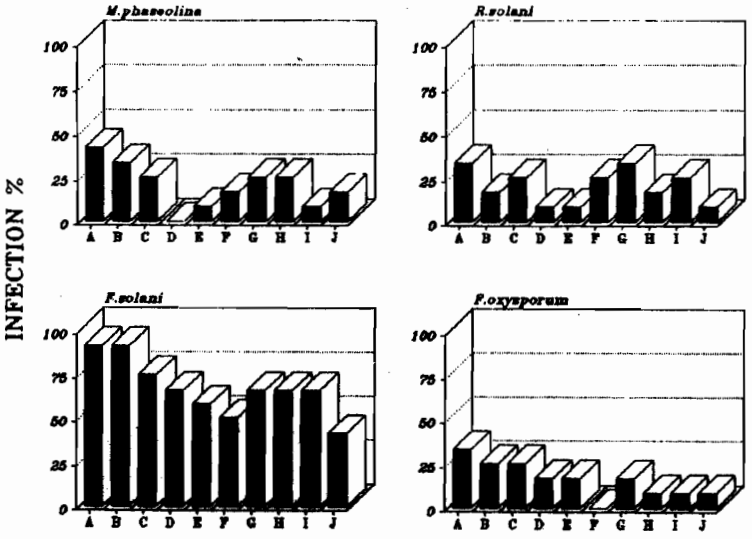

Fig. 1. Effect of biocontrol agents with *Bradyrhizobium japonicum* in the control of root-rot disease of chickpea:

A = Control, B = Rice grain, C = Wheat bran, D = *Verticillium chlamydosporium*, E = *Paecilomyces lilacinus*, F = *Talaromyces flavus*, G = *B. japonicum*, H = *V. chlamydosporium* + *B. japonicum*, I = *P. lilacinus* + *B. japonicum*, J = *T. flavus* + *B. japonicum*.

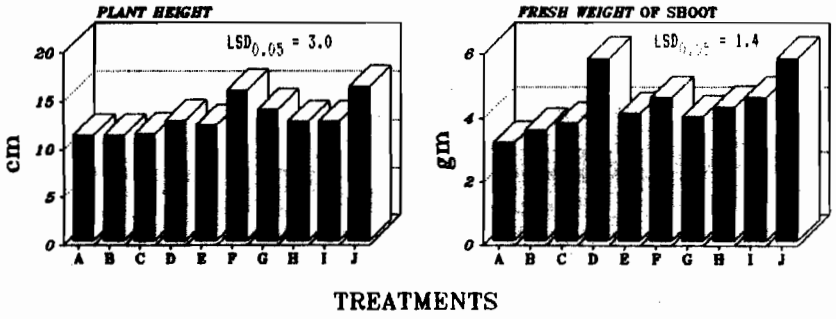

Fig. 2. Effect of biocontrol agents with *Bradyrhizobium japonicum* on fresh weight of shoot and plant height of chickpea:
A = Control, B = Rice grain, C = Wheat bran, D = *Verticillium chlamydosporium*, E = *Paecilomyces lilacinus*, F = *Talaromyces flavus*, G = *B. japonicum*, H = *V. chlamydosporium* + *B. japonicum*, I = *P. lilacinus* + *B. japonicum*, J = *T. flavus* + *B. japonicum*.

Discussion

In the present study microbial antagonists viz., *B.japonicum, V.chlamydosporium, P.lilacinus* and *T.flavus* showed significant control of *M.phaseolina, R.solani, F.solani* and *F.oxysporum* infection in chickpea. *B.japonicum* is known to secrete rhizobitoxine (Chakraborty & Purkayastha, 1984), which significantly controlled the infection of *M.phaseolina, R.solani* and *Fusarium* spp., on sunflower, okra, soybean and mungbean (Ehteshamul - Haque & Ghaffar, 1993). Similarly *P.lilacinus*, a parasite of eggs of root knot nematode (Jatala, 1985) significantly reduced the infection of root infecting fungi on sunflower, okra, soybean and mungbean (Ehteshamul - Haque *et al.*, 1990). *T.flavus* is also known as a parasite of microsclerotia of *Verticillium dahliae* (Fahima & Henis, 1990) and sclerotia of *Sclerotinia sclerotiorum* (McLaren *et al.*, 1989). In the present study *V.chlamydosporium*, a parasite of root knot and cyst nematode (deLeij, 1992) which showed antagonistic effect against *M.phaseolina, R.solani* and *F.solani in vitro* (Zaki & Maqbool, 1993b), proved as a good biocontrol agent against *M.phaseolina, R.solani, F.solani* and *F.oxysporum*, the most common root rot pathogens of crop plants in Pakistan (Ghaffar, 1992). Combined use of rhizobia with *V.chlamydosporium* also increased their efficacy against *F.oxysporum*. It would suggest that besides *B.japonicum, P.lilacinus* and *T.flavus, V.chlamydosporium* also has a good potential to control the root rot disease of chickpea caused by *M.phaseolina. R.solani, F.solani* and *F.oxysporum* under field condition.

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