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Role of *Bradyrhizobium japonicum* and *Trichoderma* spp. in the control of root rot disease of soybean

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Seed treatment of soybean with *Bradyrhizobium japonicum*, *Trichoderma harzianum*, *T. viride*, *T. hamatum*, *T. koningii* and *T. pseudokoningii* significantly controlled the infection of 30-day-old seedlingsby *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. In 60-day-old plants *Trichoderma* spp., and *B. japonicum* inhibited the grouth of *R. solani* and *Fusarium* spp., whereas the use of *B. japonicum* (TAL-102) with *T. harzianum*, *T. viride*, *T. koningii* and *T. pseudokoningii* controlled the infection by *M. phaseolina*. Greater grain yield was recorded when *B. japonium* (TAl-102) was used with *T. hamatum*.

Key words: Role of *Bradyrhizobium*, *Trichoderma*.

INTRODUCTION

Seed treatment is an effective method of introducing biocontrol agents into the soil environment. Seed coating with *Trichoderma* spp., is known to protect the seeds as effectively as chemical pesticides (H a d a r, H a r m a n, T a y l o r, 1984; H a r m a n, C h e t, B a k e r, 1980; H e n i s, G h a f f a r, B a k e r, 1978). The antagonists applied to the seeds may have the opportunity to be the first colonizer of roots (C h a o et al., 1986). In legumes the application of biocontrol agents constitutes a practical problem since some fungi inhibit (K h a n, K h a l i l, 1989) and some have a stimulatory effect on the development of rhizobia (B u t t, G h a f f a r, 1972; A b i d et al., 1992). The ability of rhizobia to inhibit certain soilborne plant pathogens (C h a k r a b o r t y, P u r k a y a s t h a, 1984; Z a k i, G h a f f a r, 1987) has increased the importance of rhizobia in addition to their use in nitrogen fixation. Since *Trichoderma* and rhizobia have no negative effect on rhizobia (H a r m a n, C h e t, B a k e r, 1981), an experiment was therefore performed to determine the role of two isolates of *Bradyrhizobium japonicum* used the role or mixed with *T. harzianum*, *T. viride*, *T. hamatum*, *T. koningii* and *T. pseudokoningii* in the control of root rot caused by *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., in soybean.

MATERIAL AND METHODS

Experiments were performed in Ferbruary, 1992 in 2 x 1 metre microplots of the Department of Botany, University of Karachi in complete randomized block design in triplicate. Five day old cultures of *B. japonicum* (KUMH 569 – TAL 102) from NifTAL, *B. japonicum* (KUMH 572 – Local isolate), *Trichoderma harzianum* (KUMH 115), *T. viride* (KUMH 656), *T. hamatum* (KUMH 29), *T. koningii* (KUMH 427) and *T. pseudokoningii* (KUMH 93) obtained from Karachi University culture collection were used. Cell suspension of rhizobia (4×10^9 cfu ml^{-1}) or conidial suspension of *Trichoderma* (5×10^8 cfu ml^{-1}) alone or mixed with each other were used as seed dressing with 1 % arabic gum as sticker. Soybean (*Glycine max*) was used as the test plant. Thirty seeds were planted in 2 metre furrows. The soil had a natural infestation of 5-11 sclerotia of *M. phaseolina* g^{-1} of soil as found by using wet seiving and dilution technique (S h e i k h, G h a f f a r, 1975), 8 % colonization of *R. solani* on sorgum seeds used as baits (W i l h e l m, 1955) and 3500 cfu g^{-1} of soil of a mixed population of *F. oxysporum* and *F. solani* as assessed by soil dilution technique (N a s h, S n y d e r, 1962).

Plants were uprooted after 30 and 60 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 (100 000 units/litre) and streptomycin (0.2 mg/litre). After the samples had been incubated for 5 days at 28°C the incidence of root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., was recorded. Dry grain yield per plant was also reocrded. Data were analysed and subjected to Factorial NOVA (FANOVA) the significance of difference (LSD) was determined according to G o m e z and G o m e z (1984).

RESULTS

No significant difference were found in seed germination. More than 50 % reduction in *M. phaseolina* infection was onserved in 30-day-old soybean seedlings when *T. harzianum, T. viride, T. koningii, T. pseudokoningii* and *B. japonicum* (KUMH 569 & KUMH 572) were used alone. The application of *B. japonicum* (KUMH 569) with *T. viride, T. koningii, T. pseudokoningii, T. hamatum* and *B. japonicum* (KUMH 572) with *T. harzianum, T. koningii, T. pseudokoningii* and *T. hamatum* inhibited the *M. phaseolina* infection by 50 % as compared to the control. In 60-day-old plants none of the antagonist used alone inhibited the growth of *M. phaseolina*, whereas the application of *B. japonicum* (KUMH 569) with *T. harzianum, T. viride, T. koningii* and *T. pseudokoningii* significantly ($p < 0.05$) reduced *M. phaseolina infection* (Fig. 1. I).

The infection of 30-day-old seedlings by *R. solani* was reduced by more than 50 % when *T. harzianum, T. koningii, T. pseudokoningii* and *B. japonicum* (KUMH 569 & KUMH 572) were used alone or when *B. japonicum* was mixed with all the species of *Trichoderma* except when *B. japonicum* (KUMH 527) was applied with

T. pseudokoningii. In 60 day old plants, *R. solani* infection was reduced by more than 50 % when *T. harzianum*, *T. viride*, *T. hamatum*, and *B. japonicum* (KUMH 569 & KUMH 572) were used alone or when *B. japonicum* (KUMH 569) was mixed with *T. harzianum*, *T. hamatum* and *B. japonicum* (KUMH 572) used with *T. hamatum*, *T. koningii* and *T. pseudokoningii* (Fig. 1. II).

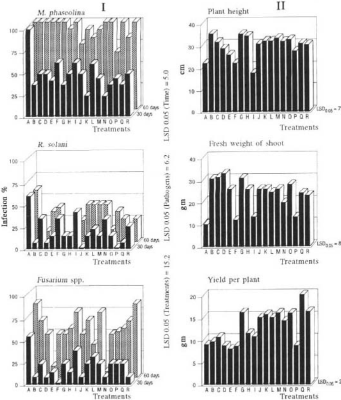

Fig. 1. I – Control of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* infection on soybean by different isolates of *Bradyrhizobium japonicum* nad *Trichoderma* spp., used as seed dressing, II – Effect of different isolates of *B. japonicum* and *Trichoderma* spp. used as seed dressing on plant height, fresh weight of shoot and yield of soybean

A – control, B – *T. harzianum*, C – *T. viride*, D – *T. pseudokoningii*, E – *T. koningii*, F – *T. hamatum*, G – *B. japonicum* (KUMH 569), H – *B. japonicum* (KUMH 572), I – B + G, J – B + H, K = C + G, L = C + H, M = D+H, O = E + G, Q = F + G, R = F + H

The infection by *Fusarium* spp., was reduced by more than 50 % in 30-day-old seedlings when *T. harzianum, T. viride, T. hamatum, T. koningii, T. pseudoconingii* and *B. japonicum* (KUMH 569 & KUMH 572) were present and when *B. japonicum* (KUMH 569) was mixed with *T. viride, T. koningii, T. pseudokoningii* and *T. hamatum* or when *B. japonicum* (KUMH 572) was used with *T. harzianum, T. koningii, T. pseudokoningii* and *T. hamatum*. In 60 day old plants the infection by *Fusarium* spp. was reduced by 50 % when *T. pseudokoningii* was used and when *B. japonicum* (KUMH 569) was mixed with *T. viride* and *T. pseudokoningii* (Fig. 1).

No significant differences were found in the fresh weight of shoot and plant height in 30-day-old seedlings whereas in 60-day-old plants greater plant height was reduced by *T. harzianum, B. japonicum* (KUMH 569) mixed with *T. pseudokoningii*. The highest fresh weight of shoot was produced by *T. harzianum, T. pseudokoningii, T. viride, B. japonicum* (KUMH 569) used alone and when *B. japonicum* (KUMH 569) was mixed with *T. koningii*. Greater grain yield was recorded in treatment when *B. japonicum* was used with *T. hamatum* (Fig. 1).

No significant difference in root nodulation were observed in treated and or untreated plants.

DISCUSSION

In the present study *B. japonicum* and *Trichoderma* spp., showed promising resluts in controlling the infection of soybean roots by phaseolina, *R. solani* and *Fusarium* spp., Harman, Chet, Baker (1980) reported that seed treatments with *T. hamatum* protect seeds and seedlings of radish and pea from the invasion of *R. solani* and *Pythium* spp. Crown rot of tomato caused by *Fusarium* spp., has been reduced in soil infested with *T. harzianum* (Sivan, Ucko, Chet, 1987). *Trichoderma koningii* reduced the damping off of pea caused by *Pythium* spp., (Lifshitz, Windham, Baker, 1986). Seed dressing or soil drench with *T. harzianum* and *T. viride* reduced the infection of *M. phaseolina, R. solani* and *Fusarium* spp. on okra, sunflower, mustard, soynbean nad mungbean (Ehteshamul-Haque, Ghaffar, Zaki, 1990; Ehteshamul-Haque, Ghaffar, 1991). It is recognized that *B. japonicum* parasitize the hyphae of *Phytophthora megasperma* (Tu, 1978) and reduced root rot of soybean, mungbean, sunflower, and okra caused by *M. phaseolina, R. solani* and *Fusarium* spp., (Ehteshamul--Haque, Ghaffar, 1993). Duffy nad Weller (1992), reported better control of *Gaeumannomyces graminis* var. *tritici* by *T. koningii* used with fluorescent *Pseudomonas* spp. In the present study, the mixed inoculum of *B. japonicum* and *Trichoderma* spp., also controlled the infection of soybean by *M. phaseolina* and *Fusarium* spp., especially in 60 day old plants. Greater yield was also recorder when *B. japonicum* was used with *T. hamatum*. There are reports which indicate that *T. viride* (Gangawane, Salve, 1987) and *Strachybotrys atra* (Butt, Ghaffar, 1972) stimulated the development rhizobia. *Bradyrhizobium* spp., and

Trichoderma did not have significant effect each other (C h a o, 1990). Treatment of pea seeds with both *Rhizobium* and *T. hamatum* also had no negativ effect on each other (H a r m a n, C h e t, B a k e r, 1981). In the rhizosphere, the population of bacteria and fungi may avoid competition by colonizing different niches and/or by some degree of spatial separation within the rhizosphere.

CONCLUSION

A combination of bacteria and fungi may therefore provide better control of seed and root rot pathogens than ether used alone (C h a o et al., 1986). *Bradyrhizobium japonicum* and *Trichoderma* spp., play an important role in the control of root rot disease of soybean resulting in increased crop productivity.

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