Pak.J.Bot., 24(2):217-221, 1992.

EFFICACY OF *TRICHODERMA* SPP., AND *RHIZOBIUM MELILOTI* IN THE CONTROL OF ROOT ROT OF FENUGREEK

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Abstract

Trichoderma viride, T. hamatum and *Rhizobium meliloti* used as seed dressing and or as soil drench reduced *Macrophomina phaseolina* infection by more than 50% on 30 day old fenugreek seedlings. Combined use of *T. koningii* and *R. meliloti* showed better control of *M. phaseolina* infection on both 30 and 60 day old plants. *T. harzianum, T. hamatum, T. pseudokoningii* and *R. meliloti* (KUMH 555 & KUMH 653) used either as seed dressing and or as soil drench completely controlled the infection of *Rhizoctonia solani* both on 30 and 60 day old plants. Combined use of *T. viride* and *T. koningii* with rhizobia were also found effective. *T. harzianum, T. koningii* alone or *R. meliloti* used with *T. viride, T. hamatum, T. koningii* and *T. pseudokoningii* completely controlled the infection of *Fusarium* spp., on 30 day old seedlings, while in 60 day old plants more than 50% reduction was found by *Trichoderma* spp., and *R. meliloti* used alone or rhizobia mixed with *Trichoderma* spp., when used either as seed dressing and or as soil drench.

Introduction

Biological control of soilborne root infecting fungi like *Sclerotium rolfsii* Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn. and *Fusarium* spp., by *Trichoderma* spp., has been reported (Chet *et al.*, 1981; Elad *et al,* 1971; Bell *et al,* 1982; Wells *et al,* 1972; Ghaffar, 1992). In legumes the application of biocontrol agents has a practical problem, since some fungi inhibit (Khan & Khalil, 1989) and some have stimulatory effect on rhizobia (Butt & Ghaffar, 1972, Abid *et al.,* 1992). The ability of rhizobia to inhibit certain soilborne plant pathogens (Chakraborty & Purkayastha, 1984; Zaki & Ghaffar, 1987) has increased the importance of rhizobia besides their use in nitrogen fixation. An experiment was therefore, carried out to study the effect of three isolates of *Rhizobium meliloti* used alone or mixed with *T. harzianum, T. viride, T. hamatum, T. koningii* and *T. pseudokoningii* in the control of root rot of fenugreek caused by *M. phaseolina, R. solani* and *Fusarium* spp.

Materials and Methods

Experiments were carried out in January, 1992 in screen house of the Department of Botany, University of Karachi. Five day old cultures of *Rhizobium meliloti* (KUMH 139, *Medicago sativa* isolate) from USDA, *R. meliloti* (KUMH 555, *Melilotus indica* isolate & KUMH 653, *Trigonella foenum-graecum* 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

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collection were used. Cell or conidial suspension of rhizobia (cfu $3x10^9$ ml^{-1}) or *Trichoderma* (cfu $5.6x10^8$ ml^{-1}) alone or mixed with each other were used as seed dressing with 1% gum arabic as sticker. Fenugreek (*Trigonella foenum-graecum* L.) was used as test plant. Eight seeds were planted in 8 cm diam., plastic pots containing 250 gm of soil. In another treatment suspension of biocontrol agents were diluted 10 times in water and 25 ml suspension of each biocontrol agent was drenched in soil and adjusted at 50% W.H.C. with water. The soil had a natural infestation of 4-9 sclerotia of *M. phaseolina* g^{-1} of soil as found by using wet seiving and dilution technique (Sheikh & Ghaffar, 1975), 5% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3500 cfu of mixed population of *F. oxysporum* and *F. solani* as assessed by soil dilution technique (Nash & Snyder, 1962). Each treatment was replicated three times and pots were randomized on a screen house bench.

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 (100000 units/litre) and streptomycin (0.2 gm/litre). After incubation for 5 days at 28°C incidence of root infecting fungi viz., *M. phaseolina*, *R. solani* and *Fusarium* spp., were recorded.

Results

More than 50% reduction in *M. phaseolina* infection in 30 day old fenugreek seedlings was observed where *T. viride, T. hamatum* and *R. meliloti* (KUMH 555) were used alone as seed dressing and or as soil drench while application of *T. hamatum* with *R. meliloti* (KUMH 139 & KUMH 653) showed more than 70% control of *M. phaseolina* infection. In 60 day old plants *T. harzianum* alone or combined use of *T. koningii* and *R. meliloti* (KUMH 555, KUMH 139 & KUMH 653) and *T. pseudokoningii* with *R. meliloti* (KUMH 555) reduced *M. phaseolina* infection by more than 50%.

Complete control of *R. solani* infection was found both in 30 and 60 day old plants where *T. harzianum, T. pseudokoningii, T. hamatum* and *R. meliloti* (KUMH 555 & KUMH 653) were used either as seed dressing or as soil drench. Combined use of *R. meliloti* (KUMH 555) with *T. viride, T. koningii*, *T. pseudokoningii*; *R. meliloti* (KUMH 139) with *T. harzianum, T. hamatum*, *T. koningii* and *R. meliloti* (KUMH 653) with *T. harzianum, T. hamatum, T. koningii* and *T. pseudokoningii* completely controlled *R. solani* infection both on 30 and 60 day old plants when used either as seed dressing and or as soil drench.

Complete control of infection by *Fusarium* spp., on 30 day old seedlings was found where *T. harzianum, T. koningii* were used alone or *R. meliloti* (KUMH 555) with *T. viride, T. koningii* and *T. pseudokoningii*; *R. meliloti* (KUMH 139) with *T. viride, T. hamatum, T. koningii* and *T. pseudokoningii*; *R. meliloti* (KUMH 653) with *T. viride, T. hamatum* and *T. koningii* were used either as seed dressing and or as soil drench. In 60 day old plants more than 50% reduction in *Fusarium* infection was produced where *T. harzianum, T. viride, T. hamatum, T. koningii, T. pseudokoningii* and *R. meliloti* (KUMH 555, KUMH 139 & KUMH 653) were used alone or rhizobia mixed with *Trichoderma* spp., and used either as seed dressing and or as soil drench.

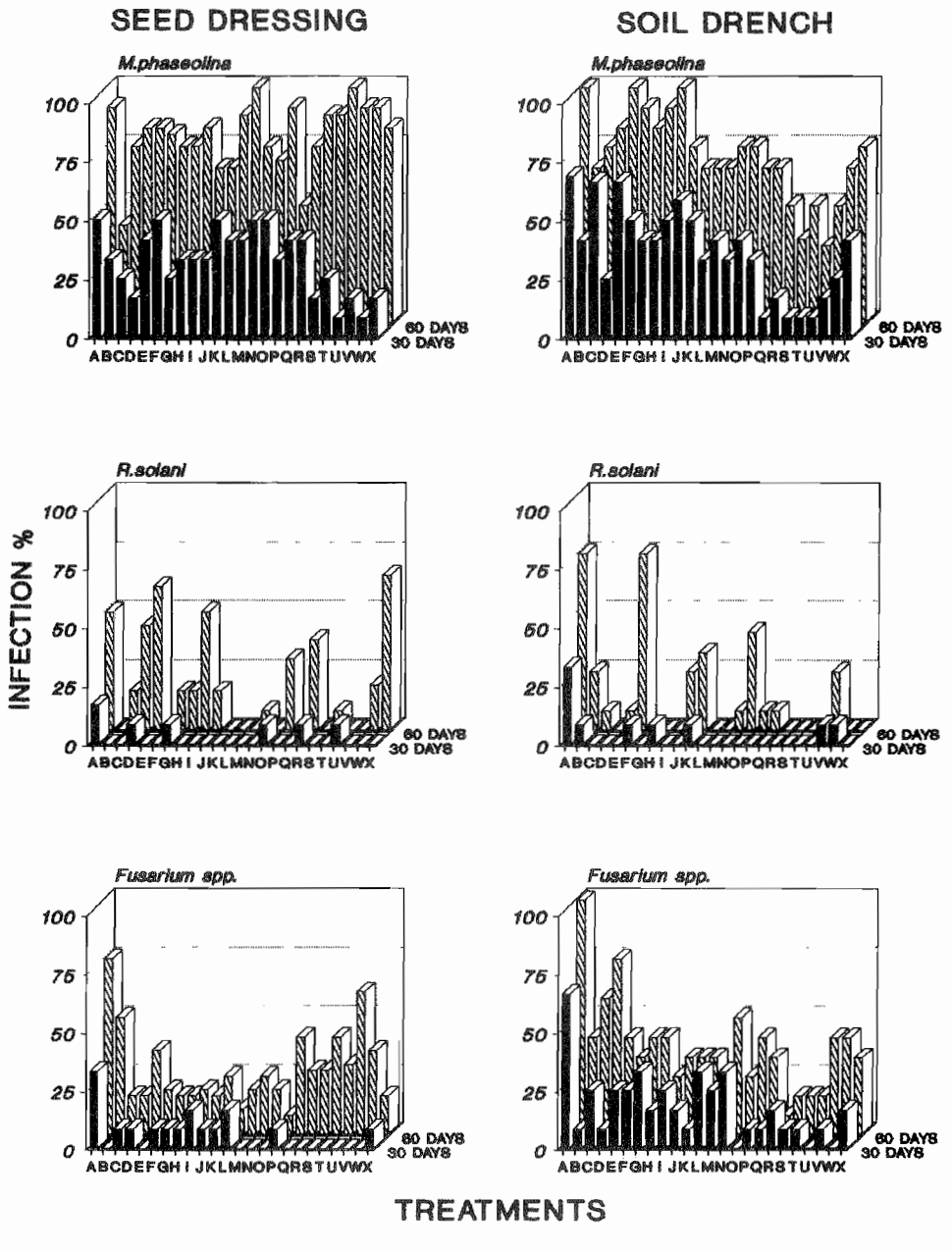

Fig.1. Control of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* infection on fenugreek by *Trichoderma* spp., and different isolates of *R. meliloti* used as seed dressing or as soil drench:
A = Control, B = *T. harzianum*, C = *T. viride*, D = *T. hamatum*, E = *T. koningii*, F = *T. pseudokoningii*, G = *R. meliloti* (KUMH 555), H = *R. meliloti* (KUMH 139), I = *R. meliloti* (KUMH 653), J = B + G, K = B + H, L = B + I, M = C + G, N = C + H, O = C + I, P = D + G, Q = D + H, R = D + I, S = E + G, T = E + H, U = E + I, V = F + G, W = F + H, V = F + I.

No significant difference was found in root nodulation among treated and or untreated plants.

Discussion

In the present study *Trichoderma* spp., and *R. meliloti* isolates showed promising results in controlling the infection of *M. phaseolina, R. solani* and *Fusarium* spp., on fenugreek roots. *Trichoderma* spp., are known to produce antibiotics that are active against pathogens *in vitro* (Dennis & Webster, 1971). Harman *et al.,* (1980) reported that seed treatments with *T. hamatum* protect seeds and seedlings of radish and pea from attack of *R. solani* and *Pythium* spp. Crown rot of tomato caused by *Fusarium* spp., have been reduced in soil infested with *T. harzianum* (Sivan *et al.,* 1987). *T. koningii* reduced preemergence damping off of pea caused by *Pythium* spp., (Lifshitz *et al.,* 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, soybean and mungbean (Ehteshamul-Haque *et al.,* 1990, Ehteshamul-Haque & Ghaffar, 1991). At a given population of *Phytophthora megasperma* and *F. oxysporum* higher level of *R. meliloti* reduced root rot of lucerne (Tu, 1980). *In vitro* hyphal growth of *F. oxysporum* was suppressed by multiplication of *R. meliloti* in a mixed culture, while in soil it reduced severity of root rot of lucerne (Sawada, 1982). Duffy & Weller (1992), reported better control of *Gaeumannomyces graminis* var. *tritici* by *T. koningii* used with fluorescent *Pseudomonas* spp. In our study use of mixed inoculum of *R. meliloti* and *Trichoderma* spp., also showed good control of infection of *M. phaseolina* and *Fusarium* spp., on fenugreek. *T. viride* is known to stimulate rhizobia (Gangawane & Salve, 1987). Treatment of pea seeds with both *Rhizobium* and *T. hamatum* had no adverse effect on each other (Harman *et al.*, 1981). It would suggest that use of *Trichoderma* spp., and *R. meliloti* has great potential to control the root rot disease of fenugreek seedlings.

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(Received for Publication 10 September 1992)