Pak. J. Bot., 22(2): 121-124, 1990.

BIOLOGICAL CONTROL OF ROOT ROT DISEASES OF OKRA, SUNFLOWER, SOYBEAN AND MUNGBEAN

SYED EHTESHAMUL-HAQUE, A. GHAFFAR AND M.J. ZAKI

Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Microbial antagonists viz, *Trichoderma harzianum, Gliocladium virens, Paecilomyces lilacinus, Bacillus subtilis* and *Streptomyces* sp., were used as seed treatment or as soil drench for the control of root rot disease caused by *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp., on okra, sunflower, soybean and mungbean under field conditions. Significant reduction in *M. phaseolina* infection of okra, sunflower, soybean and mungbean was produced by *G. virens; T. harzianum* and *P. lilacinus* reduced infection on soybean and okra; *B. subtilis* in soybean and sunflower while *Streptomyces* sp., was effective only on soybean. *T. harzianum, G. virens*; and *P. lilacinus* significantly reduced *R. solani* infection on okra, sunflower, soybean and mungbean; *B. subtilis* and *Streptomyces* sp., on okra, sunflower and soybean. Infection of *Fusarium* spp., in okra and soybean significantly reduced by *T. harzianum, G. virens, P. lilacinus, B. subtilis* and *Streptomyces* sp., on sunflower by *T. harzianum, G. virens* and *P. lilacinus*, and on mungbean by *T. harzianum* and *B. subtilis*.

Introduction

Of the various disease causing organisms, the soil borne root infecting fungi are ubiquitous and cause severe damages to crop plants world wide. During a survey of root infecting fungi *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Khun and *Fusarium* spp., were found infecting a variety of host plants in Pakistan (Ghaffar, 1988). Considering the cost of chemical pesticides and the hazards involved in their use, biological control has been suggested as an alternate method of control (Mulder, 1979). Several of the microorganisms isolated from soil appeared promising in *in vitro* tests in inhibiting growth of root infecting fungi (Ghaffar, 1988). The present report describes the effect of some of these microorganisms viz., *Trichoderma harzianum* Rifai, *Gliocladium virens* Miller, Giddings & Foster, *Paecilomyces lilacinus* (Thom) Samson, *Bacillus subtilis* Cohn and *Streptomyces* sp., used as seed dressing or as soil drench in the control of root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., on okra, sunflower, soybean and mungbean under field conditions.

Materials and Methods

Experiments were carried out during 1989 at the experimental field of the Department of Botany, University of Karachi. The soil had a natural infestation of *M. phaseolina* (3-8 sclerotia g^{-1} of soil), *R. solani* (5.7% colonization of millet seeds used

as baits), and *Fusarium* spp., (3146 conidia g^{-1} of soil including *F. oxysporum* 800 conidia and *F. moniliforme* 460 conidia g^{-1} of soil). Culture of *T. harzianum* (KUMH 115), *G. virens* (KUMH 464), *P. lilacinus* (KUMH 244), *B. subtilis* (KUMH 117) and *Streptomyces* sp., (KUMH 118) were grown on Potato Dextrose Agar (PDA) at 28°C for 5 days. Spore/cell suspensions were prepared using sterilized glass distilled water. The antagonists were either used as seed dressing (*T. harzianum* @ $80x10^8$ conidia ml^{-1}, *G. virens* @ $80x10^6$ conidia ml^{-1}, *P. lilacinus* @ $17x10^7$ conidia ml^{-1}, *B. subtilis* @ $102x10^7$ cells ml^{-1} and *Streptomyces* sp., @ $67x10^7$ conidia ml^{-1}) using 1% gum arabic as sticker, or as soil drench where 15 ml of each suspension was diluted with 300 ml of distilled water and applied in 5 feet furrows. Each treatment was replicated 3 times and randomized. Okra (*Abelmoschus esculentus* (L.) Moench), sunflower (*Helianthus annuus* L.), soybean (*Glycine max* (L.) Merr.) and mungbean (*Vigna radiata* (L.) Wilczek) were used as test plants. Plants were uprooted after 30 days growth. Ten one cm long root pieces, from each plant were surface sterilized for 2 minutes with 1% $Ca(OCl)_2$ before transfer on PDA containing penicillin (100,000 units/litre) and streptomycin (0.2 gm/litre). Plates were incubated for 4 days at 28°C for determining infection of root infecting fungi.

Results and Discussion

Of the microorganisms used, *T. harzianum* and *G. virens* gave better results than *P. lilacinus, B. subtilis* and *Streptomyces* sp., in the control of soil borne root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., in test plants of okra, sunflower, soybean and mungbean (Fig. 1). More than 50% reduction in infection of *M. phaseolina* was caused by *G. virens* on okra, sunflower, soybean and mungbean; by *T. harzianum* and *P. lilacinus* on okra and soybean, by *B. subtilis* on sunflower and soybean and by *Streptomyces* sp., on soybean when these organisms were used as seed dressing and or soil drench. Application of microorganisms as soil drench gave better results than as seed dressing (Fig. 1).

R. solani seemed to be more affected by microbial antagonists since more than 50% reduction in *R. solani* infection was observed with the use of *T. harzianum, G. virens* and *P. lilacinus* on okra, sunflower, soybean, and mungbean, and by *B. subtilis* and *Streptomyces* sp., on okra, sunflower and soybean when used as seed dressing and or soil drench.

Similarly more than 50% reduction in infection of *Fusarium* sp., was observed on okra and soybean by *T. harzianum, G. virens, P. lilacinus, B. subtilis* and *Streptomyces* sp., on sunflower by *T. harzianum, G. virens* and *P. lilacinus*, and on mungbean by *T. harzianum* and *B. subtilis* when used as seed dressing and or soil drench.

In the present study all the microbial antagonists viz., *T. harzianum, G. virens, P. lilacinus, B. subtilis* and *Streptomyces* sp., which gave promising results in the control of infection of *M. phaseolina, R. solani* and *Fusarium* spp., on okra, sunflower, soybean and mungbean are common soil saprophytes. Species of *Trichoderma* and *Gliocladium* are known to produce antibiotics that are active against pathogens *in vitro* (Dennis & Webster 1971a,b; Bell *et al.*, 1982). *Trichoderma* spp., showed direct growth towards the hyphae of *Pythium ultimum, R. solani* and *Sclerotium rolfsii* and

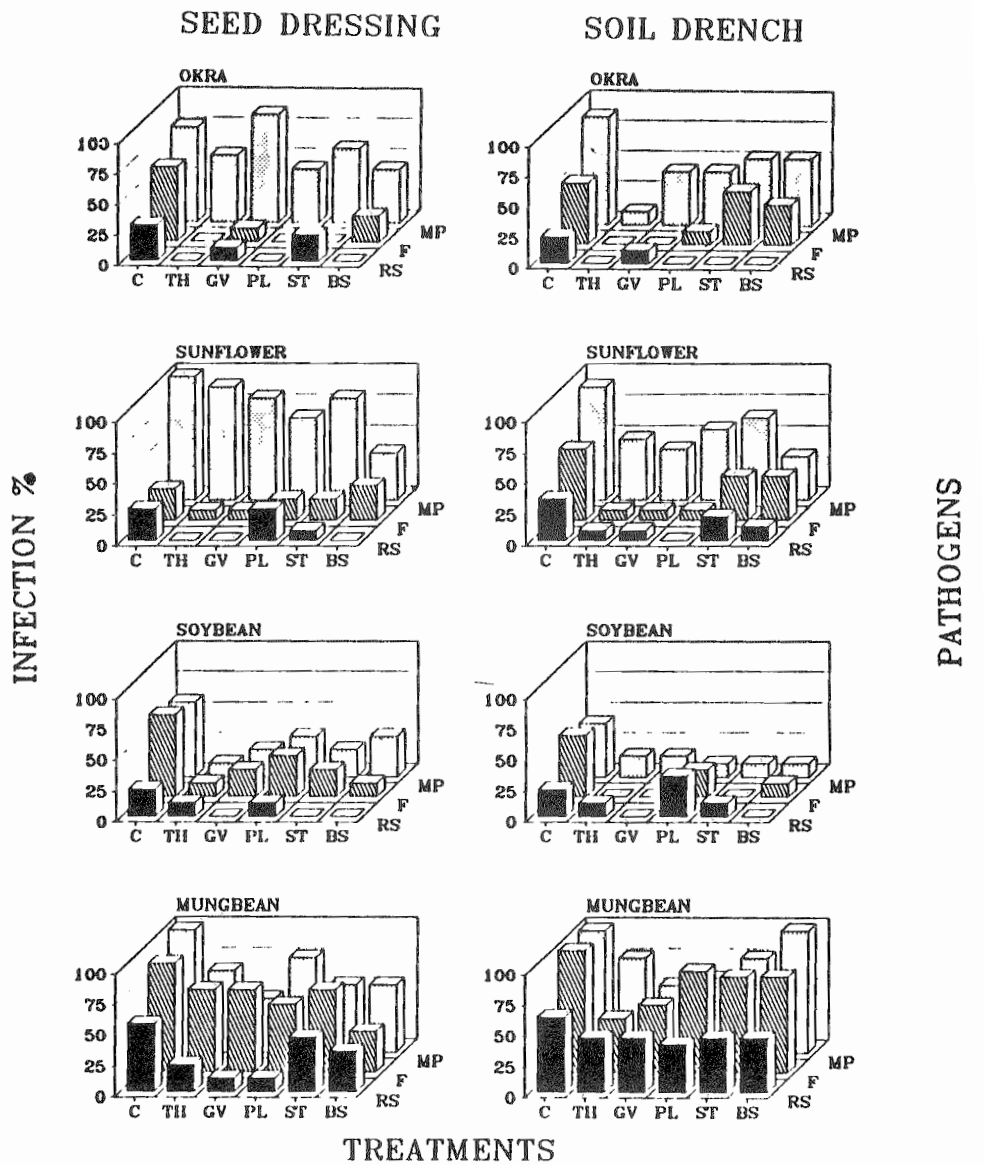

Fig.1. Effect of microbial antagonists used a seed dressing or as soil drench on infection of roots of okra, sunflower, soybean and mungbean by *Macrophomina phaseolina* (MP), *Rhizoctonia solani* (RS) and *Fusarium* spp., (F).

A = Control; B = *Trichoderma harzianum*; C = *Gliocladium virens*; D = *Paecilomyces lilacinus*; E = *Streptomyces* sp.; F = *Bacillus subtilis*.

after contact, various amounts of coiling were observed often accompanied by penetration (Chet *et al.*, 1981, Elad *et al.*, 1983). Seed dressing of cotton with *G. virens* protects seedling from damping off caused by *P. ultimum* and *R. solani* (Howell, 1982). *P. lilacinus* a parasite of *Meloidogyne* eggs significantly reduced *M. phaseolina* infection on mungbean and okra (Shahzad & Ghaffar, 1989). *B. subtilis* has been reported to produce antifungal metabolites which are inhibitory to many fungi (Swinburne *et al.*, 1975). Similarly *Streptomyces* spp., have shown increase in yield in some cases and have been used in the field on the basis of *in vitro* production of broad spectrum antibiotic (Broadbent, *et al.*, 1971).

The results of the present study would suggest that microorganisms can be used instead of chemical pesticides for the control of root rot diseases of crop plants caused either by a single fungal pathogen or by a group of root infecting fungi. Differences in the efficacy of antagonists on different crops is presumably due to certain ecological factors involved and different plant exudates. There is need to develop a methodology for large scale production of inoculum of microbial antagonists, minimum effective dose and its delivery in the soil environment for the control of soil borne root infecting fungi.

References

Bell, D.K., H.D. Wells and C.R. Markham. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal pathogens. *Phytopathology,* 72: 379-382.

Broadbent, P., K.F. Baker and Y. Waterworth. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soil. *Aust. Jour. Biol. Sci.,* 24: 925-949.

Chet, I., G.E. Harman and R. Baker. 1981. *Trichoderma hamatum*. Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microbial Ecology*, 7: 29-38.

Dennis, C. and J. Webster. 1971a. Antagonistic properties of species groups of *Trichoderma*. I-Production of non-volatile antibiotics. *Trans. Brit. Mycol. Soc.,* 57: 25-39.

Dennis, C. and J. Webster. 1971b. Antagonistic properties of species groups of *Trichoderma* II. Production of volatile antibiotics. *Trans. Brit. Mycol. Soc.,* 57: 41-48.

Elad, Y., I. Chet, P. Boyle and Y. Henis. 1983. Parasitism of *Trichoderma* spp., on *Rhizoctonia solani* and *Sclerotium rolfsii*. Scanning electron microscopy and fluorescence microscopy. *Phytopathology*, 73: 85-88.

Ghaffar, A. 1988. *Final research report of the Soilborne Diseases Research Centre*. Department of Botany, University of Karachi, Karachi-75270, Pakistan. 111 pp.

Howell, C.R. 1982. Effect of *Gliocladium virens* on *Pythium ultimum, Rhizoctonia solani* and damping off of cotton seedlings. *Phytopathology*, 72: 496-498.

Mulder, D. 1979. *Soil disinfestation*. Elsevier Scientific Publication 10, Amsterdam, 368 pp.

Shahzad, S. and A. Ghaffar. 1989. Use of *Paecilomyces lilacinus* in the control of root rot and root knot disease complex of okra and mung bean. *Pak. J. Nematol.*, 7: 47-53.

Swinburne, T.R., T.C. Barr and A.E. Brown. 1975. Production of antibiotics by *Bacillus subtilis* and their effect on fungal colonists of apple leaf scars. *Trans. Brit. Mycl. Soc.,* 65: 211-217.

(Received for publication 21 August 1990)