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EFFECT OF SEED TREATMENT WITH BIOLOGICAL ANTAGONISTS ON RHIZOSPHERE MYCOFLORA AND ROOT INFECTING FUNGI OF SOYBEAN

FARZANA ALI AND A. GHAFFAR

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

Abstract

Treatment of soybean seeds with *Paecilomyces lilacinus, Trichoderma harzianum, Gliocladium virens, Bacillus subtilis, Rhizobium meliloti* and *Streptomyces* sp., showed significant reduction in infection of root by root infecting fungi on 30 and 60 day old plants. The effectiveness of microbial antagonists declined with time and after 90 days of growth *P. lilacinus* and *T. harzianum* were effective against *M. phaseolina*, *Streptomyces* sp., against *R. solani* whereas *G. virens* and *B. subtilis* were effective against both *M. phaseolina* and *Fusarium* spp. Rhizosphere mycoflora did not show any effect on root infection or colonization.

Introduction

Soybean (*Glycine max* (L.) Merr.) an oil seed and food crop is an important source of plant proteins in the human diet (Shastri, 1956). The seeds contain 20% oil which is composed of 80% unsaturated fatty acids and 7-8% linolenic acid (Neergaard, 1977). Of the various diseases, the soilborne diseases produce severe losses to soybean plants. Seed dressing with microbial antagonists has provided significant reduction in root infection by soil borne root infecting fungi on various crops (Cheng, 1968; Liu & Vaughn, 1965; Kommedahl & Chang, 1975). Experiments were therefore carried out to study the effect of microbial antagonists in the control of root rot of soybean.

Materials and Methods

Experiments were carried out at the experimental field of the Department of Botany, University of Karachi where the soil had a natural infestation of *Macrophomina phaseolina* (3-8 sclerotia g^{-1} of soil), *Rhizoctonia solani* (5.7% colonization of seeds used as baits) and *Fusarium* spp., (3146 conidia g^{-1} of soil). Culture of *Trichoderma harzianum* (KUMH 115), *Gliocladium virens* (KUMH 464), *Paecilomyces lilacinus* (KUMH 244), *Bacillus subtilis* (KUMH 117), and *Streptomyces* sp., (KUMH 118) were grown on PDA at 28°C for 5 days. Soybean seeds were treated with a spore suspension of microbial antagonists viz., *T. harzianum* @ 8.1×10^9 conidia ml^{-1}, *G. virens* 8×10^7 conidia ml^{-1}, *P. lilacinus* 1.7×10^8 condia ml^{-1}, *B. subtilis* 1×10^9 cell ml^{-1} and *Streptomyces* sp., @ 6.7×10^8 conidia ml^{-1} using 1% gum arabic as sticker. Plants were up rooted after 30, 60 and 90 days of growth. The roots were washed in running tap water and 1 cm long root pieces with or without surface disinfection with 1% $Ca(OCl)_2$ for 2 minutes were transferred onto PDA plates containing penicillin @ 100,000 units/litre and Streptomycin @ 0.2 g/litre. The Petri plates were incubated at 28°C for 5 days to confirm root infection and colonization by pathogen in surface sterilized and non-sterilized root pieces. Rhizosphere mycoflora was recorded by volume displacement technique.

Results and Discussion

All the antagonists viz., *T. harzianum, P. lilacinus, G. virens, B. subtilis, R. meliloti* and *Streptomyces* sp., provided significant reduction in root infection by *M. phaseolina* on 30 day old plants. *R. meliloti* and *Streptomyces* sp., lost their efficacy after 90 day, whereas, remaining antagonists were still effective (Fig.1). Similarly, root infection by *R. solani* in 30 day old plants reduced significantly in all treatments except *B. subtilis* whereas, after 90 days growth none of the antagonists was found effective against *R. solani*. However, surface disinfected roots showed lesser infection than the untreated control. *Fusarium* infection on 30 day old plants also reduced significantly where seeds were treated with *P. lilacinus, T. harzianum* and *G. virens*. *R. meliloti* and *Streptomyces* sp., also produced 25% reduction in *Fusarium* infection. Infection of roots by *Fusarium* spp., on 90 day old plants reduced by *P. lilacinus* (20%), *G. virens* (40%), *B. subtilis* (40%) and *Streptomyces* sp., (20%) (Fig.1). In surface sterilized roots, 90 day old plants showed 100% infection in all treatments however, the frequency of root colonization reduced significantly in *B. subtilis, R. meliloti*, and *Streptomyces* sp., treatments (Fig.1).

Fungi isolated from the rhizosphere of soybean were *Aspergillus niger* van Tieghem, *A. terreus* Thom, *A. quadrilineatus* Thom & Raper, *A. caespitosus* Raper & Thom, *A. ungius* (Emile-Weil & Gaudin) Thom & Raper, *A. fumigatus* Fresenius, *A. wentii* Wehmer, *A. flavus* Link, *A. sulphureus* (Fres.) Thom & Church, *Aspergillus* sp., *Acremonium* sp. *Cladosporium* sp. *Chaetomium globosum* Kunze ex Staud, *Curvularia lunata* (Wakker) Boedijin, *Drechslera australiensis* (Bugnicourt) Subram & Jain ex. M.B. Ellis; Subram. & Jain, *Dendryphiella vinosa* (Berk. & Curt.) Reisinger, *Fusarium solani* (Mart.) Appel & Wollenw, *F. oxysporum* Schlecht. emend. Snyd. & Hans, *Gliocladium virens* Miller, Giddings & Foster, *Gliomastix* sp., *Helicomina caperoniae* Olive, *Monodictys putredinis* (Willr.) Hughes, *M. glauca* (Cook & Harkn), *Myrothecium roridum* Tode ex Fr., *Penicillium*, sp., *Paceilomyces lilacinus* (Thom) Samson, *Stachybotrys parvispora* Hughes, *Scolecobasidium verruculosum* Roy, Dwivedi & Mishra, *Trichoderma harzianum* Rifai, *Verrucispora proteacearum* Shaw & Alcorn and 7 unidentified species. Of the fungi isolated *S. parvispora, Monodictys levis, M. glauca, Scolecobasidium verruculosum, Verrucispora proteacearum, Dendryphiella vinosa, Helicominia caperoniae* and *Gliomastix* sp., were reported for the first time in soybean rhizosphere.

Of the antagonists used in this study, species of *Trichoderma* are known to produce non-volatile antibiotics inhibiting growth of pathogen *in-vitro* (Dennis & Webster, 1971 a,b; Bell *et al.*, 1982; Moon *et al.*, 1988). Various amounts of coiling often accompanied by penetration were also observed (Chet *et al.*, 1981; Elad *et al.*,

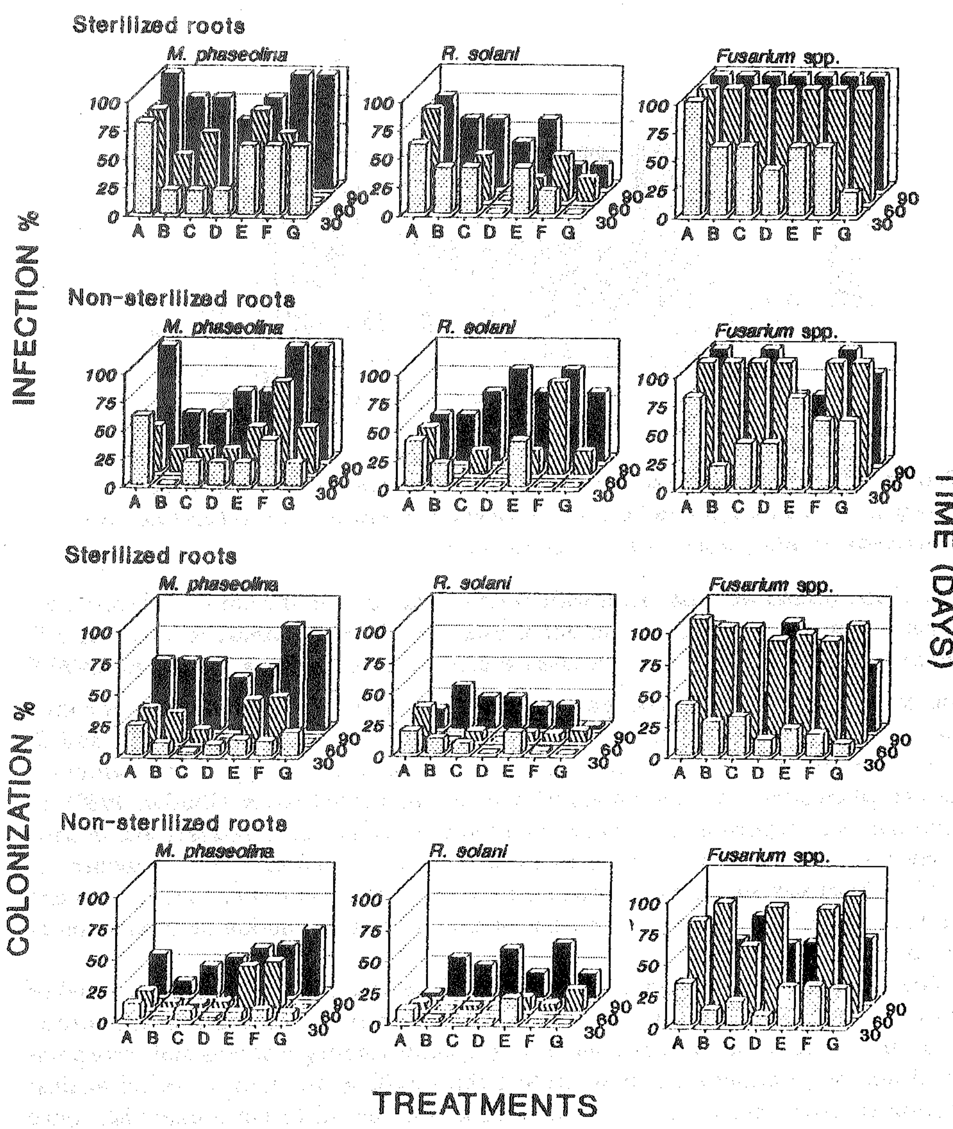

Fig.1. Effect of seed treatment with biological antagonists on infection and colonization of soybean roots by root infecting fungi.

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

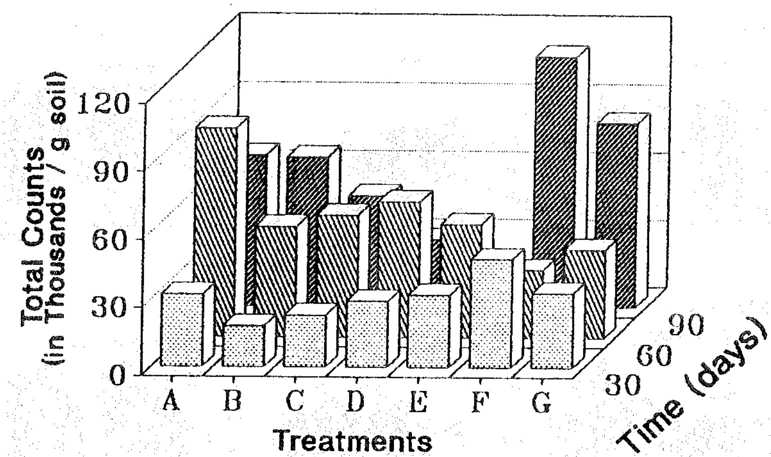

Fig.2. Effect of seed treatment with biological antagonists on rhizosphere mycoflora of soybean. A= Control, B= *Paecilomyces lilacinus*, C= *Trichoderma harzianum*, D= *Gliocladium virens*, E = *Bacillus subtilis*, F= *Rhizobium meliloti*, G = *Streptomyces* sp.

1983). Good results were obtained with *Trichoderma* spp., in the control of seed, root and early stem rot in maize and sorghum caused by *F. moniliforme, R. solani* and *P. ultimum*. *Gliocladium* sp., reduced disease incidence caused by *R. solani* to 40-70% when applied in soil at 10^6 propagules/g (Kim & Roh, 1987). Seed dressing of cotton with *G. virens* protected 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 antibiotics which act as surfactants and destroy the selective permeability of the hyphal cell membranes (Olsen, 1965; 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 broad spectrum antibiotic production *in vitro* (Broadbent *et al.*, 1971).

Antagonists were found more effective on 30 day old plants as compared to 60 or 90 day old plants. It would appear that the use of microbial antagonists as seed dressing can be helpful in the establishment of a healthy seedling whereas soil incorporation of microbial antagonists may protect the seedlings for longer period against infection by root pathogens. It was also observed that different antagonists were found effective against different pathogens on 90 day old plants. Combined use of more than one microbial antagonists may have provided better results in the control of root infection. In 60 and 90 day old plants 100% infection by *Fusarium* was observed. *Fusarium* spp., were most frequently isolated and most important pathogens on soybean roots (Spaudling, 1965). Rhizosphere population was lower in *P. lilacinus, T. harzianum* and *Streptomyces* sp., treatments. Plant age had a significant influence on rhizosphere microflora and rhizosphere microorganisms showed a gradual increase in population with age of plants and attained a peak after 60 and 90 days when plants were at flowering and fruiting stage (Fig.2), as also observed by Luke &

Devi (1975) on *Pisum sativum*, by Dayal & Srivastava (1975) on *Abelmoschus esculentus* and by Mukhopadhyay (1977) on jute. Aspergilli were isolated throughout the period of investigation from rhizosphere. Apparently the rhizosphere of *Glycine max* stimulated the growth of *A. niger*. Similar results were obtained by Kyrylenko (1966) on barley and oats. However, rhizosphere mycoflora did not show any relationship with the infection or frequency of root colonization by root infecting fungi.

The present study would suggest that seed treatment with biological antagonists are nearly as effective as chemical seed treatment. Moreover, these agents may become established in soil and could protect subsequent generation of seedlings from infection. However, there is need to develop a methodology for large scale production of inoculum of microbial antagonists and its delivery in the soil environment for the control of soilborne root infecting fungi.

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