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# EFFECT OF SEED PELLETING WITH BIOLOGICAL ANTAGONISTS IN THE CONTROL OF ROOT INFECTING FUNGI OF COWPEA AND MUNGBEAN

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**Abstract**

Effect of seed pelleting with *Stachybotrys atra*, *Memnoniella echinata* and *Rhizobium meliloti* on colonization of mungbean and cowpea roots by *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., was studied. A combined use of *S. atra* and *R. meliloti* was more effective in suppressing colonization of *M. phaseolina* and *Fusarium* spp., on mungbean and *R. solani* on cowpea roots than their separate use. Colonization of cowpea roots by all the three pathogens, and of mungbean roots by *Fusarium* spp., was greatly suppressed by a combined use of *M. echinata* and *R. meliloti* than their separate use. Use of *S. atra* showed greater phytotoxicity on cowpea as compared to mungbean.

## Introduction

Rhizobia which are known to fix atmospheric nitrogen in roots of leguminous plants have been found to inhibit growth of root infecting fungi viz., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* Kühn and *Fusarium* spp., (Zaki & Ghaffar, 1987; Ghaffar, 1988). Application of rhizobia either as seed dressing or as soil drench has shown significant suppression of root infecting pathogens on leguminous and non-leguminous plants (Zaki & Ghaffar, 1987; Ehteshamul-Haque *et al.*, 1990; Shahzad & Ghaffar, 1992a). There are reports that treatment of clover with a combination of *Rhizobium trifolii*, *Pseudomonas* sp., and *Achromobacter* sp., has resulted in increase in yield over untreated control (Allen & Allen, 1950). *Stachybotrys atra* Corda which showed inhibitory effects against several microorganisms including *M. phaseolina*, *R. solani* and *Fusarium* spp., showed stimulatory effects on *R. trifolii in vitro* (Butt & Ghaffar, 1971, 1972). Experiments were therefore carried out to study the effect of *S. atra* with root nodule bacterium, *Rhizobium meliloti* on colonization of mungbean (*Vigna radiata* (L.) Wilczek) and cowpea (*Vigna unguiculata* (L.) Walp.) roots by *M. phaseolina*, *R. solani* and *Fusarium* spp. *Memnoniella echinata* (Rivolte) Galloway, another antagonistic fungus against *M. phaseolina* (Abbas & Ghaffar, 1973), *R. solani* and *Fusarium* sp., (Ghaffar, 1988) was also used in the study.

## Materials and Methods

Microbial antagonists viz., *S. atra*, *M. echinata* and *R. meliloti* grown on PDA plates were used for pelleting mungbean and cowpea seeds. The fungi were used alone or *R. meliloti* was used with *S. atra* and *M. echinata*. The method of inoculating seeds of leguminous plants with rhizobia (Anon., 1987) was used for seed pelleting with slight

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**Table 1. Propagules of biocontrol agents per seed.**

| Treatments | Mungbean | | Cowpea | |
|---|---|---|---|---|
| | Fungus | Bacterium | Fungus | Bacterium |
| *Stachybotrys atra* | $9.3x10^4$ | - | $4.9x10^5$ | - |
| *Memnoniella echinata* | $3.2x10^5$ | - | $5.2x10^5$ | - |
| *Rhizobium meliloti* | - | $1.4x10^5$ | - | $5.9x10^6$ |
| *S. atra* + *R. meliloti* | $1.5x10^5$ | $1.1x10^5$ | $8.1x10^5$ | $3.8x10^6$ |
| *M. echinata* + *R. meliloti* | $1.7x10^5$ | $1.2x10^5$ | $8.0x10^4$ | $4.0x10^6$ |

modification. Seeds kept in polyethylene bags moistened with 40% gum arabic solution (used @ 3 ml/100 g seed) were inoculated with 7 day old spore or cell suspension of microbial antagonists @ 10 ml/100 g seeds mixed with powdered rice grain used as organic substrate @ 50 g/100 g seed. The bags were shaken to provide uniform coating of the seeds. Calcium sulphate @ 40 g/ 100 g seed was also mixed and all the seeds were evenly coated. Number of propagules of each biocontrol agent per seed is given in Table 1. Seeds were sown in 8 cm diam., plastic pots, each containing 250 g of sandy loam soil, pH 8.2, obtained from the experimental plots of the Department of Botany, University of Karachi. Soil moisture was adjusted and maintained at 50% MHC (Keen & Raczkowski, 1921). There were 5 replicates for each treatment and the pots were arranged in randomized complete block design on a screenhouse bench. Plants were uprooted after 30 days growth to assess root colonization by *M. phaseolina*, *R. solani* and *Fusarium* spp.

Roots after thorough washing in running tap water were cut into 1 cm pieces; surface disinfected with 1% calcium hypochlorite solution for 3 minutes and transferred onto PDA plates containing penicillin (@ 100,000 units/l) and streptomycin (@ 0.2 g/l). After 5 days incubation at 28°C, number of root pieces of a plant infected by *M. phaseolina*, *R. solani* or *Fusarium* spp., was recorded. On the basis of the percentage of root pieces colonized by a fungus, each plant was rated on a 0-5 Colonization Index of Shahzad & Ghaffar (1992b). An average value for each treatment was calculated.

**Results and Discussion**

***a. Effect of microbial antagonists on seed germination:*** Combined use of *M. echinata* and *R. meliloti* showed greater germination of mungbean and cowpea seeds whereas *S. atra* alone or mixed with *R. meliloti* showed a decrease in seed germination as compared to control. The phytotoxic effect of *S. atra* was more pronounced on cowpea as compared to mungbean (Fig. 1). Phytotoxicity of *S. atra* has previously been reported on cotton (Butt & Ghaffar, 1972) with significant reduction in growth of tomato, rape and flax (Domsch, 1963). An increased germination of cowpea and mungbean seeds in treatments where *M. echinata* mixed with *R. meliloti* was used would suggest a promising use of this combination of biological antagonists for plant disease control.

***b. Effect on root colonization:*** Although *M. echinata* has been reported to inhibit the growth of root infecting fungi *in vitro* (Abbas & Ghaffar, 1973), there does not appear

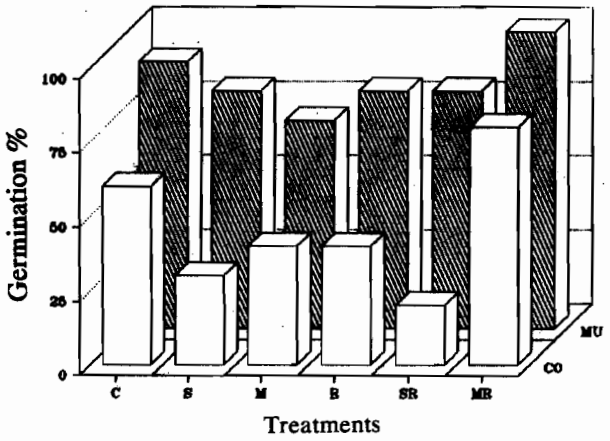


Fig. 1. Effect of seed treatment with *Stachybotrys atra*, *Memnoniella echinata* and *Rhizobium meliloti* on germination of mungbean and cowpea seeds.
C = Control, S = *S. atra*, M = *M. echinata*, R = *R. meliloti*, SR = *S. atra* + *R. meliloti*, MR = *M. echinata* + *R. meliloti*, CO = Cowpea, MU = Mungbean.

to be any report of the use of *M. echinata* as biocontrol agent. In the present study, *M. echinata* suppressed *Fusarium* and *M. phaseolina* colonization on cowpea and mung bean whereas *R. solani* colonization was suppressed on mungbean but not on cowpea. This efficacy of the biocontrol agent indicates the promise of its use in plant disease control.

Seed pelleting with *S. atra* did not suppress *M. phaseolina* colonization whereas it significantly suppressed root colonization by *R. solani* and *Fusarium* spp., on both mungbean and cowpea (Fig. 1). Butt & Ghaffar (1972) also found that *S. atra* which inhibited the growth of *M. phaseolina* in agar culture was unable to control *M. phaseolina* infection on cotton. They assumed that either the toxic metabolites effective against *M. phaseolina* were not produced in sufficient quantity in soil or the active substance was adsorbed by soil colloidal particles, or was decomposed by soil microorganisms. Suppression of *R. solani* and *Fusarium* colonization in the present study would suggest that the metabolites produced by *S. atra* in soil were effective against *R. solani* and *Fusarium* spp., but not against *M. phaseolina*. It is also possible that instead of a direct effect on root infecting fungi, *S. atra* would have stimulated other soil microorganisms which had inhibitory effect on *R. solani* and *Fusarium* spp., but not on *M. phaseolina*. Butt & Ghaffar (1972) also found that in addition to stimulation of *R. trifolii*, growth of *Aspergillus ustus*, two unidentified *Fusarium* spp., and *Pseudomonas* spp., was not inhibited by *S. atra*.

Ehteshamul-Haque & Ghaffar (1993) observed significant reduction in *M. phaseolina*, *R. solani* and *Fusarium* infection of mungbean, soybean, sunflower and okra where *R. meliloti* was used as seed dressing. In the present study, *R. meliloti* did not produce significant suppression in colonization of mungbean roots by *R. solani* and *Fusarium* sp. However, the bacterium effectively suppressed *R. solani* and *Fusarium* colonization on cowpea (Fig. 2).

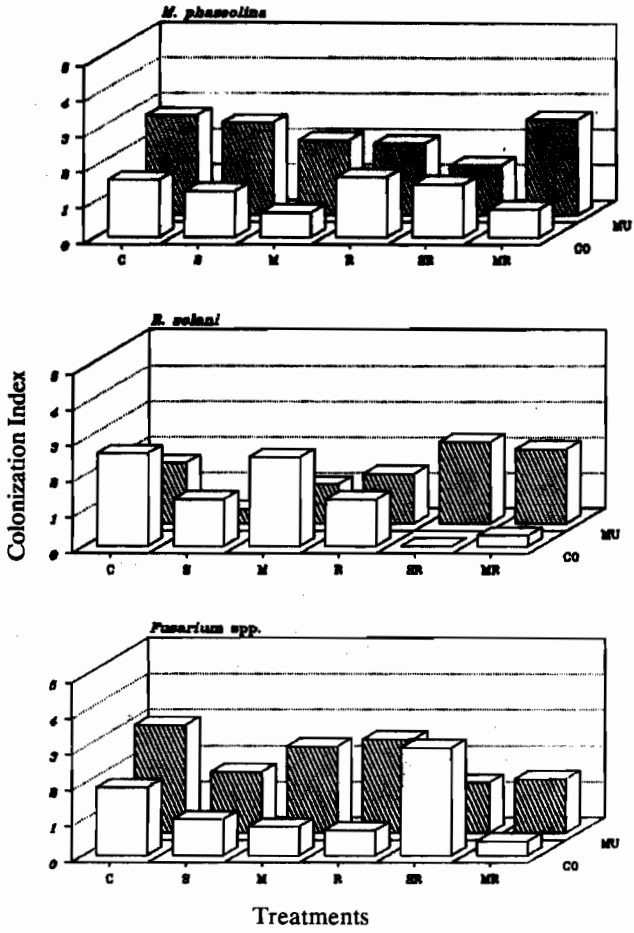


Fig. 2. Effect of seed treatment with *Stachybotrys atra*, *Memnoniella echinata* and *Rhizobium meliloti* on colonization of mungbean and cowpea roots by root infecting fungi.
C = Control, S = *S. atra*, M = *M. echinata*, R = *R. meliloti*, SR = *S. atra* + *R. meliloti*, MR = *M. echinata* + *R. meliloti*, CO = Cowpea, MU = Mungbean.

Although a combined use of *R. meliloti* with either *S. atra* or *M. echinata* showed better results than their separate use, the efficacy varied with the pathogen and the host. Seed pelleting with a mixture of *M. echinata* and *R. meliloti* was effective against all the three pathogens on cowpea roots whereas on mungbean *Fusarium* colonization was suppressed with no effects on *M. phaseolina* and *R. solani* colonization. A combined use of *S. atra* and *R. meliloti* was effective in suppressing root colonization by *M. phaseolina* and *Fusarium* spp., on mungbean but not on cowpea roots. Root colonization by *R. solani* was inhibited on cowpea roots but not on mungbean roots (Fig. 2).

Variable efficacy of a biocontrol agent or a mixture of biocontrol agents against a pathogen on different host plants growing in similar soil conditions indicated that differences in root exudates of different plants could have a stimulatory or inhibitory

effect on either the pathogen or the antagonist (Curl, 1982). Development of tolerance or ability of a pathogen to overcome the effect of inhibitory metabolites would also affect the efficacy of biocontrol agents. The growth of *Aspergillus flavus* and *R. solani* was inhibited by *S. atra* producing clear zones of inhibition which after a few days of incubation was lost and *A. flavus* slowly grew and touched the colony of *S. atra* whereas *R. solani* was found to overgrow the colony of the antagonist (Butt & Ghaffar, 1972). Shahzad & Ghaffar (1988) also observed that after three days of inhibition of *Sclerotium oryzae* by *Paecilomyces lilacinus*, *S. oryzae* overgrew the colony of *P. lilacinus*. Variation in the efficacy of biocontrol agents against different pathogens on a host would be the result of differences in metabolites effective against different pathogens. There are reports that inoculum level of *M. phaseolina* required to produce 50% infection varied with the host (Sheikh & Ghaffar, 1979). The inoculum potential of the pathogen could also affect the efficacy of a biocontrol agent on different hosts since Shahzad & Ghaffar (1993) found a correlation between the population of *M. phaseolina* in soil and the inoculum dose of *P. lilacinus* required to produce effective disease control.

The result of the present study would suggest that for a successful application of biocontrol agent compatibility between host, antagonist and pathogen is essential. Selection or development of strains of biocontrol agents which could be effective against more than one pathogens on more than one host plant could be more useful. Integration of two or more biocontrol agents could also provide better results than their separate use. There is also need to establish a correlation between the population of pathogen(s) in soil and inoculum dose of biocontrol agent(s) required for effective suppression of the disease.

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