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EFFICACY AND PERSISTENCE OF MICOBIAL ANTAGONISTS AGAINST *SCLEROTIUM ROLFSII* UNDER FIELD CONDITIONS

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Abstract

Sclerotium rolfsii showed significant negative effect on plant growth due to severe root colonization, whereas, presence of the microbial antagonists showed significant positive effect on plant growth by reducing the colonization of roots by *S. rolfsii.* Highest root colonization by *S. rolfsii* and significant reduction in plant growth were observed in sunflower and mungbean plants growing in soil artificially infested with sclerotia of *S. rolfsii.* Use of biocontrol agents in *S. rolfsii* infested soil showed significant reduction in Root Colonization Index accompanied by increase in plant growth. *Bradyrhizobium* sp., was found most effective ($p<0.01$) followed by *Rhizobium* sp., *Trichoderma harzianum, T. pseudokoningii, T. polysporum* and *T. virens.*

Efficacy of biocontrol agents was comparatively suppressed in *S. rolfsii* infested soil as compared to non-infested soil. There was no significant difference in plant length in infested and non-infested soils where biocontrol agents were used. However, plant weight was less in infested soils as compared to non-infested soils. The microbial antagonists persisted in the soil and produced similar results when mungbean and sunflowers were re-sown in the same plots.

Introduction

Sclerotium rolfsii Sacc., [teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough] is an economically important soil-borne pathogen that is very common in tropical, sub-tropical and other warm temperate regions of the world. The pathogen causes root rot, stem rot, wilt and foot rot diseases on more than 500 species of cultivated and wild plants including almost all the agricultural and horticultural crops (Aycock, 1966; Punja, 1985; Domsch *et al.,* 1980; Farr *et al.,* 1989; Cilliers *et al*., 2000; Ciancio & Mukerji, 2007). Mostly *S. rolfsii* diseases have been reported on dicotyledonous hosts, but several monocotyledonous species have also been infected (Aycock, 1966; Ciancio & Mukerji, 2007). Humid weather is conducive to sclerotial germination and mycelial growth. Consequently the diseases caused by the fungus are more serious in tropical and subtropical regions than in temperate regions (Yorinori, 1994). The large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years, as well as the profuse growth rate of the fungus make it well suited facultative parasite and a pathogen of major importance throughout the world (Punja, 1988).

The first confirmed report of losses due to the pathogen in USA was made by Rolfs (1892) on tomato (*Lycopersicon esculentum* Miller) in Florida. Aycock (1966) estimated that *S. rolfsii* produced yield depletion ranging from 1-60% in different peanut fields in southern peanut growing region of USA resulting in losses of up to US$ 10-20 million. The first report of *S. rolfsii* from Pakistan was made by Ahmed *et al.,* (1984) who isolated it from maize *(Zea mays* L.). Subsequent reports were made from oat (*Avena sativa* L.) and mash bean (*Vigna mungo* (L.) Hepper) by Shahzad & Ghaffar (1995), lentil (*Lens culinaris* (L.) Medic.) by Iqbal *et al.,* (1995), apple (*Malus sylvestris* L.) by Jahangir *et al.,* (1995), and seeds of sugarbeet (*Beta vulgaris* L.) by Ruqia (2001).

Use of chemical fungicides is no doubt the most effective method for the control of plant diseases. However, in view of the complexities arising from the use of chemical pesticides, such as harmful effects on environment and non-target organisms including man, domestic animals, beneficial insects and wild life, efforts have been focused to develop alternative approaches which are safe for all stakeholders i.e. humans, animals, environments and crops (Atlas & Bartha, 1998). Of the various methods used, the use of microorganism as biocontrol agents has provided a very promising alternative and less hazardous method for plant disease control (Papavizas & Lumsden, 1980).

Diseases caused by *S. rolfsii* continue to receive considerable attention with regard to the development of biological control strategies (Tjamos *et al*., 1992). The application of fungi as biological control agents, especially *Trichoderma* spp., to control *S. rolfsii* has been attempted (Henis, 1984; Papavizas & Lewis, 1989). *T. harzianum* reduced root rot of sugar beets (Ciccarese *et al*., 1992), stem rot of ground nut (Cilliers *et al*., 2000), damping-off and stem rot of cowpea (Adandonon, 2000; Kossou *et al*., 2001), root rot of beans and tomatoes (Elad *et al*., 1980), collar rot and seedling death of lentil (Agrawal *et al*., 1977), bulbs infection in Iris (Chet *et al.,* 1983), ground nut and tomatoes (Elad *et al.,* 1982), damping-off of beans (Henis, 1984) and damping-off and stem rot of cowpea plants (Adandonon *et al.,* 2004) and collar rot of chickpea (Maurya *et al*., 2008) caused by *S. rolfsii* and increased the yield. Application of an isolate of *T.* (*Gliocladium*) *virens* in association with solarization reduced southern blight of tomatoes (Ristaino *et al*., 1991). Mukherjee & Raghu (1997) observed that *Trichoderma* spp., were highly effective in suppressing *S. rolfsii* on ginger rhizomes and on several vegetables in storage. Similarly, Chakrabortys & Bhawmik (1985) found *T. viride* and *T. harzianum* highly effective in the control of sunflower collar rot caused by *S. rolfsii.*

Soil population of the pathogen has a direct correlation with the intensity of disease. Shokes & Gorbet (1998) reported a positive correlation of root colonization with the population of *S. rolfsii.* Similarly, Khalequzzaman (2003) observed that with a gradual increase in inoculum levels in soil, plant growth, nodulation and yield per plant reduced gradually. Chang (1994) found that the time required for the death of

Edgeworthia papyrifera in *S. rolfsii* infested soil was inversely proportional to number of sclerotia in soil. The present report describes the efficacy of microbial antagonists *viz.*, *Bradyrhizobium* sp., *Rhizobium* sp., *Trichoderma harzianum, T. pseudokoningii, T. polysporum* and *T. virens* in the control of *S. rolfsii* on mungbean and sunflower and persistence of their efficacy in soil.

Materials and Methods

A field experiment was carried out in the experimental plots of the Department of Botany, University of Karachi. Microbial antagonists *viz.*, *Bradyrhizobium* sp., *Rhizobium* sp., *Trichoderma harzianum, T. pseudokoningii, T. polysporum* and *T. virens* multiplied on sterilized rice grains for four weeks at 25°C were applied to soil @60g in 1 m long furrow (Shahzad & Ghaffar, 1989) along with 0.5 g sclerotia of *S. rolfsii*. Plots not treated with the pathogen and biocontrol agents served as control. There were three replicates for each treatment. Mungbean and sunflower seeds were sown in the field and after 30 days growth, plants were uprooted to assess plant length, plant weight and colonization of roots by *Sclerotium rolfsii*. The roots were washed in running tap water to remove soil particles. Ten randomly selected 1cm long root pieces from each plant were surface sterilized with 1% NaOCl solution for 5 min and transferred onto potato sucrose agar plates containing Penicillin (@100,000 units L^{-1}) and Streptomycin (@ 0.2 g L^{-1}). After incubation for 5 days at room temperature, root colonization (RC) by *S. rolfsii* was recorded using the following formula:

$$RC\% = \frac{\text{Number of root pieces colonized}}{\text{Total number of root pieces}} \times 100$$

Data on root colonization were converted into roots colonization index (RCI) according to a 0-5 scale of Shahzad & Ghaffar (1992) where 0=0, l=l-10, 2=11-25, 3=26-50, 4=51-75 and 5=75-100% root pieces colonized by the pathogen.

Persistence of the effect of microbial antagonists: After uprooting the plants in the above experiment, persistence of the effects of microbial antagonists in soil was examined by sowing the seeds of mungbean and sunflower in the same plots without any additional amendment of the microbial antagonists or the pathogen. Plant growth and root colonization by *S. rolfsii* were recorded after 30 days growth using the method described above.

Results

Presence of *S. rolfsii* showed significant negative effect on plant growth due to severe root colonization, whereas presence of the microbial antagonists showed significant positive effect on plant growth by reducing the colonization of roots by *S. rolfsii*. Effect of the pathogen and the antagonists varied with the host. Highest root colonization by *S. rolfsii* and significant reduction in plant growth were observed in sunflower and mungbean plants growing in soil artificially infested with sclerotia of *S. rolfsii*. The effect of *S. rolfsii* was more evident on plant weight as compared to plant length since the plants were comparatively thinner in *S. rolfsii* infested soil. Use of biocontrol agents in *S. rolfsii* infested soil showed significant reduction in RCI accompanied by increase in plant growth. *Bradyrhizobium* sp., was found most effective ($p<0.01$) followed by *Rhizobium* sp., *T. harzianum, T. pseudokoningii, T. polysporum* and *T. virens* (Figs. 1-4). Efficacy of biocontrol agents was comparatively suppressed in *S. rolfsii* infested soil as compared to non-infested soil. There was no significant difference in plant length in infested and non-infested soils where biocontrol agents were used. However, plant weights were less in infested soils as compared to non-infested soils. The reason was again the week stems of plants in *S. rolfsii* infested soil as compared to non-infested soils.

Persistence of the effect of microbial antagonist: Microbial antagonists *Rhizobium*, *Bradyrhizobium* and *Trichoderma* spp., showed a greater residual effect and reduced the infection by *S. rolfsii* and enhanced the growth of mungbean and sunflower ($p<0.05$) as compared to soil that was infested with *S. rolfsii*. Soil infested with *S. rolfsii* and amended with microbial antagonists showed greater plant growth and weight as compared to control (Figs. 5, 6).

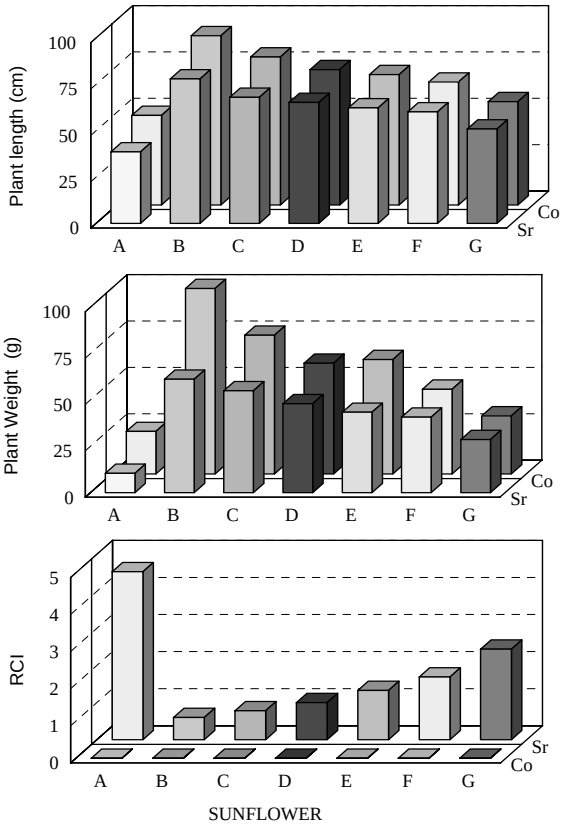

Fig. 1. Efficacy of microbial antagonists in the control of *Sclerotium rolfsii* on sunflower in microplots.

A= Control, B= *Bradyrhizobium* sp., C= *Rhizobium* sp., D= *Trichoderma harzianum*, E= *T. pseudokoningii*, F= *T. polysporum*, G= *T. virens*, Co= Control, Sr= *S. rolfsii*

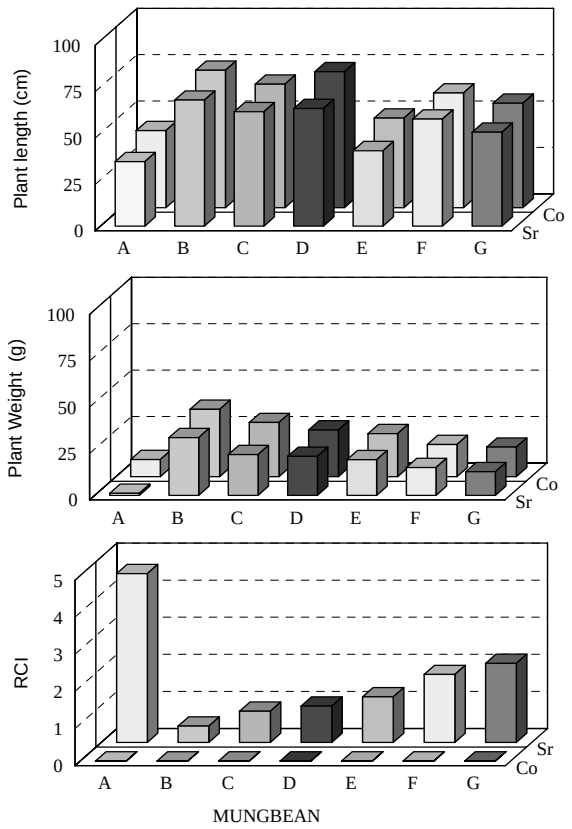

Fig. 2. Efficacy of microbial antagonists in the control of *Sclerotium rolfsii* on mungbean in microplots.

A= Control, B= *Bradyrhizobium* sp., C= *Rhizobium* sp., D= *Trichoderma harzianum*, E= *T. pseudokoningii*, F= *T. polysporum*, G= *T. virens*, Co= Control, Sr= *S. rolfsii*

Discussion

Trichoderma species are present in nearly all agricultural soils. These fungi are known to coil around the hyphae of other fungi them in a lectin-mediated reaction, and degrade their cell walls. This mycoparasitism limits growth and activity of plant pathogenic fungi. In addition to the mycoparasitism, individual strains can also produce antibiotics (Dennis & Webster, 1971). *Trichoderma* species have been used for the control of a variety of fungal pathogens like *Rhizoctonia, Sclerotinia sclerotiorum, Pythium* and *Fusarium* spp. (Harman, 1991; Lumsden & Locke, 1989; Taylor *et. al.*, 1993; Lewis & Lumsden, 2001). Production of the antifungal antibiotics, gliotoxin and gliovirin, by *T. virens* has been associated with its efficacy as a biocontrol agent of most soil-borne diseases (Highley *et al*., 1997). *T. virens* has shown promise as a preventive treatment for the control of *Rhizoctonia solani* (Howell & Stipanovic, 1995). *Trichoderma* spp., also produce organic acids, such as gluconic, citric or fumaric acids, that decrease soil pH and permit the solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium, useful for plant metabolism (Benitez et *al*., 2004; Harman *et al.*, 2004). It is well documented that some strains promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman *et al.*, 2004). Rhizobia have shown good potential as biological control agents against some plant pathogens. During the present studies, use of *Rhizobium* and *Bradyrhizobium* species promoted plant growth and provided significant reduction in colonization of roots by *S. rolfsii.* Blum *et al*., (1989) reported that *Rhizobium* strains decreased the incidence of damping-off disease in Bush bean and the index of disease severity caused by *Rhizoctonia solani.* Hossain (2000) found that treatment of seeds with biofertilizer resulted in more than 85 and 73% reduction in death of plants due to infection by *Fusarium oxysporum* in lentil and chickpea, respectively. The highest reduction (69%) of fusarial foot and root rot in chickpea over untreated control was with *Rhizobium* inoculation @ 50 g/kg seed when moisten with molasses. Hossain (2000) also found that treatment of seeds with biofertilizer also showed 76 and 87% reduction in death of plants of lentil and chickpea, respectively, due to infection by *S. rolfsii. Rhizobium* strain gave the highest promotion in plant growth. Similar results on the use of rhizobia have been reported by Hossain (2000), Kumar *et al*., (2001) and Kibria & Hossain (2002). Solaiman (1999) found that number of total nodules per plant of chickpea was significantly increased by *Rhizobium* inoculation. Strain of *R. meliloti* are antagonistic to *F. oxysporum* (Antoun *et al.*, 1998), and rhizobia antagonistic to *F. solani* isolated from commercial snap bean, appeared to have a good potential for controlling *Fusarium* rot (Buonassisi *et al.*, 1986). Ehtesham-ul-Haque & Ghaffar (1993) observed under field conditions that *R. meliloti*, *R. leguminosarum* and *B. japonicum* used either as soil drench or seed dressing reduced infection of roots by *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp., in leguminous as well as non-leguminous plants. *Rhizobium leguminosarum* significantly increased the number and growth of pea seedlings (Bardin *et al*., 2004). Attachment of rhizobia to roots of non-leguminous crops has been described by Terouchi & Syono (1990) that can explain the efficacy of rhizobia on non-leguminous crops.

Bradyrhizobium and *Rhizobium* share characteristic with plant growth promoting rhizobacteria (PGPR). Like other PGPR, these nodule inducing bacteria colonize the roots of non-legumes, produce phytohormones, siderophores and HCN and exhibit antagonistic effects towards plant pathogenic fungi (Antoun *et al*., 1998). The increment in growth parameters in response to rhizobial inoculation endorsed the fact that the test strains were having one or more growth promoting mechanisms including mobilization and efficient uptake of nutrients (Biswas *et al*., 2000 a, b), enhancement in stress resistance (Alami *et al.*, 2000), solubilization of insoluble phosphates (Alikhani *et al*., 2006), induction of systemic disease resistance (Reitz *et al.*, 2000), inhibition of fungal growth (Nautiyal, 1997), production of phytohormones (Dakora, 2003), vitamins (Dobbelaere *et al*., 2003) and siderophores (Neiland & Leong, 1986). Biswas *et al*., (2000b) reported 16% increase in number of panicles per plant of rice and suggested that the improvement was due to increase availability of nutrients and phytohormones like indole acetic acid and ethylene. Similarly, Chi *et al*., (2005) observed more than 23% increase in plant height of rice over uninoculated control and argued indole acetic acid and gibberellins production as the key mechanism for that improvement. Our results corroborate well with those of Gaur *et al.*, (1980) who found that rhizobia and bradyrhizobia, significantly increased the weight of shoot, number of pods, nodule volume and the yield of the following green gram (*Vigna radiata* (L.) Wilczek) and groundnut (*Arachis hypogaea* L.) crops. Pena–Cabriales & Alexander (1983) found that strain of rhizobia and

Fig. 3. Effect of microbial antagonists on growth of sunflower plants.
A= Control, B= Pathogen alone, C= Antagonists alone, D= Antagonist and pathogen together.

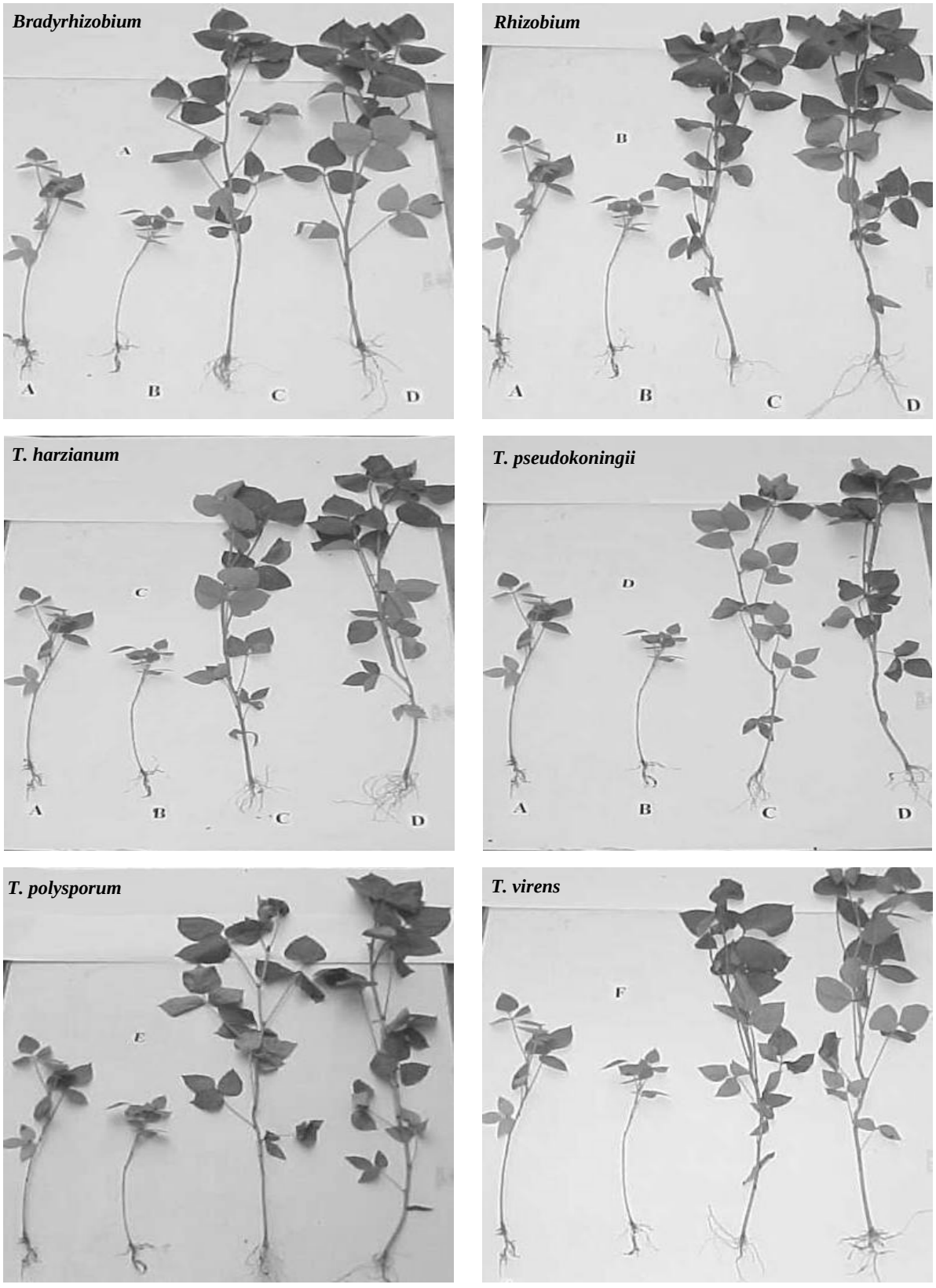

Fig. 4. Effect of microbial antagonists on growth of mungbean plants.
A= Control, B= Pathogen alone, C= Antagonists alone, D= Antagonist and pathogen together

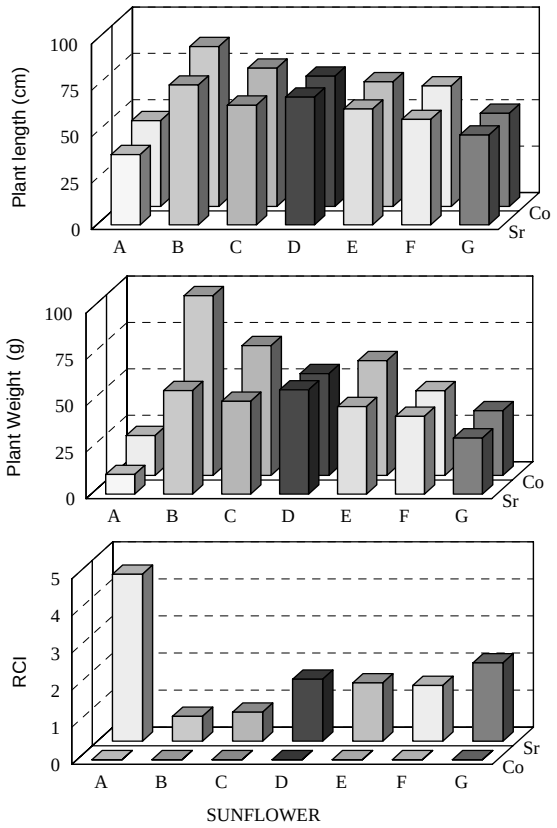

Fig. 5. Persistence of the efficacy of microbial antagonists in the control of *Sclerotium rolfsii* on sunflower in microplots.

A= Control, B= *Bradyrhizobium* sp., C= *Rhizobium* sp., D= *Trichoderma harzianum*, E= *T. pseudokoningii*, F= *T. polysporum*, G= *T. virens*, Co= Control, Sr= *S. rolfsii*

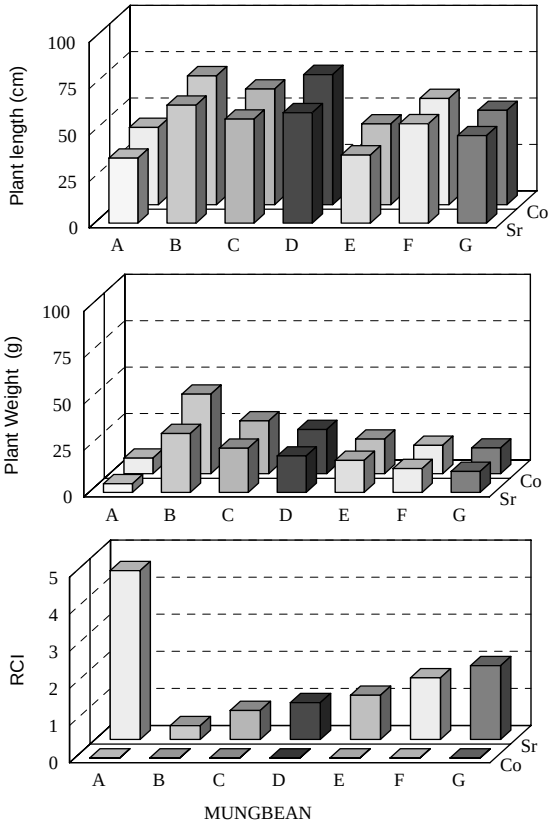

Fig. 6. Persistence of the efficacy of microbial antagonists in the control of *Sclerotium rolfsii on* mungbean in microplots.

A= Control, B= *Bradyrhizobium* sp., C= *Rhizobium* sp., D= *Trichoderma harzianum*, E= *T. pseudokoningii*, F= *T. polysporum*, G= *T. virens*, Co= Control, Sr= *S. rolfsii*

bradyrhizobia grew readily in the presence of germinating seeds and developing root systems of soybean (*Glycine max* (L.) Merr.), red clover (*Trifolium pratens* L.), kidney beans (*Phaseolus vulgaris* L.), cowpeas (*Vigna unguiculata* L.), oats, wheat and corn. Reitz *et al.,* (2000) have reported the induction of systemic resistance to the cyst nematode *Globodera pallida* in potato by *Rhizobium etli* strain G12. The beneficial effect of *Rhizobium* and *Bradyrhizobium* in legumes in terms of biological nitrogen fixation has been a main focus in the recent past. The results of the present study would suggest that use of *Trichoderma, Rhizobium* and *Bradyrhizobium* species can provide protection against *S. rolfsii* infection resulting in increased crop growth and productivity. The ability of these biocontrol agents to persist in soil can also provide protection in the next crop as well.

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