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EFFECT OF SEED PELLETING WITH *TRICHODERMA* SPP., AND *GLIOCLADIUM VIRENS* ON GROWTH AND COLONIZATION OF ROOTS OF SUNFLOWER AND MUNG BEAN BY *SCLEROTIUM ROLFSII*

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Abstract

During the present studies, *Trichoderma* species viz., *T. harzianum, T. polysporum, T. pseudokoningii* and *Gliocladium virens* were used for seed pelleting to prevent seed rot, damping-off, root rot of sunflower and mungbean caused by *Sclerotium rolfsii*. Conidial suspensions of microbial antagonists prepared either in water or 10% sugar solution effectively suppressed root colonization by *S. rolfsii* and significantly enhanced plant growth as compared to control. Growth promoted by microbial antagonist was more evident in soil when *S. rolfsii* was not present.

Introduction

More than 500 species of cultivated and wild plants are attacked by the soil-borne pathogenic fungus *Sclerotium rolfsii* Sacc., in tropical and subtropical regions of the world (Aycock, 1966; Punja, 1985; Punja & Grogan, 1983; Harlton *et al*., 1995; Mukerjee & Raghu, 1997; Cilliers *et al*., 2000). 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., and *Gliocladium* spp., to control *S. rolfsii* has been attempted in the green house (Henis, 1984; Papavizas & Lewis, 1989; Punja, 1985). *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), basal stem rot and wilt of sunflower (Okoli *et al.*, 1991) caused by *S. rolfsii* and increased the yield. Application of an isolate of *G. virens* in association with solarization reduced southern blight of tomatoes (Ristaino *et al*., 1991). The present report describes the effect of seed pelleting with three species of *Trichoderma viz., T. harzianum, T. pseudokoningii, T. polysporum* and *G. virens* on growth of mungbean and sunflower and colonization of roots by *S. rolfsii*.

Materials and Methods

a. Seed pelleting: *T. harzianum*, *T. pseudokoningii, T. polysporum* and *G. virens* grown on PSA plates were used for pelleting mungbean and sunflower seeds. Conidial suspension of *Trichoderma* spp., and *G. virens* were prepared by adding 10 ml sterilized water to a 7 days old culture of biocontrol agents in a 9cm diam., Petri plate, and rubbing the surface with the help of a sterilized spatula. Three ml of conidial suspension was added to 10 g of seeds in polyethylene bags. The bags were shaken well to provide a uniform coating. In another set, 10% sugar solution was used to make conidial suspension.

Table 1. Spore load of biocontrol agents on mungbean and sunflower seeds.

Antagonists	Host plant	Conidia in sterilized water	Conidia in 10% sugar
T. harzianum	Mungbean	$8.75x10^8$	$8.93x10^8$
T. pseudokoningii		$7.99x10^8$	$8.90x10^8$
T. polysporum		$8.17x10^8$	$8.69x10^8$
G. virens		$8.73x10^8$	$8.0x10^8$
T. harzianum	Sunflower	$9.30x10^8$	$9.27x10^8$
T. pseudokoningii		$9.12x10^8$	$9.57x10^8$
T. polysporum		$8.97x10^8$	$8.83x10^8$
G. virens		$8.99x10^8$	$8.93x10^8$

b. Spore-load per seed: Five seeds were added in a test tube containing 10 ml sterilized 0.1% water agar. The test tube was shaken well to separate conidia from seeds to get a standard suspension. A 1/10 dilution from the standard was made by transferring 1ml conidial suspension to another test tube containing 9 ml sterilized 0.1% water agar. This process was repeated to get 1/100, 1/1000, 1/10,000, 1/100,000 and 1/1000,000 dilutions. One ml from each dilution was transferred separately into Petri plates containing PSA amended with rose bengal (@ 0.1g L^{-1}), penicillin (100,000 units L^{-1}) and streptomycin (0.2 g L^{-1}). There were three replicates for each treatment. The Petri plates were incubated at room temperature for three days and the lowest dilution that showed separates growth of colonies of the antagonists was used to count the colonies. The number of colonies per plate was multiplied with the dilution factor and then divided by 5 to determine the spore-load per seed.

c. Effect of seed pelleting on pathogenecity and root colonization: Ten un-pelleted (control) and pelleted seeds were sown in pots containing 180 g soil. Pots had an artificial infestation of *S. rolfsii* @ 1 sclerotium g^{-1} soil. Soil moisture was adjusted to 50% MHC and amount of soil moisture lost was re-adjusted after each 24 hours. There were 3 replicates for each treatment. Pots were randomized on a green house bench and plants were uprooted after 30 days growth to assess plant growth, pre-emergence and post-emergence damping-off and colonization of roots by the pathogen. The data on root colonization were converted into root colonization index (RCI) according the a 0-5 scale of Shahzad & Ghaffar (1992) where 0=0, 1=1-10, 2=11-25, 3= 26-50, 4=51-75 and 5 showed 76-100% root colonization.

Results and Discussion

Seed pelleting & spore load: Use of water or sugar solution for the preparation of conidial suspension showed no significant difference in number of conidia per seed in both the host plants (Table 1). However, the number of conidia per seed of sunflower was higher as compared to mungbean. It could be attributed to the larges size of the sunflower seed. Comparatively better growth in plants when seeds were coated with conidial suspension in sugar solution supports the results of Adekunle *et al.,* (2001, 2006) who evaluated two formulations of different *Trichoderma* species as seed treatment in cowpea against *Macrophomina phaseolina* and observed that all species of *Trichoderma* performed well when seeds were coated with them in the presence of starch. El-Mohamedy *et al*., (2006) also found that incorporation of sugar cane bagasse @ 10% (w/w) in bio-priming seed treatment and soil treatment with *Trichoderma* showed a high

effect in reducing root rot incidence caused by *Fusarium solani, Rhizoctonia solani* and *M. phaseolina.* It might be possible that addition of sugar plays a dual role, on one hand it provides proper nutrition for germination of conidia of biocontrol agent present on seed surface and on the other hand, it works as a sticky agent and increases the c.f.u. per seed. Lu *et al*., (2004) also used 10% (w/v) aqueous suspension of the adhesive Pelgel for seed coating with biocontrol strains of *T. atroviride* on cucumber seeds to control *Pythium ultimum*.

Effect of seed pelleting on root colonization: Highest root colonization in sunflower and mungbean by *S. rolfsii* was observed in soil artificially infested with sclerotia of *S. rolfsii* where no biocontrol agent was used for seed pelleting. Pre- and post-emergence damping-off and stem rotting were also observed. All four biocontrol agents greatly suppressed the infection of *S. rolfsii* and significantly low RCI for sunflower were recorded in plants inoculated with biocontrol agents as compared to non-inoculated plants. Among the four biological control agents, *T. harzianum* was more effective in reducing the disease incidence followed by *T. pseudokoningii, G. virens* and *T. polysporum* (Fig. 1). Pelleting of seeds with microbial antagonists with sugar solution slightly increased the efficacy of these biological control agents (Fig. 1). Pre- and post-emergence damping-off also reduced significantly where microbial antagonists were used for seed pelleting.

The use of microorganisms as biocontrol agents has provided a very promising alternative and less hazardous method for the control of plant pathogens. Franken *et al*., (2002) observed that *Trichoderma* spp., colonize plant roots prior to stimulation of plant growth and provide protection against invasion of infectious foreign organisms. In the present study, *Trichoderma* spp., and *G. virens* significantly suppressed the infection of *S. rolfsii* on mungbean and sunflower, which is in accordance with the findings of Mukerjee & Raghu (1997) who observed that *Trichoderma* spp., and *G. virens* 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. T. harzianum* has also provided effective biocontrol agent of many diseases caused by *S. rolfsii* on different host crops such as 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).

Plant growth: Artificial infestation of soil with *S. rolfsii* resulted in reducted plant weight and shoot length of sunflower and mungbean as compared to that in control. Seed pelleting with conidia of microbial antagonists either in water or sugar solution resulted in significantly greater plant height in soil amended with sclerotia of *S. rolfsii* as compared to control where no microbial antagonists were applied (Fig. 2). Maximum plant length was recorded in plants where seeds were pelleted with *T. harzianum* followed by *T. pseudokoningii, T. polysporum* and *G. virens* in both the test crops (Fig. 2). However, the difference between the efficacy of microbial antagonist was not significant except in case of plant weight in mungbean where *T. harzianum* was significantly more effective ($p<0.05$) as compared to other antagonists used. Use of sugar solution for seed pelleting with microbial antagonists showed significant better growth as compared to conidial suspension in water but the difference was not significant. Use of microbial antagonists in non-infested soil showed significant increase in growth as compared to that in infested soil. Even in non-infested soil, use of microbial antagonists showed significant increase in plant growth as compared to plants where no antagonists were used.

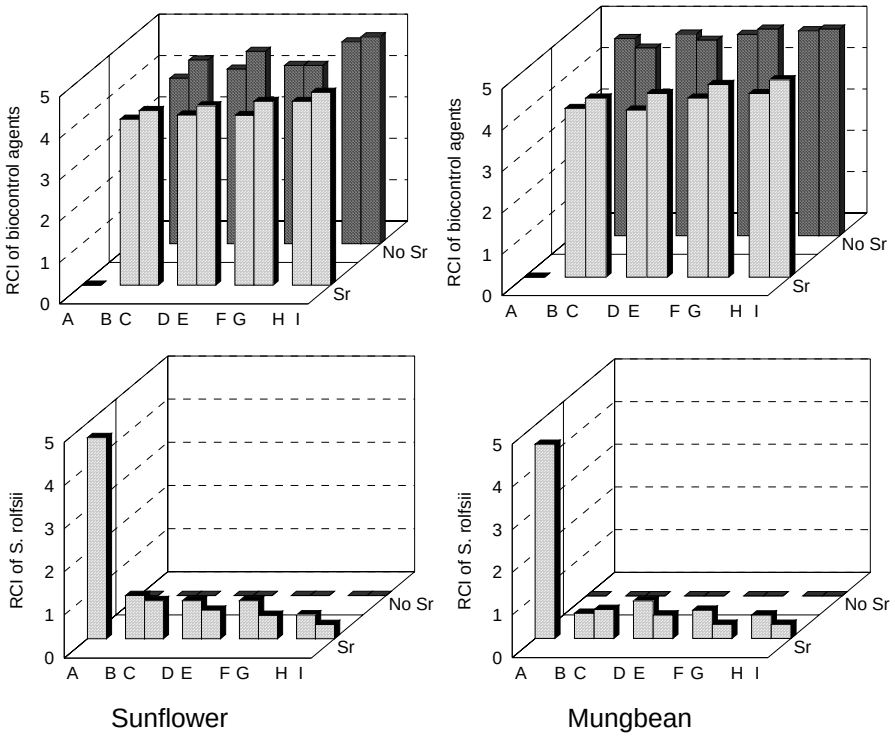

Fig. 1. Effect of *S. rolfsii* and biocontrol agents on root colonization of mungbean and sunflower.

A= control (having no biocontrol agent), B= *G. virens* without sugar solution, C= *G. virens* with sugar solution, D= *T. polysporum* without sugar solution, E= *T. polysporum* with sugar solution, F= *T. pseudokoningii* without sugar solution, G= *T. pseudokoningii* with sugar solution, H= *T. harzianum* without sugar solution, I= *T. harzianum* with sugar solution, Sr= soil infested with *S. rolfsii*, No Sr= soil not infested with *S. rolfsii.*

Several microbes including *Trichoderma* species are well known to show positive growth promoting substances. In the present study, enhancement of plant growth with microbial antagonists were in accordance with Harman *et al.*, (2004) who concluded that biocontrol agents are effective as a seed treatment since that colonize roots, increase root mass, health and consequently frequently provide yield increases. Kleifeld & Chet (1992) reported that *T. harzianum* applied to pathogen-free soil induced an increase in emergence of seedlings, plant height, leaf area and dry weight. Similarly, Arora *et al.*, (1992) observed that root colonization by *Trichoderma* frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients. Baker *et al.*, (1986) observed that radish growth was increased in raw soil by application of *T. harzianum*. There are several other reports where *Trichoderma* spp., effectively increased the plant growth, weight and root mass (Chang *et al*., 1986; Cole & Zvenyika, 1986; Sivan *et al.*, 1984; Paulitz *et al*., 1985; Ahmed & Baker, 1987; Papavizas, 1985; Kumar *et al*., 2007) that support the results of the present investigation. Use of microbial antagonists as seed treatment for better crop productivity even in absence of any disease could, therefore, be suggested.

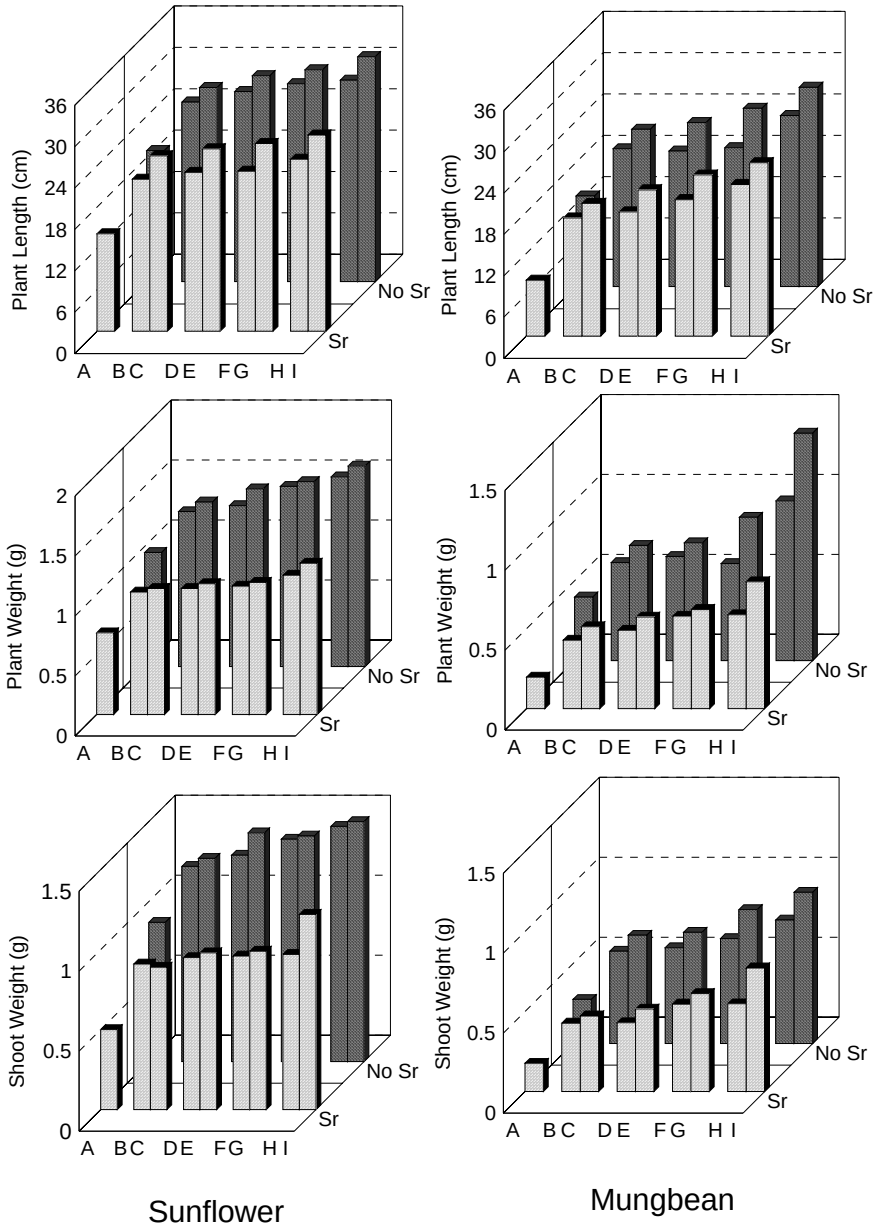

Fig. 2. Effect of *S. rolfsii* and biocontrol agents on plant growth of mungbean and sunflower.

A= control (having no biocontrol agent), B= *G. virens* w/o sugar solution, C= *G. virens* with sugar solution, D= *T. polysporum* without sugar solution, E= *T. polysporum* with sugar solution, F= *T. pseudokoningii* without sugar solution, G= *T. pseudokoningii* with sugar solution, H= *T. harzianum* without sugar solution, I= *T. harzianum* with sugar solution, Sr= soil infested with *S. rolfsii*, No Sr= soil not infested with *S. rolfsii*.

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