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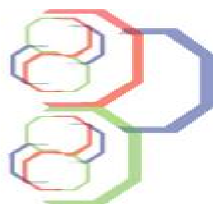

Control of *Rhizoctonia solani* in Soybean (*Glycin max* L) by seed-coating with *Trichoderma viride* and *Gliocladium virens* spores

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Abstract

Objectives: The study evaluated biocontrol of root-rot disease caused by *Rhizoctonia solani* on soybean using two antagonistic fungal species *Trichoderma viride* and *Gliocladium virens*, applied as seed coating or culture filtrate.

Methodology and Results: Pathogenic *R. solani* was added to sterile vermiculite in pots to give an inoculum density of 1- 5 g fresh weight mycelium/kg of sterile vermiculite. Sterilized soybean seeds were treated before planting by soaking for 30 min in 4 x10^6 CFU/ ml spore suspensions of either *T. viride* or *G. virens.* Coating soybean seeds with *T. viride* spores reduced the incidence of root-rot disease by up to 83% in the greenhouse experiment. Coating seeds with *G. virens* spores also decreased disease incidence but to a lesser extent than *T. viride.* Cell free culture filtrates of both *T. viride* and *G. virens* also significantly inhibited the growth of *R. solani*, with effect increasing as the concentration of the filtrates in the culture media increased. The root exudates of soybean seedlings increased the growth of the pathogenic fungus.

Potential application of findings: The results show that coating soybean seeds with the spore suspension of *T. viride* or *G. virens* can effectively control *R. solani* on soybean.

Key words: biocontrol, Gliocladium, Rhizoctonia, Soybean, Trichoderma,

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Introduction

Advances in research on biological control of soil-borne plant pathogens are occurring at a rapid rate. This progress is partly due to increased knowledge in the production, formulation and delivery of various biocontrol agents, which include fungi, bacteria and actinomycetes (Lumsden & Vaughn, 1993). The use of *Trichoderma* and *Gliocladium* as biocontrol agents of soil-borne pathogenic fungi has been known for quite a long time (Papavizas, 1992). *Trichoderma* preparation and application has already been commercialized (Pristchepa & Voitka, 1999), under the name Trichodermine® for control of root rots tomato and cucumber grown under greenhouse conditions. *Gliocladium* sp. has also been developed into a commercial product named Soil-Gard®. The biocontrol of both *Rhizoctonia solani* and *Pythium* sp. by coating radish and pea seeds with *Trichoderma hammatum* has been reported, as well as the use of *T. harzianum* as a biocontrol agent for root rot disease in beet and cucumber seedlings (Yedidia *et al.*, 2001; Viterbo *et al.*, 2004). Microorganisms that can

grow in the rhizosphere are ideal for use as biocontrol agents since the rhizosphere provides the front-line defense zone for roots against attack by pathogens. *Trichoderma* and *Gliocladium* are perhaps the two most commonly studied biocontrol agents of fungal genera (Inglis & Kawchuk, 2002).

The soil-borne pathogen *Rhizoctonia solani* is ubiquitous fungus that severely infects more than 200 crops of economic importance when environmental conditions are suitable. The pathogen causes seed and seedling rot, root and hypocotyls damage. *Trichoderma* spp. and *Gliocladium* spp. have received much attention because of their potential as biocontrol agents of many plant pathogenic fungi (Inglis & Kawchuk, 2002; Xiong *et al*., 2005). Roberts *et al*. (2005) reported successful screening of different isolates of *Trichoderma virens* for suppression *R. solani* which caused severe economic losses to field and greenhouse grown cucumber. *T. virens* isolates GL3 and GL2, provided the most effective suppression of damping-off caused by *R. solani* in greenhouse bioassays.

In this study *Trichoderma viride* and *Gliocladium virens* were assessed when applied as seed-coating or culture filtrates to control *Rhizoctonia solani* on soybean (*Glycine max*) grown in artificially infected soil.

Material and Methods

Microorganisms and culture conditions: Both *T. viride* and *G. virens* were isolated from the rhizosphere of soybean plants and were maintained on Potato Dextrose Agar (PDA). The pathogen used in this study was isolated from infected roots of soybean seedlings and maintained on PDA media. The three isolated fungi were identified at the Microbiology Department, Faculty of Science, King Abdel-Aziz University in Jeddah.

Seed coating with the biocontrol fungi: Soybean seeds were surface sterilized using 0.02% mercuric chloride for 5 min (Fadeel, 1964), rinsed several times with sterile distilled water and dried between two sterile filter papers.

Inoculum preparation: Shake cultures of *R. solani* were grown in 500 ml conical flasks containing 250 ml of liquid medium per flask (Weinhold *et al*., 1969). The mycelia were harvested after 5 days of incubation at 28 ± 1°C and washed three times using sterile distilled water, and finally collected on Buchner funnel with excess water removed by suction. The mycelia were macerated in 200 ml sterile distilled water for 30 sec using an electric blender. Inoculum was added to the sterile vermiculite in pots to give between 1 - 5 g fresh weight of mycelium per kilogram of sterile vermiculite.

The sterilized soybean seeds were soaked for 30 min in spore suspensions of both *T. viride* and *G. virens* (4 x10^6 CFU/ ml) (Berger *et al*., 1996). After soaking in spore suspensions the seeds were soaked again in 1% methyl cellulose (MC) solution, mixed at a rate of 20 ml: 50 seeds. The seeds were then removed and placed on plastic plates in front of an air current until completely dry (De-Freitas & Germida, 1991).

Effect of cell free culture filtrates (CFCF) of biocontrol agents on the growth of *R. solani*: Mycelial discs measuring 5 mm diameter were taken from the growing margins of cultures of *R. solani* (on PDA) and transferred to 250 ml conical flasks containing 50 ml liquid media mixed with the cell free culture filtrates (CFCF) of antagonists to obtain the required concentrations. The flasks were incubated for 8 days at 28 ±2°C and the fungal growth was determined after every 4 days (Walker, 1991).

Preparation of root exudates: Exudates were collected from 2 week-old soybean seedlings. About 50 g seeds were surface disinfected for 5 min in 100 ml of 0.5% sodium hypochlorite after which they were filtered through sterilized strainer and rinsed several times using sterile distilled water. The sterilized seeds were sown in 500 ml conical flasks containing sterile acid washed quartz sand wetted with suitable amount of sterile water and kept at room temperature (25°C) away from direct sunlight for two weeks. The exudates were collected and evaporated under vacuum at 50°C to a volume of 5 ml exudates/10 g fresh root, and filtered through 0.45μm bacterial filter (Dijk & Nelson, 1998).

Results and Discussion

The results showed that disease incidence increased as the density of the pathogenic inoculum increased (Table 1). There was a linear relationship between the amount of inoculum of *R. solani* applied and the incidence of rot disease on soybean seedlings. This result was similar to that obtained by Lewis *et al.* (1995); Larkin and Fravel (1999) and Viterbo *et al.* (2004). *R. solani* infection also delayed the emergence and development of soybean, also proportionally to the inoculum level. The maximum disease was obtained at the inoculum level of 0.5% (w/w: pathogen/soil). This result agreed with the results of Phillips (1989) and Conway *et al.* (1997). Due to the short incubation period (5 days), no sclerotia were present in the pathogen inoculum applied. Thus it can be argued that the infective units are the hyphae that were present in the inoculated volume of soil surrounding the infection court (in this case the soybean roots). The type of inoculum used in this study simplifies determination of the relationship between inoculum density and disease incidence, as it excludes pathogen survival structures that may not invoke infection immediately after plants are inoculated. Umechuruba & Nachukwu (1997) reported that some metabolites of pathogenic fungi decreased germination of African yam bean and could also be related to the development of seedlings and increase in disease incidence. In this study *R. solani* may have secreted enzymes such as cellulase and chitinase which might lyse the cellulose and chitin in tissues of soybean roots. Other studies have reported the potential of some *R. solani* strains to secret some toxic metabolites (Nema, 1992; Madhosingh, 1995).

Table 1: Relationship between root-rot disease incidence on soybean seedlings and the density of inoculum added to the potting soil (Mean of Replicates ± SE).

% inoculum density in soil	% of healthy seedling	% of unhealthy seedling
0.00	86.67 ± 2.98	13.33 ± 2.98
0.10	73.33 ± 2.11**	26.67 ± 2.11*
0.20	62.67 ± 3.40**	37.33 ± 3.40**
0.30	49.33 ± 1.63**	50.67 ± 1.63**
0.40	46.67 ± 2.11**	53.33 ± 2.11**
0.50	45.34 ± 1.33**	54.66 ± 1.33**

* Significant at 5 %; ** significant at 1 %

Table 2: Effect of coating soybean seeds with *Trichoderma viride* and *Gliocladium virens spores* on the incidence of Rhizoctonia rot disease. Seeds were planted in soil infested with 0.5% inoculum of *Rhizoctonia solani* and disease was assessed 4 weeks after planting (Mean of Replicates ± SE).

Treatment	% of healthy seedling	% of unhealthy seedling
Sterile soil + sterilised seeds	91.40 ± 0.5	8.60 ± 0.51
Sterile soil + seeds coated with *T. harzianum*	97.20 ± 0.37**	2.80 ± 0.37**
Sterile soil + seeds coated with spores of *G. virens*	94.20 ± 0.73*	5.80 ± 0.73*
Soil with *R.solani* + seeds coated *T. harzianum*	83.00 ± 0.95**	17.00 ± 0.95**
Soil with *R. solani* + seeds coated *G. virens*	76.00 ± 1.41**	24.00 ± 1.41**

* Significant at 5 %; ** Significant at 1 %

Further results (Table 2) revealed that treating soybean seeds with spores of the antagonistic fungi *T. viride* or *G. virens* decreased the incidence of Rhizoctonia root-rot disease. The biocontrol effect could be due to production of chitinases that hydrolyse the chitin cell wall of *R. solani* (Benhamou & Chet, 1993). Some strains of these antagonistic fungi have also been reported to secrete metabolites, e.g. mycotoxins or antibiotics that can inhibit or suppress the growth of other fungi in the rhizosphere (Ding *et al.*, 2003; Chung *et al.*, 2005).

Plant growth was also increased by dressing seeds with spores of the tested antagonistic fungi (Table 3). The increase was in both fresh and dry weight in addition to plant height and the numbers of leaves per plant. Similar plant growth promoting effects have been reported by Yedidia *et al.* (2001) and Harman *et al.* (2004). It has been suggested that some metabolites of the antagonistic microbes stimulate plant growth while others reduce population density of plant pathogens. Such metabolites may include antibiotics, siderophores, Hydrogen cyanide, enzymes, or growth stimulating hormones such as auxins and gibberellins. Some of the metabolites facilitate transformation of unavailable mineral and organic compounds into available forms to the plant (Zehnder *et al.*, 2001; Inglis & Kawchuk, 2002).

Table 3: Effect of seed coating with *Trichoderma viride* and *Gliocladium virens* spores on growth parameters of soybean seedlings in potting soil infested with 0.5% *R. solani.* Growth was assessed four weeks after planting (Mean ± SE).

Parameter	Plant height (cm)	No. of leaves/plant	Fresh weight (g/plant)	Dry weight (g/plant)
Control	23.96 ± 0.16	4.20 ± 0.37	2.61 ± 0.04	0.24 ± 0.002
Soil infested with 0.5% *R. solani*	17.26 ± 0.32**	2.67 ± 0.24**	1.85 ± 0.12**	0.17 ± 0.002**
Seeds with *T. viride* in sterile soil.	27.68 ± 0.28*	5.04 ± 0.24**	2.72 ± 0.05**	0.29 ± 0.002**
Seeds with *G. virens* in sterile soil.	26.18 ± 0.24*	4.63 ± 0.40**	2.63 ± 0.06**	0.26 ± 0.005**
Seeds with *T. viride* in soil with *R. solani*	22.10 ± 0.25*	3.85 ± 0.42*	2.47 ± 0.06**	0,23 ± 0.001**
Seeds with *G. virens* in soil with *R. solani*	21.87 ± 0.17*	4.10 ± 0.30*	2.37 ± 0.13**	0.20 ± 0.003**

*Significant at 5%; **Significant at 1%

In this study the growth of the pathogenic fungus was also significantly inhibited when cell free culture filtrates of *T. viride* or *G. virens* were included at high concentrations (Table 4). Such inhibitory effects have also been reported by Suarez *et al.* (2004) among others, and are a likely indicator that the antagonistic fungi produce some metabolite(s) that inhibit pathogen growth. The root exudates of soybean seedlings also promoted the growth of *R. solani* (Table 5). Positive responses of pathogens when exposed to the host plant or its products are well known, and have been reported for the soybean-Rhizoctonia pathosystem (Vivek *et al.*, 1999; Nagahshi & Douds, 2000). Exudates of plant roots may contain amino acids, glycosides, tannins, organic acids or carbohydrates which could increase the growth of pathogenic fungi in the rhizosphere of the plants.

Table 4: Effect of varying concentrations of cell free culture filtrates of *Trichoderma viride* and *Gliocladium virens* on the growth of *R. solani* after incubation for 8 days Data is mg of fungal biomass; Mean of replicates ±SE).

% filtrate		1st 4 days	2nd 4 days	Total 8 days
No filtrate in medium		401.0±4.5	163.0±6.83	564.0±6.87
T. viride	20	375.25±4.4	150.0±4.22**	525.25±5.71**
	40	284.75±4.07**	126.0±1.87**	410.75±5.89**
	60	219.0±5.67**	84.5±8.66*	303.5±10.65**
	80	195.25±2.78**	34.0±3.63*	229.25±4.75**
G. virens	20	376.25±6.65**	162.0±3.42**	538.25±3.44**
	40	310.75±9.23**	141.75±1.11**	452.5±10.15**
	60	261.25±5.2**	125.0±4.06**	386.25±24.99
	80	202.75±2.63**	85.25±6.66**	288.0±7.49**

*Significant at 5%; **Significant at 1%

Table 5: Effect of varying concentration of root exudates of *Glycin max* (soybean) seedlings on the growth of *R. solani* assessed at 4 day intervals (mean of replicates ±SE)

% proportion of exudates	Dry weight. (mg fungal biomass)	
	4 days	8 days
0.0	370.25±9.83**	565.75±7.34
10.0	402.25±4.33**	573.75±16.76**
15.0	421.75±12.83**	606.0±8.27**
20.0	429.75±9.71**	630.25±5.03**
25.0	467.5±3.38**	685.5±3.97**
30.0	430.0±11.63**	623.5±9.65**

*Significant at 5%; **Significant at 1%

References

Berger F, Li H, White D, Frazer R, Leifert C, 1996. Effect of pathogen inoculum, antagonist density and plant species on biological control of *Phytophthora* and *Pythium* damping-off by *Bacillus subtilis* Cot I in high humidity fogging glasshouses. Phytopathology 86: 428-433.

Chung WC, Huang JW, Huang HC, 2005. Formulation of soil biofungicide for control of Chinese cabbage (*Brassica chinensis*) caused by *Rhizoctonia solani*. Biological Control 32: 287-294.

Conway KE, Maness EM, Motes JE, 1997. Integration of biological and chemical controls for *Rhizoctonia* aerial blight and root rot of rosemary. Plant Disease 81: 795-798.

De-Freitas JR, Germida JJ, 1991. *Pseudomonas cepacia* and *Pseudomonas putida* as winter wheat inoculants for biocontrol of *Rhizoctonia solani*. Canadian Journal of Microbiology 37: 780-784.

Dertinger U, Schaz U, Schulze E, 2003. Age-dependence of the antioxidative system in tobacco with enhanced glutathione-induced production of cytokinins. Physiologia Plantarum 119: 19-29.

Ding WL, Cheng HZ, Chen J, 2003. Research on preventing medicinal plant diseases with *Trichoderma harzainum* preparation. Zhongguo Zhong Yao Za Zhi. 28: 24-27.

Fadeel AA, 1962. Location and properties of chloroplast and pigment determination in root. Physiologia Plantarum 15: 130-147.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M, 2004. *Trichoderma* species opportunistic, avirulent plant symbionts. National Review of Microbiology 2: 43-56.

Inglis GD, Kawchuk LM, 2002. Comparative degradation of oomycete, ascomycete and basidiomycete cell walls mycoparasitic and biocontrol fungi. Canadian Journal of Microbiology 48: 60-70.

Larkin RP, Fravel DR, 1999. Mechanisms of action and dose-response relationships governing biological control of *Fusarium*

wilt of tomato by non pathogenic *Fusarium* spp. Phytopathology 89: 1152 - 1161.

Lewis JA, Fravel DR, Papavizas GC, 1995. *Cladorrhinum foecundissimmn:* A potential biological control agent for the reduction of *Rhizoctonia solani.* Biological Biochemistry 27: 863 - 869.

Lumsden RD, Vaughn JL, 1993. Pest management: Biologically Based Technologies. American Chemical Society. Washington DC.

Madhosingh C, 1995. Relative wilt-inducing capacity of the culture filtrates of isolates of *Fusarium oxysporum* f.sp. *radicis-lycopersici*, the tomato crown and root-rot pathogen. Journal of Phytopathology 143: 193 - 198.

Nagahashi G, Douds DD Jr, 2000. Partial separation of root exudates components and their effects upon the growth of germinated spores of AM fungi. Mycological Research 104: 1453 - 1464.

Nema AG, 1992. Studies on pectinolytic and cellulolytic enzymes produced by *Fusarium udum* causing wilt of pigeon pea. Indian Journal of Forestry 15: 353 - 355.

Phillips AJL, 1989. Relationship of *Rhizoctonia solani* inoculum density to incidence of hypocotyl rot and damping-off in dry beans. Canadian Journal of Microbiology 35: 1132 - 1140.

Pristchepa LI, Voitka DV, 1999. Effectiveness of Trichodermin in protection of tomato and cucumber against disease. Bulletin of Polish Academy of Biological Science 47: 179 - 182.

Roberts DP, Lohrke SM, Meyer SLF, Buyer JS, Bowers JH, Baker CJ, Li W, de Souza JT, Lewis JA, Chung S, 2005. Biocontrol agents applied individually and in combination for suppression of soil-bone diseases of cucumber. Crop Protection 24: 141 - 155.

Suarez B, Rey M, Castillo P, Monte E, Lobell A, 2004. Isolation and Characterization of PRAI, a trypsin-like protease from the biocontnol agent *Trichoderma harzianum* CECT 24 displaying nematicidal activity. Applied Microbiology and Biotechnology 65: 46 - 55.

Umechuruba CI, Nwachukwa EO, 1997. The effect of filtrates of seed-borne fungi of African yam bean on seed germination and seedling development. Global Journal of Pure and Applied Science 3: 165 - 176.

Viterbo A, Harel M, Chet I, 2004. Isolation of two aspartyl proteases from *Trichoderma asperellum* expressed during colonization of cucumber roots. FEMS Microbiology Letters 238: 151 - 158.

Vivek K, Indra H, Sindhan GS, 1999. Role of seed exudates in differential behavior of barley varieties to *Drechslera graminea* infection. Journal of Mycology and Plant Pathology 29: 121 - 122.

Walker GE, 1991. Chemical, physical and biological control of carrot seedling diseases. Plant and Soil 136: 31 - 39.

Weinhold AR, Bownan T, Dodman RL, 1969. Virulence of *Rhizoctonia solani* as affected by nutrition of the pathogen. Phytopathology 59: 1601 - 1605.

Xiong H, von Weymarn N, Turunen O, Leisola M, Pastinen O, 2005. Xylanase production by *Trichoderma ressei* Rut C-30 grown on L-arabinose-rich plant hydrolysates. Bioresource Technology 96: 753 - 759.

Yedidia I, Srivastva AK, Kapulnik Y, Chet I, 2001. Effect of *Trichoderma harzianum* on microelement concentration and increased growth of cucumber plants. Plant and Soil 235: 235 - 242.

Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW, 2001. Application of rhizobacteria for induced resistance. European Journal of Plant Pathology 107: 39 - 50.