Bot. Bull. Acad. Sin. (1997) **38:** 33–39

Influence of bean seedling root exudates on the rhizosphere colonization by *Trichoderma lignorum* for the control of *Rhizoctonia solani*

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(Received March 27, 1996; Accepted November 27, 1996)

Abstract. Application of *Trichoderma lignorum* as a seed coating (8×10^6 conidia/seed) or wheat bran preparation (1×10^6 cfu/g) at a rate of 20 g/kg soil, greatly reduced the number of bean seeds infested by *Rhizoctonia solani,* and the percentage of healthy seeds reached 92%. Germination of conidia of *Rhizoctonia solani* in bean rhizosphere soil was inhibited after soil or seed application with *Trichoderma lignorum*. Bean seedling exudate increased the mycelial growth rate of *Rhizoctonia solani* and *Trichoderma lignorum* in vitro. Under greenhouse conditions, the addition of germinating bean seed exudate to soil infested with *Rhizoctonia solani* and planted with bean, reduced the disease control capability of the antagonist. Plants grown from seeds treated with *Trichoderma lignorum* had roots with lower levels of *Rhizoctonia solani* in their rhizosphere than the roots of untreated seeds. *Trichoderma* had little effect on the survival of *Rhizoctonia solani* in non-rhizosphere soil. However, application of *Trichoderma lignorum* as a wheat-bran preparation, conidial suspension or seed coating reduced the pathogen counts in the rhizosphere soil of beans.

Keywords: Biocontrol; *Rhizoctonia solani* antagonism; Soilborne plant pathogens.

Introduction

Biological control of plant diseases, especially soilborne plant pathogens, has been the subject of much research in the last two decades. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan et al., 1984 and Coley-Smith et al., 1991).

Plant roots growing in soils are a major source of carbon and energy to microorganisms in the form of root exudates, cells detached from old parts of the root, or the root itself after plant death (Cook and Baker, 1983). Competition for nutrients, primarily carbon, nitrogen, and iron may result in biological control of soilborne plant pathogens (Scher et al., 1984). Recently, Elad and Baker (1985) and Elad and Chet (1987) reported that carbon sources, either provided by synthetic substances or excreted by plant roots might be involved in the chlamydospore and oospore germination of *Fusarium oxysporum* and *Pythium aphanidermatum*, respectively. Proliferation along the developing rhizosphere is one of the most important trails for antagonists applied to seed (Cook and Baker, 1983). Most studies in this field have been dealt with antagonistic rhizobacteria (Kloepper et al., 1980; Whipps and Lynch, 1983; Ordentlich et al., 1987), but there is relatively little information involving fungi.

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Biological control of soilborne plant pathogens can be achieved by seed treatment with antagonists. Harman et al. (1980) reported the biocontrol of *Rhizoctonia solani* and *Pythium* spp. by coating radish and pea seed with *Trichoderma hamatum* (Bain.). Also, Hadar et al. (1979) and Elad et al. (1980) investigated that the application of wheat bran colonized by *Trichoderma harzianum* to soils infested by *Rhizoctonia solani* and *Sclerotium rolfsii* reduced the incidence of disease caused by these pathogens in beans.

The objectives of the present study were to study the biological control of *Rhizoctonia solani* damping-off of bean by *Trichoderma lignorum* and the relation between germinating bean seedling exudates on the potential of *Trichoderma lignorum* to inhibit conidia germination of *Rhizoctonia solani* in soils. We also examined the population dynamics and the activity of *Trichoderma* and its interactions with *Rhizoctonia solani* in the bean rhizosphere.

Materials and Methods

Soilborne Fungi

Rhizoctonia solani was isolated from diseased bean seedlings and *Trichoderma lignorum* was isolated from bean rhizosphere on potato dextrose agar (PDA, Difco). After isolation, these fungi were cultured at 27°C on yeast

extract-glucose medium (YM); containing 5 g of yeast extract (Difco, Lab., Detroit, MI), 5 g of peptone (Difco), 10 g of glucose and 20 g of agar (Difco) per liter (Sivan and Chet, 1989). Pathogenicity of *Rhizoctonia solani* to bean seedlings were tested according to the method of Sivan et al. (1984).

Production of Inhibitory Substances by Trichoderma lignorum

Excretion of inhibitory substances in liquid culture was tested by using 250 ml Erlenmyer flasks each containing 50 ml of synthetic medium (SM) (Sivan et al., 1984). The cultures were inoculated with *Trichoderma lignorum* and shaken on a rotary shaker at 150 rpm for 48 h at 30°C. The mycelium was then washed with sterile tap water and separated by centrifugation at a 9.150 g for 20 min at 4°C. The supernatant was filtrated through Whatman No. 1 filter paper, passed through a 0.45-μm Millipore filter, mixed with SM containing 3% agar (1:1 v/v), and then poured into petri dishes. SM supplemented with sterile tap water served as control. A mycelial disk of *Rhizoctonia solani* served as inoculum. Linear growth rate of *Rhizoctonia solani* was determined 24 h after inoculation.

The same technique was used to determine the effect of culture filtrate of *Rhizoctonia solani* on the linear growth of *Trichoderma lignorum*.

Seed Treatment and Experimental Design

Greenhouse Experiments — Experiments were carried out in artificially infested sandy loam soil (pH 7.3) containing 82.3% sand, 2.3% silt, 15% clay, 0.3% organic matter, 0.02% nitrogen, 0.067% potassium, 0.01% phosphorus and 0.003% extractable iron. Soil moisture-holding capacity was 12.2% (w/w). Soil was infested with a conidial suspension of *Rhizoctonia solani* (Sivan et al., 1984). Erlenmeyer flasks (250 ml) each containing 50 ml of liquid PDA, were seeded with mycelial disks from 72 h old cultures of the pathogen. Flasks were incubated at 27°C in a rotary shaker at 120 rpm for 10 days. Conidia were then separated from the mycelium by filtration through eight layers of cheesecloth. The conidia were washed in sterile tap water three times by centrifugation at 7,000 *g* for 30 min at 4°C. Ten ml of this suspension adjusted to 2×10^6 conidia per milliliter, was mixed with sandy loam soil in an electrical soil mixer.

Experiments were set up with plastic pots (9 × 4 × 10 cm) each containing 1 kg of soil sown with 10 seeds of the test plant. Seeds were not subjected to any chemical treatment. *Trichoderma lignorum* was applied as seed coating or in wheat bran preparation. A conidial suspension was prepared from cultures grown in Erlenmeyer flasks containing 20 ml of PDA. The suspension was washed three times in sterile tap water by centrifugation (15, 000 *g*) and adjusted to 6×10^8 conidia per milliliter. A seed coating was prepared from conidia collected as described above and supplemented with 0.015% (v/v) of molasses as an adhesive. Five milliliters of this suspension were used to coat 20 g of bean seeds, which were immediately dried by warm ventilation. The number of populations of *Trichoderma* on the seed surface was counted by shaking 5 g of seeds in 50 ml of sterile tap water for 1 h in 250 ml Erlenmeyer flasks in a rotary shaker at 200 rpm. Serial dilutions of the suspension were plated on a medium selective for *Trichoderma* (TSM) (Elad et al., 1981). Wheat bran at 10% moisture was amended with 40 ml of tap water per 100 grams. The mixture was then autoclaved in 250 ml Erlenmeyer flasks for 1 h at 121°C on three successive days. Homogenized mycelium from 48 h old cultures of *T. lignorum* in liquid SM was transferred to wheat bran and incubated in an illuminated chamber for 14 days at 30°C. This preparation, containing 1×10^6 colony-forming units (cfu) per gram, was mixed with soil at concentrations of 2.5, 5, 10, 15, and 20 g/kg of soil. All experiments were carried out with non-treated seeds. The incidence of disease caused by *Rhizoctonia solani* was expressed as the percentage of diseased plants. Greenhouse experiments were carried out at 27–33°C. Each treatment in all experiments contained three replicates, and the experiments were repeated at least twice.

Conidia Germination in Rhizospher Soil

Soil enrichment with conidia of *Rhizoctonia solani* was performed according to Sneh et al. (1984). Mycelial mats of *Rhizoctonia solani* were grown in liquid PDA in conical flasks at 27°C. After 7 days, mycelial mats were separated, placed in four layers of cheesecloth, and washed under distilled running water for 5 min. Twelve mats of the fungus were blended for 1 min and centrifuged for 10 min at 3,000 *g*. The pellet was resuspended in 150 ml of water and added to 1 kg of soil. The soil was mixed, placed in glass bottles, and incubated at 27°C for 4 weeks. It was then air dried to 10% moisture, sieved through a 2-mm-mesh screen, mixed well, and stored at 4°C until use. The soil contained an average of 1×10^6 cfu/kg of *Rhizoctonia solani* as determined after soil dilution and plating on PDA medium.

Samples of 1 g conidia-soil were placed in 10 ml test tubes. *Trichoderma lignorum* was added as a pregerminated conidial suspension (in water solution of 0.01% glucose). Soil was thoroughly mixed and incubated at 27°C for 22 h. After the incubation, the soil in each test tube was stained with 1 ml aliquots of 0.3% Calcoflour New M2R (American Cyanamid Company, Bound Brook, NJ) solution according to Scher et al. (1984). After 5 min, the excess solution was removed and replaced with 1 ml of distilled water. Soil was suspended and a drop of a suspension taken for observation under UV light using an epifluorescent light microscope (Olympus, Japan) at 400.

Conidia germination in the rhizosphere was tested according to Sneh et al. (1984). Bean seeds were disinfected for 3 min in 4% sodium hypochlorite and placed between four layers of wet chromatography paper, which were then wrapped in plastic bags and incubated at 30°C for 24 h. After germination the seeds were transferred to 5 g of conidia enriched soil, some samples also amended with *Trichoderma lignorum*. The mixture was placed between

two microscope glass slides, which were secured with two rubber bands. The slides were slightly slanted in moist soil in plastic pots (9 × 9 × 10 cm), wrapped in a plastic bag and incubated at 27°C for the desired period. The slides were then recovered from the soil, and the germinated seedlings, with soil adhering to roots, were carefully removed and transferred to a 10 ml test tube for microscopic observation as described above.

In both conidia germination tests (rhizosphere and non-rhizosphere soil), *Trichoderma lignorum* was also applied as seed coating. Three germinated seeds were used per treatment and the conidia were counted.

Production and Collection of Plant Exudates

Bean seeds were disinfected with 4% sodium hypochlorate for 10 min. The efficacy of disinfestation was tested by placing samples of the treated seeds on PDA or nutrient agar (Difco) plates. Eight hundred seeds were placed in a glass column (80 × 7 cm) containing 1 L of sterile tap water. The lower part of the column was connected to an air compressor and air was forced inside through a sterile glass fiber filter at a pressure that thoroughly agitated the suspended seeds. At the upper part of the column an air outlet containing an additional glass fiber filter was mounted. The column with the germinating seeds was incubated at 27°C for 4 days. The resulting seeds exudate mixture was then collected in a sterile container and kept at -30°C until use.

Rhizosphere Colonization Tests

Rhizosphere population dynamics of *Trichoderma lignorum* and *Rhizoctonia solani* were studied according to the method developed by Scher et al. (1984) as modified by Ahmad and Baker (1987). A seed of the test plant was placed at the upper part of two longitudinal halves of 50 ml polypropylene tubes measuring 11.5 × 2.8 cm filled with sandy loam soil with 15% (w/w) moisture content. *Trichoderma lignorum* was applied to soil as a seed coating, whereas *Rhizoctonia solani* was introduced to soil as conidial suspension (2 × 10^6 conidia/g of soil). One seed of bean was placed on the half tube 1 cm below the rim. The unseeded half tube was carefully placed on the first half and secured with rubber bands. Tubes of all treatments were completely randomized and placed in plastic pots (9 × 9 × 10 cm) containing sandy loan soil with the same water content. The pots were covered with polyethylene bags to maintain the same soil water content and placed in an illuminated chamber at 28°C. After 8 days of incubation tubes were removed from the pots and their two halves carefully separated. The roots with adhering soil were cut, starting from the crown into 2 cm segments. Corresponding segments from six plants were combined and shaken in a rotary shaker for 1 h in 250 ml Erlenmeyer flasks containing 30 ml of sterilized water. Serial dilutions were plated on PDA medium for *Trichoderma* and *Rhizoctonia* counts. The counts of each fungus were expressed as cfu/g of root, including adhering soil (dry weight).

Results

The effect of different treatments of *Trichoderma lignorum* in controlling damping-off disease caused by *Rhizoctonia solani* in bean seedlings is shown in Table 1. The data revealed that when the untreated bean seeds were sown in the *Rhizoctonia solani* infested soil, the number of infested seeds increased greatly and the damping-off percentage was 91%. After coating seeds with *Trichoderma lignorum* conidia (8 × 10^6 conidia/seed), the number of infested seeds decreased greatly and the damping-off percentage was 6%. Application of wheat bran preparation of *Trichoderma lignorum* (5 × 10^6 cfu/g) at a rate of 2.5 g /500 g of soil decreased the damping-off percentage to 45%. On the other hand, application of wheat bran preparation at a rate of 15 and 20 g/500 g soil, decreased greatly the damping-off percentage to 12 and 6%, respectively, as compared to untreated bean seeds.

Table 1. Effect of different treatments of *Trichoderma lignorum* in controlling damping-off disease caused by *Rhizoctonia solani* in bean seedlings.

Trichoderma treatments	(%) of damping-off*	(%) of healthy plants*
Untreated control (without *R. solani*)	0	100
Control (infested with *R. solani*)[a]	91.3 ± 0.9 x	3.0 ± 0.6 x
Seed coating[b]	5.7 ± 0.3 y	92.3 ± 0.3 y
W.B.P. g/kg soil[c]		
2.5	44.7 ± 0.9 w	55.3 ± 0.9 w
5.0	26.7 ± 0.7 w	73.3 ± 0.7 w
10.0	18.0 ± 1.2 u	82.0 ± 1.2 w
15.0	11.7 ± 0.9 u	88.3 ± 0.9 u
20.0	6.0 ± 0.6 m	92.3 ± 0.9 u

[a]Under greenhouse conditions in soil infested with 30 ml conidia of *Rhizoctonia solani*/kg soil (2 × 10^6 conidia/ml).
[b]8 × 10^6 conidia of *Trichderma lignorum* per bean seed.
[c]Wheat bran preparation (W.B.P) of *Trichoderma lignorum* consisting of 1 × 10^6 cfu/g (dry wt.).
*Each value represents the mean of three replicates of two repeated experiments ± SE. Treatments were significantly different (P=0.05) from the control, using Duncan's multiple range test.

The inhibitory effect of *Trichoderma lignorum* treatments on conidia of *R. solani* germination in bean rhizosphere soil decreased after the addition of bean seed exudate. The percentage of germinated conidia of *R. solani* increased from 15.3% to 22.7% in seed coating and from 10.3 to 23.0 in conidial suspension. Addition of bean seed exudate increased the percentage of germinated conidia of *R. solani* from 33.7% to 39.0% in absence of *T. lignorum* (Table 2). Similarly, under greenhouse conditions, the addition of bean exudate to soil infested with *Rhizoctonia solani* and planted with bean reduced the disease control obtained by *T. lignorum* as either seed coating or conidial suspension. The percentage of damping-off increased from 5.7% to 37.7% in seed coating and from 15.3% to 43.7% in conidial suspension. Addition of germinating bean seed exudates increased the percentage of damping-off from 78.3% to 83.3% (Table 2).

Addition of pregerminated conidia of *Trichoderma lignorum* to soils reduced germination of conidia of *Rhizoctonia solani* (Table 3). In non-rhizosphere and rhizosphere soil of bean seedlings previously enriched with conidia of *Rhizoctonia solani* and planted with bean seeds, the germination rate of conidia was 21 and 44 %, respectively. However, when *Trichoderma lignorum* was added as seed coating or conidial suspension, the germination rate of *Rhizoctonia solani* was reduced to 30 and 28 %, respectively in rhizosphere soil and to 12 and 8 %, respectively in non-rhizosphere soil.

To determine the colonization of rhizosphere and non-rhizosphere soils infested with *Rhizoctonia solani* and treated with *Trichoderma lignorum*, serial dilution of these soils was carried out (Table 4). After a seed coating treatment *Trichoderma lignorum* effectively colonized the rhizosphere soil (6.7×10^6 cfu/g) but failed to establish in non-rhisosphere soil (8.2×10^1 cfu/g). Application of *Trichoderma lignorum* as a conidial suspension or wheat bran preparation resulted in high colonization of both rhizosphere (7.5×10^6 and 6.8×10^6 cfu/g) or non-rhizosphere soils (3.1×10^4 cfu/g), respectively. All treatments of *Trichoderma lignorum* had little effect on the recovery of *Rhizoctonia solani* from the non-rhizosphere soil (less than 8.3×10^2 cfu/g).

Discussion

Rhizoctonia solani is considered the main soilborne pathogenic fungus capable of causing severe damage to agricultural crops such as bean, potato, and cotton (Dath, 1988; Anguiz and Martin, 1989; Lartey et al., 1991). The pathogenic properties of *Rhizoctonia solani* to bean seeds in this study are in agreement with the results obtained by Elad et al. (1980), who recorded that *Rhizoctonia solani* was capable of attacking a tremendous range of host plants causing seed decay, damping-off, stem cankers, root rot, fruit decay, and foliage disease.

Table 2. Effect of germinating seed exudate of been seeds on the inhibitory effect of *Trichoderma lignorum* on conidia germination of *Rhizoctonia solani* and on the disease of control of bean damping-off.

Trichoderma treatments	Exudates[a]	(%) Conidia germination of *R. solani**	(%) Damping-off [b,]*
None	Not added	33.7 ± 2.1 xy	78.3 ± 0.9 x
	Added	39.0 ± 2.0 x	83.3 ± 4.2 x
Seed coating[c]	Not added	15.3 ± 2.5 w	5.7 ± 0.3 w
	Added	22.7 ± 4.7 y	37.7 ± 1.5 y
Conidial suspension[d]	Not added	10.3 ± 1.5 w	15.3 ± 3.5 w
	Added	23.0 ± 5.3 y	43.7 ± 3.2 y

[a]Exudate produced by bean seeds was mixed with 50 g of soil enriched with onidia of *R. solani*.
[b]Under greenhouse conditions in soil infected with conidia of *R solani* (1×10^6 kg/soil).
[c]6×10^5 conidia of *T. lignorum* per bean seed.
[d]1×10^6 conidia of *T lignorum* per gram of soil.
*Each value represents the mean of three replicates of two repeated experiments ± SD. Values of each column followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 3. Effect of addition of *Trichoderma lignorum* on conidia germination of *Rhizctonia solani* in rhizosphere and nonrhizosphere soil.

Trichoderma treatments	Conidia germination (%)*	
	Rhizosphere soil[a]	Nonrhizosphere soil
None	43.7 ± 1.5 x	20.7 ± 1.5 x
Seed coating[b]	30.3 ± 4.2 y	11.7 ± 3.5 y
Conidial suspension[c]	27.6 ± 3.2 y	8.3 ± 1.5 w

[a]Bean seeds were germinated for 48 h in soil enriched with *R. solani*.
[b]8×10^4 conidia of *T. lignorum* per bean seed.
[c]1×10^6 pregerminated conidia of *T. lignorum* per gram of soil.
*Each value represents the mean of three replicates of two repeated experiments ± SD. Values of each column followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 4. Effect of *Trichoderma lignorum* on *Rhizoctonia solani* in bean rhizosphere and non-rhizosphere soil.*

Treatments	Colony-forming units per gram of soil			
	Trichoderma spp.		*Rhizoctonia solani*	
	Rhizosphere soil[a]	Nonrhizosphere soil[b]	Rhizosphere soil	Nonrhizosphere soil
None	8.8×10^2 [(m)]x	2.1×10^2 u	1.9×10^3 w	8.3×10^2 w
Seed coating[c]	6.7×10^6 y	8.2×10 x	3.5×10^3 y	6.6×10^2 w
Conidial suspension[d]	7.5×10^6 w	3.1×10^4 y	3.9×10^3 y	4.1×10 y
Preparation[e]	6.8×10^6 w	4.8×10^5 w	8.1×10^3 y	5.6×10 y

*The soil infested with 2×10^6 conidia per gram of soil planted with bean seeds and placed in green house for 21 days.
[a]The plants were gently uprooted and the soil adhering to the roots was collected.
[b]Combined soil samples from distance greater than 1 cm from the roots.
[c]3.8×10^4 conidia of *T. lignorum* per seed.
[d]1×10^6 conidia of *Trichoderma lignorum* per gram of soil.
[e]Wheat bran preparation of *T. lignorum* consisting of 5×10^6 cfu/g (dry wt.) was introduced into soil at rate of 5 g/kg of soil.
[(m)]Values of each column followed by the same letter are not significantly different (P= 0.05) according to Duncan's multiple range.

Trichoderma spp., are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan et al., 1984; Coley-Smith et al., 1991). In the present investigation, application of *Trichoderma lignorum* as a wheat-bran preparation, conidial suspension, or seed coating greatly decreased the number of infested seeds by *Rhizoctonia solani* as well as damping-off percentages and hence controlling the fungal disease. The findings are consistent with the results of several investigators (Sivan and Chet, 1989; Hussain et al., 1990; Roiger and Jeffers, 1991). The authors revealed that the antagonistic activity of some microorganisms against the plant pathogens may be due to the ability of these microorganisms to grow and sporulate on seed and thereafter to become established in large numbers in soils. In addition, Beagle-Ristaino and Papavizas (1985) and Hussain et al. (1990) found that colonization of the seed coat substrate and utilization of the seed exudate produced by the germinating seeds, decreased the plant seed damping-off caused by different pathogenic moulds in soil. These hypotheses may explain our present data which suggests that coated seeds by *Trichoderma lignorum* provided the seeds with high protection against seed rot or pre-emergence damping-off.

In the present study we evaluated the effect of plant exudates on the inhibition of conidia germination in vitro and on the suppression of *Rhizoctonia* damping-off of bean in vivo obtained with *Trichoderma lignorum*. In the presence of bean exudate, the reduction in *Rhizoctonia* damping-off of bean by *Trichoderma lignorum* was reduced. This indicates that competition for these or similar nutrients may occur. Indeed Rovira et al. (1974) have already mentioned that plant root exudates contain lower amounts of carbon and energy than those required by rhizosphere microflora. Similar phenomena have already been described (Elad and Baker, 1985) in the inhibition of chlamydospore germination of *F. oxysporum* F. sp. *cucumerinum* by pseudomonads. In the present study, it was reported that conidia of *Rhizoctonia solani* in the rhizosphere of bean germinated at higher levels compared with the germination obtained in non-rhizosphere soil. The same also occurred with chlamydospores of *F. solani* f. sp. *phaseoli* and f. *oxysporum* f. sp. *cucumerinum* when exposed to bean and cucumber exudates (Sneh et al., 1984). Inhibition of conidia germination of *Rhizoctonia solani* could be obtained even when the antagonists were applied as a seed coating. Apparently, effective utilization of root exudates may enable antagonists applied to seeds to proliferate along the developing rhizosphere and interfere with seed and root pathogens (Cook and Baker, 1983). After application of *Trichoderma lignorum* as a seed treatment in soil, *Trichoderma lignorum* was recovered from the rhizosphere, indicating the active proliferation of this strain along the developing roots. The highest counts of *Trichoderma lignorum* in the rhizosphere were detected on the root segment, which included the root base and tip. Because the population dynamics of *Trichoderma lignorum* were recorded from rhizosphere portions that included roots and soil, it is not clear whether *Trichoderma lignorum* colonized the rhizoplane, the rhizosphere soil, or both. The population of the *Trichoderma lignorum* could be distinguished from the resident soil population of *Trichoderma* spp. by using the natural resistance of *Trichoderma* strain to relatively high concentrations of cycloheximide. Similar findings were demonstrated by Ahmad and Baker (1987) with a benomyl-resistant mutant of *Trichoderma harzianum*. The relatively high population levels of the antagonists recovered for root tips were also demonstrated with bacteria (Scher et al., 1984; Elad and Chet, 1987).

Most of the exudates present in the rhizosphere are exudated from root tips. Thus colonization of this region in the rhizosphere might reduce infection by *Rhizoctonia solani* that penetrate the vascular system of their hosts through the undifferentiated xylem at the root tip (Cook and Baker, 1983). Rhizosphere colonization of bean by *Trichoderma lignorum* was accompanied by a simultaneous decline in *Rhizoctonia solani*. Reduction in the pathogen population in the rhizosphere should lead to a decrease in the infection rate. In soil infested with *Rhizoctonia solani*, treatments of *Trichoderma* had only a slight effect on the survival of *Rhizoctonia solani* in non-rhizosphere soil. However, application of *Trichoderma*

lignorum as a wheat-bran preparation, conidial suspension, or seed coating significantly reduced the pathogen counts in the rhizosphere soil of bean. The minimal effect of *Trichoderma lignorum* on population densities of *Rhizoctonia solani* in non-rhizosphere soil further suggests a competitive mechanism in biocontrol (Baker, 1981). These findings are in correlation with the lack of mycoparasitic and antibiotic interactions between *Trichoderma lignorum* and *Rizoctonia solani* in vitro (Abd El-Kader et al., 1984). The great reduction of the pathogen population densities in the rhizosphere soil could be a result of a lower proliferation rate of the pathogen in a rhizosphere already colonized by the antagonist. Further studies on the possible role of competition between *Trichoderma lignorum* spp. and other pathogens on rhizosphere colonization under different greenhouse environmental conditions will be carried out.

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