Original Article

EFFICACY OF *Trichoderma asperellum*, *T. harzianum*, *T. longibrachiatum* AND *T. reesei* AGAINST *Sclerotium rolfsii*

EFICÁCIA DE Trichoderma asperellum, T. harzianum, T. longibrachiatum *E* T. reesei *CONTRA* Sclerotium rolfsii

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ABSTRACT: This work was carried out to select and to evaluate efficacy of *Trichoderma* isolates to control sclerotium wilt (*Sclerotium rolfsii*) of common-bean (*Phaseolus vulgaris*). For the experiments were used two isolates of *S. rolfsii* UB 193 and UB 228. Sixty-five *Trichoderma* spp. isolates were tested and the following ones were selected *in vitro* for the *in vivo* tests: 5, 11, 12, 15, 102, 103, 127, 136, 137, 1525 (*T. longibrachiatum*), 1637 (*T. reesei*), 1642, 1643 (*T. harzianum*), 1649 (*T. harzianum*), 1700 (*T. asperellum*) and EST 5. The most promising isolates were identified by Sequencing of the internal transcribed spacer regions ITS1, ITS2, and the 5.8s rRNA genomic region, using the ITS5 and ITS4 primers and compared with sequences in the National Center for Biotechnology Information (NCBI) database. These selected isolates 1649 (*T. harzianum*), 1525 (*T. longibrachiatum*) and 1637 (*T. reesei*) were tested for evaluation of sclerotial germination inhibition under laboratory conditions, and to evaluate the effects of these on disease of bean plants under greenhouse conditions. The *Trichoderma* isolates 1649, 1525 and 1637 were more efficient in reducing sclerotial germination. In addition to 1649, 1525 and 1637, the isolates 5, 12, 102 and 1525 (*T. longibrachiatum*) significantly reduced de amount of diseased bean plants under greenhouse conditions.

KEYWORDS: *Aethelia rolfsii*. *Phaseolus vulgaris*. Biocontrol. *Trichoderma* spp.

INTRODUCTION

Sclerotium rolfsii Sacc. [Teleomorph: *Athelia rolfsii* (Cruzi) You & Kimbrough] is a pathogen of widespread distribution in soils of agricultural production areas. This basidiomycetes fungus causes a disease (Southern-blight; damping-off; sclerotium-wilt) that affects more than 500 species in over 100 plant families. This disease can be a serious problem, especially in warm regions, where the pathogen produces sclerotia in infected plant parts near the ground. The sclerotia can survive in the soil for a few months to several years, depending on environmental conditions (PUNJA, 1993; XU et al., 2008). These structures are the primary inoculum source for the development of the disease (PUNJA, 1993; XU et al., 2008). After germination of sclerotium, hyphae of *S. rolfsii* might produce oxalic acid, pectinolytic and cellulolytic enzymes that could disintegrate plant tissues (LE, 2011; PUNJA, 1993).

The control of *S. rolfsii* occurs with the aid of preventive practices such as crop rotation, deep plowing, fungicide-treated and pathogen-free seed, among others and by biological control (BLUM; LIN, 1991; SOUSA; BLUM, 2013), cultural control (BLUM; RODRIGUÉZ-KÁBANA, 2004; BLUM; RODRIGUÉZ-KÁBANA, 2006; PINHEIRO et al., 2010), and chemical control (PAULA Jr. et al., 2011; SOUSA; BLUM, 2013). Biological control of plant pathogens has a number of advantages compared to conventional fungicides. While the fungicides feature only a temporary effect and require repeated applications during the growing period of culture, biological control agents are able to establish themselves, colonize and reproduce in the ecosystem (AVILA et al, 2005; AULER et al., 2013; MELO; FAULL, 2000).

Trichoderma is well documented as effective biological control agents of plant diseases (SOUSA; BLUM, 2013). The biological control of the pathogen on bean plants by using *Trichoderma* spp. and in combination with other techniques had been investigated (SOUSA; BLUM, 2013). The genus *Trichoderma* comprises a large number of species some of which act as biological control agents through one or more mechanisms (SHARMA, 2012).

Gajera et al. (2013) reported that *Trichoderma* spp. act as bio-control agents against fungal plant pathogens either indirectly or directly. Indirect mechanism comprises competition for

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nutrients and space, modification of the environmental conditions, antibiosis and induction of plant defensive mechanisms, however direct mechanism encompasses hyper-parasitism. The studies of hyper-parasitism also have demonstrated that *Trichoderma* produce a rich mixture of antifungal enzymes, including chitinases and β-1,3 glucanases. These enzymes are synergistic with each other, with other antifungal enzymes, and with other metabolites. These indirect and direct mechanisms may act coordinately and their importance in the bio-control process depends on the *Trichoderma* species and strain, the antagonized fungus, the crop plant, and the environmental conditions including nutrient availability, pH, temperature and iron concentration (GAJERA et al., 2013).

Trichoderma spp. have been successfully used for management of diseases caused by *Rhizoctonia solani* in bean and tomato and by *S. rolfsii* in bean, cucumber, tomato, peanut, sugarbeet and soybean (BLUM; RODRIGUÉZ-KÁBANA, 2006; KOTASTHANE et al., 2015; NAEIMI et al, 2010). *Trichoderma* are multifunctional plant symbionts responsible for enhanced nutrient uptake, increased root and shoot growth, improved plant vigor and biotic or abiotic stress tolerance (HARMAN et al. 2008; HARMAN, 2011). *Trichoderma asperellum, T. harzianum, T. longibrachiatum* and *T. reesei* are among the species with promising strains for biocontrol purposes (MAYMON et al., 2004; BŁASZCZYK et al., 2014).

Due to this diversity of modes of action of *Trichoderma* spp. the constant search for new effective strains against plant pathogens is necessary. Therefore, the objective of the study was to evaluate isolates of *Trichoderma* spp. *in vivo* and *in vitro*, and, to compare *in vitro* selected *Trichoderma* spp. to *in vivo* control of sclerotium-wilt of common-bean.

MATERIAL AND METHODS

The present study was conducted at the laboratory of Mycology of the Department of Plant Pathology and at the Experimental Station of Biology of the 'Universidade de Brasília' (University of Brasília - UnB), Brasília/DF. The isolates of *Sclerotium rolfsii* (UB 193 and UB 228) and *Trichoderma* were obtained from the Mycological collection at the same University. *Sclerotium rolfsii* (UB 193 and UB 228) and *Trichoderma* spp. (Table 1) were maintained and sub-cultured (25° C; 12h light) routinely on potato dextrose agar (PDA). Sclerotia of *S. rolfsii* for *in vitro* tests were produced on PDA after 2 weeks (25° C; 12h light).

Table 1. Isolates of *Trichoderma* spp. used in experiments.

Trichoderma	**Identification code***
T. asperellum	1574 (KT001987), 1575 (KC859427), 1577 (KP340248), 1581 (KF737411), 1700 (LC057426)
T. aureoviride	1638
T. harzianum	1643 (KP263734), 1645 (KP263580), 1646 (KP263734), 1647 (KP263642), 1649 (KP418577), 1650 (KP263580)
T. koningii	1578, 1743
T. longibrachiatum	112 (KP671488), 245 (KM457631), 1168 (KC009811), 1169 (EU401554), 1523 (KP641159), 1525 (GQ203533), 1526 (KJ767090), 1576 (KP256797), 1641 (KM225906), 1644 (KP009307), 1742 (KP671488), 1744 (KC582841)
T. pilluliferum	1640
T. reesei	1168, 1637 (LC002607)
T. viride	1639 (FN868471)
***Trichoderma* sp.**	5, 7, 8, 9, 11, 12, 13, 15, 23, 24, 25, 102, 103, 104, 109, 113, 116, 118, 122, 123, 127, 131, 132, 136, 137, 518, 1330, 1528, 1529, 1642, Est02, Est05, Est06, Est07, SG, UFG

*(GenBank code - http://www.ncbi.nlm.nih.gov)

Total DNA was extracted from *Trichoderma* isolates (Table 1) analyzed samples, following the cetyltrimethyl ammonium bromide (CTAB) method (DOYLE; DOYLE, 1990). All isolates of *Trichoderma asperellum, T. harzianum,* and *T. longibrachiatum* and some the other *Trichoderma* spp. were identified by sequencing of the internal transcribed spacer regions ITS1, ITS2, and the 5.8s rRNA genomic region, using the ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (WHITE et al., 1990); and compared with

sequences in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). PCR products were treated with ExoSAP-IT enzyme and then sequenced with an 'ABI 3130xl da Applied Biosystems' sequencer at the 'Universidade Católica de Brasília' (Catholic University of Brasilia - UCB). The alignments were constructed with MEGA Version 6 (http://megasoftware.net/) and only the ones with more than 95% of identity were considered for phylogenetic purposes. Statistical significance was evaluated with a bootstrapping of 1000 repetitions (TAMURA et al., 2013).

The tests for evaluation of the *in vitro* antagonism of 65 *Trichoderma* spp. isolates (Table 1) against *S. rolfsii* were performed using the method of paired cultures described by Dennis e Webster (1971). Plates of PDA were inoculated with a 5mm disc of *S. rolfsii* 10mm from the edge of the plate. A 5mm disc of the tested *Trichoderma* isolate was placed 60mm from the *S. rolfsii* disc. As experimental control were used Petri dishes with BDA containing only culture isolates of *S. rolfsii*. The evaluations were carried out on the seventh day of incubation (25° C; 12h light), using a scale of scores according to the method of Bell et al. (1982). The mycelial interaction between pathogen and antagonist was scored from 1 to 5 according to Bell et al. (1982). This scale of classes designated by Bell et al. (1982) is the following: (1) *Trichoderma* completely overgrew *S. rolfsii*, occupying the whole 9-cm Petri-dish; (2) *Trichoderma* occupies at least 65% of the Petri dish; (3) *Trichoderma* and *S. rolfsii* grow around (40-60%) of the Petri dish; (4) *Sclerotium rolfsii* occupies at least 65% of the Petri dish without any apparent interference of *Trichoderma*; (5) *Sclerotium rolfsii* completely overgrew *Trichoderma*, occupying the whole Petri dish. A complete randomized design with 66 treatments and 4 replications was used, and after analysis of variance (F test; $P \leq 0.05$), treatments averages were compared through the Scott-Knott test ($P \leq 0.05$).

The effects of 15 *in vitro* selected *Trichoderma* spp. (Table 2) on germination of *S. rolfsii* sclerotia were evaluated by using plastic boxes (Gerbox type plastic box - 11.5 x 3.5 cm) with sterilized (121 ^{o}C; 1h) soil. The plastic boxes received 200 g of wet (70 mL of sterile water), sieved and sterilized soil [Red latosol (Oxisol); pH (H_2O) = 5.5; OM = 8.6 g/kg]. Four replications of 25 sclerotia / plastic box were inoculated with 20 µl of *Trichoderma* (1 x 10^7 conidia / mL) isolates suspension / sclerotium. Sterile water (20 µl) was applied on a set of sclerotia as experimental control. After the application of treatments, the inoculated containers were settled in an incubator (25° C; 12h light). The evaluations occurred after 7 and 14 days of incubation, by counting the number of non-germinated sclerotia. A complete randomized design with 66 treatments and 4 replications was used, and after ANOVA (F test; $P \leq 0.05$), treatments averages were compared [Scott-Knott test ($P \leq 0.05$)].

Table 2. *In vitro* antagonism of *Trichoderma* (TR) against *Sclerotium rolfsii* (UB 193; 288), using scores (1-5) according to Bell et al. (1982), after 7 days of incubation (25° C; 12h light).

TR	UB 193	UB 288	TR	UB 193	UB 288	TR	UB 193	UB 288
Test	5.0 (1) a	5.0 a	**UFG**	3.0 c	3.3 c	**1581**	2.3 d	3.0 c
518	4.5 b(2)	4.8 a	**23**	2.8 c	3.3 c	**5**	2.0 d	2.0 d
1640	4.3 b	5.0 a	**104**	2.8 c	4.3 b	**9**	2.0 d	3.5 c
109	4.0 b	5.0 a	**113**	2.8 c	3.0 c	**24**	2.0 d	3.0 c
13	3.5 c	3.0 c	**116**	2.8 c	3.0 c	**25**	2.0 d	3.0 c
118	3.3 c	3.8 b	**131**	2.8 c	2.8 c	**102**	2.0 d	2.0 d
1330	3.3 c	3.8 b	**245**	2.8 c	3.0 c	**103**	2.0 d	2.5 d
1523	3.3 c	4.0 b	**1169**	2.8 c	2.5 d	**122**	2.0 d	2.8 c
1529	3.3 c	3.0 c	**1526**	2.8 c	3.0 c	**127**	2.0 d	2.5 d
1647	3.3 c	4.0 b	**1577**	2.8 c	2.8 c	**132**	2.0 d	3.0 c
1742	3.3 c	3.3 c	**1644**	2.8 c	3.3 c	**136**	2.0 d	2.3 d
1743	3.3 c	2.8 c	**1650**	2.8 c	2.3 d	**137**	2.0 d	2.0 d
7	3.0 c	2.8 c	**1744**	2.8 c	2.8 c	**1525**	2.0 d	2.3 d
112	3.0 c	3.0 c	**Est 02**	2.8 c	2.3 d	**1637**	2.0 d	2.5 d
1168	3.0 c	3.0 c	**8**	2.5 d	3.3 c	**1643**	2.0 d	2.5 d
1575	3.0 c	3.0 c	**123**	2.5 d	3.0 c	**1700**	2.0 d	2.3 d

1576	3.0 c	3.5 c	**1638**	2.5 d	2.8 c	**Est 05**	2.0 d	2.0 d
1578	3.0 c	3.0 c	**1641**	2.5 d	3.0 c	**12**	1.8 e	2.5 d
1639	3.0 c	3.3 c	**1646**	2.5 d	3.0 c	**1642**	1.8 e	1.8 d
Est 06	3.0 c	3.0 c	**11**	2.3 d	2.0 d	**1645**	1.5 e	5.0 a
Est 07	3.0 c	2.8 c	**1528**	2.3 d	3.0 c	**1649**	1.5 e	2.0 d
S. G	3.0 c	3.00 c	**1574**	2.3 d	3.0 c	**15**	1.0 e	1.8 d
-	-	-	**-**	-	-	**CV (3)**	15, 6	14,2

(1) Average of four Petri-dishes cultures as replications. Scale of classes (Bell et al., 1982): **1**- *Trichoderma* completely overgrew the pathogen, occupying the whole petri dish; **2**- *Trichoderma* occupies at least 65% of the medium plate; **3**- *Trichoderma* and pathogen grow around (40-60%)of the Petri dish; **4**- Pathogen occupies at least 65% of the Petri dish without any apparent interference of *Trichoderma*; **5**- Pathogen completely overgrew *Trichoderma*, occupying the whole Petri dish. (2) Values in column followed by the same letter are not significantly different according to the Scott-Knott test ($P \leq 5\%$). (3) Coefficient of variation (%).

Greenhouse (20-30ºC) tests were conducted at the Experimental Station of Biology of the University of Brasilia. Seeds of bean cv. 'Pérola' were sowed in 3L black plastic pots with a capacity of 3 kg of sterilized (121ºC; 1h) soil. For each treatment, five pots with 8 bean seeds were prepared. The soil [Red latosol (Oxisol); pH H_2O, 5.5; P = 0.5 mg/dm^3; Ca = 0.5 cmol/dm^3; Mg= 0.4 cmol/dm^3; K= 0.04 cmol/dm^3; Na= 0.01 cmol/dm^3; Al= 0.1 cmol/dm3; OM= 8.6 g/kg] was previously fertilized by adding and mixing N-P-K (4-14-8; 2.5g / kg of soil). Once a day, by dripping, soil of each plastic pot received 0.5 L of water. A complete randomized block design with 15 treatments and 5 replications was used, and after ANOVA (F test; $P \leq 0.05$), treatments averages were compared through the Scott-Knott test ($P \leq 0.05$).

To inoculated the soil for the greenhouse tests, the pathogens and antagonists were grown for 10 days (25° C; 12h light) on sterilized (120ºC; 20 minutes) water soaked rice grains using a modified (Auler at al., 2013) method described by Serra e Silva (2005). The soil inoculations were made following a modified method described by Barbosa et al. (2010), where, 10 g kg^{-1} of soil of pathogen or *Trichoderma* colonized rice seeds were mixed to the pot soil content. The evaluation of the number of diseased plants was made weekly up to 42 days after the soil inoculation. After this period, the dry matter of the roots and aerial plant parts was quantified. A Pearson correlation coefficient test was made between the percentage of non-germinated sclerotia (laboratory) and amount of diseased bean plants (greenhouse), considering de results of all *Trichoderma* isolates in both *S. rolfsii* (UB 193 and 228).

RESULTS AND DISCUSSION

Most of the *Trichoderma* isolates *in vitro* inhibited mycelial growth of *S. rolfsii* (UB 193; 228) isolates (Table 2). Five *Trichoderma* isolates (12, 1642, 1645, 1649, and 15) kept under 2, on Bell´s et al. (1982) scale, the *S. rolfsii* (193) isolate. *Trichoderma* isolate (1645) inhibited one *S. rolfsii* (193) isolate but did not inhibit the other (228). Three *Trichoderma* isolates, 1642, 1649, and 15, reduced significantly the mycelial growth of *S. rolfsii*.

Castillo et al. (2011) reported the antagonism *in vitro* effect of Mexicans *Trichoderma* strains on *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*. In addition, Paica (2014) found that a strain of *T. asperellum* was characterized as an *in vitro* antagonist of *Fusarium* and *Aspergillus* isolates from corn (*Zea mays*). The type of *Trichoderma* antagonism to plant pathogenic fungi varies. Some isolates of *T. harzianum* and *T. asperellum* were reported to act by antibiosis and parasitism on different fungal pathogens (AULER et al., 2013; CASTILLO et al., 2011; ISAIAS et al., 2014; MARTINEZ et al., 2013; PAICA, 2014; PARMAR et al., 2015). Rasu et al. (2012) indicated the potential of *T. asperellum* in inhibiting the mycelial growth of *S. rolfsii* and production of cell wall degrading enzyme. Sanmartín-Negredo et al. (2012) concluded that the antagonistic activity of *T. asperellum* against *C. gloeosporioides* is due mainly to the biocidal effect of volatile metabolites.

Those *Trichoderma* isolates which *in vitro* inhibited mycelial growth (Table 2) of *S. rolfsii*, three (1637, 1525, 1694) of them were able of significantly to reduce sclerotial germination (Table 3). These three isolates were of *T. reesei* (1637), *T. longibrachiatum* (1525), and *T. harzianum* (1694). *Trichoderma asperellum* (1700) *in vitro* inhibited both *S. rolfsii* isolates, but only significantly reduced the sclerotial germination of one *S. rolfsii* isolate (UB 228). Henis et al. (1983) found that not only direct *Trichoderma* sclerotial penetration degrades sclerotium of *S. rolfsii*. These authors also indicated that enzymes and non-enzymatic toxins might be involved sclerotial germination and

degradation. Additionally, Desai e Schlosser (1999) showed that their isolates of *T. harzianum* mainly acted by post-penetration parasitism of the *S. rolfsii* sclerotia.

The isolates 1525 of *T. longibrachiatum*, 1637 of *T reesei*, 1649 of *T. harzianum*, 1700 of *T. asperellum*, 5, 11, 12, 15, Est 5, 102, 103, 127, and 137 of *Trichoderma* sp. significantly reduced the amount of diseased bean plants (Table 4). *Trichoderma longibrachiatum* (1525), T. *harzianum* (1649), and *Trichoderma* sp. (102; 103) were the most efficient in reducing *S. rolfsii* (UB 193; UB 228) diseased bean plants. With the exception of *Trichoderma* sp. (103) all other previously cited isolates were efficient in reducing sclerotium germination (Table 3). The isolate 1643 of T. *harzianum* reduced significantly *S. rolfsii* sclerotium germination (Table 3) but did not reduced disease on plants (Table 4). Considering all *Trichoderma* isolates (Tables 3 and 4) there was a significant negative correlation (r = -0,585; P = 0,017) between the percentage of non-germinated sclerotia and amount of diseased bean plants.

Table 3. Percentage of non-germinated (NG) sclerotia of *Sclerotium rolfsii* (UB 193; UB 228), 14 days after application of *Trichoderma* spp. (20 µl / sclerotium; 1 x 10^7 conidia / mL).

Treatment	*Trichoderma*	UB 193 [%NG[(1)]]	UB 228 [%NG]
1649	***T. harzianum***	77 a[(2)]	80 a
1525	***T. longibrachiatum***	53 b	73 a
1643	***T. harzianum***	37 c	34 b
1637	***T. reesei***	33 c	66 a
102	***Trichoderma* sp.**	33 c	64 a
137	***Trichoderma* sp.**	32 c	39 b
5	***Trichoderma* sp.**	24 c	73 a
Est 05	***Trichoderma* sp.**	23 c	63 a
136	***Trichoderma* sp.**	23 c	30 b
12	***Trichoderma* sp.**	22 c	70 a
15	***Trichoderma* sp.**	19 d	68 a
1700	***T. asperellum***	19 d	39 b
11	***Trichoderma* sp.**	14 d	30 b
127	***Trichoderma* sp.**	14 d	26 c
103	***Trichoderma* sp.**	9 d	33 b
None	**None**	0 d	0 d
CV (%) [(3)]	-	12	17

[(1)] Average of 4 replications of 25 sclerotia; (2) Values in column followed by the same letter are not significantly different according to the Scott-Knott test (P ≤ 5%). (3) Coefficient of variation.

Auler at al. (2013) reported that the isolates CEN155, CEN158, CEN169, CEN170, CEN194 and CEN197 of *Trichoderma harzianum* were able to inhibit the mycelial growth of *S. rolfsii* and were effective for controlling *S. rolfsii* on bean and soybean crops, affording over 88% of healthy plants. Błaszczyk et al. (2014) informed that chitinases are the most important lytic enzymes playing a key role in the degradation of cell walls of other plant pathogenic fungi by *Trichoderma* species (*T. harzianum*, *T. atroviride* and *T. asperellum*).

El-Komy et al. (2015) showed that around of 20% of their *T. asperellum* isolates were highly producer for cell-wall degrading enzymes and showed high antagonistic activity against *Fusarium oxysporum* f. sp. *lycopersici* isolates. There was a positive correlation between the antagonistic capacity of *T. asperellum* isolates towards *F. oxysporum* f. sp. *lycopersici* and their lytic enzyme production. Isolates showing high levels of chitinase and β-1,3-glucanase activities strongly inhibited the growth of *F. oxysporum* f. sp. *lycopersici*.

The isolate 1649 of *T. harzianum* was very efficient in inhibit *S. rolfsii* mycelia growth (Table 2), reduce sclerotial germination (table 3), reduce disease incidence (Table 4) and promote bean plant growth (Table 5). *Trichoderma longibrachiatum* (1525) also was efficient in inhibit *S. rolfsii* mycelia growth (Table 2), reduce sclerotial germination (Table 3), reduce disease incidence (Table 4) and promote bean plant growth (Table 5), but not as well

as *T. harzianum* (1649). Pedro et al. (2012) reported a reduction on severity of bean anthracnose (*Colletotrichum lindemuthianum*) and promotion of plant growth by isolates of *Trichoderma* under greenhouse conditions. Martínez-Medina et al. (2014) found that changes in phytohormone levels is one of the mechanisms by which selected *Trichoderma* isolates can interfere with plant growth. In addition, these authors informed an important role of auxin in controlling plant growth stimulation by *Trichoderma*, while plant-mediated mechanisms by which *Trichoderma* can control *Fusarium* (*Fusarium oxysporum* f. sp. *melonis*) wilt of melon (*Cucumis melo*) may be influenced by shoot content of ZR, ABA, and ACC.

Table 4. Percentage of diseased bean (cv. Pérola) plants after 6 weeks of the application of *Trichoderma* spp. into soil previously contaminated with *Sclerotium rolfsii* (UB 193 or 228).

TRATAMENT	*Trichoderma*	% of diseased plants		
		S. rolfsii		
		UB 193	UB 228	Average
S. rolfsii	**-**	97[(1)] aA[(2)]	98 a[(3)]A	97 A
***S. rolfsii* + 1643**	***T. harzianum***	92 aA	86 aB	89 B
***S. rolfsii* + 15**	***Trichoderma* sp.**	69 bB	83 aB	76 C
***S. rolfsii* + 11**	***Trichoderma* sp.**	58 bC	68 aC	64 D
***S. rolfsii* + 1700**	***T. asperellum***	47 bD	66 aC	57 E
***S. rolfsii* + Est 5**	***Trichoderma* sp.**	53 aC	56 aD	55 E
***S. rolfsii* + 1637**	***T. reesei***	47 bD	63 aD	55 E
***S. rolfsii* + 127**	***Trichoderma* sp.**	40 bE	68 aC	54 E
***S. rolfsii* + 137**	***Trichoderma* sp.**	63 aB	44 bE	54 E
***S. rolfsii* + 136**	***Trichoderma* sp.**	57 aC	46 bE	51 E
***S. rolfsii* + 5**	***Trichoderma* sp.**	38 aE	42 aE	40 F
***S. rolfsii* + 12**	***Trichoderma* sp.**	48 aD	17 bG	32 G
***S. rolfsii* + 1525**	***T. longibrachiatum***	29 aF	32 aF	31 G
***S. rolfsii* + 103**	***Trichoderma* sp.**	27 aF	18 bG	22 H
***S. rolfsii* + 1649**	***T. harzianum***	30 aF	9 bH	19 H
***S. rolfsii* + 102**	***Trichoderma* sp.**	21 aG	6 bH	14 I
No *S. rolfsii*	**-**	0 aH[(2)]	0 aI	0 J
CV [(4)]	**-**	-	-	12

[(1)] Average of 5 replications of 8 plants; [(2)] Values in column followed by the same capital letter are not significantly different according to Scott-Knott test ($P \leq 5\%$). [(3)] Values in row followed by the same low-case letter are not significantly different according to Scott-Knott test ($P \leq 5\%$). [(4)] Coefficient of variation; original data were transformed to arcsin {sqrt [(x+0.5)/100]}, where x is the %.

Table 5. Shoot and root fresh mass (g) of bean (cv. 'Pérola) plants grown in soil contaminated with *Sclerotium rolfsii* and *Trichoderma* spp., under greenhouse conditions.

TRATAMENT	*Trichoderma*	*S. rolfsii* UB 193		*S. rolfsii* UB 228	
		Shoot	Root	Shoot	Root
None [(1)]	-	51.7 a [(2)]	4.6 a	44.0 a	3.1 c
1649	***T. harzianum***	44.4 a	2.7 b	47.8 a	3.6 c
102	***Trichoderma* sp.**	20.0 b	3.9 a	13.7 c	1.9 d
5	***Trichoderma* sp.**	27.3 b	3.2 a	27.9 b	3.3 c
103	***Trichoderma* sp.**	33.6 b	6.1 a	34.6 b	8.2 b
127	***Trichoderma* sp.**	29.2 b	5.9 a	32.1 b	7.4 b
12	***Trichoderma* sp.**	21.9 b	2.4 b	19.9 c	1.4 d
136	***Trichoderma* sp.**	25.1 b	3.6 a	30.2 b	4.5 c
137	***Trichoderma* sp.**	25.7 b	1.8 b	34.2 b	2.7 c
1637	***T. reesei***	26.6 b	3.7 a	22.8 c	1.7 d

1700	***T. asperellum***	25.4 b	3.0 a	18.9 c	1.9 d
11	***Trichoderma* sp.**	27.2 b	3.3 a	21.6 c	1.8 d
1525	***T. longibrachiatum***	21.0 b	4.7 a	27.8 b	4.6 c
EST 5	***Trichoderma* sp.**	24.1 b	4.1 a	23.9 c	2.6 c
15	***Trichoderma* sp.**	29.5 b	4.9 a	35.7 b	11.1 a
1643	***T. harzianum***	7.6 c	0.5 c	15.4 c	0.7 d
***S. rolfsii* [3]**	**-**	0,00 c	0,00 c	0,00 d	0,00 d
CV [4]		19.2	27.2	13.9	22.0

[1] Control without *Sclerotium rolfsii* and without *Trichoderma*; [2] Average of 5 replications of 8 plants; Values in column followed by the same letter are not significantly different according to Scott-Knott test (P ≤ 5%); [3] *Sclerotium rolfsii* without *Trichoderma*. [4] Coefficient of variation; original data were transformed to sqrt(x+0.5), where x is mass (g).

CONCLUSIONS

The *Trichoderma* isolates 1649, 1525 and 1637 were more efficient in reducing sclerotial germination. In addition to 1649, 1525 and 1637, the isolates 5, 12, 102 and 1525 (*T. longibrachiatum*) significantly reduced de amount of diseased bean plants under greenhouse conditions.

The isolates 1649 of *T. harzianum* and 1525 of *T. longibrachiatum* were efficient in inhibit *S. rolfsii* mycelia growth, reduce sclerotial germination, reduce disease incidence and promote bean plant growth.

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RESUMO: O estudo teve como objetivo selecionar e testar isolados de *Trichoderma* spp. para o controle da murcha-por-esclerócio (*Sclerotium rolfsii*) em feijoeiro (*Phaseolus vulgaris*) em casa-de-vegetação. Nos ensaios realizados foram utilizados dois isolados de *S. rolfsii* (UB 193 e UB 228). Dos 65 isolados *Trichoderma* testados *in vitro* selecionaram-se os seguintes: 5, 11, 12, 15, 102, 103, 127, 136, 137, 1525 (*T. longibrachiatum*), 1637 (*T. reesei*), 1642 (*T. longibrachiatum*), 1643 (*T. harzianum*), 1649 (*T. harzianum*), 1700 (*T. asperellum*) e EST 5. Os isolados mais promissores foram identificados por sequenciamento das regiões genômicas ITS1, ITS2 e 5.8s rRNA, usando iniciadores ITS5 e ITS4, e então, tais sequencias foram comparadas com as sequências do banco de dados do "National Center for Biotechnology Information" (NCBI). Esses isolados foram avaliados quanto ao efeito sobre a germinação de esclerócios do patógeno em laboratório e sobre a doença em casa de vegetação. Os isolados 1649 (*T. harzianum*), 1525 (*T. longibrachiatum*) e 1637 (*T. reesei*) foram os mais eficientes na inibição da germinação de esclerócios de *S. rolfsii* em laboratório. Além dos isolado 1649, 1525 e 1637, os isolados 5, 12, 102 e 1525 (*T. longibrachiatum*) foram eficientes na redução da doença em plantas de feijoeiro em casa de vegetação.

PALAVRAS-CHAVE: *Aethelia rolfsii*. *Phaseolus vulgaris.* Biocontrole. *Trichoderma* spp.

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