Control of lettuce bottom rot by isolates of *Trichoderma* spp.

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ABSTRACT

Pinto, Z.V.; Cipriano, M.A.P.; Santos, A.S.; Pfenning, L.H.; Patricio, F.R.A. **Control of lettuce bottom rot by isolates of *Trichoderma* spp.** ***Summa Phytopathologica,*** v.40, n.2, p.141-146, 2014.

Bottom rot, caused by *Rhizoctonia solani* AG 1-IB, is an important disease affecting lettuce in Brazil, where its biological control with *Trichoderma* was not developed yet. The present study was carried out with the aim of selecting *Trichoderma* isolates to be used in the control of lettuce bottom rot. Forty-six *Trichoderma* isolates, obtained with baits containing mycelia of the pathogen, were evaluated in experiments carried out *in vitro* and *in vivo* in a greenhouse in two steps. In the laboratory, the isolates were evaluated for their capabilities of parasitizing and producing toxic metabolic substances that could inhibit the pathogen mycelial growth. In the first step of the *in vivo* experiments, the number and the dry weight of lettuce seedlings of the cultivar White Boston were evaluated. In the second step, 12 isolates that were efficient in the first step and showed rapid growth and abundant sporulation in the laboratory were tested for their capability of controlling bottom rot in two repeated experiments, and had their species identified. The majority of the isolates of *Trichoderma* spp. (76%) showed high capacity for parasitism and 50% of them produced toxic metabolites capable of inhibiting 60-100% of *R. solani* AG1-IB mycelial growth. Twenty-four isolates increased the number and 23 isolates increased the dry weight of lettuce seedlings inoculated with the pathogen in the first step of the *in vivo* experiments. In both experiments of the second step, two isolates of *T. virens,* IBLF 04 and IBLF 50, reduced the severity of bottom rot and increased the number and the dry weight of lettuce seedlings inoculated with *R. solani* AG1-IB. These isolates had shown a high capacity for parasitism and production of toxic metabolic substances, indicating that the *in vitro* and *in vivo* steps employed in the present study were efficient in selecting antagonists to be used for the control of lettuce bottom rot.

Additional keywords: biological control, *Rhizoctonia solani*, *Lactuca sativa*.

RESUMO

Pinto, Z.V.; Cipriano, M.A.P.; Santos, A.S.; Pfenning, L.H.; Patricio, F. R. A. **Controle da queima da saia em alface com isolados de *Trichoderma* spp.** *Summa Phytopathologica,* v.40, n.2, p.141-146, 2014.

A queima da saia, causada por *Rhizoctonia solani* AG 1-IB, é uma importante doença da cultura da alface no Brasil, para a qual o controle biológico com *Trichoderma* não foi desenvolvido até o momento no país. O presente estudo foi realizado com o objetivo de selecionar isolados de *Trichoderma* para serem usados para o controle da queima da saia em alface. Quarenta e seis isolados de *Trichoderma* obtidos com iscas contendo micélio do patógeno foram avaliados em experimentos conduzidos *in vitro* e *in vivo,* os quais foram realizados em casa de vegetação, em duas fases. Em laboratório os isolados foram avaliados com relação às capacidades de parasitismo e de produção de substâncias metabólicas tóxicas, com capacidade de inibir o crescimento micelial do patógeno. Na primeira fase dos experimentos *in vivo,* foram avaliados o número e a massa seca de plântulas de alface da cultivar White Boston. Na segunda fase, 12 isolados eficientes na primeira fase e que também apresentaram rápido crescimento e esporulação abundante em laboratório, foram avaliados com relação à sua capacidade de controlar a queima da saia em dois experimentos repetidos, e também tiveram suas espécies identificadas. A maioria dos isolados de *Trichoderma* spp. (76%) apresentou elevada capacidade de parasitismo e 50% dos isolados produzim metabólitos tóxicos capazes de inibir 60-100% do crescimento micelial de *R. solani* AG1-1-B. Vinte e quatro isolados promoveram aumento do número e 23 na massa seca de plântulas de alface inoculadas com o patógeno na primeira fase dos experimentos *in vivo*. Nos dois experimentos da segunda fase dois isolados de *T. virens,* IBLF 04 e IBLF 50, reduziram a severidade da queima da saia e promoveram o aumento do número e da massa seca das plântulas de alface inoculadas com *R. solani* AG 1-IB. Esses isolados haviam apresentado elevada capacidade de parasitismo e de produção de substâncias metabólicas tóxicas, indicando que as fases *in vitro* e *in vivo* empregadas no presente estudo foram eficientes para a seleção dos antagonistas a serem utilizados para o controle da queima da saia em alface.

Palavras-chave adicionais: controle biológico, *Rhizoctonia solani*, *Lactuca sativa*.

Lettuce is the most important leafy vegetable produced and consumed in Brazil, where it is commercialized and consumed fresh. According to the last great agricultural census conducted in Brazil in 2006, 525,602 mega grams of lettuce were produced in the country (14).

Bottom rot, caused by *Rhizoctonia solani*, is an important disease affecting lettuce and it is widespread in several producing areas of this crop in Brazil (15, 22). Losses of up to 70% have been reported for cultures produced on infested soils under conditions of high humidity and temperatures between 25 and 27 ºC during the cycle of the lettuce crop (23). The incidence of this disease is related to the amount of pathogen inocula present on the soil (12), and the control of *R. solani* is difficult because it can survive for a long time on the soil associated with organic matter or as micro-sclerotia (1).

The fungus *Rhizoctonia solani* is frequently found on soils and can be a plant pathogen to several cultures or a saprophyte. *R. solani* is composed of genetically isolated groups that are separated according to their anastomosis behavior (10). In Brazil, AG 1-IB is the anatomosis group associated with lettuce bottom rot (22); it is also the group most frequently associated with this disease in other countries (10).

Several measures have been suggested for the management of lettuce bottom rot, including deep plowing, adequate irrigation and drainage, control of weeds (23), as well as tillage or crop rotation with cereals and subsequent incorporation of the crop residues (22). On soils containing high amounts of *R. solani* inocula, chemical control with fungicides is frequently used (22). Biological control with *Trichoderma* spp. has previously demonstrated potential to control this disease (8, 17), which is very interesting, specially for this crop. Lettuce is consumed fresh and has a rather short cycle, 30 to 45 days after the transplanting of seedlings, which enhances the probability of contamination with pesticides. In 2011-2012, 41% of the lettuce samples analyzed to detect pesticides in Brazil were contaminated with pesticides not registered for this culture (2).

Trichoderma is one of the most studied antagonists for the biocontrol of soilborne pathogens, presenting the advantage of being almost innocuous to humans and having no harmful environmental effects (32). Due to the improvement in the technology developed for multiplication, formulation and storage of this fungus (30), the commercial production of *Trichoderma* has considerably expanded in Brazil in the last few years, but the products available were not developed for the control of lettuce bottom rot.

Effective antagonists can be selected by means of *in vitro* and *in vivo* experiments (6). Under *in vitro* conditions, some important biocontrol mechanisms such as parasitism and antibiosis are relatively easy to be assessed for several isolates (16), as well as the ability of the selected organisms to multiply and produce spores on inexpensive solid substrates (29). However, the effective potential for biocontrol capacity can only be properly evaluated under natural conditions (29).

Considering the relevance of the development of biological control for the management of lettuce bottom rot as an alternative to fungicides, the present study was carried out to select isolates of *Trichoderma* spp. that could be effective in controlling this disease.

MATERIAL AND METHODS

Isolates of *Trichoderma* spp. were obtained from soil samples by using baits (9) in the years of 2001 and 2003. The baits were prepared with wheat grains placed in Erlenmeyer flasks previously moistened and autoclaved for one hour at 120 ºC on two consecutive days. Each Erlenmeyer flask contained 500 g of wheat grains and received 20 discs of mycelium of *R. solani* AG 1-IB grown for seven days on potato dextrose agar medium (PDA). The flasks were kept in the laboratory at room temperature for 10 days. Petri dishes (15 cm in diameter) containing the soils received 20 baits each. After incubation from four to five days in the laboratory, the dishes were observed under a stereoscopic microscope. When mycelium and conidia characteristic of *Trichoderma* were noted over the baits, they were transferred to Petri dishes containing PDA plus streptomycin (0.2%). When needed, the colonies were transferred again to PDA until pure cultures of each isolate were obtained.

Initially, the forty-six isolates of *Trichoderma* spp. were subjected to a pairing test with *R. solani* AG 1-IB, following the method developed by Bell et al. (3), modified by May & Kimati (18). Paired colonies were allowed to grow on the surface of the medium for five days at room temperature. The development of the pathogen and the antagonist was evaluated according to Carvalho et al. (7). The experiments were carried out in a completely randomized design with four replicates per isolate.

The release of metabolic substances by the forty-six isolates of *Trichoderma* spp. was evaluated according to the cellophane method. Petri dishes containing PDA were autoclaved with a cellophane disc of 9 cm diameter placed on the medium. A disc containing the colony of the isolate of *Trichoderma* spp. was placed on the center of the plate. The colonies were allowed to grow at room temperature for 48 hours on the cellophane; then, both the colonies and the cellophane were removed and a disc containing a *R. solani* colony was placed on the center of each Petri dish. The dishes were kept at room temperature for 72 hours when the diameter of each *R. solani* colony was measured and compared with the diameter of colonies grown on PDA alone. The experiments were carried out in a completely randomized design, with four replicates for each isolate.

After the laboratorial experiments, the forty-six isolates were tested for their ability to control bottom rot in lettuce seedlings in four experiments conducted in a greenhouse from May to November 2003. The isolates of *Trichoderma* IBLF 01, IBLF 02, IBLF 03, IBLF 04, IBLF 05, IBLF 06, IBLF 07, IBLF 13, IBLF 18, IBLF 23, IBLF 24, IBLF 25 were tested in the first experiment. In the second experiment, the isolates IBLF 26, IBLF 28, IBLF 31, IBLF 32, IBLF 33, IBLF 34, IBLF 35, IBLF 36, IBLF 39, IBLF 43 were used. The isolates IBLF 31, IBLF 64, IBLF 66, IBLF 68, IBLF 70, IBLF 71, IBLF 72, , IBLF 73, IBLF 74, IBLF 75, IBLF 77, IBLF 78, IBLF 80 and the isolates IBLF 12, IBLF 15, IBLF 49, IBLF 50, IBLF 55, IBLF 56, IBLF 64, IBLF 65, IBLF 69, IBLF 81, IBLF 82, IBLF 84, IBLF 85 were evaluated in the third and fourth experiments, respectively. The pathogen and the isolates of *Trichoderma* spp. were multiplied on wheat grains prepared as previously described for the production of baits and were kept at room temperature in the laboratory for 10 days until complete colonization of the grains by the antagonists.

Pots were filled with 1.5 dm^3 of the commercial substrate Plantmax (Eucatex™), formulated with pine bark, vermiculite and turf. Before being added to the pots, the substrate was mixed with 30 g of wheat grains colonized with each isolate of *Trichoderma* spp. and 15 g of wheat grains colonized with *R. solani* AG 1-IB. The sporulation rate of each *Trichoderma* spp. isolate was evaluated in a Neubauer chamber and they contained at least 10^6 conidia mL^{-1}. Control treatments were prepared with and without the pathogen. For the positive control, pots were filled with the substrate mixed only with 15 g of wheat grains colonized with *R. solani* AG 1-IB, while for the negative control pots were filled with the substrate alone. The pots were kept at room temperature for 8 days and sown with 70 seeds of lettuce cultivar White Boston. Fifteen days after sowing, 25 seedlings were maintained in each

pot. After 30 days, lettuce plants were collected and counted, while the dry mass of total plants in each pot was weighed. Experiments were carried out in a completely randomized design, with four replicates, each of which was represented by a pot. Data were statistically analyzed and means were compared according to Scott-Knott test at 5% probability.

In the second step of the greenhouse assays, 12 isolates of *Trichoderma* spp., IBLF 01, IBLF 02, IBLF 04, IBLF 05, IBLF 13, IBLF 15, IBLF 18, IBLF 24, IBLF 26, IBLF 32, IBLF 50, and IBLF 85, were tested in two sequential experiments between May and July 2004. In these experiments, the isolates of *Trichoderma* spp. were multiplied on rice grains cooked for 7 minutes in boiling water and then autoclaved at 120 ºC for 30 minutes. Multiplication of the antagonist was modified because this method is easier to prepare and rice grains allow abundant sporulation of *Trichoderma* isolates. Similarly to the previous experiments, the substrate was mixed with 30 g of rice grains colonized with each isolate of *Trichoderma* spp. and 15 g of wheat grains colonized with *R. solani* AG 1-IB. The sporulation rate of each isolate of *Trichoderma* spp. was evaluated in a Neubauer chamber and they contained at least 10^6 conidia mL^{-1}. Control treatments were prepared with and without the pathogen. For the positive control, pots were filled with the substrate mixed only with 15 g of wheat grains colonized with *R. solani* AG 1-IB, while for the negative control pots were filled with the substrate alone. Pots were kept at room temperature for 8 days and then sown with 70 seeds of lettuce cultivar White Boston. Fifteen days after sowing, 25 seedlings were maintained in each pot in the first experiment. Unlike the first assay, all the seedlings were kept in the pots in the second experiment. Thirty days after sowing, the lettuce plants were collected and counted, while the dry mass of total plants in each pot was weighed. The severity of bottom rot was also evaluated according to a graded scale (1-4): 1 - no disease symptoms; 2 - subtle lesions on the bottom of the plant; 3 - lesions on 1/2 to 2/3 of the bottom of the plant; 4 – lesions on more than 2/3 or reaching the entire bottom of the plant, and reduction of 50% or more in the plant size. Both experiments were carried out in a completely randomized design, with four replicates, each of which was represented by a pot. Data were statistically analyzed and means were compared according to Scott-Knott test at 5% probability.

Before being tested in this step, the isolates of *Trichoderma* were identified to species level. For their identification, colonies of each isolate were transferred to Petri dishes containing 2% malt extract agar medium. The plates were incubated for one or two days at 25 ºC in the dark and then allowed to sporulate at room temperature under artificial light. For detailed examination of the branching systems of conidiophores and phialide disposition slides were prepared long before the colony reached its ultimate coloration. The size and the shape, as well as the presence or the absence of ornamentation of phialospores, were determined when the cultures were at least 2 weeks old. Species were identified based on the keys of Rifai (25), Bisset (4, 5) and Samuels et al. (26).

RESULTS AND DISCUSSION

According to the pairing tests, the 17 most effective isolates had rates between 1.0 and 2.0, 18 isolates had rates between 2.1 and 3.0, and the remaining isolates had rates between 3.1 and 4.0 (Table 1). The majority of isolates (76%) showed high capability of growing on the mycelium of *R. solani* AG 1-IB. This was expected because the method employed to obtain the isolates, using baits (9), may have favored the antagonists that were capable of parasitizing the pathogen. Grosch et al. (11) used sclerotia of *R. solani* buried in the soil to obtain three effective *Trichoderma* isolates antagonistic to this pathogen, while Patricio et al. (21) used wheat grains and obtained several isolates of *Trichoderma* antagonistic to *R. solani* and *Pythium aphanidermatum*.

In the present study, thirteen isolates released metabolites that inhibited more than 80% of the pathogen growth, 11 inhibited 61 to

Table 1. Pairing isolates of *Trichoderma* spp. with *Rhizoctonia solani* AG 1-IB.

Rating Intervals (Classes 1-5)	IBLF[1] number of isolates of *Trichoderma* spp.	Percentage of isolates of *Trichoderma* spp.
1.0-2.0	01, 02, 04, 13, 18, 23, 24, 26, 28, 31, 32, 35, 36, 50, 66, 82, 85	36.9
2.1-3.0	05, 06, 07, 15, 25, 33, 34, 39, 43, 49, 55, 56, 64, 65, 70, 75, 78, 80	39.1
3.1-4.0	03, 12, 68, 69, 71, 72, 73, 74, 77, 81, 84	24.0
4.1-5.0	-	

Class 1= *Trichoderma* spp. grows on *R. solani* and occupies the whole surface of the culture medium; Class 2 = *Trichoderma* spp. grows on at least 2/3 of the plate; Class 3 = *Trichoderma* spp. and *R. solani* grow on approximately one half of the surface of the culture medium; Class 4 = *Trichoderma* spp. grows on 1/3 of the surface of the culture medium; Class 5 = *Trichoderma* spp. does not grow and *R. solani* occupies the whole surface of the medium.
[1] The prefix IBLF refers to the number of the isolate maintained in the collection of fungi from "Laboratório de Fitopatologia do Instituto Biológico".

Table 2. Inhibition of *Rhizoctonia solani* AG 1-IB mycelial growth by toxic substances released by colonies of *Trichoderma* spp. isolates.

Inhibition of R. solani mycelial growth (%)	IBLF[1] number of isolates of *Trichoderma* spp.	Percentage of isolates of *Trichoderma* spp.
81 – 100%	05, 18, 24, 26, 33, 36, 43, 49, 50, 68, 70, 78, 80	28.3
61 - 80%	03, 04, 06, 07, 34, 35, 55, 56, 65, 73, 74	23.9
41 – 60%	23, 85	4.3
21 – 40%	02, 31, 39, 69	8.7
0 – 20%	01, 12, 13, 15, 25, 28, 32, 64, 66, 71, 72, 75, 77, 81, 82, 84	34.9

[1] The prefix IBLF refers to the number of the isolate maintained in the collection of fungi from "Laboratório de Fitopatologia do Instituto Biológico".

Table 3. First step of the *in vivo* experiments for the selection of isolates of *Trichoderma* spp. antagonistic to *Rhizoctonia solani* AG 1-IB.

Experiments	Number of tested isolates	IBLF[1] number of isolates of *Trichoderma* spp. with a larger number of seedlings and the control with the pathogen.	Percentage of isolates of *Trichoderma* spp with a larger number of seedlings and the control with the pathogen.	IBLF[1] number of isolates of *Trichoderma* spp. with greater dry weight of seedlings and the control with the pathogen	Percentage of isolates of *Trichoderma* spp. with greater dry weight of seedlings and the control with the pathogen.
Experiment 1	12	02, 03, 04, 07, 13, 18, 23, 24	66.7	01, 02, 03, 04, 05, 07, 13, 18, 24	75.0
Experiment 2	10	26, 28, 31, 32, 33, 34, 35, 36, 39, 43	100.0	26, 31, 32, 33, 35, 36, 39	70.0
Experiment 3	12	71, 72, 73, 74, 75, 78	50.0	73, 74, 75	25.0
Experiment 4	12	-	0.0	12, 15, 50, 85	30.8
Total	46	24	52.2	23	50.0

[1] The prefix IBLF refers to the number of the isolate maintained in the collection of fungi from "Laboratório de Fitopatologia do Instituto Biológico".

Table 4. Second step of the *in vivo* experiments for the selection of isolates of *Trichoderma* spp. antagonistic to *Rhizoctonia solani* AG 1-IB.

Treatments		Experiment 1			Experiment 2		
		Number of plants	Dry mass (g)	Severity of bottom rot (1-4)	Number of plants	Dry mass (g)	Severity of bottom rot (1-4)
Isolates	Species of Trichoderma						
IBLF 01[2]	*T. harzianum*	25.0 a[1]	2.7 b[1]	2.3 a[1]	52.7 a[1]	5.2 c[1]	2.3 b[1]
IBLF 02	*T.* cf. *Konigii*	17.8 b	2.2 c	2.5 a	44.0 b	4.5 c	2.2 b
IBLF 04	*T. virens*	24.8 a	3.6 b	1.5 b	54.3 a	5.6 c	1.7 c
IBLF 05	*T. virens*	25.0 a	3.1 b	3.2 a	58.5 a	5.7 c	2.3 b
IBLF 13	*T.* cf. *Konigii*	18.0 b	1.9 c	1.8 b	44.3 b	4.8 c	2.3 b
IBLF 15	*T. harzianum*	25.0 a	3.5 b	2.5 a	44.8 b	4.5 c	2.6 b
IBLF 18	*T. virens*	23.5 a	3.0 b	2.7 a	47.3 b	5.5 c	2.5 b
IBLF 24	*T. virens*	22.0 a	3.2 b	2.1 a	44.5 b	5.0 c	2.9 a
IBLF 26	*T. virens*	21.3 a	3.0 b	2.1 a	56.0 a	5.0 c	2.4 b
IBLF 32	*T.* cf. *Konigii*	17.0 b	1.7 c	2.3 a	47.5 b	5.4 c	1.9 c
IBLF 50	*T. virens*	23.5 a	2.4 c	1.3 b	60.0 a	6.0 b	1.9 c
IBLF 85	*T.* cf. *Konigii*	18.0 b	2.4 c	2.3 a	53.3 a	5.6 c	2.2 b
Control		25.0 a	4.5 a	1.0 b	56.2 a	7.0 a	1.0 d
Rhizoctonia solani [5]		10.3 c	1.2 c	2.8 a	20.2 c	2.6 d	3.2 a
Coefficient of variation (%)		21.4	21.4	25.5	17.6	15.7	17.9

[1]Means followed by the same letter do not differ according to Scott-Knott test at 5% probability.
[2] The prefix IBLF refers to the number of the isolate maintained in the collection of fungi from "Laboratório de Fitopatologia do Instituto Biológico".

80% of *R. solani* growth (Table 2) and 16 had almost no effect on the pathogen growth (Table 2). Grosch et al (11) also found differences in the capacity of *Trichoderma* strains to produce toxic metabolites that could inhibit the mycelial growth of *R. solani*.

In the first step of the *in vivo* experiments, 24 (52.2%) isolates of *Trichoderma* spp. were capable of improving the number of surviving seedlings, while 23 (50%) isolates had greater dry weight, compared to the control with the pathogen (Table 3). Considering both variables, 15 isolates (32.6% of the total of isolates) were capable of increasing the number and the dry weigh of lettuce seedlings (Table 3).

In the second step of the *in vivo* experiments, considering the first experiment, treatments with the isolates IBLF 01 and IBLF 15 of *T. harzianum* and IBLF 04, IBLF 05, IBLF 18, IBLF 24, IBLF 26 and IBLF 50 of *T. virens* had a larger number of surviving seedlings than the control with the pathogen. The same isolates, except for IBLF 50, had greater dry mass than the control with the pathogen. Only treatments with the isolates IBLF 04, IBLF 13 and IBLF 50 showed less bottom rot than the control with the pathogen (Table 4).

In the second experiment of the second step, all treatments had a larger number of surviving seedlings and greater dry mass, showing less severity of bottom rot than the control with *R. solani.* Treatments with the isolates IBLF 01, IBLF 04, IBLF 05, IBLF 26, IBLF 50 and IBLF 85 had a larger number of surviving seedlings than the remaining isolates, and treatments with the isolates IBLF 26 and IBLF 50 had greater dry mass than the other treatments. Treatments with the isolates IBLF 04, IBLF 32 and IBLF 50 showed less severity of the disease than the other treatments (Table 4).

The two isolates of *T. virens,* IBLF 04 and IBLF 50, which stood out in the second step of the *in vivo* experiments for increasing the number and the dry weight of seedlings besides reducing the severity of bottom rot in both assays, showed high capacity for parasitism with rates of 1.6 and 1.8, respectively, in the pairing test (Table 4). The capacity for mycoparasitism of *Trichoderma* isolates may play an important role in the control of *R. solani* under *in vivo* conditions. Several mechanisms can be employed by *Trichoderma* isolates to mycoparasitize the pathogenic fungi, involving the recognition of the host, and the attack, the penetration and the killing of the pathogen mycelium (31). The cell wall-degrading enzymes, such as cellulases,

chitinases, glucanases, which are capable of hydrolyzing their cell wall, are secreted in the presence of exudates of the pathogenic fungus and may be produced at different amounts by diverse *Trichoderma* strains (31), as observed in the present study.

The two *T. virens* isolates, IBLF 04 and IBLF 50, which stood out in the second step of the *in vivo* experiments, released metabolites that inhibited 80-100% of the pathogen growth. In previous studies that selected *Trichoderma* isolates antagonistic to *R. solani* and other pathogens, different results were observed. For the selection of antagonists to *R. solani, P. aphanidermatum*, *Fusarium solani*, and *Sclerotium rolfsii*, the most effective isolates were not the ones that produced the greater amounts of toxic substances (21, 24, 28). For the same pathosystem of the present study, Coley-Smith et al. (8) found that an isolate of *T. viride,* most effective for the control of lettuce bottom rot, produced only small amounts of a glucanase and a chitinase, while isolates that produced great amounts of these enzymes showed poor control of the disease. Grosch et al. (11), however, verified that isolates that released greater amounts of toxic metabolites and were capable of parasitizing *R. solani* sclerotia were effective for the control of *R. solani* in potato and lettuce seedlings.

As shown in the present study, antibiotic production has been suggested to be correlated to the biocontrol activity of various strains of *Trichoderma* (31). It is well known that *Trichoderma* strains can produce a variety of secondary metabolites showing biological activity, including antibiotics capable of inhibiting microbial growth. The production of these substances is strain-dependent and they can belong to a high variety of classes of volatile and non-volatile compounds. The latter can be water soluble compounds or peptaibols, which are linear oligopeptides (31). Several studies have shown that peptaibols could inhibit the activity of the enzyme β-glucan synthase in the host fungus and could act synergistically with β-glucanases in preventing the reconstruction of the fungus cell wall, facilitating thus the disruptive action of β-glucanases (31). In addition, previous studies have shown that *T. viride* strains may produce known antibiotics such as gliotoxin, gliovirin, and viridiol, which have antagonistic activity against several plant pathogens (31). In the present study, the toxic metabolic substances produced by the most efficient isolates were not determined but could be the subject of future studies.

Trichoderma isolates were produced on rice or wheat grains because these are low-cost substrates and the isolates exhibited rapid growth and high sporulation rates on them. Furthermore, adding the antagonist together with a food base may improve soil colonization by *Trichoderma* isolates because it favors their growth and sporulation and reduces fungistasis on the soil (20); these phenomena may also occur on the substrate.

In studies performed by other authors, smaller amounts of antagonists were added to the soil to control this disease; however, only the higher quantities were effective in controlling the disease in the field. Maplestone et al. (17) obtained the control of bottom rot with 0.5% weight/weight of one isolate of *T. harzianum*, while Coley-Smith et al. (8) achieved control with 1 kg per m^2 of *T. harzianum* in lettuce planted on beds under plastic tunnels. Recently, Lucon et al. (16) observed an improvement in the control of *R. solani* in cucumber seedlings when 1 to 4% of rice grains colonized with *Trichoderma* were added to a commercial substrate and the best control was achieved with 2 to 4% of rice grains colonized with this fungus.

In the first experiment of the second step of the *in vivo* experiments, the *Trichoderma* isolates IBLF 01, IBLF 02, IBLF 05, IBLF 13, IBLF 15, IBLF 18, IBLF24 and IBLF 26 showed a larger number and greater dry mass of lettuce seedlings than the *R. solani* treatment, despite showing high incidences of bottom rot. A similar tendency was observed in the second experiment, in which, except for the treatments IBLF 04 and IBLF 50, all other treatments had disease severity rating from 2.2 to 2.9 but showed a larger number of plants and greater dry weigh than the control with the pathogen (Table 4). This may have occurred because *Trichoderma* spp. isolates may have stimulated the plant growth, a phenomenon that has been observed by other authors for lettuce in the same pathosystem (8, 17) and has been documented in several studies for other pathosystems (31). Some *Trichoderma* strains can colonize root surfaces and promote changes in the plant metabolism, while some strains can promote plant growth and increase nutrient availability (31). This phenomenon may be related to metabolites released by *Trichoderma* isolates that enhance the efficiency of nutrient transportation from the roots, especially nitrogen, which stimulates plant growth (13, 19, 27). *Trichoderma* strains were also shown to be capable of producing organic acids such as gluconic, citric or fumaric acids that can decrease the soil pH and promote the solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium (31).

In the present study, the isolates of *T. virens* IBLF 04 and IBLF 50 were capable of reducing the severity of bottom rot and increasing the number and the dry weight of lettuce seedlings in repeated *in vivo* experiments, showing that biological control is a promising alternative for the control of lettuce bottom rot, as previously shown by other authors (8, 17). The steps employed in this study for the selection of *Trichoderma* isolates are promising, simple and could be employed for other pathosystems or to select other isolates for lettuce bottom rot. In this study, baits were used to obtain the isolates, which may favor *Trichoderma* isolates that can parasitize the pathogenic fungi, and were successfully employed in previous studies for the same (11) and similar pathosystems (21). The study of the toxic metabolic substances produced by *Trichoderma* isolates is very interesting and should be the subject of future studies, as well as the testing of the isolates under field conditions.

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