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PLANT PROTECTION
Article

The plant growth effect and biocontrol potential of *Trichoderma* sp. inoculation in tomatoes are dependent of the inoculation way

Marcia Rodrigues Sandri[1], Hélen Corso Cavião[1], Christiane Fernandes Oliveira[1], Luciana Bavaresco Andrade[1], Camille Granada[2,*], Joséli Schwambach[1]

1. Universidade de Caxias do Sul – Programa de Pós-Graduação em Biotecnologia – Caxias do Sul (RS), Brazil.
2. Universidade do Vale do Taquari – Programa de Pós-Graduação em Biotecnologia – Lajeado (RS), Brazil.

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***Corresponding author:** cegranada@univates.br

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ABSTRACT: Tomato is an important economic crop due to its consumption and production worldwide. However, like other crops, it is susceptible to pathogens, being necessary agrochemicals to prevent diseases and improve the production of fruits. Among the sustainable alternatives to crop production, microbial inoculants are used as biofungicide and biostimulants for plant development. Thus, this work aimed to evaluate the biocontrol potential of seven *Trichoderma* spp. isolates (T1, T2, T3, T4, T15, T17, and T19) against tomato pathogens *in vitro* experiments, and their potential to improve tomato growth. The *Trichoderma* spp. antagonism was investigated against *Alternaria* sp. 003/09, *Botrytis* sp. 006/13, *Fusarium* sp. 007/09, and *Stemphylium* sp. A73. Dual culture, volatile, and diffusible compounds activity tests showed that all new *Trichoderma* spp. tested could reduce the mycelial growth of all tested pathogens, highlighting T15 and T17 isolates. Seed and soil inoculation revealed very contrasting results: *Trichoderma* sp. T17 showed a beneficial effect when inoculated in soil, reducing the percentage of yellowish leaves, and increasing dry weight and stem diameter. Inoculation of *Trichoderma* sp. T17 in the seed increases hypocotyl and radicle lengths, and the seed vigor index. Finally, the strains studied present the potential to be used to develop biocontrol products.

Key words: *Lycopersicum esculentum*, biocontrol agent, phytotoxicity, seedling vigor, plant growth promotion.

INTRODUCTION

Modern agriculture requires continuously developing technologies to produce high yields in smaller spaces (Stewart et al. 2005). Chemical fertilizers and pesticides have improved crop production, playing an essential role in the last decades. However, many of these products are considered dangerous to the environment and human health (Kim et al. 2017). The concern about productivity and reducing chemical crop inputs encourages farmers to adopt integrated pest management strategies. These strategies are focused on prevention, using several plant protection methods, and considering economic and ecological benefits (Lefebvre et al. 2015). Biological control using *Trichoderma* spp. isolates is an important tool due to their biocontrol activity against plant pathogens (van Lenteren et al. 2017).

It was estimated that 60% of the registered bioinoculants comprise *Trichoderma* spp. (van Lenteren et al. 2017). Microbial inoculants can act as biopesticides when they decrease the deleterious effects of pests or diseases. They can also act as biostimulants (biofertilizers) due to their potential to promote plant growth (Calvo et al. 2014). As an example of beneficial effects, tomato seeds treated with six different *Trichoderma harzianum* strains had better germination indexes (Srivastava et al. 2010). On the other hand, Singh (2016) did not observe improvements in the germination of tomato seeds bioprimed with *Trichoderma asperellum* BHUT8, but the root and shoot weight of the seedlings showed an increment.

Different methods of enriching tomato rhizosphere with *Trichoderma* spp. have been efficient in the growth promotion of tomato seedlings. For example, Li et al. (2018) inoculated *T. asperellum* CHF78 by soil drenching four weeks after sowing, and an increase in seedling dry weight was observed. Also, Chowdappa et al. (2013) showed that inoculation of *T. harzianum* OTPB directly in the sowing cavity improved the shoot and root length and weight, leaf area, and seed vigor index. Growth promotion may also be achieved by mixing suspensions or powder products containing *Trichoderma* spp. propagules with soil/substrate before sowing the seeds or transplanting the emerged seedlings (Marín-Guirao et al. 2016).

Knowing the biotechnological potential of *Trichoderma* spp. for crop protection and growth and developing sustainable alternatives to chemical fertilizers and fungicides, this study aimed to investigate the potential of seven *Trichoderma* spp. isolates for the protection and growth promotion of tomatoes. For this purpose, biocontrol mechanisms were investigated against four tomato pathogens that cause wilt, fruit mold, and leaf spots and the effect on seed germination and seedling growth. We hypothesized that inoculation way (seed or soil) does not influence the growth promotion effect caused by *Trichoderma* spp.

MATERIALS AND METHODS

Fungal isolates, identification, and culture media

Seven *Trichoderma* spp. were isolated from vine growing soil of Caxias do Sul, Rio Grande do Sul, Brazil. The pathogens were isolated from parts of tissues of tomato plants presenting disease symptoms (Suppl. Table 1) collected at Caxias do Sul and Veranópolis cities, Rio Grande do Sul state, Brazil. All isolations were performed on potato dextrose agar (PDA) containing 1% amoxicillin. PDA was also used for the purification and storage of fungal isolates. They are maintained in the fungal collection of the Laboratory of Plant Disease Biological Control, Universidade de Caxias do Sul.

The identification was performed first based on colony and conidia morphology. After, a fragment of the ITS1-5.8S-ITS2 region and the TUB2 (β-tubulin) genes were amplified and sequenced according to the procedures described by Echeverrigaray et al. (2020). ITS region and beta-tubulin gene (accession number OL869122-OL869132) were used for phylogenetic analysis using Bayesian method described by Granada et al. (2015).

In vitro antagonism assays

Discs of 5 mm were removed from the borders of fungi colonies (*Trichoderma* spp. isolates and pathogens) with 3–10 days of growth at 25 ± 2°C, 12-h photoperiod. The dual culture test was evaluated by direct interaction of each *Trichoderma* sp. isolate and the pathogen. The pathogen was inoculated on one side of the plate, and, 48 hours later, one *Trichoderma* sp. isolate was inoculated on the opposite side, symmetrically. The diameter of pathogen colonies was calculated as an average of two crossed measurements after three, six, and nine days of pathogen inoculation.

The antagonism by diffusible compounds was performed in cellulose film (Natural produtos), placed in PDA, and one *Trichoderma* sp. was inoculated in the center. After 72 hours, the cellulose film with the *Trichoderma* sp. mycelium was removed, and the pathogen was inoculated. The diameter of the pathogen colony was measured on days 3, 7, 10, and 14 (Benítez et al. 2004). The antagonistic effect of volatile compounds produced by *Trichoderma* spp. was evaluated according to procedures described by Bruce et al. (2000). The assay was assessed using two Petri dishes containing PDA overlaid and sealed with parafilm: on the upper dish, a 6-mm diameter agar disc of the pathogen mycelium was inoculated, and on the lower dish a 6-mm diameter agar disc of the one *Trichoderma* sp. mycelium. The pathogen colony diameter was measured on days 3, 6, 9, and 14.

The growth inhibition was calculated using the control treatment (pathogen growth without the influence of *Trichoderma* spp.) and the pathogen that grew with *Trichoderma* sp. effect. The mycelial growth speed index (MGSI) was calculated according to Oliveira et al. (2016). The MGSI was determined according to Eq. 1:

$$MGSI = \Sigma [(d-dp)/N] \quad (1)$$

where: d: the mean colony diameter at the present day; dp: the mean colony diameter from the previous day; N: the number of days after dish incubation.

Viability tests were performed at the end of each experiment, transferring a disc of the pathogen fungal mycelium to a PDA to access the growth after five days of inoculation at 25°C and 12 hours of photoperiod.

Germination of inoculated seeds with Trichoderma sp.

The seeds of *Solanum lycopersicum* cultivar Micro-Tom were rinsed with 70% ethanol wash for 1 minute, followed by 10 minutes in sodium hypochlorite 1.5%, and after four washes with sterile water. Conidial suspensions of the seven new isolates of *Trichoderma* spp. with 5×10^6 conidia·mL^{-1} were prepared, and 200 seeds per treatment were soaked for 1 h in each *Trichoderma* sp. treatment. For the control treatment, 200 seeds were left for 1 h in sterile water. Ten seeds were placed in a Petri dish above one layer of filter paper moistened with 6 mL of sterile water or conidia suspension, and each treatment was composed of 10 Petri dishes with a replicate. The Petri dishes were transferred to an incubation chamber with 25°C and 12 hours of photoperiod, and the germination was evaluated after seven days. Also, the seedlings were measured, and the seed vigor index (SVI) was calculated using methods described by Abdul-Baki and Anderson (1973). The SVI was determined according to Eq. 2:

$$\text{SVI} = (\text{mean root length} + \text{mean shoot length}) \times \% \text{ germination} \quad (2)$$

Growth promotion effect of tomato seedlings by *Trichoderma* sp.

Seeds of *S. lycopersicum* cultivar Micro-Tom were germinated in 50-mL pots filled with sterilized substrate "Carolina soil padrão" (Carolina soil™), and watering was performed with 10 mL of tap water every day. Fifteen days after the first plant emergence, 20 seedlings per treatment were selected and inoculated with a conidial suspension of one *Trichoderma* sp. (5×10^5 conidia·mL^{-1}), totalizing seven inoculated treatments, or water for the control treatment. The plants were kept in a growth room with 25 ± 2°C, 50% humidity, and 12 hours of photoperiod. The seedlings were heightened and numbered. After 30 days, the following growth parameters were measured: seedling height, steam diameter, root and shoot dry weight, number of leaves, and yellowish leaves. In addition, the seedling health index (SHI) was calculated using methods described by Fan et al. (2013). The SHI was determined according to Eq. 3:

$$\text{SHI} = \left(\frac{\text{Stem diameter}}{\text{Stem height}}\right) \times \text{Dry weight} \quad (3)$$

Statistical analysis

The data were tested for normal distribution by the Kolmogorov-Smirnov test. If data distribution followed the normal distribution, analysis of variance test with means compared by Tukey's test or T3 Dunnet's test was used ($p \leq 0.01$). Non-parametric data were compared by the Kruskal-Wallis test with a Mann–Whitney post hoc and a Bonferroni's correction ($p \leq 0.01$).

Two principal component analysis (PCA) were performed: one to gathers all the new *Trichoderma* sp. potential in antagonism experiments (dual culture, diffusible, and volatile compounds), and the other one gathers growth promotion of tomato using seed inoculation (percentage of germination, hypocotyl and root lengths, SVI and rate of root/hypocotyl), and soil inoculation (number of leaves, shoot and root dry weights, growth, SHI, steam diameter, and rate of root/shoot).

RESULTS

Trichoderma spp. strains T1, T3, T4, and T15 are very similar in the macroscopic view, with rapid growth (three days in PDA, 25°C, photoperiod of 12 h) and dark green sporulation. Strain T2 also presents green sporulation and a yellow diffusible pigment, providing a yellowish color on the reverse. Strains T17 and T19 produce spores after a week (same

conditions described) and initially have yellow sporulation that becomes green in a few days. The sequencing of the ITS and beta-tubulin gene region confirmed that all antagonistic fungal isolates belonging to the *Trichoderma* spp., with the closest strains reported in Fig. 1 (data collected on National Center for Biotechnology Information). This analysis showed that the *Trichoderma* spp. isolates belong to known *Harzianum* group.

The MGSI showed that all *Trichoderma* spp. isolates present at least one type of antagonistic activity against the four pathogens studied (Table 1). However, the inhibition efficiency varied according to the type of test. The growth inhibition of *Alternaria* sp. 003/09 varied from 0% in the diffusible compounds test by strain T4 to 66% in the volatile compounds test by T1. The growth inhibition of *Fusarium* sp. 007/09 varied from 0% in the diffusible compounds test by strain T4 to 37% in the dual culture test by T17. *Botrytis* sp. 006/13 presented 1% of mycelial growth inhibition in the diffusible compounds test by strain T15 and 53% in the volatile compounds test by T2. And finally, inhibition of *Stemphylium* sp. A73 varied from 17 to 69% in the diffusible compounds test, with strains T3 and T1, respectively. Considering the efficiency of antagonism, the isolates T3, T1, and T17 stood out in the dual culture test, T2 in diffusible compounds, and the activities of all *Trichoderma* spp. were similar in the volatile compounds test, presenting highly promising results. At the end of these tests, it was possible to observe the mycoparasitism of new *Trichoderma* spp. isolates over the pathogen mycelium.

Table 1. Mycelial growth speed index (MGSI) of pathogens submitted to three tests: dual culture, diffusible compounds, and volatiles compounds@.

***Alternaria* sp. 003/09**	**Dual culture**		**Diffusible compounds**		**Volatile compounds**	
	MGSI*	**Inhibition (%)**	**MGSI**	**Inhibition (%)**	**MGSI**	**Inhibition (%)**
Control	12.8 ± 1.2 b		14.6 ± 1.5 ab		14.0 ± 2.1 a	
T1	10.6 ± 1.6 ab	31.1 ± 11.3 a	11.6 ± 1.6 bc	5.5 ± 5.5cd	6.6 ± 2.9 b	66.4 ± 12.0 a
T2	9.8 ± 1.5 a	39.3 ± 9.0 a	4.5 ± 0.7 d	66.3 ± 4.6 a	7.0 ± 2.8 b	61.2 ± 10.1 a
T3	9.0 ± 2.0 a	44.0 ± 13.3 a	8.1 ± 1.5 cd	41.0 ± 12.8 ab	6.8 ± 1.5 b	62.9 ± 4.6 a
T4	9.4 ± 1.0 a	36.4 ± 10.6 a	15.6 ± 1.0 a	0.0 ± 0.0 d	9.0 ± 0.9 b	55.3 ± 3.2 a
T15	9.7 ± 2.0 a	32.3 ± 18.4 a	9.5 ± 1.3 cd	25.5 ± 11.6 bc	8.1 ± 0.8 b	58.4 ± 5.3 a
T17	9.0 ± 1.4 a	38.9 ± 11.7 a	9.0 ± 1.2 cd	33.7 ± 8.3 ab	8.4 ± 1.8 b	60.0 ± 5.3 a
T19	9.5 ± 1.5 a	33.9 ± 12.0 a	10.0 ± 1.5 cd	17.5 ± 15.2 bcd	7.9 ± 1.2 b	59.1 ± 6.6 a
***Fusarium* sp. 007/09**	**MGSI**	**Inhibition (%)***	**MGSI**	**Inhibition (%)**	**MGSI**	**Inhibition (%)**
Control	15.4 ± 1.1 a		13.8 ± 1.8 a		16.3 ± 0.6 a	
T1	11.5 ± 0.9 bc	29.7 ± 7.8 ab	13.6 ± 2.1 a	3.5 ± 8.0 bc	12.4 ± 0.8 bc	29.0 ± 11.8 a
T2	13.0 ± 1.6 ab	17.9 ± 11.6 b	7.6 ± 1.0 c	34.3 ± 11.7 a	13.6 ± 0.7 ab	20.0 ± 8.8 a
T3	11.8 ± 0.8 bc	28.5 ± 7.0 ab	10.4 ± 0.9 bc	9.3 ± 8.5 bc	13.4 ± 1.1 bc	24.9 ± 9.2 a
T4	11.9 ± 0.7 bc	29.9 ± 7.0 ab	14.5 ± 1.8 a	0.0 ± 0.0 c	12.2 ± 1.1 bc	30.7 ± 11.4 a
T15	12.3 ± 0.4 bc	30.0 ± 5.9 ab	13.7 ± 2.1 a	3.2 ± 7.9 bc	11.9 ± 0.9 c	28.1 ± 11.1 a
T17	11.2 ± 0.9 c	37.3 ± 9.6 a	11.8 ± 0.7 ab	6.1 ± 8.3 bc	12.5 ± 1.4 bc	27.6 ± 12.2 a
T19	12.0 ± 1.2 bc	32.5 ± 11.1 a	8.8 ± 1.1 bc	12.2 ± 6.3 ab	11.7 ± 0.7 c	29.8 ± 5.9 a
***Botrytis* sp. 006/13**	**MGSI**	**Inhibition (%)***	**MGSI**	**Inhibition (%)**	**MGSI***	**Inhibition (%)***
Control	19.7 ± 1.6 a		20.5 ± 1.9 a		21.7 ± 1.2 a	
T1	13.9 ± 0.8 bc	40.2 ± 7.3 ab	14.3 ± 1.8 b	3.6 ± 8.3 b	16.7 ± 1.9 b	31.1 ± 12.2 ab
T2	16.1 ± 2.3 b	35.1 ± 11.3 ab	7.8 ± 3.0 b	53.8 ± 23.6 a	16.8 ± 2.3 b	32.7 ± 7.6 b
T3	12.8 ± 0.9 c	45.1 ± 5.7 a	7.5 ± 4.2 b	45.9 ± 37.0 a	17.0 ± 1.1 b	32.1 ± 4.2 b
T4	13.9 ± 1.8 bc	40.2 ± 5.8 ab	10.7 ± 1.4 b	40.1 ± 8.3 a	14.9 ± 2.7 bc	38.3 ± 14.3 ab
T15	15.9 ± 1.7 b	27.7 ± 11.5 b	14.3 ± 1.9 b	1.0 ± 3.1 b	15.1 ± 2.9 bc	26.8 ± 25.5 ab
T17	14.5 ± 0.8 bc	42.0 ± 3.1 a	12.2 ± 2.1 b	6.5 ± 8.2 b	14.0 ± 2.8 bc	42.1 ± 12.7 ab
T19	15.4 ± 1.4 b	32.3 ± 8.3 ab	12.2 ± 1.2 b	2.3 ± 6.2 b	12.5 ± 3.5 c	48.9 ± 13.6 a

Continue...

Table 1. Continuation...

Stemphylium sp. A73	Dual culture		Diffusible compounds		Volatile compounds	
	MGSI	Inhibition (%)	MGSI	Inhibition (%)	MGSI	Inhibition (%)*
Control	12.1 ± 2.3 a		13.5 ± 2.6 a		12.0 ± 4.4 a	
T1	8.8 ± 1.9 ab	45.3 ± 9.1 ab	3.8 ± 1.3 c	68.9 ± 11.9 a	6.7 ± 1.6 b	52.7 ± 11.2 a
T2	8.3 ± 0.7 bc	44.8 ± 5.7 ab	4.9 ± 1.2 bc	63.6 ± 8.9 a	7.5 ± 1.4 b	43.3 ± 10.9 a
T3	8.2 ± 0.4 bc	46.2 ± 4.0 a	9.4 ± 2.3 abc	17.1 ± 20.4 a	7.6 ± 1.0 b	45.7 ± 10.1 a
T4	8.8 ± 1.3 b	35.7 ± 11.3 b	10.0 ± 3.6 ab	27.2 ± 26.5 b	6.4 ± 4.0 b	58.7 ± 13.4 a
T15	5.8 ± 1.8 c	41.6 ± 14.3 ab	7.7 ± 1.4 bc	30.3 ± 12.9 b	7.1 ± 4.1 b	51.6 ± 17.7 a
T17	7.4 ± 1.0 bc	43.6 ± 17.6 a	3.9 ± 1.5 c	58.5 ± 15.3 b	8.1 ± 3.8 ab	44.1 ± 15.6 a
T19	5.0 ± 1.4 c	49.1 ± 13.1 a	9.2 ± 2.9 bc	26.7 ± 16.5 b	7.8 ± 4.5 ab	44.9 ± 21.2 a

@Data presented as mean ± standard deviation. Statistics were done comparing all *Trichoderma* spp. and control, separately for each pathogen tested; *data analyzed by analysis of variance test and means compared by Tukey's test ($p < 0.01$). Unmarked data were compared by Kruskal-Wallis test followed by a Mann–Whitney post hoc test including a Bonferroni's correction ($p < 0.01$).

Besides reuniting all mycelium measurements performed in a single image, PCA analysis also allowed comparison among the three antagonism experiments, and inference about which *Trichoderma* spp. strain was the most efficient (Fig. 2a). This analysis explained 87.8% of the total variability (principal component 1 [PC1] explained 78.3%, and principal component 2 [PC2] explained 9.5%), and highlighted the high inhibition potential of all new *Trichoderma* spp. isolates against all studied pathogens in comparison with controls. Figure 2b shows the sensitivity of the pathogens against all new Trichoderma spp. isolates. This PCA analysis explained 90.9% of data variability (PC1 explained 80.2%, and PC2 explained 10.7%). In general, the fungi *Alternaria* sp. 07/09 and *Stemphylium* sp. A73 were most affected by the *Trichoderma* sp., and *Fusarium* sp. 007/09 was the most resistant.

There was no difference in the germination rate of tomato seeds in both experiments (Table 2). It was observed a growth promotion effect in plants inoculated with T2 and T15 in all germination parameters evaluated. However, when each evaluated parameter was analysed separately, they were considered similar to the control treatment. These promising results can be observed in Fig. 2c; this analysis explained 98.5% of the data variability (PC1 = 86.3% and PC2 = 12.2%), and a significant distance from the control can be observed (highlighted in gray circle) by strains T15 and T2. These treatments were related to high root length values and root/hypocotyl length rate. The isolates T1, T4, T17, and T19 presented adverse effects on the germination parameters evaluated (Table 2 and Fig. 2c), localized on the opposite side of PC1 compared to the control, T2, and T15.

Fresh seedlings treated with the new *Trichoderma* spp. presented more than 90% survival. Besides the encouraging results of strain T2 in the germination test, plants inoculated with this strain were damaged and presented only 7.5% survival (data not shown). Seedlings treated with strain T2 became dead or very small and undeveloped, so strain T2 was excluded. In most treatments, about 30% of leaves became yellowish in four weeks, only plants inoculated with *Trichoderma* spp. T1, T3, T4, and T19 presented a low percentage of yellowish leaves in both experiments (Table 3), and inoculation with T19 increased the growth (Δ height) and shoot dry weight. PCA analysis shown in Fig. 2d explained 87.5% of the total variability (PC1 = 67% and PC2 = 20.5%). This analysis showed a group with strains T3, T17, and T19 related with higher values of all plant growth parameters evaluated, highlighting strain T19, which did not present promising results in the germination test, but was localized after control in PCA.

Thus, we could not prove our hypothesis once the inoculation technique strongly influences the growth promotion results. The data presented in this work showed that strains with high biotechnological potential using seed inoculation were not the same as those using soil inoculation. For example, the promising results presented by strain T2 with seed inoculation were opposite of those observed in soil inoculation (almost all plants died in the first days). Moreover, strains T19 and T17, which presented negative impacts when the seed was inoculated, presented the most promising results when inoculation was performed in the soil. The high biocontrol activity presented by these *Trichoderma* sp. isolates *in vitro* experiments turns them good candidates for tomato inoculation aiming biocontrol activity.

Table 2. Development of seedlings of tomato cv. Micro-tom seeds after seven days of sowing and calculated seed vigor index in two different inoculation experiments. Development of seedlings of tomato cv. Micro-tom seeds inoculated with *Trichoderma* sp. after seven days of sowing and calculated seed vigor index in two independent replicates of the experiment*.

	Treatment	Germination (%)	Hypocotyl (mm)	Radicle (cm)	Seed vigor index
Experiment 1	Control	87 ± 11.6 a	5.2 ± 1.6 b	2.7 ± 1.4 ab	2.9 ± 1.7 ab
	T1	83 ± 15.7 a	4.9 ± 2.4 b	1.7 ± 1.2 bc	1.9 ± 1.5 b
	T2	84 ± 13.5 a	5.2 ± 1.8 b	2.7 ± 1.3 ab	2.8 ± 1.6 ab
	T3	88 ± 9.2 a	6.4 ± 1.3 a	2.5 ± 1.4 abc	2.9 ± 1.4 ab
	T4	78 ± 17.5 a	4.9 ± 1.6 b	1.4 ± 0.6 bc	1.6 ± 0.8 b
	T15	90 ± 8.2 a	6.5 ± 1.6 a	3.1 ± 1.2 a	3.4 ± 1.4 a
	T17	73 ± 14.9 a	3.6 ± 1.4 b	0.4 ± 0.3 d	0.6 ± 0.4 c
	T19	84 ± 12.7 a	4.4 ± 1.4 b	1.4 ± 0. 8 bc	1.6 ± 1.0 b
Experiment 2	Control	82 ± 11.4 a	5.6 ± 1.4 b	2.6 ± 1.1 ab	2.7 ± 1.3 ab
	T1	75 ±20.1 a	4.4 ± 1.7 c	1.7 ± 0.8 b	1.7 ± 0.9 bc
	T2	90 ±12.5 a	6.8 ± 1.2 a	3.9 ± 1.1 a	4.3 ± 1.6 a
	T3	87 ± 9.5 a	4.7 ± 1.1 c	1.2 ± 0.9 bc	1.5 ± 0.9 bc
	T4	92 ± 9.2 a	6.0 ± 1.5 b	1.6 ± 0.9 b	2.1 ± 1.0 ab
	T15	83 ± 13.4 a	5.3 ± 0.9 b	1.5 ± 0.8 ab	2.6 ± 0.9 ab
	T17	81 ± 17.3 a	3.2 ± 1.1 d	0.4 ± 0.2 c	0.6 ± 0.3 c
	T19	85 ± 11.8 a	5.2 ± 1.1 b	1.4 ± 1.1 bc	1.7 ± 1.2 bc

*Data presented as mean ± standard deviation. Same letters indicate no significant difference between treatments according to Kruskal-Wallis test followed by a Mann–Whitney post hoc test including a Bonferroni's correction ($p < 0.01$).

Table 3. Tomato seedling growth parameters four weeks after transplant to 50-mL pots of soil substrate treated with *Trichoderma* sp. isolates in two different inoculation experiments. Tomato seedling growth parameters four weeks after transplant to 50-mL pots of soil substrate treated with *Trichoderma* sp. isolates in the two independent replicates of the experiment*.

	Treatment	Yellowish leaves (%)	Δ height (mm)	Shoot dry weight (mg)	Root dry weight (mg)	Total dry weight (mg)	Steam diameter (cm)	Seedling health index
Experiment 1	Control	37.3 ± 21.9ab	14.0 ± 5.5bc	48.5 ± 15.0bc	56.4 ± 19.2a	105.0 ± 21.2a	2.1 ± 0.5cba	6.5 ± 1.6a
	T1	14.8 ± 21.7d	8.3 ± 4.2de	38.7 ± 10.7c	21.4 ± 4.1b	60.2 ± 12.9b	1.8 ± 0.2cba	3.9 ± 1.1b
	T3	20.8 ± 19.2cd	15.4 ± 5.3abc	55.6 ± 10.1ab	43.0 ± 15.2a	98.7 ± 18.6a	1.9 ± 0.3cba	5.4 ± 1.2a
	T4	23.1 ± 18.3bcd	8.4 ± 3.8e	42.5 ± 16.7c	25.6 ± 5.6b	68.0 ± 17.8b	1.7 ± 0.4cb	4.1 ± 1.4b
	T15	30.3 ± 19.8abc	12.2 ± 6.2cd	48.2 ± 15.3bc	21.9 ± 5.6b	70.0 ± 17.9b	1.6 ± 0.6c	3.5 ± 1.4b
	T17	41.2 ± 26.1a	17.5 ± 6.8ab	54.7 ± 15.7ab	43.7 ± 8.5a	98.5 ± 20.4a	2.2 ± 0.4a	5.7 ± 1.8a
	T19	19.7 ± 23.2cd	23.8 ± 13.7a	66.2 ± 20.6a	50.5 ± 12.9a	116.7 ± 22.8a	2.3 ± 0.4a	6.2 ± 1.1a
Experiment 2	Control	31.9 ± 18.7bc	8.4 ± 3.4abc	34.6 ± 9.3cd	46.4 ± 14.9ab	81.0 ± 19.6a	1.7 ± 0.3a	5.2 ± 1.2ab
	T1	21.2 ± 19.3cd	10.6 ± 6.9ab	48.9 ± 17.3a	38.5 ± 11.4b	87.5 ± 27.0a	1.5 ± 0.5a	4.2 ± 1.6bc
	T3	26.2 ± 16.3bcd	9.8 ± 4.7ab	41.3 ± 12.8abc	54.1 ± 14.4a	95.4 ± 23.3a	1.6 ± 0.4a	5.8 ± 1.9a
	T4	18.8 ± 23.1d	6.1 ± 2.8c	30.1 ± 8.5d	28.2 ± 8.6c	58.3 ± 12.0b	1.2 ± 0.3b	3.3 ± 0.9c
	T15	30.7 ± 13.6bcd	7.8 ± 3.2bc	37.7 ± 11.9bc	20.1 ± 5.1c	57.8 ± 15.7b	1.5 ± 0.3ba	3.5 ± 1.1c
	T17	48.5 ± 15.2a	8.4 ± 5.2bc	40.9 ± 14.1abc	41.9 ± 10.0b	82.9 ± 19.8a	1.6 ± 0.3a	7.2 ± 0.9a
	T19	36.8 ± 20.5ab	11.2 ± 4.6c	44.0 ± 10.6ab	43.6 ± 9.3ab	87.6 ± 17.1a	1.7 ± 0.3a	5.6 ± 1.5a

*Data presented as mean ± standard deviation. Δ height (mm) means the difference between the final height (after four weeks) and the height at the day of transplant. Data presented as media ± standard deviation. Same letters indicate no significant difference between treatments according to Kruskal-Wallis test followed by a Mann–Whitney post hoc test including a Bonferroni's correction ($p < 0.01$).

DISCUSSION

Literature regarding biological control is plenty of examples of *Trichoderma* sp. isolates related to beneficial effects on plants, mainly because of their antagonism against plant pathogens (Table 1, Figs. 2a and 2b; Hewedy et al. 2020). This study tested seven *Trichoderma* sp. isolates previously described as involved in the biological control of pathogens and promotion of growth in plants (not published data). The closest *Trichoderma* spp. identified in Fig. 1, *T. harzianum, Trichoderma simmonsii*, and *Trichoderma reesei*, are found in most commercial products based on *Trichoderma* spp. around the world (van Lenteren et al. 2017). Strains belonging to *Trichoderma longibrachiatum* species were already studied regarding antifungal activity and benefits to tomato plants. De Palma et al. (2016) identified molecular mechanisms activated during the in-vitro interaction between tomato and *T. longibrachiatum* MK1, stimulating plant growth and systemic resistance. Increased transcription of genes involved in defense, cell wall reinforcement, and reactive oxygen species signaling suggests tomato pathogen resistance induced by MK1 may occur through stimulation of these mechanisms.

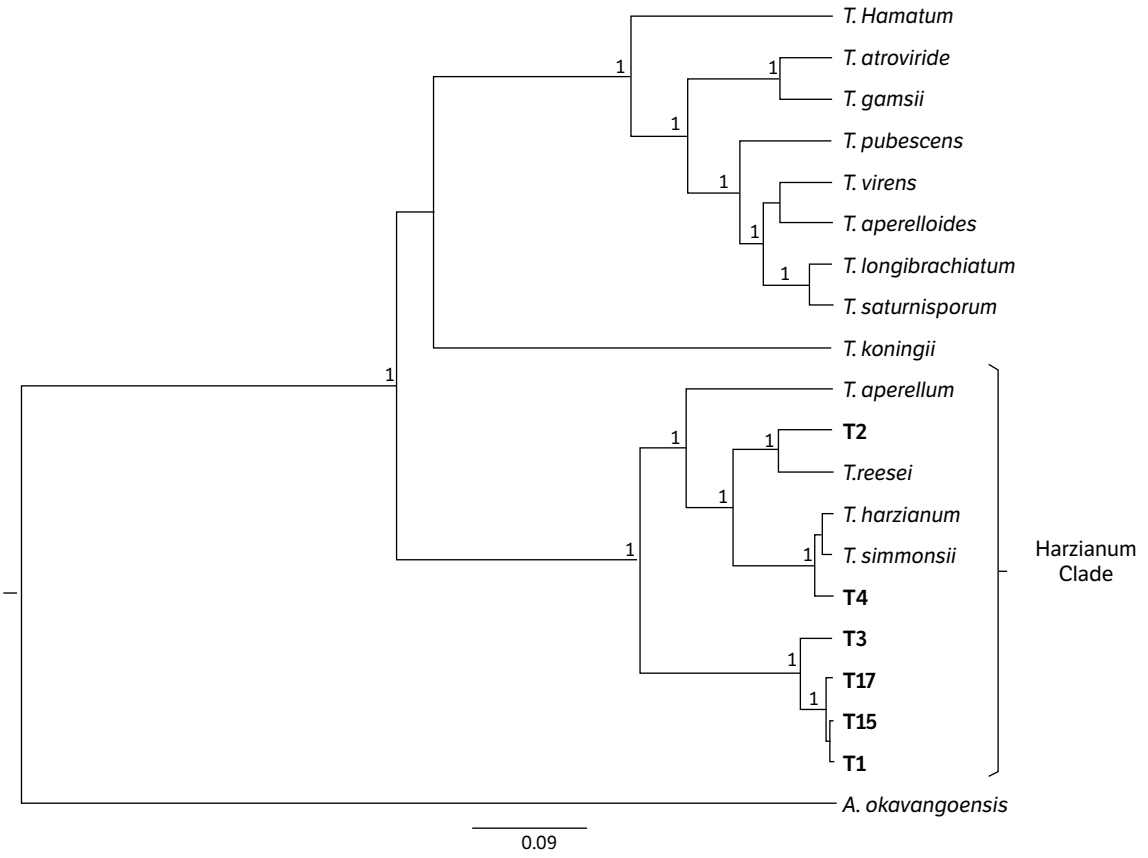

Figure 1. Phylogenetic tree of the seven new *Trichoderma* sp. isolates (T1, T2, T3, T4, T15, T17, and T19), and 19 reference strains inferred by Bayesian analysis using 533 bp of the ITS region. The significance of each branch is indicated at the nodes points by posterior probability ≥ 0.90. The scale unit represented below tree represent the number of nucleotide substitution in each branch.

However, three out of seven *Trichoderma* sp. isolates caused some detrimental interaction with tomato cv. Micro-Tom when interacting with the target plant (Figs. 2c and 2d). The capacity of microorganisms to promote plant growth has been related to the ability to solubilize nutrients, assist in plant hormonal balance, and the production and degradation of molecules related to ethylene metabolism (Gravel et al. 2007; Stewart and Hill 2014). Growth promotion is not a universal trait of all *Trichoderma* spp.; their plant inoculation can result in growth promotion, no effect at all, or cause detrimental effects on plants (Nieto-Jacobo et al. 2017). The beneficial effects on plants also depend on microbial concentration, plant type, developmental stage, the inoculation technique used, and the timing of the interaction (Rubio et al. 2017).

Tucci et al. (2011) evaluated the biocontrol effect *T. harzianum* T22 and *Trichoderma atroviride* P1 against *B. cinerea* 309 in five cultivars of tomato (lines Corbarino, M82, SM36, TA209, and of the wild *S. habrochaites* accession LA1777). These authors showed that the damage caused by *Botrytis cinerea* infection on tomato leaves could be limited by *Trichoderma* spp. rhizosphere colonization. However, several differences were identified among the five tested tomato lines. Lesion expansion was initially controlled by T22 in all tested lines. However, at later times, T22 controlled the pathogen infection only in TA209 and Corbarino.

This study used the *S. lycopersicum* cultivar Micro-Tom for *in-vivo* assessments. This miniature tomato cultivar has many advantages for molecular biology and plant physiology studies (Takahashi et al. 2005). Fiorini et al. (2016) showed that *T. harzianum* T6776 inoculation in tomato cv. Micro-Tom, seven days after sowing, could establish an endophytic relationship, alter the hormone balance, and promote the growth of seedlings. Masunaka et al. (2009) studied the potential of *Pythium oligandrum* MMR2 to suppress wilt caused by *Ralstonia solanacearum* 8242GFP in tomato cv. Micro-Tom. These authors showed that a movement of *R. solanacearum* was frequently observed in the xylem vessels of roots and stems of control plants (not inoculated).

A seed vigor test is a relevant indicator of seed quality, expressing its physiological potential. Seed vigor index was also employed to evaluate growth promotion caused by *Trichoderma* spp. inoculation in tomato seeds surface (You et al. 2016). These authors screened 72 *Trichoderma* sp. isolates, evaluating their potential to suppress *B. cinerea* growth, antifungal activity, and promote tomato seed germination. They identified isolates *T. harzianum* T-21 and T-68, and *Trichoderma koningiopsis* T-35 and T-51, which promoted tomato growth, and, on the other hand, T-21, T-51, and T-68 suppressed *B. cinerea* sporulation. *Trichoderma* sp. treatments did not affect the tomato germination rate in the present work. However, seed inoculation with *Trichoderma* sp. T17 reduced the radicles and the hypocotyl lengths, resulting in an SVI 4.7 times smaller than the control (Table 2, Fig. 2d).

Similar to this data, Ethur et al. (2008) did not find any improvement in the germination of tomato seeds treated with strains of *T. harzianum*, and all treatments caused the reduction of radicle length. In contrast, Srivastava et al. (2010) observed better germination rates and reduced time required for germination in tomato seeds treated with *T. harzianum* T35. Singh et al. (2016) showed that tomato seeds inoculated with *T. asperellum* BHUT8 did not improve germination, but the seedlings showed an increment in the radicle length and dry weight of root and shoot. The same authors claimed that the number of spores inoculated is crucial for a suitable plant growth response. For tomato seeds, these authors identified that the content of 1×10^3 conidia·mL^{-1} is ideal for improving radicle growth, and elevated spore contents (10^7–10^8 conidia·mL^{-1}) caused reduction in the radicle length and germination percentage.

Freshly germinated seedlings were cropped in a soil substrate and inoculated with the *Trichoderma* spp. isolates, and three of them (T1, T4, and T15) presented a decrease in development when compared to the untreated control (Table 2). *Trichoderma* spp. are described as producers of secondary metabolites that could be toxic to many plant pathogens and frequently to the plants (Vinale et al. 2008). For example, the peptaboil Trichokonin VI, produced by *T. longibrachiatum* SMF2 and described as a broad-spectrum antibiotic, was found to be the main responsible for inhibiting the growth of *Arabidopsis thaliana* roots through inhibition of cell division, cell elongation, and disturbing the auxin gradients at root tips (Shi et al. 2016). Similar data were observed by Vinale et al. (2008), showing that some Trichoderma spp. secondary metabolites could reduce the pea, wheat, and tomato growth.

Despite the adverse effects caused by strains T2 and T15 in soil inoculation experiments, these treatments presented highly promising results in seed inoculation experiments (Tables 2 and 3, Fig. 2d). Rejecting our hypothesis, the data presented in this work highlight that the inoculation way presented a strong influence on the plant growth effect. Environmental parameters influence the plant growth potential once different molecules are produced in soil and plates (Nieto-Jacobo et al. 2017). Howell and Puckhaber (2005) reported that some strains of *Trichoderma virens* could either act as biocontrol agents or pathogenic to cotton seedlings, depending on their capacity to induce phytoalexin synthesis on the roots. Spore content in inoculants can also change plant growth effect.

It was also reported that, using vermiculite as substrate, *Trichoderma* spp. could compete for nutrients (as nitrogen) with the plant seedling, causing worse development, yellowish, or even death of seedlings (Marín-Guirao et al. 2016). About 30% of leaves became yellowish at the end of four weeks in most treatments, probably due to the depletion of nutrients in the reduced substrate volume. However, inoculation of *Trichoderma* sp. T1, T3, T4, and T19 showed fewer yellow leaves than control, even though they presented a decrease in the root and total weight. This data may reveal a strategy of slowing down the growth and retarding the senescence (Rubio et al. 2017).

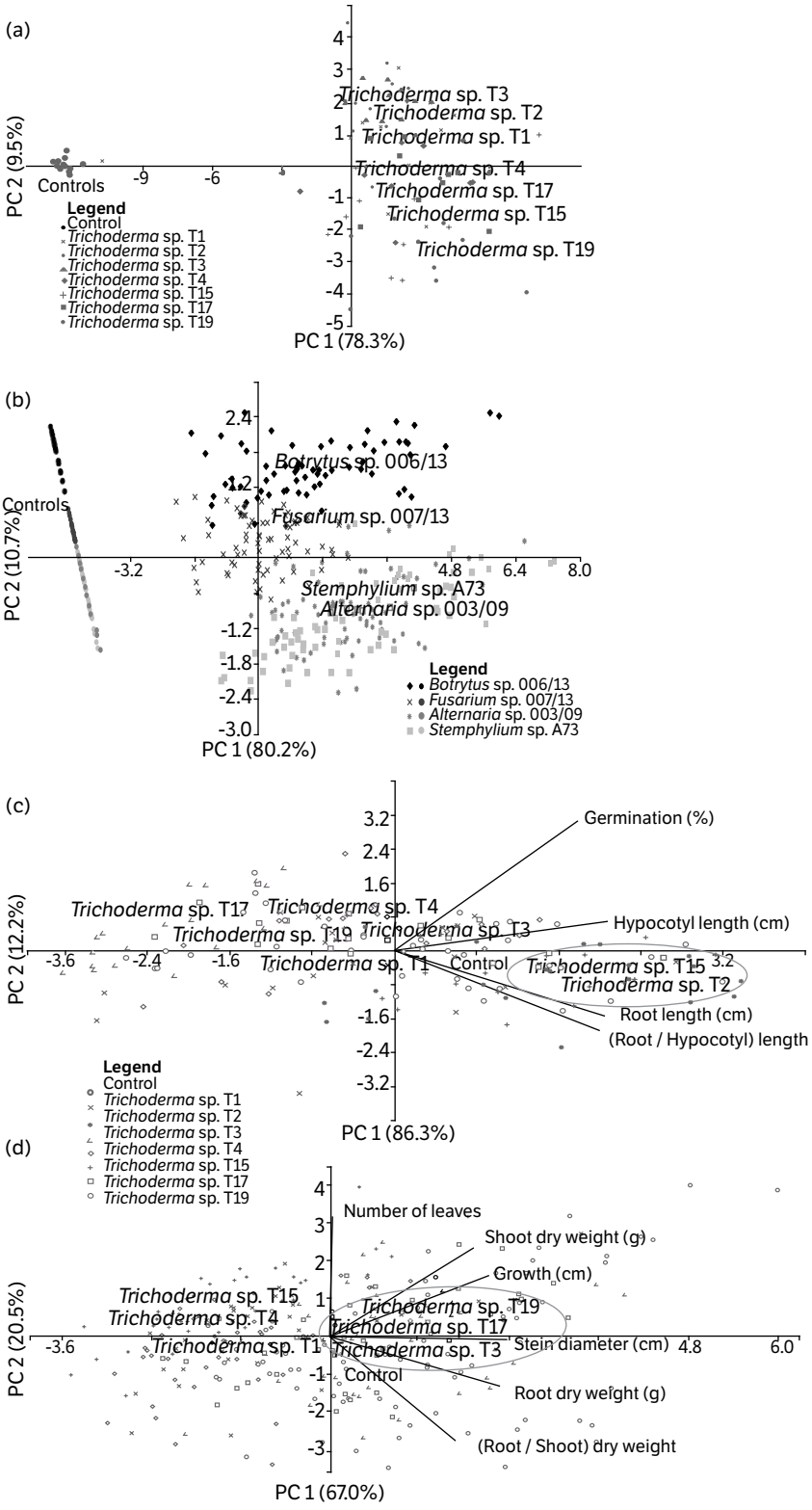

Figure 2. Principal component analysis of the antagonistic activity (dual culture, diffusible, and volatile compounds tests), determined from: (a) the seven new *Trichoderma* isolates (T1, T2, T3, T4, T15, T17, and T19); (b) the four pathogens studied (*Alternaria* sp. 003-09, *Botrytis* sp. 006/13, *Fusarium* sp. 007/09, and *Stemphylium* sp. A73); (c) seed inoculation experiment (percentage of germination, hypocotyl and root lengths, and rate of root / hypocotyl); (d) soil inoculation experiment (number of leaves, shoot and root dry weights, plant growth, steam diameter, and rate of root / shoot dry weights) for the seven new *Trichoderma* isolates (T1, T2, T3, T4, T15, T17, and T19). Most promising treatments are highlighted in a gray ellipse.

CONCLUSION

This work showed the antagonistic effect of seven *Trichoderma* sp. isolates against four tomato pathogens, showing that all can control mycelial growth, highlighting strains T17 and T19, which presented the most promising results. Furthermore, seed and soil inoculation evaluated the influence on tomato growth of the new *Trichoderma* sp., disclosing very contrasting results. Unlike most of the literature about *Trichoderma* spp., these assays described isolates that decrease the development and health of tomato plants. Among the isolates of *Trichoderma* spp. tested, T17 and T19 presented the potential to be inoculated in soil, and *Trichoderma* sp. T15 in seed. More studies about fruit productivity and economic viability of *Trichoderma* inoculation should be performed.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Schwambach, J., Granada, C. and Sandri, M. R. **Investigation:** Sandri, M. R., Cavião, H. C., Oliveira, C. F., Andrade, L. B., Schwambach, J. and Granada, C. **Methodology:** Sandri, M. R., Cavião, H. C., Oliveira, C. F. and Andrade, L. B. **Supervision:** Schwambach, J. **Writing – original draft:** Schwambach, J. and Granada, C. **Writing – review & editing:** Schwambach, J. and Granada, C.

DATA AVAILABILITY STATEMENT

Data may be made available upon request to the corresponding author.

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SUPPLEMENTARY MATERIAL

Table S1. Identification of pathogenic fungi species evaluated.

Isolate	Closest Type Strain	Length of ITS fragment	Similarity	GenBank Acession Number	Tomato tissue font
Alternaria sp. 003-09	*Alternaria angustiovoidea* CBS 195.86	512 bp	99%	OL869125	Foliar lesion
Botrytis sp. 006/13	*Botrytis californica* WSP 72753	479 bp	98%	OL869124	Tomato fruit
Fusarium sp. 007/09	*Fusarium foetens* CBS 110286	475 bp	98%	OL869122	Stem
Stemphylium sp. A73	*Stemphylium gracilariae* CBS 482.90	520 pb	98%	OL869123	Foliar lesion