

# Inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* by isolates of *Trichoderma* spp.

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## Abstract

Tomato crops have great economic importance in Brazil and worldwide; however, its economic return is dependent on the control of several diseases, including *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici*. The control of this disease is usually based on biological products, which are commonly manufactured with antagonist isolates of *Trichoderma* sp. Therefore, the antagonistic potential of *Trichoderma* sp. should be better studied against other phytopathogens, thus expanding the biofungicide options on the market. The objective of the present work was to evaluate the antagonistic potential of different isolates of *Trichoderma* against the in vitro mycelial growth of *F. oxysporum* f. sp. *lycopersici*. Four isolates of *Trichoderma* spp. were tested, three collected in litterfall and one commercial isolate (*T. asperellum*). The *Trichoderma* spp. isolates were evaluated for antagonistic potential against *F. oxysporum* f. sp. *lycopersici* using the culture pairing technique. The variables evaluated were: mean colony size (MCS), mycelial growth inhibition percentage (MGIP), mycelial growth rate index (MGRI) of phytopathogen, and antagonism of isolates of *Trichoderma* spp. through a scale of grades. All isolates of *Trichoderma* spp. tested reduced the in vitro mycelial growth of *F. oxysporum* f. sp. *lycopersici*.

**Keywords:** Antagonism. *Fusarium* wilt. Biological control.

## Introduction

Brazil is the tenth main tomato producing country, responsible for approximately 2.2 % of tomatoes produced worldwide, after China, India, Turkey, and the United States of America, that provides approximately 34.7 %, 10.5 %, 7.1 %, and 6.0 % of the world tomato production, respectively (FOOD AND AGRICULTURE ORGANIZATION CORPORATE STATISTICAL DATABASE – FAOSTAT, 2019).

The tomato production in Brazil in 2019 reached 3.92 million Mg, which were grown in an area of 54,540 ha, mainly in the states of Goiás, São Paulo, Minas Gerais, and Bahia, responsible for approximately 72 % of the national production (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE, 2020).

Tomato crops can be affected by several diseases during the plant cycle. Root pathogens are

among the most important causes of phytosanitary problems, since they present resistance structures that ensure their survival under unfavorable conditions (WONG; AMBROSIO; SOUZA, 2011).

Soil fungi are among the root pathogens, which are characterized by surviving for years under absence of a susceptible host (LOPES; MICHEREFF, 2018). According to Correia and Michereff (2018), among soil fungi, the ones that cause vascular wilting stand out, such as *Fusarium oxysporum*, *Verticillium albo-atrum*, and *Verticillium dahliae*.

*Fusarium* wilt is among the diseases that most worry tomato growers. It is caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici*, and is present in practically all areas where tomato is grown (INOUE-NAGATA *et al.*, 2016). This pathogen survives as mycelium or chlamydospores, commonly found in cultural

residues. The pathogen can also develop in tissues of several other plant species, that act as alternative hosts (BEBENDO, 2018).

According to Inoue-Nagata *et al.* (2016), the survival of the pathogen under absence of the host can be up to eight years, making it difficult to deploy management actions to reduce the initial inoculum (MELO; SERRA; NASCIMENTO, 2021). The spread of the pathogen over long distances occurs due to the use of contaminated seedlings and seeds and, in crop areas where the pathogen is present, it can spread due to the moving of propagules in soil particles, caused by plowing and harrowing, irrigation, or even rainwater (BEBENDO, 2018).

The main symptoms of the disease in tomato crops range from leaf yellowing to progressive wilting of the aerial part, which can cause the death of the plants by drying them out completely. Usually, the infection occurs unilaterally, by reaching the vascular bundles from infected root tissues. Plants that have a poorly developed root system are generally more attacked by the fungus, contributing to the progressive vigor loss and shortening of the crop cycle. The disease usually manifests in the field in spots, mainly during the growth, flowering, and fruit maturation phases. In the nursery stage, or shortly after transplanting, seedlings affected by the disease may undergo lodging or damping-off (TÖFOLI; DOMINGUES, 2018).

The control of the disease caused by *Fusarium oxysporum* can be carried out through cultural measures and practices involving adequate and balanced plant nutrition, liming, use of resistant cultivars, crop rotation, and deep plowing (INOUE-NAGATA *et al.*, 2016; MIELNICZUK; SKWARYŃO-BEDNARZ, 2020). Biological control, characterized by the use of non-pathogenic organisms on the infection site to limit the pathogen action or increase the host resistance, has also been used (TÖFOLI; DOMINGUES, 2018).

The use of biological methods to control plant diseases has grown in recent years. There has been a significant increase in products made from microorganisms approved for different cultures (MEYER; MAZARO; SILVA, 2019). In Brazil, this agribusiness segment represents 3 % to 5 % of sales of chemical products (VIEIRA *et al.*, 2016). Currently, the use of biological methods is an alternative that is not used only by agroecological-based farmers. Conventional farmers are the main consumers of biological products, as they minimize damages to the environment and human health; in addition, farmers are having a greater perception of the need to diversify tools for disease management (MEDEIROS; SILVA; PASCHOLATI, 2018).

In 2019 alone, 21 *Trichoderma*-based biofungicides were approved by the Brazilian Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA), 66 % based on *Trichoderma harzianum*, 24 % based on *Trichoderma asperellum*, 5 % based on *Trichoderma koningiopsis*, and 5 % based on *Trichoderma stromaticum* (MEYER; MAZARO; SILVA, 2019).

According to Dalacosta (2019), *Trichoderma* species have rapid growth in different media types, facilitating their mass production; thus, they have been used for biological control of fungal diseases, mainly for soil fungi, such as *Fusarium oxysporum*. The different fungus species can interfere with the phytopathogen life through different mechanisms of action, such as competition for space and nutrients, antibiosis, mycoparasitism, fungistasis, and induction of resistance, besides stimulating plants to increase their tolerance to natural stresses (INFANTE *et al.*, 2011; MASTOURI; BJÖRKMANN; HARMAN, 2012).

Töfoli and Domingues (2018) stated that when the fungus is applied via soil or in the substrate, in the case of seedling production, it significantly reduces the occurrence and severity

of diseases caused by pathogens of the genera *Fusarium*, *Sclerotium*, *Sclerotinia*, *Verticillium*, *Pythium*, and *Phytophthora*.

Therefore, the evaluation of new *Trichoderma* isolates with antagonist potential against different phytopathogens may enable the obtaining of new biofungicides to increase the supply of biocontrol products.

Thus, the objective of the present work was to evaluate the antagonistic potential of different isolates of *Trichoderma* against the in vitro mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*.

## Material and methods

The experiment was conducted at the Phytopathology Laboratory of the Federal Institute of Education, Science, and Technology of the South of Minas Gerais, Inconfidentes campus.

The isolate of *Fusarium oxysporum* f. sp. *lycopersici* used in the experiment was obtained from the mycoteca of the Sakata Company, and the isolates of *Trichoderma* spp. ( $I_3$ ,  $I_4$ ,  $I_5$ ) were obtained by Garcia (2020) from litterfall in the Olericulture Sector of the School Farm of the IFSULDEMINAS, Inconfidentes campus, and were stored in the Phytopathology Laboratory of the Inconfidentes campus. It was also used an isolate of the species *Trichoderma asperellum*, which was obtained from a commercial product (Quality®).

The antagonistic potential of the different isolates of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici* was evaluated by the technique of direct confrontation through the pairing of colonies, according to the methodology proposed by Dennis and Webster (1971). Disks of 10 mm diameter with samples obtained from colonies of the phytopathogen and isolates of the antagonist were grown for 7 days in potato-dextrose-agar (PDA) medium and incubated in a

BOD chamber at 25 °C and photoperiod of 12 hours. Petri dishes of 90 mm diameter containing 20 mL of PDA medium (pH 5.7) were used to set up the experiment. *Fusarium oxysporum* f. sp. *lycopersici* were transferred to the surface of the culture medium on Petri dishes, at 0.5 cm from the edge of the plate, and the plates were incubated in a BOD chamber at 25 °C and photoperiod of 12 hours for 48 hours, according to the methodology proposed by Dennis and Webster (1971). After the mycelial growth of *F. oxysporum* f. sp. *lycopersici* for 48 hours, colony disks of the isolates were picked on opposite sides of the Petri dishes, at 0.5 cm from the edge of the plates. Petri dishes containing paired phytopathogen and antagonist colonies were incubated in a BOD chamber at a temperature of 25 °C and a photoperiod of 12 hours during the experiment.

Each isolate of *Trichoderma* spp. was paired with a phytopathogen, and only the phytopathogen in the Petri dish without the antagonist was considered as a control, totaling 5 treatments, namely: T1 – *Fusarium oxysporum* f. sp. *lycopersici* × isolate 3 (I3); T2 – *Fusarium oxysporum* f. sp. *lycopersici* × isolate 4 (I4); T3 – *Fusarium oxysporum* f. sp. *lycopersici* × isolate 5 (I5); T4 – *Fusarium oxysporum* f. sp. *lycopersici* × *T. asperellum*; T5 – *Fusarium oxysporum* f. sp. *lycopersici*. A randomized block experimental design was used, with 7 replications; each plate was considered as a plot.

Daily evaluations started 24 hours after the subculture of *Fusarium oxysporum* f. sp. *lycopersici* for 14 days. Daily measurements of the frontal mycelial growth of the phytopathogen and antagonist colonies were made with the aid of a digital caliper. The mycelial growth inhibition percentage (MGIP) was determined on the 7<sup>th</sup> day and 14<sup>th</sup> day after incubation and calculated based on the formula described by Menten *et al.* (1976):

$$\text{MGIP} = \frac{\text{mean size of control} - \text{mean size of treatment}}{\text{mean size of control}} \times 100$$

Mean colony diameter (mm) and mycelial growth rate index (MGRI; mm day<sup>-1</sup>) were also determined, using the formula described by Oliveira and Machado (1991):

$$MGRI = \frac{\sum (S - Sa)}{N}$$

At which:

S = current mean colony size;

Sa = mean colony size on the previous day;

N = number of days after subculture.

The antagonism of the isolates of *Trichodermas* pp. was evaluated using an adaptation of the scale of grades proposed by Bell, Wells and Markhan (1982): 1 – the antagonist grows completely over the pathogen and occupies the entire Petri dish; 2 – the antagonist grows on at least 2/3 of the Petri dish, overlapping the pathogen; 2.5 – the antagonist grows for at least 2/3 of the Petri dish, but does not overlap the pathogen; 3 – antagonist and pathogen grow over half of the plate; 4 – the pathogen grows on least 2/3 of the Petri dish; 5 – the pathogen grows completely throughout the Petri dish.

The antagonism of the isolates was also determined on the 7<sup>th</sup> and 14<sup>th</sup> day of incubation.

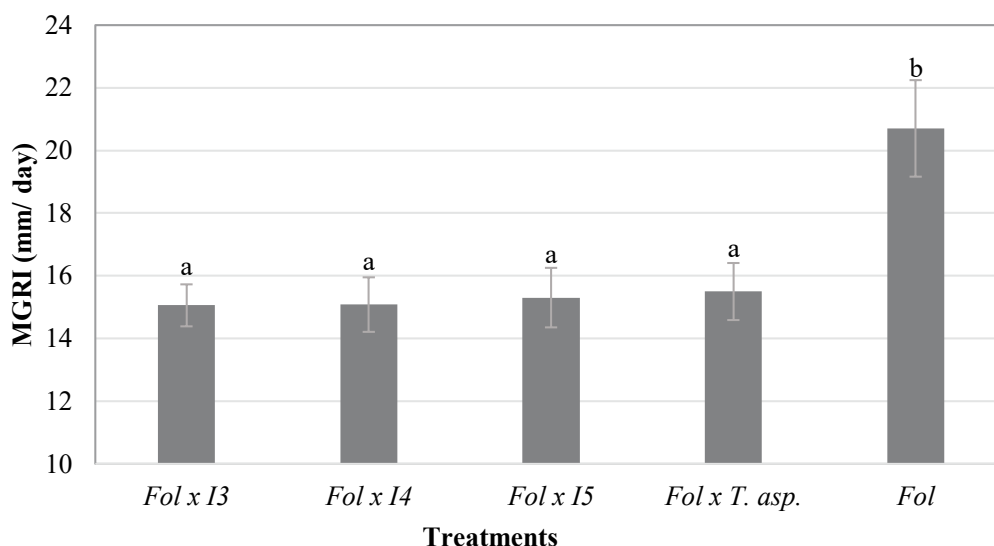
The data obtained in the experiment were subjected to analysis of variance, and the means were compared by the Scott-Knott test at 1 % probability, using the Sisvar 5.6 program (FERREIRA, 2011).

## Results and discussion

The evaluated isolates of *Trichoderma* (I3, I4, I5, and *T. asperellum*) showed a significant antagonistic effect on the mycelial growth rate index (MGRI) when compared to the control, with no difference between them, even though they were transferred to the Petri dishes 48 hours after the transfer of the pathogen (FIGURE 1). Mycelial growth rate is important for biological control, since it is connected to the speed of colonization of the substrate under competition with the phytopathogen (MEDEIROS; SILVA; PASCHOLATI, 2018).

The pathogen MGRI was 15.06 mm, 15.08 mm, 15.30 mm, and 15.50 mm in the presence of

**Figure 1.** Mycelial growth rate index (MGRI; mm day<sup>-1</sup>) of *F. oxysporum* f. sp. *lycopersici* (Fol) in the presence of *Trichoderma* spp. (I3, I4, I5 and *Trichoderma asperellum*). Inconfidentes, MG, Brazil, 2021



Bars with the same letters are not statistically different from each other by the Scott-Knott test at 1 % probability.

**Source:** Elaborated by the authors (2021).

isolates I3, I4, I5, and *T. asperellum*, respectively, while the control without the presence of the antagonist had a MGRI of 20.07 mm dia<sup>-1</sup>, which shows that the presence of the isolates inhibited the pathogen mycelial growth. The mean mycelial growth inhibition percentage of *F. oxysporum* f. sp. *lycopersici* by the isolates was 24%.

Sousa *et al.* (2017) found potential in vitro control of *R. solani* for 7 of the 9 isolates of *Trichoderma* evaluated, on average, 33% of the studied isolates reduced the pathogen MGRI in 40%, and 44% reduced the MGRI in 25%. Sá *et al.* (2019) and Soares *et al.* (2019) confirm these results in studies with *Trichoderma* spp. with reduction in mycelial growth of *Fusarium* sp. and *Fusarium solani*, which were causing damages to cowpea and watermelon crops, respectively.

The isolates of *Trichoderma* spp. showed significant differences in colony size and MGIP on the 7<sup>th</sup> and 14<sup>th</sup> day after the fungi incubation when compared to the control, with no statistical difference between the isolates in both evaluations. The isolates I3, I4, I5 and *T. asperellum* inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* on the 7<sup>th</sup> day of evaluation in 40 %, 48 %, 42 %, and 42 %, and on the 14<sup>th</sup> day in 62%, 67%, 64%, and 64%, respectively (TABLE 1).

The evaluations in the 7<sup>th</sup> and 14<sup>th</sup> day after incubation showed no increases in colony size of *F. oxysporum* f. sp. *lycopersici* in the presence of the isolates, and the isolates did not differ from each other for the evaluated parameter. This result can be explained by the frontal encounter of the pathogen colonies with the antagonist colonies, denoting its efficiency in inhibiting the pathogen mycelial growth. The reduction in mycelial growth of *F. oxysporum* f. sp. *lycopersici* may also be connected to the release of metabolites, competition for nutrients in the culture medium, and mycoparasitism by the antagonist (MATOS *et al.*, 2014). In addition, each *Trichoderma* species has its own mechanism of action to interact with each type of phytopathogen (RIBEIRO, 2017).

The MGIP increased over the days due to the continuous growth of *F. oxysporum* f. sp. *lycopersici* without the presence of the antagonist. The lowest MGIP of the isolates, compared to the control, was 40 % on the 7<sup>th</sup> day and 62 % on the 14<sup>th</sup> day of evaluation. These values were higher than those found by Fantinel *et al.* (2018), who evaluated the antagonistic potential of different isolates of *Trichoderma* and *Bacillus thuringiensis* against *Colletotrichum siamense* and found maximum MGIP of 32 % and 44.2 % on the 6<sup>th</sup> and 12<sup>th</sup> days, respectively. Hoffmann *et al.* (2015) found

**Table 1.** Mean colony size (MCS) and mycelial growth inhibition percentage (MGIP) of *F. oxysporum* f. sp. *lycopersici* (Fol) by *Trichoderma* spp. (I3, I4, I5, and *Trichoderma asperellum*) on the 7<sup>th</sup> and 14<sup>th</sup> day of incubation. Inconfidentes, MG, Brazil, 2021

Treatments	MCS (mm)		MGIP (%)	
	Day 7	Day 14	Day 7	Day 14
Fol × I3	23.18 a	23.18 a	40 a	62 a
Fol × I4	19.97 a	19.97 a	48 a	67 a
Fol × I5	22.22 a	22.22 a	1.42 a	57 a
Fol × <i>T. asperellum</i>	22.31 a	22.31 a	1.42 a	57 a
Control	38.77 b	61.66 b	0.30 b	0.30 b
CV (%)	11.43	10.53	20.18	8.38

Means followed by the same letter by in the rows are not statistically different by the Scott-Knott test at 1 % probability.

**Source:** From the author (2021)



that 3 of the 15 evaluated isolates had MGIP of 38.8 %, 44.4 %, and 48.9 % on the 7<sup>th</sup> day of pairing, and 12 isolates showed high antagonistic potential, completely inhibiting the development of *Fusarium* sp., with growth inhibition percentages between 81.2 % and 94.4 %.

According to the Bell, Wells, and Markham scale (1982), all treatments with antagonist species differed statistically from the control on the 7<sup>th</sup> day, but did not differ from each other. At the end of the experiment, the treatments that showed the greatest antagonism were those with I3, I4, and *T. asperellum* (FIGURE 2). Therefore, the evaluation of antagonism using the scale of grades enabled to differentiate the isolates only after 14 days of incubation.

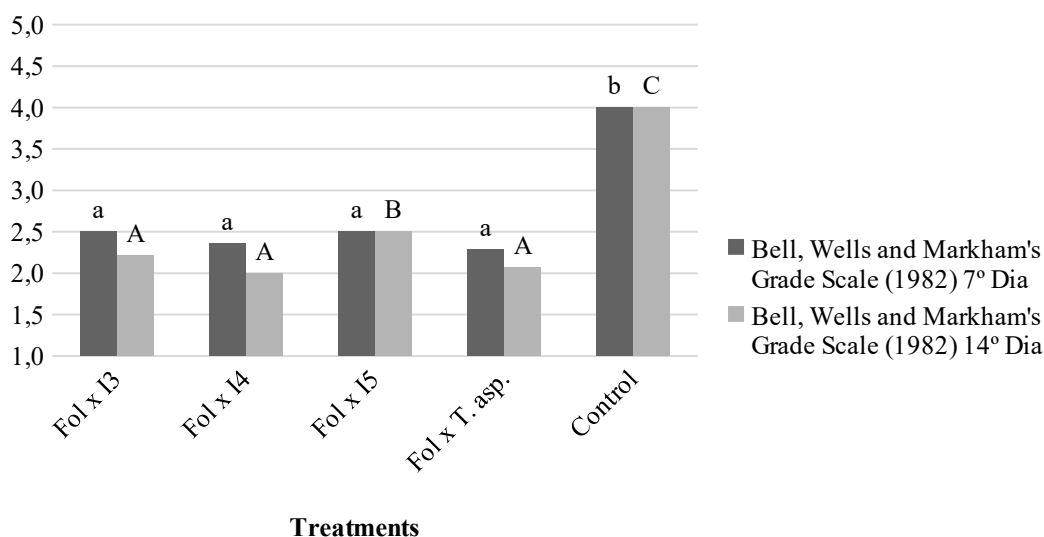
The isolate I5 grew over at least 2/3 of the plate in all replications, frontally meeting with the *F. oxysporum* f. sp. *lycopersici* colony, without colony overlap, which means it is classified as a 2.5 grade. The results showed that I5 can inhibit the pathogen growth, since it differed statistically from the control; however, it was not as efficient as I3, I4, and *T. asperellum*, which were graded as 2. These isolates had mycelial growth that

overlapped the pathogen colony, indicating a probable mycoparasitism of *F. oxysporum* f. sp. *lycopersici* hyphae by the hyphae of the isolates (I3, I4, and *T. asperellum*) of *Trichoderma* spp. According to Meyer, Mazaro, and Silva (2019), mycoparasitism is one of the most relevant characteristics of the *Trichoderma* genus.

Contrastingly, Milanese *et al.* (2013) found two isolates of *T. tomentosum* with grade 1 for antagonism of isolates of *Fusarium oxysporum*, i.e., in vitro isolates of *T. tomentosum* grew completely over the colonies of *Fusarium oxysporum*, denoting the potential for biocontrol of these species.

Although the results were significant for vitro inhibition of *F. oxysporum* f. sp. *lycopersici* by the tested isolates of *Trichoderma*, it is not possible to affirm that they would be efficient in vivo. Grigoletti Júnior, Santos, and Auer (2000) pointed out that there are some limitations in the conduction of in vitro tests for the evaluation of the antagonistic potential of a fungus, since in most cases the results obtained under controlled tests do not match or are sometimes different from those obtained in the field or in greenhouses.

**Figure 2.** Mean grades attributed to the paired cultivation of *F. oxysporum* f. sp. *lycopersici* (Fol) with different isolates of *Trichoderma* spp. (I3, I4, I5, and *Trichoderma asperellum*) on the 7<sup>th</sup> and 14<sup>th</sup> day of evaluation



Bars with the same letters are not statistically different from each other by the Scott-Knott test at 1 % probability.

Source: Elaborated by the authors (2021).

Therefore, the conduction of in-vivo evaluations of the isolates evaluated in this work is important to confirm their antagonistic potential against *F. oxysporum* f. sp. *lycopersici*.

## Conclusion

All isolates of *Trichoderma* spp. tested reduced the in vitro mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*.

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