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Evaluation of the sensitivity of *Colletotrichum* spp. to *Trichoderma harzianum*

Avaliação da sensibilidade de *Colletotrichum* spp. a *Trichoderma harzianum*

SILVA, Jackeline Laurentino da[1]; Costa, Jaqueline Figueredo de Oliveira[2]; RAMIREZ, Cecilia Hernandez[3]; SILVA, Maria Jussara dos Santos da [4]; SANTOS, Maria Hilma dos[5]; LIMA, Gaus Silvestre de Andrade[6]; ASSUNÇÂO, Iraildes Pereira[7].

[1] 0000-0003-4222-0805; Universidade Federal de Alagoas (UFAL)/Doutora do curso de Pós-graduação em Proteção de Plantas do Campus de Engenharias e Ciências Agrárias. Rio Largo, Alagoas (AL), BRASIL. jackeline.laurentino@outlook.com;
[2] 0000-0003-0121-699X; Universidade Federal de Alagoas (UFAL), Pesquisadora do Campus de Engenharias e Ciências Agrárias. Rio Largo, Alagoas (AL), BRASIL. jaquelinefigueredo@hotmail.com;
[3] 0000-0002-8639-0470; Universidade Federal de Alagoas (UFAL)/Doutoranda do curso de Pós-graduação em Proteção de Plantas do Campus de Engenharias e Ciências Agrárias. Rio Largo, Alagoas (AL), BRASIL. cecih967@gmail.com;
[4] 0000-0001-9418-854X; Universidade Federal de Alagoas (UFAL)/Doutora do curso de Pós-graduação em Proteção de Plantas do Campus de Engenharias e Ciências Agrárias. Rio Largo, Alagoas (AL), BRASIL. maria_jussara@hotmail.com.br;
[5] 0000-0001-8592-6977; Universidade Federal de Alagoas (UFAL)/Mestranda do curso de Pós-graduação em Proteção de Plantas do Campus de Engenharias e Ciências Agrárias. Rio Largo, Alagoas (AL), BRASIL. hilma2050@gmail.com;
[6] 0000-0003-2910-5896; Universidade Federal de Alagoas (UFAL), Prof. Dr. do Campus de Engenharias e Ciências Agrárias. Rio Largo, Alagoas (AL), BRASIL. gaus@ceca.ufal.br.
[7] 0000-0001-5087-0168; Universidade Federal de Alagoas (UFAL), Profª Drª do Campus de Engenharias e Ciências Agrárias. Rio Largo, Alagoas (AL), BRASIL. iraildes.assuncao@ceca.ufal.br;

[1]Setor de Fitossanidade/Campus de Engenharias e Ciências Agrárias, Universidade Federal de Alagoas, Rio Largo, AL, Brazil, 57100-000

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ABSTRACT

The objective of this study was to evaluate *in vitro* the effects of *T. harzianum* on five species of *Colletotrichum*. For the pairing test, *Colletotrichum* spp. was inoculated in synthetic PDA medium and after 72 hours the antagonist *T. harzianum* was added, in the opposite direction to the phytopathogens. To evaluate the action of metabolites, *Colletotrichum* species were inoculated in the center of Petri dishes, separately and after 48 hours *T. harzianum* was inoculated in plates containing synthetic PDA medium. The bases of the plates containing antagonist and phytopathogens were joined and sealed. The mycelial growth index (MGI) and the percentage of mycelial growth inhibition (PMGI) were estimated. When evaluating the antagonism of *T. harzianum* to *Colletotrichum* spp. in the pairing test and volatile metabolites, it was observed that all *Colletotrichum* species presented PMGI below 31% in both tests. In the MGI for the matching test, it was observed that *C. tropicale* was the most sensitive species (1.87 cm) at the concentrations of 1 and 1.5 L/ha, while in the volatile metabolites test it was *C. plurivorum* (3.21 cm). *T. harzianum* is a promising species for the reduction of *Colletotrichum* spp. associated with anthracnose in passion fruit crops.

RESUMO

O objetivo do trabalho foi avaliar *in vitro* os efeitos de *T. harzianum* sobre cinco espécies de *Colletotrichum*. Para o teste de pareamento foram inoculadas as *Colletotrichum* spp. em meio BDA sintético e após 72 horas foi adicionado o antagonista *T. harzianum*, na direção oposta aos fitopatógenos. Para avaliação da ação de metabólitos foram inoculadas as espécies de *Colletotrichum* no centro de placas de Petri, separadamente e após 48 horas foi inoculado *T. harzianum* em placas contendo meio BDA sintético. As bases das placas contendo antagonista e os fitopatógeno foram unidas e vedadas. Foram estimados o índice de crescimento micelial (IVCM) e a porcentagem de inibição de crescimento micelial (PIC). Ao avaliar o antagonismo de *T. harzianum* sobre *Colletotrichum* spp. no teste de pareamento e metabólitos voláteis observou-se que todas as espécies de *Colletotrichum* apresentaram PIC abaixo de 31%, em ambos os testes. No IVCM para o teste de pareamento observa-se que *C. tropicale* foi a espécie mais sensível (1,87 cm) nas concentrações de 1 e 1,5 L/ha, enquanto no teste de metabólitos voláteis foi *C. plurivorum* (3,21 cm). *T. harzianum* é uma espécie promissora para a redução de *Colletotrichum* spp. associadas a antracnose na cultura do maracujazeiro.

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Introduction

Brazil is one of the main food producing countries in the world. Due to the adoption of technologies, productivity has been increasing and the management of arable land is being optimized (Brazilian Institute of Geography and Statistics, 2022). Among crops of economic importance, passion fruit stands out, mainly because it is a promising alternative, with great economic return, which also plays a socioeconomic role of great relevance for small and medium producers (Meletti, 2011; Empresa Brasileira de Pesquisa Agropecuária, 2023).

The national production of passion fruit crops presents some obstacles, such as phytosanitary problems caused by phytopathogens (São José, 2015), with the genus *Colletotrichum* being the main causative agent of anthracnose disease (Dean et al., 2012). In recent years, there has been a search for the diversification of tools in the integrated management of diseases and quality products (Medeiros et al., 2018; Meyer et al., 2019).

Society's constant concern with food contamination and environmental problems, in addition to several barriers in the export market due to the use of various chemical molecules, has opened up market segments for the use of new forms of control, such as, for example, biological management (Morandi et al., 2014). According to the Brazilian Association of Biological Control Companies (ABCBio, 2021), the biocontrol industry is growing 5.3 times faster than the chemical pesticide industry. The Ministry of Agriculture and Livestock (MAPA, 2021) registered a record of 95 low-impact pesticides. Of these, fifteen pesticides are composed of species of the genus *Trichoderma*. A survey of products recommended for use in biological control, carried out by Bettiol et al. (2012), showed that more than 40 antagonist species were used to control diseases, with the genus Trichoderma responsible for almost 50%.

Several Trichoderma-based products are released, however, the *T. harzianum* species is the most commercialized, with around 38.8% of unmixed products, reaching 50% of the products available on the market. When there is a mixture of *Trichoderma* species, together with other genera of fungi, bacteria and mycorrhizae, this percentage increases, reaching 60% of biological control products released worldwide (Bettiol et al., 2019).

Species of the genus *Trichoderma* are anamorphic fungi. They have yellow or greenish sporulation and control pathogens in aerial parts, seeds and roots through the production of enzymes that degrade the cell wall (Morandi et al., 2009) and secondary metabolites responsible for antifungal activity in a wide number of fungal genera (Hermosa et al., 2014), in addition to being used as a plant growth-promoting fungus (Medeiros et al., 2018).

In view of satisfactory results in the control of several phytopathogens that cause diseases in crops of economic importance, the objective of this study was to evaluate in vitro the antagonistic effect of *Trichoderma harzianum* as a biocontrol of *Colletotrichum* spp. associated with passion fruit anthracnose.

Material and methods

Location of the experiment, obtaining Colletotrichum *species and maracuja seedlings*

The work was carried out at the Agricultural Engineering and Sciences *Campus* (CECA) of the Federal University of Alagoas, in Rio Largo (AL). Experiments were conducted in the phytosanitary clinic.

Representative isolates of five species of *Colletotrichum*, originating from the anthracnose disease in passion fruit crops, were obtained from the Phytopathogen Collection of the Federal University of Alagoas (COUFAL). Isolates were characterized by Baysean Inference, based on multi-locus analyses, using the genes glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), chitin synthetase (*CHS-1*), β – tubulin (*TUB2*) and internal transcribed space region (ITS), by Silva et al. (2022), and their partial sequences were deposited in GenBank, with the codes described in Table 1.

Table 1.

Colletotrichum isolates

SPECIES	CODES	GenBank accession numbers			
		GAPDH	***ITS***	***TUB2***	***CHS-1***
C. brevisporum	***COUFAL0271**	MT299329	MT334686	MT310545	MT314425
C. plurivorum	**COUFAL0275**	MT299333	MT334689	MT310548	-
C. fructicola	**COUFAL0279**	MT299337	MT334692	MT310551	-
C. theobromicola	**COUFAL0280**	MT299338	MT334693	MT310552	-
C. tropicale	**COUFAL0282**	MT299340	MT334695	MT310554	-

** COUFAL: Phytopathogen Collection of the Federal University of Alagoas.*

Source: Authors (2020).

Mycelial growth of Colletotrichum *and* Trichoderma harzianum *species*

The mycelial growth of *Colletotrichum* and *T. harzianum* species was evaluated, obtained from the commercial product Trichodermil SC 1306/KOPPERT, at concentrations 0.5; 1.0; and 1.5 L/ha, recommended by the manufacturer for soybean stem rot (*Sclerotinia sclerotiorum*) and pineapple rot (*Thielaviopsis paradoxa*). For this, sterilized filter paper discs (Ø 5 mm) were immersed for 30 seconds in the *Colletotrichum* spore suspensions at a concentration of 2 x 106 conidia/mL, while for *T. harzianum* a concentration of 2 x 109 conidia/mL was used (recommended by the manufacturer). Subsequently, these discs were deposited separately in the center of Petri dishes (9 cm Ø) containing synthetic Potato-Dextrose-Agar (BDA – Kasvi) culture medium. The plates were transferred to a B.O.D.

chamber. (Biochemical Oxygen Demand), at a temperature of 25 ± 2 ºC and a photoperiod of 12 h. The experiment was carried out in a completely randomized design (DIC), with five species of *Colletotrichum* and *T. harzianum*, five replications, with each replication consisting of a Petri dish.

Evaluations were obtained from daily measurements of the diameter of colonies (cm/day) taken on the back of plates in two perpendicular directions, with the aid of a millimeter ruler.

In vitro ***antagonism of*** *T. harzianum* ***on*** *Colletotrichum* ***species by pairing***

Assessments of the antagonistic activity of *T. harzianum* on the *in vitro* growth of *Colletotrichum* species were carried out according to the culture pairing methodology proposed by Dennis and Webster (1971), using concentrations 0.5; 1.0; 1.5 L/ha and the control. Initially, species of *Colletotrichum* and *T. harzianum* were cultivated separately in Petri dishes containing synthetic PDA culture medium and incubated in B.O.D., at a temperature of 25 °C, with a 12-h photoperiod, for seven days, to obtain conidial suspensions. Suspensions of *Colletotrichum* species were collected by scraping the surface of colonies with a sterile scalpel in 10 ml of ADE and filtered through layers of gauze to remove any mycelial debris.

Spores were counted in a hemocytometer and the spore count was adjusted to 2 x 106 conidia/mL. Subsequently, sterilized filter paper discs (Ø 5 mm) were moistened in a suspension of conidia of *Colletotrichum* species for 30 seconds, dried on filter paper and transferred to the end of Petri dishes containing synthetic PDA medium and incubated in B.O.D., at a temperature of 25 °C, with a photoperiod of 12 h, for 72 hours. After this period, filter paper discs were moistened with different concentrations of *T. harzianum* and subsequently deposited on the opposite end of plates, which were previously inoculated with *Colletotrichum* species, except for the control, in which only the pathogen or antagonist remained at one end of the plate. Plates were sealed again and transferred to an incubation chamber (B.O.D.), at 25 ºC with a 12-hour photoperiod, where they remained for 7 days, counting from the first inoculation of the pathogen.

The experimental design was completely randomized, in a factorial arrangement, with five replications, consisting of a Petri dish, with five species of *Colletotrichum* and concentrations of *T. harzianum*.

Evaluations were obtained from daily measurements of the diameter of colonies (*Colletotrichum* spp. and *T. harzianum*), taken on the back of plates in two perpendicular directions, with the aid of a millimeter ruler, until the seventh day. The mycelial growth speed index (IVCM; Oliveira, 1991) and the calculation of the percentage of inhibition of mycelial growth (PIC; Bastos, 1997) were determined according to formulas (1) and (2), respectively.

$$\boxed{1} \quad IVCM = \frac{C_1}{N_1} + \frac{C_2}{N_2} + \frac{C_n}{N_n}$$

Where: C1, C2 and Cn correspond to mycelial growth in the first, second and last evaluation; and N1, N2 and Nn correspond to the number of days after inoculation.

2 .

$$PIC = \frac{(Witness\ growth - Treatment\ growth) \times 100}{Witness\ growth}$$

The scale proposed by Bell et al. (1982) was used for the evaluation, which, in turn, assigns classes ranging from 1 to 5. Note 1: when the antagonist grows and occupies the entire plate; note 2: when the antagonist grows on part of the pathogen (2/3 of the plaque); note 3: when the antagonist and pathogen grow to half the plate (neither dominates the other); note 4: the pathogen grows and occupies part of the antagonist (2/3 of the plaque); and note 5: the pathogen grows and occupies the entire plate.

Action of volatile metabolites from *Trichoderma harzianum* ***on mycelial growth*** *Colletotrichum* ***spp.***

To evaluate the inhibitory potential of volatile metabolites produced by *T. harzianum* on *Colletotrichum* spp. a test adapted from the methodology described by Bomfim et al. (2010). Mycelium discs (Ø 5 mm) of phytopathogens *C. brevisporum*, *C. plurivorum*, *C. tropicale*, *C. theobromicola* and *C. fructicola* were deposited separately in the center of Petri dishes (9 cm in diameter), containing synthetic PDA culture medium. Plates were sealed and kept for 48 hours at B.O.D., at 25 ºC, with a 12-hour photoperiod. After this initial period of incubation, a disc of *T. harzianum* mycelium was transferred to the center of new Petri dishes, with the same dimensions and with synthetic PDA culture medium. Subsequently, the bases of plates containing the antagonist and pathogen were overlapped, joined and sealed. For control, the bottoms of Petri dishes containing PDA culture medium were used, where a disc of mycelium was added to the center of the plate, containing structures of pathogens, separately, and to the other bottom, only a disc of synthetic PDA medium over the culture medium, and subsequently, plates were overlapped and sealed, kept in an incubation chamber at a temperature of 25 ºC and a photoperiod of 12 h, for seven days.

The experimental design was completely randomized, in a factorial arrangement, with five replications, consisting of a Petri dish, with five species of *Colletotrichum* and *T. harzianum*. Evaluations were obtained from daily measurements of the diameter of colonies (*Colletotrichum* spp. and *T. harzianum*), taken on the back of plates in two perpendicular directions, with the aid of a millimeter ruler, until the seventh day.

The calculation of the percentage of mycelial growth inhibition (PIC; Bastos, 1997) and mycelial growth speed index (IVCM; Oliveira, 1991) was determined according to formulas described in the previous experiment.

Data analysis

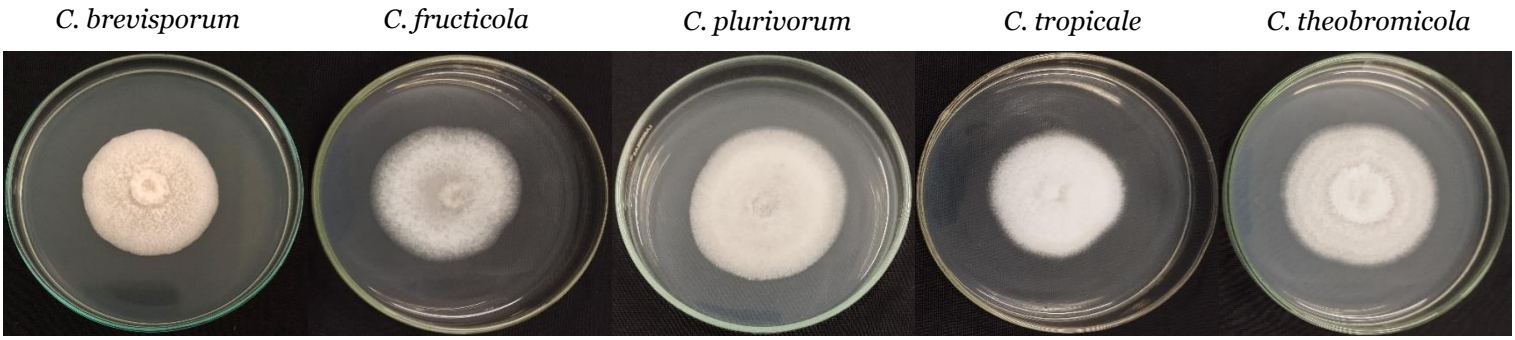
Results of experiments were subjected to analysis of variance (ANOVA) and means compared by the Tukey test, using the statistical program Assist (Silva & Azevedo, 2016).

Results

Mycelial growth of Colletotrichum and Trichoderma harzianum species

It was observed that there was uniformity in the mycelial growth of the antagonist (*T. harzianum* – Strain ESALQ 1306), at different concentrations (0.5; 1.0; 1.5 L/ha), while the *Colletotrichum* species (*C. plurivorum, C. brevisporum, C. tropicale, C. theobromicola* and *C. fructicola*) showed lower mycelial growth, when analyzed on the fourth day, the time required for the antagonist to grow completely on the surface of the PDA culture medium (Figure 1).

Figure 1.
Mycelial growth of *Trichoderma harzianum* and *Colletotrichum* spp, fourth day dia.



Trichoderma harzianum

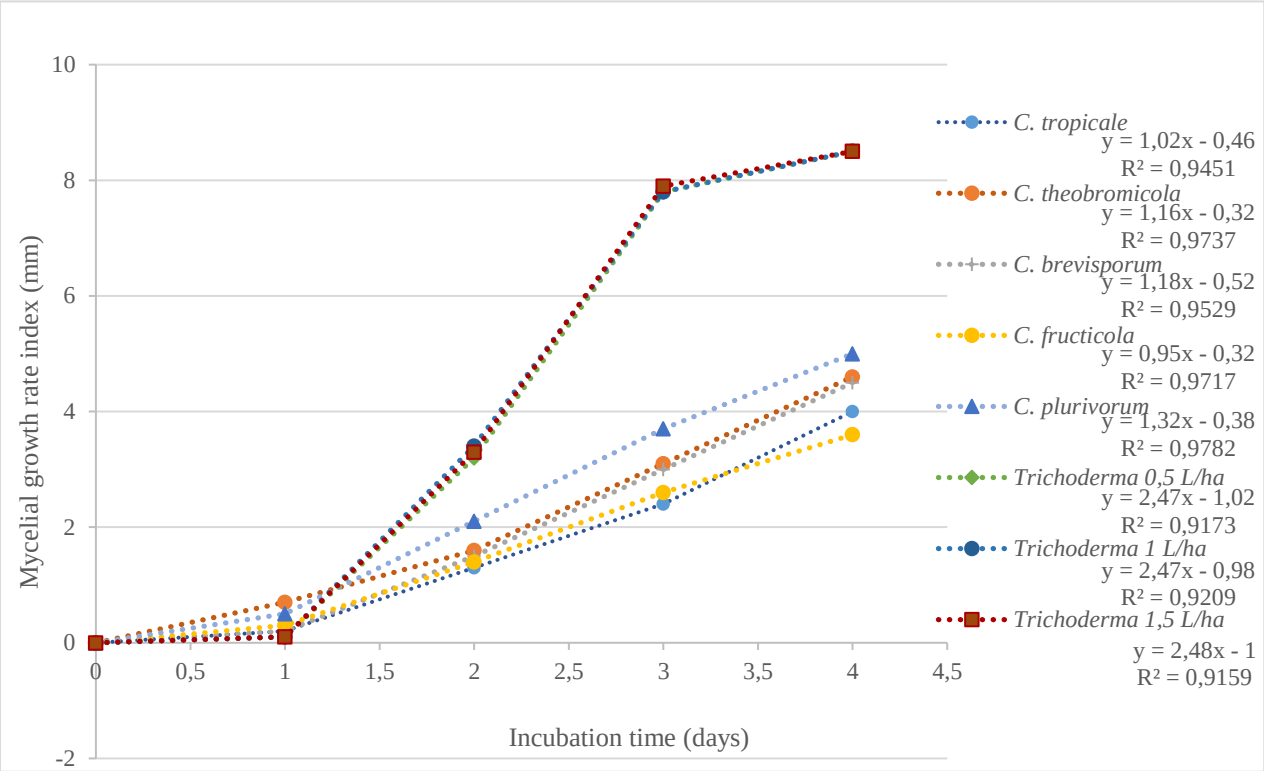
0,5 L/ha 1,0 L/ha 1,5 L/ha

Source: Authors (2023).

When evaluating the mycelial growth index, on the fourth day of incubation, it was found that the antagonist, *T. harzianum*, had a colony diameter of 8.5 cm, in the three concentrations. In the same evaluation period, *Colletotrichum* species showed different behavior, when the IVCM for *C. plurivorum* was 5.0 cm, *C. theobromicola* (4.6 cm), *C. brevisporum* (4.5 cm), *C. tropicale* (4.0 cm) and *C. fructicola* 3.6 cm (Figure 2).

Figure 2.

Regression analysis of the mycelial growth rate index of *Trichoderma harzianum* and *Colletotrichum* spp.



Source: Authors (2023).

In vitro antagonism of *T. harzianum* on *Colletotrichum* species by pairing

Analyzing the Mycelial Growth Speed Index (MCVI) of the five species of *Colletotrichum*, after seven days, without the presence of the antagonist, it was observed that there was no significant difference between species *C. plurivorum* (2.0 cm), *C. fructicola* (1.93 cm), *C. tropicale* (2.02 cm) and *C. brevisporum* (2.16 cm), except *C. theobromicola*, which presented 2.49 cm, when paper discs containing pathogen suspensions were added to ends of Petri dishes containing synthetic PDA medium (Table 2).

Species *C. plurivorum, C. brevisporum, C. fructicola* and *C. tropicale* behaved similarly in relation to IVCM, not differing significantly in the three concentrations of the antagonist (0.5; 1.0; 1.5 L/ha) and controls. However, only *C. theobromicola* showed different behavior compared to the others, at all concentrations, having the highest mycelial growth speed index (2.67 cm) at the concentration 1.5 L/ha (Table 2).

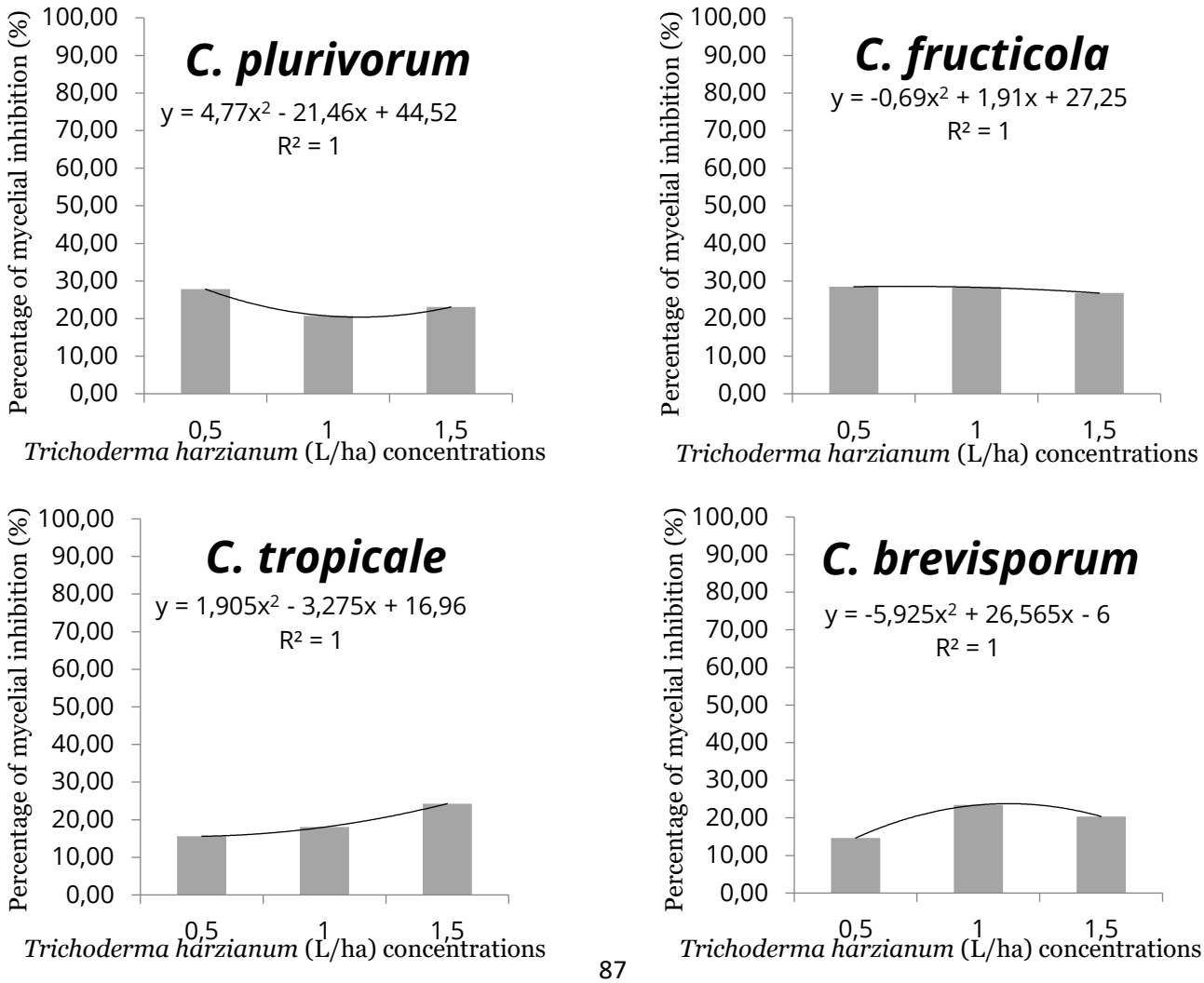
In the percentage of mycelial growth inhibition (PIC), *Colletotrichum* species presented different values compared to *T. harzianum* at concentrations 0.5; 1.0; and 1.5 L/ha, except for species *C. fructicola*, which presented the highest PIC at concentrations of 0.5 L/ha (28.47%) and 1.0 L/ha (28.31%), when compared to the other species, not statistically differing between

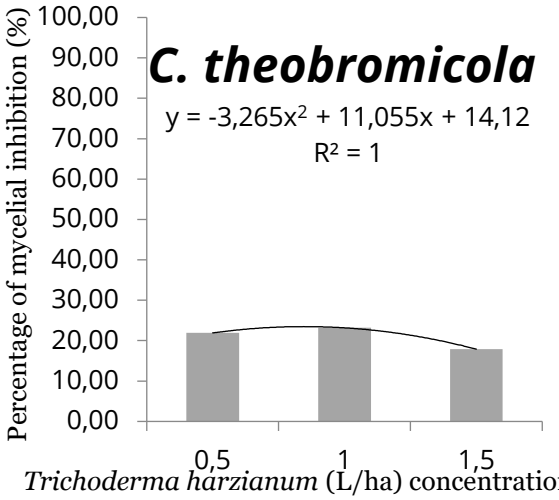
them. However, the species *C. plurivorum* and *C. brevisporum* showed similar behavior, statistically, when subjected to concentrations of 1.0 and 1.5 L/ha, and species *C. tropicale* and *C. theobromicola* when using doses of 0.5 and 1.0 L/ha. It is also observed that species *C. brevisporum* presented the lowest PIC (14.64%) and *C. fructicola* the highest PIC (28.47%) among species and concentrations tested (Table 2).

Table 2 shows that as concentrations of *T. harzianum* increased, the percentage of inhibition of mycelial growth for species *C. tropicale* also increased. Species *C. theobromicola* and *C. brevisporum* showed similar behavior at all concentrations tested, however, the highest percentage of inhibition was obtained at a concentration of 1.0 L/ha with values of 23.17 and 23.43%, respectively. In general, *C. fructicola* was the most sensitive species when subjected to confrontation with the antagonist, showing a better response at a concentration of 0.5 L/ha, with 28.47% inhibition. Species least sensitive to the antagonist was *C. brevisporum* (14.64 L/ha) at a concentration of 0.5 L/ha. However, all *Colletotrichum* species presented PIC below 30% (Figure 3 and Table 2).

Figure 3.

Percentage of mycelial inhibition (PIC) of *Colletotrichum* species using *Trichoderma harzianum*, at 7 days





Source: Authors (2023).

It was found that there was no difference between species *C. plurivorum, C. fructicola, C. tropicale* and *C. brevisporum* when evaluated with the rating scale proposed by Bell et al. (1982), with a score of 2 (2/3 of the plate occupied by the antagonist). Species *C. theobromicola* received a score of 3, at 7 and 10 days, indicating that both the antagonist and the pathogen grew by 50% of the plaque each, and one does not seem to dominate the other at concentrations 0.5; 1.0 and 1.5 L/ha (Figure 4 and Table 2).

Table 2.

Means of inhibition of mycelial growth rate and percentage of inhibition of mycelial growth at different concentrations of *Trichoderma harzianum* in the control of *Colletotrichum* spp.

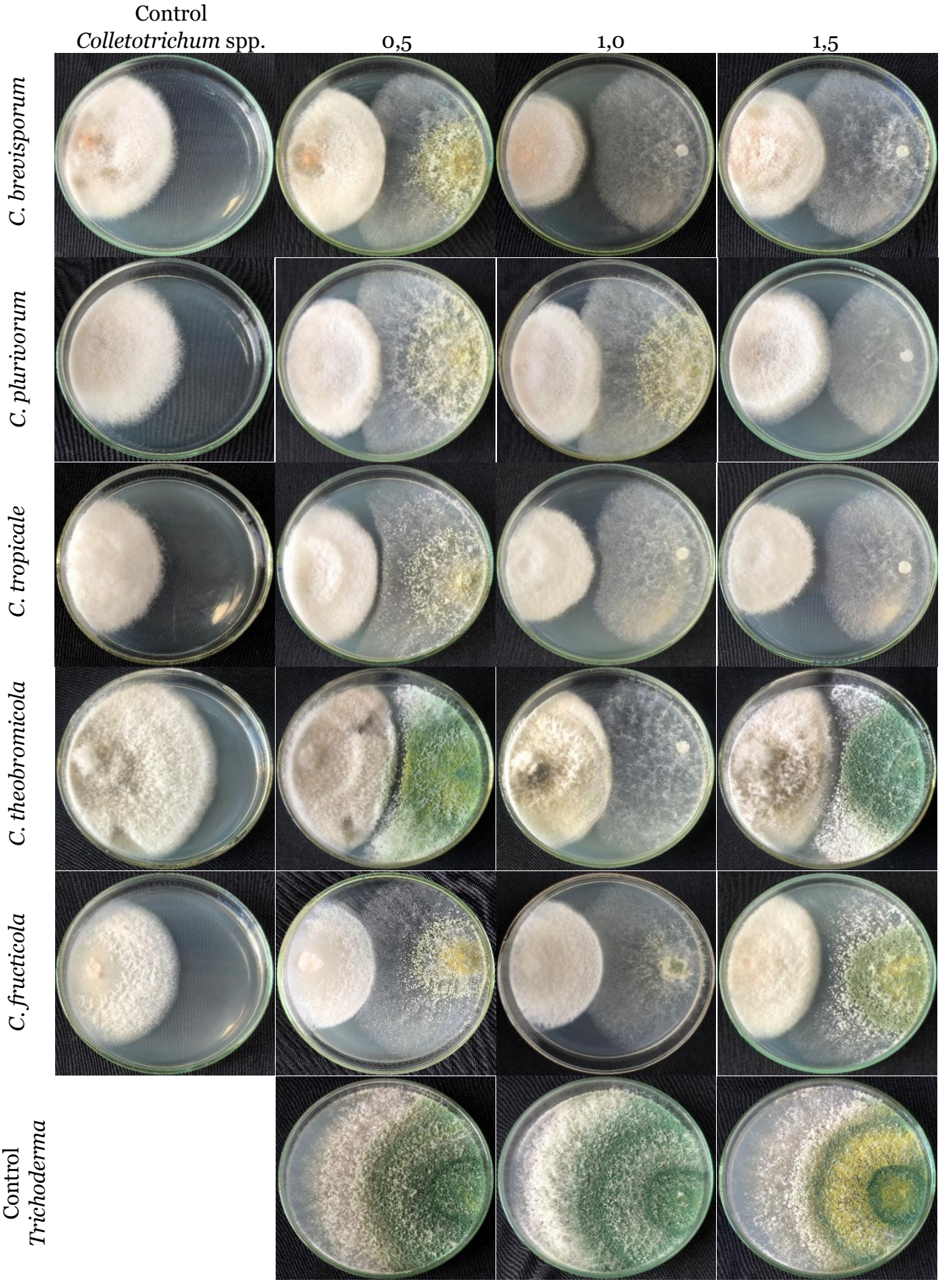
Species of *Colletotrichum*	**CONCENTRATIONS (L/ha)**							**Scale o Bell**
	IVCM (cm) (Interaction E x S)				PIC (%) (Interaction E x S)			
	Control	0,5	1	1,5	0,5	1	1,5	7 (days)
C. plurivorum	2,00 bA	2,01 bA	2,12 bA	2,19 bA	27,83 aA	20,68 bcB	23,07 abB	2
C. fructicola	1,93 bA	1,99 bA	1,98 bA	2,16 bA	28,47 aA	28,31 aA	26,77 aA	2
C. tropicale	2,02 bA	2,03 bA	1,87 bA	1,87 cA	15,59 dB	18,03 cB	24,28 abA	2
C. brevisporum	2,16 bA	1,95 bA	2,02 bA	2,01 bcA	14,64 cB	23,43 bA	20,37 bcA	2
C. theobromicola	2,49 aA	2,45 aA	2,62 aA	2,67 aA	21,91 bA	23,17 bA	17,90 cB	3
Species (S)		61.8924 **				31.9559 **		
Concentrations(C)		1.8821 ns				1.1993 ns		
Interaction E x S		2.3971 *				12.3670 **		
CV (%)		6,60				11,07		

Means followed by the same letter, lowercase in the column and uppercase in the row, do not differ significantly from each other using the Tukey test. (**) Significant at 1% probability (p < .01); (*) Significant at the 5% probability level (.01 =< p < .05); (ns) Not significant (p >= .05); CV= Coefficient of variation.

Source: Authors (2023).

Figura 4.

Pairing test using *Trichoderma harzianum* and *Colletotrihcum* spp.



Action of volatile metabolites of *Trichoderma harzianum* ***on*** *Colletotrichum*

Evaluating the IVCM, it was observed that the production of volatile metabolites of the antagonist, *T. harzianum*, proved to be significantly efficient in controlling *Colletotrichum* spp. when compared with their respective witnesses. Species *C. theobromicola* had the highest mycelial growth (4.03 cm) compared to the other species, while *C. plurivorum* was more sensitive, with 3.21 cm (Figure 5 and Table 3).

In the PIC assessment, species *C. tropicale* obtained the highest percentage of inhibition of mycelial growth (30.59%), statistically differing from the others, being the most sensitive to the action of metabolites produced by *T. harzianum*, followed by *C. plurivorum* (23.56%) and *C. brevisporum* (23.14%), which had similar behavior. Species *C. theobromicola* and *C. fructicola* had the lowest PICs, such as 22.41 and 19.02%, respectively (Table 3).

Table 3.

Mycelial growth rate index and percentage of *in vitro* mycelial inhibition of *Colletotrichum* spp. in the volatile metabolite test with *Trichoderma harzianum*

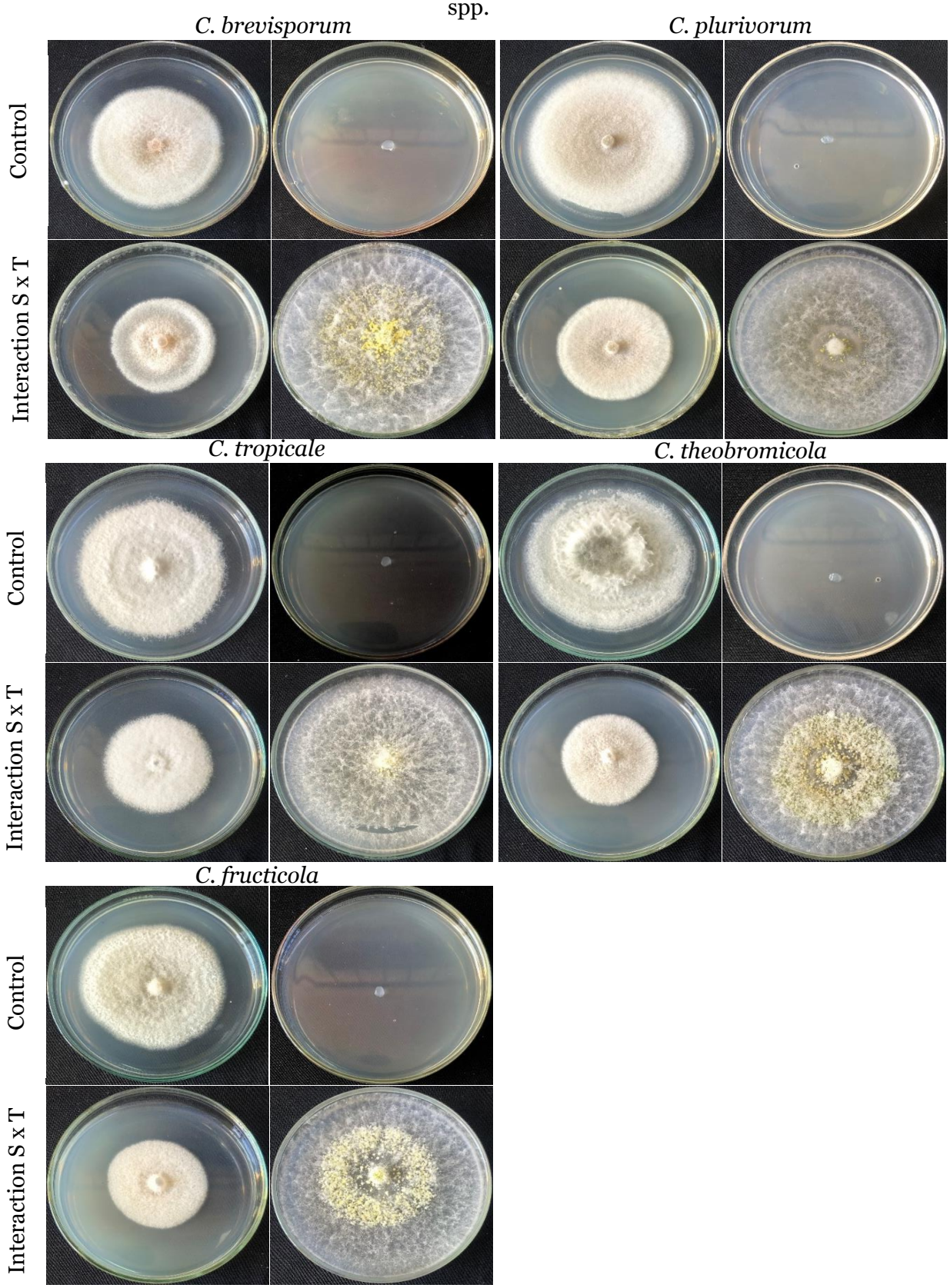
Species of *Colletotrichum*	IVCM (cm) (*Colletotrichum* spp.)	IVCM (cm) (Interaction S x T)	PIC (%) (Interaction S x T)
C. plurivorum	3,73 bA	3,21 cB	23,56 ab
C. fructicola	3,91 bA	3,56 bcB	19,02 b
C. tropicale	4,72 aA	3,61 bB	30,59 a
C. brevisporum	4,58 aA	3,92 abB	23,14 ab
C. theobromicola	4,69 aA	4,03 aB	22,41 b
Species (S)	32.0301 **		
Treatment (T)	120.8450 **		5.1740 **
Interaction S x T	4.5004 **		
CV (%)	5,28		17,49

Means followed by the same letter, lowercase in the column and uppercase in the row, do not differ significantly from each other using the Tukey test. (**) Significant at 1% probability (p < .01); CV= Coefficient of variation.

Source: Authors (2023).

Figure 5.

Test of volatile metabolites using *Trichoderma harzianum* in the control of *Colletotrichum* spp.



Discussion

The research for effective management strategies has been growing actively in recent years, taking biological control as an example. It is believed that this result is a reflection of the farmer's search for technologies to improve productivity, mainly in the control of phytopathogens (Bettiol et al., 2019). Within biological control, species of the genus *Trichoderma* stand out, which have been leveraging the management of plant diseases in Brazil (Morandi et al., 2009; Hermosa et al., 2014; Monte et al., 2019). Some authors report advantages of *T. harzianum*, in terms of competition for space and nutrients, due to its rapid mycelial growth, in the control of phytopathogens (Medeiros et al., 2018; Meyer et al., 2019; Quevedo et al., 2022).

In this study, the antifungal activity of the commercial product Trichodermil SC 1306/KOPPERT (Trichoderma harzianum) was observed against the phytopathogenic fungus *Colletotrichum* spp., originating from the anthracnose disease in passion fruit crops. It was observed that the mycelial growth of *Colletotrichum* species and the antagonist *T. harzianum* is the same based on the initial time, but it is not the same at the end of the observation when grown in PDA culture medium. This showed that the antagonist has a growth advantage in relation to *Colletotrichum* species, and it is believed that this fact occurred due to the high capacity of *Trichoderma* to colonize substrates with different characteristics (Monte et al., 2019).

Furthermore, isolated *Trichoderma* strains with potential for biocontrol of phytopathogens have been obtained from environments with high temperatures, saline or alkaline soils and low humidity conditions, thus favoring survival in environments with different conditions (Monte et al., 2019). Quevedo et al. (2022) state that a desirable characteristic is for the antagonist to have faster mycelial growth than the pathogen, because if both compete for space and nutrients, the tendency is for the microorganism that presents faster development to have an advantage over the slower growing one.

In the pairing test, macroscopically, an evident antagonism of *T. harzianum* against *Colletotrichum* species was observed. This mechanism is possibly due to competition for nutrients and space present in the culture medium, as well as mycoparasitism, which has a direct action on the phytopathogen, through infection (penetration and colonization), enzymatic degradation and the consumption of nutrients present in the fungal hyphae (Suassuna et al., 2019). According to Bell et al. (1982), species of the genus *Trichoderma* can locate hyphae of susceptible fungi, growing towards them, this may occur due to chemical stimuli produced by the host's mycelium.

Our studies are analogous to several studies on the biological control of plant pathogens that present promising results for the effectiveness of the antagonist *Trichoderma* spp. as a means of controlling phytopathogens using the confrontation technique, as, for example, in the work of Quevedo et al. (2022), in which they used the culture pairing technique to evaluate the in vitro antagonistic action of *Trichoderma* spp. in the control of *Fusarium oxysporum*, verifying that species *T. harzianum* presented an inhibition percentage of 14.24%, close to results found in this work.

Results obtained by Grano-Maldonado et al. (2021) showed that species of *C. ti* and *C. queenslandicum* presented a percentage of mycelial inhibition of around 70%. A similar result was observed by Sutarman et al. (2020), when testing the antagonism of *T. harzianum* in relation to *C. gloeosporioides* (65%) and *C. capsicum* (64.2%), pathogens associated with pepper anthracnose (*Capsicum annuum*). Teja et al. (2020) also tested the potential of *Trichoderma* spp. in relation to *C. graminicola*, the causal agent of sorghum anthracnose (*Sorghum bicolor L.*) and observed that the highest percentage of inhibition was obtained by species *T. harzianum*, with 50.8%. Ahmed and El-Fiki, (2017) reported the ability of *Trichoderma* spp. in mycoparasitizing hyphae of *Colletotrichum* spp., isolated from strawberry, *in vitro*. According to Druzhinina et al. (2011), the effectiveness in the percentage of mycelial inhibition of the action of *Trichoderma* on phytopathogens depends on a series of factors that act in coordination, among them, *Trichoderma* species and the phytopathogenic fungus stand out.

In addition to the pairing test, the rating scale proposed by Bell et al. (1982), in which our results corroborate those found by Quevedo et al. (2022), Morales-Mora et al. (2020) and Costa et al. (2019). Given answers obtained using the rating scale, the commercial product Trichodermil SC 1306/KOPPERT has biocontrol potential for at least four species of *Colletotrichum*. According to the manufacturer, the product has a mode of action through competition, parasitism and metabolites produced by *T. harzianum* 1306 (protease, lipase, glucanase and chitinase), which promote degradation of the fungal cell wall (Koppert, 2023). Tijerino et al. (2011) state that metabolites produced by *Trichoderma* species contribute to cell wall degrading enzymes in the control potential.

In vitro studies targeting the secondary metabolites of *Trichoderma* species have been the target of several researches, mainly in the control of phytopathogenic fungi of economic importance (Ramada et al., 2019). The methodology used in our research is similar to that of the work of some authors who show *Trichoderma* species acting directly to inhibit the mycelial growth of the pathogen through the action of metabolites, such as Barbosa et al. (2021), who obtained a reduction in the mycelial growth of *C. musae* through the volatile metabolites released by *Trichoderma*, with inhibition percentages that ranged from 47% to 74%. Lohmann et al. (2022) tested different species of *Trichoderma* in the control of *C. gloeosporioides*, isolated from the ornamental plant (*Cassia fistula L.*) using volatile

metabolites, and observed that the species *T. harzianum* stood out in inhibiting the mycelial growth of this phytopathogen and attributed the result to antibiosis, as through it the antagonist can control the action of the pathogen, even though it is not in direct contact with it.

Carvalho et al. (2014) state that there is a variability of response in the inhibition of phytopathogens due to different active metabolites, which may vary between the same species or fungal genera. Research shows that to a greater or lesser extent, all *Trichoderma* species are effective parasites of phytopathogenic fungi as a biotrophic nutrition strategy (Druzhinina et al., 2011; Barbosa et al., 2021; Grano-Maldonado et al., 2021; Sutarman et al., 2020).

Studies on the biological control of plant pathogens present promising results for the effectiveness of the antagonist as a means of controlling phytopathogens, with *in vitro* research being important to assist in the selection of biocontrol agents.

Conclusion

Trichodermil SC 1306/KOPPERT (*Trichoderma harzianum*) has an antagonistic action against the species of *C. brevisporum, C. plurivorum, C. tropicale, C. theobromicola* and *C. fructicola*, causal agents of passion fruit anthracnose.

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