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Trichoderma in the phytopathogenic biocontrol

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Abstract

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Fungus of the genera *Colletotrichum, Macrophomina* and *Curvularia* are among the main fungi that cause disease in plants. The fungi *Trichoderma* are an important tool in the biological control of phytopathogens due to their mechanisms of competition, antibiosis and mycoparasitism. For this reason, the present study aimed to evaluate the efficiency of the antagonism *in vitro* of species *Trichoderma* to the pathogens *Macrophomina* sp., *Colletotrichum gloeosporioides* and *Curvulária lunata*. The isolates *Trichoderma* used were: *T. harzianum* (UFT-25), *T. pinnatum* (UFT-37), *T. virens* (UFT-57), *T. asperelloides* (UFT-201) and *T. longibrachiatum* (UFT-204). In the biocontrol tests, the percentages of colonization and inhibition were evaluated and scores were assigned on an adapted scale. The experimental design was completely randomized, in quadruplicate and analyzed in a 5 x 3 factorial scheme, with five isolated from *Trichoderma* and three pathogens. In the colony matching to *C. gloeosporioides*, the isolates UFT-25 (80.39%) and UFT-204 (79.88%) obtained a higher percentage of colonization, and the isolates UFT-201 (57.05%) and UFT -204 (56.57%) were more efficient in inhibiting the pathogen. For the pathogen *Macrophomina* sp. isolates UFT-25 (70.49%) and UFT-57 (73.10%) had a higher percentage of colonization, and isolates UFT-57 (70.78%) and UFT-204 (72.69%) were superior in inhibiting the pathogen. In the confrontation with *C. lunata* sp. isolates UFT-201 (80.39%) and UFT-204 (84.52%) provided a higher percentage of colonization, and the isolate UFT-201 (80.39%) was higher in the percentage of inhibition. All *Trichoderma* isolates are considered to be efficient antagonists.

Keywords: **a**ntagonist; biological control; pathogens

Introduction

Soil phytopathogenic fungi are responsible for large losses in strategic crops with around 30% of diseases emerging plants, being a threat to food security worldwide causing loss of 125 million tons in different cultures. Losses are estimated at 78% in fruit crops, 54% in vegetable crops and 32% in cereal crops, in addition, they cause an increase in production costs, as well as making certain planting areas unfeasible (Freire, 2015; Zhang, 2018).

Phytopathogens synthesize and secrete toxic secondary metabolites as the first resources for colonization, killing host cells (Silva et al., 2019). The genus *Colletotrichum* is one of the main phytopathogenic genera responsible for anthracnose. The disease is characterized by brown spots on the leaves, stems, fruits or flowers and leading to the wilting and death of the infected plant (Ajay, 2014). The fungus of the genus *Macrophomina* is the causative agent of black root rot, also known as gray root rot or coal rot, with soy, sorghum, corn, cotton and cowpea being the main crops attacked (Ishikawa et al., 2018). Another phytopathological agent that has caused damage is the fungus *Curvularia lunata*, considered to be mitosporic fungi that cause discoloration, lesions and grain deformation mainly in rice (*Oryza sativa* L.) (Costa et

al., 2017).

Chemical control of fungal plant diseases is still widely applied, but due to the negative impacts on the environment, it is necessary to seek alternatives to control these phytopathogens (Silva et al., 2014). Research advances in the search for more effective biological control agents, with the ability to adapt the same environmental conditions as phytopathogens, facilitating the development of these antagonists with the crop without causing damage and contributing to the promotion of plant growth (Fipke et al., 2017).

Given this, biological products based on *Trichoderma* grow as an alternative to the use of chemicals, being considered of low impact on the environment, providing healthier food to the population (Junior et al., 2018). The genus *Trichoderma* appears as an important alternative in the biocontrol of phytopathogens due to the different mechanisms of action such as: competition (water, air, light and nutrients); host-parasite association, which may be physical or metabolic with digestion by hydrolytic enzymes, including chitinases, proteases, glucanases and lipases; antibiosis being the action of a microorganism that acts on another, by releasing substances that harm or kill another microorganism (Machado et al., 2012; Machado & Silva, 2013; Woo, 2014)

Thus, the objective was to evaluate the activity antagonism of isolates *Trichoderma* in the control of the pathogens *Macrophomina* sp., *Colletotrichum gloeosporioides* and *Curvularia lunata.*

Material and Methods

Obtaining the isolates

Strains *Trichoderma* were acquired at the Applied Agromicrobiology and Biotechnology Laboratory (AGRO-BIO/PPGPV) at the Federal University of Tocantins, Gurupi Campus (11°43'45"S and 49°04'07"W, 300 m.a.s.l.), where they are stored.

The isolates were characterized by the sequencing of the TEF region (Translation Elongation Factor) and identified by the access codes on the GenBank carried out by the Instituto Biológico de São Paulo (Table 1, Figure 1). The strains were harvested in a Petri dish containing PDA culture medium (potato-dextrose-agar, Kasvi, 27 g L^{-1}) and incubated in Biochemical Oxygen Demand (BOD), with a temperature of 28 ± 2ºC, and a photoperiod of 12 hours for seven days (Figure 1).

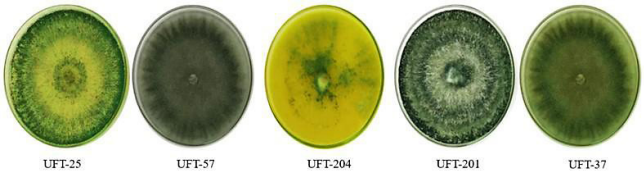

Fig. 1. Isolates Trichoderma used in the study

The biocontrol assay was performed at the AGRO-BIO-PPGPV Laboratory. The strains of *Trichoderma* were seeded in a Petri dish containing PDA (90 x15 mm) culture medium and incubated in BOD with temperature at 28 ± 2ºC, and photoperiod of 12 hours for seven days.

The experimental design was completely randomized, performed in quadruplicates and analyzed in a 5x3 factorial scheme (five isolates and three pathogens). The pathogens *Colletotrichum gloeosporioides*, *Cuvulária luneta* and *Macrophomina* sp. were obtained from the Laboratory of Phytopathology at Federal University of Tocantins (UFT - Campus Gurupi).

Colony matching test (confrontation)

The paired culture was performed in Petri (90x15 mm) dishes, where agar disks (5 mm in diameter) containing mycelium from each pathogen were placed in PDA culture medium, at a distance of approximately 1.0 cm from the edge, at the other end, the was antagonist raised in a position opposite to the pathogen colony. For the controls, they were transferred to the center of each Petri dish containing PDA medium, a 0.5 cm diameter disk of phytopathogens and antagonists, and the colonies were not paired. The plates were incubated in a BOD chamber, at 28 ± 2°C, with a photoperiod of 12 hours of light.

After seven days for the confrontation with *Macrophomina* spp., And fourteen days for the confrontations with *C. gloeosporioides* and *C. lunata*, evaluations were carried out by the percentage of colonization (%C) according to

Table 1. GenBank access codes for *Trichoderma* spp. (TEF – Translation Elongation Factor) used in this study

Isolates	Identification of species	Access GenBank	References
UFT 25	*T. harzianum* CIB T131	EU279988	Hoyos-Carvajal et al. (2009)
UFT 37	*T. pinnatum* GJS 02-120	JN175572	Druzhinina et al. (2012)
UFT 57	*T. virens* CIB T147	EU280060	Hoyos-Carvajal et al. (2009)
UFT 201	*T. asperelloides* GJS 04-217	DQ381958	Samuels et al. (2010)
UFT 204	*T. longibrachiatum* DAOM 167674	EU280046	Hoyos-Carvajal et al. (2009)

the methodology of Camporota (1985), in which: % C = DT / DE X 100, being DT, the growth radius of the colony *Trichoderma* in frontal direction to the pathogen colony and DE, the distance that separates the two colonies

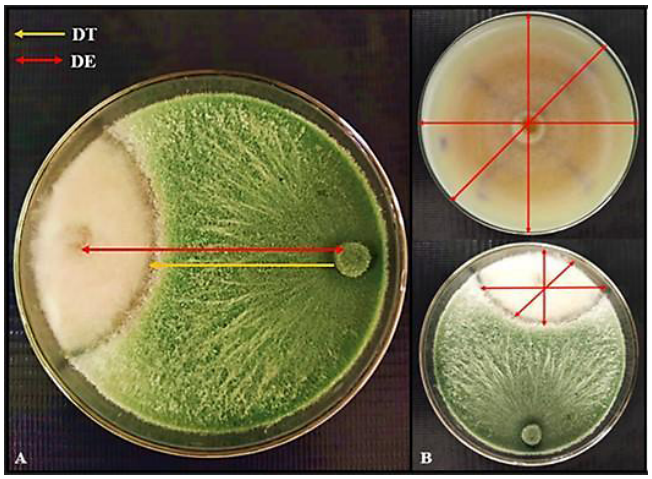

Fig. 2. Determination of the percentage of colonization (A) and percentage of inhibition (b) of isolates *Trichoderma* to pathogens in biocontrol assays

Table 2. Adapted scale used for the colony matching test proposed by Bell et al. (1982)

Notes	Scale of Assessment	Percentage
1.0	Antagonist grows across the plate and on the pathogen disk	87.5 to 100%
1.5	Antagonist grows over 7/8 of the plate	66.6 to 87.5%
2.0	Antagonist grows about 2/3 of the plate	62.5 to 66.5%
2.5	Antagonist grows about 5/8 of the plate	51 to 62.4%
3.0	Antagonist and pathogen grow to half of the plate	50%
3.5	Antagonist grows on 3/8 of the plate	37.5 to 49.9%
4	Antagonist grows on 1/3 of the plate	33.3 to 37.4%
5	Antagonist does not grow on the Petri dish	<33.2%

(Figure 2A). The percentage of inhibition (% I) = [(C - T) / C] x 100 was also carried out according to Menten et al. (1976), where: C = radial growth of the control; T = radial growth of the treatment (Figure 2B). Finally, an evaluation was also carried out according to the criteria proposed by Bell et al. (1982) with adaptation (Table 2). It was considered an efficient antagonist when its score was less than or equal to 2.0.

Statistical analysis

The data obtained in experiments were submitted to analysis of variance (ANAVA) and the means grouped by the Scott-Knott test at 5% probability, using the SISVAR software, version 5.3 (Ferreira, 2011).

Results and Discussion

Isolates *Trichoderma* were efficient in controlling pathogens, ranging from 60.84 to 84.52% of colonization, 40.88 to 80.39% of inhibition and scores of 1.5 to 2.0 on the scale of Bell (1982) (Table 3).

In the unfolding of the pathogens within treatments, the evaluation of isolated UFT-25, on %C was superior in the confrontation with the *C. gloeosporioides*. For the %I, the biggest happened with the pathogen *C. lunata*. The isolated UFT-37 got the biggest colonization in the confrontation with *C. gloeosporioides* and *C. lunata* and the larger inhibition occurred at the pathogen *Macrophomina* sp. The isolated UFT-57 didn't grant statically at %C confronting pathogens, however, the higher %I occurred to the pathogens at %C and I% when a confrontation with *Macrophomina* sp. and *C. lunata.* As regards the isolated UFT-201, that was the most efficient at %C and I% when confronted with *C. lunata*. For the isolated UFT-204, the biggest colonization happened in the confrontation with *C. lunata* the %I was higher at the confrontation with the pathogen *Macrophomina* sp. (Table 3).

Table 3. Evaluations of species for *Trichoderma in vitro* antagonism by colony matching to pathogens *C. gloeosporioides*, *Macrophomina* sp. and *C. lunata*, according to the percentage of colonization (% C), percentage of inhibition (% I), notes according to the Bell scale (1982)*

Isolated	*C. gloeosporioides*			*Macrophomina* sp.			*C. lunata*		
	%C	%I	Notes	%C	%I	Notes	%C	%I	Notes
UFT-25	80.38 aA	50.29 bC	1.0	70.49 aC	71.26 aB	1.0	75.09 bB	76.28 bA	1.5
UFT-37	76.15 bA	40.88 cC	1.5	65.02 bB	69.53 bA	2.0	76.87 bA	62.20 eB	1.5
UFT-57	76.15 bA	51.27 bB	1.0	73.10 aA	70.78 aA	1.0	75.57 bA	71.31 cA	1.5
UFT-201	76.79 bB	57.05 aC	1.0	60.84 bC	68.96 bB	2, 0	83.03 aA	80.39 aA	1.0
UFT-204	79.88 aB	56.57 aC	1.5	65.25 bC	72.69 aA	2.0	84.52 aA	65.00 dB	1.5
**CV	2.17	1.79		5.58	1.87		4.14	1.96	

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ by the Scott-Knott test (P <0.01). **CV = Coefficient of variation

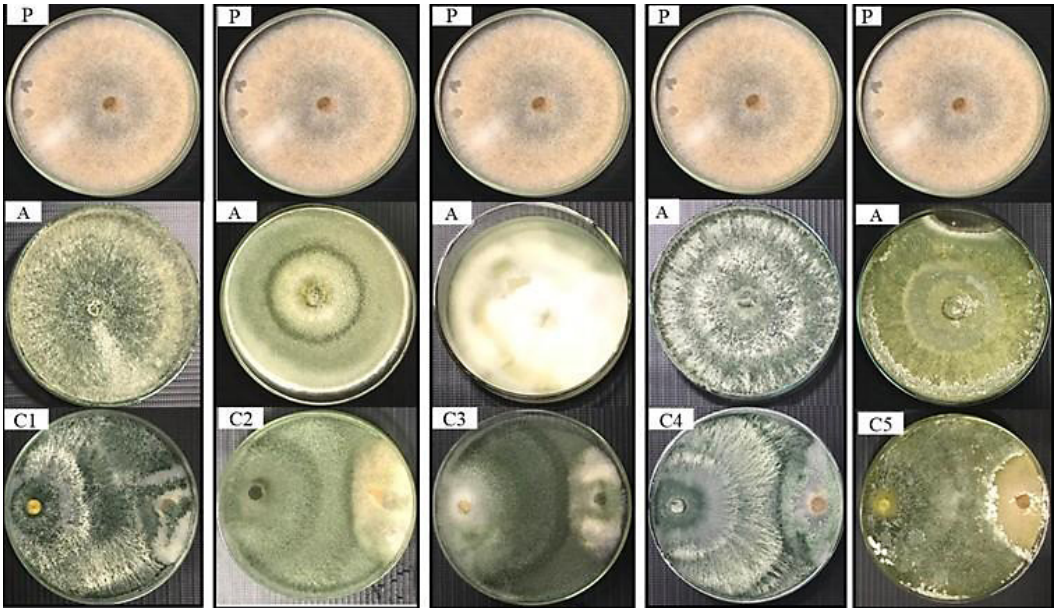

Fig. 3. Direct confrontation of the *Trichoderma* isolates to pathogen *Colletotrichum gloeosporioides:* P= Pathogen's Witness; A= Opposite's Witness; C1= Confrontation UFT-25 X Pathogen; C2= Confrontation UFT-37X Pathogen, C3=Confrontation UFT-57 X Pathogen; C4=Confrontation UFT-201 X Pathogen; C5= Confrontation UFT-201 X Pathogen

Confrontation Trichoderma spp. x C. gloeosporioides

At the evaluations of biocontrol at *C. gloeosporioides,* at the percent of colonization (%C) was a formation of two groups statically different from each other, the group of isolated UFT-25 and UFT-204 achieved higher %C about the grouping of isolated UFT-37, UFT-57, and UFT-201 (Table 3). At the %I created three groups, the group of isolate UFT-201 and UFT-204 provided the biggest inhibition to *C. gloeosporioides.* In the evaluation for grade, scales, isolates were considered antagonists efficient, assigning scores from 1.0 to 1.5.

At the evaluation of *C. gloeosporioides*'s control, the species of *Trichoderma* were considering promising on the biocontrol *in vitro* by pairing method against the pathogens. The formation of the inhibition halo was noted for the isolates UFT-25(Figure 3, C1), UFT-201 (Figure 3, C4) when the physical contact between the mycelia of *Trichoderma* occurred and pathogens. The *Trichoderma*'s opposite activity was usually associated with hydrolytic enzymes, chitinase, and glucanases produced in the interaction with the *Trichoderma*, and antibiotic production, as viridine glycotoxin suzucacillin and tricordermin.

In the biocontrol, by isolates, UFT-25 and UFT-201, had an increasingly more aggressive, overlapping and sporulating at pathogen's colony (Figure 3, C1, C3 and C4), the same stressed in pathogen's inhibition, with %I of 76.79 to 79.88%. The results with these species corroborate with the results obtained by La Cruz et al. (2018) in the fungus *C. gloeosporioides* biocontrol by pairing, which showed inhibition of 77.5 to 78.1%.

Alvindia (2018) studying the efficiency of strain DGA01 by *T. harzianum* against the *C. gloeosporioides* causing anthracnose in mango, dipping fruits in conidia suspension, observed an 87.90% reduction of the anthracnose gravity, assigning the control for the mycoparasitic mechanisms and metabolites production. The biocontrol of *Trichoderma* spp. to *C. gloeosporioides* already reported in citrus (Ferreira et al., 2020), grape (Narkar et al., 2012), bell paper (Nurbailis et al., 2019), açai palm (Costa et al., 2019).

***Confrontation Trichoderma* spp. x *Macrophomina* sp.**

In the *in vitro* biocontrol evaluations of the pathogen *Macrophomina* sp., the isolates were grouped together forming two statistically different groups. In the percentage of colonization (%C), the group of isolates UFT-25 and UFT-57 provided better averages (Table 3). For inhibition percentage (%I), the highest inhibition averages were obtained by the UFT-25, UFT-57, UFT-204 group. In the assignments of grades, all isolates were considered efficient antagonists with grades from 1.0 to 2.0 (Table 3).

The results of the present study demonstrated the efficacy of *Trichoderma* isolates in controlling of *Macrophomina* sp., it occurred inhibition of mycelial growth of the pathogen (Figure 4, C1 to C5), formation inhibition zone (Figure 4, C1 and C4) and colonization on the pathogen (Figure 4, C1 and C3).

These behaviors in the confrontation, suggests that the action of each isolate depends on the species of the antagonist and their different biocontrol mechanisms adopted, such as parasitism, predation and antibiosis. The action of the

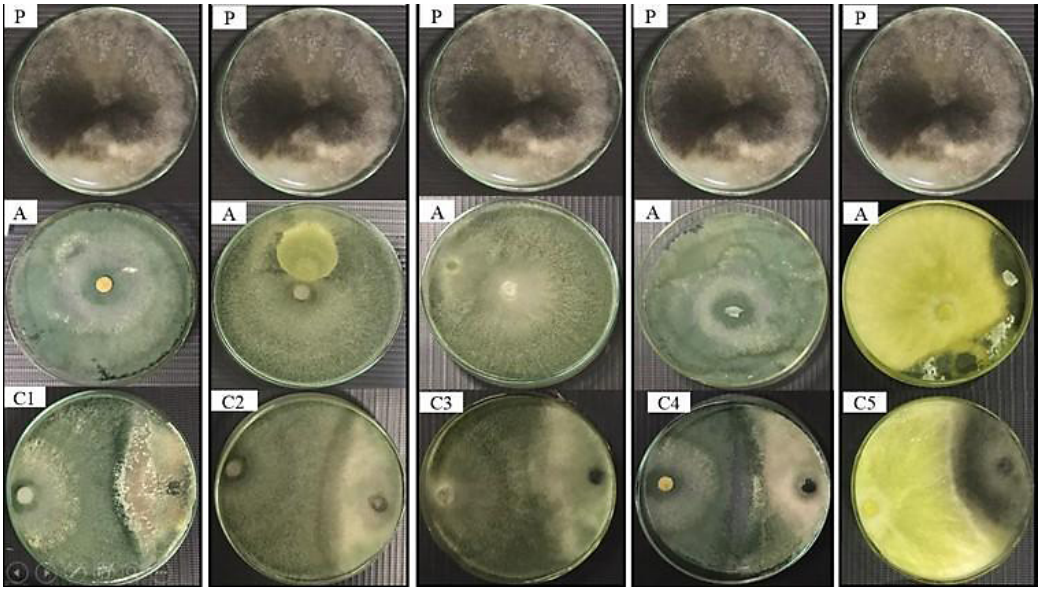

Fig. 4. Direct confrontation of *Trichoderma* isolates with the pathogen *Macrophomina* sp.: P = Witness of the pathogen; A = Witness of the antagonist; C1 = UFT-25 x Pathogen Confrontation; C2 = Confrontation UFT-37 x Pathogen; C3 = Confrontation UFT-57 x Pathogen; C4 = Confrontation UFT-201 x Pathogen; C5 = Confrontation UFT-201 x Pathogen

antagonist in biocontrol can vary depending on the isolates, within and between species, and present different behaviors in relation to the pathogens due to the origin of the isolated fungus, the nutritional status of the culture medium, pH, and thus, influencing the different mechanisms of action *Trichoderma* that can act more aggressively in certain cases (Dennis & Webster, 1971).

The UFT-25, UFT-57 and UFT-204 isolates inhibited the growth of *Macrophomina* sp. from 71.26 to 72.69% (Table 3). These results are in accordance with that found by Khalili et al. (2016), who, when testing isolates of *T. harzianum* as biological control agents to *Macrophomina phaseolina*, found an inhibition of 72.31% in comparison, and 63.26% by volatile compounds. The inhibition of the pathogen occurs due to the production of hydrolytic enzymes produced by *Trichoderma*, which are responsible for the degradation of the fungal cell wall (Haddad et al., 2017; Carvalho et al., 2018). And as the genus *Macrophomina* belongs to the *Ascomycota* division and they have a cell wall composed of chitin and β-1,3-glucan which is inhibited by the production of antibiotics or by chitinases and β-1,3-glucanases (Broetto et al., 2014).

Sreedevi et al. (2011) obtained a satisfactory result when paired with *Trichoderma* spp. for the *in vitro* biocontrol of *M. phaseolina* that causes peanut root rot, the species *T. viride* and *T. harzianum* reduced mycelial growth by 61.1% and 64.4%, respectively. Pastrana et al. (2016) found the efficiency of *Trichoderma* in the field against coal rot in strawberry cultivation, evaluating the application of *T. asperellum* by root immersion, reduced the incidence of coal rot by up to 44% in a growth chamber, and even 65% in field conditions. Khan et al. (2019) evaluating the performance of *Trichoderma* in the management of root rot in mung beans, observed a higher percentage of germination of seeds treated with *T. viride* (92 and 90%), *T. hamatum* (88 and 87%) or *T. harzianum* (86 and 85%) and reduction in the severity of root rot from 40 to 59%.

Confrontation Trichoderma* spp. x *C. lunata

In the percentage of colonization (% C) of biocontrol *in vitro* to the pathogen *C. lunata* sp. they formed two statistically different groups, the group of isolates UFT-201 and UFT-204 provided better colonization averages. For the percentage of inhibition (%I), five groups were formed, the UFT-201 group provided the highest average, followed by the UFT-25, UFT-57, UFT-204 and UFT-37 groups (Table 3).

The present study demonstrated the efficiency of the species to the pathogen *C. lunata* in an *in vitro* test by colony matching. The species of *T. asperellum* (UFT-201) stood out in the inhibition and colonization (Table 3), occurring overlapping the pathogen colony more aggressively (Figure 5 C4) in relation to the other isolates. A plausible explanation for the relevance of the results obtained may be the joint action of the different mechanisms of action (antibiosis, parasitism, predation, competition) of *Trichoderma*. Baiyee et al. (2019) obtained satisfactory results evaluating the efficacy of *T. spirale* in the control of *C. aeria* with inhibition of 85.64 to 93.03%.

The isolates of *Trichoderma* showed inhibition of the pathogen greater than 50%, with emphasis on the isolate

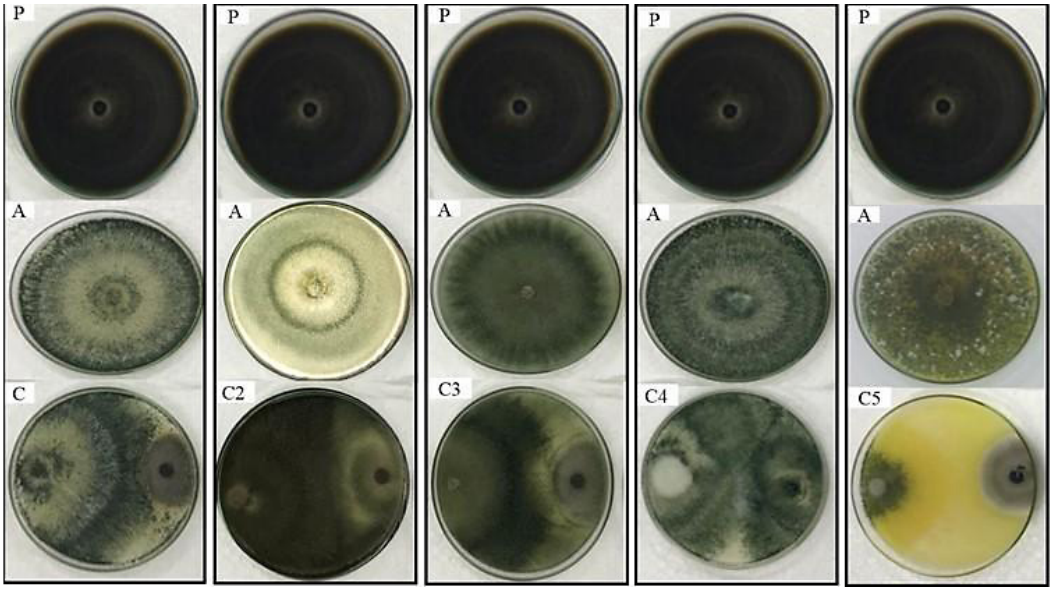

Fig. 5. Direct confrontation of *Trichoderma* isolates with the pathogen *Curvularia lunata:* P = Witness of the pathogen; A = Witness of the antagonist; C1 = UFT-25 x Pathogen Confrontation; C2 = Confrontation UFT-37 x Pathogen; C3 = Confrontation UFT-57 x Pathogen; C4 = Confrontation UFT-201 x Pathogen; C5 = Confrontation UFT-201 x Pathogen

UFT-201 (*T. asperellum*), which obtained a higher %I for *C. lunata* (Table 3). The results of this work are in accordance with those obtained by Ramírez *et al*. (2019), where they evaluated the efficiency of *Trichoderma* isolates in paired culture and obtained results with inhibition of *C. lunata* by more than 50%, with *T. asperellum* (GRB-HA1 and GRB-HA2) providing greater inhibition of the pathogen with 83.87%. Whereas Iftikhar et al. (2017) evaluated the inhibition of *Trichoderma* to *C. lunata* isolated from tomato fruits; they found an inhibition of 93.8% (*T. viride*) and 90.2% (*T. harzianum*) covering the colony of the pathogen.

The reduction in the mycelial growth of *C. lunata* probably occurred due to the capacity of these antagonists to produce volatile metabolites with inhibitory effects to the growth of the pathogen. Fungi of the genus *Trichoderma* are capable of producing a variability of volatile metabolites with antifungal action, such as 6-pentylpyran-2na (6PP), 3,4-dimethyl-1-hydroxybutanoic acid, 2-methylpropan-1-ol, oct-1-en-3-ol, octan-3-one, octan-3-ol and oct-1-en-3-one, 2-heptanone, 1-pentanol, 2-heptylfuran (Dias, 2014; Monti et al., 2020). In addition, among these metabolites produced by *Trichoderma*, 6PP acts as a growth promoter in plants (Lee et al., 2016).

Conclusions

Most of the *Trichoderma* isolates were considered efficient antagonists by Bell's score criteria.

The *T. harzianum*, *T. asperellum* and *T. longibrachiatum* species have greater potential in the biocontrol of *C. gloeosporioides*.

The *T. harzianum*, *T. virens* and *T. longibrachiatum* species have greater potential in the biocontrol of *Macrophomina* sp.

The *T. asperellum* and *T. longibrachiatum* isolates are more efficient in biocontrol of *C. lunata*.

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