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Antagonism of *Trichoderma* on the control of *Fusarium* spp. on *Phaseolus lunatus* L.

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Abstract

Biological control strategies have become an important tool in the sustainable management of plant diseases. This paper aims to report the *Fusarium* species that affect fava beans (*Phaseolus lunatus* L.) grown in Paraíba, Brazil, and determines the potential of *Trichoderma* isolates to control these fungi. Two *Trichoderma* and ten *Fusarium* isolates from fava bean seeds were selected. The beans were obtained from cultivated areas in the municipalities of Remígio, Alagoa Grande and Campina Grande, in Paraíba state. Phylogenetic analyzes based on DNA sequences of the translation elongation factor 1-α (*TEF1*) gene resolved the *Fusarium* isolates into four species belonging to the *F. fujikuroi* and *F. incarnatum-equiseti* species complexes. *In vitro* tests showed that the two isolates of *Trichoderma* tested presented antagonistic potential against the pathogens from the fava beans evaluated. In the direct comparison test, the growth of the pathogens was reduced from the seventh day in both treatments. Sporulation also showed a reduction, but only for 40% of *Fusarium* isolates. This work demonstrates that *Trichoderma* isolates can be used as a sustainable alternative to manage *Fusarium* spp. infection of fava beans.

Keywords: Antagonistic activity, biological control, fava beans, seed pathology.

Antagonismo de *Trichoderma* no controle de *Fusarium* spp. sobre *Phaseolus lunatus* L.

Resumo

O controle biológico para manejo de doenças de plantas tem sido ampliado a fim de reduzir os impactos ambientais negativos. Os objetivos deste trabalho foram reportar espécies de *Fusarium* que acometem sementes de feijão fava (*Phaseolus lunatus* L.) cultivadas no estado da Paraíba, e determinar o potencial de isolados de *Trichoderma* para o controle desses fungos. Foram selecionados dois isolados de *Trichoderma* e dez isolados de *Fusarium* obtidos de sementes de feijão fava em áreas de cultivo nos municípios de Remígio, Alagoa Grande e Campina Grande, no estado da Paraíba. Análises filogenéticas baseadas em sequências de DNA do gene fator de alongamento de cadeia 1 alfa (*TEF1*) dividiram os isolados de *Fusarium* em quatro espécies pertencentes aos complexos *F. fujikuroi* e *F. incarnatum-equiseti*. Os testes *in vitro* mostraram que os dois isolados de *Trichoderma* utilizados apresentaram potencial de controle sobre *Fusarium* em feijão fava. No teste de confronto direto, o crescimento do patógeno foi reduzido a partir do sétimo dia de cultivo, com ambos os isolados de *Trichoderma*. A esporulação apresentou redução para apenas 40% dos isolados de *Fusarium*. Isolados de *Trichoderma* podem ser usados como uma alternativa sustentável para o manejo de *Fusarium* spp. no feijão fava.

Palavras-chave: Atividade antagônica, controle biológico, feijão fava, patologia de sementes.

Introduction

Fava bean seeds (*Phaseolus lunatus* L.) are grown worldwide, due to their high protein and nutrient content. It is one of the species of greatest economic and social importance for the Brazilian Northeast (Santos, 2008). The Paraíba state accounts for almost 50% of the production of this legume in the Northeast region and is cultivated mainly by family farmers (Gomes, Nunes, Nascimento, Souza & Porcino, 2016). However, fava bean productivity has been reduced by diseases (Gomes & Nascimento, 2018). The seeds can carry pathogens and introduce them into new production areas (Silva-Flávio, Sales, Aquino, Soares, Aquino & Catão, 2014; Nascimento & Medeiros, 2015).

The *Fusarium* genus is an important plant pathogen that infects many economically important crops (Summerell, 2019). Vascular wilts, cankers, stem, and root rots are some of the symptoms caused by *Fusarium* in plants (Summerell & Leslie, 2011). *Fusarium* currently comprises hundreds of species distributed in 23 species complexes (Summerell, 2019). Many of these species are morphologically cryptic and require molecular methods for their identification (O'Donnell *et al*., 2015). The inability to distinguish cryptic species among pathogens complicates disease management (Bickford, Lohman, Sodhi, NG, Meier, Winker, Ingram & Indraneil, 2007). Thus, accurate identification of *Fusarium* species is essential for control strategies to be implemented properly (Santos, Trindade, Lima, Barbosa, Costa, Carneiro-Leão & Tiago, 2019).

Biological control is used as an effective alternative for plant pathogens control. Microorganisms used in biological control exhibit antagonistic characteristics and can increase resistance against pathogens (Bhattacharryya, Goswami & Bhattacharyya, 2016). *Trichoderma* species are promising to plant pathogen management strategies because they are eco-friendly and sustainable (Mousumi Das, Haridas & Sabu, 2019), avirulent plant symbionts, and common on rhyzosphere. They are known by their antagonism against phytophatogens and mycotoxin production (Harman, Howell, Viterbo, Chet & Lorito, 2004; Zhang *et al.*, 2017). There are many reported compounds associated with *Trichoderma*, such as acetylorcinol, alternariol, cerevisterol and scytalones, that causes inhibitory effect on other microrganisms (Zhang *et al*., 2017). Nowadays, more than 60% of biopesticides contain *Trichoderma* isolates in their composition, which put them among the most explored biocontrol agents (López-Bucio, Pelagio-Flores & Herrera-Estrella, 2015).

In addition, *Trichoderma* makes the plant produces compounds on reponse to the fungal invasion, such as phytoalexins, chitinases, glucanases and other metabolites, which induce local or systemic defense responses, consequently promoting seed protection against invasors, enhancing plant growth, and assisting their development and metabolism (Benítez, Rincón, Limón & Codón, 2004; Harman *et al*., 2004; Hermosa, Viterbo, Chet & Monte, 2012).

The aim of this work was to identify and report species of *Fusarium* obtained from fava beans seeds in the state of Paraíba, Brazil, and to evaluate the *in vitro* potential of *Trichoderma* as a biological control agent against *Fusarium*

Materials e Methods

Origin of fava bean seeds

Creole fava beans seeds, variety "Orelha de Vó", were obtained from Alagoa Grande, Remígio, and Campina Grande counties, Paraíba, Brazil, from local producers and commercial markets.

Isolation and identification of Fusarium isolates

The health seeds were evaluated by the Blotter-test method using 10 Petri dishes (15 cm) previously sterilized, with filter paper moistened in distilled and sterilized water, containing 20 seeds per plate, totaling 200 seeds per lot. After seven days of incubation at 25 ± 2 ºC in a Biochemical Oxygen Demand (BOD) incubator, the somatic and reproductive structures of *Fusarium* were identified by optical and stereomicroscope and isolated on PDA medium. Ten *Fusarium* isolates used in this research, from common beans seeds (*Phaseolus vulgaris* L.), and described as Isolate 01 through Isolate 10.

For molecular identification of *Fusarium* isolates, the DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Partial sequences of the chain elongation factor 1 alpha (*TEF1*) were amplified using primers EF1α and EF2 (O'Donnell *et al*., 1998) and the cycling conditions were 8 min at 95 C, followed by 35 cycles of 15s at 95 C, 20 s at 53 C, and 1 min at 72 C, and a final step for 5 min at 72 as described on Santos *et al*. (2019). The sequences generated were deposited in Genbank under accession numbers MW846625, MW846627, MW846631, MW846632, MW846628, MW846624, MW846633, MW846629, MW846626, MW846630, for the isolates 01 to 10, respectively. These sequences were used to perform BLASTn searches in NCBI's GenBank database to determine the complexes to which they belong. After that, sequences were obtained from the databases and aligned using MAFFT v. 7 (Katoh,, Rozewicki & Yamada, 2019). Phylogenetic reconstructions of maximum likelihood were performed using RAxML-HPC2 on XSEDE (8.2.10) (Stamatakis, 2014) on the portal CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2012), with 1000 replications of bootstrap and GTR nucleotide replacement model.

Obtaining Trichoderma isolates

Two *Trichoderma* isolates were obtained from the Laboratory of Phytopathology of the Federal University of Paraíba: *Trichoderma* sp., isolated from cotton seeds (*Gossypium* sp.), and an isolate from the commercial product Trichodel® (*Trichoderma asperellum*).

Evaluation of Trichoderma antagonism in vitro

The antagonism of *Trichoderma* against ten *Fusarium* isolates was evaluated *in vitro* by a direct confrontation assays in Petri dishes, according to Carvalho, Mello, Júnior & Silva. (2011). Discs (5mm) containing the pathogen, were transferred to Petri dishes (90 mm) with PDA medium, three days before antagonist incubation, in opposite positions on the plate. The plates were incubated at 25 ± 2 ºC and a 12-hour photoperiod with fluorescent light.

The colony diameters were measured daily and, based on the index by Bell, Wells & Markham (1982), scores ranging from 1 to 5 were attributed: 1. Antagonist grows and occupies the entire plate; 2. Antagonist grows and occupies part of the pathogen, around 2/3 of the Petri dish; 3. Antagonist and pathogen grow to half of the plate where neither dominates the other; 4. The pathogen grows and occupies part of the antagonist, around 2/3 of the Petri dish; 5. The pathogen grows and occupies the entire Petri dish.

The mycelial growth rate index was evaluated according to Oliveira (1991). The *Fusarium* spore production was evaluated after the 13th day of incubation. The spores were

released with ADE and Tween 20, with subsequent filtering in sterile gauze and adjusting the conidia concentration thru 1.0 x 10^4 conidia / mL^{-1} in the hemacitometer (Alfenas & Mafia, 2016).

The experimental design was completely randomized, with four replicates of both *Fusarium* and *Trichoderma* isolates (10x4). Four Petri dishes for each *Fusarium* isolate were used as a control, with 120 plates in total.

Results e Discussion

Ten *Fusarium* isolates were obtained from fava beans seeds and purified. In BLASTn searches on GenBank, using the generated *TEF*1 sequences, three isolates showed greater similarity with species sequences of the *F. incarnatum-equiseti* species complex (FIESC), while the other seven isolates showed greater similarity with sequences of the *F. fujikuroi* species complex (FFSC). Phylogenies were prepared for each one of these complexes.

In the FIESC, the phylogenetic analysis (Figure 1) showed two isolates in the *F. sulawesiensis* species (= FIESC 16) and one isolate formed a genealogically exclusive lineage that may represent a new phylogenetically species belonging to *F. incarnatum* clade of the FIESC, however, further studies are necessary to present a proper morphological description and species delimitation. The FIESC comprises 38 species, and a few phylogenetically distinct strains (Xia *et al*., 2019), isolated from various biological sources, and often from soil and plants. Among these species are found mycotoxins producers that can cause food contamination and impact the humans and animals health (Avila *et al*., 2019).

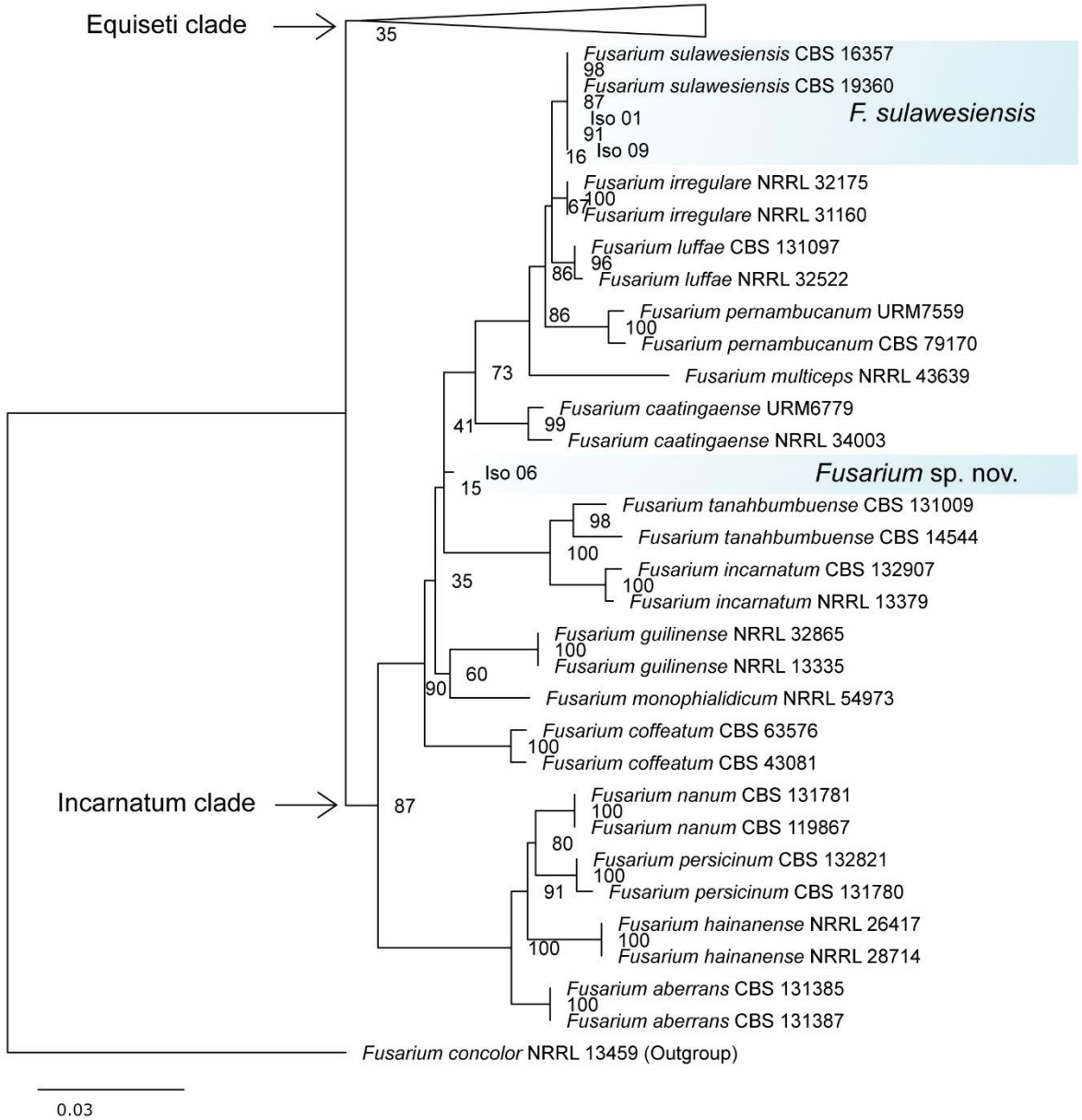

Figure 1. Maximum-likelihood tree inferred from partial *TEF1* sequences from members of *Fusarium incarnatum-equiseti* species complex (FIESC). *Fusarium concolor* (NRRL 13459) was used as outgroup. Numbers of the nodes are Parsimony bootstrap values.

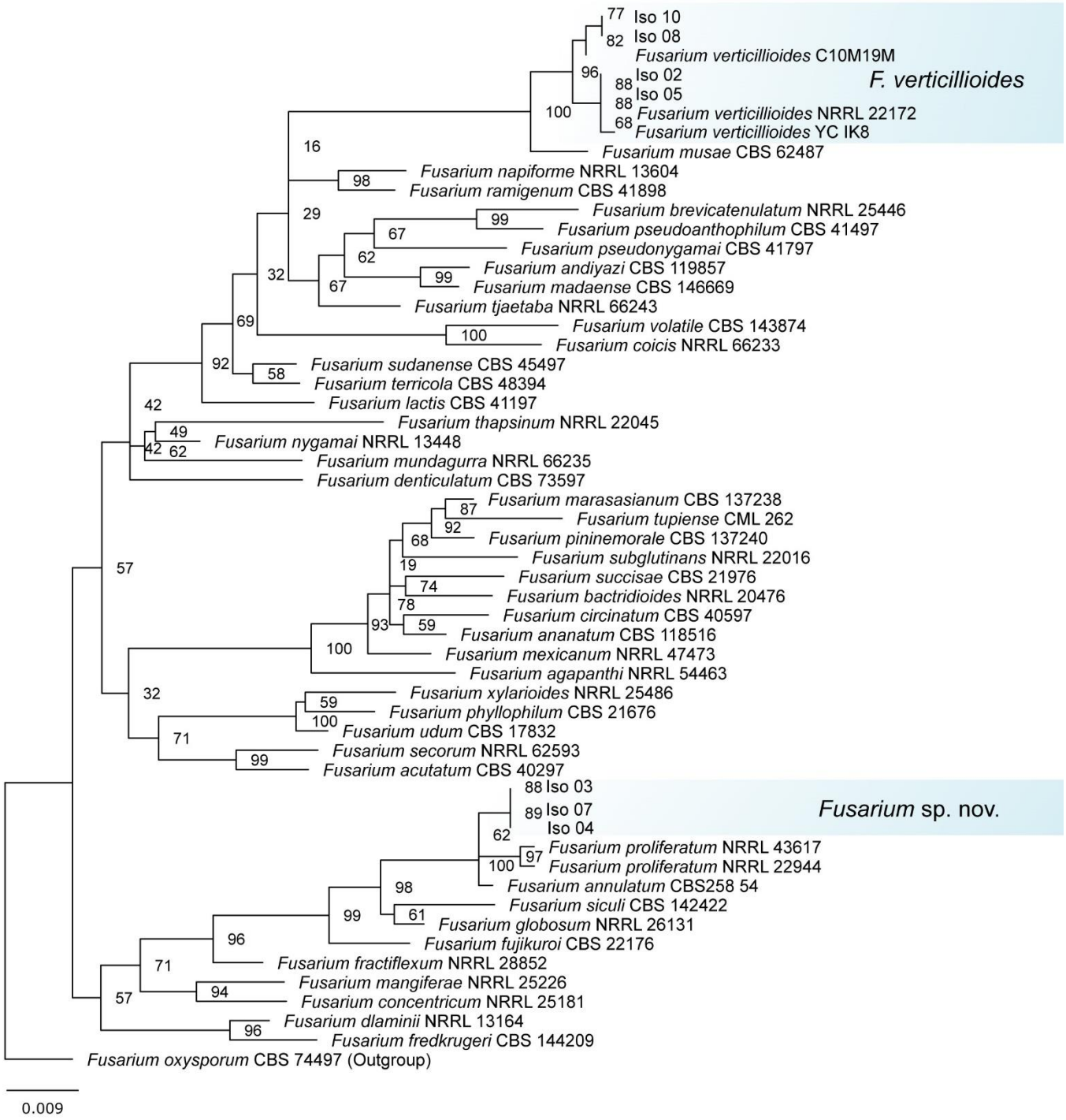

Figure 2. Maximum-likelihood tree inferred from partial *TEF1* sequences from members of *Fusarium fujikuroi* species complex (FFSC). *Fusarium oxysporum* (CBS 74497) was used as outgroup. Numbers of the nodes are Parsimony bootstrap values.

In Brazil, *F. sulawesiensis* has been reported associated with rice (*Oryza sativa* L.) (Avila *et al*., 2019) and melon (*Cucumis melo* L.) (Lima *et al*., 2020). This paper represents the first record of *F. sulawesiensis* associated with fava beans. Other species of the FIESC complex have been recorded in other countries associated with Fabaceae species, for example, *F. equiseti* in *Vicia faba* seeds in Poland (Sadowski, 1988) and *F. duofalcatisporum* in *P. vulgaris* seeds in Sudan (O'Donnell *et al*., 2009).

In the FFSC, the phylogenetic analysis supported four isolates in a clade with sequences of specimens of *F. verticillioides*, a member of the African subclade of the FFSC; and three isolates as a possible new phylogenetic species, genealogically related to *F. proliferatum* and *F. annulatum*, Asian subclade of the FFSC members (Figure 2). Further studies are necessary to present a proper morphological description and species delimitation. Fungi of this complex are known to causes diseases in grains, such as

ear and stalk rot of maize (*Zea mays* L.), bakanae disease of rice (*Oryza sativa* L.), and deterioration of other crops, such as barley (*Hordeum* vulgare L.), soybean (*Glycine max* L.), in addition to producing mycotoxins (Choi *et al.*, 2018).

Fusarium verticillioides is known as one of the main pathogens of maize plants, reducing grain productivity worldwide (Leslie & Summerell, 2006; Watson, Burgess, Summerell & O'keeffe, 2014). Mota *et al*. (2017) and Sousa *et al*. (2020) reported that *F. verticillioides* infects fava beans in Brazil, however, the diagnosis was made based only on morphological characteristics. Thus, this paper comprises the first record of *F. verticillioides* in fava beans in Brazil, based on phylogenetic analyzes.

Trichoderma antagonism analysis

In the direct confrontation test, the two *Trichoderma* isolates inhibited the total growth of *Fusarium* isolates, from the fifth or sixth day of incubation. On the 13th day, the two antagonists occupied the plate completely, in contrast to the most of *Fusarium* isolates (Table 1). Similar results were observed by Rodrigues, Magalhães, Costa & Luz (2018) where *Trichoderma* isolates grew faster than the pathogen, stabilizing their growth in a few days.

All *Fusarium* isolates were inhibited by treatments with *Trichoderma* compared to the control, both on the 7th and 13th day. Comparing the effect of *Trichoderma* sp. and *T. asperellum* on *Fusarium* isolates, on the 7th day, for isolates 04 (*Fusarium* sp. of the FFSC), 06 (*Fusarium* sp. of the FIESC) and 10 (*F. verticillioides*) the greatest inhibition was obtained using *T. asperellum*, and for isolates 01 (*F. sulawesiensis*) and 07 (*Fusarium* sp. of the FFSC), it was using *Trichoderma* sp. For the other five isolates, there was no statistically significant difference between the two treatments with *Trichoderma*. On the 13th day, there was a significant difference among the two treatments just for isolates 01 (*F. sulawesiensis*), 03 and 06 (*Fusarium* sp. of FIESC), which showed the highest inhibition when treated with *Trichoderma* sp (Table 1).

Comparing *Fusarium* isolates, on the seventh day, isolates 08 and 10 of *F. verticillioides* were highly inhibited by *Trichoderma* sp.; and isolate 08 by *T. asperellum*. On the 13th day, all *Fusarium* isolates responded to treatment with *Trichoderma* sp. similarly, but when *Fusarium* isolates were treated with *T. asperellum* differences were observed with the highest inhibitions showing on for all isolates of *F. sulawesiensis* from FIESC and, F. verticillioides, and for two of the three isolates of Fusarium sp. of the FFSC (Table 1).

Table 1. Growth (cm) of *Trichoderma* and *Fusarium* isolates on the paired cultured test method on PDA media, obtained from the notes scale.

Code	*Fusarium* spp. isolate	7 Days		13 Days	
		Trichoderma sp.	*T. asperellum*	*Trichoderma* sp.	*T. asperellum*
1	*F. sulawesiensis*	4.5 ± 0.2^{aA}	2.2 ± 0.8^{bcB}	1.0 ± 0.0^{aB}	1.5 ± 0.2^{cbA}
2	*F. verticillioides*	2.2 ± 0.8^{cA}	2.0 ± 0.0^{cA}	1.0 ± 0.0^{aA}	1.0 ± 0.0^{cA}
3	*Fusarium* sp. (FFSC)	2.0 ± 0.0^{cA}	2.0 ± 0.0^{cA}	1.0 ± 0.0^{aB}	2.0 ± 0.0^{bA}
4	*Fusarium* sp. (FFSC)	2.0 ± 0.0^{cB}	2.7 ± 0.8^{baA}	1.0 ± 0.0^{aA}	1.0 ± 0.0^{cA}
5	*F. verticillioides*	3.0 ± 0.1^{bA}	3.0 ± 0.0^{aA}	1.0 ± 0.0^{aA}	1.0 ± 0.0^{cA}
6	*Fusarium* sp. (FIESC)	2.0 ± 0.0^{cB}	3.0 ± 0.0^{aA}	1.0 ± 0.0^{aB}	3.0 ± 0.0^{aA}
7	*Fusarium* sp. (FFSC)	2.5 ± 0.9^{cbA}	2.0 ± 0.0^{cB}	1.0 ± 0.0^{aA}	1.0 ± 0.0^{cA}
8	*F. verticillioides*	1.0 ± 0.0^{dA}	1.0 ± 0.0^{dA}	1.0 ± 0.0^{aA}	1.0 ± 0.0^{cA}
9	*F. sulawesiensis*	2.0 ± 0.0^{cA}	2.0 ± 0.0^{cA}	1.0 ± 0.0^{aA}	1.0 ± 0.0^{cA}
10	*F. verticillioides*	1.0 ± 0.0^{dB}	2.0 ± 0.0^{cA}	1.0 ± 0.0^{aA}	1.0 ± 0.0^{cA}

Averages followed by the same lowercase letter in the columns and uppercase in the rows, do not differ statistically from each other for the Tukey test at the level of 1% probability ($p < 0.01$). The Tukey test was carried out independently for each evaluated period (7 days and 13 days). The averages of the control on the seventh and thirteenth days were the same (5,00 ± 0,00), reaching the maximum growing on the seventh day.

Based on the paired culture method, the mycelial growth (Table 2) was evaluated until 13th day, the isolates 02(*F. verticillioides*), 03 (*Fusarium* sp. of the FFSC), 04 (*Fusarium* sp. of the FFSC), 05 (*F. verticillioides*), 08 (*F. verticillioides*) and 09 (*F. sulawensiensis*) had their growth significantly reduced by the two treatments when compared to the control. Isolates 01 and 10 had growth affected only by *T. asperellum*, while the growth of the isolates 06 and 07 was not influenced by the treatments.

Carvalho *et al*. (2011) observed similar results, comparing *T. harzianum* and *Fusarium oxysporum* f.sp. *phaseoli* by the pairing culture method, where the averages on the seventh day were lower than control; and on the 13th day, the total colonization of *T. harzianum* was higher than the pathogen.

Due to the aggressive and faster growth, *Trichoderma* colonizes primarly the substrate and shows a better efficiency to absorb nutrients than other fungi (Sood *et al*., 2020) and the competition for carbon and nitrogen, together with competition for position on the substrate, can limit the growth of *Fusarium* (Vinale *et al*., 2008). In addition to affect mycelial growth, the antagonist can also impacts the penetration, progression and colonization of pathogen on the substrate, allied with the production of compounds with fungitoxic response (Harman *et al*., 2004; Boughalleb-M'Hamdi, Salem & M'Hamdi, 2018).

By pairing the culture of *T. harzianum* and *Sclerotium rolfsi*, Jana & Mandal (2017), found that up to 71% growth inhibition of *S. rolfsi*. Mousumi Das *et al*. (2019) confronting *T. harzianum* with *F. oxysporum*, observed inhibition of

78.3% of pathogen growth. The efficiency of *Trichoderma* as an antagonist is due to its rapid development and production of large amounts of conidia, ability to survive in unfavorable environmental conditions; and efficiency in nutrient use. This genus is highly aggressive against pathogens, preventing their development (Benítez *et al*., 2004; Carreras-villaseñor, Sánchez-Arreguín & Herrera Estrella, 2012).

Based on sporulation values (Table 3) of *Fusarium* isolates paired with *Trichoderma*, it was observed that the sporulation of 03 (*Fusarium* sp. of the FFSC), 05 (*F. verticillioides*) and 07 (*Fusarium* sp. of the FFSC) isolates was reduced by *Trichoderma* isolates, whereas isolate 04 (*Fusarium* sp. of the FFSC) a significant reduction was observed when treated only with *T. asperellum*. For the other *Fusarium* isolates, none of the treatments significantly interfered with spore production compared to the control.

The inhibition of sporulation on *Fusarium* isolates, can be explained by the production of compounds associated with growth inhibition, such as koninginins, viridins and trichoviridins (Reino, Guerrero, Hernández-Galán & Collado, 2008). As sporulation is strategic for the spread of fungi in the environment (Huang & Hull, 2017), reduce it increases the efficiency of management and biological control of plant pathogens.

Table 2. Mycelial growth rate index of *Fusarium* spp. submitted to biological control with *Trichoderma* in vitro in PDA medium.

Code	*Fusarium* spp. isolate	Control	*Trichoderma* sp.	*T. asperellum*
1	*F. sulawesiensis*	17.4 ± 0.3^{aA}	13.8 ± 1.0^{bA}	11.6 ± 0.4^{bB}
2	*F. verticillioides*	20.1 ± 2.3^{aA}	4.9 ± 1.3^{cD}	10.1 ± 1.4^{bC}
3	*Fusarium sp.* (FFSC)	15.2 ± 0.8^{aB}	9.7 ± 1.2^{bC}	9.0 ± 1.1^{bC}
4	*Fusarium sp.* (FFSC)	17.1 ± 1.0^{aA}	8.8 ± 1.1^{bC}	15.0 ± 3.2^{bC}
5	*F. verticillioides*	16.0 ± 0.3^{aB}	9.2 ± 0.9^{bC}	9.4 ± 0.8^{bC}
6	*Fusarium sp.* (FIESC)	18.3 ± 0.0^{aA}	14.3 ± 0.3^{bA}	15.1 ± 2.6^{bA}
7	*Fusarium sp.* (FFSC)	10.1 ± 4.2^{aC}	8.8 ± 1.5^{aC}	9.9 ± 0.8^{aC}
8	*F. verticillioides*	17.9 ± 2.2^{aA}	12.6 ± 1.6^{bB}	12.5 ± 0.6^{bB}
9	*F. sulawesiensis*	17.7 ± 0.2^{aA}	11.6 ± 1.1^{bB}	7.0 ± 1.6^{cD}
10	*F. verticillioides*	17.5 ± 0.0^{aA}	14.5 ± 1.1^{bA}	9.7 ± 0.7^{cC}

Averages followed by the same lowercase letter in the columns and uppercase in the rows, do not differ statistically from each other by the Tukey test ($p < 0.05$).

Table 3. Sporulation of *Fusarium* spp. isolates, with paired culture method with *Trichoderma* on the 13th day of in vitro culture (PDA).

Code	*Fusarium* spp. isolates	Control	Conidial number ($1{,}0x10^4$)	
			Trichoderma sp.	*T. asperellum*
1	*F. sulawesiensis*	4.0 ± 0.8^{edA}	0.5 ± 0.2^{cA}	2.5 ± 0.6^{aA}
2	*F. verticillioides*	17.6 ± 3.9^{cebdBA}	31.5 ± 11.9^{bA}	13.1 ± 0.9^{aB}
3	*Fusarium sp.* (FFSC)	129.5 ± 16.1^{aA}	15.9 ± 6.7^{cbB}	12.3 ± 4.1^{aB}
4	*Fusarium sp.* (FFSC)	27.0 ± 5.5^{cbdA}	11.4 ± 6.1^{cbBA}	1.3 ± 0.3^{aB}
5	*F. verticillioides*	38.0 ± 22.9^{cbB}	59.9 ± 10.7^{aA}	4.3 ± 2.4^{aC}
6	*Fusarium sp.* (FIESC)	2.7 ± 0.5^{eA}	0.5 ± 0.5^{cA}	0.0 ± 0.0^{aA}
7	*Fusarium sp.* (FFSC)	39.9 ± 41.1^{bA}	1.8 ± 3.2^{cB}	9.9 ± 1.3^{aB}
8	*F. verticillioides*	16.0 ± 15.5^{cedA}	0.5 ± 0.9^{cA}	0.1 ± 0.1^{aA}
9	*F. sulawesiensis*	5.0 ± 1.4^{edA}	0.7 ± 0.7^{cA}	2.7 ± 1.6^{aA}
10	*F. verticillioides*	1.3 ± 0.6^{eA}	0.06 ± 0.1^{cA}	0.1 ± 0.1^{aA}

Averages followed by the same lowercase letter in the columns and uppercase in the rows do not differ statistically from each other for the Tukey test ($p<0{,}01$).

Rodrigues *et al*. (2018) demonstrated that *Trichoderma* spp. were effective in reducing sporulation in eight of the 12 *Ceratocystis* isolates. Zivkovic, Stojanovic, Ivanovic, Gavrilovic & Popovic (2010) observed that spore suspensions of *T. harzianum* and *Gliocladium roseum* significantly reduced *Colletotrichum acutatum* and *C. gloesporioides* sporulation at 86% and 89%, respectively. The sporulation reduction was observed for some isolates tested in this study. However, the sporulation of six *Fusarium* isolates was not reduced by the two *Trichoderma* isolates tested.

Conclusion

Fusarium isolates obtained from fava beans seeds in Paraíba, Brazil, belong to four species - *F. sulawesien*sis, a possible new phylogenetic species in the *F. incarnatum-equiseti* species complex (FIESC), *F. verticillioides* and a possible new phylogenetic species in the *F. fujikuroi* species complex (FFSC). Further studies are necessary to present a proper morphological description and delimitation of these species. *Trichoderma* sp. and *T. asperellum* isolates show antagonistic potential against *Fusarium* isolates. The two *Trichoderma* isolates tested had a significant impact inhibiting *Fusarium* isolates growth, but had a significant impact on sporulation of only a few isolates. *T. asperellum* is indicated as the most promising for the biological control of most *Fusarium*

isolates obtained from fava beans.

Fava beans, cultivated mainly in semiarid areas by family farmers in Brazil, represents an important source of income for the country. In this study, we identified *Fusarium* species associated with fava beans and indicated ecofriendly alternatives for their control. As we discussed, the treatment with *Trichoderma* consists of an efficient management strategy, which could enhance the potential of this crop, which is mainly affected by phytopathogens. Further, it is important that studies verify the action of these *Trichoderma* isolates on the growth of fava bean seedlings.

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