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

Gabriel Ginane Barreto*, Ana Carla da Silva Santos*, Mirelly Miguel Porcino*, Patrick Materatski*, Carla Varanda*, Maria do Rosário Félix*, Luciana Cordeiro do Nascimento*

* Programa de Pós-Graduação em Botânica, Universidade Estadual de Feira de Santana, Feira de Santana, 44036-900, Bahia, Brasil.
* ginanebarretog@gmail.com
* Programa de Pós-Graduação em Biologia de Fungos, Universidade Federal de Pernambuco, Recife, 50670-901, Pernambuco, Brasil.
* Programa de Pós-Graduação em Agronomia, Universidade Federal do Paraíba, Areia, 58397-000, Paraíba, Brasil.
* Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

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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-a (*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, Souhi, 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 (Bhattacharyya, 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 rhizosphere. They are known by their antagonism against phytopathogens and mycotoxin production (Harman, Howell, Viterbo, Chet & Lorito, 2004; Zhang et al., 2017). There are many reported compounds associated with Trichoderma, such as acetylornicol, alternariol, cerevisterol and scytalone, that causes inhibitory effect on other microrganisms (Zhang et al., 2017). Nowadays, more than 60% of biocerticides 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 response 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, Remigio, 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 °C 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 15 s 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 Trichode³ (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 °C 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

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released with ADE and Tween 20, with subsequent filtering in sterile gauze and adjusting the conidia concentration thru $1.0 \times 10^4$ conidia / mL 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 & Discussion**

Ten *Fusarium* isolates were obtained from fava beans seeds and purified. In BLASTn searches on GenBank, using the generated TEF1 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](image-url). 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 cause diseases in grains, such as...
ear and stalk rot of maize (Zea mays L.), bokanae 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 |
|------|--------------------------|--------|--------|
|      | *F. sulawesiensis*       | 4.5 ± 0.2bA | 2.2 ± 0.8bB |
| 1    | *F. verticillioides*     | 2.2 ± 0.8cA | 2.0 ± 0.0aB |
| 2    | *Fusarium* sp. (FFSC)   | 2.0 ± 0.0aA | 2.0 ± 0.0aA |
| 3    | *Fusarium* sp. (FFSC)   | 2.0 ± 0.0bA | 2.7 ± 0.8bA |
| 4    | *F. verticillioides*     | 3.0 ± 0.1ba | 3.0 ± 0.0aA |
| 5    | *Fusarium* sp. (FIESC)  | 2.0 ± 0.0bB | 3.0 ± 0.0aA |
| 6    | *Fusarium* sp. (FIESC)  | 2.5 ± 0.9bBa | 2.0 ± 0.0bB |
| 7    | *F. verticillioides*     | 1.0 ± 0.0aB | 1.0 ± 0.0aB |
| 8    | *F. sulawesiensis*      | 2.0 ± 0.0aB | 2.0 ± 0.0aA |
| 9    | *F. sulawesiensis*      | 1.0 ± 0.0bB | 2.0 ± 0.0aA |
| 10   | *Fusarium* sp. (FFSC)   | 1.0 ± 0.0dA | 1.0 ± 0.0aA |

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 FIESC), 05 (*F. verticillioides*), 08 (*F. verticillioides*) and 09 (*F. sulawesiensis*) 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 primarily 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* (Vinales 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 rolfsii*, Jana & Mandal (2017), found that up to 71% growth inhibition of *S. rolfsii*. 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-Arreguin & 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 koningins, 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. sultawesiensis*       | 17.4 ± 0.3aA | 13.8 ± 1.0bA | 11.6 ± 0.4bB |
| 2    | *F. verticillioides*      | 20.1 ± 2.3aA | 4.9 ± 1.3cd           | 10.1 ± 1.4bc          |
| 3    | *Fusarium* sp. (FFSC)    | 15.2 ± 0.8bB | 9.7 ± 1.2bc           | 9.0 ± 1.1bc          |
| 4    | *Fusarium* sp. (FFSC)    | 17.1 ± 1.0aA | 8.8 ± 1.1bc           | 15.0 ± 3.2bc          |
| 5    | *F. verticillioides*      | 16.0 ± 0.3bB | 9.2 ± 0.9bc           | 9.4 ± 0.8bc          |
| 6    | *Fusarium* sp. (FIESC)   | 18.3 ± 0.0aA | 14.3 ± 0.3ab          | 15.1 ± 2.6ab          |
| 7    | *Fusarium* sp. (FFSC)    | 10.1 ± 4.2bc | 8.8 ± 1.5ac           | 9.9 ± 0.8ac          |
| 8    | *F. verticillioides*      | 17.9 ± 2.2aA | 12.6 ± 1.6bB          | 12.5 ± 0.6bB          |
| 9    | *F. sultawesiensis*       | 17.7 ± 0.2aA | 11.6 ± 1.1bB          | 7.0 ± 1.6dD          |
| 10   | *F. verticillioides*      | 17.5 ± 0.0aA | 14.5 ± 1.1bA          | 9.7 ± 0.7cC          |

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 (1.0x10⁶) |
|------|--------------------------|---------|--------------------------|
|      |                          |         | *Trichoderma* sp. | *T. asperellum* |
| 1    | *F. sultawesiensis*       | 4.0 ± 0.8bA | 0.5 ± 0.2aA       | 2.5 ± 0.6A |
| 2    | *F. verticillioides*      | 17.6 ± 3.9bB      | 31.5 ± 11.9bA    | 13.1 ± 0.9bB         |
| 3    | *Fusarium* sp. (FFSC)    | 129.5 ± 16.1tA | 15.9 ± 6.7tB     | 12.3 ± 4.1tB         |
| 4    | *Fusarium* sp. (FFSC)    | 27.0 ± 5.5bB      | 11.4 ± 6.1tB     | 1.3 ± 0.3tB          |
| 5    | *F. verticillioides*      | 38.0 ± 22.9bB     | 59.9 ± 10.7tA    | 4.3 ± 2.4tA          |
| 6    | *Fusarium* sp. (FIESC)   | 2.7 ± 0.5aA       | 0.5 ± 0.5tA      | 0.0 ± 0.0tA          |
| 7    | *Fusarium* sp. (FFSC)    | 39.9 ± 41.1bA     | 1.8 ± 3.2tB      | 9.9 ± 1.3tB          |
| 8    | *F. verticillioides*      | 16.0 ± 15.5tA     | 0.5 ± 0.9tA      | 0.1 ± 0.1tA          |
| 9    | *F. sultawesiensis*       | 5.0 ± 1.4cA       | 0.7 ± 0.7tA      | 2.7 ± 1.6tA          |
| 10   | *F. verticillioides*      | 1.3 ± 0.6tA       | 0.06 ± 0.1tA     | 0.1 ± 0.1tA          |

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, Gavriloivc & Popovic (2010) observed that spore suspensions of *T. harzianum* and *Gliocladium roseum* significantly reduced Colletotrichum acutatum and *C. gloeosporioides* 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. sultawesiensis*, 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.

References
Alfenes, A.C., & Mafia, R.G. (2016). Métodos em Fitopatologia. (2a ed.) – Viçosa (MG): Ed. UFV.

Avila, C. F., Moreira, G. M., Nicollo, C. P., Gomes, L. B., Abreu, L. M., Pfennig, L. H., et al. (2015). *Fusarium incarnatum-equiseti* species complex associated with Brazilian rice: Phylogeny, morphology and toxigenic potential. *International Journal of Food Microbiology*, 306. doi: 10.1016/j.ijfoodmicro.2019.108267

Benítez, T., Rincón, A.M., Limón, M.C. & Codón, A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology*, 7(4), 249-260. doi: 10.4172/2169-0111.1000e110

Bell, D.K., Wells, H.D., Markham, B.A. (2015). *Trichoderma* and of its genes. *Scientia Horticulturae*, 196, 109-123. doi: 10.1016/j.scienta.2015.08.043

Miller, M.A., Pfiffeir, W. & Schwartz, T. (2012). The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In: Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the extreme to the campus and beyond, 1-8. Association for Computing Machinery, USA.

Mota, J.M., Melo, M.P., Silva, F.F.S., Sousa, E.M.J., Souza, E.S., Barguil, B.M. et al. (2017). *Fusarium* species complex isolated from Korean cereals. *Brazilian Journal of Biosystems Engineering*, 11(1), 79-87. doi: 10.18011/bioeng2017v11n1p79-87

Nascimento, L.C., Medeiros, J.G.F. (2015). Patologia das sementes: noções básicas. João Pessoa: Editora da UFPB.

O’Donnell, K., Kistler, H.C., Cigelnik, E., Ploetz, R.C. (1998). *Fusarium oxysporum* species complex isolated from Korean rice. *Phytopathology*, 88, 35-44. doi: 10.1094/phyto-67-392

Oliveira, J.A. (1991). *Trichoderma* as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. *Science Horticulturae*, 196, 109-123. doi: 10.1016/j.scienta.2015.08.043

O’Donnell, K., Sutton, D.A., Rinaldi, M.G., Gueidan, C., Crous, P.W., & Geiser, D.M. (2009). Novel Multilocus Sequence Typing Scheme Reveals High Genetic Diversity of Human Pathogenic Members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology*, 47(12), 3851-3861. doi: 10.1128/jcm.01616-09

Oliveira, J.A. (1991). Efeito do tratamento fungicida em sementes no controle de tombamento de plântulas de pepino (Cucumis sativus L.) e pimentão (Capsicum annuum L.). *Revista Brasileira de Fitopatologia*, 47(5), 583-595. doi: 10.1590/S1516-89132007000500022

Reino J.L., Guerreiro R.F., Hernández-Galán R. & Collado I.G. (2008). Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochemistry Reviews*, 7, 89-123. doi: 10.1007/s11101-006-9032-2

Rodrigues, G.S., Magalhães, D.M.A., Costa, A.M. & Luz, E.D.M.N. (2018). Antagonismo de *Trichoderma* spp. ao agente etiológico da Marcha de Ceratoctis em cacau. *Anna Pl. *Biocatalysis and Agricultural Biotechnology*, 17, 177-183. doi: https://doi.org/10.1016/j.cbab.2018.11.021

Rodrigues, G.S., Magalhães, D.M.A., Costa, A.M. & Luz, E.D.M.N. (2018). Antagonismo de *Trichoderma* spp. ao agente etiológico da Marcha de Ceratoctis em cacau. *Anna Pl. *Biocatalysis and Agricultural Biotechnology*, 17, 177-183. doi: https://doi.org/10.1016/j.cbab.2018.11.021
Barreto, et al. – control of Fusarium spp. on Phaseolus lunatus

Sadowski, S. (1988). Occurrence of broad bean (Vicia faba L.) diseases in Olsztyn-Elb ag and Bydgoszcz Provinces. Acta Agrobotanica. 41(2), 245–255. doi: 10.5586/aa.1988.014

Santos, A.C.S., Trindade, J.V.C., Lima, C.S., Barbosa, R.D.N., Costa, A. F., Cameiro-Leão M. P. & Tiago P. V., (2019). Morphology, phylogeny, and sexual stage of Fusarium caatingaense and Fusarium pernambucanum, new species of the Fusarium incarnatum-equiseti species complex associated with insects in Brazil. Mycologia, 111(2), 244-259. doi: 10.1080/00275514.2019.1573047

Silva-Flávio, N. S. D. S., Sales, N.L.P., Aquino, C.F., Soares, E.P.C., Aquino, L.F.S. & Catão H. C. R. M. (2014). Qualidade sanitária e fisiológica de sementes de sorgo tratadas com extratos aquosos e óleos essenciais. Semina: Ciências Agrárias, 35(1), 7-20. doi: 10.5433/1679-0359.2014v35n1p7

Sousa, M.J.O., Almeida, F.A., Leite, M.L.T., Fonseca, W.L.F., Lopes, K.P., Gomes C. D. L., Sampaio E. G., Santos, E. da N. & Gondim A. R. de O. (2020). Biocidal potential of some organic by-products on sanitary and physiological quality of red and white fava beans seeds. Australian Journal of Crop Science, 14(3), 462-468. doi: 10.21475/ajcs.20.14.03.p1997

Sood, M., Kapoor, D., Kumar, V., Sheteiwy, M. S., Ramakrishnan, M., Landi, M. et al. (2020). Trichoderma: The “Secrets” of a Multitalented Biocontrol Agent. Plants, 9(6), 762. doi: 10.3390/plants9060762

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), 1312–1313. doi: 10.1093/bioinformatics/btu033

Summerell, B. A., Leslie, J. F. (2011). Fifty years of Fusarium: how could nine species have ever been enough? Fungal Diversity, 50(1), 135-144. doi: 10.1007/s13225-011-0132-y

Summerell, B. A. (2019). Resolving Fusarium: Current status of the genus. Annual Review of Phytopathology, 57(1), 323-339. doi: 10.1146/annurev-phyto-082718-100204

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Wooka, S.L., Lorio, M. (2008). Trichoderma–plant–pathogen interactions. Soil Biology & Biochemistry, 40(1), 1-10. doi: 10.1016/j.soilbio.2007.07.002

Xia, J.W., Sandoval-Denis, M., Crous, P.W., Zhang, X.G. and Lombard, L. (2019). Numbers to names—restyling the Fusarium incarnatum-equiseti species complex. Persoonia: Molecular Phylogeny and Evolution of Fungi, 43(1), 186-221. doi: 10.3767/persoonia.2019.43.05

Watson, A., Burgess, L. W., Summerell, B. A., & O’keeffe, K. (2014). Fusarium species associated with cob rot of sweet corn and maize in New South Wales. Australasian Plant Disease Notes, 9(1). doi: 10.1007/s13314-014-0142-1

Zivkovic, S., Stojanovic, S., Ivanovic, Z., Gavrilovic, V. & Popovic, T. (2010). Screening of antagonistic activity of microorganisms against Colletotrichum acutatum and Colletotrichum gloeosporioides. Archive of Biological Science, 62 (3), 611-623. doi: 10.2298/abs1003611z

Zhang, J., Chen, G.-Y., Li, X.-Z., Hu, M., Wang, B.-Y., Ruan, B.-H. Hao Z., Li-Xing Z., Zhou J., Ding, Z. & Yang,., (2017). Phytoxic, antibacterial, and antioxidant activities of mycotoxins and other metabolites from Trichoderma sp. Natural Product Research, 31(23), 2745–2752. doi: 10.1080/14786419.2017.1295235

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