

## In vitro confrontation of *Trichoderma* spp. isolates with phytopathogenic and non-phytopathogenic fungi

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### ABSTRACT

The aim of this study was the confrontation of *Trichoderma* spp. with pathogenic and non-pathogenic fungi. The *Trichoderma* spp. and filamentous fungi (*Fusarium solani*, *Alternaria* sp., *Colletotrichum gloeosporioides*, and *Aspergillus niger*) were transferred to potato-dextrose-agar (PDA). The confrontation was performed using Petri dishes inoculated with mycelial disks in opposite position (9 mm) of *Trichoderma* spp. and filamentous fungi. After inoculation the plates remained under 25 ° C regime to 12 hours light for a period of seven days. After this period was evaluated qualitatively according to the scale of Bell et al. (1982), which consists of the suitability scores for the area percentage of the culture medium under expression of antagonism. The isolate of *C. gloeosporioides* (jatropha) was isolated which had statistically slightly inhibited growth by *Trichoderma* spp., Unlike the isolated *Alternaria* sp. (parsley) was isolated micelial fungi suffered the most antagonism. Statistically the *Trichoderma* isolate derived from pineapple promoted the highest antagonistic activity against isolates of pathogenic fungi tested, being a candidate for use in biocontrol.

**Palavras-chave:** plant, biological control, antagonism.

### Confronto *in vitro* de isolados *Trichoderma* spp. com fungos fitopatogênicos e não fitopatogênicos

#### RESUMO

O objetivo deste trabalho foi realizar o confronto de isolados de *Trichoderma* spp. com fungos fitopatogênicos e não fitopatogênicos. Os isolados de *Trichoderma* spp. e de fungos filamentosos (*Alternaria* sp., *Aspergillus niger*, *Bipolaris* sp., *Colletotrichum gloeosporioides* e *Fusarium solani*) foram repicados em meio batata-dextrose-ágar (BDA). O confronto foi realizado utilizando placas de Petri, sendo inoculado em posições opostas discos de micélio (9 mm) de *Trichoderma* spp. e fungos filamentosos. Após a inoculação as placas permaneceram sob regime de 25 oC, à 12 horas de luz, por um período de sete dias. Após este período avaliou-se qualitativamente de acordo com a Escala de Bell, que consiste na adequação de notas para a porcentagem de área do meio de cultura sob de expressão do antagonismo. O isolado de *C. gloeosporioides* (pinhão manso) foi o isolado que estatisticamente teve seu crescimento pouco inibido pelos isolados de *Trichoderma* spp., ao contrário do isolado de *Alternaria* sp. (salsa) foi o isolado filamentoso que mais sofreu antagonismo. Estatisticamente o isolado de *Trichoderma* oriundos de abacaxi promoveu a maior atividade antagonista contra os isolados de fungos fitopatogênicos testados, sendo um candidato para uso no biocontrole

**Key words:** planta, controle biológico, antagonismo.

## 1. Introduction

Fungi belonging to the genus *Trichoderma* sp. Pers. (1794) have significant economic importance for agriculture, for its use as a biological control agent of many cultivated plants, and also acting in promoting growth by inducing plant resistance to diseases (MOHAMED; HAGGAG, 2006). This fungus has the ability to degrade cell walls from other fungi using their enzymes, as well as the ability to produce antifungal substances. Such survival strategies make them competitive and proliferating in the rhizosphere (RESENDE et al., 2004; SAMUELS, 2006; BELL et al., 1982; CHET et al., 1997).

The 297 species of *Trichoderma* already described (INDEX FUNGORUM, 2015) are opportunistic, symbionts of plant roots, large producers of antibiotics (KUMAR et al., 2012), strong soil competitors, saprophytic agents (MOREIRA; SIQUEIRA, 2006), and many of these species are antagonists of other fungi and bacteria, and that includes plant pathogens, with their morphological structures (AMORIM; PASCHOLATI, 2011), which are hardly attacked by other microorganisms (BEDENDO et al., 2011).

These fungi have several ways of interacting like antibiosis, hyperparasitism, competition and growth promotion (MELO, 1996). Lorito et al. (1993) point out that these characteristics are essential for their use as biocontrol agents.

Biological control methods should be based on the selection of antagonistic microorganisms and may be in vitro or in vivo. In vitro tests allow us to recognize the growth and the mechanisms of action, which facilitates the observation of antagonistic interactions between the fungus and the plant pathogen (LIU et al., 2009; LOUZADA et al., 2009; CARVALHO et al., 2011)

Biocontrol can be made indirectly through competition for spaces and nutrients absorption niches by promoting growth in plants, activating defense mechanisms, production of antibiotics or by direct mycopredatism (AGRIOS, 1997; AMORIM et al., 2011).

The biocontrol process depends not only on the antagonist but on the types of crops and environmental conditions such as nutrient availability, pH, temperature and humidity. The activation mechanism is connected to the production of metabolites and other specific compounds as plant growth, hydrolytic enzymes, antibiotics, and permeases C and N (BENITEZ et al., 2004).

Phytopathogenic microorganisms have coexisted with plants since the beginning of agriculture, causing economic damage and losses. Traditional methods to control these pathogens has been the use of chemical pesticides and fungicides, which has caused drastic environmental impacts. One of the most promising ways

of achieving the reduction of the use of these chemicals until a future replacement is the use of biological control agents (ACBs) (HOWELL, 2002; ZIMAND et al., 1996).

The search for more sustainable alternatives for the exploitation of natural resources (agriculture) makes the replacement of chemical control tactics by technically appropriate options, economically viable and socially acceptable (MACEDO, 2000). There are 55 registered commercial products in the world that have as active ingredient strains of *Trichoderma* spp. representing an improvement and expansion of control possibilities using biological control tools (BETTIOL et al., 2012).

The use of biological products against pathogens has been widely studied (KUMAR et al., 2012; CARVALHO et al., 2011; LIU et al., 2009; LOBO JUNIOR et al., 2009; LOUZADA et al., 2009; HOITINK et al., 2006; FOSTER, 1950), because these can reduce the human actions of conventional agriculture, allowing for a more sustainable system of agricultural cultivation. The use of biocontrol agents reduces this environmental impact by the biodegradability and the no aggression of the environment (BETTIOL et al., 2012).

Most commercial products with *Trichoderma* spp. as active ingredient are being released or in registration phase at the Ministry of Agriculture of Brazil (MAPA, 2014). Hence, the importance of this antagonist as a sustainable alternative to regular use and long-term action to control plant pathogenic fungi.

The objective of this research was to evaluate the in vitro antagonism by *Trichoderma* isolates on pathogenic and non-pathogenic fungi.

## 2. Material and Methods

The tests were performed in 2014 in the Microbiology and Plant Pathology Laboratory at the Federal Institute of Goiás, Campus Urutaí, Urutaí-GO, using isolates belonging to Mycological Reference Collection.

### 2.1. Preparation and incubation of isolated filamentous fungi

Fourteen isolates belonging to Mycological Reference Collection were used in this study. These isolates had different hosts as a source of origin. The isolates that were maintained on silica gel were transferred to Petri dishes containing potato-dextrose-agar (PDA) to obtain patterns cultures, being 7–10 days of growth under conditions of 25 ° C and photoperiod of 12 hours of light. The *Trichoderma* isolates are listed in Table 1. During the pairing were used as positive control, two commercial isolates of *Trichoderma* (SC Trichodermil 1306<sup>®</sup> and Quality WG<sup>®</sup>), also listed in

Table 1. The matrices dishes of *Trichoderma* were cultured, and then disks of 9 mm diameter were cut out to be deposited next to the edges of the Petri plate containing PDA culture medium.

**Table 1.** List of *Trichoderma* isolates and yours origin host using this experiment\*

Number.	Origin host by <i>Trichoderma</i> isolates
1	Soy (cod. ND)
2	Cotton (cod. ND)
3	Pineapple (cod. 589)
4	Eucaliptus (cod. ND)
5	Algodãozinho (cod. 210)
6	Corn (cod. 15)
7	Jackfruit (cod. ND)
8	Peach palm (cod. ND)
9	“Guatambu do Cerrado” (cod. 62)
10	“Araticumzinho” (cod. 215)
11	Ficus (cód. 208)
12	“Sucupira” (cod. 563)
13	<i>Trichoderma asperellum</i> (Sólido - Quali WG®) (cod. 597)
14	<i>Trichoderma harzianum</i> (líquido - Trichodermil SC1306®) (cod. 588)

\* Cod. ND isolate without register in Collection; Cod. number represent de number registered in collection.

## 2.2. Preparation and incubation of isolates filamentous fungi

In order to obtain matrices, monosporic cultures in PDA medium were peaked from: a) *Fusarium solani* – peach palm (Code 607); b) *Alternaria* sp. – parsley (Code 593.); c) *Colletotrichum gloeosporioides* - Jatropa (without register); d) *C. gloeosporioides* – peach palm (Code 067).; e) *Bipolaris* spp. – braquiaria, f) *Aspergillus niger* – jack bean (Code 377). The first four of these isolates are phytopathogenic and last one is non-pathogenic. These fungal strains served as matrices for delivery of mycelium, and after incubation for a period of 7-10 days at 25 °C under a photoperiod of 12 hours, disks were cut and delivered on the opposite side of a Petri plate containing PDA medium, consisting of two mycelia discs, an antagonist (*Trichoderma* spp.) and other filamentous fungus.

## 2.3. In vitro pairing test between mycelial fungi

The confrontation was performed using mycelium disks, except for the *Aspergillus niger* isolate, which used the method of "Direct Attach" - (ALFENAS; MAFIA, 2007) for of the opposing plates to *Trichoderma* isolate. The experiment consisted of two replicates represented by 14 isolates of *Trichoderma* spp., matched with five strains of phytopathogenic and

non-phytopathogenic fungi, being a completely randomized design, represented by 140 experimental units, or 140 Petri dishes containing combinations of *Trichoderma* isolates and filamentous fungi.

The experiment was evaluated using the scale of Bell et al. (1982) for analyzing the proportion of antagonism between two paired fungi in culture medium, which was represented by grade 1, when *Trichoderma* pathogen grows completely over the covering surface of the medium; grade 2 when *Trichoderma* covered at least two thirds of the surface of the culture medium; grade 3 when *Trichoderma* and pathogen colonizing each half of the petri dish, with no dominance of microorganisms; grade 4 when the pathogen colonizes at least 2/3 of the surface of the culture medium, but *Trichoderma* resists colonization; and grade 5, the pathogen grows over *Trichoderma* and occupies the entire surface of the culture medium.

## 2.4. Data analysis

Data analysis consisted in qualitative interpretation of notes and the frequency of repetitions which have ratings in different grades analyzed by phytopathogenic and non-phytopathogenic fungi (Figure 1) and frequency rating of antagonism between the pairs of isolates (Figure 3), and the use of factorial ANOVA hypothesis testing factor 1 types of filamentous fungi and factor 2 source hosts of *Trichoderma* isolates and mean comparison by Tukey test of the values of antagonism notes generated from the scale of Bell et al. (1982). The values of the notes when they had normal distribution were transformed by  $\sqrt{(x + 10)}$ .



**Figure 1.** Example growth and different confrontation classified according Bell scale (Bell et al., 1982). Note 1. The *Trichoderma* sp. isolate grow up grown completely over the filamentous fungus covering the surface of the medium; Note 2. *Trichoderma* sp. isolate covered at least two thirds of the surface of the culture medium; Note 3. The *Trichoderma* sp. isolate and filamentous fungi colonized each half of the Petri dish, with no dominance; Note 4. The filamentous fungus colonized at least 2/3 of the surface of the culture medium, wherein the *Trichoderma* sp. resists colonization.

### 3. Results and Discussion

Although belonging to the same species, both *Colletotrichum gloeosporioides* isolates showed statistically different behavior from each other, being the isolate from jatropha that presented more aggressive to the micelial growth of *Trichoderma* (Figure 2). Isolates from peach palm statistically suffered the most antagonistic effect, but the *Alternaria* sp. isolate from salsa was statistically most affected by the tested *Trichoderma* isolates (Figure 2).

The *Trichoderma* isolates from milkweed, pineapple and eucalyptus showed a statistically higher antagonistic activity against the phytopathogenic fungi tested (Figure 2), which are important candidates for use in field tests for the identification of antagonist effect efficiency. Under the experimental conditions, commercial *Trichoderma* isolates and isolates from cotton, "araticunzinho", ficus, corn and soy showed the minorantagonistic effects, or are not candidates for effective control of in vitro growth of filamentous fungi (Figure 2).

Isolates of *Fusarium* sp. and *Bipolaris* sp. had their growth strongly inhibited by *Trichoderma* spp. This is equivalent, when considering the area occupied in the culture medium by *Trichoderma* was 66.6% (44.4% for *Fusarium* sp.) hence inhibiting the growth of the phytopathogen. As for *Trichoderma* from milkweed and *Fusarium* sp. from peach palm, the antagonist grew 100% of the area, totally inhibiting the growth of the phytopathogen.

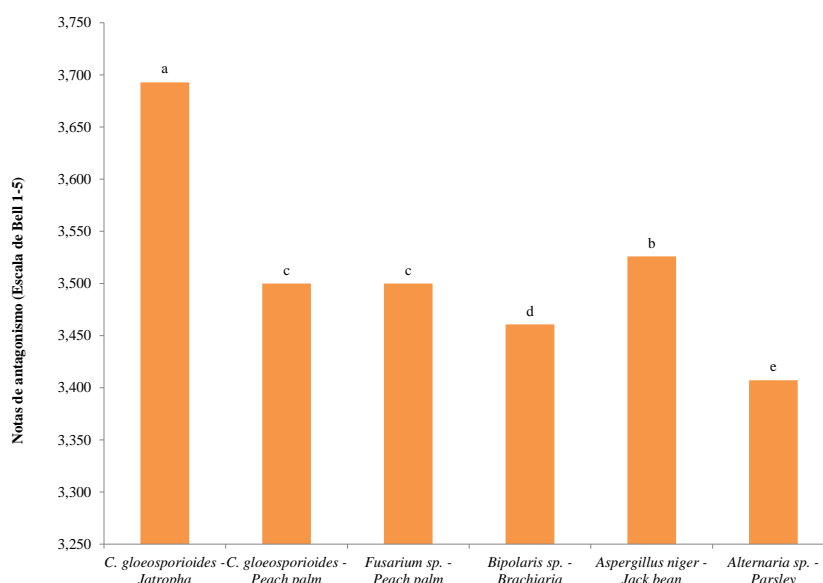
As for *Trichoderma* come from milkweed and *Fusarium* sp. come from peach palm, the antagonist grew by 100 % of the area totally inhibiting the growth of the pathogen.

Isolated *Bipolaris* sp. from braquiaria and *Trichoderma* sp. occupied 66.6% of the culture medium, inhibiting the growth of the phytopathogen, after verifying the original evaluation marks antagonism. Differently, *Trichoderma* sp. derived from eucalyptus and pineapple, grew 100 % of the area, inhibiting the full growth of the phytopathogen.

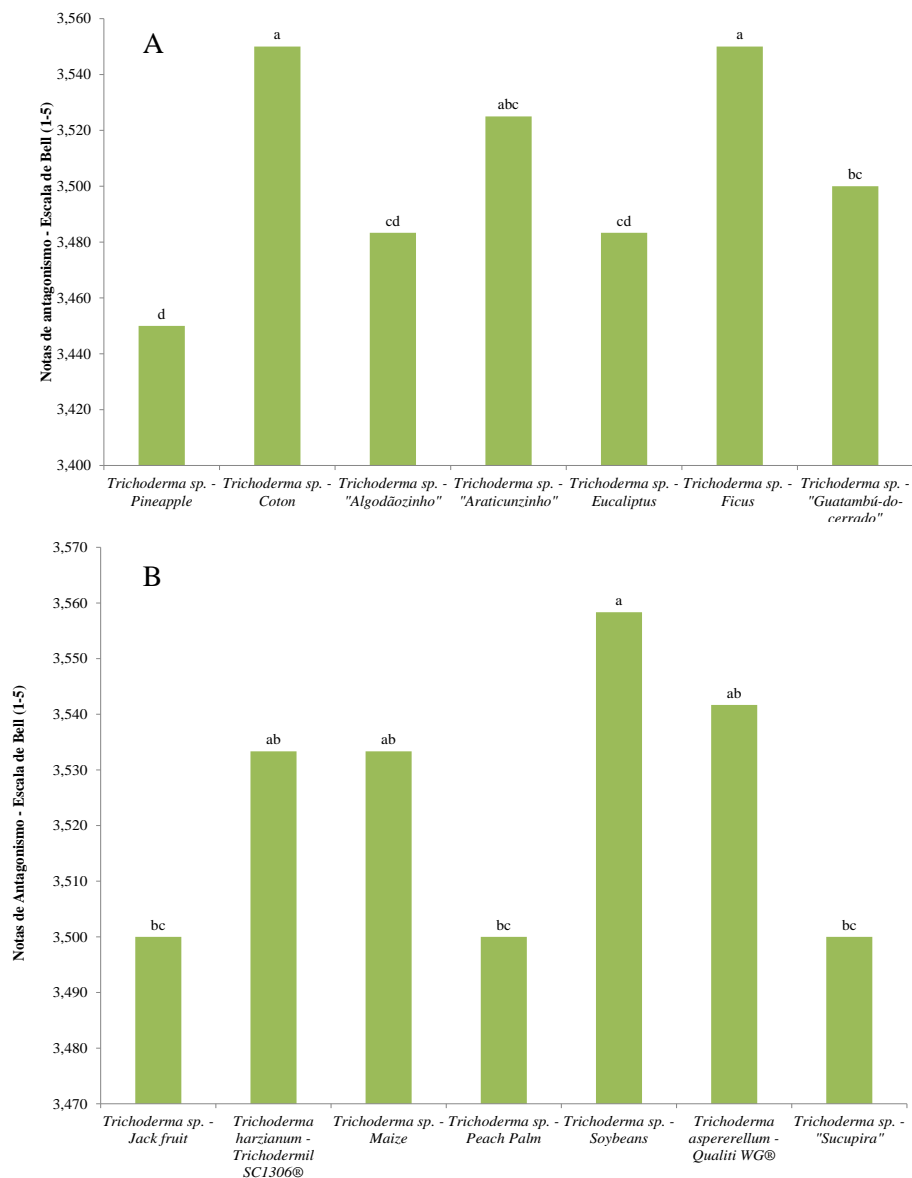
When analyzing horizontally the interactions (Table 2) of the filamentous fungi isolates, we observed there was no significant difference between *Colletotrichum gloeosporioides* (Jatropha), *C. gloeosporioides* (peach palm), *Fusarium* sp. (peach palm); being the opposite for others. As for *Bipolaris* sp. from braquiaria and *Trichoderma* sp. (pineapple), *Trichoderma* sp. (milkweed), *Trichoderma* sp. (eucalyptus), *Trichoderma* sp. ("guatambú-do-cerrado") they showed statistically higher antagonistic effect against phytopathogenic fungi. For *Aspergillus niger* combinations showed statistically higher antagonistic effects.

The isolate *A. niger* derived jack bean, a fungus considered a weak parasite (AMORIM; PASCHOLATI, 2011) derived, demonstrated a variable antagonistic interaction (original data) with the tested *Trichoderma* isolates, behavior not found for phytopathogenic fungi tested. Highlighting the variations of antagonism, *Trichoderma* sp. grew 66,6% in the surface of culture medium in most pairings, inhibiting the growth of *A. niger* and *Alternaria* sp.

Isolated called *Trichoderma* Solid isolated from commercial product Qualiiti WG<sup>®</sup> showed a variation in their antagonism, where *A. niger* derived from jack bean colonized 66.6% of plaque inhibiting *Trichoderma* action that resisted colonization.



**Figure 2:** Average of the transformed ( $\sqrt{x+10}$ ) of notes by antagonism of pathogenic and non-pathogenic fungi studied. (Bars followed by the same letter in Tukey test did not differ in the probability of 5 %).



**Figure 3.** Average of the transformed notes ( $\sqrt{x} + 10$ ) antagonism of all isolates of *Trichoderma* spp. used for comparison (A and B representing a continuation of the data). Values followed by the same letter do not differ in the Tukey test at 5 % probability.

*Trichoderma* by formulation solid isolated from the commercial product in formulation solid Qualiiti WG® showed a variation in its antagonism where *A. niger* derived from jack bean, colonized 66.6% of the plate, thus inhibiting the action of *Trichoderma* that resisted colonization.

With regard to *Trichoderma* sp. from pineapple, ficus, "araticunzinho", eucalyptus, *Trichoderma* formulation liquid (Trichodermil SC1306®), soybean, *Trichoderma* formulation solid (Qualiiti WG®), maize and cotton occupied a surface area of 66.6 % through the plate culture and 33.3 % were colonized by *Alternaria* sp.

Antagonism of *Trichoderma* spp. is explained by the production of broad spectrum antibiotics, such as gliotoxin, viridin, trichodermina, suzucacilina, alamethicin and dermadina, which have the ability to

inhibit the growth of other fungi (DENNIS; WEBSTER, 1971).

In addition to antibiotics, the microorganisms produce enzymes such as cellulase and hemicellulase, lignocellulolytic materials which degrade and cause lysis in the cell wall of pathogenic fungi (MELO, 1996).

Most *Trichoderma* spp. expanded on the plate surface and any combination or repetition was classified by grade 5, which is the score given when filamentous fungus grows on the entire surface of the culture medium by preventing the growth of *Trichoderma* spp. The *Trichoderma* isolates sold and marketed (named solid T and T net) did not express or had highlighted the antagonism.

There was no better *Trichoderma* spp. isolate with antagonistic effect on *Colletotrichum* come from jatropa and peach palm.

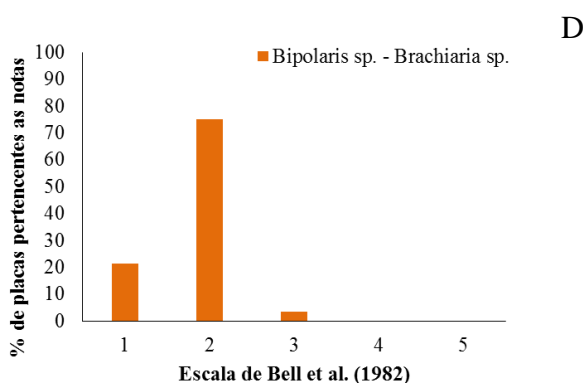
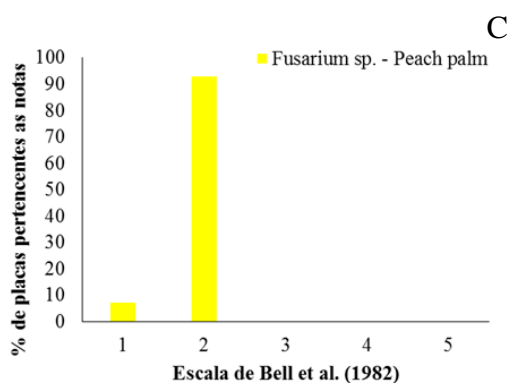
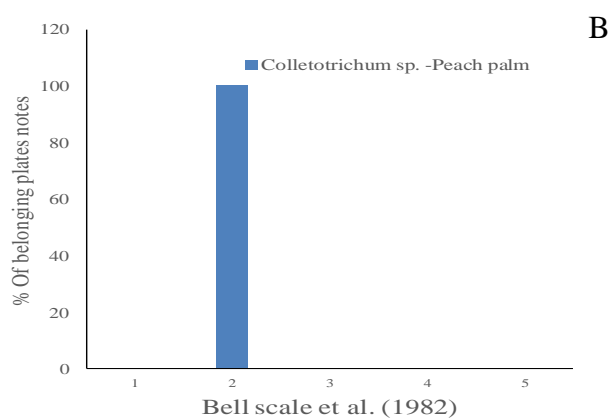
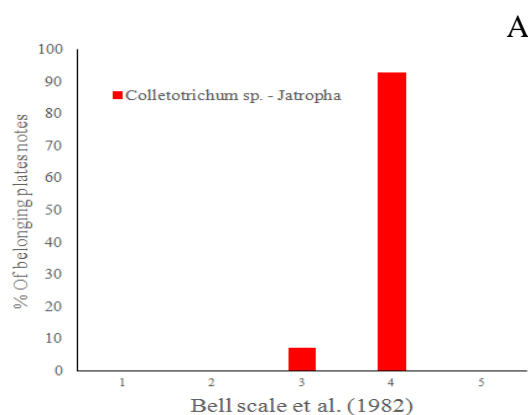
**Table 2.** Averages transformed interaction ( $\sqrt{x + 10}$ ) of the factors types of pathogens and types of *Trichoderma*\*

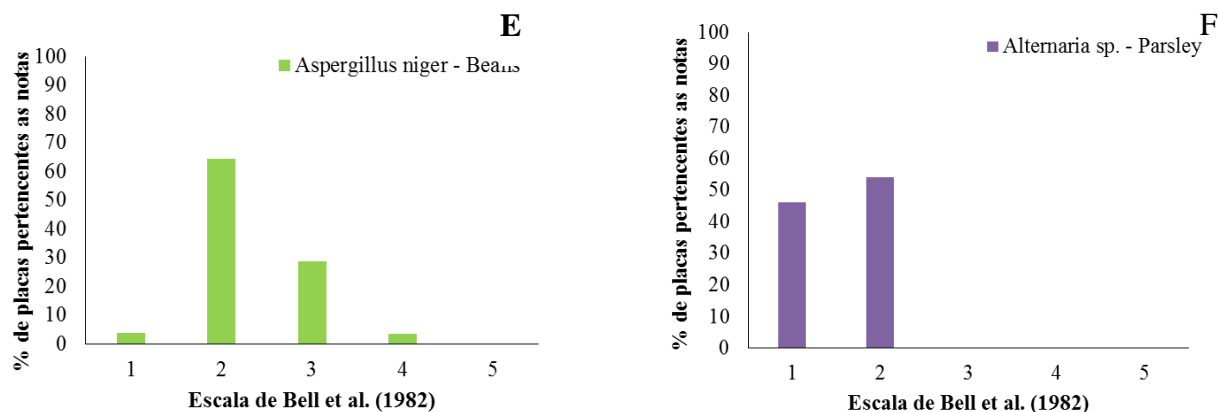
<i>Trichoderma</i> sp.	Pineapple	Cotton	Algodãozinho	Araticunzinho	Eucalyptus	Ficus	Guatambu-do-cerrado
<b>Plant pathogen</b>							
<i>Colletotrichum gloeosporioides</i> - Jatropha	3,70 aA	3,70 aA	3,70 aA	3,65 aA	3,70 aA	3,70 aA	3,70 aA
<i>Colletotrichum gloeosporioides</i> - Peach palm	3,50 bA	3,50 cA	3,50 cA	3,50 bA	3,50 bA	3,50 cA	3,50 bA
<i>Fusarium</i> sp.- Peach palm	3,50 bA	3,50 cA	3,50 cA	3,50 bA	3,50 bA	3,50 cA	3,50 bA
<i>Bipolaris</i> sp.-Braquiária	3,30 dB	3,50 cA	3,30 dB	3,50 Aa	3,30 dB	3,50 bA	3,50 bA
<i>Aspergillus Níger</i> -Jack bean	3,40 cB	3,60 bA	3,60 bA	3,50 bAB	3,50 bAB	3,60 bA	3,50 bAB
<i>Alternaria</i> sp.- Parsley	3,30 dB	3,50 cA	3,30 dB	3,50 bA	3,40 cAB	3,50 cA	3,30 cB

## Continuation

<i>Trichoderma</i> sp.	Jackfruit	<i>Harzianun</i> .- Trichodermil SC 1306®	Corn	Peach palm	Soybean	<i>Asperellum</i> - “Sucupira” Qualiti WG®	
<b>Plant pathogen</b>							
<i>Colletotrichum gloeosporioides</i> - Jatropha	3,70 aA	3,70 aA	3,70 aA	3,70 aA	3,70 aA	3,65 aA	3,70 aA
<i>Colletotrichum gloeosporioides</i> - Peach palm	3,50 bA	3,50 bA	3,50 bA	3,50 bA	3,50 cA	3,50 bA	3,50 bA
<i>Fusarium</i> sp.- Peach palm	3,50 bA	3,50 bA	3,50 bA	3,50 bA	3,50 cA	3,50 bA	3,50 bA
<i>Bipolaris</i> sp.-Braquiária	3,50 bA	3,50 bA	3,50 bA	3,50 bA	3,55 bcA	3,50 bA	3,50 bA
<i>Aspergillus Níger</i> -Jack bean	3,50 bAB	3,50 bAB	3,50 bAB	3,50 bAB	3,60 bA	3,60 aA	3,50 bAB
<i>Alternaria</i> sp.- Parsley	3,30 cB	3,50 bA	3,50 bA	3,30 cB	3,50 cA	3,50 ba	3,30 cB

\*Averages following lowercase in columns followed by the same letter do not differ by Tukey test at 5% probability; Medium uppercase in columns followed by the same letter do not differ by Tukey test at 5% probability.





**Figure 4.** Percentage of antagonistic response classes according to the range of Bell et al. (1982) for the different pathogenic and non-pathogenic fungi. A. *Colletotrichum* sp. Jatropha, B. *Colletotrichum* in Peach palm, C. *Fusarium* sp. in Peach Palm, D. *Bipolaris* sp. in Brachiaria sp., E. *Aspergillus niger* in beans, F. *Alternaria* sp. in Parsley.

#### 4. Conclusions

*Trichoderma* isolates from “algodãozinho”, pineapple and eucalyptus showed higher antagonistic activity against the tested phytopathogenic fungi.

There was no better isolate from *Trichoderma* spp. with antagonistic effect on *Colletotrichum* come from jatropha and peach palm.

For the *Fusarium* isolate from peach palm, the best isolate of *Trichoderma* sp. with antagonistic effect came from “algodãozinho”.

For *Bipolaris* isolate from braquiaria, the best isolate of *Trichoderma* sp. with antagonistic effect came from pineapple and eucalyptus.

For *Aspergillus niger* derived from jack bean, the best *Trichoderma* sp. with antagonistic effect came from pineapple.

For *Alternaria* sp. from parsley, the best *Trichoderma* sp. with antagonistic effects we as isolated from pineapple.

For *Alternaria* isolate from parsley was the most susceptible to the antagonism promoted by *Trichoderma* spp.

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