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ACTION OF TRICHODERMASPP. IN THE CONTROL OF FUNGI THAT CAUSE DISEASES IN PLANTS OF THE CERRADO OF TOCANTINS BIOME, NORTHERN BRAZIL

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ABSTRACT

Fungi of the genus *Trichoderma* are able to act as agents to control the growth of pathogenic fungi that cause diseases in several cultivated plants. Therefore, this work aimed to select and test *Trichoderma*isolates capable of inhibiting, in vitro, the mycelial growth of soil pathogens. 50 isolates of *Trichoderma* spp. were tested against the pathogenic fungi *Sclerotiumrolfsii*, *Rhizoctoniasolani* and *Fusarium*sp. The in vitro culture pairing technique was used, with scores on grades according to the criteria proposed by Bell and percentage of colonization. In the percentage of inhibition of the growth of the pathogen, 23 isolates with more than 60% inhibition were more efficient for *S. rolfssi*, five with 100% inhibition for *R. solani* and 32 isolates with more than 70% for *Fusarium*sp.

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INTRODUCTION

Diseases caused by soil fungi are one of the main causes of low productivity in agricultural crops, and among the main ones are the fungi of the genus Sclerotium, Rhizoctonia and *Fusarium*. The fungus *SclerotiumrolfsiiSacc*.is the cause of Scleroderma or Scleroderma, a disease that damages several species of host plants. *RhizoctoniasolaniKühn* is the main pathogenic fungus responsible for tipping and root rot diseases. Of the genus *Fusarium* the most common species are *F. solani*, *F. oxysporum*, *F. moniliforme* and *F. verticillioidis*. It is a pathogen that destroys the roots of plants such as soybean, such as red rot of the root, which has been causing significant losses in Brazil (ALMEIDA, SEIXAS, 2010, REALE *et al*., 2012; MADALOSSO *et al*., 2015). The use of chemicals in plant disease control is still widely used. However, limiting chemicals as to the efficiency and quantity of products that can be used for this purpose hinders control, since most products are also effective only when applied preventively.

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In addition to environmental damage, due to the high residual power these products present, they can contaminate the soil and water sources (MICHEREFF *et al*., 2005). Fungi of the genus *Trichoderma* spp. has been widely used as an alternative to the use of chemical products and is considered of great economic importance for agriculture, since they are capable of acting as control agents for disease-causing fungi in several cultivated plants, such as growth promoters and resistance inducers of plants to diseases (HOFFMANN *et al*., 2015, CHAGAS *et al*., 2016). *Trichoderma* has become one of the most researched fungi in laboratory, greenhouse and field conditions (Chagas JR *et al*., 2012, TANČIĆ *et al*., 2013, HOACHMANN *et al*., 2015, PACHECO *et al*., 2016, CHAGAS *et al*. 2016) as an alternative agent for the biological control of plant diseases, in the search for native isolates with potential for biological control and formulation of inoculants. However, the selection of isolates of *Trichoderma* spp. must be constant, especially in regions of economically important crops such as soybean, corn, rice and common bean, in the Cerrado region, where studies are scarce. The objective of this work was to select isolates of *Trichoderma* spp. originating from areas of the Cerrado of Tocantins to inhibit the growth of plant pathogens from the same region of the state of Tocantins.

MATERIAL AND METHODS

We used 50 *Trichoderma* isolates, originally obtained from soil samples collected in areas at the experimental station of the Federal University of Tocantins (UFT), Gurupi University Campus (11 ° 43'45 "S and 49 ° 04'07" W, 300 m of mean altitude) and in floodplain areas of the municipality of Lagoa da Confusão - TO (10 ° 47'37 "S and 49 ° 37'25" W, 200 m average altitude), withdrawn at a depth of 0-10 cm in the soil profile of different crops and planting forms. The isolates were identified taking into account only the morphological characteristics (BARNETT; HUNTER, 1998) and kept in a refrigerator in BDA medium and preserved in water, according to Castellani methodology (PIRES *et al*., 2012). The fungi *Sclerotiumrolfsii*, *Fusarium* sp. and *Rhizoctoniasolani* isolated from soybeans from plants cultivated in the southern region of Tocantins State and with typical symptoms of the disease were obtained from the laboratory of Phytopathology Laboratory of UFT - Campus de Gurupi, To, compare the antagonistic potential of *Trichoderma* spp. A standard *Trichodermaharzianum* strain (CIB T44) was obtained from the InstitutoBiológico de São Paulo (ICB).In order to evaluate the antagonistic potential, the pathogen colonization technique and biological controller were used, described by Mariano (1993). Aseptically transferred to Petri dishes containing 20 mL of the BDA culture medium, a 0.4 cm diameter disk of each phytopathogen and the antagonist *Trichoderma* spp. Placed 1.5 cm from the edge of the plate on opposite sides. For the control, a 0.4 cm diameter disk of the phytopathogen or the antagonist was transferred to the center of the petri dishes containing BDA medium and the colonies were not matched. Plates were incubated in a BOD type chamber at 25 ± 2 ° C, with photoperiod of 12 hours. After seven days, the percentage of colonization according to Camporota (1985) methodology was evaluated, where:% C = DT / DE x 100, where DT is the growth rate of the colony of *Trichoderma* spp. in the frontal direction to the colony of the pathogen and DE, the distance separating the two colonies. An evaluation was also performed according to the criteria proposed by Bell *et al*. (1982) and adaptations with scales varying from 1 to 5: Note 1 antagonist grows throughout the Petri dish (87.6 to 100%); note 1.5 antagonist grows on 7/8 of the plaque (66.6 to 87.5%); note 2 antagonist grows on 2/3 of the plaque (62.5 to 66.5%); note 2.5 antagonist grows on 5/8 plaque (51 to 62.4%); note 3 antagonist and pathogen grow to the middle of the plaque (50%); 3.5 antagonist grows on 3/8 of the plate (37.5 to 49.9%); Note 4 antagonist grows on 1/3 of the plaque (33.3 to 37.4%) and note 5 antagonist does not grow on petri dish (percentage below 33.2%). The isolate was considered as antagonistic or efficient when its score was less than or equal to 2.0. All treatments were conducted in triplicate with a completely randomized design. The percentage results estimated according to the growth fraction on the plaque were submitted to analysis of variance and in case of significance, the means were compared by the Scott-Knott test at 1% probability, using the statistical program ASSISTAT version 7.6 beta.

RESULTS AND DISCUSSION

Of the 50 isolates of *Trichoderma spp*. and *Trichodermaharzianum* (standard), 19 inhibited the mycelial growth of *S. rolfsii*, by the crop pairing test (grades less than or equal to 2.0), being significantly ($p < 0.01$) higher than the others, besides the other four isolates were also significantly ($p < 0.01$) higher, even though they had a 2.5 mark, colonizing more than 60% of the petri dish, according to Table 1.

Identification	*S. rolfsii*		*R.solani*		*Fusarium*sp.	
	Note	%	Note	%	Note	%
UFT 06	1,5	69,3 a	2,0	62,7 d	1,5	76,5 a
UFT 09	1,5	66,7 a	2,5	59,7d	1,5	75,2 a
UFT 10	2,0	66,3 a	3,5	37,7 f	1,5	72,5 a
UFT 12	2,0	62,7 a	2,5	61,0 d	1,5	85,1 a
UFT 14	2,0	65,3 a	2,5	54,0 d	1,5	79,1 a
UFT 15	4,0	37,0 d	2,0	63,0 d	3,5	38,8 c
UFT 18	2,5	53,7 b	2,5	56,3 d	1,5	75,6 a
UFT 19	2,5	62,3 a	2,0	63,0 d	1,5	81,5 a
UFT 20	2,0	64,3 a	5,0	31,3 f	1,5	83,5 a
UFT 22	3,5	40,7 d	2,5	55,7 d	1,5	84,7 a
UFT 23	1,5	71,0 a	2,5	57,3 d	1,5	81,0 a
UFT 24	3,5	40,0 d	4,0	34,7 f	1,5	81,8 a
UFT 26	2,0	63,0 a	3,5	45,3 e	1,5	76,7 a
UFT 28	2,0	63,7 a	1,0	100 a	1,5	72,2 a
UFT 32	2,5	56,0 b	1,0	100 a	3,5	44,4 c
UFT 33	3,5	49,7 c	1,5	75,3 c	1,5	82,6 a
UFT 34	1,5	69,7 a	3,5	40,3 f	1,5	81,2 a
UFT 35	2,5	57,0 b	1,5	79,7 c	1,5	79,9 a
UFT 36	2,0	63,3 a	4,0	37,3 f	1,5	77,5 a
UFT 37	1,5	69,0 a	4,0	34,3 f	1,5	66,8 b
UFT 38	4,0	33,7 d	5,0	27,3 f	3,5	49,3 c
UFT 41	3,5	48,3 c	1,5	71,7 c	1,5	78,4 a
UFT 45	2,5	61,3 a	1,5	76,3 c	1,5	78,6 a
UFT 46	2,0	64,3 a	3,5	41,0 f	1,5	73,1 a
UFT 48	2,5	58,0 b	2,0	64,0 d	1,5	81,3 a
UFT 56	4,0	33,7 d	1,5	73,0 c	1,5	78,9 a
UFT 57	2,5	58,3 b	1,5	72,0 c	2,0	65,3 b
UFT 63	1,5	67,0 a	1,0	88,3 b	1,5	76,4 a
UFT 67	2,5	54,7 b	2,5	54,7 d	1,5	76,9 a
UFT 70	2,5	60,7 a	1,5	68,0 c	3,5	49,7 c
UFT 74	3,0	50,7 b	1,5	70,3 c	3,5	49,0 c
UFT 78	2,5	56,7 b	1,0	88,3 b	3,5	49,4 c
UFT 79	2,0	64,0 a	2,5	56,7 d	3,5	48,9 c
UFT 80	2,0	64,0 a	3,5	38,3 f	1,5	81,4 a
UFT 85	2,0	66,0 a	1,5	87,0 b	1,5	73,5 a
UFT 86	1,5	72,3 a	2,5	61,3 d	1,0	94,4 a
UFT 87	2,5	59,3 b	3,5	45,0 e	3,5	48,6 c
UFT 92	2,5	58,3 b	1,5	85,3 b	1,5	82,0 a
UFT 95	3,5	37,7 d	2,5	52,3 d	1,5	81,5 a
UFT 96	2,5	62,3 a	1,0	100 a	1,5	66,9 b
UFT 99	5,0	28,3 d	3,5	48,3 e	2,0	63,7 b
UFT 100	2,5	53,7 b	3,5	38,3 f	2,0	63,6 b
UFT 102	2,5	56,7 b	1,0	100 a	2,0	65,4 b
UFT 104	2,0	63,0 a	3,5	39,3 f	1,5	81,4 a
UFT 110	2,5	58,0 b	1,0	100 a	2,0	63,9 b
UFT 111	2,5	53,0 b	1,5	82,3 c	1,5	84,0 a
UFT 201	2,5	59,3 b	3,5	45,3 e	2,0	65,1 b
UFT 204	3,5	47,7 c	3,5	48,3 e	1,5	72,8 a
UFT 205	2,5	55,3 b	3,5	45,3 e	2,0	63,0 b
T. harzianum	2,5	52,0 b	3,5	47,7 e	3,0	50,8 c

* Means followed by the same lowercase letter in the column do not differ statistically from one another by the Scott-Knott test ($p < 0.01$).

The matched culture test between the pathogen *S. rolfsii* and the antagonist showed that each *Trichoderma* isolate used different forms of antagonism. In the UFT 37 isolate, a halo (dark color) appeared along the contact line between the colonies of the antagonist and the pathogen (Figure 1A). Tančić *et al*. (2013), also obtained positive results of inhibition of *S. Rolfsii* in most of the studied isolates, colonizing more than 70% of the pathogen. Pacheco *et al*. (2016) also verified the inhibition of S. rolfsii*sclerotia* germination by *Trichoderma* spp. in the laboratory. Some *Trichoderma* strains also sporulated abundantly when grown on the *S. rolfsii* pathogen colony as occurred with the UFT 70 isolate (Figure 1B), which appears with less sporulation when grown alone on the plate and well sporulated when in contact with the pathogen.

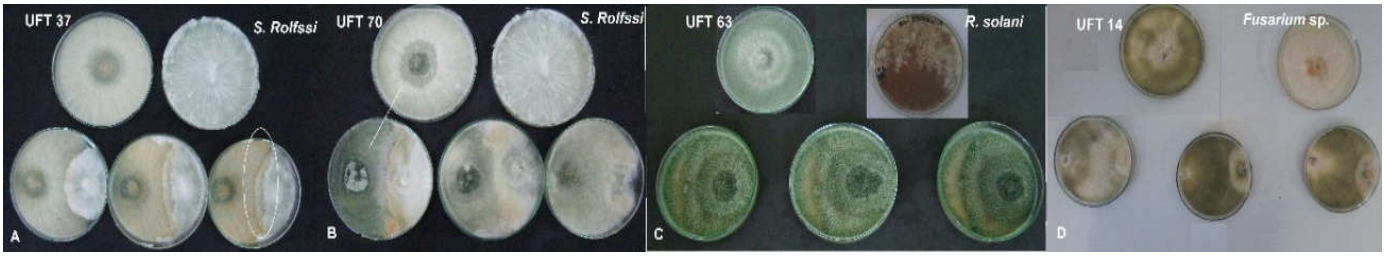

Figure 1. A) *Trichoderma* UFT 37 isolate (left) on the control of *S. rolfsii* (right) presenting a darker coloration halo along the line of contact between the colonies of the antagonist and the pathogen. B) *Trichoderma* UFT 70 isolate (on the left) showing greater sporulation when in contact with the *S. Rolfsii* pathogen (on the right). C) *Trichoderma* UFT 63 isolate (left) in *R. solani* control (right). D) *Trichoderma* UFT isolate 14 (left) in the control of *Fusarium* sp. (on the right)

This has shown the reproduction capacity of *Trichoderma*spp. even during competition for space, and depending on the form of attack that the pathogen suffers. Isaías *et al*. (2014) observed that *T. harzianum* presented a maximum grade (note 1) of antagonism of the scale of Bell *et al*. (1982), totally inhibiting the in vitro growth of *S. rolfsii* and *V. dahliae*. Among the isolates of *Trichoderma* spp., 22 inhibited the mycelial growth of *R. solani* (less than or equal to 2.0) (Figure 1C). The other 27 isolates plus the standard strain were considered inefficient with grades larger than 2.0, ie, occupying less than 62.7% of the petri dish (Table 1). The isolates UFT 110, UFT 102, UFT 32, UFT 28 and UFT 96 were significantly ($p < 0.01$) antagonistic efficient when compared to the others, occupying 100% of the plaque and receiving a grade equal to 1.0. In addition, the isolates UFT 63 and UFT 78 also obtained a grade equal to 1.0, with mycelial growth occupying 88.3% of the plaque. This mycelial growth efficiency confirms the agility of some strains in space competition. Space competition may induce the development of parasitic mechanisms by *Trichoderma* spp. accelerating its growth against the pathogen (BRITO *et al*., 2010). According to these same authors, the sexual phase of *T. harzianum*, denominated *Hypocrealixii*, presented better performance in *R. solani* control than *T. asperellum*, of a commercial product, due to the rapid colonization of the medium.

For *Fusarium* sp., 41 isolates of *Trichoderma* spp. inhibited the mycelial growth of this fungus, with grades less than or equal to 2.0. Of these, 32 isolates were significantly ($p < 0.01$) antagonistic efficient, with a grade varying from 1.0 for the UFT 86 isolate and 1.5 for the others (Table 1). Nine isolates were considered inefficient with a mark higher than 2.0, occupying less than 60% of the petri dish. Moraga-Suazo *et al*. (2011) also observed a higher growth rate on the plaque by *Trichoderma* spp. evaluating its antagonistic effect, in vitro, on *Fusariumcircinatum*. Some isolates of *Trichoderma*spp. also sporulated more when in contact with the pathogen *Fusarium* sp. (Figure 1D). In a control study of *Fusariumoxysporum*, in vitro, Carvalho *et al*. (2011) also observed that three isolates of *T. harzianum* after contact with the colonies of *Fusariumoxysporum*, totally invaded the colony of the pathogen, producing spores on them, showing the potential of some species of *Trichoderma* in the antagonism of this pathogens. In the tests of antagonism in paired culture it can be observed that by the evaluation of note by the method of Bell *et al*. (1982), four isolates (UFT 06, UFT 28, UFT 63 and UFT 85) were efficient antagonists for all phytopathogens studied in this study, with a score of less than or equal to 2.0. It was also observed that some isolates were efficient (less than or equal to 2.0) for one pathogen and showed inefficiency for others, such as UFT 79 and UFT 206 isolates that were efficient for *S. rolfsii* and inefficient for the others pathogens. The isolates UFT 15, UFT 32, UFT 70, UFT 74 and UFT 78 were efficient for the control of R. solani, but inefficient for the other pathogens studied. And the isolates UFT 18, UFT 22, UFT 24, UFT 67, UFT 95, UFT99, UFT 100, UFT 201, UFT 204 and UFT 205 efficient in the control of *Fusarium* sp. and inefficient for the other isolates. These results show that fungi *Trichoderma* spp. are used of different forms of antagonism, presenting greater or less aggression in the control of soil phytopathogens depending on the defense activity exerted by the phytopathogen on it and their physical structure and also by the production of inhibitory substances. The results obtained demonstrate the potential of Trichoderma species in the biological control of plant pathogens commonly found in the region, such as *S. rolfsii*, *R. solani* and *Fusarium* sp. Perspectives on the use of these species in commercial formulations designed to control plant diseases are promising, since edaphoclimatic differences are directly related to the biological activity efficiency of these fungi.

Conclusion

23 isolates of *Trichoderma* spp. were efficient with more than 60% inhibition against *S. rolfsii*, five isolates were efficient with 100% inhibition against *Rhizoctoniasolani* and 32 isolates were efficient with more than 70% inhibition against *Fusarium sp*.

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