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RESEARCH ARTICLE

TRICHODERMAASPERELLUM UFT 201 ON BIOCONTROL, GROWTH PROMOTION AND SOYBEAN PRODUCTIVITY AT FIELD CONDITIONS

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ABSTRACT

Considering the difficulties the producers have to control the diseases and maintain soybean crop stand, this study aimed to evaluate the efficiency of inoculation of *Trichoderma asperellum* UFT 201 in control of *Rhizoctoniasolani* for the maintenance of stand and productivity of soybean at field conditions. The experiments were conducted during crops 2014/15 and 2015/16. Evaluations were performed at 25 and 50 days after sowing (DAS). Counts were performed in the number of plants in order to check the initial stand and final stand, treatment efficiency with *Trichoderma* and productivity. For the experiments with different dosages of the inoculant based on *Trichoderma asperellum* UFT 201, there were differences between the doses with the best results for the treatments with doses of 2 to 4 kg ha^{-1} at crop 2014/2015 and all doses at crop 2015/2016 and for stand characteristics, *Rhizoctonia* control, plant biomass, evidencing the efficiency of the inoculation of *Trichoderma asperellum* UFT 201, reflecting the increase on productivity.

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INTRODUCTION

Soybean is the most used protein regarding animal feed and the second highest source of vegetable oil in the world (Usda, 2015). According to the 2015/16 world harvest, the USDA estimates a record soybean production of 317.6 million tons, surpassing by 4.8 million tons the last cycle production. This performance is presented at USA, Brazil and Argentina, the largest world producers of it, Brazil had a production of 96.2 million tons in 2014/2015 (Conab, 2015). According to IBGE (2015), soybean crop had increases on productivity in the last Brazilian crops, showing an average productivity of approximately 3.021 kg ha^{-1}, using an area of 31.406 million ha and final production of 94.878 million tons. Despite this increase on overall productivity, there were decrease on productivity in some areas, several factors contribute to this decrease. Disease is one of the main factors. Approximately 40 diseases caused by fungi, bacteria, nematode and virus were identified in Brazil. Among the main diseases, there are those caused by pathogens living in soil, such as *Rhizoctonia* (Madalosso *et al*., 2015). This pathogen may cause several damage with relevant losses in stand and, consequently, in productivity, these losses got larger throughout the last years. *Rhizoctoniasolani* Kühn (teleomorph *Thanatephoruscucumeris* Basidiomycota) is the main pathogenic fungus, responsible for the diseases damping off and root rot. This pathogen has been highlighting its importance, mainly in floodplain and drylandplantings, with high occurrence at Tocantins. This fungus is found in rotation areas of irrigated rice, with common bean and soybean, it became one of the main factors regarding the productivity decrease (Lobo *et al*., 2007). *Trichoderma* fungi are one of the main microorganisms with importance for the biocontrol of diseases in plants. These fungi have great economic importance for agriculture, since they are capable of act as disease control agents of several cultivated plants, as inducers of disease resistance in plants, and as promoters of vegetable growth (Contreras-Cornejo *et al*., 2009; Silva*et al*., 2012; Asuming-Brempong, 2013). Considering the difficulties the producers have to control the diseases and maintain soybean crop stand, this study aimed to evaluate the efficiency of inoculation of *Trichodermaasperellum* UFT 201 in control of *Rhizoctoniasolani* for the maintenance of stand and productivity of soybean at field conditions, in Cerrado, Gurupi – TO.

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MATERIALS AND METHODS

Two field experiments were performed at the Experimental Station of Federal University of Tocantins (Universidade Federal do Tocantins), Campus Gurupi, in harvests 2014/15 and 2015/16. The geographic coordinates of the experimental station are 11º43'45'' S and 49º04'07'' W, average altitude of 287 meters. The local climatic characterization is tropical humid climate, classification type Aw, according to Köppen and Geiger. The experiments were conducted during crops 2014/15, from November 2014 to April 2015; and 2015/16, from December 2015 to April 2016. The preparation of the area was done through the conventional method, using harrow, two leveling operations, in order to standardize the area with the use of leveling grid and furrowing, adopting furrow depth of 10 cm and spaced 50 cm between lines. For the experiment I, crop 2014/2015, before planting, it was collected a composite soil sample and it was performed the chemical and granulometric characterization; in which were found the following values: 0.5 $cmol_c$ dm^{-3} ofCa; 0.2 $cmol_c dm^{-3}$ of Mg; 27.6 mg dm^{-3} of K; 1.5 mg dm^{-3} of P; 0.0 $cmol_c$ dm^{-3} of Al; 0.8 $cmol_c$ dm^{-3} of SB; 19.6% of V; pH in water 5.6 ; 1.0% of organic matter; texture of 72.3, 8.2 and 19.5% of sand, silt and clay, respectively. The chemical attributes of 0-20 cm depth were determined as follows: pH in water – Ratio 1:2:5; P and K – extractor Mehlich 1; Al^{3+}, Ca^{2+} and Mg^{2+} – extractor KCL (1 mol L^{-1}); H + Al – Extractor SMP; SB = sum of exchangeable bases; (T) = Cation Exchange capacity at pH 7.0; V – Basis saturation index; and OM = organic matter (oxidation: $Na_2Cr_2O_7$ 4N + H_2SO_4 10N) (Embrapa, 2009). Based on soil sample, it was performed an application of dolomitic limestone filler with PRNT of 100%, 90 days before planting, for the soil correction at a dosage of 1.2 ton ha^{-1}. In this area, sowing fertilization was performed, based on soil analysis: application of 400 kg ha^{-1} of formulation 5-25. At 30 days after germination, an application of KCl, in coverage, at a dose of 65 kg ha^{-1}, was performed. This fertilization was done manually in planting lines, a day before planting. Each experimental plot consisted of 8 rows of 6 linear meters, space between lines of 0.5 m; totaling 24 m². The space between blocks was 1 meter. Seeds were treated a day before planting, with product based on pyraclostrobin + methyl triophanate + fipronil, being used 100 g for each 50 kg of seed. At the planting day, seeds were inoculated with bacteria of genus *Bradyrhizobiumjaponicum* (SEMIA 5079 and SEMIA 5080), at a dosage of 80 g (one dose)/50 kg seed. Were used treatments with diferente dosages (1, 2, 3 and 4 kg ha^{-1}) of inoculant based on *T. asperellum* UFT 201, and also treatments witness without inoculation (negative control) and a treatment considered positive control, with the inoculation of comercialinnoculant, based on *Trichodermaharzianum* Rifai ESALQ 1306 (2 x 10^{10} viable conidia mL^{-1}, dosage of 1 L ha^{-1}, liquid formulation, with recommendation of 1 L ha^{-1}), registered as a microbiological fungicide (Register MAPA nº 002007) for the control of *Sclerotinia*, *Fusarium* and *Rhizoctonia.*

For the treatment with the use of different dosages, was used the granulated product with the active microorganism *Trichodermaasperelum* UFT201, selected as potential for biocontrol of *Rhizoctoniasolani* (Mela or Damping off) and as growth promoter, formulated with minimal concentration of viable conidia of 2 x 10^8 g^{-1},the application vehicle for it is the sterile millet, applied directly in furrow during planting. The commercial product used as a positive control was used in liquid form, also applied directly in the furrows during planting. The cultivar used was Monsoy 7739 iPro. Fifteen seeds per linear meter were sown, aiming to a final stand of 13 plants per linear meter. During the development of the crop, all necessary phytotechnological and phytosanitary managements were performed according to recommendations of Henning (2009).The control of invasive plants was performed 20 days after planting, in which the soybean was in the V3 stage, using the herbicide Roundup WG at the dose of 1.5 $kgha^{-1}$. In the same application, the control of caterpillars attacking soybean at the initial stage was performed by using the insecticides based on gammacyhalotrin (150 g L^{-1}) and diflubenzuron (240 g L^{-1}) at doses of 50 mL ha^{-1} and 120 $mLha^{-1}$, respectively. For the control of anthracnose (*Colletotrichumtruncatum*) and Asian rust (*Phakopsorapachyrhizi*), the application of the fungicide azoxystrobin + cyproconazole at the dose of 500 mL ha^{-1} was performed in R1. Evaluations were performed at 25 and 50 days after sowing (DAS).Counts were performed in the number of plants in order to check the initial stand and final stand in the useful plot of10 m^2 of the experimental plots of 24 m^2, corresponding to five rows of five linear meters.

With the initial and final stand data, the efficiency in the use of *Trichoderma* in the maintenance of the stand was calculated, using the equation: E (%) = {1. [Ti / Tc]} x100 in which E% = treatment efficiency; Ti = average % of the final stand in treatment i; Tc = average % of the final stand in treatment control (Gava and Menezes, 2012). Grain production was obtained in the central rows of each plot with a useful area of $10m^2$, after the physiological maturation of the plants, when approximately 80% of the pods were dry.T hen, the pods were threshed manually correcting the grain moisture to 14%, being determined the productivity in the same useful area of the experimental plots, estimated for kg ha^{-1}andbags ha^{-1}. The Experiment II, crop 2015/2016 was also performed in the same area of the Experimental Station of the Federal University of Tocantins (Universidade Federal do Tocantins - UFT), Campus Gurupi. The area was also previously prepared through conventional method, with plowing and harrowing. Then, again, liming was performed 60 days before planting, with 1.2 Ton ha^{-1} of dolomite limestone filler with 100% of PRNT. The cultivar used was Msoy 8349 iPro. Twelve seeds per linear meter were used, aiming to a final stand of 10 plants per linear meter. In this experiment II, the fertilization, experimental plots, treatments, experimental design, seed treatment, inoculation with rhizobia, phytotechnical and phytosanitary management were the same ones used for the experiment I.

The evaluations of stand, treatment efficiency with *Trichoderma* and productivity were also similar to experiment I. The evaluations of severity of *Rhizoctoniasolani* and of initial and final stand were also performed. For the evaluation of severity in the plots, the scale of notes for incidence and percentage with symptoms of "Mela" (Van Schoonhovemand Pastor-Corrales, 1987) was used, determining the incidence (0 = no incidence, 1 = with Mela symptom), the assignment of scores as a function of the symptom percentage (1 = no symptom; 3 = up to 30%; 5 = 31 to 60%; 7 = 61 to 90%; 9> 90%) and the severity that determines the percentage of plants with Mela symptoms, in the useful area (4.5 m^2). The plant height, quantity of internodes, number of pods and number of grains per pod, at stage R8, were determined in the lines near the useful area of the experimental plots, using 10 plants per experimental plot, totaling 40 plants per treatment.

For the two experiments, the data were submitted to analysis of variance and to Scott-Knott grouping of averages test, at 5% probability, using the statistical program ASSISTAT version 7.6 beta (Silva, 2008).

RESULTS AND DISCUSSION

For the experiment I, crop 2014/2015, the treatments using *T. asperellum* UFT 201 at dosages of 3 and 4 kg ha^{-1} were superior ($p<0.01$) for the initial stand in relation to the other treatments and to the witness without inoculation. For the final stand, the treatments using the inoculation with *T. asperellum* UFT 201 at dosages of 2, 3 and 4 kg ha^{-1} were superior ($p<0.01$) to the others (Table 1). Regarding survival, treatments with inoculation of *T. asperellum* UFT 201 at different doses presented averages varying from 94.5 to 97.4, being above 7% superior in relation to witness and positive control treatment (Table 1). The efficiency of these treatments with inoculation of different doses of *T. asperellum* UFT 201 varied from 7.7 to 11.9%.The positive control treatment showed negative efficiency in relation to the survival of the plants at stand.

Table 1. Initial stand (IS), final stand (FS), survival, efficiency and productivity of soybean cv. Monsoy 7739 iPro, inoculated with different doses (1, 2, 3 and 4 kgha^{-1}) of inoculant based on T. *asperellum* UFT 201, cultivated at Cerrado in the experimental area of Federal University of Tocantins, CampusGurupi, TO. Crop 2014/2015.[1]

Trichoderma	IS 25 DAP[2]	FS 50 DAP	Surviv.[3] (%)	E (%)[4]	Prod.(Kg ha^{-1})	Bags (ha)
1 kg ha^{-1}	319,0 b	307,0 b	94,5	7,7	2712 a	45,2 b
2 kg ha^{-1}	323,0 b	316,5 a	95,9	11,1	3057 a	51,0 a
3 kg ha^{-1}	328,5 a	319,0 a	97,4	11,9	2916 a	48,6 a
4 kg ha^{-1}	331,5 a	315,0 a	96,9	10,5	3130 a	52,2 a
Witness	295,5 d	285,0 c	87,7	-	1948 b	32,5 d
Positive control[5]	304,5 c	284,0 c	87,4	- 0,4	2387 b	39,8 c
CV (%)[6]	2,2 **	2,4 **	-	-	10,6 **	11,1**

[1] Means followed by the same lowercase letter in the columns do not differ from one another by the Skott-Knott test at 1% significance. [2] DAP = Days after planting. [3] Surviv. = Percentage of plant survival in relation to the expected stand of 325 plants per 10 m^2 (325 thousand plants ha^{-1}). [4] Efficiency in the use of *Trichoderma* in the maintenance of the stand. [5]Commercial product, based on *T.harzianum* propagules (2 x 10^{10} UFC mL^{-1}, dosage of 1 L ha^{-1}). [6]Coefficient of variation.

Regarding productivity, treatments with different dosages of the inoculant *T. asperellum* UFT 201 were superior ($p<0,01$) in relation to treatments positive control and witness without inoculation. Treatments with different dosages of the inoculant *T. asperellum* UFT 201 obtained productivity averages varying from 2712 (45.2 bags ha^{-1}) to 3130 (52.2 bags ha^{-1}) kg ha^{-1}, representing an increase of more than 39% in relation to witness and of 13% in relation to positive control treatment (Table 1). For experiment II, crop 2015/2016, initially was performed the evaluation of incidence of Mela, in which the severity caused by *Rhizoctoniasolani* was significantly influenced by treatments with inoculation of *Trichoderma*. However, even with Mela incidence, the symptoms of occurrence were the lowest in the evaluation scale (up to 30%) (Table 2). Although no significant differences were observed among treatments with inoculation at different dosages of *Trichoderma* and of the commercial product for the control of Mela, these treatments were superior in relation to the witness which showed the highest percentage of the disease incidence.

For the characteristics of height, internodes and number of pods, treatments with *Trichoderma* inoculation and thetreatment control were superior ($p<0.01$) to witness without inoculation (Table 3). Regarding the number of pods, there was no significant difference between the treatments. For the number of grains per plant, treatments with *Trichoderma* inoculation at doses of 2, 3 and 4 kg ha^{-1} were superior ($p<0.05$) in relation to the other treatments (Table 3).

Table 2. Incidence and severity of Mela (*Rhizoctoniasolani*) of soybean plants, cv. Msoy 8349 iPro, inoculated with different dosages of inoculant based on *T. asperelum* UFT 201. Gurupi, TO. Crop 2015-2016.[1]

Treatments	INC[2]	Grade (%)[3]	SEV[4]
1 kg ha^{-1}	1	3	4,8 b
2 kg ha^{-1}	1	3	4,0 b
3 kg ha^{-1}	1	3	4,6 b
4 kg ha^{-1}	1	3	5,7 b
Witness	1	3	11,3 a
Positive control[5]	1	3	6,7 b
CV (%)[6]			13,2 *

1 Means followed by the same lowercase letter in the columns do not differ from one another by the Skott-Knott test at 5% significance.2 INC:Incidence (0 = no incidence, 1 = with mela's symptom); 3Grade: (1 = no symptom, 3 = up to 30%, 5 = 31-60%, 7 = 61-90%, 9> 90%); 4Sev: Severity - percentage of plants with Mela symptom;5 Commercial product based on (2 x 1010 of viable conidia mL-1, dosage of 1 L ha-1). 6 Coefficient of variation.

Table 3. Plant height, quantity of internodes, number of pods and number of grains per pod, at R8, of soybean cv. Msoy 8349 iPro, inoculated with different doses of *Trichodermaasperelum*UFT 201, Gurupi, TO. Crop 2015/2016.[1]

Treatments	Height	Internodes	N pods	N Grains
1 kg ha^{-1}	71,5 a	11,8 a	54,0 a	100,0 b
2 kg ha^{-1}	65,0 a	12,0 a	58,3 a	117,3 a
3 kg ha^{-1}	64,5 a	11,8 a	59,5 a	119,0 a
4 kg ha^{-1}	68,3 a	11,5 a	59,3 a	116,3 a
Witness	56,3 b	10,3 b	48,8 a	81,3 b
Positive control[2]	61,5 a	11,8 a	50,8 a	101,5 b
CV (%)[3]	11,4	4,3	12,0	9,2

[1] Means followed by the same lowercase letter in the columns do not differ from one another by the Skott-Knott test at 5% significance.Average of 10 plants per experimental plot. [2]Commercial product based on *T. harzianum*(2 x $10^{10}$of viable conidia mL^{-1}, dosage of 1 L ha^{-1}). [3]Coefficient of variation.

Treatments using *T. asperelum* UFT 201 at different dosages and the commercial product were superior ($p<0.01$) for the initial and final stand compared to the treatment witness without inoculation (Table 4). But at the initial stand the treatments with the doses of 1, 2 and 3 kg ha^{-1} were superior ($p<0.05$) among treatments with inoculation. The same occurred for the survival percentage, providing efficiency of the treatments with the different doses of *T. asperelum* UFT 201, varying from 17.2 to 21.7% in relation to the witness (Table 4).

Table 4. Initial stand (IS), final stand (FS), survival, efficiency and productivity of soybean cv. Msoy 8349 iPro, inoculated with different dosages of *Trichodermaasperelum* UFT 201, Gurupi, TO. Crop 2015/2016.[1]

Treatments	IS 20 DAP[2]	FS 50 DAP	Surviv.[3] (%)	E (%)[4]	Prod. (Kg ha^{-1})	Bags (ha)
1 kg ha^{-1}	223,5 a	220,2 a	86,7 a	19,3	3018,3 a	50,3 a
2 kg ha^{-1}	226,5 a	221,0 a	87,7 a	20,6	2834,7 a	47,3 a
3 kg ha^{-1}	226,5 a	221,7 a	88,5 a	21,7	3139,0 a	52,3 a
4 kg ha^{-1}	219,5 b	219,0 a	85,2 a	17,2	2848,0 a	47,5 a
Witness	214,2 c	208,9 b	72,7 b	-	2213,3 b	36,9 b
Positive control[5]	218,8 b	216,8 a	82,5 a	13,5	3029,0 a	50,5 a
CV (%)[6]	7,9	5,8	6,7	-	8,6	8,6

[1] Means followed by the same lowercase letter in the columns do not differ from one another by the Skott-Knott test at 5% significance. [2] DAP = Days after planting. [3] Surviv. = percentage of plant survival in relation to the expected stand of 250 plants per 10 m^2 (250 thousand plants ha^{-1}). [4] Efficiency in the use of *Trichoderma* in the maintenance of the stand. [5] Commercial product based on *T. harzianum* (2 x $10^{10}$of viable conidia mL^{-1}, dosage of 1 L ha^{-1}).[6] CV = Coefficient of variation.

Regarding productivity, treatments with different doses of *T. asperelum* UFT 201 and control were superior ($p<0.05$) to the witness treatment (Table 4). The treatments with the different doses of *T.asperelum* UFT 201 and the control treatment obtained averages of productivity varying from 2834.7 (47.3 bags ha^{-1}) to 3139 (52.3 bags ha^{-1}) kg ha^{-1}, representing an increase of 28.1% in relation to the witness (Table 4). The promotion of plant growth through species of *Trichoderma* is not only related to the control of pathogens, since the improvement of plant growth was observed in the absence of any detectable disease and in sterile soil, demonstrating to be an ability independent of the antifungal abilities (Topolovec-Pintariã *et al*., 2013). This can be observed at the experiment in Gurupi, crop 2015/2016 (Table 3), with the inoculation of different doses of *T.asperelum* UFT 201 in soybean, observing superior responses to the plant characteristics of height, internodes, number of pods and number of grains, in relation to thetreatment witness without inoculation. Several metabolic factors produced by *Trichoderma*, such as the solubilization of phosphates, siderophores and auxin may be responsible for the promotion of plant growth, as reported in several studies about the action of *Trichoderma* spp. as growth promoters in crops such as corn, tomato, cucumber, cowpea bean and rice (Gravel *et al*., 2007; Oliveira *et al*., 2012; Asuming-Brempong, 2013; Kotasthane *et al*., 2014). However, the results presented for the experiment in crop 2015/2016 show the efficiency of the treatments with *Trichoderma* inoculation for the biological control of *Rhizoctonia,* in relation to the treatment witness without inoculation. Fungi of the genus *Trichoderma* spp. commonly present mechanisms to control phytopathogens of three types: direct competition for space or nutrients (Vinale *et al*., 2008; Martínez *et al*., 2013), production of antibiotic metabolites of volatile or non-volatile nature (Hermosa *et al*., 2012; Pereira *et al*., 2014) and direct parasitism on other phytopathogens (Kotasthane *et al*., 2014; Carvalho *et al*., 2015). These characteristics make fungi efficient on the biocontrol of diseases caused by soil pathogens that influence the establishment of plants, such as *Rhizoctoniasolani,* that cause damping off, generating losses at initial stand and, consequently, reduction of productivity (Madalosso *et al*., 2015).

The use of microorganisms as biocontrol agents of plant diseases, although much studied lately, it still needs to be better understood in order to achieve its ideal efficiency. The application of microorganisms present in different products is conditioned by several factors, including soil, composition, organic matter and soil nutrients that influence the colonization and survival of these microorganisms in the soil. Isolated samples of *Trichoderma* are beneficial for antagonism and/or promoters of plant growth these characteristics made possible to develop biological products with favorable environmentally characteristics. But the success of these as a product is supported by a precise selection of the strain(s), not only from the physiological point of view, but also by the specificity of use and by a quality system for its production. The versatility, the range of biological mechanisms and the ecological plasticity that these fungi have made them excellent biological controllers.

Conclusion

For the experiments with different dosages of the inoculant based on *Trichoderma asperellum* UFT 201, there were differences between the doses with the best results for the treatments with doses of 2 to 4 kg ha^{-1}at crop 2014/2015 and all doses at crop 2015 / 2016 and for stand characteristics, *Rhizoctonia* control, plant biomass, evidencing the efficiency of the inoculation of *Trichoderma asperellum* UFT 201, reflecting the increase on productivity.

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