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Trichoderma asperellum (UFT201) functions as a growth promoter for soybean plant

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This work is aimed at evaluating the efficiency of *Trichoderma asperellum,* UFT 201 as a growth promoter of soybean plant in the Cerrado biome in Tocantins, Brazil. Two field experiments were performed in two different cities using different concentrations (1, 2, 3 and 4 kg ha^{-1}) (Porto Nacional and Formoso do Araguaia). For inoculation treatments with *Trichoderma asperellum* UFT 201 (comprising the granulated product), there were differences between the doses, with positive results for treatments with doses of 2 to 4 kg ha^{-1}. In two experiments, a dose of 2 kg ha^{-1} was recommended, with similar results to the highest doses used. The strain of *T. asperellum* UFT 201 can be recommended as a plant growth promoter in soybean.

Key words: Bioagent, bioinducer, growth promoter, *Glycine max*.

INTRODUCTION

Among the use of growth promoters, biological treatments have been discussed in scientific circles because these organisms contribution both increased productivity and biological control (Contreras-Cornejo et al., 2016). In the rhizosphere, several microorganisms can promote plant growth and protect the root system from infection by pathogens; included in this group is the fungal genus *Trichoderma* (Machado et al., 2012; Martinez et al., 2013). Species of *Trichoderma* have received scientific and agricultural economic attention for their beneficial actions, such as the ability to promote plant growth through biomass accumulation (Contreras-Cornejo et al., 2016).

Trichoderma, a naturally occurring soil fungus widely studied and frequently used in agricultural production may be formulated as bio-fertilizer and soil inoculant. Some species of *Trichoderma* can promote the growth of plants and increase the germination and emergence rates of seeds (França et al., 2017; Herrera-Jiménez et al., 2018). This is done with a seemingly symbiotic rather than parasitic relationship between the fungus and the plant where the fungus occupies a nutritional niche, such as meeting nutritional needs through the solubilization of phosphates (Contreras-Cornejo et al., 2016) and the

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plant may be impacted through growth or development, there by affecting the productivity of the culture. The fungus' ability to colonize the roots is a key factor for its interference in the growth and productivity of the plant (Rubio et al., 2017; Herrera-Jiménez et al., 2018).

Some strains of *Trichoderma* increase the total area of the root system, enabling greater access to mineral elements present in the soil. Others are able to solubilize and make phosphate rock, iron, copper, manganese and zinc available to the plant (Chagas et al., 2015; Herrera-Jiménez et al., 2018). Additionally, they can improve the active mechanisms of macro and micronutrient absorption and increase plant efficiency in the use of important nutrients such as nitrogen (Machado et al., 2012; Iq and Zhao, 2013; Vinale et al., 2013; Chagas et al., 2015) as well as improve the synthesis of phytohormone growth (Oliveira et al., 2012; Zhang et al., 2013). These processes not only increase the growth of plants but also stimulate respiration, thereby enhancing photosynthesis or photosynthetic efficiency, as well as increase the plant's ability to withstand abiotic stresses such as drought, salinity and high temperature.

Significant results in increased plant biomass have been reported in crops such as soybean (Chagas et al., 2017a) and cowpea bean (Chagas et al., 2016) with the use of *Trichoderma* spp. found in various formulated products as the basis of this fungus on the world market (Woo et al., 2014).

In particular, this result has been observed in terms of root growth promotion, although significant increases have also been observed in aerial vegetative growth, such as the length and thickness of the stem, leaf area, chlorophyll content and yield (size and/or number of flowers and/or fruit) (Hermosa et al., 2013; Studholme et al., 2013). Numerous of hypotheses have been proposed to explain this observation, including improved chemical solubilization and availability (Oliveira et al., 2012) and nutrient uptake by the plant (Contreras-Cornejo et al., 2016).

To achieve higher economic returns for the increase in biomass and productivity of strategic crops, such as soybeans, it is necessary to continue the process of generating information from targeted research that seeks and evaluates innovative management practices. Therefore, this work is aimed to verify the efficiency of *Trichoderma asperellum* UFT 201 as a promoter of plant growth and contributor to soybean productivity in the Cerrado in Tocantins, Brazil.

MATERIALS AND METHODS

Location of experiments

Two field experiments were conducted in the state of Tocantins, Brazil. The first was in the city of Porto Nacional in December 2015 to May 2016 at the Research Station ALX Farias Agro Agricultural Research of the Cerrado LTDA (23°36'45.1 "S to 51°11'01.4" W). The local climatic characterization is defined as a humid tropical climate with classification Aw by Köppen and Geiger, with an average temperature of 26.1°C and 1.622 mm average annual rainfall. The second experiment was conducted in the city of Formoso do Araguaia (11°47'45"S to longitude 49°31'43" W) from May to September 2016. The local climatic characterization is a humid tropical climate with water deficiency (B1wA'a') by Köppen and Geiger. The Formoso do Araguaia region had an annual average temperature of 24.0°C and average rainfall below 80 mm for the period of the experiment.

Soil analysis and fertilization

Before planting, a composite soil sample was collected and physical and chemical characteristics were determined. The following values were found in Porto Nacional: 1.75 cmolc dm^{-3}Ca; 1.15 cmolc dm^{-3} Mg; 66.3 mg dm^{-3} K; 9.4 mg dm^{-3} P; 0.0 cmolc dm^{-3} Al; 1.49 cmolc dm^{-3} SB; 62% V; pH 5.5 in water; 20 g dm^{-3} organic matter, and 73.3, 7.2 and 19.5% sand, silt and clay, respectively. The following values were found in the Formoso do Araguaia: 4.13 cmolc dm^{-3} Ca; 1.96 cmolc dm^{-3} Mg; 35.0 mg dm^{-3} K; 10.7 mg dm^{-3} P; 0.0 cmolc dm^{-3} Al; 6.99 cmolc dm^{-3} SB; 62.3% V; pH 5.7 in water; 3.12% organic matter; and 79.0, 8.8 and 16.2% sand, silt and clay, respectively (Embrapa, 2009). The soil of the experimental area in Porto Nacional was classified as an Oxisol with dystrophic medium texture and in Formoso do Araguaia was rated Hominid Plinth (Embrapa, 2009).

The areas were previously prepared by conventional plowing and harrowing methods. In the experimental station in Porto Nacional, irrigation was performed when necessary by spraying. The irrigation system in the area of the Formoso do Araguaia was subirrigation.

Mineral fertilization was carried out before sowing. In Porto Nacional, 540 kg ha^{-1} 5-25-00 formulation and 100 kg ha^{-1} KCl in the V3 stage was applied. In the area of Formoso do Araguaia, 80 kg P_2O_5 was applied in the form of superphosphate, K_2O, and 60 kg as KCl, based on the soil test and the culture need.

In both experiments, the plots consisted of eight rows at six meters long and spaced 0.5 m (24.0 m²) apart. A useful area of the three-axis portion with a length of 3 m and a total of 4.5 m² was employed.

Design of experiments

The treatments used in the experiments were as follows: Four treatments with different doses of product formulated with *Trichoderma asperellum* UFT201 (1, 2, 3 and 4 kg ha^{-1}) and two control treatments, one a positive control using a commercial product based on *Trichoderma harzianum* (ICB Nutrisolo the BioAgrotec Ltd., with 2 x 10^9 spores mL^{-1}, 1 L ha^{-1} dosage) according to the manufacturer's recommendations and one negative control (no inoculation). For treatment with granular *T. asperellum* UFT201 based product formulated with a minimum concentration of 2 × 10^8 CFU g^{-1}, with the composition millet was directly applied to the planting lines.

The cultivars used in the experiments were TMG 132 in Porto Nacional and CD 2851 IPRO in Formoso do Araguaia. In the experiments, 12 seeds per meter was used to develop a final stand of nine plants per meter. The experimental design was a randomized block design with four replications.

Treatment of soybean seeds

The seeds were treated one day prior to planting with Standak® Top (thiophanate methyl + pyraclostrobin + fipronil) at 50 grams for 100 kg of seeds. On the day of planting, the seeds were inoculated with rhizobia (*Bradyrhizobium japonicum* strain SEMIA 5079 and SEMIA 5080) in a recommended liquid inoculant for soybean with 10^9 cell g^{-1}. The inoculant was applied at a ratio of 500 g of inoculant per 50 kg of seed.

During culture development, all necessary phytotechnical and phytosanitary management was carried. Weed control was conducted 25 days after planting when soybean was at the V3 (three trifoliate) stage using Roundup herbicide at a dose of 1.5 kg ha^{-1}. The same application was performed for caterpillars' infestation of soy at an early stage using Nexide insecticide (Gamacialotrina 150 g L^{-1}) and Diflubenzuron 240 SC (diflubenzuron, 240 g L^{-1}) at doses of 50 and 120 mL ha^{-1}, respectively. For the control of anthracnose (*Colletotrichum truncatum*) and Asian rust (*Phakopsora pachyrhizi*), R1 (beginning flowering) was applied with the fungicide Priori Xtra (azoxystrobin + cyproconazole) at a dosage of 500 mL ha^{-1}.

Evaluations

The initial stand was evaluated at 20 DAP, and the end stand was evaluated at 50 DAP in the floor area (4.5 m^2). The efficiency (E%) of *T. asperellum* UFT 201 inoculation was calculated using the following equation: E (%) = {1 - [Ti / Tc]} x 100, where E% = efficiency of the treatment; % Ti = average final stand in the treatment i; and Tc = average final stand% in the control treatment (Gava and Menezes, 2012).

The plant height, number of internodes, number of pods and number of seeds per pod at stage R8 were determined, by collecting 10 plants per experimental plot, totaling 40 plants per treatment.

Grain yield was obtained in the central rows of each plot with an area of 4.5 m^2 after physiological maturation of plants when approximately 80% of the pods had dried up. Then, the beans were used to manually correct the threshed grain moisture to 14%, with productivity quantified in the useful area and estimated in kg ha^{-1}.

Statistical analysis

Data were subjected to analysis of variance and the Scott-Knott mean test at 5% significance level using the statistical probability ASSISTAT program version 7.6 beta.

RESULTS

Experiment in Porto Nacional

For yield characteristics, the number of pods in treatments inoculated with *T. asperellum* UFT 201 and the positive control (commercial product) were greater ($p < 0.01$) than those in the negative control (control without inoculation). In addition, the number of grains per plant inoculation treatments with doses of 2, 3 and 4 kg ha^{-1} were higher ($p < 0.05$) than those in the other treatments (Table 1).

For the initial and final stand treatments using *T. asperellum* UFT 201 at different dosages and the positive control, the values were greater ($p < 0.01$) than those in the control treatment (Table 2). The same was true for the percentage of survival, showing efficiency of the treatments with different doses of *T. asperellum* UFT 201 ranging from 11.5 to 22.3% compared to that in the control treatment (Table 2). The productivity with treatments with different doses of *T. asperellum* UFT 201 and the positive control was greater ($p < 0.05$) than that of the absolute control treatment (Table 2). The treatments with different doses of *T. asperellum* UFT 201 and the positive control obtained an average productivity ranging from 2983.3 to 3358.4 kg ha^{-1} (Table 2).

Experiment in Formoso do Araguaia

The height characteristics in treatments inoculated with *T. asperellum* UFT 201 at doses of 2, 3 and 4 kg ha^{-1} of *T. asperellum* UFT 201 product were greater ($p < 0.01$) than those of the positive control and the absolute control (Table 3). For the number of pods, treatments inoculated with *T. asperellum* UFT 201 at doses of 1, 2 and 3 kg ha^{-1} of *T. asperellum* UFT 201 were greater ($p < 0.05$) than those in the other treatments. The number of grains per plant treatment with *T. asperellum* UFT 201 inoculation at doses of 2, 3 and 4 kg ha^{-1} was higher ($p < 0.05$) than that of the other treatments (Table 3).

For the initial and final stand treatments using *T. asperellum* UFT 201 at different dosages and the positive control, the values were greater ($p < 0.01$) than that of the absolute control. The same was true for the percentage of survival, showing efficiency of the treatments with different doses of *T. asperellum* UFT 201 varying from 3.4 to 9.2% compared to that of the absolute control (Table 4). The productivity with treatments with different doses of *T. asperellum* UFT 201 and the positive control were also higher ($p < 0.05$) compared to that of the absolute control treatment (Table 4). The treatments with different doses of *T. asperellum* UFT 201 and the positive control obtained medium productivity achieved ranging from 2833 at 3400 kg ha^{-1}, an increase of 7.5% compared to that of the absolute control (Table 4).

DISCUSSION

Inoculation of *T. asperellum* UFT 201, which comprises the granulated product *T. asperellum* UFT 201, at doses 2 to 4 kg ha^{-1} induced increases in plant growth parameters, such as height, which may have favored increased pods per plant and consequently the number of kernels per plant (Tables 1 and 3) in the two experiments in two different regions. These positive effects associated with maintenance of the stand may have favored increased productivity (Tables 2 and 4). These positive effects may be associated with the ability of this strain to promote plant growth due to its solubility capacity and IAA synthesis in the rhizosphere (Oliveira et al., 2012;

Table 1. Growth parameters of soybean cv. TMG 132, inoculated with different doses of *Trichoderma asperellum* UFT 201, Porto Nacional (Crop 2015/2016[1]).

Treatment	Height[2]	Internodes[2]	Npod[2]	Ngrains[2]
1 kg ha^{-1}	56.5[a]	12.3[a]	64.0[a]	95.0[b]
2 kg ha^{-1}	56.0[a]	12.3[a]	60.8[a]	112.0[a]
3 kg ha^{-1}	56.8[a]	13.5[a]	65.8[a]	102.0[a]
4 kg ha^{-1}	54.5[a]	11.8[a]	63.3[a]	103.8[a]
Positive control[3]	54.0[a]	11.5[a]	57.3[b]	89.0[b]
Negative control[4]	49.5[b]	10.5[a]	57.3[b]	93.5[b]
CV (%)[5]	7.7	8.0	13.6	12.5

[1]Means followed by the same letter in the column do not differ by Scott-Knott test at 5% significance level. [2]Mean of 10 plants per experimental plot. [3]Commercial product, *T. harzianum* - based (2 × 10^9 viable conidia mL^{-1}, a dosage of 1 L ha^{-1}).[4]negative control without inoculation of *Trichoderma*.[5]CV = Coefficient of Variation.

Table 2. Initial status (EI), end status (FE), survival (Surv.), efficiency (E) and productivity (Prod.) of soybean cv. 132 TMG inoculated with *Trichoderma asperellum* UFT 201, Porto Nacional (Crop 2015 / 2016[1])

Treatment	EI (20 DAP[2])	FE (50 DAP)	Surv.[3] (%)	E[4] (%)	Prod. (kg ha^{-1})
1 kg ha^{-1}	75.5[a]	70.0[a]	86.4[a]	11.5	3015.4[a]
2 kg ha^{-1}	75.5[a]	74.5[a]	92.0[a]	18.7	3358.4[a]
3 kg ha^{-1}	78.3[a]	76.8[a]	94.8[a]	22.3	3169.2[a]
4 kg ha^{-1}	76.8[a]	72.3[a]	89.3[a]	15.2	3170.0[a]
Positive control[5]	75.3[a]	71.0[a]	87.7[a]	13.2	2983.3[a]
Negative control[6]	73.0[b]	62.8[b]	77.5[b]	-	2527.5[b]
CV (%) [7]	6.8	7.1	8.1	-	8.4

[1]Means followed by the same letter in the column do not differ by the Scott-Knott test at the 5% significance level. [2]DAP = Days after planting. [3] Survival = Plant survival percentage compared to the expected 81 plants stand in 4.5 m^2 (270 hectares^{-1} plants). [4] Efficient use of *Trichoderma* in maintaining the stand. [5] Commercial product, *T. harzianum*-based (2 x 10^9 viable conidia mL^{-1}, a dosage of 1 L ha^{-1}). [6] Negative control without inoculation of *Trichoderma*.[7]CV = Coefficient of Variation.

Table 3. Growth parameters of soybean CD 2851 IPRO inoculated with different doses of *T. asperellum* UFT 201 in Formoso do Araguaia (Crop 2016[1])

Treatment	Height [2]	Internodes[2]	N pod[2]	N grains[2]
1 kg ha^{-1}	49.0[b]	11.2[a]	55.3[a]	90[b]
2 kg ha^{-1}	58.3[a]	11.5[a]	56.0[a]	99[a]
3 kg ha^{-1}	56.0[a]	10.0[a]	56.3[a]	98[a]
4 kg ha^{-1}	55.0[a]	10.3[a]	52.0[b]	95[a]
Positive control[3]	50.0[b]	10.0[a]	52.3[b]	90[b]
Negative control[4]	43.0[c]	9.5[a]	48.3[c]	80[c]
CV (%)[5]	8.1	8.0	8.6	12.5

[1] Means followed by the same letter in the column do not differ by Scott-Knott test at 5% significance level. [2]Average of 10 plants per experimental plot.[3]Commercial product, *T. harzianum*-based (2 x 10^9 viable conidia mL^{-1}, a dosage of 1 L ha^{-1}).[4] Negative control without inoculation with *Trichoderma*.[5]CV = Coefficient of Variation.

Asuming-Brempong, 2013; Contreras-Cornejo et al., 2016).

Some authors report positive results with *Trichoderma* inoculation on soybean, such as Milanesi et al. (2013), Chagas et al. (2017a) and Gonçalves et al. (2018), which concluded that *Trichoderma* act as soybean seedling growth promoters. Guareschi et al. (2012) studied *Trichoderma* spp. via seed treatments (200 mL of 50 kg

Table 4. Initial status (EI), end status (FE), survival (Surv.), efficiency (E) and productivity (Prod.) of soybean cv. CD 2851 IPRO inoculated with *Trichoderma asperellum* UFT201. Formoso do Araguaia, TO. Crop 2016.[1]

Treatment	HEY (20DAP[2])	EF (50DAP)	Surv.[3] (%)	E[4] (%)	Prod. ($kgha^{-1}$)
1 $kg\ ha^{-1}$	80.5[a]	75.0[a]	93.2[a]	6.3	3000[a]
2 $kg\ ha^{-1}$	82.5[a]	79.0[a]	95.8[a]	9.2	3400[a]
3 $kg\ ha^{-1}$	79.3[a]	73.8[a]	93.1[a]	6.2	2833[a]
4 $kg\ ha^{-1}$	78.8[a]	73.5[a]	93.3[a]	6.4	3067[a]
Positive control [5]	78.3[a]	71.0[a]	90.7[a]	3.4	3000[a]
Negative control[6]	75.0[b]	65.8[b]	87.7[b]	-	2633[b]
CV (%)[7]	8.8	8.1	8.1	-	9.4

[1]Means followed by the same letter in the column do not differ by the Scott-Knott test at the 5% significance level. [2] DAP = Days after planting. Survival = Plant survival percentage compared to the expected 81 plants stand in 4.5 m^2 (270 $hectares^{-1}$ plants). [4]Efficient use of *Trichoderma* in maintaining the stand.[5]Commercial product (ICB, 2 x 10^9 viable conidia mL^{-1}, a dosage of 1 L ha^{-1}).[5] Negative control without inoculation of *Trichoderma*.[6] CV = Coefficient of Variation.

seed) and via soil application (1 L ha^{-1}) at 10 days after emergence concluded that with the experimental conditions, the application of *Trichoderma* spp. promoted shoot growth and sunflower and soybean roots.

Pedro et al. (2012) reported that the species *T. harzianum*, *T. theobromicola* and *T. strigosum* were efficient in plant growth promotion and protection against bean anthracnose. Similarly, Santos et al. (2010) observed that the use of *Trichoderma* spp. gave positive results in the increase of fresh and dry matter of passion fruit plants from cuttings.

Other research has demonstrated that seeds treated with *Trichoderma* have played an important role in increasing plant biomass, such as in soybean (Chagas et al., 2017a; Gonçalves et al., 2018), cowpea (Chagas et al., 2016) and rice (Chagas et al., 2017b).

The increase in biomass production may vary according to species and the *Trichoderma* isolate used in the culture as well. The colonization of roots often increases root growth, crop yield, resistance to abiotic stresses and improves the use of nutrients (Rubio et al., 2014).

Studies have shown that strains of *Trichoderma* efficiently promote plant growth via colonization of the rhizosphere in natural and axenic conditions or by providing nutrients to the plant (Machado et al., 2012; Martinez et al., 2013). These findings help in the understanding of the role of *Trichoderma* in natural or cultivated ecosystems and promote their use in agriculture.

Most species of *Trichoderma* behave differently as biocontrol agents and as plant growth promoters. Among the factors that determine the success of the inoculation of *Trichoderma* are the abiotic and biotic conditions. Thus, selected isolates for biological control of pathogens in controlled experimental conditions may be unable to produce the same results under field conditions, similar to the selected isolates as the ability to promote plant growth. This relates to the fact that the establishment and development of conditions on the ground are critical for the organism, as they are subject to different reactions of the host and the environment, which may lead to more effective control or promotion of more variable plant growth than could be obtained with chemicals. In the present study, using the microorganism *T. asperellum* UFT 201 showed significant results regarding the characteristics of plant growth and productivity stands and provided examples of benefits to soybean crop growing areas with these cultures.

Conclusion

For inoculation treatments with *T. asperellum* UFT 201, there were differences between the doses, with positive results for treatments with doses of 2 to 4 kg ha^{-1}. In two experiments, a dose of 2 kg ha^{-1} was recommended, with similar results to the highest doses used. The strain of *T. asperellum* UFT 201 can be recommended as a plant growth promoter in soybean.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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