

***Trichoderma* efficiency in the maintenance and productivity of soybean plants in producing savanna regions, Tocantins, Brazil**

Eficiência de *Trichoderma* na manutenção e produtividade de plantas de soja em regiões produtoras no cerrado, Tocantins, Brasil

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A B S T R A C T

Trichoderma is a natural soil fungus that has been extensively studied and used in agricultural production as a biofertilizer and soil inoculant. Thus, the objective of this work was to evaluate the efficiency of *Trichoderma* in maintenance and productivity of soybean plants in producing savanna regions, Tocantins, Brazil. Experiments were carried out in three regions in the state of Tocantins, Gurupi, Alvorada and Crixas. The experimental design was performed in a randomized block with four replicates. The treatments were composed by the control without inoculation and inoculation of *Trichoderma* (*Trichoderma asperellum*) at the dose of 2 kg ha⁻¹. The inoculation of *Trichoderma asperellum*, strain UFT 201, promoted the initial and final maintenance of plants in all the experiments in the different regions and increased also the productivity in the experiments in Gurupi and Crixas regions. For the experiment in Gurupi, the treatment with *Trichoderma* inoculation was also higher in relation to control, with production of 2514 kg ha⁻¹, which represented an increase above 34.5%. In Crixas the treatments with the inoculation of *Trichoderma* presented a production of 3840 kg ha⁻¹, 33% higher than the control that was of 2880 kg ha⁻¹.

Keywords: biofertilizer, fungus, agricultural production.

R E S U M O

Trichoderma é um fungo natural do solo que tem sido amplamente estudado e utilizado na produção agrícola como um biofertilizante e inoculante do solo. Assim, o objetivo deste trabalho foi avaliar a eficiência de *Trichoderma* na manutenção de plantas de soja e produtividade, em regiões produtoras no cerrado em Tocantins. Experimentos foram realizados em três regiões do Estado de Tocantins, designadamente Gurupi, Alvorada e Crixas. O delineamento experimental foi em blocos casualizados com quatro repetições. Os tratamentos foram compostos pela testemunha sem inoculação e inoculação de *Trichoderma* (*Trichoderma asperellum*) na dose de 2 kg ha⁻¹. A inoculação de *Trichoderma asperellum*, estirpe UFT 201, promoveu a manutenção inicial e final de plantas em todas os experimentos nas diferentes regiões e também, o aumento da produtividade nos experimentos nas regiões de Gurupi e Crixas. Para o experimento em Gurupi, o tratamento com inoculação de *Trichoderma* foi superior ao controle, com produção de 2514 kg ha⁻¹, o que representou um aumento acima de 34,5%. Em Crixas, o tratamento com a inoculação de *Trichoderma* apresentou uma produção de 3840 kg ha⁻¹, 33% superior ao controle que foi de 2880 kg ha⁻¹.

Palavras-chave: biofertilizante, fungo, produção agrícola.

INTRODUCTION

Since the 1970s, soybean agribusiness has been responsible for innumerable productive specializations in the Brazilian agrarian space (Santos e Silveira, 2012). It is still the main agricultural product of the Brazilian exports and the main responsible for the increase of the national grain harvest. The soybean has advanced in territorial expansion by the North and Northeast, due to the new technologies involved in its cultivation. The use of management techniques such as no-tillage and irrigation, although it is largely used, it also brings new challenges to cultivation, especially regarding diseases caused by soil pathogens. The use of chemicals as fungicides to control soil diseases has a very high cost. So the integration between biological control techniques and cultural practices that inhibit the pathogen are the best alternatives (Woo *et al.*, 2014).

In the literature, it is possible to find a large number of works mentioning the properties of *Trichoderma* inoculation and its use in some cultures (Guareschi *et al.*, 2012; Rahman *et al.*, 2014; Chagas *et al.*, 2015). *Trichoderma* is a naturally occurring soil fungus that is widely studied and frequently used in agricultural production that can be formulated as a biofertilizer and soil inoculant. The mechanisms for promoting plant growth by soil microorganisms can be direct and indirect. The direct ones can be the production of phytohormones, or another substance analogous to these, that influence the growth or development of the plant, or that even supply its nutritional needs by the solubilization of phosphates (Gravel *et al.*, 2007; Machado *et al.*, 2011). The indirect benefits may occur due to the action of microorganisms through the control of pathogens (Harman *et al.*, 2004; Silva *et al.*, 2011; Gava e Menezes, 2012).

Thus, the objective of this work was to evaluate the efficiency of *Trichoderma* in maintenance and productivity of soybean plants in the producing savanna regions, Tocantins, Brazil.

MATERIAL AND METHODS

Experiments were carried out in three regions in the state of Tocantins, called Gurupi, Alvorada and Crixas.

The experiment in Gurupi, was conducted during the crop year of 2013/2014, (i.e. from December 2013 to April 2014) at the experimental station of the Federal University of Tocantins, Campus of Gurupi. The geographic coordinates of the experimental station correspond to 11°43'45" S and 49°04'07" O, with an average altitude of 287 meters. The local climatic characterization is of tropical humid climate with classification of type Aw according to Köppen and Geiger.

Before sowing, a composite soil sample was collected and the chemical and granulometric characterization was performed where the following values were found: 0.5 cmol_c dm⁻³ of Ca; 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 5.6 in water; 1.0% of organic matter; texture of 72.3, 8.2 and 19.5% of sand, silt and clay respectively. The chemical attributes of the 0-20 cm depth were determined as follows: pH in water – Ratio 1:2,5; P and K – Mehlich Extractor 1; Al³⁺, Ca²⁺ and Mg²⁺ – KCl Extractor (1 mol L⁻¹); H + Al – SMP Extractor; SB = Sum of Exchangeable Bases; (T) = Cation-exchange capacity at pH 7.0; V – Base Saturation Index; and OM = Organic Matter (oxidation: Na₂Cr₂O₇ 4N + H₂SO₄ 10N) (Embrapa, 2009).

Based on the results of soil samples, the application of dolomite limestone filler with 100% of PRNT, 90 days before sowing, for soil correction was performed in the amount of 1.2 Ton ha⁻¹.

In this area, the sowing fertilization was carried out, based on the soil analysis, being applied 400 kg ha⁻¹ of the formulation 5-25. At 30 days after germination, the application of KCl was performed in coverage at the dose of 65 kg ha⁻¹. This fertilization was performed manually in the sowing lines one day before sowing.

The preparation of the area was done by the conventional method, using a harrow, two leveling operations in order to standardize the area using leveling grid and the furrow, adopting furrow depth of 10 cm and spaced 50 cm between rows.

The experimental design was performed in a randomized block with four replicates. The treatments were composed by the control without inoculation

and by the inoculation of *Trichoderma* (*Trichoderma asperellum*) at the dose of 2 kg ha⁻¹.

For the treatment with *Trichoderma*, the granulated inoculant with active microorganism *Trichoderma asperellum* (strain UFT 201) was used. This strain was selected due their potential for *Rizoctonia solani* biocontrol (Tipping) and also as plant growth promoter. This inoculant was formulated with a minimum conidial concentration of 2×10^8 g⁻¹, with the sterile millet being the vehicle of application.

The inoculant was mixed directly into the fertilizer box and applied directly to the fertilization line at the dosage used.

The cultivar used was the Monsoy 7739 iPro. The sowing was performed on December 6, 2014. Fifteen seeds per linear meter were sown, aiming at a final stand of 13 plants per linear meter. Each experimental plot consisted of 8 lines of 6 linear meters, with spacing between lines of 0,5 meters, with a total of 24 m². The spacing between blocks was 1 meter. The seeds were treated one day before sowing with product based on Piraclostrobin + Thiophanatomethyl + Fipronil, using 100 grams for each 50 kg of seeds. On the day of sowing, the seeds were inoculated with the bacteria belonging to the genus *Bradyrhizobium japonicum* (SEMIA 5079 and SEMIA 5080), with a dosage of 80 grams (one dose) / 50 kg seeds. During the development of the crop, all phytotechnical and phytosanitary managements were performed according to recommendations of Henning (2009). The control of invasive plants was carried out 20 days after sowing, when the soybean was in the V3 stage, using the Roundup WG herbicide at the dose of 1.5 kg ha⁻¹. In the same application, the control of caterpillars that attacked the soybean in the initial stage was performed using the insecticides based on Gacianthothrin (150 g L⁻¹) and Diflubenzuron (240 g L⁻¹) at doses of 50 mL ha⁻¹ and 120 mL ha⁻¹, respectively. The control of anthracnose (*Colletotrichum truncatum*) and Asian rust (*Phakopsora pachyrhizi*) was performed in R1 with the application of the fungicide based on Azoxystrobin + Ciproconazole at the dose of 500 mL ha⁻¹.

For the evaluation of the initial and final maintenance of plant and productivity it was used the useful central area of 10 m². The efficiency (E%) of

the use of *Trichoderma* in the stand maintenance, was calculated using the equation: $E\% = \{1 \cdot [Ti / Tc]\} \times 100$. (Gava e Menezes, 2012). The productivity in the same useful area of the experimental plots was determined, being estimated for kg ha⁻¹.

At Alvorada, two experimental areas were used in Fazenda Santa Rita lot. 57, glebe 04, Field (12°28'39''S – 49°7'39''): one in the first year and another in second year of soybean planting. The experiments were conducted in the crop year 2014/2015, from December 2014 to April 2015. The local climatic characterization is an humid tropical climate with classification of type Aw according to Köppen and Geiger, the average temperature was 25.2 °C and 1586 mm the average annual rainfall value.

Before sowing, a soil sample from each area was collected and the physical and chemical characterization was performed. The following values were found: First year area: 1.2 cmol_c dm⁻³ of Ca; 0.4 cmol_c dm⁻³ of Mg; 0.1 cmol_c dm⁻³ of K; 2.8 mg dm⁻³ of P; 0.08 cmol_c dm⁻³ of Al; 7.1 cmol_c dm⁻³ of CTC; 1.7 cmol_c dm⁻³ of SB; 30% of V; pH 4.9 in water; 1.0% of organic matter; texture of 73.1, 9.0 and 17.99% of sand, silt and clay, respectively. Second year area: 1.4 cmol_c dm⁻³ of Ca; 0.6 cmol_c dm⁻³ of Mg; 0.3 cmol_c dm⁻³ of K; 5.8 mg dm⁻³ of P; 0.07 cmol_c dm⁻³ of Al; 7.1 cmol_c dm⁻³ of CTC; 2.3 cmol_c dm⁻³ of SB; 31% of V; pH 5.3 in water; 1.0 % of organic matter; texture of 74.4, 8.2 and 17.4% of sand, silt and clay, respectively (EMBRAPA, 2009).

Liming with dolomite limestone filler with 100% of PRNT, 70 days before sowing for soil correction, at the amount of 1.5 Ton ha⁻¹ was performed. The preparation of the area was also done by the conventional method, using a harrow, two leveling operations to standardize the area with the use of leveling grid and grooving, adopting furrow depth of 10 cm and spacing 50 cm between rows. The fertilization (NPK) was done at the time of sowing, following the recommendation of the soil analysis, using 400 kg of 00-05-25. Coverage fertilization with potassium chloride (KCl) was performed at the amount of 65 kg ha⁻¹, at 30 days after sowing.

The treatments were composed by the control without inoculation and inoculation of *Trichoderma asperellum* UFT 201 at a dose of 2 kg ha⁻¹. For the treatment with *Trichoderma*, the granulated

inoculant was formulated with a minimum viable conidial concentration of $2 \times 10^8 \text{ g}^{-1}$.

The soybean varieties, Soytec 820 RR, were used in these experiments. The sowing was performed on December 10, 2014. Fifteen seeds were sown per linear meter, aiming at a final stand of 11 plants per linear meter.

The treatments, design and experimental plots, seed treatment, phytotechnical and phytosanitary management and evaluations were similar to the experiment in Gurupi.

In the experiment performed in Crixas, an experimental area was used at the Fazenda California, Crixas Allotment, Glebe 2 to 5 km from the center of the city of Crixas do Tocantins ($11^{\circ}6'3'' \text{ S e } 48^{\circ}54'59'' \text{ O}$). The experiment was conducted in the crop 2014/2015, from December 2014 to April 2015. The local climatic characterization is an humid tropical climate with classification of type Aw according to Köppen and Geiger, the average temperature was $26.6 \text{ }^{\circ}\text{C}$ and 1665 mm the average annual rainfall value. Before sowing a soil sample was collected and the physical and chemical characterization was performed. The following values were found: $1.7 \text{ cmol}_c \text{ dm}^{-3}$ of Ca; $0.9 \text{ cmol}_c \text{ dm}^{-3}$ of Mg; $0.2 \text{ cmol}_c \text{ dm}^{-3}$ of K; 7.8 mg dm^{-3} of P; $0.05 \text{ cmol}_c \text{ dm}^{-3}$ of Al; $7.6 \text{ cmol}_c \text{ dm}^{-3}$ of CTC; $2.8 \text{ cmol}_c \text{ dm}^{-3}$ of SB; 32% of V; pH 5.7 in water; 1.5% of organic matter; texture of 68.1, 7.2 and 24.7% of sand, silt and clay, respectively (EMBRAPA, 2009).

The preparation of the area was also done by the conventional method according to the previous experiments. The liming was, performed four months before sowing in order to reduce the soil acidity level, using 2.8 kg of limestone ha^{-1} .

The area used was the second year area of cultivation. Soybean varieties, Soytec 820 RR, were used in these experiments. The sowing was performed on December 2, 2014. Fifteen seeds were sown per linear meter, aiming at a final stand of 12 plants per linear meter.

The treatments, fertilization, the design and experimental plots, seed treatment, phytotechnical and phytosanitary management and the evaluations were similar to the experiment in Gurupi.

In all experiments the data were submitted to analysis of variance and Duncan's average test at 1 and 5% of probability using the statistical program ASSISTAT version 7.6 beta (Silva, 2008).

RESULTS AND DISCUSSION

For the experiment in Gurupi, the treatment with *Trichoderma* inoculation was superior ($p < 0.01$) for the initial and final maintenance of plants in relation to the control treatment without inoculation (Table 1). Regarding the plant survival in the stands, the treatment with inoculation of *Trichoderma* presented a survival percentage of 97.4, with an inoculation efficiency of 15.1% (Table 1).

Table 1 - Initial (I) and final (F) maintenance of plants, survival, efficiency (E) and productivity of soybean Monsoy 7739 iPro, inoculated with *Trichoderma asperellum* strain UFT 201, cultivated in the cerrado in the experimental area of Gurupi, during the crop year 2013 / 2014 ¹

Treatments	I 25 DAP ²	F 50 DAP	Surviv. ³ (%)	E (%) ⁴	Prod. (Kg ha ⁻¹)
Control	297.0 b	275.0 b	84.6	-	1745 b
<i>Trichoderma</i>	329.5 a	316.5 a	97.4	15.1	2514 a
CV (%) ⁵	2.8 **	2.9 **	-	-	11.6 *

¹ Average followed by the same lowercase letter in the columns do not differ by Duncan's test at 1 or 5% significance. ² DAP = Days after planting. ³ Surviv. = Percentage of plant survival in relation to the expected stand of 325 plants in 10 m² (13 plants per linear meter). ⁴ Efficiency in the use of *Trichoderma* in the maintenance of the stand. ⁵ CV = Coefficient of Variation. * Significant at 5%. ** Significant at 1%.

Regarding productivity, estimated in kg ha^{-1} , the treatment with *Trichoderma* inoculation was also significantly higher ($p < 0.05$) in relation to control with production of 2514 kg ha^{-1} , which represented an increase above 34.5% (Table 1).

For the experiments in Alvorada, the results of the initial and final maintenance of plants, for the treatment with *Trichoderma* inoculation, were higher ($p < 0.01$) than the control without inoculation. Even though it was an area of first year of planting, the survival rate of 84.6% was higher for the treatment with *Trichoderma* inoculation in relation to the witness that was 74.7% and 13.3% of efficiency, indicating the potentiality in the maintenance of this stand for the areas that received the

inoculation with *Trichoderma* (Table 2). There was no significant difference between the treatments, but for the treatment with *Trichoderma* inoculation there was a 6% increase in productivity compared to the control without inoculation (Table 2).

Table 2 - Initial (I) and final (F) maintenance of plants, survival, efficiency (E) and productivity of soybean Soytec 820 RR, inoculated with *Trichoderma asperellum* strain UFT 201, cultivated in the cerrado in Alvorada, in a first year area of soybean planting, during the crop year 2014 / 2015¹

Treatments	I 25 DAP ²	F 50 DAP	Surviv. ³ (%)	E (%) ⁴	Prod. (Kg ha ⁻¹)
Control	207.5 b	205.5 b	74.7	-	2966 a
<i>Trichoderma</i>	261.5 a	232.5 a	84.6	13.3	3144 a
CV (%) ⁵	6.2 **	6.4 *	-	-	8.6 ns

¹ Average followed by the same lowercase letter in the columns do not differ by Duncan's test at 1 or 5% significance. ² DAP = Days after planting. ³ Surviv: = Percentage of plant survival in relation to the expected stand of 275 plants in 10 m² (11 plants per linear meter). ⁴ Efficiency in the use of *Trichoderma* in the maintenance of the stand. ⁵ CV = Coefficient of Variation. * Significant at 5%. ** Significant at 1%. ns Not significant

In the second year area of planting, the results of the initial and final stands were similar to those found in the first year planting area (Table 3). Even with a higher percentage of plant survival for the treatment with *Trichoderma* and with an efficiency of 36.1 in relation to the control treatment, there was no significant difference in productivity, but there was a 3.7% increase in productivity compared to the control without inoculation (Table 3).

Table 3 - Initial (I) and final (F) maintenance of plants, survival, efficiency (E) and productivity of soybean Soytec 820 RR, inoculated with *Trichoderma asperellum* strain UFT 201, cultivated in the cerrado in Alvorada, in second year area of soybean planting, during the crop year 2014/2015¹

Treatments	I 25 DAP ²	F 50 DAP	Surviv. ³ (%)	E (%) ⁴	Prod. (Kg ha ⁻¹)
Control	214.0 b	177.5 b	64.5	-	3000 a
<i>Trichoderma</i>	261.5 a	241.5 a	87.8	36.1	3110 a
CV (%) ⁵	7.9 **	8.4 *	-	-	7.1 ns

¹ Average followed by the same lowercase letter in the columns do not differ by Duncan's test at 1 or 5% significance. ² DAP = Days after planting. ³ Surviv: = Percentage of plant survival in relation to the expected stand of 275 plants in 10 m² (11 plants per linear meter). ⁴ Efficiency in the use of *Trichoderma* in the maintenance of the stand. ⁵ CV = Coefficient of Variation. * Significant at 5%. ** Significant at 1%. ns Not significant

For the experiment in Crixas, the treatment with inoculation of *Trichoderma* was superior to the initial (p<0.01) and final (p<0.05) maintenance of plants in relation to the control treatment without inoculation. The survival percentage, considering the expected final stand was 100% with the efficiency of the treatment with inoculation of 12.7% (Table 4).

Table 4 - Initial (I) and final (F) maintenance of plants, survival, efficiency (E) and productivity of soybean Soytec 820 RR, inoculated with *Trichoderma asperellum* strain UFT 201, cultivated in the cerrado in Crixas, during the crop year 2014/2015¹

Treatments	I 25 DAP ²	F 50 DAP	Surviv. ³ (%)	E (%) ⁴	Prod. (Kg ha ⁻¹)
Control	299.0 b	267.0 b	89	-	2880 b
<i>Trichoderma</i>	317.0 a	301.0 a	100	12.7	3840 a
CV (%) ⁵	7.3 **	8.1 *	-	-	7.5 **

¹ Average followed by the same lowercase letter in the columns do not differ by Duncan's test at 5% significance. ² DAP = Days after planting. ³ Surviv: = Percentage of plant survival in relation to the expected stand of 300 plants in 10 m² (12 plants per linear meter). ⁴ Efficiency in the use of *Trichoderma* in the maintenance of the stand. ⁵ CV = Coefficient of Variation. * Significant at 5%. ns Not significant

For productivity, there was a significant difference between the treatments, with higher productivity (p<0.01) for the treatments with *Trichoderma* inoculation with the production of 3840 kg ha⁻¹, 33% higher than the control with 2880 kg ha⁻¹, referring to an increase of 960 kg ha⁻¹ (Table 4).

The positive results observed in different soybean experiments can be explained by the action of the inoculant used, considering that fungi of the genus *Trichoderma* are used in the biological control of phytopathogens and in the promotion of plant growth due to its versatility of action, such as parasitism, antibiosis and competition, besides acting as inducers of resistance to plants against diseases and to produce growth hormones (Guareschi *et al.*, 2012; Kumar *et al.*, 2012; Milanesi *et al.*, 2013; Chagas Junior *et al.*, 2015; Chagas, 2015). These fungi are found in the rhizosphere, are growth promoters in plant species (Machado *et al.*, 2012), and are also a rich source of secondary metabolites, presenting a vast repertoire of genes supposedly involved in the biosynthesis of non-ribosomal peptides, polypeptides, terpenoids and pironas (Mukherjee *et al.*, 2012). So, the inoculation

with a high concentration of these microorganisms can provide positive results on the biocontrol of phytopathogens and, consequently, the promotion of plant growth.

This positive effect on the initial and final maintenance of plants in the present study can be directly associated to the biocontrol of this pathogen, as observed in the different experiments and in the different regions studied. Possibly, treatments with the inoculation of *Trichoderma* may have provided an initial control of this pathogen, as observed in other studies evaluating the biocontrol of *Trichoderma* against *Rhizoctonia solani* (Verma *et al.*, 2007; Lucon *et al.*, 2009; Kotasthane *et al.*, 2014; Pereira *et al.*, 2014; Rahman *et al.*, 2014).

Different *Trichoderma* isolates obtained from different places have different responses regarding the biocontrol of phytopathogens (Martínez *et al.*, 2013), and have also an antagonistic capacity against *R. solani*, as was observed in isolation studies and selection of *Trichoderma* for biological control of phytopathogens (Garcia *et al.*, 2008; Reyes *et al.*, 2008; Kotasthane *et al.*, 2014; Chagas,

2015). This ability depends on the strain specificity and its mode of action, as well as on the sporulation capacity and establishment of the fungus in the field, which will also depend on the selection, as a biocontrol agent, for one or more target phytopathogens and the elaboration of the final product for commercial use.

The application of *Trichoderma* in field conditions is a promising practice, since the biological control offers greater durability, safety and better cost-effectiveness than the chemical fungicides applied in the soil (Yaqub e Shahzad, 2011; Kumar *et al.*, 2012).

CONCLUSION

The inoculation of *Trichoderma asperellum* UFT 201 promoted the initial and final maintenance of plants in all the experiments in the different regions and also, the increase of productivity in the experiments in the Gurupi and Crixas regions. However, these results are preliminary and were only based on one year of field trials.

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