

Efficiency of the inoculation of *Trichoderma asperellum* UFT-201 in cowpea production components under growth conditions in field

Eficiência da inoculação de *Trichoderma asperellum* UFT-201 em componentes de produção de feijão-frade sob condições de crescimento no campo

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ABSTRACT

The rhizosphere contains many microorganisms that can promote plant growth and protect the root system against infection by pathogens. Fungi of *Trichoderma* sp. are included in this group. The aim of this study was to evaluate efficiency of the inoculation of *Trichoderma* in agronomic performance of cowpea under field conditions, in the savanna in Gurupi, Tocantins, in harvests 2013/2014 to 2014/2015. The treatments consisted in seed treatment, with and without inoculation with *Trichoderma asperellum* UFT 201 with minimum concentration of viable conidia of 2×10^9 L⁻¹, at a dose of 20 g kg⁻¹ of seed. Biomass evaluations were made on efficiency, initial and final stands and survival in the use of *Trichoderma* in the stand maintaining and productivity. The results indicated that the ability of bio-protection and plant growth promotion were of great importance for the biomass production, the stand maintaining and cowpea productivity. In both harvests, survival was superior in the plants treated with *Trichoderma*, 23.0 and 13.2%, respectively, and yields were 9.5% higher compared to control for the two harvests. In general, the inoculation of *Trichoderma* provided the best results in the biomass variables analyzed for the two crops with higher productivity.

Keywords: biomass, productivity, *Trichoderma*, *Vigna unguiculata*.

RESUMO

A rizosfera contém muitos microorganismos que podem promover o crescimento vegetal e proteger o sistema radicular de infecções por agentes patogênicos. Neste grupo incluem-se fungos de *Trichoderma* sp. O objetivo deste estudo foi avaliar a eficiência da inoculação de *Trichoderma* na performance agronômica de feijão-frade em condições de campo, na savana em Gurupi, Tocantins, nas campanhas de 2013/2014 a 2014/2015. Os tratamentos consistiram sementes inoculadas (ou não) com *Trichoderma asperellum* UFT 201 com uma concentração de conídios viáveis mínima de 2×10^9 L⁻¹, numa dose de 20 g kg⁻¹ de semente. Foram efetuadas avaliações de biomassa em termos de eficiência, populações iniciais e finais e sobrevivência de *Trichoderma*, e na manutenção e produtividade da cultura. Os resultados indicam que a capacidade de bioproteção e promoção do crescimento vegetal foram de grande importância para a produção de biomassa, a manutenção da cultura e a produtividade de feijão-frade. E ambas as campanhas a sobrevivência foi superior nas plantas tratadas com *Trichoderma*, 23,0 e 13,2% respectivamente, e as produtividades foram 9,5% superiores ao controle em ambas as campanhas. Em termos globais, a inoculação com *Trichoderma* providenciou os melhores resultados nas diversas variáveis ilustradoras de biomasse para as duas campanhas, conduzindo a uma maior produtividade.

Palavras-chave: biomassa, produtividade, *Trichoderma*, *Vigna unguiculata*.

INTRODUCTION

The cowpea plant (*Vigna unguiculata* (L.) Walp) is considered a subsistence crop, that acquires great socio-economic importance in agriculture in the North and Northeast of Brazil, whereas composes the main low-cost source of protein for human consumption with a great variability among cultivars (Bertini *et al.*, 2009). It is known for presenting hardiness and adaptability in different agro-ecosystems. However, historically, this culture has low productivity due to crop conditions without adoption of advanced technologies.

Diseases are one of the main causes of low productivity. Thus, according to the climatic conditions, some pathogens have the ability to reduce or prevent the production of the grain. The use of management techniques such as tillage and irrigation although welcome, on the other hand brings new challenges to the culture, especially regarding diseases caused by soil-borne pathogens (Paula Júnior & Zambolim, 2006). The use of chemicals such as fungicides for the control of soil borne diseases has a very high, cost: therefore, the integration between biological control techniques and cultural practices that inhibit the pathogen is the best alternatives (Azarmi *et al.*, 2011; Woo *et al.*, 2014).

In the rhizosphere, several microorganisms, such as the case of fungi of the genus *Trichoderma*, can be found promoting plant growth and protecting the root system from infection by plant pathogens (Machado *et al.*, 2012; Martínez *et al.*, 2013). The *Trichoderma* strains have received scientific and agro-economic attention by presenting antagonistic actions against various pathogens such as parasitism, antibiosis and competition (Verma *et al.*, 2007; Martínez *et al.*, 2013).

Some strains of *Trichoderma* increase the total area of the root system, enabling greater access to mineral elements. Others are able to solubilize and provide rock phosphate, iron, copper, manganese and zinc to the plant. Also the active mechanisms for the absorption of macro and micronutrients can be improved by increasing the plant efficiency to use some important nutrients, such as nitrogen (Lucon, 2009; Schuster & Schmoll, 2010; Shores *et al.*, 2010; Machado *et al.*, 2012).

In order to achieve higher economic returns by the increase in the biomass and productivity of

strategic crops for the Tocantins savannah, such as cowpea, continuity in the process of generating information is needed, from the targeted research, seeking and evaluating innovative management practices such as the application of inoculants using *Trichoderma* as plant growth promoting organism and aiming at efficiency in the biological control of diseases.

In this sense, the objective of the present works was to evaluate the efficiency of the inoculation of *Trichoderma* in the agronomic performance of cowpea plants under field conditions.

MATERIAL AND METHODS

Experiments were carried out at the Experimental Station of the Federal University of Tocantins (UFT), Campus Gurupi (11°43'45" south latitude and 49°04'07" west longitude, altitude 280 m). Evaluations of biomass and productivity were conducted in the Microbiology Laboratory, located in the Business Incubator. Two field experiments were performed with the cowpea crop, the first harvest in 2013/2014 and the second in 2014/2015, both in the period between December and March.

The soil of the experimental area was classified as dystrophic oxisol of medium texture (Embrapa, 1997). The local climatic characterization is tropical humid with little water deficiency (B1wa'a') as Savannah Tropical according to Köppen- Geiger (Peel *et al.*, 2007).

Chemical analysis of the soil before the experiment was carried out in the first agricultural year (first experiment), where the following values were found: 2.04 cmol_c dm⁻³ Ca; 0.32 cmol_c dm⁻³ Mg; 0.12 cmol_c dm⁻³ K; 2.09 mg dm⁻³ P; 0.04 cmol_c dm⁻³ Al; 5.9 cmol_c dm⁻³ CTC; 2.48 cmol_c dm⁻³ SB; 30% V; pH 5.4 in water; 1.0% organic matter; texture 72.3, 8.2 and 19.5% sand, silt and clay, respectively (Embrapa, 1997).

The sowing fertilization (N-P-K+Micro) was based on soil analysis and following recommendations of Freire Filho *et al.* (2005). Fertilization was the same in all experiments: 80 kg P₂O₅, 60 kg K₂O and 30 kg FTE BR 12 powder, calcium (Ca): 7.1%, sulfur (S): 5.7%, boron (B): 1.8%, copper (Cu): 0.8%, manganese (Mn): 2.0%, molybdenum (Mo): 0.1%, zinc (Zn): 9.0%), per ha. The fertilization was done in

row using as phosphorus source superphosphate ammoniated composed of 3% N and 17% P₂O₅, and as potassium source KCl (58% K₂O).

The preparation of the area was made by the conventional method, a harrowing with use of disc harrows, two leveling operations in order to standardize the area using disking and furrower of rows with a furrow depth of 10 cm and spaced 50 cm between rows.

The cultivar Fradinho type was used in the experiments, with particular growth habit, upright plant, medium-slow cycle (70-90 days) with husk grain white color (Freire Filho *et al.*, 2005). Sowing was performed using 15 seeds per meter, aiming to have 10 established plants per meter.

The design was a randomized blocks with four replications. The treatments were composed by seed treatment, with and without inoculation of *Trichoderma*. Each plot consisted of nine lines of 6 m, with line spacing of 0.5 m, 1 m between plots and 1 m between blocks, each plot totaling 24 m².

The cowpea seeds were inoculated with rhizobia (*Bradyrhizobium* sp., SEMIA 6462 strain) at the time of sowing with commercial inoculant recommended for cowpea, using peat, previously inoculated with 10⁹ cells g⁻¹. The application of the inoculant was conducted in a ratio of 500 g of inoculant per 50 kg of seed.

The treatment with *Trichoderma* was developed using the inoculant Trichoplus JCO powder that has as active principle the strain *Trichoderma asperellum* UFT 201, with the potential for biocontrol and as plant growth promoter. This product was formulated with a minimal concentration of viable conidia of 2 x 10⁹ L⁻¹ by JCO Fertilizantes using kaolin as a vehicle. The inoculant was applied directly on the seeds at a dose of 20 g per kg of seed, according to the recommended concentration for seed treatment using *Trichoderma* powder.

During the development of the plants, cultural management was carried out according to Freire Filho *et al.* (2005).

Biomass evaluations were made in two times, at 25 and 50 days after planting (DAP), on the previous lines of the border. The central useful area related to 10 m² was used for the initial and final stand

assessments and productivity. The effectiveness or efficiency in the use of *Trichoderma* to maintain the stand was calculated using the equation: E% = {1 - [Ti / Tc]} x 100, where E=% efficacy of treatment; Ti=% mean of final stand in the treatment i; Tc=% mean of final stand in the control treatment, adapted from Gava & Menezes (2012). Six plants in each plot were collected for each evaluation of biomass. The roots were carefully washed in potable water with the aid of a sieve to remove impurities and to recover the roots and nodules. The shoot was separated from the roots with a cut made in the stem base, and the nodules were removed and counted. Subsequently, the shoot, root, and nodules were placed in a paper bag and dried in a forced circulation oven at 65 °C until constant weight.

Biomass was assessed by the parameters: dry matter of the aerial part (DMAP), root dry matter (RDM), total dry matter (TDM), number of nodules (NN) and dry matter of nodules (DMN). The relative efficiency (RE) was determined with the biomass of the aerial part of the last evaluation (50 DAP) according to formula: RE = (DMAP inoculated with *Trichoderma* / DMAP without *Trichoderma*) x 100 (Lima *et al.*, 2005). Grain production was obtained in the useful area of 10 m², after physiological maturity of the plants, when approximately 80% of the beans have dried. The beans were manually threshed and grain moisture was adjusted to 14%. After the harvest the productivity per hectare was quantified.

The data were analyzed by ANOVA and Duncan mean test at 1 and 5% probability using the statistical program ASSISTAT version 7.7 beta (Silva, 2008).

RESULTS AND DISCUSSION

In the first experiment with cowpea in the 2013/2014 harvest, the results showed no significant difference (p < 0.05) between treatments with and without *Trichoderma* to the dry matter of the aerial part (DMAP), root dry matter (RDM), total dry matter (TDM), number of nodules (NN) and the dry matter of nodules (DMN) at 25 days after planting (DAP). In the assessment 50 DAP, the DMAP and TDM were higher (p < 0.05) for treatment with *Trichoderma*. In the case of RDM, NN and DMN no significant differences were found (Table 1).

Table 1 - Dry matter of aerial part (DMAP), root dry matter (RDM), total dry matter (TDM), number of nodules (NN) and nodules dry matter (NDM) in cowpea 'Fradinho' with and without *Trichoderma* inoculation, 25 and 50 days after planting (DAP). Gurupi, TO. Harvest 2013/2014 ¹

Treatments	DMAP	RDM	TDM	NN	NDM
	(g)	(g)	(g)		(mg)
25 DAP					
Without <i>Trichoderma</i>	2.9 a	0.4 a	3.3 a	18.3 a	45 a
With <i>Trichoderma</i>	4.3 a	0.5 a	4.8 a	26.1 a	79 a
CV (%) ²	26.1 ^{ns}	28.4 ^{ns}	26.1 ^{ns}	25.6 ^{ns}	45.4 ^{ns}
50 DAP					
Without <i>Trichoderma</i>	7.7 b	0.8 a	8.5 b	29.0 a	101 a
With <i>Trichoderma</i>	11.4 a	1.0 a	12.4 a	38.4 a	139 a
CV (%)	14.7 [*]	32.2 ^{ns}	11.1 [*]	12.5 ^{ns}	31.7 ^{ns}

¹ Averages followed by same lower case letter in columns do not differ by Duncan test at 1 and 5 % significance. * Significant at 5%, ** Significant at 1%, ns not significant.

² Coefficient of variation.

For the 2014/2015 harvest, the results of biomass and nodulation 25 DAP also had no significant differences between treatments. At 50 DAP, there was significant differences for DMAP parameters, RDM and TDM being higher ($p < 0.01$) for the treatment inoculated with *Trichoderma* (Table 2).

In the initial stage of crop development (25 DAP) there was no response from *Trichoderma* inoculation, which can be explained by the fact that in the initial stage of the crop the fungus could still not have fully established in the area. Oliveira *et al.* (2012) obtained similar results, with no significant differences for any of the characteristics evaluated at 25 DAP for cowpea inoculated with *Trichoderma* in savanna soils. According to Chagas Jr. *et al.* (2012), in a similar study, using the cultivar cowpea Vinegar and evaluating the double inoculation of *Rhizobium* and *Trichoderma*, there was a significant difference for biomass in treatments inoculated with *Trichoderma* in the seed.

Machado *et al.* (2011) in experiments with *Lotus corniculatus* L. (birdsfoot trefoil) and *Avena strigosa* Schreb (black oat), observed that all strains of rhizobia tested together with a commercial product consisting of *Trichoderma harzianum* Rifai were able to promote plant growth of black oat, increasing the plant mass of the aerial part.

Ethur *et al.* (2006), in a study with turnip seeds, found that the treatment with *Trichoderma* sp. resulted in an increase of plants height, showing the performance of the fungus as a plant growth promoter.

On the other hand, the relative efficiency (RE), which relates the biomass of the aerial part of the treatments inoculated with *Trichoderma* against the control treatment without inoculation, was a higher ($p < 0.01$) for the treatment using *Trichoderma*, with an increase of 48 to 49% for harvests 2013/2014 and 2014/2015, respectively, compared to control (Figure 1). Similar results were reported by Chagas Jr *et al.* (2012, 2014), also with double inoculation of *Rhizobium* and *Trichoderma* in cowpea.

For the initial stand (25 DAP) for 2013/2014 harvest, there was significant difference between treatments with superior response to treatment using *Trichoderma*, similarly to the final stand (50 DAP) (Table 3). As for the survival and efficacy of treatment with *Trichoderma* inoculation, it was evident the inoculation with superior values ($p < 0.05$) the treatment without inoculation, with efficiency of 23% in the number of plants effectively in production. For the 2014/2015 harvest, also the treatment with *Trichoderma* inoculation was higher ($p < 0.05$) for the variables as initial stand, final stand and percentage of survival, with a percentage of 13.2% higher efficiency in relation to control.

Table 2 - Dry matter of aerial part (DMAP), root dry matter (RDM), total dry matter (TDM), number of nodules (NN) and nodules dry matter (NDM) in cowpea 'Fradinho' with with and without *Trichoderma* inoculation, 25 and 50 days after planting (DAP). Gurupi, TO. Harvest 2014/2015 ¹

Treatments	DMAP	RDM	TDM	NN	NDM
	(g)	(g)	(g)		
25 DAP					
Without <i>Trichoderma</i>	2.9 a	0.4 a	3.3 a	18.3 a	45 a
With <i>Trichoderma</i>	4.3 a	0.5 a	4.8 a	26.1 a	79 a
CV (%) ²	26.1 ^{ns}	28.4 ^{ns}	26.1 ^{ns}	25.6 ^{ns}	45.4 ^{ns}
50 DAP					
Without <i>Trichoderma</i>	7.7 b	0.8 a	8.5 b	29.0 a	101 a
With <i>Trichoderma</i>	11.4 a	1.0 a	12.4 a	38.4 a	139 a
CV (%)	14.7 [*]	32.2 ^{ns}	11.1 [*]	12.5 ^{ns}	31.7 ^{ns}

¹ Averages followed by same lower case letter in columns do not differ by Duncan test at 1 and 5 % significance. * Significant at 5%, ** Significant at 1%, ns not significant.

² Coefficient of variation.

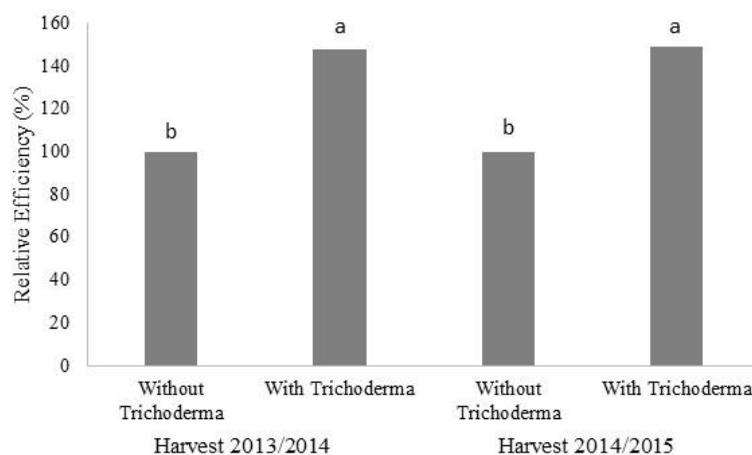


Figure 1 - Relative efficiency of *Trichoderma* inoculation in biomass production of the aerial part of cowpea at 50 day after planting (DAP) compared to control without inoculation. A: Harvest 2013/2014. B: Harvest 2014/2015. Different letters indicate statistically significant differences, as indicated by Duncan test at 5 % probability.

These results demonstrate the maintenance of the stand due to the control of diseases commonly related to stand loss in the initial stage of plant development, such as Mela caused by *Rhizoctonia solani* J.G. Kühn. However, during both harvests symptoms of this disease were found to occur in levels below 10%, which is considered a low level of disease severity, according to the grading scale for incidence and severity of Mela, according to Van Schoonhoven & Pastor-Corrales (1987).

Similar results were reported by Gava & Menezes (2012) and Chagas Jr. *et al.* (2014), for the treatment of seeds with *Trichoderma* isolates showing increased plant stand at the end of the experiment compared to the control treatment, leading to increased fruit yield of melon plant (*Cucumis melo* L.), and cowpea, respectively.

Regarding productivity, the 2013/2014 harvest was higher ($p < 0.05$) for the treatment inoculated

Table 3 - Initial stand (IS), final stand (FS), survival, efficiency and productivity of cowpea 'Fradinho' with and without *Trichoderma* inoculation, 25 and 50 days after planting (DAP), in Gurupi, TO, Brazil in the harvests 2013/2014 and 2014/2015 ¹

Treatments	IS	FS	Survival (%) ²	Efficiency (%) ³	Prod. (Kg ha ⁻¹)
	25 DAP (plants m ⁻²)	50 DAP (plants m ⁻²)			
Harvest 2013/2014					
Without <i>Trichoderma</i>	20.0 b	19.0 b	63.3 b	-	1548.7 b
With <i>Trichoderma</i>	25.9 a	23.3 a	77.7 a	23.0	1695.3 a
CV (%) ⁴	11.8 *	12.7 *	12.1 *	-	9.3 *
Harvest 2014/2015					
Without <i>Trichoderma</i>	26.0 b	24.3 b	81.0 b	-	1690.3 b
With <i>Trichoderma</i>	28.9 a	27.5 a	91.6 a	13.2	1850.3 a
CV (%)	9.8 *	11.1 *	9.0 *	-	8.47 *

¹ Averages followed by same lower case letter in columns do not differ by Duncan test at 1 and 5 % significance. * Significant at 5%, ** Significant at 1%, ns not significant. ² Plants survival compared to expected stand 30 plants m⁻², considering three lines of a meter. ³ Efficiency of *Trichoderma* in maintaining the stand. ⁴ Coefficient of variation.

using *Trichoderma* (1695.3 kg ha⁻¹) compared to control (1548.7 kg ha⁻¹), a production of 28.3 bags ha⁻¹ and 9.5% higher than control with 25.8 bags ha⁻¹, related to an increase of 2.5 bags ha⁻¹ (Table 3). In the harvest 2014/2015 the value was also higher (p<0.05) for treatment with *Trichoderma* (1850.3 kg ha⁻¹) compared to control (1690.3 kg ha⁻¹), a production of 30.8 bags ha⁻¹, and also 9.5% higher than the control with 28.2 bags ha⁻¹, related to an increase of 2.6 bags ha⁻¹.

Positive results for the productivity of cowpea in response to double inoculation of *Rhizobium* and *Trichoderma* were also reported by Chagas Jr *et al.* (2012, 2014) to cultivar vinegar, indicating that there was a significant increase in productivity in treatments with inoculation of *Trichoderma*.

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

Further research has shown that seeds treated with *Trichoderma* have played an important role in increasing the biomass of plants such as papaya, tomato (Gravel *et al.*, 2007; Tavares, 2009; Fontenelle *et al.*, 2011), corn (Resende, 2004), eucalyptus (Fortes

et al., 2007; Filho *et al.*, 2008), chickpeas (Srivastava *et al.*, 2008), beans (Hoyos-Carvajal *et al.*, 2009) and rice (Almança, 2005).

The increase in plant biomass production can vary according to the species *Trichoderma* used as well as with the culture. Significant increases were reported by Carvalho Filho *et al.* (2008) for *Eucalyptus urophylla* S.T. Blake, an increase ranging between 57 and 136%. Tavares (2009) observed increases in biomass of papaya up to 110%. Increases in tomato biomass above 1000% were reported by Fontenelle *et al.* (2011).

Several authors cited *Trichoderma* spp. as a plant growth promoters and numerous researches have been conducted because these fungi produce antimicrobial substances which ensure a wide spectrum of activity against various plant pathogens. Moreover, some strains of *Trichoderma* promote plant growth by increasing the availability of nutrients and production of growth hormones (Filho *et al.*, 2008; Louzada *et al.*, 2009). The root colonization often increases the development, crop yield, resistance to abiotic stresses and improves nutrient use (Rubio *et al.*, 2014).

Most species of *Trichoderma* behave differently in the actions as biocontrol and as promoters of plant growth. Among the factors that determine the success of the inoculation of *Trichoderma*, there are the

abiotic and biotic. Thus, selected isolates for biological control of pathogens in controlled experimental conditions may be unable to produce the same results under field conditions, similarly to the selected according to ability to promote plant growth. This relates to the fact that the establishment and development of soil conditions are critical to the microorganism, because they are subject to different reactions of the host and the environment, which may lead to a more variable effectiveness compared to the control obtained with chemical fungicides (Harman, 1991). Thus, it is almost impossible to quantify separately in field experiments the disease control responses and the probable promotion of growth, but the results of biomass, stand and productivity are examples of benefit to the cowpea in cultivation area of this culture.

Biological control has been researched using the knowledge of genetics and biochemistry. However, little is known about the real effects and/or growth-promoting mechanisms of plants inoculated with *Trichoderma*. Studies show efficient strains for biocontrol of various pathogens and other efficient promoting plant growth through colonization of the rhizosphere on natural and axenic conditions (Pomella & Ribeiro, 2009) or providing nutrients to the plant (Machado *et al.*, 2012;

Martínez *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 (Pomella & Ribeiro, 2009). The researches carried out using isolates with potential for antagonism have shown ability to control diseases. However, the level of efficacy observed in many field works is lower than expected. According to Pomella & Ribeiro (2009), some factors are important to obtain effective results with biocontrol agents: effective strains in the field against various pathogens, low cost of production involving the efficient formulations and form, dose and time of application.

CONCLUSION

The inoculation using *Trichoderma* positively influenced the production of biomass, stand maintenance and the productivity of cowpea under field conditions in Tocantins savannah.

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