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Combined inoculation of rhizobia and *Trichoderma* spp. on cowpea in the savanna, Gurupi-TO, Brazil

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ABSTRACT

The goal of this study was to evaluate the combined inoculation of rhizobia and *Trichoderma* spp. in cowpea, their ability to promote growth and use as a biological control agent for foliar blight (*Rhizoctonia solani*). The study was conducted in a field experiment in a randomized block design with four replications. The treatments consisted of seed-applied rhizobia with and without *Trichoderma* spp., and to the soil at planting time and 15 days after planting (DAP). The rhizobia inoculation was performed with a mixture of strains INPA 03-11B and UFLA 03-84. For the treatments with *Trichoderma* spp., a commercial powder product, Trichoplus JCO was used. The application of *Trichoderma* spp. and rhizobia at 15 DAP, in both the seed and in the soil, showed the best results ($p < 0.05$). The application of *Trichoderma* spp. did not inhibit the nodulation process, and the best results were obtained when the fungus was inoculated with rhizobia. The inoculation treatments were positive for *Trichoderma* spp. The *Trichoderma* spp. treatments showed improvements in stand counts, survival and effectiveness against *R. solani*, reinforcing that the usage of *Trichoderma* spp. as a seed and soil treatment.

Key words: biocontrol, biological nitrogen fixation, *Rizoctonia solani*, *Vigna unguiculata*

Inoculação combinada de rizóbio e Trichoderma spp. em feijão-caupi no cerrado, Gurupi-TO, Brasil

RESUMO

O trabalho teve, como objetivo, avaliar a inoculação combinada de rizóbio e *Trichoderma* em feijão caupi quanto à capacidade de promoção de crescimento e biocontrole da mela (*Rizoctonia solani*). O experimento foi conduzido em campo, em delineamento experimental blocos ao acaso com quatro repetições. Os tratamentos utilizados foram inoculações simples e combinadas de rizóbio e *Trichoderma* na semente e no solo, no momento do plantio e *Trichoderma* aos 15 dias após o plantio (DAP). A inoculação com rizóbio foi realizada com a mistura das estirpes INPA 03-11B e UFLA 03-84. Para os tratamentos com a utilização de *Trichoderma* sp., foi utilizado o inoculante comercial Trichoplus JCO em pó. A aplicação de rizóbio e *Trichoderma* aos 15 DAP apresentou, tanto na semente quanto no solo, os melhores resultados ($p < 0,05$). A aplicação de *Trichoderma* não prejudicou o processo de nodulação sendo que os melhores resultados foram obtidos quando este fungo foi inoculado com rizóbio. Os tratamentos com inoculação de *Trichoderma* foram positivos para a manutenção de estande e sobrevivência e eficácia contra a *R. solani*, evidenciando o efeito da inoculação do *Trichoderma*, principalmente na semente e no solo.

Palavras-chave: biocontrole, fixação biológica do nitrogênio, *Rizoctonia solani*, *Vigna unguiculata*

Introduction

The cowpea, *Vigna unguiculata* (L.) Walp, is a plant belonging to the family Fabaceae, which is distinguished by being a rich source mainly in protein and iron (Santos et al., 2009). It has become one of the alternative sources of income and a staple food for the population of savannah areas like in the North and Northeast of Brazil, by consuming it in the form of mature grains and greens. In these areas, adaptability and stability are very important characters, which can determine the success or failure of a crop, especially in regions where environmental conditions are greatly influenced by the amount and distribution of rainfall, that vary from location to location and the time of year (Freire Filho et al., 2005).

The inoculation of cowpea starts with an inoculant from a product or formulation containing certain micro-organism in order to introduce or increase certain microbial community in the environment of interest, making it possible to increase this type of fixed N in agro ecosystems. In this case the FBN is manipulated by the use of inoculant strains that were selected based on and adapted to the environmental conditions (Zilli et al., 2009). In the savannah such as in Tocantins, the use of inoculants on cowpea is still very limited, requiring further studies on biological nitrogen fixation in this crop and the agronomic efficiency of rhizobia strains in terms of climate and soil of the savannah in southern Tocantins.

This legume, though well adapted to these regions, is still susceptible to various diseases. One of the most important foliar blights affecting it is known as web blight, initiated by the fungus *Rhizoctonia solani*. The fungus *R. solani* features three ecological types, according to the part that it infects: aerial, surface and underground. The type of blight of that affects cowpea is isolated to the shoot area, which, besides reducing photosynthesis due to the occurrence of necrotic lesions on the leaf, and foliar breakage, can cause seedling death when transmitted by seeds. Under the conditions of the humid tropics and in some regions of semi-arid conditions, the combination of temperature and high relative humidity favors the development of the disease, as a result decreases the yield. This disease can be responsible for up to 50% losses in yield, causing it to become economically important to find a solution, as it is a fungus with genetically diverse soil pathogens that occur in various species of plants worldwide (Sartorato et al., 2006).

As with a majority of plants, there are no effective fungicides available against the *R. solani* disease, even though Chlorothalonil, Thiophanate-Methyl, Iprodione and some other chemicals are sometimes recommended. Furthermore, the use of chemicals to control diseases is a growing concern for environmentalists. In this context, the main task would be to develop a program of biological control. The literature on biological control of soil-borne pathogens with fungi mycoparasitas is huge and various fungi have been reported to be good antagonists of *R. solani*. The most prominent are the fungi *Trichoderma* spp. which are considered important as inoculants for crops, which are among the most studied and known biocontrol agents in the world (Verma et al., 2007).

Currently, species of *Trichoderma* spp. are the biological control agents most commercially used in Brazil (Lopes, 2009). Formulated as biopesticides, biofertilizers and soil inoculants (Harman et al., 2004), and *T. harzianum* species have been studied further. Some species of *Trichoderma* spp. can promote plant growth, increase seed germination and emergence. This happens in a seemingly symbiotic relationship and not parasitic, between the fungus and the plant, where the fungus occupies the niche and the plant nutrient is protected from diseases. The ability of the fungus to colonize the roots is a key factor in its growth and plant productivity (Samuels, 2006).

The increase in plant growth caused by *Trichoderma* spp. may involve some factors still unclear, such as the production of hormones and vitamins, converting materials to a form useful for plant absorption, the transport of minerals and especially the control of pathogens (Harman et al., 2004; Chagas Jr et al., 2012, Oliveira et al., 2012; Silva et al., 2012), as in the control of blight (*R. solani*) in cowpea.

Therefore, inoculation of rhizobia and *Trichoderma* spp can exert antagonistic action against blight, act as a growth promoter and is effective against the infective activity and nitrogen fixation of rhizobial strains. The purpose of this work was to evaluate the combined inoculation of rhizobia and *Trichoderma* spp. as a viable substitute for industrial chemicals by seeking biological sustainability and increased productivity of the plant cowpea grown in the fields of the savannahs of Brazil.

Material and Methods

The study was conducted at the Experimental Field of the Federal University of Tocantins Campus Gurupi - TO, located at 11º43' south latitude and 49º04' west longitudes, 280 m altitude. Characterization of the local climate is a humid tropical climate with little water deficiency (B1wA'a') as classified Tornthwaite.

Before planting, we collected a soil sample composite and performed the physical and chemical characterization according to Embrapa (1997), and the following levels were found: 1.5 $cmol_c$ dm^3 of Ca; 0.7 $cmol_c$ dm^3 of Mg; 0.1 $cmol_c$ dm^3 of K; 2.8 mg dm^3 of P; 0.07 $cmol_c$ dm^3 of Al; 7.4 $cmol_c$ dm^3 of CTC; 2.3 $cmol_c$ dm^3 of 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.

Mineral fertilization was performed before sowing by applying 80 kg of P_2O_5 of superphosphate, and 60 kg of K_2O in the form of KCl, based on soil analysis and crop needs.

The experiment was conducted during the 2012/2013 harvest (December 2012 to March 2013). Each experimental plot consisted of a length of five meters and a width of four meters in nine planting rows of cowpea (vinegar variety), the spacing between the planting rows was 0.50 m. The size of each plot was 20 m^2.

The treatments were: T1: Rhizobia inoculation only; T2: *Trichoderma* spp. inoculation only in the seed; T3: Rhizobia inoculation and *Trichoderma* spp. in the seed; T4: Rhizobia seed inoculation and *Trichoderma* sp. in soil; T5 Rhizobia seed inoculation and *Trichoderma* spp. at 15 days after planting (DAP), T6: Rhizobia inoculation and *Trichoderma* spp. the seed and *Trichoderma* spp. at 15 DAP, T7: Rhizobia inoculation on seed and *Trichoderma* spp. in soil and *Trichoderma* spp. at 15 DAP; T8: Control fertilized with nitrogen and T9: Control without nitrogen and non-inoculated. The experiment was a randomized block with four replications.

Rhizobia strains were used after growing on YMA medium (yeast extract, agar and mannitol) for five days, then were suspended individually in a saline solution (0.2% $MgSO_4$) and these suspensions (10^9 cells mL^{-1}) were added together with the seed two hours before sowing, using 50 mL kg^{-1} of seeds. An inoculation was done with strains INPA 03-11B and UFLA 03-84 characterized as *Bradyrhizobium* sp. obtained from the Laboratory of Microbiology, of the Federal University of Lavras (UFLA) and approved as inoculants for the cultivation of cowpea by the Ministry of Agriculture, Livestock and Supply (MAPA).

For the treatments with *Trichoderma* spp. the commercial inoculant Trichoplus JCO was used with a dose of 10 g kg^{-1} of seeds. Treatment with direct application to the soil was done by applying 3 kg of Trichoplus JCO powder per hectare, corresponding to 4 g per plot. The commercial product Trichoplus JCO formulated with *Trichoderma* spp. with minimal concentration of 2 x 10^{12} L^{-1} of viable conidia was applied as indicated by the manufacturer (JCO fertilizantes) directly to seed and fertilizer treatments mixed into the soil with *Trichoderma* spp. and coverage for treatments with applications of 15 DAP. The distribution of 4 g Trichoplus JCO covered at 15 DAP was made after diluting Trichoplus JCO in 10 L of water for each plot, applied to the rows using a backpack sprayer.

For the treatment using nitrogen (urea), 50 kg ha^{-1} of N, divided into two applications of 20 kg ha^{-1} at planting and 30 kg ha^{-1} coverage 25 days after plant emergence.

The emergence occurred starting from the third day after sowing. We performed thinning of the seedlings at 15 days after planting, leaving 10 plants per linear meter.

Assessments were made at 25 and 50 DAP. For each evaluation we collected six plants from each plot, being held washing the roots under running water to remove all objectionable material taking care not to lose the roots and nodules, with the aid of a sieve. Shoots were separated from roots with a cut made to the base of the stem, and the nodules were removed and counted. Subsequently the shoot, root and nodules were placed in paper bags and taken and placed in a drying oven for 72 hours at 65 ºC until constant weight was achieved. The evaluation was done using shoot dry matter (SDM), root (RDM), and total (TDM), as well as the number of nodes (NN) and dry mass of nodules (MSN). With the aboveground biomass of the last evaluation (50 DAP) the relative efficiency was determined (RE) and calculated according to the formula: ER = (MSPA inoculated / N with MSPA) x 100 (Lima et al., 2005). The N content in the shoot was determined by the use of the Kjeldahl method (Bremner & Mulvaney, 1982). The nitrogen accumulation in shoots (NAS) was calculated by multiplying the content by dry weight of shoot. Based on the values of nitrogen accumulated (total N) determined the symbiotic efficiency, calculated using the formula: ES = [(total N fixed - Ntotal TS / N) / (total N TC / N - Ntotal TS / N) x 100], where N = Total fixed Total Nitrogen treatment; Ntotal TS / N = Total Nitrogen control without nitrogen, total N TC / N = Total Nitrogen of N-control (Lima et al., 2005).

We assessed the incidence of foliar blight (*R. solani*) and initial and final stand counts. To evaluate the incidence of the disease in the population, we used the percentage with symptoms of blight in the study population. Disease severity was determined by the percentage of diseased area in the plant. At 25 DAP was rated the initial stand and final stand at 50 DAP in ground area (6 m^2), which represent the five central lines (2 m^2) per 3 m long, 1 m distant from the border. The efficacy (E%) or control efficiency of foliar blight by treatments was calculated using the following equation: E% = {1 - [Ti / Tc]} x 100, where E% = efficiency of the treatment; Ti = % average final stand count in treatment i, Tc =% average final stand count in the control treatment (Gava & Menezes, 2012).

The grain yield was obtained in the central rows of each plot with an area of 6 m^2, after maturation of plants. The crop was harvested when approximately 80% of the pods had dried up. Then the pods were threshed manually correcting the grain moisture to 14%, and then the yield was calculated.

Data was subjected to analysis of variance and treatment means compared by the Duncan test at 5% using the statistical program ASSISTAT version 7.1 beta.

Results and Discussion

A SDM at 25 DAP was higher ($p < 0.05$) for treatments inoculated with rhizobia and *Trichoderma* spp. on seed, rhizobia and *Trichoderma* spp. on seed more application at 15 DAP and rhizobia and *Trichoderma* spp. in the soil more application at 15 DAP (Table 1) . For the RDM there was no

Table 1. Shoot dry matter (SDM), root (RDM), total (TDM), nodule number (NN) and dry mass of nodules (NDM) in cowpea cv. vinegar inoculated with rhizobia and *Trichoderma* spp.[1]

Treatments	SDM	RDM	TDM	NN	NDM
		(g)			(mg)
			25 DAP[2]		
Rhizobia	2.0 b	0.4 a	2.4 a	33 a	157 a
Tricho. (seed)	2.1 b	0.4 a	2.5 a	17 b	60 b
Riz. + *Tricho*. (seed)	2.4 a	0.4 a	2.8 a	43 a	193 a
Riz. + *Tricho* (soil)	2.0 b	0.5 a	2.5 a	35 a	167 a
Riz. + *Tricho*. 15 DAP	1.6 b	0.4 a	2.0 b	43 a	137 a
Riz. + *Tricho*. (seed) e 15 DAP	2.4 a	0.4 a	2.8 a	47 a	173 a
Riz. + *Tricho*. (soil) e 15 DAP	2.6 a	0.4 a	3.0 a	29 a	177 a
Control with N	1.7 b	0.4 a	2.1 b	11 b	83 b
Control without N	0.3 c	0.2 b	0.5 c	10 b	56 b
CV (%) [3]	13.9	28.0	13.8	36.4	51.9
			50 DAP (Flowering)		
Rhizobia	11.8 a	2.0 b	13.8 a	111 a	350 a
Tricho. (seed)	12.8 a	2.8 a	15.6 a	37 b	150 b
Riz. + *Tricho.* (seed)	11.4 b	2.8a	14.2 a	120 a	333 a
Riz. + *Tricho* (soil)	9.4 b	2.7 a	12.1 b	115 a	253 a
Riz. + *Tricho*. 15 DAP	8.3 b	2.5 a	10.8 b	119 a	276 a
Riz. + *Tricho.* (seed) e 15 DAP	12.9 a	3.0 a	15.9 a	141 a	250 a
Riz. + *Tricho.* (soil) e 15 DAP	13.4 a	3.5 a	16.9 a	116 a	293 a
Control with N	11.7 a	2.0 b	13.7 a	25 b	110 b
Control without N	3.1 c	0.5 c	3.6 c	18 b	50 b
CV (%)	14.5	33.5	15.4	22.9	33.7

[1] Means followed by the same lowercase letter in columns do not differ by the Duncan test at 5%.
[2] CV: coefficient of variation

difference between treatments, only in relation to the control. Only for TDM treatment rhizobia more *Trichoderma* spp. at 15 DAP showed means inferior to the other inoculation treatments. For NN and NDM we found a higher mean ($p < 0.05$) in most treatments compared to the fertilized control and control, except for treatments only in *Trichoderma* spp. seed.

For the evaluation at 50 DAP (Table 1), the SDM was significantly higher ($p < 0.05$) for most of the treatments inoculated with rhizobia, however the rhizobia treatments more *Trichoderma* in the seed, rhizobia more *Trichoderma* in the soil and rhizobia more *Trichoderma* at 15 DAP, had significantly lower MSPA than the other treatments at inoculation. For the RDM no significant differences between the inoculation treatments were found, except for the treatment with only rhizobia. The best results for TDM were the inoculated treatments, with the exception of the rhizobia and *Trichoderma* spp. treatments on soil and rhizobia and *Trichoderma* spp. at 15 DAP. NN relative to the lowest means in the treatments were found only in *Trichoderma* spp. fertilized and seed control, and control, which also occurred for the variable NDM.

Regarding à shoots (SDM, RDM and TDM), treatments that stood out were the inoculation of rhizobia and *Trichoderma* spp. on seed and *Trichoderma* spp. at 15 DAP and inoculation of rhizobia and *Trichoderma* spp. and *Trichoderma* spp. in the soil at 15 DAP, the two assessments, overcoming results obtained with nitrogen. Saber et al. (2009) also found increased biomass of *Vicia faba* with the co-inoculation of *T. harzianum* and rhizobia, where the co-inoculation action of bacteria stimulated and facilitated infection of root hairs and root winding of the *V. faba* due to the chitinase and cellulase production by the fungus.

Diniz et al. (2006) found that seeds of lettuce (*Lactuca sativa*) inoculated with *Trichoderma viride* had higher dry matter production of shoots. Machado et al. (2011), in experiments with *Lotus corniculatus* L. and *Avena strigosa* Schreb observed that all strains and rhizobia strains tested in conjunction with a commercial product based on *T. harzianum* were able to promote the growth of oat plants, increasing plant mass of shoots. Chagas Jr et al. (2012) reported also that the inoculation of rhizobia and *Trichoderma* spp. in cowpea yielded significantly higher results for shoot biomass.

Considering nodulation for the first assessment (25 DAP) that was lower ($p < 0.05$) were for the treatments without rhizobia inoculation. This demonstrates that the application of *Trichoderma* spp. did not inhibit the nodulation process. The same happened to the second assessment (50 DAP).

Regarding the relative efficiency, which relates the shoot biomass at 50 DAP, the treatments in relation to the fertilized control (Figure 1), highlighting the treatments with inoculations and fertilized control, except for treatments with rhizobia and *Trichoderma* in the soil and rhizobia and *Trichoderma* at 15 DAP. Considering the symbiotic effectiveness (Figure 1), the highest averages ($p < 0.05$) were found for the treatments inoculated with rhizobia, rhizobia and *Trichoderma* spp. on seed and 15 DAP and rhizobia and *Trichoderma* spp. in soil and 15 DAP, showing the efficiency in the ability to assimilate atmospheric nitrogen by inoculated strains. A symbiotic efficiency of rhizobia strains have been reported in other

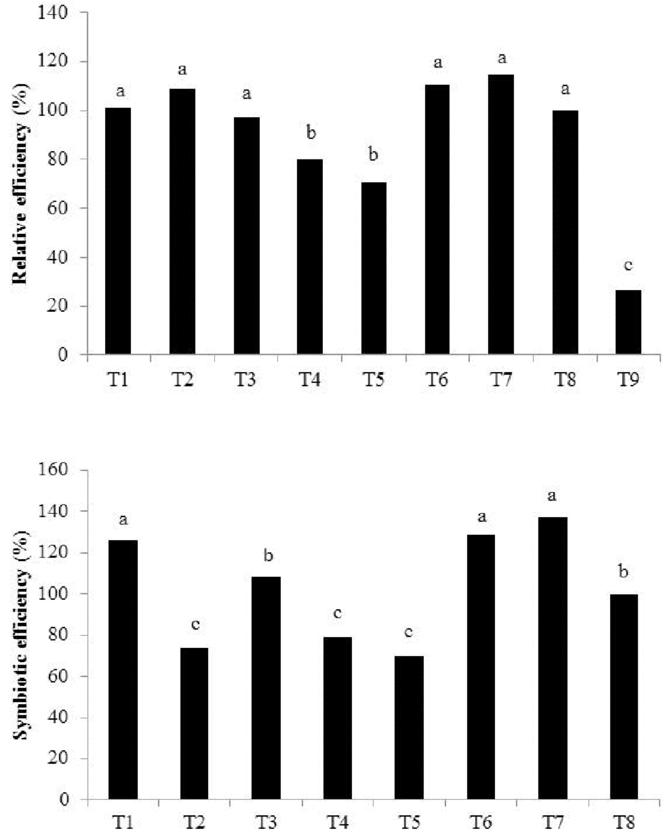

Figure 1. Relative efficiency and symbiotic efficiency rhizobia inoculation in cowpea cv. vinegar. T1: rhizobia (seed), T2: *Trichoderma* spp. (seed), T3: rhizobia (seed) and *Trichoderma* spp. (seed), T4: rhizobia (seed) and *Trichoderma* spp. (soil), T5: Rhizobia (seed) and *Trichoderma* spp. (15 DAP), T6: rhizobia (seed) and *Trichoderma* spp. (seed) and 15 DAP, T7: rhizobia (seed) and *Trichoderma* spp. (soil and 15 DAP), T8: Control with N; T9: Control without N

studies with different cultivars of cowpea (Zilli et al., 2009; Chagas Jr et al., 2010a,b).

For the N content in shoots (Table 2), the treatments inoculated with rhizobia produced significantly greater without differ from fertilized control treatment, which confirms the high efficiency of the strains to fix the nitrogen. The nitrogen accumulation in shoots (NAS), the highest values ($p < 0.05$) were found for the treatments inoculated with only rhizobia, rhizobia and *Trichoderma* spp. in the seed and *Trichoderma* spp. at 15 DAP and rhizobia and *Trichoderma* spp. in the soil and *Trichoderma* spp. at 15 DAP.

The limitation in the formation of nodules in the treatments without rhizobia inoculation restricted N accumulation (Table 2). Regarding variable NAS, treatments with rhizobia and *Trichoderma* spp., both on the soil and in the seed, plus the application of *Trichoderma* spp. at 15 DAP and treatment only with rhizobia, obtained the best results, which realizes the effect, especially inoculating rhizobia strains selected for the bean cowpea. The N content and NAS were also significantly higher in cowpea inoculated with strains of rhizobia as described by Melo & Zilli (2009), Almeida et al. (2010) and Chagas Jr et al. (2010a). According to Brito et al. (2009) the symbiotic fixation provided the largest amount of N accumulated in plants of beans and cowpea, followed, in descending order, by soil and fertilizer.

Table 2. Content of Nitrogen (CN), nitrogen accumulation in shoots (NAS), and yield in cowpea cv. Vinegar inoculated with rhizobia and *Trichoderma* spp.[1]

Treatments	CN (mg g^{-1} plant^{-1})	NAS (mg plant^{-1})	Yield (kg ha^{-1})
Rhizobia	40.5 a	956 a	1.223.9 b
Tricho. (seed)	25.0 b	638 c	1.008.0 c
Riz. + *Tricho.* (seed)	37.5 a	855 b	1.449.8 a
Riz. + *Tricho* (soil)	36.0 a	673 c	1.339.2 b
Riz. + *Tricho*. 15 DAP	37.3 a	616 c	1.205.4 b
Riz. + *Tricho.* (seed) e 15 DAP	38.1 a	983 a	1.617.9 a
Riz. + *Tricho.* (soil) e 15DAP	38.6 a	1035 a	1,486.8 a
Control with N	34.3 a	803 b	1,455.3 a
Control without N	28.7 b	178 d	285.2 d
CV (5)[(2)]	11.8	10.2	11.5

[1] Means followed by the same lowercase letter in columns do not differ by the Duncan test at 5%. [2] coefficient of variation

Table 3. Incidence of blight (*Rhizoctonia solani*) plant cowpea cv. vinegar inoculated with rhizobia and *Trichoderma* spp. Gurupi TO[1]

Treatments	25 DAP[(2)]		50 DAP	
	Incidence	Severity	Incidence	Severity
	(%)			
Rhizobia	25 a	9.7 b	13	10.0 b
Tricho. (seed)	12 d	5.3 c	7	6.0 c
Riz. + *Tricho.* (seed)	11 d	3.3 c	6	5.7 c
Riz. + *Tricho* (soil)	15 c	5.0 c	9	6.0 c
Riz. + *Tricho*. 15 DAP	16 c	8.7 b	10	10.0 b
Riz. + *Tricho.* (seed) e 15 DAP	5 e	4.0 c	1	6.0 c
Riz. + *Tricho.* (soil) e 15 DAP	10 d	5.0 c	3	5.0 c
Control with N[(3)]	22 b	8.0 b	11	8.0 b
Control without N[(4)]	27 a	15.0 a	14	22.3 a
CV (%)[(5)]	16.8	21.9	17.3	20.4

[1] Means followed by the same lowercase letter in columns do not differ by the Duncan test 5%. [2] DAP = Days after planting. [3] Control fertilized with mineral nitrogen. [4] control without inoculation and without fertilization. [5] Coefficient of Variation

As for productivity, especially rhizobia and *Trichoderma* spp. treatments on seed, rhizobia and *Trichoderma* spp. on the seed and *Trichoderma* spp. at 15 DAP and rhizobia and *Trichoderma* spp. in the soil and *Trichoderma* spp. at 15 DAP and control fertilized with superior productivity ($p < 0.01$) to other treatments (Table 2).

We observed grain yields of cowpea ranging from 1205.4 to 1617.9 kg.ha^{-1} for the treatments inoculated with rhizobia, with productivity between 16% and 38% higher compared to treatments with an inoculation of *Trichoderma* spp. only between 76% and 82% compared to the control.

Chagas Jr et al. (2012), in an experiment with cowpea inoculated with rhizobia and *Trichoderma* spp., the productivity was found to be significantly higher in the treatment with the dual inoculation of rhizobia and *Trichoderma* spp. in the seed and soil in relation to the treatment without inoculation of *Trichoderma* spp. on the seed.

The evaluation of the intensity of blight caused by *R. solani* was significantly influenced by the use of inoculation of *Trichoderma* spp. However, even with the incidence of blight in the study population, the occurrence of symptoms was less than 30% at both times (Table 3). Possibly, the treatments with inoculation of *Trichoderma* spp. can provide initial control of pathogens. The lowest percentage of severity was found for treatments with *Trichoderma* spp. on seed and soil. Treatments only inoculated with rhizobia and treatment only at *Trichoderma* spp. at 15 DAP were significantly lower between treatments with inoculations.

For the variable initial stand count (25 DAP), the largest number of plants were found in the treatment inoculated with rhizobia and *Trichoderma* spp. in both on the seed and in the soil, which highlights the control of Blight using *Trichoderma* spp., as noted in the evaluation incidence and severity (Table 4).

In general, the application of *Trichoderma* spp. resulted in a significant reduction in the incidence of cowpea leaf blight. Treatments inoculated with *Trichoderma* spp. an seed and soil and booster at 15 DAP had the highest effectiveness to control blight, ranging from a 49% to 61% reduction in the incidence of disease and consequently the stand count.

As for the survival and efficiency of the treatments inoculated with *Trichoderma* spp. it was evident the effect of inoculation of *Trichoderma* spp., especially in the seed and soil, with values higher than the control without N (Table 4) and control with N. Gava & Menezes (2012) in a study with muskmelon observed that inoculation with *Trichoderma* spp. isolates showed higher plant stand counts at the end of the experiment compared to the control treatment, leading to higher fruit production.

Beyond efficiency as promoters of plant growth, the action of *Trichoderma* spp. as a biocontrol of pathogens may be due to the induction of disease resistance, as is probably due to the treatments inoculated with *Trichoderma* spp., where there was a lower incidence and severity for blight (Table 3) and a higher stand count (Table 4). The efficiency as inducers of

Table 4. Initial and final stand counts, survival and effectiveness of the control of Blight (*Rizhoctonia solani*) in cowpea cv. vinagre inoculated with rhizobia and *Trichoderma* spp.[1]

Treatments	Initial stand 25 DAP[(2)]	Final stand 50 DAP	Survival	Effectiveness
	(plants m^{-2})		(%)	
Rhizobia	26.0 b	24.3 b	81.0 b	35.0
Tricho. (seed)	28.0 a	27.3 a	91.0 a	52.0
Riz. + *Tricho.* (seed)	28.7 a	26.9 a	89.7 a	49.0
Riz. + *Tricho* (soil)	24.3 b	22.3 b	74.3 a	24.0
Riz. + *Tricho*. 15 DAP	25.3 b	24.3 b	81.0 b	35.0
Riz. + *Tricho.* (seed) e 15 DAP	29.3 a	29.0 a	96.7 a	61.0
Riz. + *Tricho.* (soil) e 15 DAP	30.0 a	29.0 a	96.7 a	61.0
Control with N[(3)]	25.0 b	24.7 b	82.0 b	37.0
Control without N[(4)]	21.0 c	18.0 c	60.0 c	-
CV (%)[(5)]	11.8	17.7	12.1	

[1] Means followed by the same lowercase letter in columns do not differ by the Scott-Knott test 5%. [2] DAP = Days after planting. [3] Control fertilized with mineral nitrogen. [4] control without inoculation and without fertilization. [5] Coefficient of Variation

anthracnose resistance by *Trichoderma* spp. was reported by Silva et al. (2011) on cucumber.

Analyzing the results, it can be observed that the application of *Trichoderma* spp. on the seed and 15 DAP significantly favored the variables analyzed. This can be explained by the fact that in seed germination and seedlings at 15 DAP are more susceptible to disease incidence, and a second dose of the *Trichoderma* spp. favored bioprotection of the plants, thus increasing the resistance of the same. However regardless of the occurrence of the disease, the dual inoculation of rhizobia and *Trichoderma* spp. also resulted in greater growth in cowpea. These results may be considered positive for the use of these isolates , since the colonize the plant's root system , these micro-organisms can enhance plant growth, and biocontrol and biological nitrogen fixation, and may be related to the ability of phosphate solubilization and IAA production, as observed in vitro surveys (Chagas Jr et al., 2010b; Oliveira et al., 2012).

Conclusions

The application of rhizobia and *Trichoderma* spp. on the seed followed by an application of *Trichoderma* spp. at 15 DAP in both the seed and in the soil, showed the best results in most of the variables analyzed.

Treatments inoculated with *Trichoderma* spp. were positive for the maintenance of the stand count, and survival and efficiency against blight, showing the effectiveness of inoculation of *Trichoderma* spp., especially in the seed and soil.

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