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Inoculation of *Rhizobium* associated with *Trichoderma asperellum* on the development and yield of cowpea

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ABSTRACT: This study aimed to evaluate the efficiency of simultaneous inoculation of isolates from rhizobia and *Trichoderma* in cowpea and their effects on its growth, nodule quality and grain yield. In the experiment under greenhouse conditions, the cowpea cv. Vinagre, of a medium-late cycle, was used in a test with a completely randomized design. In the field test, three cowpea cultivars (Vinagre, Fradinho and Sempre Verde) were used in a randomized block design, with seven treatments and three replicates. The variables evaluated in the greenhouse were biomass, nodulation and relative efficiency (RE) while the yield was evaluated in the field. Seeds inoculation of cowpea with *Trichoderma* and rhizobia, in the greenhouse, followed by an additional *Trichoderma* shoot application at 25 days after sowing (DAS), was relevant for biomass accumulation, with no difference in the nitrogen application and nodulation. Applying rhizobia was important for the root development. Seed inoculation with rhizobia and *Trichoderma* followed by another Trichoderma application at 25 DAS, provided increased yield in the three studied varieties, under field conditions.

Key words: biomass; nodulation; *Vigna unguicula*

Inoculação de *Rhizobium* associado a *Trichoderma asperellum* no desenvolvimento e produtividade no feijão-caupi

RESUMO: O trabalho teve por objetivo avaliar a eficiência da inoculação simultânea de isolados de rizóbio e *Trichoderma* em feijão-caupi e seus efeitos no crescimento, qualidade de nódulos e na produtividade de grãos. No experimento em casa de vegetação, foi utilizado o feijão-caupi, cv. Vinagre, de ciclo médio-tardio, em ensaio com delineamento inteiramente casualisado. No ensaio de campo, foram utilizadas três cultivares de feijão-caupi, Vinagre, Fradinho e Sempre Verde, com delineamento em blocos casualizados, sete tratamentos e três repetições. Foram avaliadas, em casa de vegetação, as variáveis biomassa, nodulação, eficiência relativa (ER) e em campo, a produtividade. Em casa de vegetação, a inoculação do feijão-caupi com *Trichoderma* e rizóbio nas sementes, seguido de aplicação adicional de *Trichoderma*, aos 25 Dias Após a Semeadura (DAS), na parte aérea, foi importante para o acúmulo de biomassa, não havendo diferença da aplicação de nitrogênio e nodulação. A aplicação de rizóbio foi importante para o desenvolvimento da raiz. A inoculação de sementes com rizóbio e *Trichoderma*, seguido de *Trichoderma*, aos 25 DAS proporcionou aumento de produtividade nas três variedades estudadas, em condições de campo.

Palavras-chave: biomassa; nodulação; *Vigna unguicula*

Introduction

There is today a rising need in using biological products, which have, beyond the lesser environmental impact, a beneficial effect on the microbiota from soil and plant alike, in addition to stimulating an increased yield. Few studies on the cowpea crop are available, despite its importance both economic and social, and the demand for sustainable technologies, as an essential factor for the strengthening of this crop cultivation.

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important leguminous species in the Brazilian diet, especially in the North and Northeast regions, where its production is concentrated (Almeida et al., 2017). This plant adapts itself to extreme environmental conditions, such as high temperatures, water deficit and low fertility (Santos et al., 2008).

The increase in the cowpea-cultivated area has been intensified in the Cerrado biome, where the species is inserted as crop rotation, followed by soybean, rice and maize. Cowpea is usually grown when there is no expectation for rains that would enable growing a second crop (Oliveira et al., 2016). The low mean cowpea yield in Brazil results from the low technological level employed in its cultivation, despite states such as Amazonas, Goiás, Mato Grosso and Mato Grosso do Sul all amassing yields of over 1,000 kg ha^{-1} (Conab, 2019). In the 2018/2019 harvest, around 36 thousand hectares were cultivated in Tocantins, making it the third largest producer state in the North region (Conab, 2019).

This leguminous species can associate symbiotically with microorganisms in the soil, mainly bacteria from the genus *Bradyrhizobium*, *Rhizobium* and *Sinorhizobium*, which perform the biological nitrogen fixation (BNF) and supply the plant with nitrogen compounds. Moreover, they can have symbiosis with fungi and increase the soil exploration capacity, an important propriety for the nutrient recycling (Tampakakia et al., 2017).

During BNF, the atmospheric nitrogen (N_2) is transformed into ammonia (NH_3) at the expense of plant-supplied energy. Furthermore, the plants provide carbohydrates for the nitrogen-fixing bacteria, which leads to an extra metabolic energy expenditure. In addition to some grasses, the legumes are able to perform BNF and contribute by improving soil fertility and increasing production in reducing problems caused by lack of nitrogen. Increasing the efficiency from the nodulation process and, consequently, the BNF is one of the ways to increase yield in some crops (Benício et al., 2011). Wagatsuma et al. (2011) obtained a biomass increase in maize inoculated with *T. harzianum* in combination with *Azospirillum brazilense*, a bacterium capable of performing BNF.

Promoting the plant growth via microorganisms can be through direct mechanisms, such as producing growth-stimulating hormones and phosphate mobilization. Among the microorganisms with this potential are the fungi from the genus *Trichoderma*, which are naturally present in the soils and act as colonizers of the plant roots, stimulating their growth (Haddad et al., 2017) and allowing a greater nutrients availability and increasing the yield (Nieto-Jacobo et al., 2017). Some *Trichoderma* isolates are also able to improve the photosynthetic rate and the plants respiratory activity; others are able to stimulate the growth and production of plant biomass (Hermosa et al., 2012). Promoting the plant growth by applying *Trichoderma* isolates may relate to the phytohormones production, as well as the control of microorganisms that are harmful to the plant in the rhizosphere (Kumar et al., 2017).

In light of the foregoing, this study aimed to evaluate the inoculation efficiency of isolates from rhizobia and *Trichoderma* on cowpea, evaluating as well their effects on growth, nodule quality and grain yield.

Materials and Methods

Nodule quality and growth of cowpea plants inoculated with rhizobia and *Trichodema*

The conduction of the experiment was in a greenhouse, at the Federal University of Tocantins, Gurupi *Campus*, in Tocantins. The employed design was the completely randomized, with 4 replicates and totaling 28 pots (experimental unit).

Treatments description

All cowpea seeds used in the experiments were from the cultivar Vinagre (grain with a reddish-colored tegument).

The treatments used in all experiments, both under controlled conditions as well as in field tests were the following: seed inoculation of *Trichoderma asperellum* (isolate UFT201) and rhizobia (T1); seed inoculation with *T. asperellum* and rhizobia plus additional *T. asperellum* shoot application at 25 days after sowing (DAS) (T2); seed inoculation of rhizobia plus *T. asperellum* shoot application at 25 DAS (T3); seed-only inoculation of *T. asperellum* (T4); seed-only inoculation of rhizobia (T5); nitrogen control (T6) and control with no inoculation and nitrogen application (T7). For the nitrogen control, 50 kg ha^{-1} of nitrogen (urea) was applied in parts inside the pots, with 20 kg ha^{-1} supplied during the planting and 30 kg ha^{-1} during the top-dressing, at 25 days after emergence. Rhizobia inoculation was performed with a commercial inoculant produced with *Bradyrhizobium elkanni* (INPA 03-11B) mixed with *B. viridifuturi* (UFLA 03-84), at the 10^9 UFC mL^{-1} concentration. Seed inoculation was with the 0.5 g mL^{-1} dose of the inoculant diluted in water for 200 g of seeds. Regarding the *T. asperellum* treatments (Isolate UFT 201), 4.8 x 10^8 UFC g^{-1} from the commercial inoculant was used for 200 seeds. In the treatments where an additional *T. asperellum* application occurred, 6.24 g L^{-1} of the inoculant diluted in water and applied via backpack sprayer were used on the shoot at 25 DAS.

The soil used in the experiment was from an area with no cowpea-growing history and previously cultivated with vegetable crop plants. With the collected soil samples, the following physicochemical characteristics were determined: pH 5.6 (in water); 2.8 $cmol_c$ dm^{-3} of Ca; 0.70 $cmol_c$ dm^{-3} of Mg; 0.24 $cmol_c$ dm^{-3} of K; 33.0 mg dm^{-3} of P; 5.2 $cmol_c$ dm^{-3} of CEC;

73% of base saturation; 2.3% of organic matter; and sandy texture (Embrapa, 1997). Based on both the soil analysis and the crop recommendation, a mineral fertilization prior to the sowing was held by applying 80 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O.

Five cowpea seeds (Vinagre variety) were sown in 1.7 L pots, at a 2.0 cm depth, with an equal soil volume in all treatments. At ten days after sowing, the thinning performed left two plants per pot (experimental plot). Then, the plants were daily irrigated with enough water to keep the soil moisture, totaling a daily volume of 400 ml/pot. This volume was sufficient in keeping the soil close to field capacity (FC), which indicates the approximate upper limit of the water amount available to the plants (Jong, 2000).

Collection of the plants was during the flowering (PF), at 43 days, where the number and dry weight of nodules, as well as the dry weight of the shoot (SDW) and root (RDW) were all evaluated. Calculation of the rhizobia relative efficiency (RE) was according to the formula: RE = (inoculated SDW/SDW with N) x 100 (Lima et al., 2005). During collection, the plants in each pot had their roots carefully washed under running water, using a sieve in order to remove all undesirable material and not losing and/or damaging the roots and nodules. The shoot was separated from the roots with a cut made with a utility knife at the stem base and the nodules were manually removed and counted. Subsequently, roots and nodules were separately transferred into paper bags and kept in an oven at 65 °C for drying until constant weight.

Data were subjected to the analysis of variance (ANOVA) by the F test and the means comparison was by the Tukey test, both at 5% probability. All analyses were performed with the statistical program ASSISTAT version 7.6 beta.

Yield of cowpea cultivars inoculated with rhizobia and *Trichoderma* and grown under Cerrado conditions

Three experiments were conducted, in the same experimental area, at the Federal University of Tocantins (Gurupi *Campus*), located at 11°43’ South latitude and 49°04’ West longitude, at an altitude of 280 m. Local climate is of the Aw type (humid climate with moderate water deficiency) as according to the Köppen classification. The conduction of experiments that had the same methodology was in randomized blocks, with three replicates. The treatments were the same described previously in experiment 1, differing only in the used varieties, with adoption of the ones most cultivated in the region, with the cultivars Vinagre, Fradinho and Sempre Verde among them. The tests took place simultaneously with each experimental plot having a useful area of 6.0 m^2 and containing five rows of 3.0 meters in length each, spaced 0.50 m apart. In these plots, 20 seeds were sown per linear meter and, 10 days after germination, the thinning performed left 10 plants per linear meter. These plants were daily irrigated in order to keep the soil moisture.

The soils used in the experimental plots of the three experiments were the same in where the collection for conducting the controlled conditions test occurred (already previously described in item 1.1), in which there was no cowpea-growing history, but a previous ornamental plants cultivation. Based on the chemical soil analysis and on the crop recommended, a mineral fertilization was held prior to the sowing by applying 80 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O. Simple soil samples were taken from the experimental plots for forming a composite sample in order to determine the following physicochemical characteristics: pH 5.6 (in water); 2.8 cmol$_c$ dm^{-3} of Ca; 0.7 cmol$_c$ dm^{-3} of Mg; 0.24 cmol$_c$ dm^{-3} of K; 33.0 mg dm^{-3} of P; 5.2 cmol$_c$ dm^{-3} of CEC; 73% of base saturation; 2.3% of organic matter and sandy texture (Embrapa, 1997).

The harvesting was performed when about 80% of the pods were dry. Following this, these harvested pods were manually threshed and the following parameters were determined in each treatment: grains number, length of five pods, weight of 100 grains and grain yield. During yield calculation, the grain moisture was corrected to 14% and the data were expressed in kg ha^{-1}.

Data were subjected to the analysis of variance (ANOVA) by the F test and the means comparison was with the Scott-Knott test, both at 5% probability. All analyses were performed with the statistical program ASSISTAT version 7.6 beta.

Results and Discussion

Nodule quality and growth of cowpea plants inoculated with rhizobia and *Trichoderma*

During this study, the observed was that, for the cv. Vinagre, generally there were no statistical differences in the treatments with rhizobia and/or *Trichoderma* in relation to the non-inoculated control on the shoot biomass (Figure 1A), assuming values close to the obtained in the nitrogen control. Regarding the dry root biomass, however, the treatment that had seed-only inoculation of rhizobia, followed by the treatment with *T. asperellum* and rhizobia in the seed plus additional *T. asperellum* shoot application at 25 DAS, differed from the other treatments (Figure 2B). According to Chagas Junior et al. (2010), inoculation of leguminous seeds with rhizobia and *Trichoderma* not only protect plants against various diseases, but also improves the nodulation and its growth. Thereby, according to the obtained results and considering the non-inoculated control, it is possible to infer that, regarding the cv. Vinagre, these two treatments were effective in increasing the root biomass, but not having the same efficiency in respect to the shoot biomass.

Machado et al. (2011) observed that joint-applying rhizobia with *Trichoderma harzianum* promoted the growth of black oat plants (*Avena strigosa*) with increased shoot biomass. The same study mentioned does not corroborate with the results obtained in this own, since where the simultaneous inoculation was made in the seed, followed by *Trichoderma* shoot application at 25 DAS, did not result in increased shoot biomass.

In this present study, there was a favorable increase in the dry root biomass for cowpea plants inoculated with

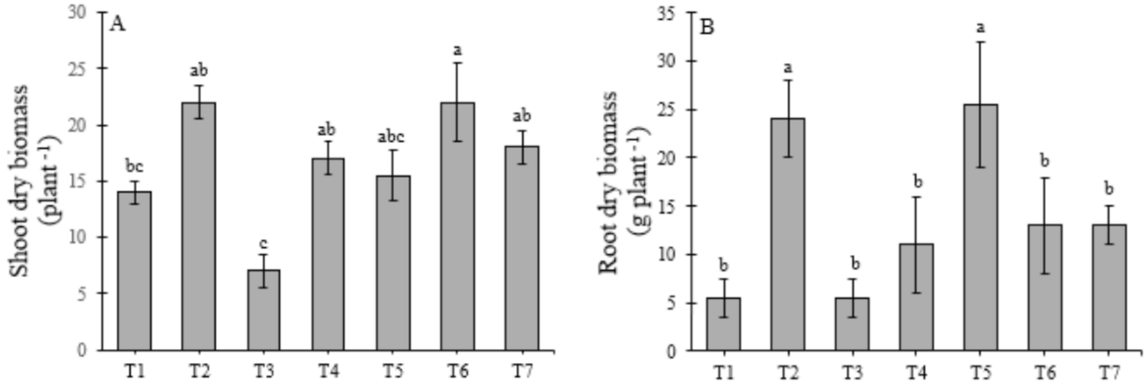

Figure 1. Dry biomass from the (A) shoot (CV* = 21.79) and (B) root (CV = 29.88) in cowpea plants (cv. Vinagre) subjected to the following treatments: (T1) seed inoculation of *Trichoderma* and rhizobia; (T2) seed inoculation of *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 days after sowing (DAS); (T3) seed inoculation of rhizobia and *Trichoderma* shoot application at 25 DAS; (T4) seed-only inoculation of *Trichoderma*; (T5) seed-only inoculation of rhizobia ; (T6) nitrogen control; and (T7) non-inoculated control. Same lowercase letters do not differ from each other by the Tukey test ($p \geq 0.05$). *CV (%) = Coefficient of Variation

Trichoderma and rhizobia in their seeds, followed by a *Trichoderma* shoot application at 25 DAS, statistically similar to that registered for plants only inoculated with rhizobia (Figure 1B). This increased root biomass indicates that there may have been a greater resistance to abiotic stresses in the rhizobia treatments, such as pH and temperature, which are incident on the rhizosphere.

A greater nodule production occurred in the treatments with only inoculation of rhizobia and in the treatment with seed inoculation of rhizobia followed by the *Trichoderma* shoot application at 25 DAS (Figure 2A); however, the nodules from this last treatment had lesser dry biomass (Figure 2B). This probably resulted in the lowest dry biomass from shoot and roots alike (Figure 1). Silva et al. (2007) found lower root dry biomass values in cowpea plants inoculated with the *Paenibacillus polymyxa* rhizobacterium, and this may be related to the increased energy expenditure caused by the bacteria in the plant. On the other hand, Guareschi et al. (2012) verified increased shoot and root biomass in soybean after inoculating its seeds with a *Trichoderma* suspension.

It is important emphasizing that, in this study, the nodules were also registered both in the treatments with inoculation of rhizobia and in those with only *Trichoderma* inoculation, the nitrogen control and the non-inoculated control (Figure 2). This response is probably due to the presence of a native rhizobia population previously existing in the soil, with the nodulation-inducing ability. This fact can also explain some responses verified in the plants, such as the increased biomass of the shoot, roots and nodules (Figures 1 and 2).

Increased nodule dry biomass occurred in the plants that had their seeds inoculated with *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 DAS (Figure 2B). This treatment also differed from the non-inoculated control with respect to the number of nodules (Figure 2A). The close relationship between *Trichoderma* and the plant species results in beneficial effects for the plants, such as increased efficiency in using nitrogen through the mechanisms of reduction and assimilation of this nutrient (Lorito et al., 2010). This increased

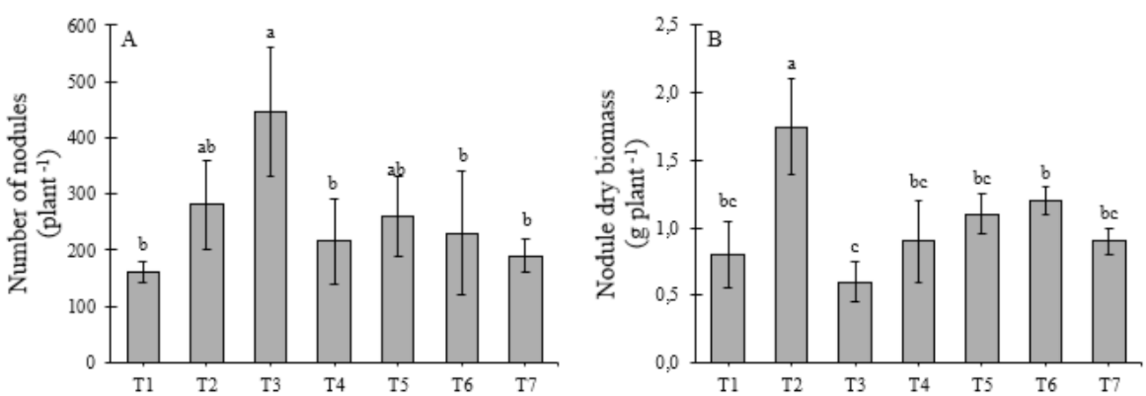

Figure 2. (A) Number (CV* = 30.66) and (B) dry biomass (CV = 21.33) of nodules in cowpea plants (cv. Vinagre) subjected to the following treatments: (T1) seed inoculation of *Trichoderma* and rhizobia; (T2) seed inoculation of *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 days after sowing (DAS); (T3) seed inoculation of rhizobia and *Trichoderma* shoot application at 25 DAS; (T4) seed-only inoculation of *Trichoderma*; (T5) seed-only inoculation of rhizobia; (T6) nitrogen control; and (T7) non-inoculated control. Same lowercase letters do not differ from each other by the Tukey test ($p \geq 0.05$). *CV (%) = Coefficient of Variation.

number of nodules in the cowpea plants subjected to seed inoculation with *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 DAS is probably responsible for the significant increased biomass of the shoot and roots, as demonstrated in Figure 1. The nodule development is strongly correlated with the shoot due to the need in partitioning the photoassimilates produced by the plant (Voisin et al., 2010). Co-inoculation with *T. harzianum* and rhizobia stimulates the action of the nitrogen-fixing bacteria and can cause an increased number of root hairs. Moreover, the efficiency and the capacity in surviving and forming nodules of the nitrogen-fixing bacteria also depends on the interaction with edaphoclimatic factors (Guareschi et al., 2011).

As for the relative efficiency from the shoot biomass, during the flowering, at 43 DAP (Figure 3), significant differences were found in the following treatments: seed inoculation with *T. asperellum* and rhizobia plus additional *T. asperellum* shoot application at 25 days after sowing (DAS); seed-only inoculation *T. asperellum*; and the non-inoculated control and no mineral nitrogen application control, having the highest values ($p < 0.05$) but not differing from the control treatment fertilized with mineral nitrogen. Other treatments had significantly lower results, such as the following: seed inoculation of *T. asperellum* and rhizobia; seed inoculation of rhizobia plus additional *T. asperellum* shoot application at 25 DAS; seed-only inoculation of rhizobia, respectively represented by T1, T3 and T5, where occurred a reduction of between 32.9 and 67.1% in relation to the nitrogen control.

Regarding the seed-only inoculation of rhizobia, a lesser efficiency was observed, statistically different from the control using chemical fertilization (Figure 3). Hence, stating that the tested strains demonstrated efficiency in stimulating

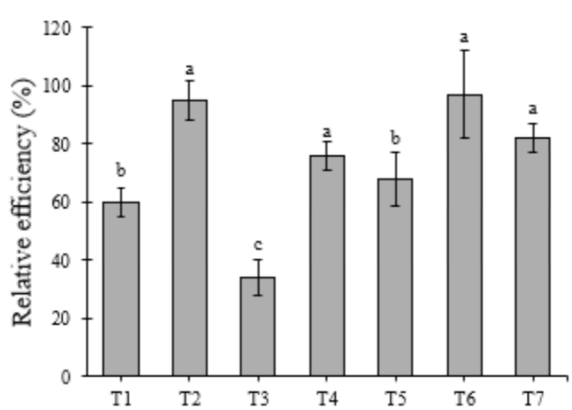

Figure 3. Relative efficiency (CV% = 21.79) in cowpea plants (cv. Vinagre) subjected to the following treatments: (T1) seed inoculation of *Trichoderma* and rhizobia; (T2) seed inoculation of rhizobia and *Trichoderma* plus additional *Trichoderma* shoot application at 25 days after sowing (DAS); (T3) seed inoculation of rhizobia and *Trichoderma* shoot application at 25 DAS; (T4) seed-only inoculation of *Trichoderma*; (T5) seed-only inoculation of rhizobia; (T6) nitrogen control; and (T7) non-inoculated control. Same lowercase letters do not differ from each other by Tukey test ($p \geq 0.05$). *CV (%) = Coefficient of Variation.

nodulation and biomass formation was thus not possible. Chagas Junior et al. (2010), under greenhouse and field conditions, found different results from those discussed when evaluating the biomass of cowpea inoculated with rhizobia strains.

Agronomic efficiency of cowpea cultivars inoculated with rhizobia and *Trichoderma* and grown under Cerrado conditions

Mean yield values of the cowpea plants (cultivars Vinagre, Fradinho and Sempre Verde) subjected to different treatments are available in Table 1. Among the studied variables, there was a significant difference ($p < 0.05$) in yield for all cultivars.

This study registered significant differences in grain yield for cowpea plants from the cv. Vinagre, highlighting following treatments as the best: seed inoculation of *Trichoderma* and rhizobia, seed inoculation with *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 DAS, seed-only inoculation of rhizobia and the nitrogen control. These treatments recorded mean yield of 702.22; 715.55; 686.66 and 517.77 kg ha^{-1}, respectively, higher values than those registered for the national mean yield of cowpea, which is from 130 to 500 kg ha^{-1} (Freire Filho et al., 2011). In light of the obtained results, and taking the good availability of soils and water into account, it is likely that the cowpea crop in the central-northern region of Brazil may contribute to increasing the national mean yield, bearing in mind the good conditions that favorable to the plants development.

The *Trichoderma* interference with plant growth and increased yield may happen due to its ability in colonizing roots (Carvajal et al., 2009). Currently, allied to the possible beneficial effects on plants, there is a great environmental appeal for using biological products that can benefit the production of healthier foods and help in controlling diseases, with positive effects on the agricultural production.

Table 1. Yield of cowpea (cultivars Vinagre. Fradinho and Sempre Verde) subjected to the following treatments: (T1) seed inoculation of *Trichoderma* and rhizobia; (T2) seed inoculation with *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 days after sowing (DAS); (T3) seed inoculation of rhizobia and *Trichoderma* shoot application at 25 DAS; (T4) seed-only inoculation of *Trichoderma*; (T5) seed-only inoculation of rhizobia; (T6) nitrogen control; and (T7) non-inoculated control.

Treatments	cv. Vinagre	cv. Fradinho	cv. Sempre Verde
	Yield (kg ha^{-1})		
T1	702.22 a	446.66 d	1725.92 b
T2	715.55 a	2280.00 a	2875.55 a
T3	286.66 b	2095.55 a	1634.07 b
T4	406.66 b	733.33 c	1315.55 b
T5	686.66 a	1281.11 b	1666.66 b
T6	517.77 a	668.14 c	1073.33 c
T7	180.00 b	326.66 d	588.88 c
CV[1](%)	24.13	14.86	19.27

Same lowercase letters do not differ from each other by Scott-Knott test at 5% probability.
[1] CV (%) = Coefficient of variation.

Regarding the yield from cv. Fradinho, the following treatments: seed inoculation with *T. asperellum* and rhizobia plus additional *T. asperellum* shoot application at 25 days after sowing (DAS), followed by the treatment with seed inoculation of rhizobia plus additional *T. asperellum* shoot application at 25 DAS, had statistical difference ($p < 0.05$) in comparison with the other treatments, thus demonstrating a superiority over them, with the highest yield means, producing 1611.86; 1427.41 kg ha^{-1} more than the nitrogen control and 1954.66; 1768.89 kg ha^{-1} more than the absolute control (Table 1). Hermosa et al. (2012), state that some *Trichoderma* spp. isolates have the ability of interacting with plant roots, promoting vegetative growth, disease resistance and tolerance to abiotic stresses, thus resulting in healthier and more productive plants. The treatment containing only rhizobia also proved to be efficient when compared to the nitrogen control, producing about 612.97 kg ha^{-1} more than it. These results show that, depending on the interaction with the cultivar and the local edaphoclimatic conditions, using rhizobia in the cultivar Fradinho can replace mineral fertilization in terms of yield. The following treatments: seed inoculation of *T. asperellum* and rhizobia and seed-only inoculation of *T. asperellum* had the lowest means in relation to the treatments with inoculation, differing ($p < 0.05$) from each other and from the other inoculated treatments, inferring that, for this cultivar, it may be necessary using *Trichoderma* reinforcement on the shoot of the plant. Chagas et al. (2016), studying the growth stimulation in common bean (*P. vulgaris* L.) by *Trichoderma*, verified an improvement in plant growth and development, where the growth stimulation was evidenced by the increased biomass and yield.

For the experiment using the Sempre Verde variety (Table 1), a statistical difference ($p < 0.05$) was found regarding the yield. The best treatment was seed inoculation with *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 days after sowing. This treatment produced 2286.67 kg more than the absolute control, with no rhizobia inoculation and no nitrogen. The results for this cultivar corroborate with the data obtained by Bernardes et al. (2010), who verified that the bean seeds inoculation with only *Trichoderma* did not provide a statistical difference in the crop yield components. Lobo Júnior et al. (2009) obtained significant increases in the production of the common bean cultivar Pérola, when applying *Trichoderma* spp. isolates via boom sprayer. This treatment had a production of 1802.22 and 2286.67 kg ha^{-1}, higher than the treatments of nitrogen control and non-inoculated control.

Species from the genus *Trichoderma* can act as organisms that decompose organic matter and cycle nutrients, thus causing positive effects on the nutrients flow in the development of pods and filling of grains (Haddad et al., 2017). Although these effects have already been verified in several studies, they are influenced by factors such as the synergistic and antagonistic interactions with other microorganisms, soil moisture, temperature, nutrients, etc. *Trichoderma* fungi, in addition to controlling phytopathogens, can also be used as growth-promoting agents. This mechanism refers to the plants development in general, including the beneficial effects on seed germination, emergence, seedling development, and production of grain and fruit. Regarding the effects of this fungus, Chagas et al. (2016) describe that the solubilized nutrients become available for absorption by the roots, thus reducing the fertilization need of the plant.

As for the yield, in general, the treatments seed inoculation of *Trichoderma asperellum* and rhizobia, seed inoculation of rhizobia plus additional *T. asperellum* shoot application at 25 DAS; seed-only inoculation of *T. asperellum* and seed-only inoculation of rhizobia and with no nitrogen application were classified as the best, with means superior to the nitrogen control treatments (no inoculation and no nitrogen application). Such results suggest there is a specificity between symbiotic microorganisms with the cowpea, in a similar fashion of the best treatments, while applying only rhizobia in the seeds from cultivar Vinagre was enough to increase yield when compared with the non-inoculated and no nitrogen controls. In the cultivars Fradinho and Sempre Verde, it was demonstrated the efficiency of using *Trichoderma* and rhizobia in the seeds treatment associated with the subsequent spraying of the fungus on the cowpea plant shoot. The benefits of using the biological agent were also observed by Chagas Junior et al. (2010), who found higher yield in cowpea plots, whose seeds were treated with *Trichoderma* spp. and rhizobia, compared to non-inoculated seeds or only inoculated with rhizobia alone.

Conclusions

Seed inoculation of cowpea with *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 days after sowing (DAS) was important for the accumulation of biomass and nodulation, evaluated during the flowering.

No inhibitory effect from *Trichoderma* was verified, when it was inoculated into the seeds on the rhizobia strains in cowpea.

Seed inoculation of cowpea with rhizobia and *Trichoderma*, followed by *Trichoderma* at 25 DAS provided increased yield in the cultivars Sempre Verde and Fradinho.

In cultivar Vinagre, the best treatments were the seed inoculation of *Trichoderma* and rhizobia, seed inoculation with *Trichoderma* and rhizobia plus additional *Trichoderma* shoot application at 25 DAS, seed-only inoculation of rhizobia and the nitrogen-only control.

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