

## **Biomass efficiency and productivity of soybean inoculated with Trichoderma**

### **Eficiência de biomassa e produtividade de soja inoculada com Trichoderma**

DOI:10.34117/bjdv7n11-563

Recebimento dos originais: 12/10/2021

Aceitação para publicação: 30/11/2021

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**ABSTRACT**

This work aimed to evaluate the efficiency of the product TrichoPlus (*Trichoderma asperellum*) as a plant growth promoter in soybean and the productive performance at the field in a floodplain in the municipality of Formoso do Araguaia, Tocantins, Brazil. Four treatments with different doses of the TrichoPlus product (2, 4, 6 and 8 g per kg of seeds) were used in the experiment, plus two treatments being a positive control with a commercial product based on *Trichoderma asperellum* and an absolute control (without inoculation). Biomass, stand and productivity data were evaluated. For the treatment with the TrichoPlus product, a powder formulation was used, with active ingredient based on *Trichoderma asperellum* 201, formulated with a minimum concentration of  $2 \times 10^8$  UFC g<sup>-1</sup>, having graphite in the composition as inert, being applied directly to the seeds before planting, aiming better adherence and protection of the *Trichoderma* in direct contact with the seed and protection right after planting. Significant results were observed for the treatments with different doses of TrichoPlus in the characteristics of biomass, stand maintenance and productivity. The best dose for productivity was achieved in 5.1 g kg<sup>-1</sup> of seed, with gains 12.6 and 6.0% higher compared with the absolute control and positive control treatments, proving its effectiveness in promoting plant growth and, consequently, in the productivity, in the cultivation in floodplain soil.

**Keywords:** Fungus, *Trichoderma asperellum*, *Glycine max* L. Merr., Yield components.

**RESUMO**

Este trabalho teve como objetivo avaliar a eficiência do produto TrichoPlus (*Trichoderma asperellum*) como promotor de crescimento vegetal em soja e o desempenho produtivo no campo em uma planície de inundação no município de Formoso do Araguaia, Tocantins, Brasil. Quatro tratamentos com diferentes doses do produto TrichoPlus (2, 4, 6 e 8 g por kg de sementes) foram utilizados no experimento, mais dois tratamentos sendo um controle positivo com um produto comercial à base de *Trichoderma asperellum* e um controle absoluto (sem inoculação). Dados de biomassa, estande e produtividade foram avaliados. Para o tratamento com o produto TrichoPlus, foi utilizada uma formulação em pó, com princípio ativo à base de *Trichoderma asperellum* 201, formulado com concentração mínima de  $2 \times 10^8$  UFC g<sup>-1</sup>, tendo grafite na composição como inerte, sendo aplicado diretamente nas sementes antes do plantio, visando melhor aderência e proteção do *Trichoderma* no contato direto com a semente e proteção logo após o plantio. Resultados significativos foram observados para os tratamentos com diferentes doses de TrichoPlus nas características de biomassa, manutenção do estande e produtividade. A melhor dose para produtividade foi alcançada em 5,1 g kg<sup>-1</sup> de semente, com ganhos 12,6 e 6,0% maiores em relação aos tratamentos controle absoluto e controle positivo, comprovando sua eficácia na promoção do crescimento das plantas e, conseqüentemente, na produtividade, na região. cultivo em solo de várzea.

**Palavras-chave:** Fungo, *Trichoderma asperellum*, *Glycine max* L. Merr., Componentes de produção.

## 1 INTRODUCTION

The high productivity of crops such as soybean (*Glycine max* L. Merr.) is generally associated with the use, among other factors, of high doses of fertilizers. These, in its turn, represent a significant portion of the crop production costs, in addition to being industrially obtained from non-renewable sources, and potentially environmental pollutants.

The use of beneficial microorganisms in large agricultural commodities has been gaining space in Brazil. With the intensification of the problems encountered in the field of breaking the resistance of chemical products, the use of microorganisms in agriculture is showing good results and it is becoming the target of large companies that work with microorganisms for biological control and plant growth promoters (Brazil, 2019), such as *Trichoderma* (Bettiol et al., 2019; Barbosa et al., 2021).

As an additional advantage, these microorganisms are referred to as non-toxic to humans and animals (Mertz et al., 2009), affordable, the production does not depend on a limited natural resource and some, still, can persist in the soil or plants, and may dispense reapplications (Suassuna et al., 2019). Such fungi present high genetic diversity (Bissett et al., 2015; Quin and Zhuang, 2016; Plessis et al., 2018) and can be used to produce a wide variety of products of interest.

Some lineages of *Trichoderma* increase the total root system surface, allowing a greater access to the mineral elements present in it. Others are capable of solubilizing and making available to the plant rock phosphate, iron, copper, manganese and zinc. In addition, they can improve the active mechanisms of absorption of macro and micronutrients, as well as increase the efficiency of the plant to use some important nutrients, such as nitrogen (Das et al., 2017; Woo and Pepe, 2018; Mendoza-Mendoza et al., 2018).

These fungi can positively influence seed germination, development and crop yield, also due to the production of growth-promoting substances and improvement in plant nutrition, mainly through phosphorus solubilization, synthesis of indole acetic acid (Oliveira et al., 2012; Silva et al., 2012; Chagas et al., 2017 a, b, c), as disease control

agents of various cultivated plants and disease resistance inducers in plants (Asuming-Brempong, 2013; Das et al., 2017; Woo and Pepe, 2018; Mendoza-Mendoza et al., 2018)

The use of *Trichoderma* for biocontrol and as a plant growth promoter presents numerous advantages, however some complications have limited its use, among them the sensitivity of the organism to radiation, inadequate humidity and low thermal stability, factors that cause low bio persistence of the organisms in the environment (Vemmer and Patel, 2013). Therefore, especially in relation to the climatic conditions in Brazil, the persistence of these organisms is, on average, only three days and this factor ends up reducing efficiency in the field (Fraceto et al., 2019), making it necessary to develop technologies that can increase persistence.

Other issues faced are some unsatisfactory results that they present, there is often the need to make several applications, or even complementation with non-biological pesticides and use of fertilizers, and in most cases, this need is due to problems in the application technology used, and not due to the product inefficiency. Thus, besides the search for new products, the adequacy of the application technology is a crucial step in the management of biological products use.

This work aimed to evaluate the efficiency of the product TrichoPlus (*Trichoderma asperellum* 201) as a plant growth promoter in soybean and its productive performance in the field.

## 2 MATERIAL AND METHODS

The experiment was conducted in the municipality of Formoso do Araguaia, Tocantins, in a floodplain system, in the Formoso Project third stage, under the geographical coordinates latitude 11 47'45" S and longitude 49 31'43" W, 240 meters altitude.

The experiment was conducted in the period from May to August 2018. The local climate is characterized by a humid tropical climate with a small water deficit (B1wA'a) according to Köppen and Geiger (Peel et al., 2007). The area of Formoso do Araguaia had an average annual temperature of 24.0 °C and average rainfall for the experimental period of less than 80 mm.

The chemical characterization of the soil was made, depth 0-20 cm (Table 1), presenting the following granulometric characteristics: 79.8, 8.1 and 12.1% of sand, silt and clay, respectively (Embrapa, 2011). The soil was classified as Plintossolo Háplico (Embrapa, 2011) with soybean and rice use records.

Table 1. Chemical analysis of floodplain soil used for soybean cultivation. Formoso do Araguaia, TO. 2018 harvest.

pH	P	K	Al <sup>3+</sup>	H+Al	Ca <sup>2+</sup>	Mg <sup>2+</sup>	SB	T	V	MO
H <sub>2</sub> O	mg dm <sup>-3</sup>		-----cmol <sub>c</sub> dm <sup>-3</sup> -----						%	%
5.5	14.1	35.0	0.0	4.0	4.30	2.2	6.5	11.2	62.3	3.20

Chemical attributes of the 0-20 cm depth; pH in water - Ratio 1: 2.5; P and K - Mehlich 1 extractor; Al<sup>3+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> - KCl extractor (1 mol L<sup>-1</sup>); H + Al - SMP extractor; SB = Sum of Exchangeable Bases; (T) = Cation Exchange Capacity at pH 7.0; V - Bases Saturation Index; and MO = organic matter (oxidation: Na<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 4N+H<sub>2</sub>SO<sub>4</sub>10N).

The irrigation system on the property is by subirrigation, carried out when necessary, using surface irrigation, with infiltration directly through the soil surface.

Mineral fertilization was conducted at planting with 300 Kg ha<sup>-1</sup> of the formulation 4-28-10 and 100 Kg ha<sup>-1</sup> of KCl at the V3 stage, 25 days after sowing, based on soil analysis and crop requirements.

Each portion consisted of nine soybean planting lines, six meters long by four meters wide, and the spacing between planting lines was 0.50 m. The size of each experimental portion was 24 m<sup>2</sup>. The CD 2851 IPRO cultivar was used.

The seeds were treated on the same day as planting with fungicides based on Thiofanto-methyl and Azoxystrobin, and the bacteriostatic insecticide based on Chlorantraniliprole, using a dosage of 100 grams for every 50 kg of seeds, as recommended by the manufacturer.

The soybean seeds were inoculated with rhizobium bacteria belonging to the Bradyrhizobium genus (SEMIA 5079 and SEMIA 5080), with a dosage of 400 mL (four doses of 100 mL, according to the manufacturer), for each 50 kg of seeds. Initially, 18 seeds were used per linear meter, with a final stand of 14 plants per linear meter.

The treatments used in the experiment were: four treatments with different doses of TrichoPlus (2, 4, 6 and 8 g per kg of seeds), plus two control treatments, being a positive control with a commercial product based on Trichoderma asperellum (1x10<sup>10</sup> UFC g<sup>-1</sup>, dispersible granules formulation, dosage of 100 g per 100 kg of seeds, according to the manufacturers' recommendations), and an absolute control (without inoculation).

For the treatment with the product TrichoPlus, the powder formulation was used, with active ingredient based on Trichoderma asperellum 201, formulated with a minimum concentration of 2 x 10<sup>8</sup> UFC g<sup>-1</sup>, having in the composition the graphite as inert, being applied directly in the seeds before planting, aiming at better adherence and protection of the Trichoderma in direct contact with the seed and the protection right after planting.

The experiment was in randomized blocks with four repetitions. During the development of the crop, all the necessary phytotechnical and phytosanitary management were carried out according to Henning (2009) recommendations. Initially, weeds were controlled 28 days after planting, using Roundup herbicide at a dose of 1.5 kg ha<sup>-1</sup>. The same application was used to control the caterpillars that attacked the soybean at the initial stage, using insecticides based on Gamma-cyhalothrin, 150 g L<sup>-1</sup> and Diflubenzuron, 240 g L<sup>-1</sup>, at doses of 50 mL ha<sup>-1</sup> and 120 mL ha<sup>-1</sup>, respectively. To control anthracnose (*Colletotrichum truncatum*) and Asiatic rust (*Phakopsora pachyrhizi*), a fungicide based on Azoxystrobin and Cyproconazole was applied at R1, at a dosage of 500 mL ha<sup>-1</sup>.

Biomass assessments were made in two seasons, at 28 and 55 days after emergence (DAE), in the lines preceding the border. The biomass assessments were made through the shoot dry mass. The aerial part was separated from the roots by a cut made at the base of the stem. Subsequently, the aerial part was placed in paper bags and taken for drying in a greenhouse at 65 °C until reaching constant weight.

For the initial stand evaluation, final stand and productivity, the central useful area of 6 m<sup>2</sup> was used. The plant's height, quantity of internodes, quantity of pods and quantity of grains per pod were determined at the R8 stage, 125 days after the sowing, in the lines next to the useful area of the experimental plots, using 15 plants per experimental plot, totaling 60 plants per treatment. Grain production was determined in the 6 m<sup>2</sup> area, after the physiological maturity of the plants, when approximately 80% of the pods were dry. Subsequently, the pods were threshed by hand, correcting the humidity of the grains to 13%. After the harvest, the productivity per hectare was quantified.

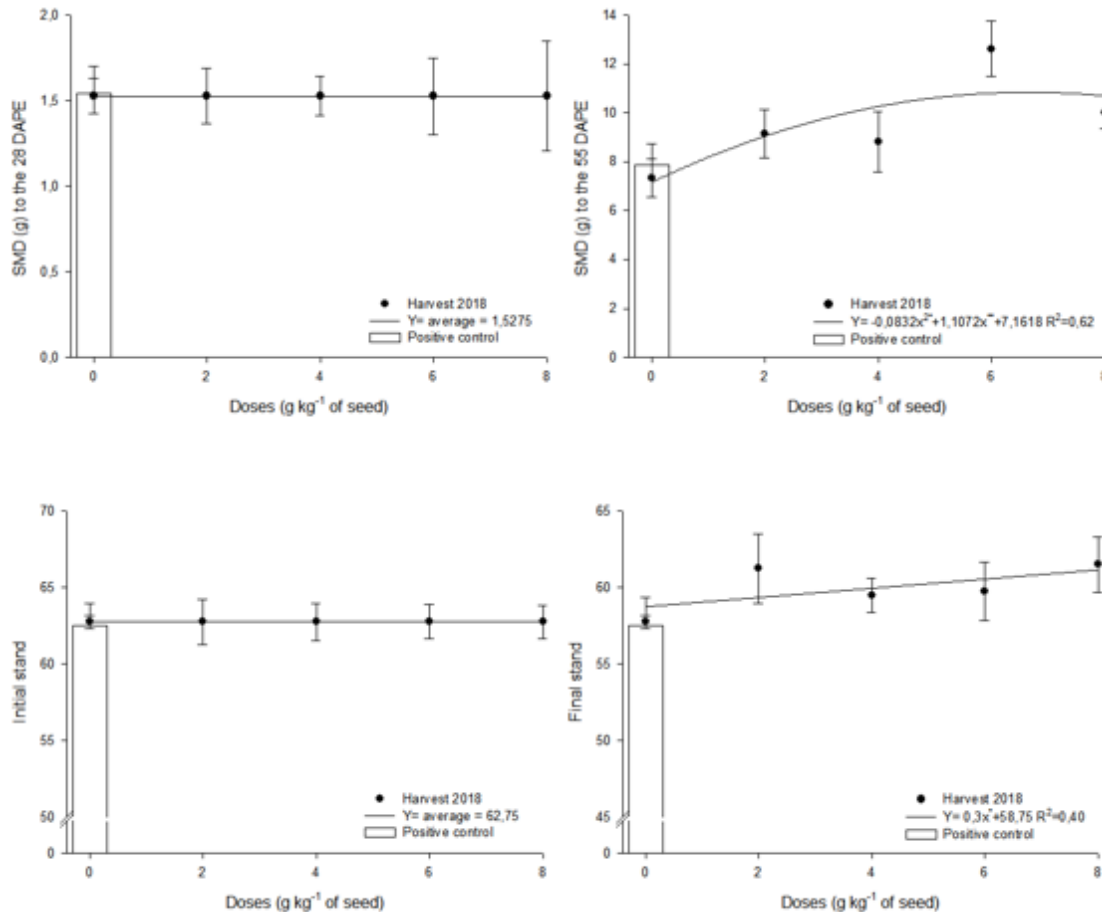
The data were submitted to the variance analysis F test. For efficiency effects the data were submitted to Tukey's test ( $p \leq 0.05$ ), for dose effects the regression test was applied and the selection of the models were based on the significance of the betas and the highest coefficient of determination (R<sup>2</sup>), using the SISVAR statistical program. The graphs were plotted using the SigmaPlot version 14.0® program.

### 3 RESULTS AND DISCUSSION

Most of the evaluated characteristics showed that the doses of the product TrichoPlus significantly influenced the shoot dry mass (SDM) characteristics at 55 DAE (Figure 1), final plant stand (Figure 1), plant height (Figure 2), number of internode (Figure 2), number of pods per plant (Figure 2), number of grains per plant (Figure 2) and

grain productivity (Figure 2). The characteristics of the initial stand (Figure 1) and shoot dry mass portion at 28 DAE (Figure 1A) were not significant by the F test ( $p \leq 0.05$ ).

Figure 1. Shoot dry mass (SDM) at 28 and 55 days after emergency (DAPE), initial stand and final stand of soybean cv. CD 2851 inoculated with different doses of TrichoPlus (*Trichoderma asperellum*), in Formoso do Araguaia-TO, Brazil. 2018 Harvest.



For SDM at 28 DAE, there were no regression curve adjustments between treatments ( $Y = \text{mean}$ ). The polynomial model that best fits the behavior of the shoot dry mass variable at 55 DAE as a function of the doses of the TrichoPlus product was the linear one (Figure 1). In the evaluation of SDM at 55 DAE, all the doses used were higher than the positive control (Figure 1), thus demonstrating the potential of the product TrichoPlus in floodplain soil, where the plants had an increase of more than 27%, at the seed's dose of 6.6 g kg<sup>-1</sup>, when compared with the positive control.

In the evaluation of the initial stand, for the averages evaluated there was no statistical difference in the betas significance ( $p \leq 0.05$ ) (Figure 1). For the final stand, a linear effect was observed under the doses of TrichoPlus, and there was no difference

between the positive and negative control, according to the standard deviation of the average evaluated (Figure 1).

The polynomial model that best fitted the behaviour of height and internode variables as a function of TrichoPlus doses was linear (Figure 2). For the height characteristic the soybean plants had a height of 94 cm at the dosage of 8 g kg<sup>-1</sup> of seed, which had better performance when compared to the positive control used in the experiment. The difference between the best observed dose and the positive control was 8 cm, which represents a percentage of 8.5%. As for the number of internodes, this percentage increased to 13.4%, where it is observed that all doses used in the experiment were higher when compared to the positive control (Figure 2).

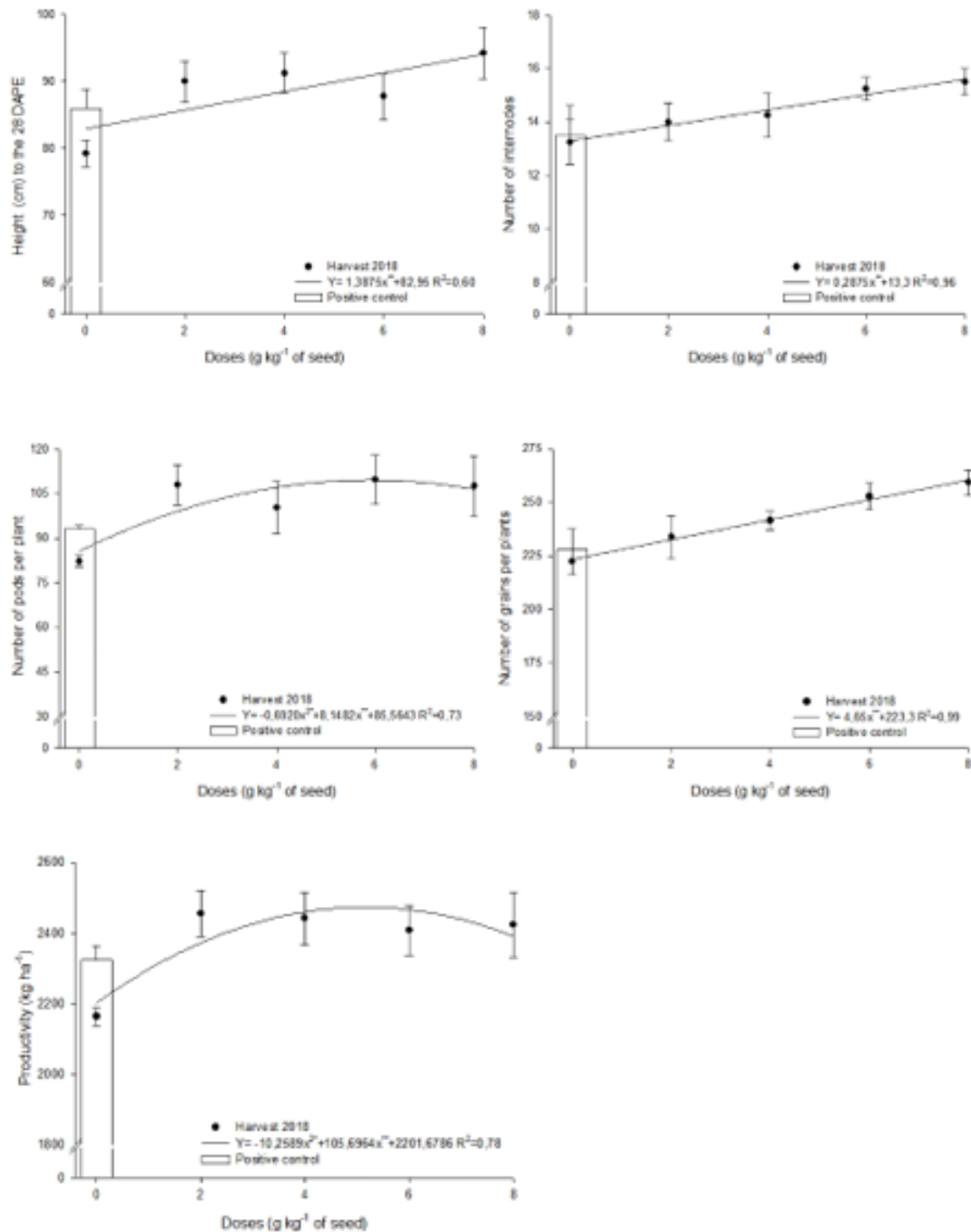
The number of pods per plant showed a quadratic response as a function of the doses of the TrichoPlus product, by the regression test (Figure 2). The maximum technical efficiency for number of pods per plant was obtained at the dosage of 5.9 g kg<sup>-1</sup> of seed with an average of 109 pods per plant, which represents an increase of 15.1% higher than the positive control.

As for the number of grains per plant, the polynomial model that best fit the evaluated means was linear (Figure 2). The maximum efficiency of the TrichoPlus product was reached at a dose of 8 g kg<sup>-1</sup> of seed. There is an increase between the evaluated dosages, which corresponded to 14.7% when compared to the negative control. However, when compared to the positive control, there was an increase of 12.5% in number of grains per plant.

The productivity of soybean seeds showed a significant quadratic response as a function of the doses of the TrichoPlus product inoculated into the seeds (Figure 2). The best dosage obtained by the TrichoPlus product for productivity was reached in 5.1 g kg<sup>-1</sup> of seed, where the productivity would reach 2473 kg ha<sup>-1</sup> (41.2 bags ha<sup>-1</sup>) of seed (Figure 2). The increase in relation to plants that did not receive a dose of any Trichoderma-based product represents a rise of about 12.6% (5.2 bags ha<sup>-1</sup>). When the comparison is between the use of TrichoPlus and the positive control, there was an increase in seed productivity of 2.5 bags ha<sup>-1</sup>, which represents a 6% increase.



Figure 2. Plant height, number of internodes, number of pods per plant, number of grains per plant and productivity of soybean cv. CD 2851, inoculated with different doses of TrichoPlus (*Trichoderma asperellum*), in Formoso do Araguaia-TO, Brazil. 2018 harvest.



Significant results were observed for the treatments with different doses of TrichoPlus in the characteristics of biomass, stand maintenance and productivity. The best dose for productivity was achieved at 5.1 g kg<sup>-1</sup> seed, with gains 12.6 and 6.0% higher compared to the absolute control and positive control treatments.

The positive results observed for the different experiments with soybean can be explained in function of the action of the inoculant used, keeping in mind that fungi of

the genus *Trichoderma* are used not only in the biological control of phytopathogens, but as promoters of plant growth, due to its versatility of action, as parasitism, antibiosis and competition, besides acting as inducers of resistance to plants against diseases and produce growth hormones, solubilization of phosphate, siderophores and secondary metabolites (Milanesi et al, 2013; Chagas Junior et al., 2015; Chagas et al., 2015; Contreras-Cornejo et al., 2016; Bononi et al., 2020). These fungi are found in the rhizosphere, are growth promoters in plant species, and produce a rich source of secondary metabolites, presenting a vast repertoire of genes supposedly involved in the biosynthesis of non-ribosomal peptides, polyketides, terpenoids and pyrones (Mukherjee et al., 2012), and inoculation with a high concentration of these microorganisms can provide positive results regarding the biocontrol of phytopathogens and, consequently, the promotion of plant growth.

Root colonization by *Trichoderma* often increases root development, crop productivity, resistance to abiotic stresses and improves nutrient use (Rubio et al., 2017). Most of the *Trichoderma* spp. species present different behavior in the actions as biocontrol and as promoters of the vegetable growth. Among the factors that determine the success of the *Trichoderma* inoculation, there is a biotics and the biotics. Therefore, isolates selected for biological control of pathogens under controlled experimental conditions may be unable to produce the same results under field conditions, as well as those selected for their ability to promote plant growth. This relates to the fact that the conditions of establishment and development in the soil are critical for the microorganism, as they are subject to the differentiated reactions of the host and the environment, which can lead to a more variable control efficacy than that obtained with chemical fungicides. That way, it is not possible to quantify the responses of disease control and probable growth promotion separately in field experiments, but the results of biomass, stand and productivity are examples of the benefit to soy cultivation, as observed in the present experiment with the inoculation of the seeds with the product *TrichoPlus*.

Studies show strains with efficiency for biocontrol of various pathogens and others efficient in promoting plant growth, via colonization of the rhizosphere making nutrients available to the plant (Martínez et al., 2013). In addition to *Trichoderma*'s ability to control phytopathogens through the production of antibiotics, some compounds produced by *Trichoderma* can alter host plant metabolism (Azevedo et al., 2020). The increase in plant productivity is evident when seeds exposed to conidia, but separated by cellophane paper, without contact, show an increase in their growth, suggesting that

metabolites of *Trichoderma* spp. act not only as growth promoter, but also as signaling molecules (Ramada et al., 2019).

Therefore, the fungi belonging to the genus *Trichoderma* besides being recognized biofungicides, can also be classified as biofertilizers, biostimulants and potentiators of resistance against biotic and abiotic stresses (Li et al., 2015; Sellal et al., 2020). The result of all interactions is usually the promotion of plant growth (Woo and Pepe, 2018), that is, even when there is no disease, when the product is used, the producer will have the benefit of increased nutrient use efficiency and productivity (Bononi et al., 2020; Steffen, et al., 2021).

These findings help to understand the role of *Trichoderma* in natural or cultivated ecosystems and promote its use in agriculture. The present work carried out with the product TrichoPlus as a plant growth promoter in soybean demonstrated its ability, which is reflected in gains of productivity.

Some factors are important to obtain effective results with plant growth promoting inoculants: effective strains in the field against various phytopathogens and as plant growth promoters, low production costs involving efficient formulations and the form, dose and time of application. This was developed for the product TrichoPlus (*Trichoderma asperellum*) aiming the seeds inoculation, providing the adherence and protection of the *Trichoderma* in direct contact with the seed and the protection right after planting. This adequate formulation aims to maintain the viability of the active principle aiming at the protection of the structures, the persistence and the effectiveness in the field, presenting an adequate concentration of structures (conidia) of the fungus and the ease of the product application (inoculation).

The technology of inoculation of microorganisms, such as the fungus *Trichoderma*, of biotechnological interest in agriculture, is a resource of great economic importance, in addition to the contribution with the reduction of the use and consequent impact of agrochemicals. Sustainable agriculture requires the use of a strategy that increases productivity without harming the environment, opening up new perspectives to contribute to the development of new technologies, methods and strategies in agribusiness. The processes mediated by microorganisms become essential in the preservation and conservation of natural resources.

#### **4 CONCLUSIONS**

The inoculation of soybean with TrichoPlus promoted an increase in the characteristics of biomass, efficiency in the maintenance of the stand and productivity with the best doses of 4 and 6 g kg<sup>-1</sup> of seeds, proving its effectiveness in promoting plant growth and, consequently, productivity in the cultivation of floodplain soil.

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