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Agronomic efficiency of soybean inoculated with *Trichoderma* and *Purpureocillium* in cerrado condi-tions, Tocantins, Brazil

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INFO

Keywords
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ABSTRACT

The objective of this study was to evaluate the action of *Trichoderma* and *Purpureocillium* on the productivity of soybean. Three independent experiments were performed in the region of Porto Nacional, Brazil, using three different varieties of soybean: precocious (Soytec 820 RR), intermediate (Syn13840 IPRO) and late (Sambaiba RR). The treatments used were witness without inoculation, inoculation of TrichoPlus (*Trichoderma*) at a dose of 2 kg ha^{-1} and a dose of 4 kg ha^{-1}, inoculation of TrichoMix (*Trichoderma* and *Purpureocillium*) at a dose of 2 kg ha^{-1} and a dose of 4 kg ha^{-1}. The results of productivity, for the first experiment with precocious soybean, were superior ($p<0.01$) with the inoculation of TrichoPlus at a dose of 4 kg ha^{-1}, followed by the treatments with TrichoMix at a dose of 4 kg ha^{-1} and TrichoPlus at a dose of 2 kg ha^{-1}. For experiments with intermediate and late soybean, the productivities were superior ($p<0.01$) for all treatments with inoculation in relation to the witness.

RESUMO

Eficiência agronômica da soja inoculada com Trichoderma e Purpureocillium em condições de cerrado, Tocantins, Brasil

O objetivo deste estudo foi avaliar a ação de *Trichoderma* e *Purpureocillium* sobre a produtividade da soja. Foram realizados três experimentos independentes na região do Porto Nacional, no Brasil, utilizando três variedades de soja: precoce (Soytec 820 RR), intermediário (Syn13840 IPRO) e tardio (Sambaiba RR). Os tratamentos utilizados foram testemunhas sem inoculação, inoculação de TrichoPlus (*Trichoderma*) em uma dose de 2 kg ha^{-1} e uma dose de 4 kg ha^{-1}, inoculação de TrichoMix (*Trichoderma* e *Purpureocillium*) em uma dose de 2 kg ha^{-1} e uma dose de 4 kg ha^{-1}. Os resultados da produtividade, para o primeiro experimento com soja precoce, foram superiores ($p <0,01$) com a inoculação de TrichoPlus em uma dose de 4 kg ha^{-1}, seguidos pelos tratamentos com TrichoMix a uma dose de 4 kg ha^{-1} e TrichoPlus com uma dose de 2 kg ha^{-1}. Para os experimentos com soja intermediária e tardia, as produtividades foram superiores ($p <0,01$) para todos os tratamentos com inoculação em relação à testemunha.

Palavras-chaves
glycine max
biocontrole
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INTRODUCTION

The world agricultural segment becomes more advanced each day, with the insertion of new technologies, management and applicability to the process of production. The rural producer has been searching for techniques that make possible its production, aiming cost reduction, environmental sustainability and techniques that increase the productivity, without burdening production.

Brazil's economy is based on agribusiness, with emphasis on intensive and extensive livestock, crops of corn, sugar cane and soybean, which are favored for edaphoclimatic factors and for its vast territory, that comprehends a significant interval of latitude, what allows the production of these commodities (CONAB, 2020).

Nowadays, soybean production [*Glycine max* (L.) Merril] and the cultivated area with this crop in Brazil were estimated in 114,8 millions of tons and 35,8 millions of hectares, for 2018/2019 harvest (CONAB, 2019). In relation to that amount, it is highlighted the expansion of cultivation of this oleaginous in the region of Cerrado conditions of Tocantins. Despite being a very expressive crop, there are phytosanitary problems that occur during the cycle, this situation is recognized as one of the factors that affect the most the production cost.

Diseases are one of main causes of low productivity, and, depending on climatic conditions, some pathogens have the ability to reduce or even stop the production of grains (Madalosso et al., 2015). The utilization of management techniques, such as no-till farming and irrigation, bring beyond benefits, new challenges to the cultivation, mainly regarding diseases caused by soil pathogens. Given the various techniques in agriculture the use of chemicals, such as fungicides for soil disease control, has a high cost; therefore, the integration between techniques of biological control and cultivation practices that inhibit the pathogen is the best alternative (Woo et al., 2014; Meyer et al., 2019).

Fungi of the genus *Trichoderma* (teleomorph Hypocrea) are opportunistic, symbionts of plants, strong competitors in the environment of soil, constitute sources of enzymes that degrade other fungi walls, and also are important producers of antibiotics and parasites of pathogenic fungi (Kumar et al., 2012; Woo et al., 2014; Monte et al., 2019; Suassuna et al., 2019). Species of the genus *Trichoderma* have received agro-economic and scientific attention since they show actions of antagonism against several pathogens, such as parasitism, antibiosis and competition (Martínez et al., 2013; Druzhinina et al., 2018; Woo & Pepe, 2018). Some strains of *Trichoderma* increase the total surface of root system, what allows higher access to the mineral elements present in it (Chagas et al., 2015; Mendoza-Mendoza et al., 2018). Other strains are capable of solubilize and provide for plant the phosphate of rock, iron, copper, manganese and zinc. Beyond that, they can improve the active mechanisms of absorption of macro and micronutrients, as well as, increase the efficiency of plant in using some important nutrients, such as nitrogen (Shoresh et al., 2010; Machado et al., 2012; Das et al., 2017; Woo & Pepe, 2018; Mendoza-Mendoza et al., 2018; Bononi et al., 2020).

The genus *Purpureocillium* is a cosmopolitan fungus that lives in saprophytism in soil, air and decaying vegetables, such as grains and food. Many studies suggest the fungus use as a biocontrol agent, since it is described as parasite of insects (Marti et al., 2006) and nematodes (Monfort et al., 2005). The parasitism of *Purpureocillium* lilacinum is optional, it can infect nematodes in juvenile stages, sedentary females that cause galls or more aggressively eggs (Nunes et al., 2010). Species of the genus *Purpureocillium* are capable of producing many secondary metabolites of different chemical classes, such as: xanthones, peptides, alkaloid, polyketides, α-pyrones, and with various biological activities (cytotoxic, antibacterial and immunostimulants) (Elbandy et al., 2009; Teles; Takahashi, 2013). Studies involving the selection of isolated elements of *P. lilacinum* for nematode control are important in the search for efficient microorganisms, adapted to different regions.

Several products based on *Trichoderma* and *Purpureocillium* are commercialized all around the world. Thus, the objective of this study was to evaluate the effect of fungi *Trichoderma* and Purpureocillum, which are components of the products TrichoPlus based on *Trichoderma* and TrichoMix based on *Trichoderma* and *Purpureocillium*, in the establishment of soybean crop and productivity at field conditions, in Cerrado conditions of Tocantins.

MATERIAL AND METHODS

The experiments were carried out at the municipality of Porto Nacional, in the Research Station ALX Farias Agro Pesquisa Agropecuária dos Cerrados LTDA (23º36´45,1" S - 51º11'01,4" W), in Tocantins, 2014/2015 harvest. The local climatic characterization is humid tropical climate with classification of type Aw, according to Köppen and Geiger. The average temperature was 26.1 ºC and the average annual rainfall was 1622 mm.

Before planting, a composite sample of soil was collected and was performed physical and chemical

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characterization, in which were found the following values (Table 1): texture of 73.3, 7.2 and 19.5% of sand, silt and clay, respectively (EMBRAPA, 2009). The soil was classified as medium yellow dystrophic oxisol (EMBRAPA, 2011).

Tabela 1 - Chemical analysis of the soil used for soybean cultivation, at the ALX Experimental Station, in Porto Nacional, Tocantins, Harvest 2014/2015.

Soil	pH	P	K	Al^{3+}	H+Al	Ca^{2+}	Mg^{2+}	SB	T	V	MO
Cm	$CaCl_2$	mg dm^{-3}		--------------$cmol_c$ dm^{-3}----------						%	$g.dm^{-3}$
0-20	5.56	9.4	66.3	0.0	1.5	1.75	1.15	1.49	2.9	62	20

Chemical attributes of 0-20 cm depth; pH in CaCl2; P and K – Mehlich 1; Al3+, Ca2+ and Mg2+ - KCl extractor (1 mol L-1); H + Al – SMP extractor; SB = sum of exchangeable bases ; (T) = cation exchange capacity at pH 7.0; V – base saturation index; and MO = organic matter (oxidation: Na2Cr2O7 4N + H2SO4 10N).

The mineral fertilization was performed before sowing, according to recommendations regarding soil analysis.

Three soybean varieties were used: precocious (Soytec 820 RR), intermediate (Syn 13840 IPRO) and late (Sambaiba RR), these were independent experiments.

The design used was randomized blocks with four replications. The treatments consisted of: witness without inoculation; inoculation of TrichoPlus (based on *Trichoderma*) at a dose of 2 kg ha^{-1}; inoculation of TrichoPlus at a dose of 4 kg ha^{-1}; inoculation of TrichoMix (based on *Trichoderma* + *Paecilomyces*) at a dose of 2 kg ha^{-1} and inoculation of TrichoMix at a dose of 4 kg ha^{-1}. The doses were used according to recommendations of different products/producers. Each experimental plot was constituted by nine rows of 6 m; spacing 0.5 m between rows, 1 m between plots and 1 m between blocks, totaling each experimental plot 24 m².

For the treatment with use of *Trichoderma*, the inoculant TrichoPlus (granulated with the active ingredient based on *Trichoderma* asperelum) was used, selected with potential for biocontrol and as the plant growth promoter, formulated with minimum concentration of viable conidia of 1×10^8 g^{-1}, and its main vehicle is millet. For treatment using TrichoMix, the inoculant used was granulated with the active ingredient based on *Trichoderma* asperelum (UFT 201) and *Purpureocillium lilacinum*, selected with potential for nematode control, formulated in the same way with concentration of 7×10^8 g^{-1}.

RESULTS AND DISCUSSION

For the experiment with soybean in precocious cycle, all treatments were superior ($p<0.01$) for initial and final stand in relation to the treatment witness without inoculation; however, for these characteristics, the averages were significantly superior for treatment with inoculation of TrichoMix at a dose of 4 kg ha^{-1} (Table 2). Regarding survival of plants of treatments with inoculation of TrichoMix and TrichoPlus, the effect of inoculation was evident, with percentage of survival of plants at final stand varying from 73.2 to 80.2%, values that are 8.2 to 15.1% higher in relation to the witness.

Table 2 - Initial Stand (IS), Final Stand (FS), survival, effectiveness and productivity of soybean of precocious cycle Soytec 820 RR, inoculated with TrichoMix and TrichoPlus granulated, cultivated in Cerrado conditions at Porto Nacional, Tocantins. Harvest 2014/2015.(1)

Treatment	IS 25 DAP(2)	FS 50 DAP	Surviv.3 (%)	Effectiv4 (%)	Prod. (Kg ha^{-1})	Bags (ha)
Witness	195.0 c	179.0 d	65.1	-	1925 c	32.1 c
TrichoMix 2	212.5 b	211.5 b	76.9	18.1	1980 c	33.0 c
TrichoMix 4	231.0 a	220.5 a	80.2	23.2	2420 b	40.0 b
TrichoPlus 2	215.0 b	201.5 c	73.3	12.6	2365 b	39.4 b
TrichoPlus 4	204.0 b	204.0 bc	74.2	14.0	2805 a	46.8 a
CV (%)(5)	7.2 **	4.4 *	-	-	8,6 **	9.1*

(1) Means followed by same lower case letter, in columns, do not differ by Duncan test at 1 or 5% of significance. (2) DAP = Days after planting (3) Survival = percentage of survival of plants in relation to the expected stand of 275 plants in 10 m² (11 plants per linear meter) (4) Effectiveness or efficiency in utilization of products for stand maintenance (5) CV = Coefficient of Variation * Significant at 5%. ** Significant at 1%.

The effectiveness in the utilization of products for maintenance of stand varying from 12.6 to 23.2%. In relation to productivity, there was significant difference among treatments, with production of 46.8 bags ha^{-1} (2805 kg ha^{-1}) for treatment with inoculation of TrichoPlus at a dose of 4 kg ha^{-1}, 45.8% superior to the witness with 32.1 bags ha^{-1} (1925 kg ha^{-1}), an increase over 14 bags

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ha^{-1}. Treatment with TrichoMix at a dose of 4 kg ha^{-1} and treatment TrichoPlus at a dose of 2 kg ha^{-1} were also superior to the witness.

Regarding the experiment with intermediate cycle soybean, for initial stand all treatments with inoculation of TrichoMix and TrichoPlus were superior ($p<0.01$) in relation to witness (Table 3). For final stand, the treatment with inoculation of TrichoPlus at a dose of 4 kg ha-1 was superior ($p<0.05$) in relation to other treatments. Regarding survival, in treatments with inoculation, it is possible to highlight treatments with inoculation of TrichoMix and TrichoPlus, both at a dose of 4 kg ha-1, with percentage of plant survival at final stand of 82.4 and 87.8%, respectively, an increase of 8.9 and 14.3% in relation to the witness, with percentage of effectiveness of 12.1 and 19.5%, respectively.

For productivity, there was significant difference among treatments, with higher values ($p<0.01$) for treatments with inoculation of TrichoMix and TrichoPlus at a dose of 2 kg ha-1 and TrichoMix at a dose of 4 kg ha-1. With a production of 51.7 (3100 kg ha-1), 55.0 (3300 kg ha-1) and 49.3 (2950 kg ha-1) bags ha-1, respectively, an increase of 64.1, 74.6 and 56.5% in relation to the witness with 31.5 bags ha-1 (1890 kg ha-1), respectively. These values mean an increase of more than 17 bags ha^{-1}. The treatment with inoculation of TrichoPlus at a dose of 4 kg ha^{-1}, also presented productivity superior to the witness without inoculation (Table 3).

Table 3 - Initial Stand (IS), Final Stand (FS), survival, effectiveness and productivity of soybean of intermediate cycle Syngenta 3840, inoculated with TrichoMix and TrichoPlus granulated, cultivated in Cerrado conditions at Porto Nacional, Tocantins. Harvest 2014/2015.[1]

Treatment	**IS 25 DAP[2]**	**FS 50 DAP**	**Surviv.[3] (%)**	**Effectiv[4] (%)**	**Prod. (Kg ha^{-1})**	**Bags (ha)**
Witness	219.0 b	202.5 c	73.5	-	1890 c	31.5 c
TrichoMix 2	276.5 a	203.5 c	74.0	0.7	3100 a	51.7 a
TrichoMix 4	259.0 a	226.5 b	82.4	12.1	3300 a	55.0 a
TrichoPlus 2	259.0 a	206.5 c	75.1	2.2	2950 a	49.3 a
TrichoPlus 4	262.5 a	241.5 a	87.8	19.5	2550 b	42.5 b
CV (%)[5]	8.1 **	5.4 **	-	-	8,1 **	9.3**

(1) Means followed by same lower case letter, in columns, do not differ by Duncan test at 1 or 5% of significance. (2) DAP = Days after planting (3) Survival = percentage of survival of plants in relation to the expected stand of 275 plants in 10 m² (11 plants per linear meter) (4) Effectiveness or efficiency in utilization of products for stand maintenance (5) CV = Coefficient of Variation * Significant at 5%.** Significant at 1%.

For experiment with late cycle soybean, initial and final stands in treatments with inoculation of TrichoMix and TrichoPlus were superior ($p<0.01$) in relation to the witness (Table 4); however, for final stand the treatment with inoculation of TrichoMix at a dose of 2 kg ha^{-1} was superior among treatments with inoculation. Regarding survival, treatments with inoculation showed percentage varying from 75.5 to 88.2%, an increase of 7.7% in relation to the witness, showing effectiveness of treatments with percentage varying from 11.4 to 30.1%. Regarding productivity, there was significant difference among treatments, with superior productivities ($p<0.01$) for treatments with inoculation, with production of 45.8 (2748 kg ha^{-1}), 51.2 (3100 kg ha^{-1}), 54.2 (3250 kg ha^{-1}) and 51.2 (3100 kg ha^{-1}) bags ha^{-1}, respectively. These values represented an estimated increase of 27.9, 43.0, 51.4 and 43.0% in relation to the witness with 35.8 bags ha^{-1} (2150 kg ha^{-1}), respectively. These values represent an increase varying from 10 to 18.4 bags ha^{-1} (Table 4).

Table 4 - Initial Stand (IS), Final Stand (FS), survival, effectiveness and productivity of soybean of late cycle Sambaiba RR, inoculated with TrichoMix and TrichoPlus granulated, cultivated in Cerrado conditions at Porto Nacional, Tocantins. Harvest 2014/2015.[1]

Treatment	**IS 25 DAP[2]**	**FS 50 DAP**	**Surviv.[3] (%)**	**Effectiv[4] (%)**	**Prod. (Kg ha^{-1})**	**Bags (ha)**
Witness	212.5 b	186.5 c	67.8	-	2150 b	35.8 b
TrichoMix 2	264.0 a	242.5 a	88.2	30.1	2750 a	45.8 a
TrichoMix 4	264.0 a	207.5 b	75.5	11.4	3100 a	51.2 a
TrichoPlus 2	265.0 a	214.0 b	77.8	14.8	3250 a	54.2 a
TrichoPlus 4	261.5 a	207.5 b	75.5	11.4	3100 a	51.2 a
CV (%)[5]	7.2 **	6.4 **	-	-	7.1 **	6.3**

(1) Means followed by same lowercase letter, in columns, do not differ by Duncan test at 1 or 5% of significance. (2) DAP = Days after planting. (3) Survival = percentage of survival of plants in relation to the expected stand of 275 plants in 10 m² (11 plants per linear meter). (4) Effectiveness or efficiency in use of *Trichoderma* for stand maintenance. (5) CV = Coefficient of Variation. * Significant at 5%. ** Significant at 1%.

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Positive results observed for soybean cultivars can be explained due to action of the inoculations used, since fungi of genus *Trichoderma* are used as biological control of plant pathogens and to promote vegetal growth, due to its versatility of action as parasitism, antibiosis and competition. They also act as inducers of plant resistance against diseases and they produce hormones for growth (Guareschi et al., 2012; Kumar et al., 2012; Milanesi et al., 2013; Chagas Junior et al., 2015; Chagas et al., 2015). These characteristics make them the fungi most researched at laboratory, greenhouse and field, mainly regarding the diseases caused by soil pathogens that influence the establishment of plants, such as *Fusarium* sp. and *Rizoctonia solani*, these pathogens cause red root rot and damping off (Madalosso et al., 2015), what generate losses at initial stand and reduction of productivity. This positive effect in the maintenance of initial and final stands may be directly associated with the utilization, as biocontrol, of these pathogens, as observed for all three studied cultivars (Tables 2, 3 and 4).

There are few studies reporting the use of *Trichoderma* and *Purpureocillium* jointly; however, the results presented are similar to those found by some authors who report positive results with the inoculation of *Trichoderma* in soybean, such as Milanesi et al. (2013), they concluded that isolated elements of *Trichoderma* act as growth promoters of soybean seedlings. Guareschi et al. (2012) in a study with inoculation of *Trichoderma* spp. via seed treatment (200 mL for each 50 kg of seed) and application via soil (1 L ha^{-1}) at 10 days after emergency, concluded that, in the experiment conditions, the application of *Trichoderma* spp. promoted growth of aerial part and roots of sunflower and soybean. Chagas Junior et al. (2012) working with cowpea inoculated with *Trichoderma* in seed and soil reported the increase on values of biomass, nutrient content and productivity approximately 20% higher than the treatments control, given by the biocontroller action of *Trichoderma*.

The promotion of plant growth through species of *Trichoderma* is not only related to the control of pathogens, because the improvement in plant growth was observed in absence of any detectable disease and in sterile soil, thus, that ability is independent of antifungal capabilities (Topolovec-Pintarić et al., 2013).

Low specificity of hosts and its adaptation to a wide range of pH indicate the potential of *Purpureocillium lilacinum* in the control of plant diseases. Hashem e Abo-Elyousr (2011) in a study with tomato, reported that *P. lilacinum* reduced significantly the population of *M. incognita* of tomato roots, maintaining the productivity.

The use of microorganisms as biocontrol agents of plant diseases, despite being studied a lot lately, still needs to be better comprehended, so, it will be possible to achieve its optimal efficiency. The application of microorganisms that exist in different products is subjected to various factors, among them, soil, composition, organic matter and soil nutrients that influence on colonization and survival of these microorganisms in soil.

Applications of control agent combinations should also be analyzed for reduction of population of plant pathogens, such as nematodes. Fungi with different mechanisms of actions are able to live and work in the same plant in order to, not only reduce population density of pathogens, but also promote plant growth, maintenance of stand and increase on productivity. Thus, these results indicate the viability of the associated use of these biological control agents in applications via soil.

The studies performed with *Trichoderma* and *Purpureocillium* with potential for antagonism have shown its ability to control diseases. However, the level of effectiveness verified in several field studies is lower than expected. According to Pomella e Ribeiro (2009), some factors are important for obtaining effective results with biocontrol agents: effective strains at field against different plant pathogens, low production cost involving the efficient formulations and form, dose and time of application.

CONCLUSIONS

The inoculation of *Trichoderma* and *Purpureocillium*, present in the TrichoPlus and TrichoMix products, provided positive results for the maintenance of the stand and in the productivity of early, intermediate and late cycle soybean cultivares in field conditions, in Cerrado, Tocantins, Brazil.

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