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ICB NUTRISOLO TRICHODERMA® AS GROWTH PROMOTER IN SOYBEAN CROPS

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ABSTRACT - Soybean is the most important crop in the world due to growing external demand. Nevertheless, its production requires a high use of pesticides. *Trichoderma* is a fungal genus with the potential to promote plant growth because of its broad metabolic arsenal. Because it increases efficiency in several cultures, *Trichoderma* spp. have the potential to be used as an alternative to chemicals. Given this context, this study aimed to evaluate the performance of the ICB Nutrisolo Trichoderma® inoculant on parameters related to soybean growth and production. Crops with five different soil and climate conditions were evaluated in Rio Grande do Sul State (southern Brazil) using parameters such as average plant height, average plant population, average number of grains/pod, number of pods/plant average, and average weight of one thousand grains, which revealed the results of productivity. The ICB Nutrisolo Trichoderma® increased plant height, number of pods, and grain weight, consequently increasing productivity in soybean plants. In this way, it was possible to characterize the product ICB Nutrisolo Trichoderma® as a growth-promoting inoculant for the soybean crop, increasing the scope of product registration.
Keywords: consortium, inoculant, productivity, *Trichoderma* spp.

ICB NUTRISOLO TRICHODERMA® COMO PROMOTOR DE CRESCIMENTO NA CULTURA DA SOJA

RESUMO - A soja é a cultura de maior importância mundial, devido à crescente demanda externa. Porém, seu cultivo requer elevado uso de pesticidas e agrotóxicos. *Trichoderma* é um gênero fúngico com potencial promotor de crescimento em plantas, devido a seu amplo arsenal metabólico. Em virtude do aumento de eficiência em diversas culturas, *Trichoderma* spp. possuem potencial a serem utilizados como alternativa ao uso de químicos. Com isso, o trabalho teve como objetivo avaliar o desempenho do inoculante ICB Nutrisolo Trichoderma® sobre parâmetros relacionados ao crescimento e a produção na cultura da soja. Foram avaliadas cinco condições edafoclimáticas diferentes no estado do Rio Grande do Sul (Brasil), através de parâmetros como altura média de plantas, população média de plantas, número médio de grãos/vagem, número médio de vagens/planta e peso médio de mil grãos, que levaram à resultados de produtividade. ICB Nutrisolo Trichoderma® aumentou a altura da planta, número de vagens e peso de grãos, elevando consequentemente a produtividade em plantas de soja. Desta forma, foi possível caracterizar o produto ICB Nutrisolo Trichoderma® como inoculante promotor de crescimento para a cultura da soja, aumentado assim o escopo do registro do produto.
Palavras-chave: consórcio, inoculante, produtividade, *Trichoderma* spp.

INTRODUCTION

Soybeans [*Glycine max* (L.) Merr] are one of the most important crops today, with Brazil being the world's largest producer. With a planted area of 38.502 million hectares during the 2020-2021 crop year, Rio Grande do Sul State produced an impressive 20.164 million tons of soybean (EMBRAPA, 2021). This crop is considered one of the leading world commodities as it is an important source of lipids and proteins and due to its myriad of applications in human and animal food.

In the search for high productivity with the lowest possible losses, pesticides and agrochemicals have proven efficient. However, these compounds produce numerous negative effects on the health of humans, ranging from nausea to cancer (MORAES, 2019), on the environment, such as surface water pollution, impacts on biodiversity and ecosystem functions (NORDBORG et al., 2016), and on long-term productivity due to increased phytopathogen resistance (GAINES et al., 2020). Given the significant demand for grain, it is pivotal for research to seek alternatives to reduce this compound in the field and thus collaborate with modern and sustainable agriculture.

Soybean is a source of vegetable protein, and it depends on the plant's high biological value of plant compounds (BAGALE, 2021). One of the ways to obtain these nutrients is through fixation mediated by microorganisms. Certain *Trichoderma* spp. strains have multiple beneficial functions for plants (WOO; PEPE, 2018), promoting growth by using different mechanisms to increase solubilization and nutrient availability, including

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phosphate, iron, phosphorus, copper, manganese, and zinc, in addition to systemically stimulating plant defenses against pathogens and environmental stress, mitigating undesirable microorganisms by attenuating their growth through competition, antibiosis, and mycoparasitism (MONTE et al., 2019).

In the Brazilian market of bioformulated products, different products containing *Trichoderma* species as an active principle are commercialized. However, the use of potentially functional strains in relation to their applicability must be used and genetically adapted to each region where they will be used. The consistency of the performance of bioproducts is still considered a restriction to their widespread adoption concerning the Brazilian soil and climate variability, along with biotic and abiotic factors (STEWART; HILL, 2014). The commercial use of the fungus *Trichoderma* as a plant growth promoter is already a reality in some countries, including Japan, South Africa, Portugal, and Russia (BETTIOL et al., 2019). In the case of Brazil, the first product based on eight strains and three species of *Trichoderma* spp. was registered as ICB Nutrisolo Trichoderma® under no. RS-12734 10000-4 as a growth promoter inoculant for lettuce culture (*Lactuca sativa* L.), and it is already being seen as a reference product in other crops (CHAGAS JUNIOR et al., 2019). Given this context, this study aimed to evaluate the performance of the product ICB Nutrisolo Trichoderma® as a growth promoter for soybean cultivation in order to include this crop in the increased scope of the product registration.

MATERIAL AND METHODS

Five soybean (*Glycine max* L. Merr.) producing regions with distinct soil and climate conditions were selected: Pantano Grande, Cruz Alta, Ibirubá, Santa Maria, and Tupanciretã in Rio Grande do Sul State, southern Brazil (Table 1). The evaluations were performed during the 2013/2014 agricultural season. The seeds (cultivar NA 5909) were planted in the no-till system over black oat straw, with fertilization performed according to the crop recommendation for the production ceiling of 4 tons per ha. A 0.5-meter spacing was used between rots, with a plant population of 290,000 per ha, totaling 14.5 seeds per linear meter. Weed, pest, and disease management were performed according to the recommendations for the crop (COSTAMILAN et al., 2012).

We applied 100 mL of ICB Nutrisolo Trichoderma® (ICB BIOAGRITEC Ltda, Brazil) for every 50 kg of seeds, a consortium constituted of eight strains of three *Trichoderma* spp. species (*Trichoderma harzianum*, *T. asperellum*, and *T. koningiopsis*) at the concentration of 1.0×10^{11} CFU mL^{-1}. As a positive control, we used the rhizobium-based inoculant (Rhizomax®, Novozymes BioAg) with the *Bradyrhizobium japonicum* strains SEMIA 5079 and 5080 at a concentration of 2.0×10^{9} cels L^{-1}; only water was used as the negative control.

TABLE 1 - Description of the experimental areas with their respective soil types in Rio Grande do Sul State, southern Brazil.

Areas	Municipalities	Altitudes	Regions	Soils
Area 1	Pantano Grande, RS	119 m	Central depression	Typical red-yellow Argisol, sandy texture
Area 2	Cruz Alta, RS	414 m	Middle plateau	Typical red dystrophic Latosol, medium texture
Area 3	Ibirubá, RS	426 m	Middle plateau	Typical red Dystrophic Latosol, clayey texture
Area 4	Santa Maria, RS	115 m	Central depression	Sandy dystrophic red-yellow Argisol
Area 5	Tupanciretã, RS	420 m	Middle plateau	Typical red dystrophic Latosol, sandy texture

Three readings were taken to evaluate the stand: the first at seven days after planting (DAP), the second at fifteen DAP, and the third at the physiological maturity stage. The readings were performed by counting the number of plants every two meters in the four central rows, totaling eight meters. The data from the first two readings were expressed as the number of plants per linear meter, and the data from the last reading were expressed as the average number of plants per hectare.

The average plant height was evaluated at 30 and 85 DAP by measuring the distance (cm) from the ground to the last trefoil. The average number of pods per plant was measured by randomly collecting 20 plants in the physiological maturity stage in the four central rows. The plants collected to determine the number of grains per pod were threshed by hand. The grains obtained after the opening of the pods collected in each plot were mixed, and six random samples of one thousand grains were weighed on an analytical balance, generating six data points for each treatment to verify the parameter thousand-grain weight (g). To evaluate the productivity, the useful area of each plot was harvested and threshed. The grain moisture content was determined using an Agrologic AL-120 apparatus, and the production value was expressed in kg ha^{-1} with moisture corrected to 13%.

A randomized block experimental design was used with six repetitions. Each plot was composed of eight 6-meter long rows totaling a 24-m^2 area, with the useful plot being composed of four 4-meter long central rows totaling 16 m^2. The mean values were submitted to analysis of variance (ANOVA), and in order to identify the effects of treatments on the parameters evaluated, Duncan's test was applied at 5% probability of error using the statistical package SPSS (version 16.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Trichoderma is considered a multitalented microorganism due to its vast metabolic arsenal positively expressed on the interaction plant-fungus. It is an extremely versatile fungus for possessing the ability to develop several functions in phytosanitary management. Its symbiotic association promotes plant development by increasing nutrient absorption; hence, it acts indirectly in controlling phytopathogens (RAWAT et al., 2011; SOOD et al., 2020).

Considered one of Brazil's main constituents of biological products, it is economically competitive against other compounds given its productive efficiency and specificity, which ensures health at the environmental level and for the worker and final consumer (MASCARIN et al., 2019).

At 30 and 85 days, the treatment with ICB Nutrisolo Trichoderma® was statistically superior in the parameter average plant height in the five areas evaluated (Table 2; Figure 1A). This parameter is pivotal for the final calculation of productivity because larger plants have a higher number of nodes and, consequently, more pods per plant (BALBINOT JUNIOR et al., 2015). The increased plant size stems from the increased nutrient availability by *Trichoderma* spp. as the strains that compose the product have biochemical mechanisms related to plant growth promotion, including phosphate solubilization, production of enzymes that enhance the mineralization of organic matter, and siderophore production (DONI et al., 2014; ZHAO et al., 2014). In addition, *Trichoderma* spp. affects rhizogenesis, increasing root biomass (especially secondary roots) (CONTRERAS-CORNEJO et al., 2015).

TABLE 2 - Average height of soybean plants (cm) in the five distinct areas and three treatments.

		Area 1	Area 2	Area 3	Area 4	Area 5	CV(%)
30 DAP	C+	$18.21 \pm 2.02^{b*}$	20.61 ± 2.57^{b}	22.21 ± 2.67^{a}	19.3 ± 1.26^{c}	21.01 ± 2.07^{b}	7.65
	INT	23.32 ± 2.47^{a}	25.78 ± 2.75^{a}	24.7 ± 2.59^{b}	23.1 ± 1.60^{a}	24.64 ± 1.95^{a}	4.54
	C-	18.53 ± 2.17^{b}	20.55 ± 2.35^{b}	22.51 ± 2.56^{a}	19.8 ± 1.08^{b}	21.16 ± 1.85^{b}	7.25
85 DAP	C+	68 ± 3.53^{b}	66.16 ± 2.78^{b}	72.08 ± 5.29^{a}	72.1 ± 5.29^{b}	72.07 ± 1.68^{c}	4.02
	INT	72.88 ± 2.86^{a}	72.66 ± 1.86^{a}	74.92 ± 4.35^{b}	74.9 ± 4.35^{a}	75.96 ± 1.69^{a}	1.93
	C-	67.69 ± 2.89^{b}	65.88 ± 2.43^{b}	71.06 ± 5.48^{a}	71.1 ± 5.48^{b}	73.15 ± 2.17^{b}	4.20

*Means followed by equal letters in the column do not differ statistically, by Ducan test, at 5% error probability. INT = ICB Nutrisolo Trichoderma®, C+ = positive control, C- = negative control, DAP = days after planting, CV = coefficient of variation.

Although the number of grains per pod in plants treated with ICB Nutrisolo Trichoderma® did not show a higher statistical value compared to the other treatments (Table 3; Figure 1C), the average number of pods per plant expressed statistically significant differences in most areas treated with the consortium (Table 3; Figure 1D). Consortia can provide a more consistent level of growth promotion and a broader spectrum of activity, assisting in increasing yield potential in the field (STEWART; HILL, 2014). Dalchiavon and Carvalho (2012) reported that the number of pods and grain mass per plant is the main components for estimating yield. In this study, the number of pods per plant ranged from 39 to 48 in the treatment with *Trichoderma* spp. (Table 3).

The parameter weight of one thousand grains was superior in most areas treated with ICB Nutrisolo Trichoderma® (Table 2; Figure 1E). However, it should be considered that in area 1, the treatment did not stand out, nor for the average number of grains per pod (Table 2; Figure 1C). Even so, area 1 differed statistically in the productivity parameter of the other plants without treatment (Table 2; Figure 1F). This fact can be explained by the increased average height of the plant, which consequently developed a greater number of pods, which directly reflected in higher productivity. Larger plants have more leaves, and this greater photosynthetic area reflects the superior formation of compounds directed to grain and pod production (PETTER et al., 2016).

The action of *Trichoderma* spp. as a growth promoter has been reported in different plants. Similar findings to those described herein were reported by Chagas et al. (2017) in soybean, cowpea, rice, and maize treated with *T. asperellum*. The authors obtained 60% more growth in all crops compared to the control. Growth promotion in plants by *Trichoderma* spp. can be influenced by gibberellin and auxin production, such as indoleacetic acid, which develops lateral roots. This development increases crop productivity, allowing greater adaptation to abiotic conditions and improving nutrient uptake (CHAGAS JUNIOR et al., 2019). In soybeans, productivity increased by 13.02% with *Trichoderma* spp. application (CHAGAS JUNIOR et al., 2021); França et al. (2017) demonstrated growth promotion by *Trichoderma* strains in cherry tomatoes and associated its application to greater phosphate solubilization.

Despite no statistically significant difference being observed in the average plant population for the treatment with the consortium (Table 3; Figure 1B), there was a significant increase in final productivity in the area treated with the inoculant based on *Trichoderma* spp., reaching up to 3397.6 kg ha^{-1} (Table 3; Figure 1F). Therefore, it can be concluded that increased productivity verified in this area depends on the physiological improvement of plants treated with ICB Nutrisolo Trichoderma® and does not refer to the increase in the stand.

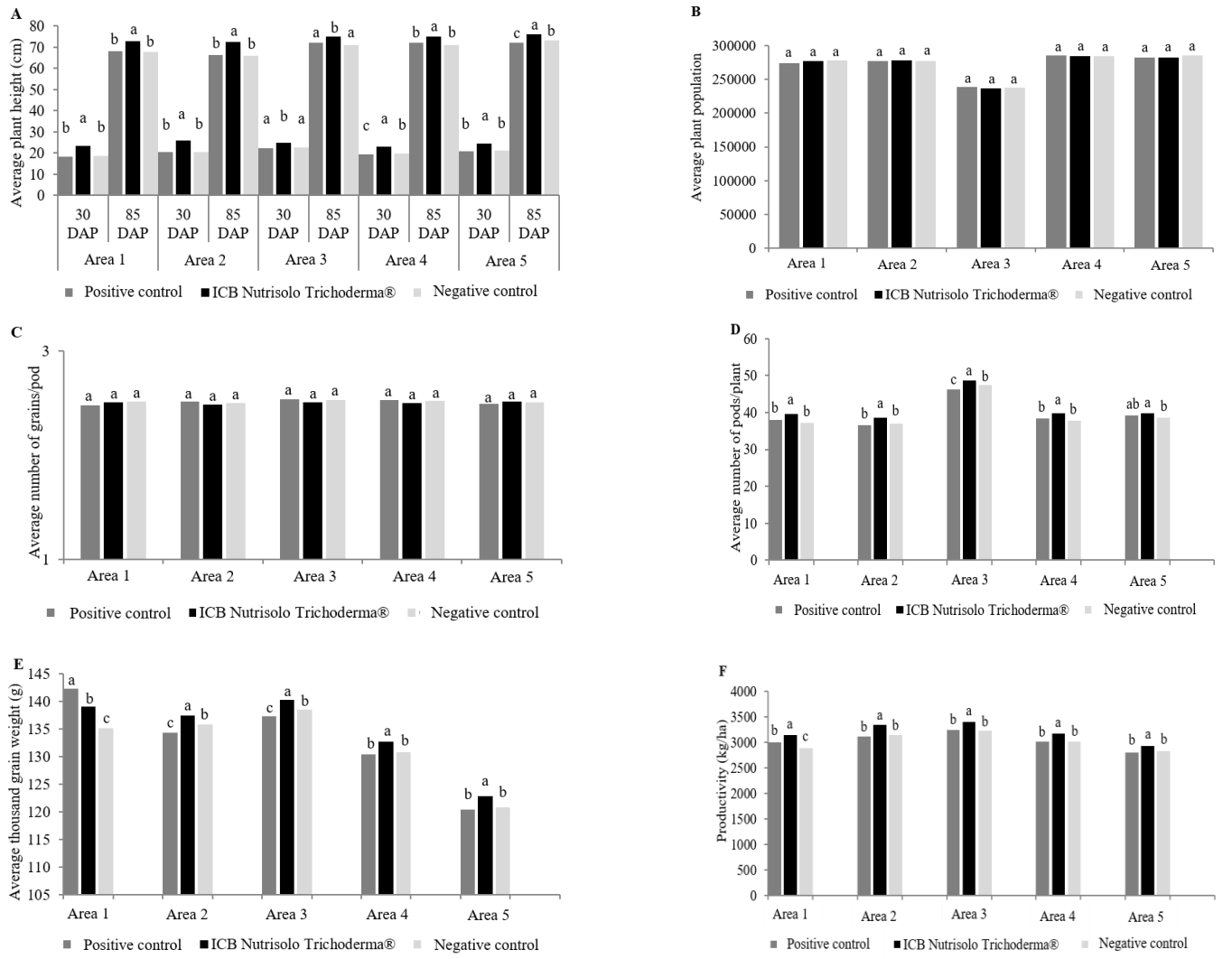

FIGURE 1 - A = Average plant height (cm), DAP = days after planting, B = average plant population, C = average number of grains/pods, D = average number of pods/plants, E = average thousand grain weight (g), F = productivity (kg ha^{-1}). *Means followed by the same lower-case letter are not statistically different using Duncan's test at 5% probability of error.

The homogeneity of the results obtained with the application of the product ICB Nutrisolo Trichoderma® under the different soil and climate conditions tested demonstrates that the beneficial action of the product on the growth of soybean plants is consistent. This bioactive compound, consisting of eight strains and three different *Trichoderma* species, each possessing their own characteristics associated with survival, may have favored their permanence in the soil and plants and, consequently, the crop's development. This metabolic diversity allows a microbial consortium to perform multiple and more complex tasks than an isolated microorganism by using the nutritional resources available in the environment more efficiently and making different effector molecules available (WOO; PEPE, 2018).

Therefore, soybean plants treated with ICB Nutrisolo Trichoderma® presented higher rates of average plant height, average number of pods per plant, and productivity in all areas tested. The weight of one thousand grains showed higher results than the negative control, and although the average plant population and the average number of grains did not show the most significant statistical differences compared to the other treatments, ICB Nutrisolo Trichoderma® promoted an increase in the average height of the plant, which consequently developed a greater number of pods, directly reflecting in higher productivity under all soil and climate conditions evaluated.

TABLE 3 - Parameters evaluated in the five distinct areas and three treatments.

	Treatments	Area 1	Area 2	Area 3	Area 4	Area 5	CV (%)
$\bar{\chi}$ APP	C+	274583.3 ± 5781.5^{a*}	276875 ± 1311^{a}	238958.3 ± 293.5^{a}	285416.6 ± 1020.6^{a}	282500 ± 3162.2^{a}	6.92
	INT	277500 ± 2500^{a}	277916.6 ± 1290.9^{a}	236875 ± 036.2^{a}	284791.6 ± 1461.3^{a}	282916.6 ± 2922.6^{a}	7.31
	C-	278125 ± 2592^{a}	277500 ± 2091.6^{a}	237708.3 ± 461.3^{a}	284583.3 ± 3129.1^{a}	285208.3 ± 2425.9^{a}	7.28
$\bar{\chi}$ NGP	C+	2.25 ± 0.65^{a}	2.3 ± 0.66^{a}	2.33 ± 0.70^{a}	2.32 ± 0.65^{a}	2.27 ± 0.59^{a}	1.35
	INT	2.29 ± 0.57^{a}	2.26 ± 0.60^{a}	2.29 ± 0.67^{a}	2.28 ± 0.60^{a}	2.30 ± 0.64^{a}	0.66
	C-	2.3 ± 0.65^{a}	2.28 ± 0.59^{a}	2.32 ± 0.69^{a}	2.31 ± 0.61^{a}	2.29 ± 0.64^{a}	0.69
$\bar{\chi}$ NPP	C+	38 ± 5.19^{b}	36.48 ± 3.64^{b}	46.22 ± 4.2^{c}	38.3 ± 1.66^{b}	39.24 ± 1.60^{ab}	9.60
	INT	39.61 ± 4.27^{a}	38.67 ± 2.12^{a}	48.57 ± 2.89^{a}	39.8 ± 1.96^{a}	39.7 ± 4.28^{a}	9.95
	C-	37.20 ± 5.57^{b}	37.03 ± 3.5^{b}	47.4 ± 4.08^{b}	37.7 ± 3.28^{b}	38.62 ± 2.29^{b}	11.14
$\bar{\chi}$ AWT	C+	142.3 ± 9.55^{a}	134.29 ± 2.6^{c}	137.25 ± 1.26^{c}	130.3 ± 2.18^{b}	120.37 ± 1.31^{b}	6.22
	INT	139.06 ± 1.98^{b}	137.4 ± 1.47^{a}	140.2 ± 1.60^{a}	132.6 ± 3.16^{a}	122.8 ± 0.98^{a}	5.29
	C-	135.04 ± 1.97^{c}	135.82 ± 3.63^{b}	138.4 ± 2.01^{b}	130.8 ± 2.81^{b}	120.8 ± 0.67^{b}	5.24
PROD	C+	2995.3 ± 121.04^{b}	3121.9 ± 48.44^{b}	3240.2 ± 46.72^{b}	3014.8 ± 43.5^{b}	2802.9 ± 67.6^{b}	6.10
	INT	3150.07 ± 31.28^{a}	3348.3 ± 59.08^{a}	3397.6 ± 136.9^{a}	3168.4 ± 70.5^{a}	2935.4 ± 53.6^{a}	5.73
	C-	2887.07 ± 74.86^{c}	3142.4 ± 45.06^{b}	3224 ± 46.76^{b}	3022.1 ± 84.9^{b}	2830.4 ± 44.07^{b}	5.49

*Means followed by equal letters in the same column do not differ statistically by Ducan's test at 5% error probability. INT = ICB Nutrisolo Trichoderma®, C+ = positive control, C- = negative control, $\bar{\chi}$ APP = average plant population, $\bar{\chi}$ NGP = number of grains per pod, $\bar{\chi}$ NPP = average number of pods/plant, $\bar{\chi}$ AWT = average weight of one thousand grains, PROD = productivity (kg ha^{-1}), CV = coefficient of variation.

CONCLUSIONS

ICB Nutrisolo Trichoderma® increased plant height, number of pods, and grain weight, consequently increasing soybean productivity. Hence, it was possible to characterize the product ICB Nutrisolo Trichoderma® as a growth-promoting inoculant for soybean cultivation, thereby increasing the scope of the product registration.

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