# CHEMICAL AND BIOLOGICAL SEED TREATMENT AND THEIR EFFECT ON SOYBEAN DEVELOPMENT AND YIELD<sup>1</sup>

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**ABSTRACT** - In the search for homogeneous planting density and satisfactory results in soybean development and yield, the treatment of seeds can be carried out using a combination of chemical fungicides, growth stimulators, and other biological agents. The objective of this study is to evaluate the effect of chemical and biological inputs for soybean seed treatment on crop development and yield. Experiments were carried out in the 2010/2011 growing season in three municipalities of Rio Grande do Sul using the following treatments: fungicide fludioxonil, *Trichoderma* spp., fludioxonil + *Trichoderma* spp., fludioxonil + *Trichoderma* spp. + the growth stimulator Biozyme®, and a control (untreated). The total number of pods in treatment with *Trichoderma* spp. and the other treatments were up to 77% higher than that of the control. The grain yield of treatments with fludioxonil, fludioxonil + *Trichoderma* spp., and fludioxonil + *Trichoderma* spp. + Biozyme® was up to 14% higher than that of the control. These results indicate that fludioxonil combined with the fungus *Trichoderma* spp. and Biozyme® is indicated for treating soybean seeds and increases crop development and grain yield.

**Keywords**: *Glycine max*. Plant growth. *Trichoderma* spp.

# INSUMOS QUÍMICO E BIOLÓGICO NO TRATAMENTO DE SEMENTES E SUA INTERFERÊNCIA NO DESENVOLVIMENTO E PRODUÇÃO DE SOJA

**RESUMO** - Na busca por estande de plantas homogêneo e resultados satisfatórios no desenvolvimento e produção de soja, pode ser realizado o tratamento de sementes combinando fungicidas químicos, bioestimuladores e outros produtos biológicos. Dessa forma, objetivou-se avaliar a interferência de insumos químico e biológico em tratamento de sementes no desenvolvimento e produção de soja. Para isso, foram conduzidos experimentos no ano agrícola 2010/2011 em três localidades do Rio Grande do Sul, utilizando-se os seguintes tratamentos de sementes: fungicida fludioxonil; *Trichoderma* spp.; fludioxonil + *Trichoderma* spp. + Biozyme<sup>®</sup>; e testemunha (sem tratamento). Para o número total de vagens, o tratamento com *Trichoderma* spp. e os demais tratamentos conjugados apresentaram até 77% mais vagens do que a testemunha. Os tratamentos fungicida fludioxonil, fungicida fludioxonil + *Trichoderma* spp. e fungicida fludioxonil + *Trichoderma* spp. e Biozyme<sup>®</sup> apresentaram até 14% maior produtividade do que a testemunha. O fungicida fludioxonil foi indicado para tratamento de sementes de soja conjugado com o fungo *Trichoderma* spp. e o bioestimulador Biozyme<sup>®</sup>, por promoverem o desenvolvimento de plantas e o aumento da produtividade de soja.

Palavras-chave: Glycine max. Crescimento de plantas. Trichoderma spp.

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#### INTRODUCTION

Soybean is the most important crop in Brazilian agribusiness, which is the basis of the primary sector economy and trade balance in Brazil. Brazil is the world's second largest producer and exporter of grains, and the state of Rio Grande do Sul accounts for 16% of the national production, with an average of 3,013 kg ha<sup>-1</sup> (CONAB, 2019).

In the search for homogenous plant density and satisfactory results in soybean development and grain yield, other crop protection products, and growth stimulators are used in conjunction with fungicides in seed treatment. Seed treatment with fungicides is considered essential for managing seedborne pathogens and soil-borne fungi, which can cause seed rot and seedling damping-off (HENNING et al., 2010).

Integrated disease management using biological control agents such as antagonistic fungi combined with chemical fungicides can be effective to control pathogens and reduce the use of pesticides (PANDOLFO, 2007). Mertz, Henning and Zimmer (2009) reported that several bioprotective agents are being commercialized and recommended for treating soybean seeds. However, there is limited research on seed treatment with biological fungicides.

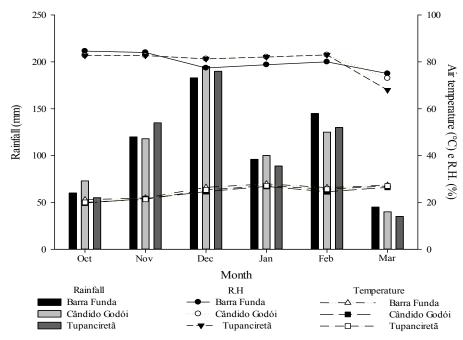
At present, formulations based on *Trichoderma* spp. are being used to treat soybean seeds, and the fungus acts as an antagonist to the pathogens present in the seeds or soil and as a plant growth stimulator. *Trichoderma* spp. are antagonistic fungi widely used in the biological control of pathogenic fungi (MACHADO et al., 2012) and seed treatment (ETHUR et al., 2005; MASTOURI;

BJÖRKMAN; HARMAN, 2010) against a wide range of disease-causing pathogens in different cultures. In addition, this fungus stimulates plant growth and development (HARMAN et al., 2004). In this respect, the TA1 isolate of *Trichoderma asperellum* promoted an increase in shoot development in cucumber (ETHUR et al., 2012), and an isolate of *T. harzianum* increased the number of pods per plant and grain yield in soybean (GÖRGEN et al., 2009).

The objective of this study is to evaluate the use of chemical and biological inputs in soybean seed treatment and their effect on crop development and yield.

## MATERIAL AND METHODS

The field experiments were conducted in three municipalities during the 2010/2011 growing season: Barra Funda (27°55'56"S and 53°31'58"W), with typical Dystrophic Red Latosol; Cândido Godói (27°93'13.72"S and 54°71'20.04"W), Eutrophic Red Nitosol; and Tupanciretã (29° 07'012"S and 53°57'56.8"W), with Dystrophic Yellow Red Argisol (EMBRAPA, 2013), in soybean -producing regions 102 and 101, respectively, in Rio Grande do Sul, Brazil (KASTER; FARIAS, 2011). According to the classification of Köppen-Geiger, the climate of the region is type Cfa, subtropical humid with hot summers and without a defined dry season. Data on rainfall, air temperature, and relative air humidity in these municipalities from October 2010 to March 2011 are presented in Figure 1.



**Figure 1**. Rainfall accumulation (mm), mean temperature (°C), and relative air humidity (RH%) in the study period in the 2010/2011 growing season in the municipalities of Barra Funda, Cândido Godói, and Tupanciretã, Rio Grande do Sul, Brazil

The study used a completed randomized block design, with five treatments and four replications. The experiments were organized in four blocks of  $25.00 \times 2.25$  m, each block consisting of five plots of  $5.00 \times 2.25$  m ( $11.25 \text{ m}^2$ ), and each plot consisting of five rows spaced at 0.45 m.

The fungicide fludioxonil (phenylpyrrole) in combination with *Trichoderma* spp. was used for treating soybean seeds. This fungicide was selected because it has been shown to promote 100% fungal survival in the seed treatment tests (PAZINI et al., 2013). The *Trichoderma* spp. isolate T2 was obtained from the sclerotia of *Sclerotinia sclerotiorum*, which were used as baits in the soil of Itaqui, Rio Grande do Sul. The *Trichoderma* spp. powdered formulation was obtained as described by Ethur et al. (2005). Before seed treatment, the viability of *Trichoderma* spp. was assessed by germination in Petri dishes containing potato dextrose agar medium.

The chemical treatment of soybean cultivar NS6411RR was carried out manually in plastic bags one day before sowing. One of the treatments contained Biozyme® (Arysta Lifescience do Brazil Indústria Química e Agropecuária SA, Salto de Pirapora, SP, Brazil), which is a mixed mineral fertilizer (N,  $K_2O$ , B, Fe, Mn, S, Zn, and total organic carbon), and was applied on seeds and leaves in the proportion of 2 mL<sup>-1</sup> kg of seed.

Treatment with *Trichoderma* spp. was performed at the time of sowing, and the fungus was mixed to the seeds in the proportion of 600 g of powder to 100 kg of seeds. Therefore, the seed treatments used in the three localities were: T1, control (untreated seeds); T2, 0.435 g *Trichoderma* spp.; T3, 0.087 mL fludioxonil + 0.435 mL water; T4, 0.087 mL fludioxonil + 0.435 mL water + 0.435 g *Trichoderma* spp.; T5, 0.087 mL fludioxonil + 0.435 mL water + 0.435 g *Trichoderma* spp. + 0.174 mL Biozyme.

The cultivation system used was no-tillage, and black oats (Avena strigosa) were cultivated in

the winter in the three experimental areas. Black oats were desiccated 60 days after sowing (DAS), and fertilization at planting was 350 kg ha<sup>-1</sup> of the formulation 2-18-18 NPK. During the crop cycle, three fungicide applications and insect and weed control were performed according to the needs of each experimental area.

Plant height was measured in three central rows of each plot (area of 4.05 m<sup>2</sup>) at 30 DAS (PH30DAS) and 60 DAS (PH60DAS). Other evaluated variables were the number of pods with one grain per plant (NP1G), number of pods with two grains per plant (NP2G), number of pods with three grains per plant (NP3G), number of pods per plant (NPP), number of filled grains (NFG), number of infected grains (NIG), thousand-grain mass (TGM), and grain yield per hectare (GY).

Only the variables PH30DAS, PH60DAS, NSG, NIG, TGM, and GY presented valid assumptions for analysis of variance. Therefore, the square root transformation was applied to NPP and NPOG, and the inverse transformation was applied to NPDG and NPTG. The data from the three municipalities were subjected to combined analysis. Analysis of variance was performed using the F-test, and the means were compared using the Scott-Knott test at a level of significance of 5%. All analyses were performed using the software R (R DEVELOPMENT CORE TEAM, 2015)

#### RESULTS AND DISCUSSION

The interaction between seed treatment (ST) and each study area (SA) was significant only for PH30DAS and NPP (Tables 1 and 2), and therefore the isolated effect of each factor on the other variables was then evaluated. There were significant differences in NP2G, NFG, NIG and GY between treatments, and significant differences in PH60DAS, NP1G, NP2G, NP3G, NFG, TGM, and GY between the SAs (Table 1 and 2).

**Table 1**. Summary of the analysis of variance of plant height at 30 days after sowing [DAS] (PH30DAS), plant height at 60 DAS (PH60DAS), number of pods with one grain (NP1G), number of pods with two grains (NP2G), and number of pods with three grains (NP3G) in soybean seeds treated with chemical and biological inputs in the municipalities of Barra Funda, Cândido Godói, and Tupanciretã, Rio Grande do Sul, Brazil, in the 2010/2011 growing season.

	Mean square											
SV	DF	PH30DAS		PH60DAS		NP1G		NP2G		NP3G		
			cm			number						
ST	4	15.00	ns	20.68	ns	0.25	ns	0.00014	*	0.0002	ns	
SA	2	485.09	*	752.22	*	5.01	*	0.00064	*	0.0081	*	
ST*SA	8	10.04	*	2.92	ns	0.36	ns	0.00006	ns	0.0001	ns	
Block (SA)	9	2.29	ns	8.30	ns	0.18	ns	0.00007	ns	0.0001	ns	
Residual	36	3.49		14.39		0.26		0.00004		0.0001		
CV (%)		7.16		4.06		22.48		23.60		24.18		

SV, sources of variation; DF, degrees of freedom; CV, coefficient of variation; ST, seed treatment; SA, study areas (Barra Funda, Cândido Godói, and Tupanciretã); "snon-significant; \*significant at p<0.05 using the F-test.

**Table 2**. Summary of the analysis of variance of the number of pods per plant (NPP), number of filled grains (NFG), number of infected grains (NIG), thousand-grain mass (TGM), and grain yield (GY) of soybeans seeds treated with chemical and biological inputs in the municipalities of Barra Funda, Cândido Godói, and Tupanciretã, Rio Grande do Sul, Brazil, in the 2010/2011 growing season.

	Mean square										
SV	DF	NPP		NFG NI		NIG	G TGM			GY	
		number					g		kg ha <sup>-1</sup>		
ST	4	2.87	*	636.36	*	180.15	*	11.02	ns	383895.81	*
SA	2	66.68	*	6823.95	*	22.85	ns	967.70	*	466542.60	*
ST*SA	8	2.33	*	52.91	ns	10.85	ns	14.40	ns	76023.12	ns
Block (SA)	9	0.99	ns	46.02	ns	6.84	ns	11.05	ns	101312.23	ns
Residual	36	0.89		33.73		8.01		19.94		95939.56	
CV (%)		11.06		2.96		12.60		2.71		11.69	

SV, sources of variation; DF, degrees of freedom; CV, coefficient of variation; ST, seed treatment; S, study areas (Barra Funda, Cândido Godói, and Tupanciretã);  $^{ns}$ non-significant; \*significant at p<0.05 using the F-test.

PH30DAS was highest in Barra Funda, and there were significant differences between the treatments only in Tupanciretä, where fungicide + *Trichoderma* spp. + Biozyme<sup>®</sup> was more effective, and plant height was higher than that in the other treatments (Table 3). Mertz, Henning and Zimmer (2009) observed that the height of soybean plants

obtained from seeds treated with different fungicides and *Trichoderma*-based bioprotective agents under field conditions was not significantly different at 28 DAS. However, shoot and root growth was comparatively higher in treatments with *Trichoderma* spp. in sunflower and soybean crops (GUARESCHI et al., 2012).

**Table 3**. Mean plant height at 30 days after sowing [DAS] (PH30DAS) and the number of pods per plant (NPP) in soybean seeds treated with chemical and biological inputs in the municipalities of Barra Funda, Cândido Godói, and Tupanciretã, Rio Grande do Sul, Brazil, in the 2010/2011 growing season.

			Stud	dy area						
Treatment	PH30DAS (cm)									
Treatment	Barra F	unda	Cândido	Godói	Tupai	nciretã				
Control	32.3	aA <sup>1</sup>	22.1	aC	26.1	bB				
Tric	29.4	aA	20.5	aB	27.7	bA				
fdx	29.8	aA	19.6	aC	26.2	bB				
fdx + Tric	30.0	aA	20.4	aC	24.2	bB				
fdx + Tric + Bz	30.6	aB	21.0	aB	31.1	aA				
	NPP (number)									
	Barra F	unda	Cândido	Godói	Tupai	Tupanciretã				
Control	54.3	aВ	53.5	aВ	77.1	bA				
Tric	65.8	aB	44.6	aC	128.7	aA				
fdx	76.5	aA	43.4	aB	99.4	bA				
fdx + Tric	72.5	aB	52.4	aB	136.2	aA				
fdx + Tric + Bz	62.8	aВ	48.6	aB	123.7	aA				

SA, study areas (Barra Funda, Cândido Godói, and Tupanciretã; C, control; *Tric, Trichoderma* spp.; fdx, fludioxonil; Bz, Biozyme<sup>®</sup>; <sup>1</sup>The means followed by the same lowercase letter in each column and the same uppercase letter in each row were not different from each other using the Scott-Knott test at a level of significance of 5%.

Harman et al. (2004) found that *Trichoderma* spp. increased the absorption of nitrogen, arsenic, cobalt, cadmium, chromium, nickel, lead, vanadium, magnesium, manganese, copper, boron, zinc, aluminum, and sodium. However, in most cases, the increase in the absorption of these elements was small in agricultural systems. Biozyme<sup>®</sup>, which was used in combination with other control agents to treat soybean seeds, has been shown to increase the vigor of maize seedlings up to a dose of 10 mL kg<sup>-1</sup> of seeds (NICCHIO et al. al., 2013).

There were significant differences in NPP

between the treatments only in Tupanciretã, and the NPP in treatments with *Trichoderma* spp., fludioxonil + *Trichoderma* spp., and fludioxonil + *Trichoderma* spp. + Biozyme® was increased by 67%, 77%, and 60%, respectively, relative to the control (Table 3). However, the NPP responded differently to treatments in each municipality and was significantly higher in Tupanciretã in all treatments relative to the other regions. Fungicides combined with biological control agents and/or growth stimulators can benefit soybean cultivation by reducing the risk of development of fungicide-

resistant pathogens and broadening the control spectrum (PESQUEIRA; BACCHI; GAVASSONI, 2016).

There were no significant differences in NP1G and NP3G between the treatments (Table 1). This result is because this yield component is

strongly affected by cultivars, which, to date, are selected to produce three eggs per pod (KRUTZMANN et al., 2013). However, the NP2G in treatment with *Trichoderma* spp. and *Trichoderma* spp. + fludioxonil was 23.8% and 22.4% higher than that of the control, respectively (Table 4).

**Table 4**. Mean number of pods with two grains (NP2G), number of filled grains (NFG), number of infected grains (NIG), and grain yield (GY) in soybean seeds treated with chemical and biological inputs in the municipalities of Barra Funda, Cândido Godói, and Tupanciretã, Rio Grande do Sul, Brazil, in the 2010/2011 growing season.

Treatment	NP2	NP2G		NFG		NIG		
Treatment			number				kg ha	1
Control	37.0	$b^1$	183.7	c	29.3	a	2,461	b
Tric	45.8	a	195.8	b	21.0	b	2,451	b
fdx	37.0	b	201.4	a	20.3	b	2,800	a
fdx + Tric	45.3	a	201.4	a	20.2	b	2,754	a
fdx + Tric + Bz	35.5	b	197.8	b	21.3	b	2,788	a

C, control; *Tric*, *Trichoderma* spp.; fdx, fludioxonil; Bz, Biozyme<sup>®</sup>, <sup>1</sup>The means followed by the same lowercase letter in each column were not different from each other using the Scott-Knott test at a level of significance of 5%.

The control treatment presented a increase in the NIG when compared to the other treatments and a 8.8 decrease in the NFG relative to treatment with fludioxonil and fludioxonil + Trichoderma spp. (Table 4). This result is related to the lower incidence of foliar diseases and the lower percentage of defoliation of soybean plants when ST is performed (GOULART; ROESE; MELO, 2015) by increasing the production of photoassimilates and, consequently, grain filling.

The NFG was 8.8% higher in the treatments fludioxonil and fludioxonil + Trichoderma spp. than was that in the control. The NFG in treatments with Trichoderma spp. and fludioxonil + Trichoderma spp. + Biozyme® was 2.8% and 1.8% lower than treatment with fludioxonil and fludioxonil + Trichoderma spp., respectively. In addition to the lower defoliation and lower incidence of diseases (GOULART; ROESE; MELO, 2015), combination of a fungicide, Trichoderma spp., and Biozyme<sup>®</sup> may have supplied the required micronutrients for plant development, which favored grain filling (AVELAR et al., 2011).

GY using *Trichoderma* spp. was not significantly different from that of the control.

Nonetheless, GY using a fungicide, fungicide + *Trichoderma* spp., and fungicide + Trichoderma spp. + Biozyme<sup>®</sup> increased by 14%, 12%, and 13%, respectively, relative to the control (Table 4). This finding may be because chemical treatments provide greater protection to seeds and seedlings against the attack of plant pathogens (CONCEIÇÃO et al., 2014), favoring plant emergence and increasing GY. Although *Trichoderma* spp. alone did not increase GY, Görgen et al. (2009) observed the isolate 1306 of *Trichoderma harzianum* increased GY in soybeans.

There were no significant differences in the TGM in the presence or absence of chemical and biological treatment of soybean seeds. These results corroborate those of Goulart, Roese and Melo (2015), in which, in 2 years, ST with and without fluquinconazole did not affect the TGM regardless of whether fungicide was applied to soybean stems. However, in the present study, there were significant differences in the TGM between the municipalities, and the TGM was 6.1% and 8.4% higher in Tupanciretã than in Cândido Godói and Barra Funda, respectively (Table 5).

**Table 5**. Mean values of plant height at 60 days after sowing [DAS] (PH60DAS), number of pods with one grain (NP1G), number of pods with two grains (NP2G), number of pods with three grains (NP3G), number of filled grains (NFG), thousand-grain mass (TGM), and grain yield (GY) in soybean seeds treated with chemical and biological inputs in Barra Funda, Cândido Godói, and Tupanciretã, Rio Grande do Sul, Brazil, in the 2010/2011 growing season.

Maniainalita	PH60DAS	NP1G	NP2G	NP3G	NFG	TGM	GY
Municipality	cm		nur	nber		g	kg ha <sup>-1</sup>
Barra Funda	86.3 b <sup>1</sup>	3.8 b	38.8 b	23.4 b	180.7 c	159.2 c	2,518 b
Cândido Godói	97.7 a	4.1 b	28.9 c	15.6 c	190.9 b	162.7 b	2,617 b
Tupanciretã	96.0 a	8.7 a	52.7 a	51.2 a	216.5 a	172.6 a	2,818 a

<sup>&</sup>lt;sup>1</sup>The means followed by the same lowercase letter in each column are not different from each other using the Scott-Knott test at a level of significance of 5%.

In Tupanciretã, the soybean cultivar and treatments used were adequate to local conditions because there was an increase in PH60DAS, NFG, NP1G, NP2G, NP3G, TGM, and GY compared to the other two regions (Table 5). This difference in response to ST may be related to the edaphoclimatic conditions in each region. However, these differences were expected because of the agroclimatic zoning of each region.

#### CONCLUSIONS

The fungicide fludioxonil combined with the fungus *Trichoderma* spp. and the growth stimulator Biozyme<sup>®</sup> is indicated for treating soybean seeds and increases crop development and yield.

Soybean development and grain yield responded differently to treatment with chemical and biological inputs, regardless of the study area.

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