IMPACTO CIENTÍFICO EDITORA

HEALTH AND GERMINATION OF SOYBEAN SEEDS AS A FUNCTION OF BIOLOGICAL TREATMENT WITH BACILLUS IN SOIL WITH THE HYDRORETAINING POLYMER

10.56238/edimpacto2025.015-006

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ABSTRACT

The use of seeds of good physiological and sanitary quality is one of the main factors for obtaining higher grain yields in soybean. Seed treatment is a management technique that aims to ensure sanitary quality, being efficient in the control of phytopathogens and especially fungi associated with seeds or present in the soil. Among the biological control options, the use of bacteria of the genus Bacillus has been used. In addition to the phytosanitary problems of the soybean crop, the climate risk has been a cause of concern for producers. With the occurrence of dry spells in the Cerrado regions, the use of synthetic water-retaining polymers is another alternative for water maintenance in the producing regions. Therefore, few studies have been developed associating the use of various inputs including seed treatment, application of polymers in the soil, with the fungal microflora in the seeds. Thus, this study aimed to evaluate the incidence of fungi in the seeds of the soybean cultivar M8644 IPRO, as a function of the biological treatment of seeds and subsequent influence of the treatments on the fungi present in harvested grains. Seed lots produced in

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the municipality of Lagoa da Confusion, TO, were used. The laboratory experiment was conducted in a completely randomized design, with four replications and five treatments, as follows: 1-fungicide, 2-Bacillus 02, 3-Bacillus BRSW, 4-Bacillus 02 + Bacillus BRSW, 5-Control (seeds treated with sterile water). In the field, the same treatments were used, with the presence and absence of hydrogel in the ripening line, with 04 replications. For the survey of the fungal population, sanitary analysis was carried out in Blother Test and germination in sand. The experimental data were submitted to analysis of variance and when significant by the F test, Tukey's test was applied using the SISVAR program. It was concluded that seed treatment with bioprotective effect was favorable to be used.

Keywords: Glycine max. Seed treatment. Bioprotective. Hydrogel.

INTRODUCTION

Soybean (*Glycine max* (L.) Merril) is a crop of great global importance, one of the main commodities of the Brazilian agricultural market. Research data prove that soybean crops originated with high quality seeds provide higher yields (França Neto et al., 1984; Kolchinski et al., 2005), with increases of 20% to 35% in grain yield in relation to the use of low-vigor seeds.

Among the practices used for this purpose, seed treatment is a management technique that aims to ensure sanitary quality, being efficient in the control of phytopathogens and especially fungi associated with seeds or present in the soil. When a pathogen is passively transported, it is a contaminant or weed. In this case, the pathogen is located on the surface of the seed (Agarwal; Sinclair, 1987). The attack of pathogens on soybean seeds can be considered as one of the causes that lead to the loss of physiological quality of the seeds, causing a reduction in germination.

Among the pathogens transmitted by seeds, fungi are considered the most important, not only due to the greater number, but also due to the damage caused both in yield and seed quality. In soybean crops, there are several pathogens that cause damage to the quality of the seeds present in the soils, which can contaminate the seeds externally and internally, including: *Cercospora kikuchii*, *Cercospora sojina, Fusarium semisectum, Phomopsis* and *Colletotrichum truncatum* (Embrapa, 2002; Goulart, 1997).

Among the biological control options, the use of bacteria of the genus *Bacillus* has been used, both in seed treatment (Bashan, 1998), as well as in foliar applications. In addition to improving crop yields*, B. subtilis* also induces resistance to fungal phytopathogens (Clemente et al., 2016; Son; Guenther, 2015). Strains of *B. amyloliquefaciens* and *B. subtilis* are known to compete, colonize plants, and can act simultaneously as biofertilizers and biopesticides to microorganisms such as bacteria and fungi (Khatun et al., 2018; Souza et al., 2015; Chen et al., 2007). Although chemical fungicides reduce the level of fungal infection in seeds, recurrent treatment with these products can select lines resistant to some fungicide groups (Souza et al., 2015).

In addition to the phytosanitary problems of the soybean crop, the climatic instability in the Cerrado regions increases the probabilities of climatic events limiting plant production, such as lower rainfall and longer interval between rainfall, resulting in the occurrence of dry spells in essential phases for the development of the crop, which may then cause crop failure due to lack of rainfall and, or, to low rainfall during the establishment phase of the crop (Borghi et al., 2014). Adding technologies that allow greater efficiency in the use of water could reduce the risks of losses in the implementation phase of rainfed crops.

Synthetic water-retaining polymers, also known as hydrogels, are hygroscopic materials made up of acrylamide monomers. The carboxylic group along the polymer chain confers a high water absorption capacity, although the cross-links present in the chain prevent its complete solubilization (Bortolin et al., 2012). Polyacrylamide is the main polymer used in agriculture and absorbs water through the formation of hydrogen bonds (Ahmed, 2015). In this case, the polymer can absorb up to 400 times its weight in water and increase its size by up to 100 times, polymers generally do not pose a risk of residual effect on the agricultural environment.

Few studies have been developed associating the use of various inputs, including seed treatment, application of polymers in the soil, with the fungal microflora in the seeds. Thus, the objective of this work is to evaluate the incidence of fungi in the seeds of the soybean cultivar M8644 IPRO, as a function of the biological treatment of seeds and subsequent influence of the treatments on the fungi present in harvested grains.

METHODOLOGY

Seed lots produced in the municipality of Lagoa da Confusion, TO, under the geographic coordinates 10°49'34.78"S and 49°54'0.33"W and 200 m of altitude, were used. The laboratory experiment was conducted in a completely randomized design, with four replications and five treatments, as follows: 1-fungicide, *2-Bacillus* 02, 3-Bacillus *BRSW, 4-Bacillus 02* + Bacillus BRSW, 5-Control (seeds treated with sterile water). The biological treatment of the seeds with the bacterium of the genus *Bacillus* was done at a concentration of 2 x 105 UFC.ml. For the chemical treatment of the seeds, the a.i. Methyl Thiophanate + fluazine was used, at the dosage of 215 ml/100 kg of seeds and i.a. chlorantraniliprole, at the dosage of 50 ml/ha.

In the field, the same treatments mentioned above were used, with the presence and absence of hydrogel in the sowing line, with 04 replications. To survey the fungal population associated with soybean seeds, sanitary analysis was performed on moistened Germitest paper or Blother Test (Brasil, 2009) and germination in sand. The seeds from each lot were submitted to asepsis in a sequence of 50% alcohol solution for 30 seconds, 2% sodium hypochlorite for 1 minute and later, two sequences of washes in sterilized distilled water. The seeds were arranged individually and then stored in an incubation room, at a temperature of 25 °C and a photoperiod of 12 hours for five days, where they remained until the evaluation. The evaluation was performed individually in each gerbox, with the aid of a magnifying glass and optical microscope. To identify the fungi, slides were prepared and visualized under an optical microscope, noting the incidence of the genera found. Specialized literatures such as Barnett and Hunter (1998) and Watanabe (2010) were used in the identification.

The germination test was carried out in plastic trays of 40 cm long, 30 cm wide and 10 cm high, containing autoclaved sand, seed lots were distributed per treatment containing 100 seeds for each tray, with 4 replications, maintaining the moisture of the substrate daily through manual irrigation, being evaluated daily after the initial emergence for ten days.

For the Germination calculation:

$$G = \left(\frac{N}{100}\right) x100$$

(LABOURIAU; VALADARES, 1976)

Where:

- N is the number of seeds germinated at the end of the test;
- 100 is the total number of seeds placed to germinate.

The experimental data were submitted to analysis of variance and when significant by the F test, Tukey's test was applied at 1% and 5% probability using the SISVAR software (Ferreira, 2011). For analysis of variance, the data in percentage were previously transformed by (root ((x+0.5) /100)).

RESULTS and DISCUSSION

The analysis of variance (Table 01) showed a significant effect for treatment in all fungi evaluated. When germination was analyzed, it also showed significance.

Table 01. Analysis of variance of fungi in seeds submitted to different treatments and germination for the soybean cultivar M8644 IPRO.

F V	GL	Medium Square					
		Fusarium sp [1]	*Cladosporium* sp [1]	*Penicillium* sp [1]	*Aspergillus* sp [1]	*Rhizoctonia* sp [1]	Germination
Rep	3	0,0152	0,0265	0,0104	0,0036	0,0070	150,1333
Trat	4	0,293**	0,1636 **	0,4191**	0,4433**	0,0282**	186.8000 *
Error	12	0,0196	0,0225	0,0122	0,0076	0,0048	42.8000
Average		0,34	0,43	0,31	0,57	0,13	57,80
CV		41,23	34,65	34,65	15,12	53,42	11,32

[not] significant; ** significant for P ≤ 0.01; *Significant for P ≤ 0.05 by the F test.
1Values in percentage transformed by (root ((x+0,5)/100)).

Source: Author himself.

Analyzing the fungal incidence in the seeds (Table 02), some differences were found between the treatments. The fungicide was the most efficient in controlling the genera, with 100% of the incidence eliminated. Only Cladosporium grew in the seeds treated with Methyl Thiophanate, but in a smaller percentage than in the control. In general, the treatment with Bacillus 02 and BSRW, isolated and associated, reduced the percentage of fungal genera in the seeds, in relation to the control. Bacillus BSRW and 02 were similar to fungicide treatment in the control of *Cladosporium, Penicillium* and *Rhizoctonia*. The association of the two strains of Bacillus in seed treatment was similar to the fungicide in the control of *Fusarium, Cladosporium, Penicillium* and *Rhizoctonia.* Thus, the biological treatments statistically demonstrated efficiency equivalent to the chemical treatment.

Table 02. Means of fungal genera present in seeds submitted to different treatments and germination for the soybean cultivar M8644 IPRO.

Treatments	Variables					
	Fusarium sp [1]	*Cladosporium* sp [1]	*Penicillium* sp [1]	*Aspergillus* SP [1]	*Rhizoctonia* SP [1]	Germination (%)
Fungicide	0.00 c	7.00 b	0.00 b	0.00 c	0.00 b	56.00ab
Bacillus 02	27.00ab	10.00 b	10.00 b	30.00 b	0.00 b	57.00ab
Bacillus BSRW	2.00 c	27.00ab	2.00 b	69.00a	5.00ab	59.00ab
B02+BSRW	9.00 bc	14.00 b	6.00 b	28.00 b	0.00 b	68.00A
Witness (water)	54.00a	58.00A	77.00a	86.00a	6.00A	49,00 b
CV	41,23	34,65	34,65	15,12	53,42	11,32

Means followed by the same letter do not differ by Tukey's test at 5% probability of error.
1Values in percentage transformed by the root ((x+0,5)/100)).

Source: Author himself.

Regarding the control treatment, a large amount of fungi was found in the seeds. Thus, the importance of seed treatment before planting is proven.

Remuska et al. (2007) tested *Bacillus thuringiensis* for the control of mycelial growth of several pathogens, obtained positive results with the use of the antagonist in the control *Rhizoctonia solani* , corroborating with the data demonstrated here, where in treatment with *Bacillus* it presented lower fungal incidence when compared to untreated seeds (Witness).

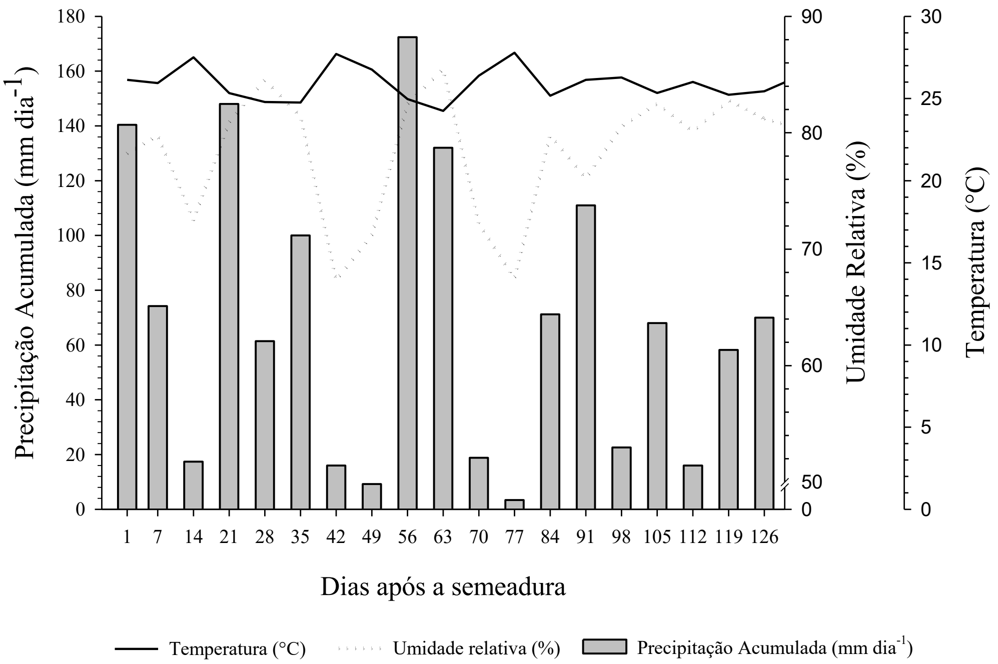
Regarding the percentage of inhibition of the fungus *Fusarium* sp. (Table 2) by the treatments using as a reference its presence in untreated seeds, it was observed that the fungicide treatment showed efficiency of 100% in the elimination of this pathogen, the biological treatment B02 showed efficiency of 50%, the biological BSRW alone and associated showed efficiency above 80%. It is also worth mentioning that these pathogens found can often be present in the outer integuments (hull) of soybean seeds.

Regarding seed germination with the biological treatment associated with the two *Bacillus*, it presented the best result with 68 % germination, which differed statistically from the control (Table 2). The biological treatments with *Bacillus* 02 and BSRW, in isolated treatment, together with the fungicide, despite having decreased the incidence of fungi and having numerically higher germination, did not differ statistically from the control in the germination rate.

According to Marroni et al. (2012), germination can be influenced by the chemical treatment and the initial quality of the lots. Menten (1995) points out that the effects of seed treatment on germination occur in the medium and long term, as happens, for example, with the decrease in the advance of disease development and/or the introduction of pathogens in the area.

During the conduct of the test in the field, the relative humidity remained with a minimum variation of 67.33% and a maximum of 85.66%. The average air temperature ranged between 24.4 and 27.8o C. The occurrence of precipitation was relatively high during the period of execution of the experiment, presenting a total of 1078 mm in this period (Figure 1).

Figure 1. Average climatic variables of weekly temperatures (^{C}), relative humidity (%) and rainfall (mm), from November 2018 to March 2019, in the municipality of Gurupi, Tocantins.



Source: Inmet, 2019.

The analysis of variance of the sanitary sample of fungi from the harvested seeds (Table 3) showed no significant effect on the interaction of the seed treatment VS hydrogel for any of the fungi evaluated. Therefore, evidencing the independence of the factors should therefore be studied in isolation. Regarding the source of variation of the hydrogel, there was a significant difference only for the fungus *Cercospora* sp. Regarding the source of variation in treatment, there was no significant effect.

Table 03. Analysis of variance for fungal genera present in seeds harvested for the soybean cultivar M8644 IPRO. Gurupi, Tocantins, 2018/2019 harvest.

FV	GL	Medium Square			
		Fusarium sp [1]	*Cladosporium* sp1	*Cercospora* sp.[1]	*Rhizoctonia* sp1
Rep	3	0,0074	0,0783	0,0341	0,0028
Treatment	4	0.0001 ns	0.0189 ns	0.0128 ns	0.0010 ns
Hydrogel	1	0.0001 ns	0.0024 ns	0,0257 *	0.0028 ns
TS x H	4	0.0020 ns	0.0020 ns	0.0058 ns	0.0010 ns
Error	27	0,0012	0,0108	0,0065	0,0012
Average		0,97	0,62	0,16	0,07
CV%		3,68	16,80	49,71	45,71

not significant; ** significant for $P \leq 0.01$; *Significant for $P \leq 0.05$ by the F test.
1Values in percentage transformed by (root ((x+0,5)/100)).

Source: Author himself.

Most of the fungal genera identified in this work (*Fusarium, Rhizoctonia, Aspergillus, Penicillium, Cladosporium,* etc.) are considered typical soil inhabitants and are active in the decomposition of organic matter. Fungi of the genus *Aspergillus* and *Penicillium*, in addition to inhabiting the soil, are also common in environments containing stored grains and causing food degradation and biodeterioration. In the present work, the high percentages of fungi found in soybean seeds may have as one of the causes, the favorable climatic conditions of the place where they were produced, in addition to the management adopted. In the region of Lagoa da Confusion, intense cultivation prevails, both in the harvest and in the off-season. This continuous cultivation in the same area favors the increase in the incidence of these pathogens due to the increase of inoculum in the area (Fernandes et al., 2005).

Among all treatments evaluated (Table 04) for the fungi *Fusarium* sp. and *Cladosporium* sp. there was no significant difference, either for the presence or absence of hydrogel. In this way, it is proven that the use of seeds treated with Bacillus in soil that received the hydrogel in the sowing rows, does not interfere with the health of the seeds, microorganisms with a protective effect on the seed at sowing may be associated with the use of hydrogel.

Table 04. Averages for fungal evaluations of harvested soybean seeds, cultivar M8644 IPRO, submitted before planting to different fungicide and biological treatments.

Treatments	Variables			
	Fusarium sp [1]	*Cladosporium* sp1	*Cercospora* sp1	*Rhizoctonia* sp1
	CH SH Medium	CH SH Medium	CH SH Medium	CH SH Medium
Chemist	93.2 97.0 95.1 A	29.0 32.7 30.8 A	2.5 1.0 1.7 A	0.0 1.2 0.6 A
B02	96.7 90.0 93.7 A	34.0 40.2 37.1 A	3.7 2.7 3.2 A	0.0 1.5 0.7 A

BSRW	90.5 98.5 94.5 A	45.7 44.5 45.1 A	6.5 1.5 4.0 A	0.0 0.0 0.0 A
B02+BSRW	96.2 91.5 93.8 A	41.5 39.0 40.2 A	1.5 1.5 1.5 A	0.0 0.0 0.0 A
Control	92.5 94.2 93.7 A	43.0 48.0 45.5 A	6.0 4.7 5.3 A	0.0 0.0 0.0 A
Average	93.8 A 94.2 A	38.6 A 40.9 A	4.0 A 2.3 B	0.0 to 0.5 A

Means followed by the same letter, uppercase for column (with and without hydrogel) and lowercase for the line (treatment), do not differ by Tukey's test at 5% probability of error.
1Values in percentage transformed by (root ((x+0,5)/100)). CH-with hydrogel and SH-without hydrogel.
Source: Author himself.

For the genus *Cercospora* sp., there was a statistical difference in the treatment with Bacillus BSRW, where in the presence of hydrogel it had a higher incidence, evidencing an unfavorable interaction between the bacterium and the hydrogel in the case of *Cercospora* sp. On the other hand, for *Rhizoctonia* sp., no difference was found between the use of hydrogel.

It is important to note that the presence of the phytopathogen *Fusarium* sp. both in the evaluation of planted and harvested seeds is due to the fact that this fungus may be endophytic and thus probably remain inside the seed. The high incidence of *Fusarium* sp. is also related to temperature indices, which are conducive to the production of spores in a range ranging from 25°C to 35ºC, corroborating the climatic data of this study (Desai et al., 2003; Gupta et al., 2010).

CONCLUSION

The biological and chemical seed treatments were efficient in controlling phytopathogens present in the seeds.

The biological treatment associated with the two *Bacillus* resulted in higher seed germination when compared to the control.

The presence of the hydrogel does not promote the development of phytopathogenic fungi.

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