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Physiological and sanitary quality of soybean seeds under different chemical treatments during storage

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ABSTRACT
The purpose of this study was to evaluate the effect of the chemical treatment with fungicide, insecticide, micronutrient and polymer on physiological and sanitary quality of soybean seeds during storage. The treatments were arranged in a 3 x 5 x 5 factorial scheme (cultivars x seeds treatment x storage period), in completely randomized design with four replicates. Three cultivars were used: NA 4823RG, BMX TurboRR and Fundacep 62RR. The treatments were: T1: no chemical treatment, T2: fungicide, insecticide and micronutrient; T3: fungicide, insecticide, micronutrient and polymer, T4: fungicide; T5: insecticide. After the chemical treatment, the seeds were stored under environmental conditions from May to December 2012, and seed quality was evaluated at 0, 2, 4, 6 and 8 months of storage. Seeds water content and physiological quality were determined through tests of germination, accelerated aging, seedling length, seedling dry weight and sanity. The treatment with fungicides, insecticides, micronutrients and polymer did not affect seed quality over eight months of storage and promoted the control fungi associated with the seeds.

Palavras-chave:
Glycine max
tratamento químico
viabilidade

Qualidade fisiológica e sanitária de sementes de soja sob diferentes tratamentos químicos durante o armazenamento

RESUMO
Objetivou-se avaliar o efeito do tratamento químico de sementes de soja com fungicida, inseticida, micronutriente e polímero na qualidade fisiológica e sanitária durante o armazenamento. Os tratamentos foram dispostos em um esquema fatorial 3 x 5 x 5 (cultivares x tratamento de sementes x período de armazenamento), em delineamento inteiramente casualizado com quatro repetições. Foram utilizadas três cultivares NA 4823RG, BMX TurboRR e Fundacep 62RR. Os tratamentos foram: T1: sem tratamento químico; T2: fungicida, inseticida e micronutriente e T3: fungicida, inseticida, micronutriente e polímero, T4: fungicida e T5: inseticida. Após o tratamento as sementes foram armazenadas sob condições ambientais de maio a dezembro de 2012, sendo as avaliações da qualidade das sementes realizadas aos 0, 2, 4, 6 e 8 meses de armazenamento. Determinou-se o teor de água das sementes e a qualidade fisiológica através do teste de germinação, envelhecimento acelerado, comprimento e massa seca de plântulas, além da sanidade. O tratamento com o fungicida, inseticida, micronutriente e polímero não prejudicou qualidade fisiológica das sementes ao longo de oito meses de armazenamento e promoveu o controle de fungos associados às sementes.

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Introduction

Soybean seed quality can be affected by innumerous biotic and abiotic factors, including storage (Carvalho et al., 2014), which, under uncontrolled conditions, exposes the seeds to oscillations of temperature and relative humidity, besides the attack of pest insects and fungi, contributing to the reduction of quality (Ludwig et al., 2011). Thus, the chemical treatment of the seeds with fungicides, insecticides and the coating with polymers, for being efficient in the control of pathogens and insects (Pereira et al., 2010; Conceição et al., 2014), can contribute to maintaining their quality during the storage period, besides helping to control diseases in the initial period of crop establishment, favoring the emergence and development of seedlings (Balardin et al., 2011).

Aiming to protect the seeds during storage, some companies have adopted the technique of industrial seed treatment (IST). In this process, the seeds are treated immediately after processing and, later, bagged and stored until the moment of sowing. However, it is important that, besides the efficiency of the products (for the control of pathogens, insects and supply of nutrients), they do not affect seed physiological quality negatively. Hence, Brzezinski et al. (2015), evaluating the effect of anticipated treatment of soybean seeds with different combinations of chemical products, concluded that they damage crop performance. Likewise, Dan et al. (2013) observed reduction in the emergence of seedlings derived from soybean seeds treated with the insecticide thiamethoxam after three months of storage.

Given the above, the objective of this study was to evaluate the physiological and sanitary quality of soybean seeds treated with fungicide, insecticide, micronutrients and polymer, during eight months of storage.

Material and Methods

The study was carried out at the Laboratory of Didactics and Research on Seeds, in the experimental area of the Plant Science Department of the Federal University of Santa Maria and at the Seed Processing Unit (SPU) of the company Imex Sul Insumos Agrícolas Ltda., with headquarters in the municipality of Santa Maria-RS, where the seeds were stored under uncontrolled conditions. The environmental conditions during the storage period are described in Figure 1.

The experiment used two lots of seeds for each of the three soybean cultivars, NA 4823RG, BMX TurboRR and Fundacep 62RR, in a total of six lots. Initial water content was determined in the seeds, which was on average 12.4 and, then, they were subjected to the following treatments: 1) Control – without treatment; 2) Fungicide + insecticide + micronutrients; 3) Fungicide + insecticide + micronutrients + polymer; 4) Fungicide + micronutrient; and 5) Insecticide + micronutrient. The volume of mixture used in the treatments was 600 mL 100 kg^{-1} of seed, which was replenished with distilled water when necessary.

The utilized products were: fungicide Carbendazim 30 g a.i.·100 kg^{-1} of seed + Thiram 70 g a.i.. 100 kg^{-1} of seed (Derosal Plus®) at the dose of 200 mL·100 kg^{-1} of seed; insecticide Imidacloprid 90 g a.i.. 100 kg^{-1} of seed + Thiodicarb 30 g a.i.. 100 kg^{-1} of seed (Cropstar), at the dose 300 mL.100 kg^{-1} of seed; the treatment with micronutrients used fertilizer of liquid formulation with Mo – 12%, Co – 1% and B – 1% (GRAP 180 JE), at the dose of 100 mL.100kg^{-1} of seed; and the polymer Laborsan of liquid formulation, at the dose 100 mL.100 kg^{-1} of seed. The seeds were treated in plastic bags with capacity for 3 L, using 500 g of seeds per bag. After treatment, the initial quality of the lots was evaluated. The seeds were placed in the SPU in raffia sacks and their quality was monitored after 2, 4, 6 and 8 months of storage.

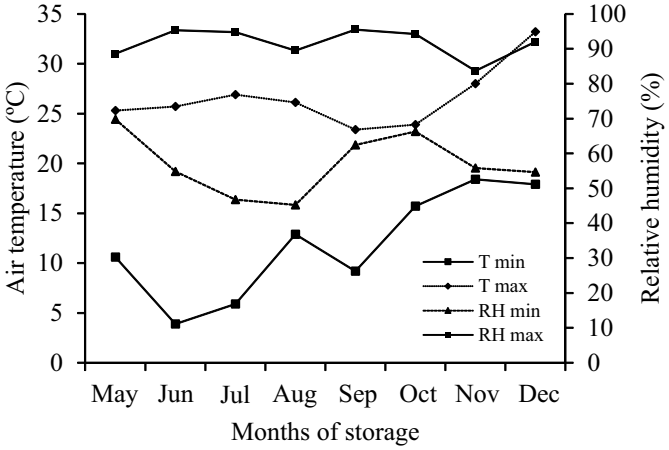

Figure 1. Monthly maximum and minimum values of temperature and relative humidity in 2012 along the storage period

For the evaluation of physiological and sanitary quality, the seeds were subjected to the following tests and determinations: Water content: determined by the method of the oven at 105 ºC ± 3 for 24 h, using four samples for each lot, according to the Rules for Seed Analysis-RSA (Brasil, 2009); b) Germination: four samples of 100 seeds were used for each lot of the cultivars, sown in rolls of paper towel moistened to 2.5 times the weight of the dry paper and maintained in germinator regulated at 25 ºC. The evaluations were performed at 4 and 8 days, after the beginning of the test, according to RSA (Brasil, 2009), and the results were expressed in percentage of normal seedlings; c) Accelerated aging test: Gerbox-type plastic boxes received 40 mL of water and a galvanized wire screen, on which the seeds were distributed. The boxes were closed with masking tape and taken to an incubating oven for a period of 48 h, at temperature of 41 ºC, as described in Marcos Filho (1999). Then, the seeds were subjected to the germination test using four samples of 100 seeds per lot, following the same methodology previously described (Brasil, 2009); d) Seedling length: evaluated in four samples of 20 seeds from each lot of the cultivars. The seeds were placed to germinate at temperature of 25 ºC, using moistened roll of paper as substrate. The evaluations were performed at 7 days after sowing by measuring the length (shoots, roots and total) of 15 normal seedlings per sample, which were randomly removed. The results were expressed in mean length per seedling in centimeters; e) Seedling dry matter: the seedlings used for length evaluation were separated using a scalpel in order to remove the cotyledons, placed in Kraft paper bags and dried in a forced-air oven at 80 ºC, for 24 h. After this period, the samples were removed from the oven, placed in a desiccator and then weighed, determining the total dry

matter of the seedlings, with results expressed in mg seedling^{-1} (Nakagawa, 1999); f) Sanity test: performed through the filter paper test ("blotter test"), using four samples of 100 seeds of each lot of the cultivars, which were individually arranged on a layer of moistened filter paper and placed in Gerbox-type boxes (20 seeds per box). The samples were incubated at temperature of 20 ± 2 ºC with photoperiod of 12 h, for 8 days. Then, the seeds were individually examined using magnifying lens or microscope, to detect the occurrence or not of fungal growth. The results were expressed in percentage of infected seeds (Henning, 2004); g) Tetrazolium test: performed according to the methodology described by França Neto et al. (1998), using 200 seeds per treatment, distributed in four samples of 50 seeds of each lot of the cultivars, pre-conditioned in moistened paper and incubated for 16 h at 25 ºC. Subsequently, the seeds were placed in glass containers, immersed in solution of tetrazolium salt (0.075%) and maintained in a dark chamber at 40 ºC for 3 h. After this period, the tetrazolium solution was removed and the seeds were washed with water. The seeds were individually analyzed through a longitudinal cut along the embryonic axis, and the viability, vigor and percentage of damages by moisture, mechanical damages and damages by bedbugs were determined based on the location, size and type of damage.

The treatments were arranged in a 3 x 5 x 5 factorial scheme (cultivars x seed treatment x storage period) in split plot in time. The plots corresponded to the cultivars combined with the seed treatments and the subplot, in time, to the storage periods, adopting a completely randomized design with two replicates (two lots per cultivar) constituted by the mean of the four samples of each lot of the test, performed in laboratory in the different storage periods. The variables that showed significance by F test (Anova) were subjected to the Scott-Knott test for the comparison of seed treatments and the storage periods. When the interaction Seed treatment x Storage period was significant, a follow-up analysis was performed for the effect of the seed treatments in each storage period, and vice versa. When the interaction was not significant, the main effects of each factor were analyzed individually. For the sanity, the data were transformed to $\sqrt{y+0.5}$ and, for the variables water content and germination, the utilized transformation was according to the Box-Cox methodology (Box & Cox, 1964), with lambda values of -2.5 and 2.5, respectively, in order to meet the assumption of homogeneity of the experimental errors. The statistical analyses were performed at 0.01 probability level, using the computational program Sisvar® (Ferreira, 2008).

Results and Discussion

It is observed that, only for germination percentage, there was significant effect of the interaction between seed treatments and storage periods, while for the other variables there was a significant effect of the storage periods (Table 1). The water content showed difference lower than 1.0% and the values remained between 10.3 and 11.1% along the entire storage period (Table 1), evidencing that the treatments do not have effect on this factor. The seeds showed a safe water content for storage (lower than 12%) during the entire studied period. Similar results were observed by Pereira et al. (2011), who

Table 1. Water content (%), germination (%), accelerated aging (%), shoot length (cm), root length (cm), total length (cm) and seedling dry matter (g) in seeds of the soybean cultivars NA 4823RG, BMX Turbo RR and Fundacep 62RR subjected to five different chemical treatments of seeds in five storage periods

Seed treatments[1]	Storage period - Months					
	0	2	4	6	8	Mean
			Water content			
Control	11.1*	10.6	10.6	10.0	10.0	10.5
F + I + M	11.1	10.8	10.5	10.0	10.5	10.6
F + I + M + P	11.0	10.8	11.0	10.1	10.3	10.6
F + M	11.2	11.0	10.8	10.3	10.3	10.7
I + M	11.0	11.0	10.6	10.1	10.5	10.6
Mean	11.1 B	10.8 B	10.7 B	10.1 A	10.3 A	
CV (%)	0.04					
			Germination			
Control	92 a A	91 a A	85 a B	69 a C	55 b D	78
F + I + M	87 a A	91 a A	84 a A	74 a B	73 a B	82
F + I + M + P	87 a A	89 a A	86 a A	71 a B	79 a B	83
F + M	88 a A	93 a A	85 a B	77 a C	77 a C	84
I + M	88 a A	90 a A	80 a B	70 a C	47 b D	75
Mean	88	91	84	72	66	
CV (%)	13.19					
			Accelerated aging			
Control	73	70	79	53	37	62
F + I + M	73	74	76	64	44	66
F + I + M + P	73	74	75	57	39	64
F + M	76	78	66	66	49	67
I + M	70	75	73	57	37	62
Mean	74 A	74 A	73 A	60 B	41 C	
CV (%)	5.81					
			Shoot length			
Control	15.7	13.3	12.3	12.1	11.1	12.9
F + I + M	16.3	13.5	10.3	11.0	11.6	12.5
F + I + M + P	15.6	12.6	11.6	11.6	11.6	12.6
F + M	15.5	13.0	11.6	11.7	12.3	12.7
I + M	16.8	12.3	9.6	11.6	9.8	11.9
Mean	16.0 A	12.9 B	11.1 B	11.4 B	11.3 B	
CV (%)	13.95					
			Root length			
Control	17.0	15.3	16.1	17.5	16.0	16.4
F + I + M	18.3	17.5	14.3	17.1	16.5	16.7
F + I + M + P	17.8	17.3	14.8	18.5	16.3	16.9
F + M	17.6	17.1	16.0	17.6	16.3	16.9
I + M	19.1	16.6	14.6	18.6	15.0	16.8
Mean	18.0 A	16.8 A	15.2 B	17.9 A	16.0 B	
CV (%)	6.48					
			Total length			
Control	32.8	28.8	28.4	29.4	27.2	29.3
F + I + M	34.7	30.6	24.6	28.2	28.3	29.3
F + I + M + P	33.6	30.1	26.3	30.1	27.9	29.6
F + M	33.2	30.0	27.5	28.8	28.6	29.6
I + M	35.7	28.9	24.1	29.8	24.6	28.6
Mean	34.0 A	29.7 A	26.2 B	29.2 A	27.3 B	
CV (%)	10.04					
			Total dry matter			
Control	0.62	0.56	0.51	0.52	0.49	0.54
F + I + M	0.62	0.54	0.52	0.54	0.52	0.55
F + I + M + P	0.62	0.54	0.49	0.53	0.49	0.53
F + M	0.63	0.51	0.46	0.52	0.52	0.53
I + M	0.63	0.53	0.47	0.51	0.45	0.52
Mean	0.62 A	0.52 B	0.49 B	0.52 B	0.49 B	
CV (%)	7.18					

*Means followed by the same letter, lowercase in column, uppercase in row and inside each variable do not differ by the Scott-Knott test, $p < 0.01$; [1]Seed treatments: Control – Without chemical treatment; F – Fungicide (Carbendazim 30 g a.i. kg^{-1} + Thiram 70 g a.i. kg^{-1}), I: Insecticide (Imidacloprid 90 g a.i. kg^{-1} + Thiodicarb 30 g a.i. kg^{-1}), M - Micronutrient (fertilizer of liquid formulation with Mo - 12%, Co - 1% and B - 1%); and P – Polymer (liquid formulation); CV% - Coefficient of variation

also did not detect significant differences in the water content between soybean seeds treated and not treated with fungicides and polymer during storage.

In regard to seed germination, until the sixth month of storage, there was no difference between the control (without chemical treatment) and the other treatments (Table 1). However, from this period on (Table 1), the treatments with application of fungicides, associated or not with the polymer, showed superior performance compared with the control and the treatment with only insecticides. In addition, along the storage, the treatments without the use of fungicide showed sharper reductions for this variable, reaching values of 55 and 47%, respectively (Table 1). This occurs because, as described below, there was a higher incidence of fungi in this period. Thus, it can be noted that the seed treatment did not damage the physiological quality of the seeds during storage, besides controlling the proliferation of fungi, thus decreasing the speed of the deterioration process.

Similar results were observed by Pereira et al. (2010), who concluded that soybean seeds treated or not with thiram+thiabendazole and carbendazin+thiram associated with polymer coating showed differences in germination lower than 5% until the sixth month of storage, and the distinct treatments did not differ. Thus, it is evident the protecting effect of the fungicide on the potential of storage of soybean seeds.

Regardless of the utilized chemical treatment, the seeds showed germination percentage above the minimum standards required for marketing (80%) until the fourth month of storage. From this point on, there was a decrease in germination and vigor (Table 1). This accentuated speed in the process of seed deterioration can be explained by the wide fluctuations of relative air humidity and air temperature during the storage period (Figure 1) and by the high percentage of damages by moisture found in the lots (Table 2). Thus, despite the high values of initial germination, the potential of storage of the seeds was low, mainly because of the climatic conditions, which were not favorable to the production of seeds for the South region of Brazil in the 2011/12 season, especially for problems of drought, which occurred in the beginning of the crop cycle, and excess of rains in the harvest period (CONAB, 2012). These damages can increase in extension, thus reaching critical regions of the seeds, decreasing vigor and viability, as observed by Krohn & Malavasi (2004), who reported that the soybean seeds that remained treated for a period longer than four months showed inferior performance in comparison to those treated in the other periods. These authors also point out that the chemical treatment has no effect on factors such as mechanical damage, deterioration by moisture, attack of bedbugs and inadequate storage, which cause reduction in the physiological quality of the seeds.

In the evaluation of seed sanitary quality, there was significant effect of the interaction between seed treatments and storage periods for *Aspergillus* sp. and *Penicillium* sp., whereas for *Macrophomina* sp. only the effect of seed treatments was significant (Table 3). The observed incidence of fungi was low, except for the storage fungi *Aspergillus* sp. and *Penicillium* sp., which showed an increase during the studied period, reaching maximum values of 17.66 and 10%, respectively (Table 3). This occurs because these pathogens find favorable conditions for proliferation during the storage.

Table 2. Percentage of mechanical damage, damage by moisture and damage by bedbug for three soybean cultivars

Cultivar	Vigor	Viability	Mechanical damage	Damage by	
				moisture	bedbug
BMX Turbo RR	77	86	18	53	8
Fundacep 62 RR	81	89	16	52	2
NA 4823RG	81	92	16	68	2

Table 3. Percentage of infection for the sanity test in seeds of the soybean cultivars NA 4823RG, BMX Turbo RR and Fundacep 62 RR, subjected to five different chemical treatments of seeds in five storage periods

Seed treatments[1]	Storage period - Months					
	0	2	4	6	8	Mean
			Aspergillus sp.			
Control	1.00 b A*	7.33 b B	9.00 b B	13.16 b C	17.16 b D	9.53
F + I + M	0.16 a A	0.16 a A	0.15 a A	0.16 a A	0.00 a A	0.13
F + I + M + P	0.00 a A	0.16 a A	0.16 a A	0.00 a A	0.17 a A	0.10
F + M	0.16 a A	0.00 a A	0.00 a A	0.00 a A	0.16 a A	0.60
I + M	4.16 b A	8.16 b B	10.16 b B	11.33 a B	17.66 b C	10.30
Mean	1.10	3.16	3.90	4.93	7.03	
CV (%)	21.13					
			Cercospora kikuchii			
Control	4.66 c B	3.00 b A	3.00 b A	2.66 b A	3.83 b B	3.43
F + I + M	0.16 a A	0.00 a A	0.16 a A	0.33 a A	0.16 a A	0.16
F + I + M + P	0.00 a A	0.00 a A	0.16 a A	1.00 a B	0.16 a A	0.26
F + M	0.16 a A	0.16 a A	0.16 a A	0.16 a A	0.00 a A	0.13
I + M	2.50 b A	3.33 b A	3.16 b A	2.83 b A	4.83 b B	3.33
Mean	1.50	1.30	1.33	1.40	1.80	
CV (%)	35.06					
			Fusarium sp.			
Control	2.16	1.83	1.16	1.50	0.66	1.46
F + I + M	1.16	0.16	0.83	0.33	0.00	0.50
F + I + M + P	0.50	0.00	0.16	0.00	0.16	0.16
F + M	0.00	0.00	0.00	0.00	0.00	0.00
I + M	2.16	2.00	1.50	1.66	1.00	1.66
Mean	1.22	0.80	0.73	0.70	0.36	
CV (%)	33.63					
			Macrophomina sp.			
Control	1.66	1.33	1.00	0.33	0.50	0.86 b
F + I + M	0.00	0.00	0.00	0.00	0.00	0.00 a
F + I + M + P	0.00	0.16	0.16	0.50	0.16	0.20 a
F + M	0.00	0.00	0.16	0.16	0.16	0.10 a
I + M	1.16	1.06	0.66	0.50	0.66	0.83 b
Mean	0.46	0.53	0.40	0.30	0.30	
CV (%)	37.18					
			Phomopsis sp.			
Control	1.00	0.50	1.16	0.83	0.33	0.70
F + I + M	0.50	0.16	0.16	0.16	0.00	0.20
F + I + M + P	0.33	0.16	0.50	0.50	0.00	0.03
F + M	0.00	0.00	0.00	0.16	0.00	0.30
I + M	0.50	0.83	1.16	0.50	0.00	0.76
Mean	0.46	0.33	0.60	0.50	0.16	
CV (%)	24.26					
			Penicillium sp.			
Control	0.33 a A	4.33 b B	5.33 b B	9.16b C	10.00 b C	5.83
F + I + M	0.00 a A	0.00 a A	0.16 a A	0.16 a A	0.33 a A	0.13
F + I + M + P	0.00 a A	0.16 a A	0.16 a A	0.16 a A	0.16 a A	0.13
F + M	0.00 a A	0.00 a A	0.16 a A	0.33 a A	0.16 a A	0.13
I + M	0.32 a A	5.33 b B	5.66 b B	8.50 b C	9.16 b C	5.80
Mean	0.13	1.96	2.30	3.66	3.96	
CV (%)	26.25					

**Means followed by the same letter, lowercase in column, uppercase in row and inside each variable do not differ by the Scott-Knott test, $p < 0.01$; [1]Seed treatments: Control – Without chemical treatment; F – Fungicide (Carbendazim 30 g a.i. kg^{-1} + Thiram 70 g a.i. kg^{-1}), I: Insecticide (Imidacloprid 90 g a.i. kg^{-1} + Thiodicarb 30 g a.i. kg^{-1}), M - Micronutrient (fertilizer of liquid formulation with Mo - 12%, Co - 1% and B - 1%); and P – Polymer (liquid formulation); CV% - Coefficient of variation

Similar behavior was observed by Pereira et al. (2011), who worked with soybean seeds and concluded that the field fungi associated with the seeds decreased during the storage period, while storage fungi increased. In addition, it should be pointed out the importance of detecting phytopathogenic fungi of the crop still in the seeds, because they are able to cause reduction in germination and initial development of soybean seedlings, besides efficiently spreading diseases such as stem and pod canker, purple seed stain, and anthracnose and fusariosis caused by *Phomopsis* sp., *Cercospora kikuchii* and *Fusarium* spp. (Henning, 2004). On the other hand, *Aspergillus* sp. and *Penicillium* sp. are the main pathogens responsible for the deterioration of soybean seeds during the storage (Costa et al., 2013).

The highest incidence of *Aspergillus* sp., *Penicillium* sp. and *Cercospora kikuchii* was observed in the treatments in which fungicide was not used along the storage period (Table 3). This higher infestation in the seeds, especially of storage fungi, may have contributed to the reduction of germination and vigor observed in these treatments (Table 1).

The use of fungicides associated or not with insecticides and polymers in the control of fungi present in the seeds was efficient to reduce the rate of infestation of the seeds, regardless of the pathogen and degree of incidence, reaching values lower than 1% during the entire storage period. These results highlight the viability of using these products in combination, without affecting the performance of the fungicide in the control of fungi, guaranteeing safety to the producer at the moment of sowing with the use of the anticipated treatment of the seeds. Similar results were reported by Ludwig et al. (2011), who studied the behavior of the fungi *Rhizoctonia* sp., *Fusarium* sp., *Colletotrichum sp*., *Phomopsis* sp., *Alternaria* sp. and *Cercospora kikuchii* subjected to chemical treatment.

Conclusions

1. The treatment of soybean seeds with fungicide (Carbendazim + Thiram), insecticide (Imidacloprid + Thiodicarb), micronutrient and polymer did not damage the physiological quality of the seeds along eight months of storage.

2. The treatment of soybean seeds with fungicide (Carbendazim + Thiram) promoted the control of fungi associated with the seeds.

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