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Effect of the storage of soybean seeds treated with agrochemicals on the physiological quality and on the seedlings morphology

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ABSTRACT

Seed treatment is the most efficient technique for controlling pests and pathogens that can influence the initial development of the crop. However, storing treated seeds may damage the physiological quality. Thus, the objective of this study was to evaluate the influence of storage on the physiological quality of treated soybean seeds and on the morphology of soybean seedlings. The treatments were carried out with Fludioxonil, Imidacloprid and Anthranilic Diamide, in the dosage of 200, 150 and 100 mL of the commercial product 100 kg of seeds, respectively, from the cultivar BRSMG752S. The experimental designs used were randomized blocks (greenhouse) and completely randomized (laboratory), in a simple factorial 4x4scheme, with 4 sowing times (30, 20, 10 and 0 days after treatments) and 4 treatments (3 products and untreated seeds) with 4 repetitions. The treatment of soybean seeds with Anthranilic Diamide submitted to different storage periods was the one with the closest physiological quality to the treatment without application of agrochemicals. The soybean seeds treated with Imidacloprid showed a reduction in physiological quality with the storage time, reduction (in general) in the root length and presented a lower seedling length at 20 days of storage.

Keywords: *Glycine max*, fludioxonil, germination, seed quality, seed technology.

INTRODUCTION

The cultivation of soybean (*Glycine max* (L.) Merr.) has great importance in the national scenario, both for social and economic reasons. The attraction for this consumption of soybean is determined mainly by price, among others, and by fatty acid composition (Águila, 2018). The economic reasons causes more techniques to appear in order to increase the productivity and profitability of the crop. One of the most used and important practices today is the seed treatment (ST), which aims to protect the seed, considered the most important input of the crop (Gomes, Barrozo, Souza, Sader, & Silva, 2009), the improvement of performance, the uniformity of the initial stand, the vegetative development and productivity of the crop (Camara, 2015). In addition, to the largest Brazilian soybean growing region, the Midwest, the main growers' demand is for early and productive cultivars that make second season cultivation possible (Bezerra et al, 2017).

Seed treatment has a relatively low cost and the environmental impact is reduced due to the small area that receives the product compared to the application of agrochemicals in the aerial part of the plant (Cunha et al., 2015). Although ST is advisable to protect seeds from attack by insect pests and microorganisms, Brazil does not have standards that make it mandatory (Peske, Lucca-Filho, & Barros, 2006).

The ST is divided into two possibilities, industrial treatment (TIS) and conventional seed treatment. The conventional treatment is that which can be carried out within the property where the crop will be installed in the future, using a rotating drum, treatment machine or concrete mixer. Despite being low cost and providing significant results to farmers, today, the conventional treatment is being replaced by TIS, which is gaining space by reducing labor and allowing the producer to receive the seeds ready for sowing. In addition, TIS is feasible for ensuring that the amount of product added to the seed is appropriate with the recommendation of the manufacturer.

Although the ST is undoubtedly beneficial for obtaining uniformity at the beginning of ploughing, there is much discussion about how the storage of treated seeds should be carried out and whether it influences the physiological quality. Zorato and Henning (2001) found that there is no influence on the quality of soya beans from anticipated fungicide treatments (thiabendazole+thiram; carbendazin+thiram and carboxin+thiram). However, they emphasized that the adoption of this practice requires precaution, since the treated and non-commercialized lots cannot be stored, thus causing damage, because treated seeds are unfit for consumption.

For Fessel, Mendonça, Carvalho and Vieira (2003) the chemical treatments applied tend to increase dosages, generating latent effects, unfavorable to the performance of the seeds, intensified with the extension of the storage period. Dan, Dan, Barroso and Braccini (2010) concluded that the application of the insecticides carbofuran and acephate is harmful to the physiological quality of the soybean cultivar (M-SOY 6101) for a storage period of up to 45 days. Bortoletto, Macedo, Oliveira and Souza (2017), when evaluating soybean seeds with pesticides in different storage times, observed that the time of stored and treated seeds should not be extended for more than 90 days. Silva, Camargo, Souza, Teixeira and Kikuti (2019) reported that the effects of chemicals and physiological quality of soybeans are reduced during storage.

Thus, the objective was to evaluate the influence of the storage period of soybean seeds treated with agrochemicals on the physiological quality seeds and on the seedlings morphology of soybean.

MATERIALS AND METHODS

The experiments were conducted in a greenhouse at the Biostatistics Laboratory of the Institute of Exact and Technological Sciences and at the Plant Production Physiology and Metabolism Laboratory of the Federal University of Viçosa, UFV - Rio Paranaíba *Campus*, in the city of Rio Paranaíba, located in the state of Minas Gerais. The soybean cultivar that was used was the BRSMG752S. Four treatments were carried out:

Treatment 1 - Seeds treated with the fungicide Fludioxonil (200mL of the commercial product per 100kg of seeds);

Treatment 2 - Seeds treated with the insecticide Imidacloprid (150mL of the commercial product per 100kg of seeds);

Treatment 3 - Seeds treated with the insecticide Anthranilic Diamide (100mL of the commercial product per 100kg of seeds); and

Treatment 4 - Untreated seeds. The treatments were performed in four different seasons which were 30, 20, 10 and 0 days before sowing.

The treated seeds were stored in paper bags and packed under uncontrolled conditions in order to simulate a possible storage reality. The greenhouse experiment was carried out in a tray containing a substrate composed of sterilized sand, according to the sterilization process proposed by Ghini (2004), where they were evaluated.

Germination test and first germination count: performed by counting normal seedlings at 5 and 8 days after sowing according to the Seed Analysis Rule (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009). The first germination count was evaluated along with the percentage of normal seedlings obtained on the 5th day after the start of the germination test (MAPA, 2009). The data from both tests were expressed as percentage.

Speed of germination (SG): the number of emergent seedlings was recorded until the emergency stabilization occurred, to calculate the index proposed by Maguire (1962) was used:

$$SG\colon \frac{E_1}{N_1}+\frac{E_2}{N_2}+\cdots+\frac{E_n}{N_n}$$

Being:

E_n: Number of normal plants computed on the umpteenth count

N_n: Number of days after sowing on the umpteenth count

Seedling and root size: 25 normal seedlings were randomly selected per plot of the experiment between sand and the measurement was performed three days after emergency stabilization, using a millimeter ruler.

The experiment in the greenhouse was carried out according to the design in randomized blocks in the simple factorial scheme AxB, being factor A composed of 4 sowing seasons and factor B by the 4 seed treatments (3 agrochemicals and no treatment), with 4 repetitions of 100 seeds per plot. In the laboratory experiment, the germination test was evaluated at 5 and 8 days after the experiment was assembled. It was conducted according to the experimental design and was entirely randomized in a 4x4 simple factorial scheme, with 4 repetitions of 50 seeds per plot. In the Biostatistics Laboratory, the variance analysis was carried out, paying attention to the analysis of the residues regarding normality using the Lilliefors Test and variance homogeneity according to Bartlett. Subsequently, the Tukey Test was performed to compare the means. In the statistical analysis, 5% significance was considered, using Program R (R Core Team, 2019).

RESULTS AND DISCUSSION

For the first count results of the sand bed germination test, the transformation ($ARCSEN(X/100)^{0.5}$) was necessary in order to meet the normality and homogeneity of variance. Through the analysis of variance, significant effects were observed for the interaction, treatments and planting times.

Fludioxonil, Anthranilic Diamide and the untreated seeds showed no difference for the first germination count between the four storage seasons. While Imidacloprid in the 20 and 30-day storage provided lower averages (Table 1).

Table 1. First germination count (in %) of soybean seeds evaluated in sand bed according to 4 treatments and 4 storage seasons

Treatments	Storage Periods[1]				Averages
	30	20	10	0	
Fludioxonil	29.7bA	29.0bA	53.5bcA	53.0bcA	41.3
Imidacloprid	5.0cB	0.0cB	48.7cA	37.5cA	22.8
Anthranilic Diamide	76.7aA	78.5aA	78.0abA	78.7aA	77.9
Untreated seeds	79.7aA	76.5aA	78.2aA	76.5abA	77.7
Averages	47.7	46.0	64.6	61.4	
C.V.(%)			17.1		

[1]Averages followed by the same lower case letter in the columns and upper case letter in the row do not differ by the Tukey test at 5% significance.

For the comparison of agrochemicals within each storage season, the insecticide Imidacloprid obtained the lowest first germination count averages in the 30 and 20 days storage seasons, while the Anthranilic Diamide and the untreated seeds presented the highest averages and did not differ from each other. At 10 and 0 days of storage, lower averages were observed for Imidacloprid, however, they did not differ from Fludioxonil (Table 1).

The first count of germination is considered a simple vigor test, with the assumption that the most vigorous seeds germinate first. In this perspective, as storage time was prolonged, there was a decrease in seed vigor, indicating the effect of agrochemicals on the quality of soybeans, especially Imidacloprid.

In the sand bed germination test there was no significance in the interaction of the factors. However, there was a difference when analyzing the isolated factors. The treatments with Fludioxonil and Imidaclopride did not statistically differentiate from each other, but they presented lower averages than the other treatments. The treatments with Anthranilic Diamide and the untreated seeds did not differ, indicating that this agrochemical did not interfere with the germination of soybean seeds. During the periods, seeds treated 0 days before sowing had the highest germination average, not differing from the 30 and 10-day seasons, the treatment season 20 days before sowing had the lowest mean, not differing from the 30 and 10-day seasons (Table 2).

For the speed of germination the results were similar to those presented in the first germination count, where Fludioxonil, Anthranil Diamide and the untreated seeds did not present differences in the evaluated storage times, and Imidacloprid expressed less vigor in the times of 30 and 20 days of storage (Table 3).

When analyzing 30, 20 and 0 days of storage, the treatments with Fludioxonil and Imidacloprid showed the lowest values of speed of germination. However, at 20 days the averages differed among themselves; for the 10 days of storage, Fludioxonil showed lower speed of germination and did not differ from Imidacloprid (Table 3). Then, it is evident that more vigorous seeds with better physiological performance, produce seedlings with higher rates of development and mass gain, due to the higher capacity of tissue transformation (Amaro et al., 2015).

Table 2. Germination (in %) of soybean seeds in the sand bed according to 4 treatments and 4 storage periods[1]

Treatments	**Germination (%)**	**Period (days)**	**Germination (%)**
Fludioxonil	74.6b	30	78.4ab
Imidacloprid	76.6b	20	76.6b
Anthranilic Diamide	81.6a	10	80.2ab
Untreated seeds	82.9a	0	80.7a
Averages	78.9	Average	79.0
C.V.(%)	5.2	C.V.(%)	5.2

[1]Averages followed by the same lower case letter in the columns do not differ by the Tukey test at 5% significance.

Table 3. Speed of germination in the sand bed according to 4 treatments and 4 storage periods

Treatments	**Storage Days[1]**				**Averages**
	30	20	10	0	
Fludioxonil	13.1bA	13.1bA	14.5bA	14.7bA	13.9
Imidacloprid	12.5bB	11.3cB	15.1abA	14.7bA	13.4
Anthranilic Diamide	17.1aA	16.8aA	16.9aA	16.9aA	16.9
Untreated seeds	17.2aA	16.7aA	16.9aA	16.9aA	16.9
Averages	14.9	14.5	15.9	15.8	
C.V.(%)		6.3			

[1]Averages followed by the same lower case letter in the columns and upper case letter in the row do not differ by the Tukey test at 5% significance.

For the length of seedlings, there was no significant difference in the periods within each agrochemical (Table 4). When analyzing products within periods, there was no difference in the agrochemicals at times of 30, 10 and 0 days of storage. At 20 days of storage, Imidacloprid presented a lower average seedling length compared to the other treatments, but it did not differ from the Diamide Anthranil treatment (Table 4).

The interaction of factors (Treatments *vs.* Storage Seasons) was not significant for root length. When the factors were analyzed in isolation, it was possible to observe that seeds treated with Imidacloprid had smaller roots and there was no difference when compared to Anthranilic Diamide (Table 5).

Based on the results obtained in the present greenhouse experiment, it was observed that seeds treated with Anthranilic Diamide can be stored for up to 30 days, which showed the same performance in germination and vigor as the untreated seeds. For the use of Imidaclloprido for seed treatment, it is recommended to "treat and plant", i.e. avoid storage, as this may cause reductions in germination rates and vigor. According to Dan et al. (2010), seeds treated with Imidacloprid at the end of the 45 day storage period showed germinated rate above 80%, however, they observed a reduction in the speed of germination as the storage period increased.

For the first germination count test on germitest® paper, there was significance in the interaction of the factors. When analyzing treatments within periods, seeds treated with Fludioxonil and the untreated seeds showed the highest averages, except for 20 days, when the agrochemical was used. The treatment with Anthranilic Diamide expressed lower averages at 30 and 20 days after storage, while Imidacloprid showed high vigor only on day 0. For treatments with agrochemicals within each period, there were differences, i.e., Imidacloprid was the product that provided less vigor to soybeans, regardless of the period of storage evaluated, although at 20 and 0 days after storage, Imidacloprid was no different from Anthranilic Diamide (Table 6).

Table 4. Length (in cm) of soybean seedlings in the sand bed according to 4 treatments and 4 storage periods

Treatments	Storage Days[1]				Averages
	30	20	10	0	
Fludioxonil	16.5aA	18.2aA	17.6aA	16.9aA	17.3
Imidacloprid	16.2aA	15.4bA	17.3aA	16.5aA	16.4
Anthranilic Diamide	18.3aA	16.4abA	16.4aA	16.8aA	16.9
Untreated seeds	16.9aA	17.6aA	17.8aA	17.4aA	17.4
Averages	16.9	16.9	17.3	16.9	
C.V.$_{(\%)}$	6.6				

[1]Averages followed by the same lower case letter in the columns and upper case letter in the row do not differ by the Tukey test at 5% significance.

Table 5. Length of roots (in cm) of soybean seedlings treated at different times. in sand bed 3 days after emergency stabilization. depending on 4 treatments and 4 storage times[1]

Treatments	Length of roots	Period (days)	Length of roots
Fludioxonil	11.9a	30	11.3a
Imidacloprid	10.7b	20	11.3a
Anthranilic Diamide	11.4ab	10	11.6a
Untreated seeds	11.6a	0	11.4a
Average	11.2	Average	11.4
C.V.$_{(\%)}$	7.5	C.V.$_{(\%)}$	7.5

[1]Averages followed by the same lower case letter in the columns do not differ by the Tukey test at 5% significance.

In the germination test, performed on germitest® paper, Fludioxonil reduced the germination of soybean seeds at 20 days of storage. For Imidaclloprido and Anthranilic Diamide, germination was lower at 30 and 20 days, but for Imidacloprid there was no difference between 30 and 10 days after storage; the untreated seeds did not show any distinction for the different evaluated periods (Table 7).

The germination averages did not show differences between the treatments in the day 0 period, which indicates the choice of any treatment or the absence of it, for the germination process. At 30 days after storage, Fludioxonil and the untreated seeds expressed greater germination; at 20 days all agrochemicals provided germination reduction, with lower results for Imidacloprid, it was observed at 10 days, except for Fludioxonil which was similar to the untreated seeds (Table 7).

In the laboratory experimente, a better performance of seeds treated with fungicide (Fludioxonil) was observed in relation to seeds treated with insecticides, which may have occurred due to the proliferation of fungi in the germinator, which maintained high humidity and temperature with little oscillation, near 25ºC. However, further research should be conducted to substantiate this claim, as untreated seeds showed similar or even superior behavior (for 20 days of storage) compared to Fludioxonil treated seeds.

Table 6. First germination count (in %) of soybean seeds. sown on germitest® paper. according to 4 treatments and 4 storage seasons.

Treatments	**Storage Days**[1]				**Averages**
	30	20	10	0	
Fludioxonil	59.0aA	43.0bB	59.5abA	69.5aA	57.6
Imidacloprid	19.5cC	21.0cC	32.5cB	53.0bA	31.5
Anthranilic Diamide	30.0bB	28.0cB	54.5bA	61.0abA	43.4
Untreated seeds	62.5aA	66.5aA	66.5aA	64.0aA	64.9
Averages	42.7	39.6	53.2	61.7	
C.V.$_{(\%)}$		11.1			

[1]Averages followed by the same lower case letter in the columns and upper case letter in the row do not differ by the Tukey test at 5% significance.

Table 7. Germination (in %) of soybean seeds. sown on germitest® paper. according to 4 treatments and 4 storage seasons

Treatments	**Storage Days**[1]				**Average**
	30	20	10	0	
Fludioxonil	76.0aA	61.5bB	75.5abA	84.5aA	74.4
Imidacloprid	40.5bBC	31.5dC	48.0cB	73.0aA	48.2
Anthranilic Diamide	44.5bB	48.0cB	68.0bA	74.0aA	58.6
Untreated seeds	74.5aA	80.5aA	82.5aA	76.5aA	78.5
Average	58.9	55.4	68.5	77.0	
C.V.$_{(\%)}$		9.8			

[1]Averages followed by the same lower case letter in the columns and upper case letter in the row do not differ by the Tukey test at 5% significance.

For Amaro et al. (2015), tests of first germination count, speed of germination and seedling length show sensitivity to identify different levels of vigor in bean seeds. These, together with the germination test, are also indicated to elucidate the best treatment of soybean seeds with agrochemicals and their storage times, to ensure physiological quality.

CONCLUSIONS

The treatment of soybean seeds with Anthranilic Diamide submitted to different storage periods was the one with the closest physiological quality to the treatment without application of agrochemicals.

Soybean seeds treated with Imidacloprid showed a reduction in physiological quality with the storage time, reduction (in general) in the root length and presented a lower seedling length at 20 days of storage.

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