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Physiological quality of colza seeds (*Brassica napus* L.) after coating and seed treatment during storage

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Seed coating is a technique widely used by growers to increase, standardize and improve seed germination conditions. Despite this, there is lack of information about the physiological quality of coated seeds during storage. The objective of this study is to evaluate the physical and physiological quality of colza (*Brassica napus* L.) seeds coated with bentonite and treated with fungicide and black pepper plant extract within 120 days of storage. Colza seeds were coated with bentonite as a filler material and polyvinyl acetate (PVA) glue as cementing material. For treatment of the seeds a fungicide (Carboxin + Thiram) and black pepper (*Piper nigrum* L.) plant extract were added together with the cementing material. Seeds with no coating or treatment were used as controls. After coating, the seeds were stored for 120 days, and the first germination counting, germination, third counting, shoot dry matter and water content were evaluated every 30 days. Germination and vigor of canola seeds coated and treated with fungicide or black pepper plant extract decreased significantly throughout the storage period. At 120 days of storage, the germination of the seeds coated with bentonite + glue and bentonite + plant extract was at an average of 33.6% in the third counting.

Key words: *Brassica napus* L, water content, seed coating, bentonite.

INTRODUCTION

Colza seeds (*Brassica napus* L.) are the third most-produced oilseeds in the world. It is an annual herbaceous plant belonging to the Brassicaceae family, which produces high quality oil-rich grains. This culture is responsible for 15% of the world's edible vegetable oil production, although it is also used in the production of biodiesel and animal feed (Tomm, 2007).

The use of machines in agriculture is of known importance. They are responsible for the expansion of areas of cultivation and productivity. According to Beltrão

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and Vieira (2001) one of the obstacles in mechanized sowing is the seed with small size, irregular shape and light. Colza seeds, according to Angelotti-Mendonça et al. (2016) have such characteristics. Seeds no longer represent only a propagule of a crop; they also carry a new way of managing agricultural technology. The value added to the seeds, methods and technologies of production of seed coating are the main requirements of an increasingly competitive market. For this, seeds with high germination/emergence uniformity and production of seedlings with high growth potential are required (Baudet and Peres, 2004).

According to Lopes and Nascimento (2012) coating/pelleting is a technology that is based on the sedimentation of dry inert materials with fine granulometry on the surface of the seeds with the aid of a cementing material. According to Nascimento et al. (2009), this technology allows the uniformization of the form, increase the size and weight of the seeds. Thus, sowing is facilitated, when it is done manually or through machines (Nascimento et al., 2009). Another important practice used during the coating process is the application of chemical products for the treatment of seeds, aiming the control and/or protection against insects, microorganisms and rodents (Sampaio and Sampaio, 1994). The process consists basically of applying successive layers of an inert solid material to the seeds in constant movement within a concrete mixer, alternating the application of the filler material with the spraying of a water-soluble binder (Silva, 1997; Silva and Nakagawa, 1998).

Although the technique has been developed for several years, information on the composition of the materials used and the preparation of the coatings are not well known, since this technique remains inaccessible to the seed and seed conditioning companies (Silva et al., 2002). Even with potential for utilization, there is lack of information available on seed coatings, encrustation or pelleting, especially regarding coating composition and seed performance during storage (Oliveira et al., 2003).

Based on that, the study of materials used for the seed coating, in association or not with products for treatment, whether chemical or natural, is of fundamental importance, supplying information in this area of research, especially when dealing with the storage of coated seeds. The objective of this study is to evaluate the physical and physiological quality of colza (*B. napus* L.) seeds during storage, coated with bentonite and treated with fungicide or plant extract.

MATERIALS AND METHODS

Locale of the experiment

The experiment was carried out at the Laboratório de Armazenamento e Processamento de Produtos Agrícolas, of the Universidade Federal de Campina Grande, Campus of Campina Grande, Paraíba, Brazil.

Plant extract preparation

Fruits of Black Pepper (*Piper nigrum* L.) were purchased at the central fair of the city of Campina Grande, Paraíba, Brazil. The aqueous extract was obtained from the fruits powder, which were weighed, moistened with distilled water, and placed for maceration for 72 h, at room temperature of 24.0 ± 4.0°C, in the absence of light and with daily agitation for five minutes. The amount of powder used corresponded to 20% of the volume of water used. The final solution was filtered on filter paper; the extract was stored in an amber glass container with of 0.5 L volume (Almeida et al., 2004).

Seed coating and storage of the seeds

The colza seeds (*B. napus* L.) were submitted to the coating process that occurred by alternating application between cementing material and filler material. This process was repeated until a complete utilization of the materials. These seeds were coated with bentonite and treated with fungicide (Carboxin + Thiram) and aqueous black pepper plant extract *(P. nigrum* L.) (the volume of vegetal extract and fungicide corresponded to 10% of the volume of cementing material). After 24 h of coating, the seeds were stored under ambient conditions of temperature (24.02 ± 5.85°C) and relative humidity (82.08 ± 5.85%) in acrylic containers (thickness 1.5 mm), with capacity for 2000 seeds (50 ml), for 120 days. The germination tests and vigor were performed every 30 days. Seeds with no coating or treatment were used as controls.

Germination test

The germination test was performed with four sub-samples of 50 seeds, sowing them in plastic trays with vermiculite, moistened with distilled water corresponding to 60% of the retention capacity of the substrate. These were maintained under ambient conditions of temperature, relative humidity and photoperiod. Germination was evaluated on the seventh day after the start of the test and the first counting was performed on the fourth day after sowing (Brasil, 2009). A third germination counting was also performed at 14 days after sowing to evaluate whether there was a decrease or delay of germination.

Shoot dry matter

To determine the shoot dry matter, the seedlings were cut at the substrate level and placed in *kraft* paper bags. The bags were placed in a lab stove with forced air circulation for drying, at a temperature of 65 °C until a constant weight was reached. After that, the plant material was weighed in a precision digital scale and the data expressed in milligrams (mg).

Water content

The water content was determined by the standard lab stove method at 105 ± 2°C, where four sub-samples of 2.0 g of seeds were placed in metal containers previously dried in a greenhouse and weighed. After 24 h the containers with the samples were placed in a desiccator for 20 min, until they reached room temperature and weighed, obtaining the final weight (containers plus the dry sample). The results were expressed as percentage of weight in wet basis according to the equation contained in the Rules for Seed Analysis (Brasil, 2009).

Experimental design and statistical analysis

The experiment was arranged in a completely randomized design and arranged in a 4 x 5 factorial scheme (coating materials x storage periods); each treatment had four replications. The data were submitted to Analysis of Variance ($P \leq 0.05$). For the quantitative factor (storage periods); the data were submitted to regression in the Variance Analysis to determine the models for each material. For the qualitative factor (coating materials) the means, when necessary, were compared by the Scott-Knott test ($P \leq 0.05$). In addition, the germination data were corrected, considering the control in time 0 days as 100%. For all statistical analyses the software Assistat, version 7.6, was used.

RESULTS

Table 1 shows the mean squares values for water content (WC), first germination counting (FGC), germination (G), third germination counting (TGC) and shoot dry matter (SDM). The effect of interaction between the factors (coating materials × storage periods), except for FGC, was observed for all variables under study, where a highly significant effect was observed for the isolated factors. The observed interactive effects reveal statistical differences between factor treatments. When comparing the coating materials within each storage period, it was observed that for 0 and 30 days of storage the highest water content was verified, when the seeds were coated with bentonite + fungicide. On the other hand, the lowest water contents, for these same periods of storage, were verified in the control. When the seeds were coated with bentonite + glue and bentonite + plant extract, the water contents were intermediate to the other materials (Table 2).

At 60, 90 and 120 days of storage it is observed that there was no statistical difference between the coating materials for water content. However, the water content has statistically different values when the variants with coating materials and the control are compared (Table 2).

Figure 1 shows the water content of seeds coated with bentonite and treated with fungicide or black pepper extract during 120 days of storage. According to the regression analysis of variance, for bentonite + glue and bentonite + fungicide the analysis did not reveal any significant model. For the other combinations, the only significant models were of second degree; however, they presented values of R^2 very low (0.19 and 0.50), being chosen graphically to present the behavior of the data and the means. In general, no significant variations were observed in the water content of the coated seeds or not during storage.

No statistical difference was observed for the coating materials within each storage period for the first germination counting. However, a statistical difference was found between the means of the factor "coating materials", regardless of storage periods. There was a higher germination for the control, differing statistically from the germination of seeds that were coated with bentonite + PVA glue and bentonite + plant extract. The seeds coated with bentonite + fungicide had the lowest germination compared to the others treatments studied, differing statistically from the other coating materials and the control (Table 3). When comparing the coating materials within each storage period, it was observed that for the periods 0, 30, 60 and 90 days, the lowest germinations were verified for the seeds coated with bentonite + fungicide. On the other hand, the highest germinations, for these same periods of storage, were verified in the control. The germinations observed in seeds coated with bentonite + PVA glue and bentonite + plant extract, presented an intermediate behavior, differing statistically among them, in all storage periods mentioned above. At 120 days of storage, the highest germination was verified in the control, differing statistically from the germination of seeds coated with bentonite + PVA glue and bentonite + plant extract, which were statistically similar to each other, and different from the germination of seeds coated with with bentonite + fungicide, with no germination (Table 4). The models of highest degree that best fit the germination data of coated seeds or not according to storage periods were first degree for bentonite + fungicide and bentonite + plant extract, being significant at 1%, and of second degree for bentonite + PVA glue, significant at 5%. For the control, the analysis revealed no significant model. The models presented coefficients of determination ranging from 0.9275 to 0.9831, representing the experimental data satisfactorily.

It can be observed that in the control, the germination had low influence by the storage period. The germination of the seeds coated with bentonite + PVA glue practically remained until 60 days of storage, however, after this period there was a decrease of germination as the storage time increased. The seeds coated with bentonite + fungicide and bentonite + plant extract had their germination decreased after 30 days of storage, continuing until the end of the storage period (Figure 2).

The means of the third counting of germination of the seeds coated with bentonite and treated with fungicide or black pepper extract are presented in Table 5. Comparing the coating materials within each storage period it can be verified for the time 0 (zero) that the greatest germination was found in the control, differing statistically from the germination percentage observed in coated seeds, which did not differ statistically among them. At 30 days of storage, the highest germination was verified in the control. On the other hand, the lower germinations, in the third counting, were observed for the seeds coated with bentonite + fungicide and bentonite + plant extract. Seeds that were coated with bentonite + PVA glue exhibited intermediate performance, differing statistically from the other coating materials and the control (Table 5). At the periods of 60 and 90 days of storage it could be verified that all the coating materials were statistically different from each other. The lowest germinations were observed in seeds coated with bentonite + fungicide. Differently, the highest germinations were observed in the control. The other treatments had intermediate values, differing statistically

Table 1. Mean squares for water content (WC), first germination counting (FGC), germination (G), third germination counting (TGC) and shoot dry matter (SDM) of colza seedlings coated with different filler materials (FM) during the storage period (SP).

SV	DF	Average square				
		WC	FGC	G	TGC	SDM
FM	3	32.339**	9021.916**	12352.983**	7708.850**	49741.666**
SP	4	0.007*	504.325**	31.782**	2010.550**	42.861**
FM x SP	12	0.005**	1.568ns	3.059**	300.683**	3.729**
Error	60					

**, *, [ns] Significant at 1, 5% and not significant, respectively.

Table 2. Water content (%) of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

Coating material	Storage period (days)				
	0	30	60	90	120
T	5.07±0.10[c]	5.12±0.07[c]	5.06±0.08[b]	5.03±0.16[b]	5.07±0.07[b]
B + G	7.40±0.03[b]	7.44±0.07[b]	7.48±0.12[a]	7.44±0.04[a]	7.45±0.24[a]
B + F	7.84±0.19[a]	7.91±0.16[a]	7.86±0.17[a]	7.83±0.12[a]	7.78±0.12[a]
B + PE	7.43±0.17[b]	7.46±0.12[b]	7.50±0.11[a]	7.44±0.09[a]	7.53±0.13[a]

* Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 4.25. (T) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

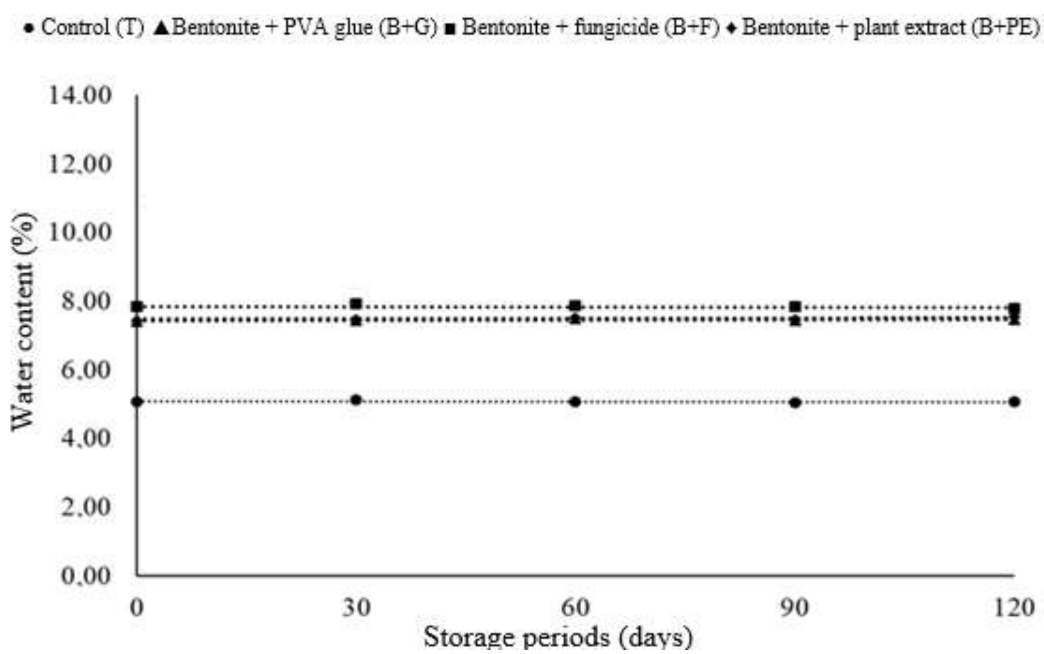

Figure 1. Water content of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage, compared to control.

from each other and the other treatments (Table 5). At 120 days of storage, the highest germination occurred in the control, differing statistically from the germinations observed in seeds coated with bentonite + PVA glue and bentonite + plant extract, which were statistically equal to each other and different from the germination verified of seeds coated with bentonite + fungicide, which was the coating material that provided lowest germination among

Table 3. First germination counting (%) of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days storage.

Filler material	Storage period (days)					Average
	0	30	60	90	120	
T	57.5 ± 0.83 -100	58.0 ± 1.22 -100	57.0 ± 0.50 -99.1	55.0 ± 2.05 -95.7	46.5 ± 0.83 -80.9	54.80[a] -95.3
B + G	34.5 ± 2.49 (60.0)	30.5 ± 1.64 -53	26.0 ± 1.58 -45.2	18.0 ± 1.87 -31.3	16.0 ± 2.74 -27.8	25.00b -43.5
B + F	8.0 ± 1.58 -13.9	8.0 ± 2.83 -13.9	6.0 ± 2.35 -10.4	1.0 ± 0.50 -1.7	0.0 ± 0.00 0	4.60[d] -8
B + PE	26.0 ± 2.45 -45.2	23.0 ± 1.12 -40	18.0 ± 2.83 -31.3	12.5 ± 1.64 -21.7	10.0 ± 0.71 -17.4	17.90[c] -31.1
Average	31.5 -55	29.88 -52	26.75 -47	21.63 -37.6	18.13 -31.5	

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 16.21 (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract. *Means in parentheses corrected to 100%.

Table 4. Germination (%) of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

Filler material	Storage period (days)				
	0	30	60	90	120
T	71.5±1.92[a] -100	71.5±1.92[a] -100	71.0±2.50[a] -93.9	68.5±2.68[a] -95.8	67.0±2.69[a] -93.7
B + G	54.0±2.12[b] -75.5	50.5±2.38[b] -70.6	49.0±3.50[b] -68.5	41.0±4.03[b] -57.3	24.0±4.95[b] -33.6
B + F	23.0±2.50[d] -32.2	15.5±1.09[d] -21.7	13.0±1.50[d] -18.2	6.5±3.49[d] -9.1	0.0±0.00[c] 0
B + PE	44.0±1.22[c] -61.5	30.0±1.87[c] -42	29.0±2.69[c] -40.6	19.5±0.83[c] -27.3	16.0±1.58[b] -22.4

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 15.17. (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

the materials (Table 5).

According to the regression in the analysis of variance, the highest degree model, that best fit the data of the third germination counting as a function of the storage periods, was the second degree, for all the coated seeds, being significant at 1% for bentonite + plant extract and 5% for bentonite + glue and bentonite + fungicide. The determination coefficients for these models ranged from 0.9658 to 0.9999, representing reliably the experimental data from the third germination counting (Figure 3). It was verified that in the control there was no significant decrease in the third counting of germination during the storage period. For the seeds coated with bentonite and PVA glue, the germination tended to decrease up to 60 days of storage, but slowly. After that, an accentuated decrease of germination was verified. For the seeds coated with bentonite + fungicide a decrease of the germination was verified at 30 days of storage, maintaining this behavior until the end of storage. It can be verified that the seeds coated with bentonite + plant

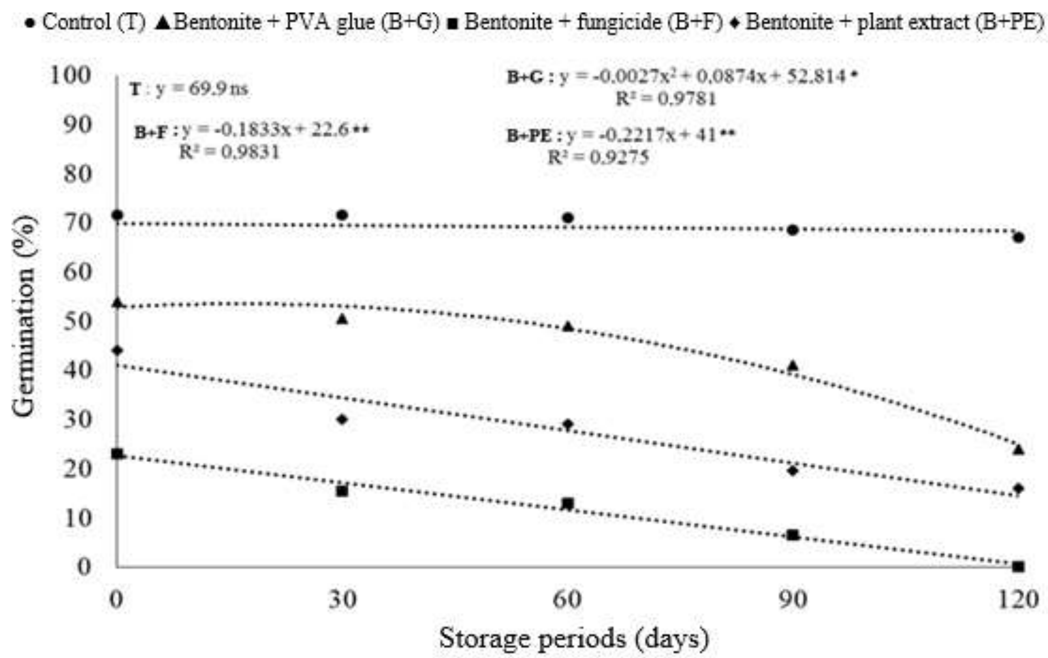

Figure 2. Germination of colza seed coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage, compared to control.

Table 5. Third germination counting (%) of bentonite-coated colza seeds and treated with fungicide and black pepper extract during 120 days of storage.

Filler material	Storage periods (days)				
	0	30	60	90	120
T	72.0±0.71[a] -100	72.0±1.58[a] -100	71.5±2.17[a] -99.3	69.0±2.29[a] -95.8	67.0±2.69[a] -93.1
B + G	59.0±1.66[b] -81.9	54.0±1.00[b] -75	50.0±3.03[b] -69.4	43.5±3.96[b] -60.4	24.5±5.31[b] -34
B + F	55.5±1.92[b] -77.1	37.0±0.43[c] -51.4	22.5±1.09[d] -31.3	11.0±3.77[d] -15.3	4.0±1.22[c] -5.6
B + PE	49.5±0.43[b] -68.8	33.5±1.92[c] -46.5	30.0±3.03[c] -41.7	23.0±1.80[c] -31.9	24.0±1.09[b] -33.3

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test ($P \leq 0.05$). CV% = 12.68. (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

extract had their germination reduced at 30 days of storage which continued until up to 90 days of storage. After this period the germination in the third counting tended to remain (Figure 3).

Comparing the coating materials within each storage period, it could be verified that at the time 0 (zero), the highest value for dry matter was verified in the control, differing statistically from the dry matter of seedlings from seeds coated with bentonite + PVA glue and bentonite + plant extract, which were statistically equal to each other and different from bentonite + fungicide, which had the lowest value for dry matter (Table 6). For the other storage periods, it was verified that all the coating materials were statistically different, with the highest values for shoot dry matter being verified in the control. On the other hand, the lowest values for shoot dry matter were verified when the seeds were coated with bentonite + fungicide (Table 6). The regression of the analysis of

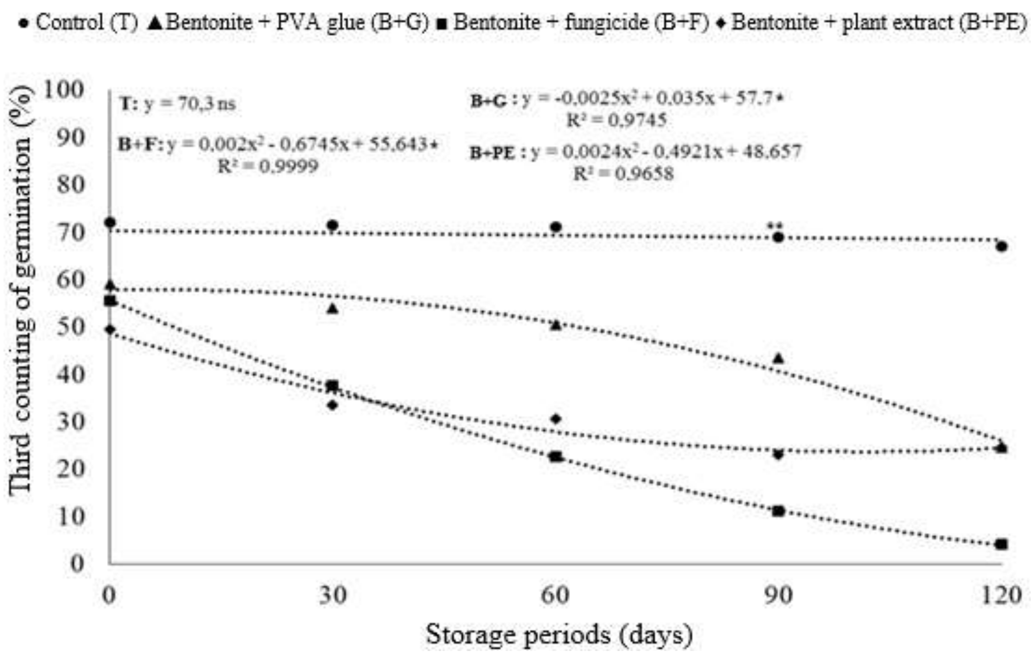

Figure 3. Third counting of germination of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

Table 6. Shoot dry matter of seedlings (mg) of colzacoated with bentonite and treated with fungicide and black pepper extract during 120 days storage.

Filler material	Storage periods (days)				
	0	30	60	90	120
T	140.0±3.54[a]	137.5±2.17[a]	135.0±2.50[a]	127.5±4.15[a]	127.5±8.93[a]
B + G	92.5±2.17[b]	82.5±5.45[b]	80.0±7.07[b]	62.5±4.15[b]	35.0±5.59[b]
B + F	37.5±2.17[c]	22.5±5.45[d]	15.0±2.50[d]	10.0±3.54[d]	0.0±0.00[d]
B + PE	80.0±3.54[b]	52.5±4.15[c]	45.0±5.59[c]	27.5±2.17[c]	15.0±2.50[c]

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 15.16. (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

variance reveals that for the control and for seeds coated with bentonite + PVA glue, the model of highest degree that best fit the experimental data of the shoot dry matter as a function of storage periods was the second degree, where the first treatment was significant at 1% and for the second treatment, significant at 5%, having determination coefficients of 0.9245 and 0.9797, respectively. For bentonite + fungicide and bentonite + plant extract the model of highest degree that best fit the experimental data was the first degree, with determination coefficients of 0.9661 and 0.9678, respectively. Regarding the determination coefficients, all of them reliably represent the experimental data, with R^2 above 92% (Figure 4). For all treatments, a decrease in shoot dry matter was observed during the storage period. For bentonite + PVA glue, a slight decrease was verified up to 60 days of storage, with a more accentuated decrease after this period (Figure 4). For the seeds coated with bentonite + fungicide and bentonite + plant extract, a decrease of shoot dry matter of the seedlings was observed as the time of storage increased (Figure 4).

DISCUSSION

It could be observed that the water content varied for bentonite at 0 and 30 days, having higher percentages when the bentonite was used together with the fungicide. For the other storage periods, no difference was observed between products of treatment with bentonite. It can be seen that all combinations of bentonite and treatment product had higher water contents than the

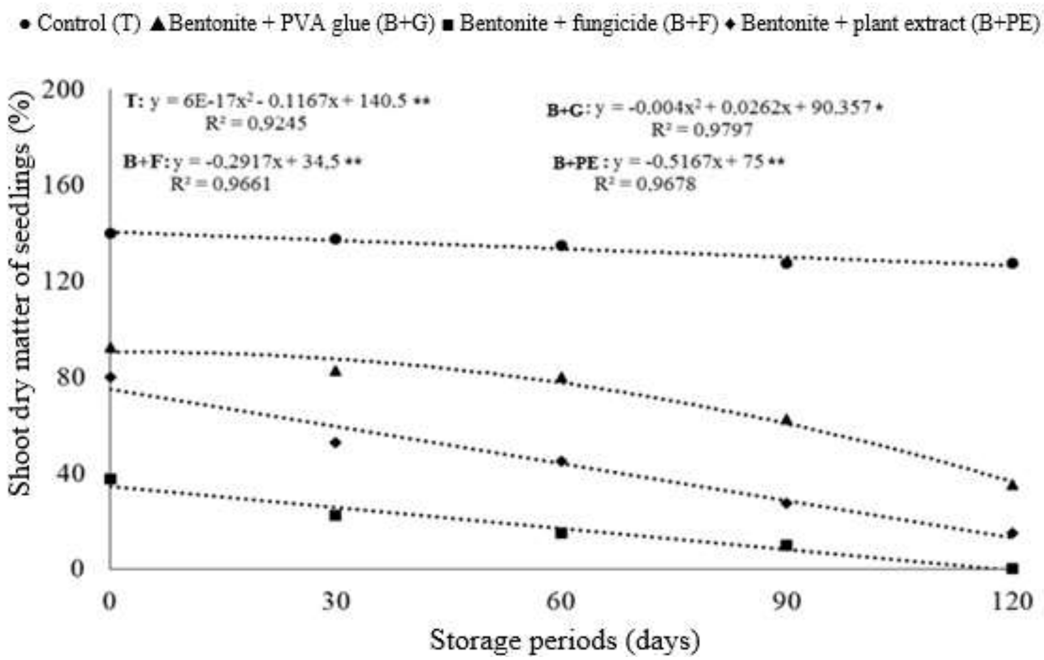

Figure 4. Shoot dry matter of seedlings of colza coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

control. This fact is explained by the high swelling capacity of bentonite in the presence of water, since it is a material that can retain water (Silva and Ferreira, 2008). Another aspect observed is that the water contents of the seeds, coated or not, presented little variation during the storage. This can be explained by the fact that they were stored in acrylic containers and in laboratory conditions, where there is little variation of climatic conditions.

All parameters of physiological seed quality evaluated in this studied were negatively affected by the coatings during storage, especially those seeds coated with bentonite + fungicide. The fungicide caused an acceleration in the deterioration of the seeds during storage, observing total inhibition of germination at 120 days of storage. Differently, non-coated seeds exhibited a decrease in germination and vigor in a slight manner during storage. The non-coated seeds had an average germination of 70%, which accentuated the effect of the treatments. However, colza seed is a difficult material to obtain in our region, being the only material available at the time of installation of the experiment. Another aspect noticed, is that in the third counting of germination the seeds had a greater recovery of the germination in the time 0 (zero), differently from what was observed at 120 days of storage. In the second and third counting, at time 0 (zero), a decrease of approximately 20% occurred when compared with the control. On the other hand, at 120 days of storage this decrease was approximately 60% when compared to the control.

This shows that the coating materials reduce seed germination and vigor, and this effect is intensified during storage. It is observed that the coating process reduced on average of 25% the germinations at time zero, and also occurred an average decrease of 25% of germination throughout the period of 120 days of storage. This suggests that other materials or combinations of materials should be tested in order to minimize such negative effects. It is observed that the coating, independently of the treatment product, negatively modified the physiological quality of the seeds during storage. Similar results were also observed by Roos (1979), Silva (1997), Silva and Nakagawa (1998), and Pereira et al. (2001). They found that seed coating may reduce the seed storage potential. Kim et al. (2000), studying different types of coating, among them the pelletizing, on the physiological quality of lettuce seeds, during nine months of storage, verified losses in germination and vigor. Pereira et al. (2004) also found losses in the physiological quality of pelleted seeds of *Brachiaria decumbens* during 12 months of storage.

Regarding the use of fungicide, Oliveira et al. (2003), studying different pelleting materials with or without fungicide in tomato seeds (*Lycopersicon esculentum* L.) during 24 months of storage, found that the fungicide associated with pelleting materials reduced the physiological quality of seeds.The physiological quality of coated seeds during storage may have been influenced by the climatic conditions in which they were stored. Roos (1979), studying different temperature and relative humidity conditions combined with four filler materials during storage in lettuce seeds, found that temperatures lower than 10% and relative humidity less than 70% retained the physiological quality of these seeds for up to three years. The same author verified that the relative humidity above 70% and the temperature rises to 21°C caused a faster deterioration of the seeds as compared to non-coated seeds.

Another factor that may have negatively affected the physiological quality of the coated seeds was that they had higher water contents. This probably caused an increase in the respiratory rate of the seeds leading to loss of germinative potential. Different behavior was observed in the control, where the water contents of the seeds were inferior to the coated seeds.

Conclusion

Based on the results it can be concluded that Colza seeds coated with bentonite + fungicide have higher water contents, followed by bentonite + plant extract, bentonite + PVA glue and non-coated seeds; the water content of the seeds, independently of the filler material and treatment product are little influenced during the storage period. Coated seeds have the germination and vigor reduced more rapidly during storage. The bentonite + fungicide used for coating of colza seeds totally inhibits germination at 120 days of storage. Non-coated seeds statistically maintain germination at 120 days of storage. The third counting indicates further studies of longer periods for germination of coated colza seeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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