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Physiological response of colza (*Brassica napus* L.) seeds coated and treated with alternative materials

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The coated seeds have multiple advantages in relation to the common seeds. In addition to the increase in size and uniformity in their shape, treatment products can be incorporated in them helping the germination process. Against the foregoing, the objective of this work was to evaluate the physiological quality of the colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide (carboxin+thiram) and plant extract of black pepper (*Piper nigrum* L.). Colza seeds were encrusted with bentonite, gypsum and kaolin and received or not treatment with fungicide (carboxin + thiram) and aqueous extract of black pepper (*Piper nigrum* L.) which were added together with the cementing mixture (30% of glue of polyvinyl acetate). Germination was evaluated in three periods (4, 7 and 14 days after sowing). The experiment was organized in a completely randomized design and arranged in a factorial scheme (filling materials × treatment products). The means, when necessary, were compared by the Scott-Knott test ($p \leq 0.05$). The seeds coated with gypsum presented the best results for germination and vigor, followed by kaolin and bentonite. The seed coating with gypsum + plant extract presents the same germination and vigor of the seeds without coating.

Key words: Coating, bentonite, physiological response.

INTRODUCTION

The colza/canola (*Brassica napus* L.) is the third most produced oleaginous in the world and it is an annual herbaceous plant belonging to the Brassicaceae family which produces high quality oil-rich grains. This crop is responsible for 15% of the world's edible vegetable oil production, although it is also used in the production of

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biodiesel and animal feed (Tomm et al., 2007). The use of machines in the agriculture is of known importance. They are a responsible part for the expansion of the areas of cultivation and productivity. According to Beltrão and Vieira (2001) one of the obstacles in the mechanical sowing are the seeds with small size, light weight and irregular shape. Colza seeds, according to Angelotti-Mendonça et al. (2016), present such characteristics. The sowing is facilitated when the small seeds are coated, aiming to increase their weight and size, so they flow easily in the seeder (Queiroga and Silva, 2008).

According to Lopes and Nascimento (2012) coating/ pelletizing is a process which consists of depositing dry inert materials of fine granulometry on the surface of the seeds with the assistance of an adhesive material (cementing). This treatment enables to standardize the shape, size and weight of the seeds. Due to this, the distribution of seeds is facilitated, be it manual or mechanized (Nascimento et al., 2009).

In addition, there is the possibility of incorporation of nutrients, growth regulators and other agrochemicals (insecticides and fungicides) during the encrustation / pelletizing process, which may constitute improvements in seed health and seedling establishment (Silva et al., 2002). The use of plants with bioactive potential, in the form of extracts, oils and powders, against various organisms has been increasingly encouraged. Researchers such as Bomtempo (2007), Bong (2010), Cardoso et al. (2005) Abbasi et al. (2010) and Khan et al. (2010) state that piperine, the main compound found in black pepper (*Piper nigrum* L.), has recognized cytotoxic, anti-inflammatory, antipyretic, analgesic, antioxidant, antitumor, antifungal and bactericidal activity.

The process consists basically on applying successive layers of an inert solid material over the seeds in constant movement inside a concrete mixer, alternating the application of the filling material with the spraying of a water-soluble cementing material (Silva, 1997; Silva and Nakagawa, 1998). It is necessary to attend to the fact that the main objective of the seed coating technique is to optimize the behavior of the seeds, both from the physiological and economic point of view. Consequently, the choice of the coating materials is important so they do not affect negatively the seeds vigor and germination (Oliveira et al., 2003).

According to Baudet and Peres (2004) the specific details of this methodology are not informed as they are considered trade secrets, and thereby only the general description of processes and inputs used are available. Thus, it is essential to study the behavior of the seeds through the use of different materials for the filling, as well as the treatment products through tests of germination and vigor. In light of the foregoing, the objective of this work was to study the performance of the colza seeds (*B. napus* L.) coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper (*Piper nigrum* L.) as for the germination and vigor.

MATERIAL AND METHODS

Plant material

The seeds were purchased from the local market of the city of Campina Grande, Paraíba, Brazil. After acquisition, the seeds were taken to the Laboratory for cleaning to remove all strange material that came along with the seeds. The experiment was conducted at the Laboratório de Armazenamento e Processamento de Produtos Agrícolas (LAPPA), in the Universidade Federal de Campina Grande, Campina Grande Campus, Paraíba, Brazil.

Preparation of the plant extract

The fruits of black pepper (*P. nigrum* L.) were purchased at the central fair of the city of Campina Grande, Paraíba, Brazil. The aqueous extract was obtained from the powder of the fruits, which were weighted, moistened with distilled water and kept in an infusion for 72 h, at ambient temperature of 24.0 ± 4.0°C, in the absence of light and with daily shaking for five minutes. The amount of powder used corresponded to 20% of the volume of water used. Posteriorly, the solutions were filtered on filter paper and the extract was stored in an amber glass container with capacity for 0.5 L (Almeida et al., 2004).

Materials and process of seed coating

Three filling materials were used: (1) bentonite, (2) gypsum and (3) kaolin. As cementing material, the PVA (polyvinyl acetate) glue was used at the percentage of 30% (Melo et al., 2016), for each filling material. As treatment products, the aqueous extract of black pepper (*P. nigrum* L.) and a fungicide (carboxin+thiram) were used, corresponding to 50% of the mixture (Table 1).The seed coating process occurred by the alternating application of cementing material and filling material in a machine developed for this purpose. This process was repeated until all the material destined to the process has been totally used.

Germination test

The germination test was conducted with four sub-samples of 50 seeds; they were sown in plastic trays with vermiculite, moistened with distilled water with volume corresponding to 60% of the holding capacity. These were kept in ambient conditions of temperature, relative air humidity and photoperiod. The germination percentages were recorded after 4 and 7 days form beginning of the treatments (Brasil, 2009). A third germination count was also performed at 14 days after sowing to evaluate whether there was reduction or delay in germination.

Dry matter of the aerial part

To determine the dry matter of the aerial part, the seedlings, at the second count, were cut at the height of the substrate surface and put in *kraft* paper bags. After that, they were subjected to drying in an oven with forced air circulation, at a temperature of 65°C until reaching constant weight. Thereon, the plant material was weighted in a precision digital scale and the data were expressed in milligrams (mg).

Table 1. Treatments used in the encrustation process.

Treatments	PVA glue	Fungicide	Vegetable extract
Seeds without encrustation	-	-	-
Bentonite Gypsum Kaolin	Cementing material (30% PVA glue + 70% distilled water)	Cementing material (30% PVA glue + 50% fungicide + 20% distilled water)	Cementing material (30% PVA glue + 50% vegetable extract + 20% distilled water)

Experimental design and statistical analysis

The experiment was organized in a completely randomized design and arranged in a 4 × 3 factorial scheme (filling materials x treatment products). Each treatment was repeated four times. The data were submitted to Analysis of Variance ($P \leq 0.05$) and the means, when necessary, were compared by the Scott-Knott test ($P \leq 0.05$).

RESULTS

Table 2 organized the values of the mean squares for the first germination count (FCG), germination (G), third germination count (TCG) and dry matter of the aerial part (DMAP) of the seedlings from the colza seeds coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper. It is verified for all the studied variables a highly significant effect for the isolated or interacting factors, revealing statistical differences between the treatments. Comparing the treatment products within each filling material, the mean germination for the control was of 57.50%. For the bentonite, the highest germination was observed when the glue was used, followed by the plant extract and the fungicide, these presenting statistical difference between them. For gypsum and kaolin, the highest germinations were observed when the plant extract was used. On the other hand, the lower germinations, for these two materials, were observed when the fungicide was used. For these materials, the glue presented intermediate behavior (Table 3).

When comparing the filling materials within each treatment product, it is verified that for the glue the highest germinations occurred in the control and when the gypsum was used, being statistically equal to each other, and different from the germination observed when using kaolin and bentonite. When the fungicide was used it can be verified that all the treatments were statistically different among them, with higher germination in the control and lower in the bentonite. The kaolin and the gypsum presented intermediate behaviors (Table 3).

When the plant extract of black pepper was used, it was verified that the gypsum and the control presented the highest germinations. On the other hand, the lowest germination was observed when the seeds were coated with the bentonite. Seeds that were coated with kaolin showed germinations with intermediate behavior in relation to the other treatments (Table 3). When comparing the filling materials within each treatment product, it is verified that the highest germination was verified in the control, differing statistically from the gypsum. On the other hand, the lowest germinations were observed when bentonite and kaolin were used, not differing statistically from each other. In relation to the fungicide, it is verified that the highest germination was verified in the control and the lowest one when the bentonite was used. The coating of colza seeds with gypsum and kaolin provided seeds with intermediate germinations in relation to the other materials (Table 4).

When using the black pepper plant extract, it was verified that there was no statistical difference between the germination of the seeds of the control and those coated with gypsum. These germinations were statistically different from those observed when the seeds were coated with kaolin and when bentonite was used (Table 4).

It can be verified that in the control the mean germination was of 71.50%. For the bentonite, it was observed that the glue provided a higher germination, differing from the plant extract of black pepper and the fungicide, which presented the lowest germination among the treatment products for this filling material. As for the gypsum and kaolin, the greatest germinations occurred when the black pepper plant extract was used, differing from the glue. The fungicide, in these two materials, provided the lowest germinations (Table 4). When comparing the treatment products within each filling material, it was verified that in the control the mean germination was of 72.0%. In relation to the bentonite, it is observed that the glue and the fungicide provided the highest germinations, which were significantly different from the germination observed when the plant extract was used. Regarding the gypsum, it is verified that the three products differed from each other. When using the plant extract the germination was the highest among the three products, followed by the germination observed when the glue and the fungicide were used. As for the kaolin, the highest germination was verified when the plant extract was used, differing from the glue and the fungicide, being these statistically equal (Table 5).

On the other hand, when comparing the filling materials within each treatment product, it can be verified that for the glue the highest germination occurred in the control, which differed from the germination when the gypsum

Table 2. Mean squares referring to the first count (FCG), second count (G), third count (TCG) and dry matter of the aerial part (DMAP) of the seedlings from colza (*B. napus* L.) seeds coated with different filling materials (FM) and percentages of cementing material (PC).

Source of variation	Mean squares				
	FD	FCG	SCG	TCG	DMAP
FM	3	2496.66**	1994.05**	772.08**	10257.63**
PC	2	1723.58**	1540.18**	121.33**	6543.75**
FM x PC	6	307.91**	292.15**	110.00**	1015.97**
Error	36	15.38	13.62	12.91	107.63

**Significant at 1%.

Table 3. Means of the first germination count (%) of colza (*B. napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper.

Filling materials	Treatment products		
	PVA glue	Fungicide	Plant extract
Control	57.50±0.83^{aA}	57.50±0.83^{aA}	57.50±0.83^{aA}
Bentonite	34.00±2.49^{cA}	8.00±1.58^{dC}	26.00±2.45^{cB}
Gypsum	54.00±1.87^{aB}	24.00±2.12^{cC}	60.00±0.71^{aA}
Kaolin	44.50±1.92^{bB}	32.00±1.87^{bC}	52.50±1.48^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 9.27.

Table 4. Means of the second germination count of seeds (%) of colza (*B. napus* L.) coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper.

Filllingmaterials	Treatment products		
	PVA glue	Fungicide	Plant extract
Control	71.50±1.09^{aA}	71.50±1.09^{aA}	71.50±1.09^{aA}
Bentonite	54.50±2.28^{cA}	23.00±2.50^{cC}	44.00±1.22^{cB}
Gypsum	63.50±1.48^{bB}	39.50±2.17^{bC}	70.75±0.82^{aA}
Kaolin	53.00±1.50^{cB}	42.75±0.82^{bC}	60.50±1.92^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 6.65.

was used, and also differed from the bentonite and kaolin, which did not presented statistical difference between them (Table 5).

Regarding the fungicide, the highest germination was verified in the control, differing from the other materials. In the case of the black pepper plant extract, it is verified that the germination when the gypsum was used did not differ from the control. However, they differed from the germination registered when the kaolin was used, and also when the bentonite was used, with the lowest germination among the materials (Table 5).

In Table 6 are the mean values for the dry matter of the aerial part of seedlings from colza seeds coated with bentonite, gypsum and kaolin and treated with fungicide and black pepper plant extract. When comparing the filling materials within each treatment product, it is verified that for the PVA glue the highest value for dry matter was observed in the control. On the other hand, the lowest values for dry matter of the aerial part were registered when the seeds were coated with bentonite and kaolin. However, the coating of seeds with gypsum showed intermediate values for the dry matter of the aerial part.

Comparing the filling materials within the fungicide, it can be verified that the highest value of dry matter was found in the control, differing statistically from the values of dry matter for seeds coated with gypsum and kaolin, which were statistically equal but different from the dry matter of the aerial part observed when the seeds were coated with bentonite. As for the plant extract, it can be

Table 5. Means of the third germination count (%) of colza (*B. napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and black pepper plant extract.

Filling materials	Treatment products		
	PVA glue	Fungicide	Plant extract
Control	72.00±0.71^{aA}	72.00±0.71^{aA}	72.00±0.71^{aA}
Bentonite	59.00±1.66^{cA}	55.50±1.30^{bA}	49.50±0.43^{cB}
Gypsum	66.00±1.87^{bB}	56.50±2.86^{bC}	72.50±1.09^{aA}
Kaolin	56.50±1.92c^{B}	51.50±1.09^{bB}	61.50±2.28^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 5.79.

Table 6. Means of the dry matter of the aerial part (mg) of seedlings from colza (*B. napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper.

Filling materials	Treatment products		
	PVA glue	Fungicide	Plant extract
Control	140.00±3.54^{aA}	140.00±3.54^{aA}	140.00±3.54^{aA}
Bentonite	92.50±2.17^{cA}	37.50±2.17^{cB}	80.00±3.54^{cA}
Gypsum	120.00±6.12^{bA}	72.50±4.15^{bB}	132.50±9.60^{aA}
Kaolin	95.00±4.33c^{B}	70.00±3.54^{bC}	117.50±2.17^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 10.06.

observed that the highest values of dry matter were registered in the control and when the seeds were coated with gypsum, not differing statistically between each other. On the other hand, they differed statistically when the kaolin and the bentonite were used, which differed statistically from each other (Table 6).

When comparing the treatment products within each filling material, it is verified that in the control the mean value for dry matter of the aerial part was 140.0 mg. For bentonite and gypsum, the highest values of dry matter of the aerial part were registered when only the glue was used and in combination with the plant extract of black pepper. For the kaolin, the highest value of dry matter of the aerial part was registered when using the black pepper plant extract, differing statistically from the one observed when using the glue and the fungicide, which was the lowest dry matter value among the treatment products (Table 6).

DISCUSSION

The reductions of germination and vigor of the coated seeds were also verified by Oliveira et al. (2003), who reported that some materials, among them the fungicide, used in the coating of the seeds, as well as their dosage, may cause immediate phytotoxic effects in the germination or reduce the physiological quality of the seeds. Franzin et al. (2004) also verified reductions in the germination and vigor of pelleted lettuce (*Lactuca sativa* L.) seeds in relation to non-pelleted seeds, and they also suggested that these seeds should be evaluated by means of specific tests. Ferreira et al. (2015) while evaluating the physiological quality of six batches of coated seeds of hybrid brachiaria, cv. Mulato II, verified that the coated seeds presented a significant reduction in relation to the bare seeds. These authors suggest that such reduction was due to the physical barrier created by the materials used in the coating process. In this respect, Sachs et al. (1981), reported that most of the coating materials used hinder the penetration of oxygen in the seed. Giménez-Sampaio and Sampaio (1994), support the opinions above by stating that the obtained results for coated seeds and bare seeds cannot be interpreted in the same way, since the tests used to evaluate the germination capacity and vigor of the coated seeds are the same.

In general, the gypsum stood out in relation to the germination and vigor of the seeds. Despite the germination observed with the gypsum, alone, it is not a decisive parameter for the choice of the coating material. It must have a capacity to increase the diameter of the seed in a satisfactory way, and in addition to other characteristics, it must have resistance sufficient for the use in the mechanized planting. The bentonite is a clay stone that can be calcium or sodium with high capacity of water absorption and low permeability and, when moistened, makes a viscous gel that expands it by increasing its volume (Tonnesen et al., 2012). The high viscosity attributed to the bentonite causes a resistance to flow (Menezes et al., 2009), which explains a decrease in the germination of the colza seeds coated with this material. Despite the lower germination of seeds covered with the bentonite in relation to the other materials, it was promising in the coating process of colza seeds, resulting in a reduction of 20% of the germination in relation to the control, counted in the third count. Also, the bentonite presents the characteristics of increased size, weight and greater resistance required for a good coating covering. The germination result suggests testing other cementing materials and/or percentages of them, and a finishing polymer that can promote a decrease of this difference of germination with the control.

Conclusions

In view of the above, it can be concluded that the germination and the vigor are affected by coating. Seeds coated with gypsum present the best results for germination and vigor, followed by kaolin and bentonite. The coating of the seeds with gypsum + plant extract presents the same germination and vigor of seeds without coating. The fungicide damages the germination and vigor of the colza seeds regardless of the coating material used.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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