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PHYSIOLOGICAL QUALITY OF CRAMBE SEEDS SUBMITTED TO DESICCATION WITH GLYPHOSATE AND STORED IN PAPER BAGS

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Abstract–Crambe (*Crambe abyssinica* Hochst) is an oilseed species with great economic potential, which is characterized by adapting to antagonistic environmental conditions and good rusticity. However, uneven seed maturation is a limiting factor for obtaining quality seeds. The use of desiccants for anticipating harvest can reduce seed exposure in the field after physiological maturation. Thus, the aim of this work was to evaluate the effect of different glyphosate desiccant doses applied before harvest on the physiological quality of crambe (*Crambe abyssinica* Hochst) seeds at different storage times. A randomized block experimental design in a 5 x 5 factorial scheme was used, with five Roundup® glyphosate doses (0.0, 0.5, 1.0, 1.5 and 2.0 g L^{-1}) applied before harvest and five storage times (0, 3, 6, 9 and 12 months). After harvesting and processing, seeds were placed in paper bags and submitted to storage. During storage, seeds were submitted to physiological quality determination, where variables germination, seedling emergence, emergence speed index, first count, fresh mass and dry mass of seedlings and electrical conductivity were evaluated. The variables analyzed demonstrated that the physiological quality of crambe seeds was reduced with increasing herbicide doses.

INTRODUCTION

Crambe (*Crambe abyssinica* Hochst) is an oilseed species that can have up to 38% oil content in its seeds (Pitol *et al.,* 2010). It is a plant characterized by adapting to antagonistic environmental conditions and good rusticity. It has indeterminate flowering habit, that is, it starts from the base to the tips, with the same plant having seeds at different maturation stages (Oliveira *et al*., 2014), making harvest difficult.

Harvesting at the right time is essential for seed quality preservation, since early harvest can lead to increased deterioration due to the high moisture content, while late harvest makes seeds exposed to unfavorable environmental conditions, compromising their quality (Cangussu *et al.,* 2018). In this sense, it is necessary to use alternative methods to overcome adversities, allowing the early removal of seeds from the field.

The use of desiccants applied in the pre-harvest period has shown to be a promising alternative, as demonstrated in studies carried out with *Phaseolus vulgaris* (DE Barros *et al.,* 2019) and *Sorghum bicolor* (Assis *et al.,* 2019), which obtained harvest uniformity and anticipation. This technique promotes the drying and fall of leaves, provides quick loss of water from seeds, allowing the harvest of lots with reduced moisture levels, in a period closer to the point of physiological maturity, when vigor, germination and dry matter levels are high (Silva *et al.,* 2016). However, there are gaps in knowledge about the best desiccant dose to be applied in order to obtain better quality grains.

However, not only harvest at the right time influences the physiological quality of seeds. Another point that must be considered is the storage of seeds after harvest, whose basic function is to preserve their physiological quality. In this context, the aim of the present work was to evaluate the effect of different Roundup® glyphosate doses applied before harvest on the physiological quality of crambe (*Crambe abyssinica* Hochst) seeds at different storage times.

MATERIALS AND METHODS

The experiment was carried out at the Laboratory of Seed Analysis of the Department of Agricultural Sciences, State University of Montes Claros (DCA/UNIMONTES), campus of Janaúba, Minas Gerais, from January 2019 to January 2020. Crambe seeds 'FMS Brilhante' cultivar produced in the experimental area of UNIMONTES and stored in paper bags were used.

The experimental design used was in randomized blocks, in a 5 x 5 factorial scheme, with four replicates, with five Roundup® glyphosate doses (0.0, 0.5, 1.0, 1.5 and 2.0 g L^{-1}) applied to plants before harvest and five storage times (0, 3, 6, 9 and 12 months). The glyphosate herbicide used was the original Roundup® commercial product with formulation containing 480 $g.L^{-1}$ of N-(phosphonomethyl) glycine isopropylamine salt, 360 $g.L^{-1}$ of the acid equivalent (a.e.) of N-(phosphonomethyl) glycine (GLYPHOSATE) and 648 $g.L^{-1}$ of inert ingredients.

For plots that received the application of this treatment, plants were sprayed with Roundup® herbicide (0.0, 0.5, 1.0, 1.5 and 2.0 g L^{-1}) at 74 days after sowing (DAS), established according to the maturation of seeds based on their color, when 80% of brown seeds were verified. Seven days after herbicide application, seeds were manually harvested, and those from plots of plants without desiccation were also collected.

After harvest, seeds were transported to the laboratory to be manually extracted from racemes, processed to remove impurities and submitted to water content determination. The water content was determined by the standard method in an oven at 105 ± 3 °C for 24 hours, with four replicates of 5 g of seeds, with results expressed as percentage (Brazil, 2009).

Then, seeds were placed in plastic trays and kept in laboratory environmental conditions (± 25 °C and 65% of Relative Air Humidity [RAH]), whose water loss was monitored by means of periodic weighing until reaching water content of approximately 10%. Subsequently, the initial quality of seeds was evaluated, and then, seeds were submitted to storage.

For storage, seeds were placed in permeable packaging (paper bags), sealed and kept in laboratory environmental conditions for 12 months. Initially and after each storage time, in addition to determining the water content, the physiological quality of seeds was evaluated through the following analyses:

Germination test: four replicates of 50 seeds were sown on Germitest paper, moistened with distilled water at volume (mL) equivalent to 2.5 times its dry weight and placed in plastic Gerbox boxes (11.5 x 11.5 x 3.5 cm). Boxes were kept in digital germinator previously regulated at temperature of 25 °C with constant photoperiod, and evaluations were carried out on the seventh day after sowing, which consisted of counting the number of normal seedlings, with results expressed in percentage (Brazil, 2009).

First germination count: consisted of recording the number of normal seedlings obtained on the fourth day after the beginning of the germination test, with results expressed as percentage (Brazil, 2009).

Seedling emergence: conducted under laboratory environmental conditions using four replicates of 50 seeds. Sowing was carried out at depth of 1 cm in plastic Gerbox boxes containing washed sand, sterilized and moistened with distilled water in an amount equivalent to 50% of the holding capacity (Brazil, 2009). Evaluations were carried out on the seventh day after the test installation, and results were expressed in percentage of normal seedlings, adopting as evaluation criterion, seedlings emerged above the substrate with expanded cotyledonary leaves.

Emergence speed index (ESI): consisted of daily counts of the number of normal seedlings until the seventh day after sowing. At the end of the test, ESI was calculated using the formula proposed by Maguire (1962).

Fresh mass of seedling: performed using normal seedlings resulting from the emergence test. At the end of the test, to obtain the fresh matter mass, normal seedlings from each replicate were weighed on precision scale with accuracy of 0.0001 g, and results were expressed in grams per replicate.

Dry mass of seedlings: normal seedlings obtained from the emergence test were placed in paper bags, identified and taken to dry in an oven with forced air circulation, at 55 ºC, for 72 hours, for the dry matter determination. After this period, samples were placed to cool in the desiccator and were again weighed on a precision scale, and the average results were expressed in grams per replicate.

Electrical conductivity: performed by the mass method, using four replicates of 50 physically pure seeds for each treatment. The previously weighed seeds were placed in plastic cups with capacity of 200 mL containing 75 mL of distilled water. Cups containing seeds were placed in B.O.D chamber regulated at temperature of 25 °C, and after 24 hours of imbibition, readings were performed using digital conductivity meter (Digimed DM 31), with results expressed in $\mu S\ cm^{-1}\ g^{-1}$ (Marcos-Filho *et al.*, 1987).

Data obtained were submitted to assumptions of adherence to normality, independence and homoscedasticity of residues and, after meeting assumptions, the other analyses were performed. Variables were submitted to multiple regression to fit regression models that expressed this biological factor and were significant ($p<0.05$). For these analyses, the SISVAR (Ferreira, 2015) and Sigma Plot 12.0, demonstrative version, statistical programs were used.

RESULTS AND DISCUSSION

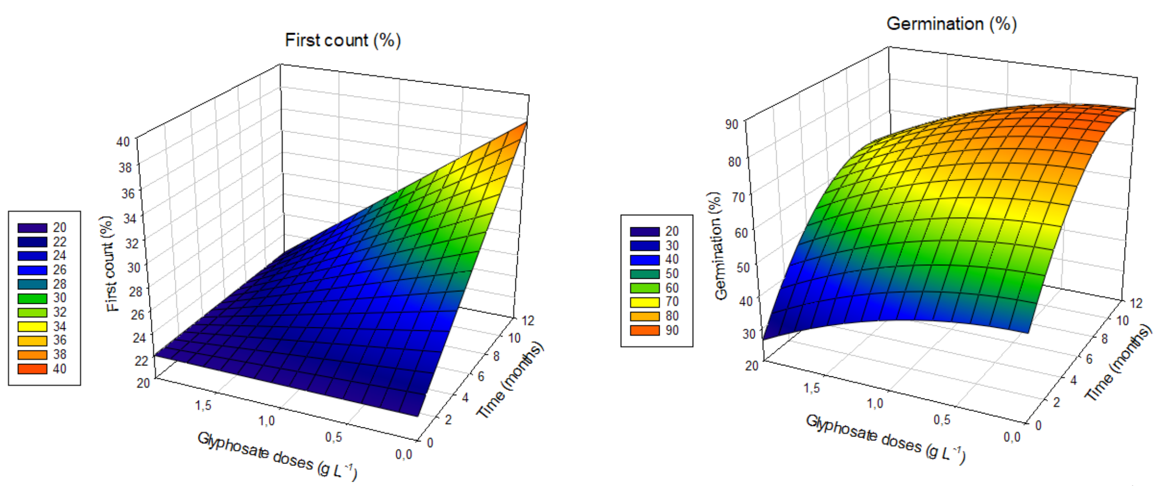
From results obtained, significant effect for the interaction of variables First Count (FC), Germination (G), Seedling Emergence (SE), Emergence Speed Index (ESI), Fresh Mass (FM), Dry Mass (DM) and Electrical Conductivity (EC) was observed.

The results of the FC germination test showed decreasing response with increasing glyphosate doses (Figure 1), significantly inhibiting the germination of crambe seeds, obtaining better results with storage time of twelve months for the first count and nine months for germination, both without the application of glyphosate. This effect may have occurred because glyphosate causes a deficit of aromatic amino acids and secondary phenolic compounds, resulting in inhibitory effect on organogenesis, preventing root differentiation, according to Nagata *et al.* (2000).

In the present work, reduction in the germination of Crambe seeds was observed with increasing herbicide doses during the storage period, ranging from 83.6% to 26.49%. Similar results were observed by Oliveira *et al.* (2020) and Bervald *et al.* (2010), who analyzed the performance of soybean seeds submitted to glyphosate doses and found that, with increasing herbicide doses, reduction in germination and in the size of normal seedlings of the respective seeds was observed. Oliveira *et al.* (2013), working with bean and Barbosa *et al.* (2017) with *Zea mays*, also found reduction in seed germination when treated with higher glyphosate doses.

When not treated with glyphosate, crambe seeds showed better SE and ESI, with better results with storage time of nine months for SE and six months for ESI (Figure 2).

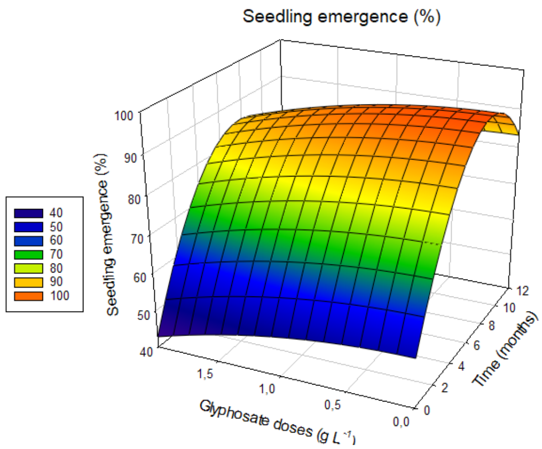
The SE results corroborate results obtained by Daltro *et al.* (2010), who evaluated the effect on the



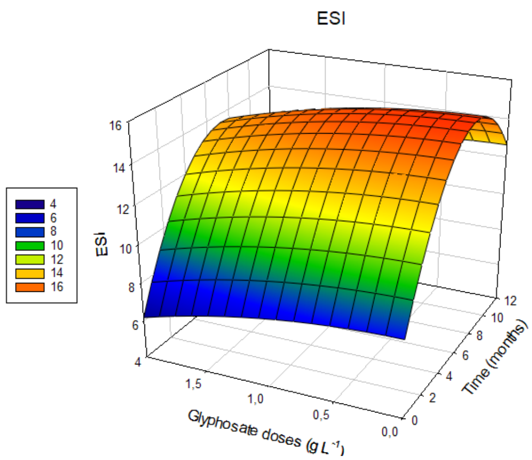
FC = 22.05+1.3128*time-0.6511*time *doses

$G = 46.1375 + 7.6876*time-0.3984*time^2- 4.9126*doses^2$

Fig. 1. A - First Count (FC) and B - Germination (G) of crambe seeds, 'FMS Brilhante' cultivar, submitted to different times and glyphosate doses stored in paper bags.

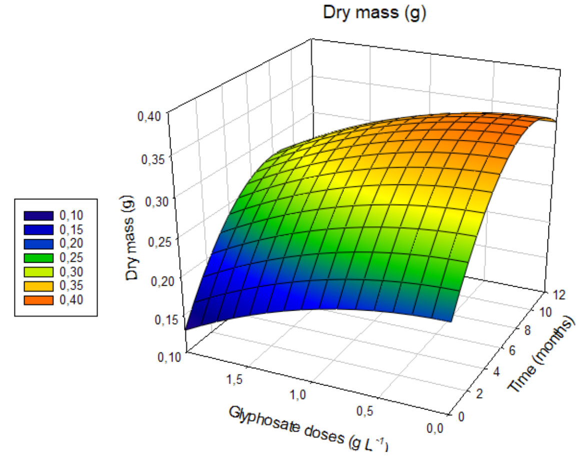


$SE = 52.9085+10.7295^{*}\ time-0.6841^{*}time^{2}-2.5218^{*}$

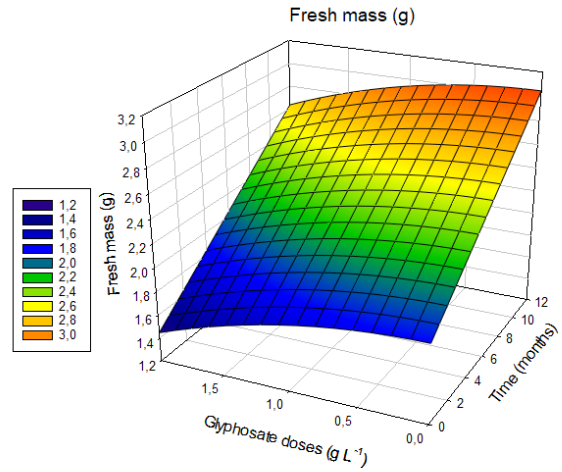


$ESI = 8.0183+1{,}9850^{*}\ time\ -0.1333^{*}\ time^{2}-0.4623^{*}doses^{2}\ doses^{2}$

Fig. 2. A - Seedling Emergence (SE) and B - Emergence Speed Index (ESI) of crambe seeds 'FMS Brilhante' cultivar submitted to different times and glyphosate doses stored in paper bags.



$FM = 1.8635+0.0987^{*}time-0.1039^{*}doses^{2}$



$DM = 0.2154+0.0351^{*}time\ -0{,}0021^{*}time\ ^{2}-0.0214^{*}doses^{2}$

Fig. 3a. Fresh Mass (FM) and B - Dry Mass (DM) of crambe seedlings 'FMS Brilhante' cultivar submitted to different times and glyphosate doses stored in paper bags.

physiological quality of soybean seeds and found that seeds desiccated with glyphosate showed seedlings with stunted and poorly developed root systems.

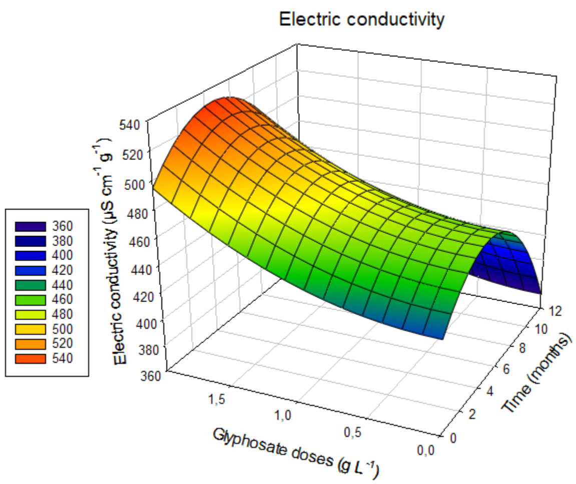
The reduced emergence speed with increasing glyphosate dosages influenced the rapid establishment of seedlings, as seedlings with higher ESI have greater ability to withstand stresses that may occur during emergence (Dan *et al.*, 2010). Similar results were found by Bervald *et al.* (2010), who analyzed the performance of conventional and transgenic soybean seeds when submitted to glyphosate doses and found that with increasing herbicide doses, reduction in ESI was observed. The percentage reduction of these variables can be explained by the phytotoxic action of the herbicide.

According to Figure 3, better results were obtained with storage time of twelve months for FM and nine months for DM, both without glyphosate application, corroborating Funguetto *et al.* (2004), who observed that, in the presence of glyphosate, the physiological processes capable of guaranteeing the development of seedlings of non-genetically modified cultivars are affected.

Studies carried out by Kappes *et al.* (2012) showed that bean seedling biomass accumulations were affected by pre-harvest paraquat application. Similar results were observed by Bellé *et al.* (2014), who verified reduction in the vigor of wheat seeds after chemical desiccation of plants in the pre-harvest period with glyphosate and paraquat, resulting in lower DM of seedlings. Vanzolini *et al.*

(2007) highlighted that the study regarding the DM of seedlings or their parts is effective to detect subtle differences in seed vigor. Seeds with high physiological potential result in seedlings with higher DM in relation to those with low physiological potential (Höfs *et al*., 2004).

Figure 4 presents the results obtained for EC, which showed increasing response with increasing glyphosate doses (2.0 g L^{-1}) and in six months of storage, electrical conductivity of 527.17 µS cm^{-1} g^{-1} was observed. Bervald *et al.* (2010) found that electrical conductivity values increased significantly with increasing glyphosate doses in soybean seeds.



EC = 429.0788+15.5962*time-1.6964* $time^2$ + 16.3976* $doses^2$

Fig. 4. Electrical conductivity (EC) of the germination of crambe seedlings 'FMS Brilhante' cultivar submitted to different times and glyphosate doses stored in paper bags.

According to Marcos Filho (1999), the electrical conductivity test evaluates seed quality by determining the amount of leachate in the seed soaking solution, with lower values corresponding to lower release of exudates. This indicates high physiological potential (greater vigor), revealing less intensity of disorganization of cell membrane systems. Thus, it was observed that glyphosate was able to deteriorate the quality of seeds.

CONCLUSION

The physiological quality of crambe seeds is drastically reduced by increasing glyphosate doses, stored in paper bags at different times. Glyphosate promotes reduction in seed germination, ESI, fresh and dry mass, in addition to increasing electrical conductivity, affecting the normality of seedlings.

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