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Hydroalcoholic Extract of Crambe on *Sitophilus zeamais* Insects and Maize Seed Quality

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Abstract

The objective of this work is to evaluate the insecticidal and attractiveness of concentrations of hydroalcoholic extract of crambe grains on Sitophilus zeamais, and its effect on the physiological quality of corn seeds. The experiments were conducted at the Laboratory of Entomology and Seeds of the Assis Gurgacz University Center, in Cascavel, PR. The evaluation of attractiveness and insecticidal effect were evaluated using DIC, with 4 treatments (0, 5, 15 and 25% extract concentration) and 10 or 5 replications, respectively, totaling 40 experimental plots for the insect attractiveness test and 20 Experimental plots for the test of the insecticidal effect. For the experiment on the physiological quality of corn seeds submitted to the extracts, a DIC was set up in a 4 × 4 factorial scheme, factor 1 being the storage time of the seeds (0, 30, 60 and 90 days) and factor 2, the concentrations (0, 5, 15 and 25%), with 4 replicates, totaling 64 plots. Data were submitted to ANAVA, and means adjusted to regression or submitted to the Tukey test at 5% of probability, using the statistical program ASSISTAT®. The results evidenced the treatment with a hydroalcoholic extract in the concentration of 25% as the one with the highest insecticidal effect, and extract at 15% concentration yields a higher percentage of germination, normal seedlings, and mass of seedlings than the control. At 25% concentration, the extract do not negatively influence any of the parameters analyzed. Storage time above 60 days stimulates germination, mass and length of maize seedlings.

Keywords: *Zea mays*, insecticide, *Crambe abyssinica*, attractiveness

1. Introduction

Maize is produced in many continents and can be used in several ways, from human and animal nourishment to the high technology industry, in the production of films, biodegradable packaging, and other products. According to Paes (2006), about 70% of the world's maize production is destined to animal feed. In developed countries this figure may reach 85%. However, only 15% of all world production is destined for human consumption, directly or indirectly.

Travaglia (2011) states that grains often need to be stored for more than a year due to off-seasons and drought periods. The purpose of storage is to preserve the characteristics and quality of the grains over time in order to meet the market demands. However, if storage conditions are not adequate the grains become susceptible to deterioration and exposed to possible contamination.

Seed or grain quality may be affected by several factors, including storage pests, such as *Sitophilus zeamais*, which may be responsible for the physical deterioration of the stored batch (Lorini Kryzanowski, França-Neto, & Henning, 2010).

Improper storage conditions lead to severe attacks of storage pests, which might make these grains unfit for consumption (Michelraj & Sharma, 2006).

Currently, storage pests are commonly controlled by applying chemical products, however, Barbosa (2004) states that the residues of these chemical insecticides can be found not only in the grains, but also in the processed products in different concentrations. Thus, according to Viebrantz, Radunz, and Dionello (2016), due to the need to improve food quality and safety, the use of chemical methods has been replaced by alternative methods.

Crambe (*Crambe abyssinica*) is an oilseed that belongs to the brassicaceae family. It presents rapid growth and short cycle. It is highly tolerant to pests, which only attack the crop during the seedling phase. Some pests that have already been reported for attacking crambe crops are the Cabbage Aphid (*Brevicoryne brassicae*), Cucurbit Beetle (*Diabrotica speciosa*) and *Agrotis* sp. (Bezerra et al., 2011).

The brassica family has been studied due to the production of secondary metabolites, such as glucosinolates (Merah, 2015). Pitol, Broch, and Roscoe (2010) explain that crambe presents low incidence of pests due to the presence of glucosinolates. Pal Vig, Rampal, Thind, and Arora (2009) also mention studies that point out that this compound has several biological activities, such as protection against pathogens and weeds. Tsao, Peterson, and Coats (2002) suggest that remnants of plants that contain glucosinolates, when incorporated into the soil, can control soil pests.

Studies on crambe extracts have demonstrated its efficiency in controlling nematodes (Coltro-Roncato et al., 2016), whereas other studies incorporated crambe residues in soils colonized with nematodes (Nascimento, Jaime, G. E. Silva, A. R. Silva, & Alves, 2016).

According to Pal Vig et al. (2009), glucosinolates accelerate insect respiration, which consequently increases their need for ATP while blocking its production. This leads to the exhaustion of energy sources and culminates in the insect's death.

The objective of this research was to evaluate the insecticidal effect and attractiveness of different concentrations of hydroalcoholic extract of crambe on *Sitophilus zeamais*, as well as its effect on the physiological quality of stored maize seeds throughout the storage period.

2. Material and Methods

This experiment was conducted at the Laboratory of Entomology of the Assis Gurgacz University Center in Cascavel, Paraná, Brazil, at a temperature of 25±2 °C and relative humidity of 60±5%. The insects used in the tests were obtained from the insect farm kept in the laboratory, with maize kernels placed in containers measuring 8 cm in diameter × 6 cm in height. Grains of the maize hybrid AM 4003 were obtained from Melhoramento Agropastoril, a grain-producing company. Crambe grains were obtained from the experimental fields at the School Farm of the Assis Gurgacz Foundation University Center, Cascavel, Paraná, Brazil, in 2014, and stored away from light and heat.

The crambe grains were ground in an IKA A11 Basic 2500 1/min IP43 mill in order to obtain powder, which was mixed with 100 mL of the hydroalcohol composed of 50% water and 50% alcohol, homogenized in a blender in previously determined concentrations, and kept in a beaker with film and foil for light protection, for 48 h.

2.1 Evaluation of Repellency/Attractiveness on Sitophilus zeamais Insects

The experiment was set up in a completely randomized design, consisting of 4 treatments (concentrations 0%, 5%, 15% and 25%) and 10 replications of each, totaling 40 experimental plots.

For the evaluation of the repellency on *Sitophilus zeamais*, two MDF feeding arenas measuring 45 × 45 × 3 cm were used, having a central hole with a diameter of 8 cm and four lateral holes with a diameter of 6 cm each. They were interconnected symmetrically by four 10-cm paths that connected the central and lateral holes, all with a depth of 2 cm, coated with contact paper and covered with perforated paper to allow aeration.

Ten grams of maize kernels were placed in each container. Container #1 was the control (maize kernels only). Container #2 had maize kernels mixed with 0.5 mL of hydroalcoholic extract of crambe at 5% concentration. Container #3 had maize kernels mixed with 0.5 mL of hydroalcoholic extract of crambe at 15% concentration. Container #4 had maize kernels mixed with 0.5 mL of hydroalcoholic extract of crambe at 25% concentration.

Ten *Sitophilus zeamais* insects were released into the central container of each arena. After 1 hour and 48 hours, the number of insects in each container was counted in order to assess the attractiveness at the first moment of exposure and two days later.

Data were subjected to analysis of variance (ANOVA) and means were fit to the regression in the statistical program ASSISTAT® version 7.7 (Silva & Azevedo, 2016).

2.2 Insecticide Evaluation

The assay was performed on Petri dishes, in a completely randomized design consisting of 4 treatments, as follows: Treatment #1 - control (distilled water); Treatment #2 - hydroalcoholic extract of crambe at 5% concentration; Treatment #3 - hydroalcoholic extract of crambe at 15% concentration; and Treatment #4 -

hydroalcoholic extract of crambe at 25% concentration. There were also 5 replications of each, totaling 20 experimental plots.

Each Petri dish was lined with two sheets of germination test paper. One ml of distilled water or one ml of the hydroalcoholic extract of crambe was added to the Petri dishes with a syringe in the different concentrations determined. The dishes were then infested with 10 non-sexed adult insects of *Sitophilus zeamais* and sealed with film paper with microapertures to allow air to enter. The 20 Petri dishes were placed in a BOD chamber, at a temperature of 25±2 °C, photoperiod of 14hL and relative humidity of 60±5%.

The evaluations were carried out 12 h and 24 h after beginning the experiment by obtaining the number of dead insects. Data were subjected to ANOVA and means were fit to the regression in the statistical program ASSISTAT® version 7.7 (Silva & Azevedo, 2016).

2.3 Germination Test

The germination test in maize kernels was conducted at the Seeds Laboratory of the Assis Gurgacz Foundation University Center, in Cascavel, Paraná, Brazil. The experiment was set up in a completely randomized design consisting of a 4 × 4 factorial scheme. Factor 1 was seed storage time (0, 30, 60 and 90 days), and factor 2 was the concentration of the hydroalcoholic extract of crambe (0, 5, 15 and 25%). There were 4 replications, totaling 64 experimental plots.

Each replication included 50 seeds of the maize hybrid AM 4003. The parameters evaluated were percentage of germination, percentage of normal seedlings, and seedling mass and length at day 7 after sowing (Brasil, 2009).

The data were subjected to analysis of variance and the means were compared by Tukey's test at 5% probability in the statistical program ASSISTAT® version 7.7 (Silva & Azevedo, 2016).

3. Results and Discussion

Table 1 shows the analysis of variance of the regression of data related to the insecticidal action of the hydroalcoholic extract of crambe on *Sitophilus* insects after 12 and 24 h of exposure. The number of dead insects after 12 h of exposure fit the linear regression, whereas the number of dead insects after 24 h fit the cubic regression, both determined based on the R^2.

Data transformation was necessary to determine the most adequate coefficient of variation, which is justified by Haddad and Vendramim (2000), who state that the transformation of data by using $\sqrt{x}$ is usual in entomology, since it homogenizes the experimental variance, which is a statistical requirement for the validation of the tests of significance and confidence intervals for the means of the treatments.

Table 1. Regression in the analysis of variance of the insecticidal action of different concentrations of hydroalcoholic extract of crambe on the insect *S. zeamais*, for 12 h and 24 h, with transformation of using $\sqrt{x}$

Treatments	Number of dead insects after 12 h	Number of dead insects after 24 h
F-statistics	40.22	28.25
CV (%)	16.80	18.07
L.R.	*	n.s.
C.R.	n.s.	*

Note. n.s = non-significant; * = Significant at 5% probability; CV = Coefficient of Variation; L.R. = Linear Regression; C.R. = Cubic Regression.

Figure 1 shows the percentage of dead insects as the concentration of hydroalcoholic extract of crambe increased. After 12 hours of exposure, the extracts provided approximately 35% of dead insects, whereas in the control, all insects remained alive. These results point out that the alternative control of these insects is possible. Some studies have demonstrated the resistance of *Sitophilus zeamais* to chemical insecticides that have not yet been released for the control of storage pests in Brazil, such as indoxicarb (Haddi, Mendonça, Santos, Guedes, & Oliveira, 2015), thus, having an alternative method for controlling them is necessary.

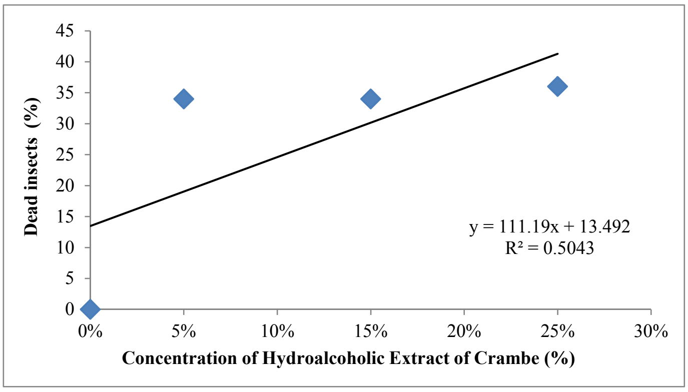

Figure 1. Evaluation of the insecticidal action after 12 h of exposure of *Sitophilus zeamais* to different concentrations of hydroalcoholic extracts of crambe under controlled conditions of temperature and photoperiod

Nascimento, Diniz Filho, Mesquita, Oliveira, and Pereira (2008) found 96 to 100% of dead *Sitophilus* insects exposed to *Tagetes patula* extract in vapor form. Restello, Menegatt, and Mossi (2009) studied the effect of the essential oil of *T. patula* on *Sitophilus* mortality and found 100% of dead insects. Silva, Melo, Pessoa, Almeida, and Gomes (2012) observed a raise in *Sitophilus* mortality as the rates of *Momordica charantia* (L.) extract increased, reaching 100% mortality when applying 10 mL of the extract.

Figure 2 shows the percentage of dead insects after 24 hours of exposure to different concentrations of hydroalcoholic extracts of crambe. The data did not fit the regression. In the control, 100% of the insects remained alive. In the treatment with the extract at 5% concentration, about 65% of the insects died. This number decreased to 45% when concentration was 15% and increased to about 60% when extract concentration was 25%.

According to Glaser (1996), the increase in *Sitophilus* mortality due to the presence of crambe extract happens because the glucosinolates found in brassicas might act as control for pests such as nematodes, flies, maggots and mites.

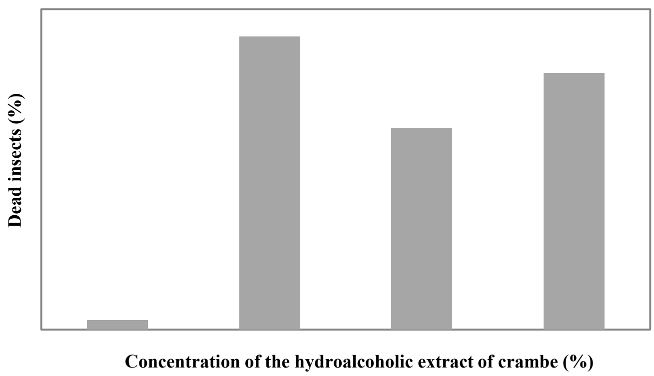

Figure 2. Analysis of the insecticide action on *Sitophilus zeamais* after 24 hours of exposure to different concentrations of hydroalcoholic extract of crambe under controlled conditions of temperature and photoperiod

Table 2 shows the insects' behavior in the feeding arena containing maize kernels with the 4 different concentrations of hydroalcoholic extract of crambe, at 1 h and 48 h of exposure. Just as the insects were exposed, it was observed that 44.2% of them were attracted to maize kernels with no addition of hydroalcoholic extract, what differs statistically from the other treatments. Concurrently, 23% of the insects were attracted to kernels with extract at 15% concentration, 19.2% were attracted to kernels with extract at 5% concentration and 15% were attracted to kernels with extract at 25% concentration, showing no statistical difference among treatments.

After 48 h of exposure, Treatment #1 remained as the most attractive to insects, with 30% preference; however, it was statistically equal to Treatment #3, with 25.4% preference, and Treatment #4, with 28% preference. Treatment #2 attracted only 16.6% of the insects, what differs statistically from the control (T1). Cruz, Sousa, Medeiros, Silva, and Gomes (2012) studied the action of different essential oils in the control of *Sitophilus zeamais* insects and concluded that lavender oil had the best effect in repelling weevils from maize. Nonetheless, further research is required in order to establish the ideal concentration.

Table 2. Percentage of *Sitophilus zeamais* insects attracted to maize with different concentrations of hydroalcoholic extract of crambe, in a free-choice feeding arena, assessed at 1h and 48h of exposure

Treatment	% Attracted Insects	
	1 h	48 h
T1 (0%)	44.2a	30.0a
T2 (5%)	19.2b	16.6b
T3 (15%)	23.0b	25.4ab
T4 (25%)	15.0b	28.0ab
CV (%)	32.79	32.48
MSD	12.24	14.8

Note. Means followed by the same letter in the columns are not significantly different (Tukey, $P < 0.05$).

Figure 3 shows that the percentage of insects attracted to maize kernels with hydroalcoholic extract of crambe (45% attracted to control) after 1 h of exposure reduced with higher concentrations of the extract; only 17% of the insects were attracted to treatment #4 – 25% extract concentration. Fernandes and Favero (2014) studied the attractiveness/repellence of the essential oil of *Schinus molle* on *Sitophilus zeamais* at 1 h and 24 h of exposure and observed repellent action of the lethal concentrations of 5 and 10 ppm.

Almeida, Silva Junior, Silva, Lino, and Silva (2012) studied the control of *Sitophilus zeamais* with a hydroalcoholic extract made from pinecone and black pepper and reported that the infestation percentage of the insect-pest on maize seeds decreased with higher concentrations of the extract, what was corroborated in this experiment.

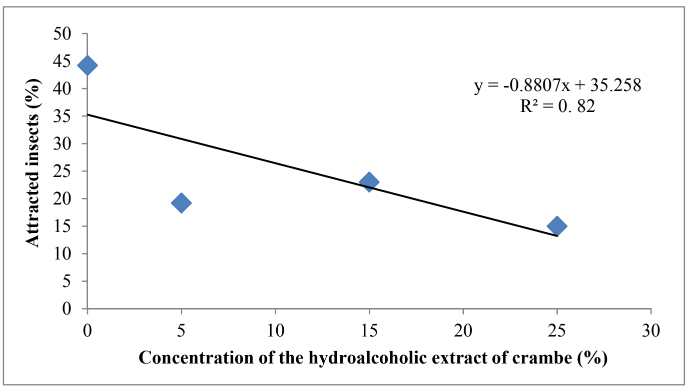

Figure 3. Percentage of *Sitophilus zeamais* attracted to maize kernels treated with different concentrations of hydroalcoholic extract of crambe, after 1 h of exposure in a free-choice feeding arena

Concerning to preference, after 48 h of exposure (Figure 4), 30% of the insects were attracted to maize kernels in the control treatment. This percentage was lower in treatments #2 and #3 and showed a tendency of increase in treatment #4 – 25% extract concentration (28% of insects attracted). Guimarães et al. (2014) noticed that aqueous extracts from seeds of *Capsicum baccatum* showed repellent activity on maize weevils whereas the alcoholic extract from the pulp of *Capsicum baccatum* showed 34.6% attractiveness on adult *Sitophilus zeamais* insects. Such result is similar to those found in this experiment, which indicates that the substances released in alcohol might be different than those released in water.

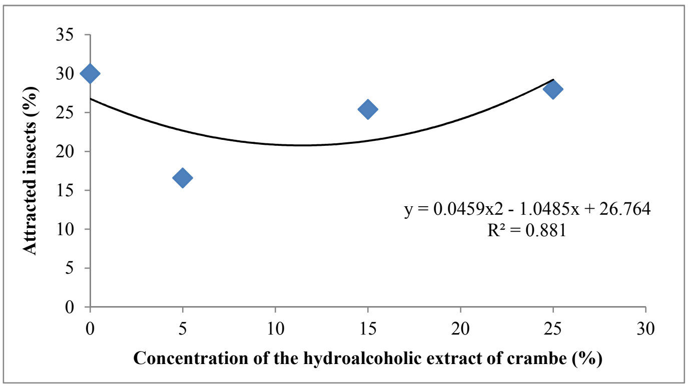

Figure 4. Percentage of *Sitophilus zeamais* attracted to maize kernels treated with different concentrations of hydroalcoholic extract of crambe, after 48 h of exposure in a free-choice feeding arena

Table 3 shows the percentage of germination and normal seedlings, mass of 10 seedlings (g) and seedling length (cm) of maize kernels treated with different concentrations of hydroalcoholic extract of crambe (0, 5, 15 and 15%) under different storage times (0, 30, 60 and 90 days).

The coefficients of variation found in every parameter assessed point to the homogeneity of the data, since the percentage of germination, normal maize seedlings and mass of 10 seedlings (g) presented CV below 10% (3.99, 4.69 and 6.12%, respectively) whereas seedling length had a 14.18% CV. According to Gomes (2002), coefficients of variation up to 10% suggest low heterogeneity, that is, the data is reliable. Ten to 20% represent medium homogeneity.

Table 3. Percentage of germination and normal seedlings, mass of 10 seedlings (g) and seedling length (cm) of maize kernels treated with different concentrations of hydroalcoholic extract of crambe, under different storage times and controlled conditions

Treatment	Germination at 7 days (%)	Normal seedlings (%)	Mass of 10 seedlings (g)	Seedling length (cm)
Storage time (T)				
0 day	88.00b	88.00a	4.48d	3.30c
30 days	92.75a	87.62a	6.03c	12.09b
60 days	91.50a	85.50a	7.64a	16.16a
90 days	91.75a	85.87a	6.77b	16.21a
Extract concentration (C)				
0%	90.00b	84.5b	6.01b	11.22a
5%	91.75ab	87.37ab	6.11b	12.03a
15%	93.50a	90.75a	6.47a	12.10a
25%	88.75b	84.37b	6.33ab	12.40a
CV (%)	3.99	4.69	6.12	14.18
MSD	3.41	3.83	0.36	1.59
F-statistics				
T-factor	*	ns	*	*
C-factor	*	*	*	ns
T * C	ns	*	*	ns

Note. *Significant at 5% probability; ns: non-significant; CV (%) = Coefficient of variation. MSD = minimum significant difference.

Concerning to the germination percentage, there was no interaction between the factors storage time and extract concentration, what shows the independence of the factors analyzed. Regarding the storage time, at 0 day the germination rate (88%) was statistically inferior to those under other storage times, all above 90% and statistically equal.

In relation to the extract concentration, at 15% there was higher germination than at 0% and 25%, despite being statistically equal to the treatment with 5% extract concentration, what demonstrates that the extract stimulates maize seed germination. However, Brow and Morra (2005) reported that plants that have glucosinolates might affect successive crops. Some reports state that the presence of brassicas reduces the establishment of seedlings of different crops. *Brassica napus* straw, for instance, decreases the emergence of wild oat (*Avena sterilis*) and black mustard (*Brassica nigra*) and inhibits the germination of grasses and broccoli.

As for seedling length, there was no interaction among the factors and the extract concentration did not influence this parameter. Such result is in accordance with those of Renosto, Vonz, Paiva, Marostica, and Viecelli (2014), who studied static extracts of crambe at 2.5 and 10% concentration and did not find significant difference in the length of the aerial part of the maize plants.

Storage time significantly influenced length. The longer the kernels exposed to the extracts were stored, the longer the length of the seedlings. Thus, it is concluded that storage time positively influences growing.

Table 4 shows the relationship between storage time and different concentrations of the hydroalcoholic extract of crambe and its effect on the normality of the maize seedlings. Regardless of the storage time, the percentage of normal seedlings was statistically the same. However, if each storage time is analyzed separately, only at 0 day the extract concentration did not influence the percentage of normal seedlings. At 30 and 60 days, the percentage of normal seedlings was higher than in the control only at 15% concentration (94.5% and 91.5%, respectively). Menegusso and Simonetti (2015) studied aqueous extracts made from the root and aerial part of crambe plants and found that at 10% concentration, the number of normal maize seedlings was higher than in the control, however, at higher concentrations (20 and 30%), the number of normal seedlings decreased.

After 90 days of storage, the percentage of normal seedlings was statistically equal to that of the control at every concentration, except at 25%, which was lower (Table 4).

Table 4. Relationship between storage time and concentrations of the hydroalcoholic extract of crambe and its effect on the percentage of normal maize seedlings under controlled conditions

Storage time (T)	Hydroalcoholic extract of crambe concentration (%)			
	0%	5%	15%	25%
0 day	86.0aA	90.0aA	90.0aA	86.0abA
30 days	84.0aB	84.5aB	94.5aA	87.5aAB
60 days	80.5aB	85.5aAB	91.5aA	84.5abAB
90 days	87.5aA	89.5aA	87.0aAB	79.5bB

Note. Same lowercase letter in the same column and same uppercase letter in the same row are not significantly different (Tukey, $P < 0.05$).

Table 5 depicts the relationship between storage time and extract concentration as well as its effect on the mass of 10 seedlings seven days after sowing. At 60 days of storage, regardless of the concentration to which the maize kernels were exposed, the mass of 10 seedlings was higher than in any other storage time, except at 0% and 90 days, which was statistically equal to the mass at 60 days of storage.

The extract concentration did not significantly influence the mass of 10 seedlings at 0 or 90 days of storage, however, it influenced at 30 and 60 days. The best result was achieved at 15% concentration and 30 days of storage. Such results differ from what was found in experiments using crambe hay over maize, in which the researchers reported a reduction in the mass of the aerial part of maize plants (Spiassi, Fortes, Pereira, Senem, & Tomazoni, 2011).

Table 5. Relationship between storage time and concentration of the hydroalcoholic extract of crambe and its effect on the mass of 10 maize seedlings under controlled conditions

Storage time (T)	Hydroalcoholic extract of crambe concentration (%)			
	0%	5%	15%	25%
0 day	4.62cA	4.45dA	4.55cA	4.30dA
30 days	5.62bB	5.75cB	6.85bA	5.90cB
60 days	7.15aB	7.67aAB	7.80aAB	7.92aA
90 days	6.65aA	6.55bA	6.67bA	7.20bA

Note. Same lowercase letter in the same column and same uppercase letter in the same row do not differ among themselves at 5% significance by Tukey's test.

In summary, besides making maize kernels less attractive to *Sitophilus* insects, the hydroalcoholic extract of crambe also improved the germination parameters of the stored maize seeds, what indicates the feasibility of the development of a hydroalcoholic extract of crambe-based product.

4. Conclusions

Considering the proposed goals and the results obtained in this experiment, it is concluded that:

- The hydroalcoholic extract of crambe at 25% concentration applied on maize kernels results on an attractiveness rate (28%) statistically equal to that of the control treatment (30%) but on a higher mortality rate (60%-0%).
- Concerning to the parameters related to the quality of the maize seeds, the hydroalcoholic extract of crambe at 15% concentration yields a higher percentage of germination, normal seedlings, and mass of 10 seedlings than the control. At 25% concentration, the extract do not negatively influence any of the parameters analyzed.
- Storage time above 60 days stimulates germination, mass and length of maize seedlings.

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