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Effects of Crambe-based products (*Crambe abyssinica*) on the control of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize

Ana Paula Morais Mourão Simonetti[1,2]*, Viviane Prata Dall`oglio[2], Tais Regina Strieder[2], Hingrit Mazurek Siqueira[2], Silvia Renata Machado Coelho[1] and Divair Christ[1]

[1]UNIOESTE/CASCAVEL/CCET/PGEAGRI – State University of West Paraná. Rua Universitária, 2069, CEP: 85.819-110 - Bairro Faculdade, Cascavel, Paraná, Brazil.
[2]Centro Universitário Assis Gurgacz, Av. das Torres, 500, CEP: 85806-095 Bairro FAG, Cascavel, Paraná, Brazil.

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Maize is one of the most consumed cereals in the world, but its consumption is not always immediate. Thus, its storage is an important logistical factor. During the storage period, the grains may be exposed to several pests, including *Sitophilus zeamais*. The aim of this study was to test different *Crambe abyssinica*-based products as an alternative control of this pest. The experiment was conducted in the Laboratory of the Assis Gurgacz University Center in Cascavel, PR, Brazil and divided into three stages (survival, emergence and mortality), all arranged in a completely randomized design in a 2×5 factorial scheme. Factor 1 was the type of crambe-based product (crambe powder or aqueous extract) and factor 2 was the product concentration (0, 1, 2, 3 and 4%). The attractiveness, repellency and insecticidal effect were evaluated in the three treatments (control, crambe powder and aqueous extract). It was concluded that between the crambe-based products assessed, the aqueous extract was the one that caused the lowest survival and emergence of *S. zeamais* and the lowest consumption of maize, proving to be repellent at first contact with the insect.

Key words: Maize weevil, plant extract, brassicas.

INTRODUCTION

Brazil is one of the largest producers of maize in the world. In the country, it is cultivated in two crop seasons. Data published by Conab (2016) revealed that the area sown for the 2015/2016 season was equivalent to 15,215,900 hectares, with an estimated yield of 82,327,400 tons of grains and an average production of 5,411 kg per hectare.

Not all harvested grains are used immediately and several tons of grains are stored after harvesting. Improper storage conditions lead to severe pest attacks on stored grains, making them unsuitable for consumption (Michelraj and Sharma, 2006) or for

*Corresponding author. E-mail: anamourao@fag.edu.br. Tel: (55) 45 -99115949.

marketing in Brazil when more than 4% of the grain samples present weevils, as stated in the IN No. 60 (Botelho et al., 2013).

Among the many pests that attack stored grains, *Sitophilus zeamais* stands out for being responsible for the physical deterioration of the stored batch (Lorini et al., 2010). Adult and immature insects feed on the grain and cause large losses of mass, germinating power, seed vigor, as well as nutritional and commercial values of the grain (Santos, 2008).

S. zeamais is considered an internal primary pest, as it attacks whole grains, perforating them and developing within them, and may present cross infestation, that is, infest both the seeds in the field and in the grain storage (Gallo et al., 2002; Lorini et al., 2010).

According to Benhalima et al. (2004), *S. zeamais* control is performed chemically by the application of contact insecticides, which may cause intoxication to applicators and the selection of resistant populations, besides leaving toxic residues on the grains. Kljajic and Peric (2005) also highlighted the increasing cases of resistance developed by some insect species to certain formulations.

Krinski et al. (2014) stated that the use of plant-based products, such as extracts and essential oils, against insects has increased in Brazil. Thus, numerous studies on the alternative control of *Sitophilus* species with the use of plant-based extracts, powders and oils have been developed (Guimarães et al., 2014; Almeida et al., 2014a, b; Fernandes and Favero, 2014; Silva et al., 2013).

Crambe abyssinica Hochst belongs to the botanical family of brassicas or cruciferous. It is native to the Mediterranean region, but is well adapted to different climatic conditions (Souza et al., 2009). Crambe is highly tolerant to pests, which attack the plant only during the seedling stage. Some of these pests are the Cucurbit Beetle (*Diabrotica speciosa*) and *Agrotis* species (Knights, 2002; Bezerra et al., 2011).

Several cruciferous plants have been studied for being sources of chemical protectors, such as glucosinolates, which are capable of synthesizing (Fahey et al., 2001). Chew (1988) states that crambe (*C. abyssinica* Hochst) produces glucosinolate, which is a secondary metabolite that helps plants defend against insects and fungi.

The objective of this study was to assess the repellent and insecticidal effect of *C. abyssinica* Hochst powder and aqueous extract, as well as their effects on the survival, emergence and mortality of weevils (*S. zeamais*) in maize (*Zea mays*).

MATERIALS AND METHODS

This experiment was conducted at the Entomology Laboratory of the Assis Gurgacz University Center in Cascavel, Paraná State, Brazil, at a temperature of 25 ± 2°C, relative humidity of 60 ± 5%. The insects used in the experiment were obtained from the insect farm kept in the laboratory in containers measuring 8 cm of diameter by 6 cm of height containing maize grains (hybrid AM 4003), which were obtained from the company Melhoramento Agropastoril. *C. abyssinica* grains were obtained from experimental fields of FAG's School Farm in the 2014 season and stored away from light and heat.

The crambe grains were ground in a mill (IKA A11 Basic 2500 1/min IP43) to obtain powder. The liquid crambe extract was obtained by grinding the crambe grains in a blender and then mixing 100 ml of distilled water at certain concentrations, being 1 g of grains in 100 ml of distilled water to obtain the concentration of 1%, 2 g in 100 ml of distilled water to obtain the concentration of 2%; 3 g to obtain the concentration of 3% and 4 g to obtain concentration of 4% followed by storage in a beaker with plastic film and aluminium foil for light protection for 48 h.

Effects of the crambe-based products on *S. zeamais* survival, emergence and mortality

The experiments were conducted in a completely randomized design in a 2×5 factorial scheme. Factor 1 was the type of crambe-based product (crambe powder or aqueous extract) and factor 2 was the concentration of the products (0, 1, 2, 3 and 4%). Eight replications were performed of each treatment, totalizing eighty experimental plots.

The treatments were performed with 400 g of maize, which were exposed to 10 ml of the aqueous extract in different concentrations and homogenized in a plastic bag for 2 min. The volume of aqueous extract was based on preliminary tests with dyestuff in different amounts to achieve the maximum coating of the maize grains. The same was performed for setting the number of grams of crambe powder to be used.

The plastic containers were used in the experiment had a diameter of 8 cm and a height of 6 cm. Their covers were perforated for aeration and protected by a tissue to prevent the escape of insects. Each container kept 50 g of maize, weighed in analytical balance, and 20 non-sexed inoculated insects aged up to 15 days.

The number of dead insects was observed daily until the seventh day of experiment. At 40 days of exposure of insects to the grains containing the products, the number of dead and live insects and the mass (g) of the remaining grains were obtained. After these assessments, all insects were discarded and the containers with the grains were kept and analyzed after 60 days of experiment to investigate the number of hatched insects.

Effects of repellency/attractiveness on *S. zeamais* insects

To assess the repellency of the crambe-based products on *S. zeamais*, two feeding stations made of MDF were used, measuring 45×45×3 cm with a central hole with a diameter of 8 cm and four side holes with a diameter of 6 cm each, symmetrically connected by four paths connecting the central and lateral holes with a length of 10 cm each, all with a depth of 2 cm, lined with contact paper and covered with plastic film perforated to allow aeration.

The experiment was conducted in a completely randomized design (CRD) with 3 treatments (T1 - maize grains, T2 - maize grains mixed with crambe powder, and T3 - maize grains mixed with aqueous extract of crambe at 4% concentration) with 10 replications.

Each treatment was tested separately from the control. Ten grams of maize grains were placed in each container of the station. Containers 1 and 2 (control treatments) had only maize grains, and containers 3 and 4 had maize grains mixed with 0.5 g of crambe powder. Just as in the other station, containers 3 and 4 were filled with 10 g of maize grains mixed with 0.5 ml of aqueous extract of crambe, whereas containers 1 and 2 were the control treatments.

Table 1. Number of dead *Sitophilus zeamais* insects, number of emerged insects and mass of maize grains (g) at 40 days; and number of emerged insects at 60 days of exposure to different concentrations of crambe powder and aqueous extract of crambe – Cascavel, PR.

Treatments	Number of dead insects at 40 days	Number of emerged insects at 40 days	Mass of grains (g)	Number of emerged insects at 60 days
Crambe-based product				
Powder	0.75	18.87[a]	48.81[b]	4.59[a]
Aqueous extract	0.95	14.92[b]	49.68[a]	3.02[b]
Concentration				
0%	0.44	15.94	49.66	3.66
1%	0.44	17.12	49.16	3.98
2%	1.62	15.19	48.75	3.99
3%	0.75	17.87	49.25	3.94
4%	1.00	18.37	49.41	3.46
CV (%)	17.92	18.70	2.02	27.79
F-Test				
Type of product × Concentration	n.s	n.s	n.s	n.s
Type of product	n.s	*	*	*
Concentration	n.s	*	n.s	n.s

n.s: Not significant. *Significant at 5% probability by Tukey's test.

Ten non-sexed *S. zeamais* insects were released into the central container of each station. After 1 and 48 h, the number of insects per container was counted.

The Preference Index (PI) for each of the times (1 and 48 h) was calculated using the data obtained in the tests, based on the formula proposed by Procópio et al. (2003): PI = (percentage of insects in treated container) – (percentage of insects in non-treated container) / (percentage of insects in treated container) + (percentage of insects in non-treated container); where PI = -1.00 to -0.10 indicating repellent plant; PI = -0.10 to +0.10 indicates neutral plant; and PI = +0.10 to +1.00 indicates attractant plant.

Contact toxicity assay

The evaluation was carried out in Petri dishes, in a completely randomized design with three treatments: T1 – control (distilled water), T2 – aqueous extract of crambe, and T3 – crambe powder; with 10 replications each, totaling 30 plots.

Each Petri dish was lined with two sheets of germination paper and either 1 ml of distilled water, 1 ml of extract of crambe at 4% concentration or 0.4 g of crambe powder was added. Then, each dish was infested with 10 non-sexed adult *S. zeamais* insects. Dishes were sealed with plastic film perforated for aeration. The 30 dishes were subsequently accommodated in a B.O.D incubator, at 25 ± 2°C temperature, photoperiod of 14 h and relative humidity of 60 ± 5%. At 12 and 24 h, the number of dead insects was recorded.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the averages were compared by Tukey's test at 5% significance level in the statistical software ASSISTAT® version 7.7 (Silva and Azevedo, 2009).

RESULTS AND DISCUSSION

Survival, emergence and mortality of *S. zeamais*

According to the number of dead and live insects at 40 days of exposure to different amounts of crambe-based products (Table 1), there was no significant interaction between factor 1 (type of crambe-based product) and factor 2 (concentration). The number of dead insects was also not significantly influenced (at 5% probability) by the type of product or concentration. In a study by Lima-Mendonça et al. (2013), adult *Sitophilus* spp. insects had 89 and 100% mortality when subjected to powders of *Piper nigrum* L. and *Chenopodium ambrosioides*, respectively. However, the number of live insects after exposure to crambe powder was significantly superior (18.87) to the number of live insects after exposure to the aqueous extract of crambe (14.92), regardless of the concentration, what indicates that insects lived longer in maize mixed with crambe powder than in maize mixed with aqueous extract. Such results agree with those by Silva et al. (2013), who achieved a high mortality rate (95.83%) using hydroalcoholic extract of *Cinnamomum zeylanicum* in a study on the control of *Sitophilus* with different vegetable extracts.

The mass of the maize grains was also significantly higher (49.68 g) when they were mixed with aqueous extract than when mixed with crambe powder (48.81 g), which means that the insects consumed less maize grains mixed with aqueous extract than those mixed with crambe powder. Fazolin et al. (2010) also reported

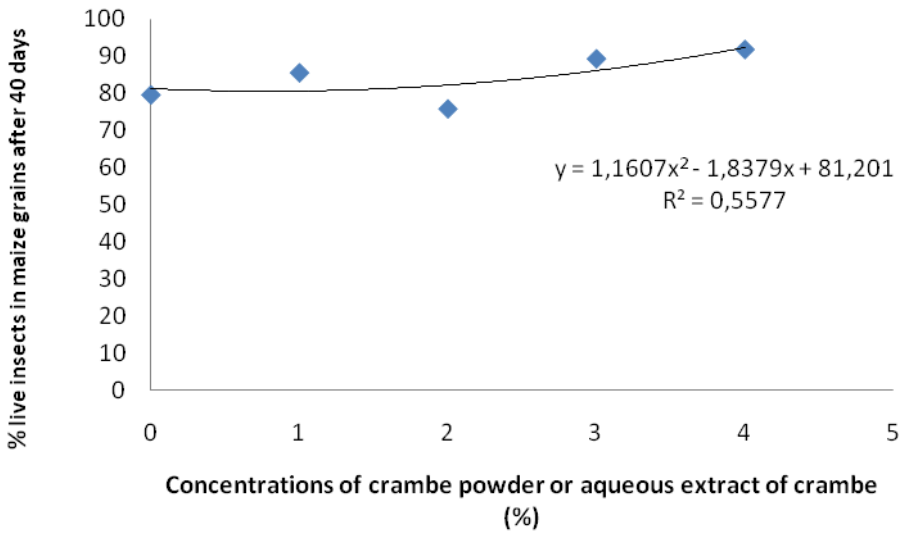

Figure 1. Percentage of live insects in maize grains after 40 days of exposure to different concentrations of crambe powder or aqueous extract of crambe – Cascavel, PR.

significant differences in the mass of maize grains treated with *Tanaecium nocturnum* and exposed to *Sitophilus* in comparison to other treatments.

The coefficient of variation (CV) of the mass of maize grains was 2.02%. According to Pimentel-Gomes (2009) coefficients are low when below 10%, medium when between 10 and 20%, and high when between 20 and 30%.

The number of emerged insects at 60 days was higher in maize grains mixed with crambe powder (4.59) than in maize grains mixed with aqueous extract (3.02), thus, a better condition for egg and larvae development was observed in the treatment with crambe powder.

Coitinho et al. (2010) studied the effects of oils from different vegetable species on stored maize exposed to *Sitophilus* and also reported lower emergence of this insect in maize mixed with *Piper marginatum* than in other treatments.

Figure 1 shows that the percentage of live insects at 40 days was lower in the treatment with 2% concentration (T3) and in the control (T1) than in the other treatments. Therefore, the regression fitted a second order polynomial, with the lowest number of dead insects at 2% concentration.

When using crambe powder, the highest mortality rate at the seventh day of exposure was found with the concentration of 2% (T3), followed by the concentrations of 4% (T5), 3% (T4), 1% (T2) and control (T1). When using aqueous extract of crambe, the highest mortality rate was found with the concentration of 1%, followed by the concentrations of 4, 3 and 2% and control (Figure 2).

Siqueira and Simonetti (2015) obtained 97.5% mortality when *Sitophilus* adults were exposed to maize grains mixed with crambe for 15 days, whereas only 5% mortality was observed for the insects in maize grains without treatment.

Repellency/Attractiveness on *Sitophilus* spp. insects

Only the aqueous extract of crambe after 1 h of exposure provoked repellency on *Sitophilus* adults (Table 2). Crambe powder after 1 and 48 h of exposure as well as the aqueous extract after 48 h of exposure were considered neutral, as their preference indices ranged from -0.10 to +0.10 (Procópio et al., 2003).

Initial repellency superior to that assessed after 48 h was also observed in a study by Pedotti-Striquer et al. (2006) on *Sitophilus* control with vegetable powders of *Ocimum gralissimum* and *Mikania glomerata*, in concentrations of 100 and 400 ppm. However, several different vegetable powders assessed in other researches did not cause repellency on *Sitophilus* spp., such as powders of *Azadirchta indica*, *Melia azedarach*, *Ricinus communis*, *C. ambrosioides* (Procópio et al., 2003), *P. nigrum*, *Anadenanthera colubrina*, *Annona muricata*, among others (Lima-Mendonça et al., 2013).

Gott et al. (2010) concluded that the repellent effect of *Curcuma longa* on *Sitophilus* spp. disappears with time probably due to the evaporation of its compounds in charge of the repellent activity. Such evidence backs up the result of this study, which showed that after 48 h of exposure the crambe extract went from repellent to neutral.

Insecticidal effect

Figure 3 shows the insecticidal effect. The statistical

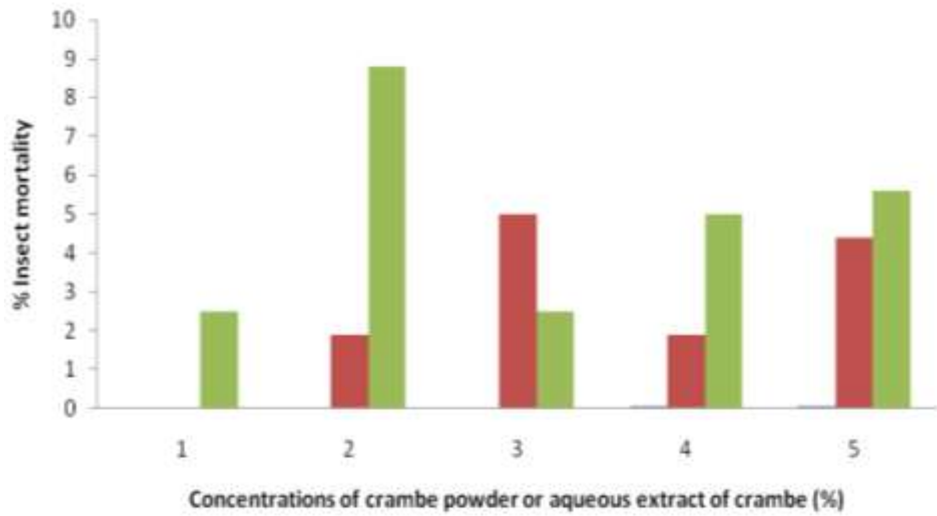

Figure 2. Insect mortality at the seventh day of exposure to different concentrations of crambe powder or aqueous extract of crambe – Cascavel, PR.

Table 2. Comparison of the effect of the repellency of the different crambe-based products on *Sitophilus* sp adults through the Preference Index (PI) and percentage of adults attracted, calculated in free-choice tests with two different exposure times.

Treatment	PI (1 h)	Classification	PI (48 h)	Classification
Crambe powder Control	0.01	N	0.1	N
Aqueous extract of Crambe Control	-0.26	R	0.02	N

PI: Preference index; Classification: N: Neutral, R: Repellent.

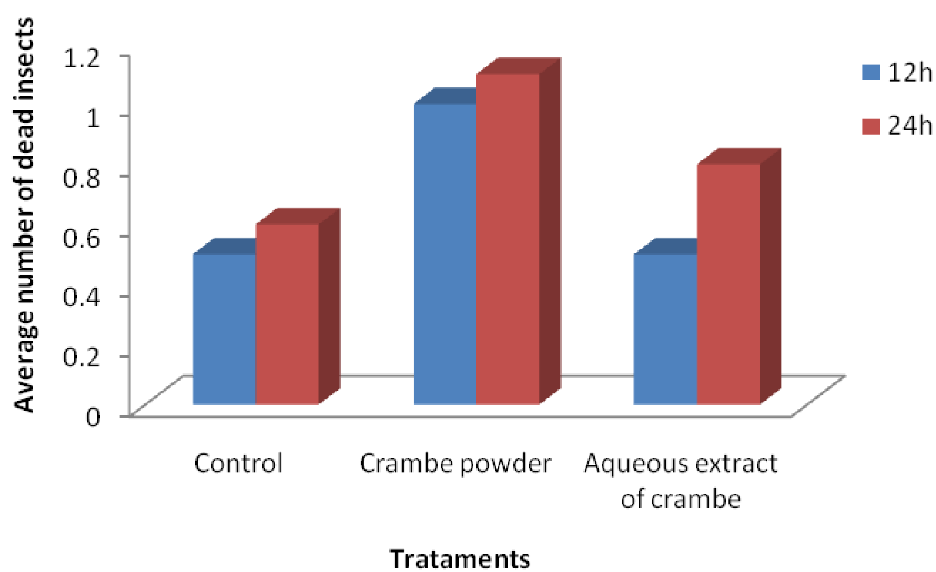

Figure 3. Average number of dead insects after 12 or 24 h of exposure to crambe powder or aqueous extract of crambe, at controlled temperature and photoperiod, Cascavel – PR. 12 h of exposure: CV= 9.67%; 24 h of exposure: CV = 8.86%. n.s: Non-significant at 5% probability by Tukey's test.

analysis did not present significant differences between the control and treatments with crambe powder and aqueous extract of crambe at 5% significance by Tukey's test. The treatment with crambe powder was slightly superior to the others at 12 and 24 h of exposure to the insect.

Krinski et al. (2014) states that several plants have insecticidal capability and can be considered as alternatives in insect control, which was not the case of crambe in the forms of powder or aqueous extract.

Conclusion

It was concluded that between the crambe-based products assessed, the aqueous extract was the one that caused the lowest survival and emergence of *S. zeamais* and the lowest consumption of maize, proving to be repellent at first contact with the insect.

Conflict of Interests

The authors have not declared any conflict of interests.

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