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Original Article

Compounds from Brazil nuts and their effects on *Sitophilus zeamais* (Coleoptera: Curculionidae) survival in bulk ground feed

Compostos de castanha-do-brasil e seus efeitos na sobrevivência de *Sitophilus zeamais* (Coleoptera: Curculionidae) em ração moída a granel

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Abstract

Sitophilus zeamais is a pest in several stored agricultural products, but presents difficulty feeding on Brazil nuts (*Bertholletia excelsa* H.B.K*).* Even when certain bulk bran feeds are unsuitable for its development, it may be found in these products, especially when stored near corn grain. Feed used for young pigs requires special care due to possible contamination, with *S. zeamais* being an important vector for microorganisms, especially fungi. The objective was to investigate aspects related to fractions or compounds present in Brazil nut that can interfere with *S. zeamais* feeding. A corn based commercial feed combined with Brazil nut compounds in different formulations was offered to adults of *S. zeamais* to evaluate the survival of this insect. Treatments consisted of: T1 (control = animal feed (from market) for pigs); T2 (animal feed + *in natura* nuts); T3 (animal feed + 60% added nuts oil); T4 (animal feed + defatted nuts); T5 (animal feed + fixed mineral residue from nuts). The presence of nuts oil was found to reduce *S. zeamais* survival, with high mortality rates, especially in treatments that contained higher concentrations of these compounds, since the raw chestnut also contains high levels of oil. In treatments T1(control = animal feed (from market) for pigs) and T5 (animal feed + fixed mineral residue from nuts), that contain no nut oil, insects survived longer. After elemental analyses, given the dynamics observed in each treatment and their association with the potential toxic effects, none of the compounds present in the respective nut fractions could be associated with insect mortality, leaving only the association with the presence of nut oil in the substrates. Further research into nut oil should are necessary, as it demonstrates potential for the control of *S. zeamais* in bulk animal feed and in stored corn, aiming for the development of sustainable and less toxic alternatives for the management of this pest.

Keywords: *Bertholletia excelsa*, mortality, oil, pest.

Resumo

Sitophilus zeamais é uma praga de diversos produtos agrícolas armazenados e que tem apresentado dificuldades em se alimentar da castanha-do-brasil (*Bertholletia excels* H.B.K). Mesmo as rações não sendo adequadas para o seu desenvolvimento, esse curculionídeo é encontrado nesses produtos farelados a granel, principalmente se estiverem estocadas próximo a depósitos de milho em grãos. As rações utilizadas para alimentar suínos em fase de creche requerem cuidados especiais devido a contaminação e, *S. zeamais* é um importante veiculador de microrganismos, principalmente fungos toxigênicos. O objetivo foi investigar aspectos relacionados as frações ou compostos presentes na castanha que podem dificultar que esse coleóptero se alimente. Uma ração comercial a base de milho e compostos de castanha-do-brasil compuseram substratos com diferentes formulações e essas foram oferecidas à adultos de *S. zeamais* para verificar a sobrevivência desse inseto. Os tratamentos consistiam em: T1 (controle= ração comercial de suínos na fase inicial); T2 (ração + amêndoa *in natura*); T3 (ração mais 60% de óleo da amêndoa adicionado); T4 (ração + amêndoa desengordurada); T5 (ração + resíduo mineral fixo da amêndoa). Foi verificado que a presença do óleo da castanha pode reduzir o tempo de sobrevivência de *S. zeamais*, com taxas de mortalidade elevadas, especialmente nos tratamentos que continham maiores quantidades destes compostos, já que a castanha crua também contém altos níveis de óleo. Nos tratamentos T1 (controle= ração comercial de suínos na fase inicial) e T5 (ração + resíduo mineral fixo da amêndoa) que não apresentam o óleo da castanha os insetos sobreviveram por mais tempo. Após as análises elementares nenhum dos elementos presentes nas respectivas frações da castanha puderam ser associados as mortes devido a dinâmica observada em cada tratamento e, a associação com as presenças de potenciais elementos com efeitos de toxicidade, restando apenas a associação que se observa devido aos percentuais de óleo de castanha nos substratos. Estudos complementares

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com o uso do óleo da castanha precisam ser desenvolvidos, pois esse pode auxiliar no controle de *S. zeamais* em rações a granel e até para milho armazenado visando o desenvolvimento de alternativas sustentáveis e menos tóxica para controle desta praga.

Palavras-chave: *Bertholletia excelsa*, mortalidade, óleo, praga.

1. Introduction

Sitophilus zeamais Mostchulsky, 1985 (Coleoptera: Curculionidae), also known as corn weevil, is a primary pest that attacks grains in the field and warehouses, causing major post-harvest losses worldwide (Lorini et al., 2015). Both adults and larvae feed on the grain mass, causing significant losses in the quantity and quality of the cereal (Ojo and Omoloye, 2012).

Sitophilus zeamais has been reported in the commercial bulk ground for animal rearing (Aquino and Potenza, 2013; Lorini, 2002). This insect stands out for its voracity with resulting economic loss in stored products. It has also been reported as a potential vector for microorganisms such as fungi, some of which are toxicogenic (Aquino and Potenza, 2013; Lorini, 2002). However, *Sitophilus* spp. has difficulty to surive on crushed seeds or flour and adapt better to substrates formed by whole, healthy grains (Sinha and Waters, 1985). A study carried out to verify whether *S. zeamais* is capable of adapting to Brazil nuts (*Bertholletia excelsa* H.B.K) as a food substrate showed that this insect should not be considered a pest for this nut (Pires et al., 2020)

The storage of feed containing primary and secondary pests, associated with toxigenic fungi, represents a potential risk for the emergence of mycotoxins in the substrates, which can affect the health of domestic animals (Aquino and Potenza, 2013; Stringhini et al., 2000).

Infestations the *S. zeamais* can occur in the field (Likhayo and Hodges, 2000; Boiça Junior et al., 1996) and during transportation or storage due to its high capacity to attack grains of various species (Lorini et al., 2015; Gallo et al., 2002) and biotic potential and survival at great deep in the grain masses (Alves and Poltronieri, 2024; Radünz et al., 2024; Antunes et al., 2011; Fragoso et al., 2005; Caneppele et al., 2003; Faroni, 1992). Insects of this species also attack cereal crops such as rice (*Oryza sativa* L.) (Colares et al., 2016; Fontes et al., 2003), sorghum (*Sorghum bicolor* (L.) Moench.) (Link et al., 1985; Waquil and Nakano, 1979) and millet (*Pennisetum glaucum*) (Lale and Yusuf, 2000). However, it is unclear whether they are adapting to the substrate or whether their presence is only on bulk ground for domestic animals.

Among the control methods for *S. zeamais*, the use of alternative products has become increasingly necessary, which is mainly due to the resistance that insects have been developing to conventional chemical and physical control methods, leading to a search for safer alternatives that present fewer environmental impacts (Nta et al., 2024; Paixão et al., 2009).

Studies on insect-plant interactions have been important for the development of strategies for integrated pest management, including the use of bioactive compounds extracted from plants (Pinto-Zevallos et al., 2013), which may affect oviposition, larval development, and mortality (Pinto-Zevallos et al., 2013).

The Brazil nut, a fruit of the chestnut tree, is obtained through extractive practices that occur in the Amazon Biome (Thomas et al., 2015). Its nuts have high concentrations of lipids (60-70%), proteins (17%) and minerals of high nutritional value, and are also an important source of selenium and unsaturated fatty acids (Chunhieng et al., 2004; Freitas and Naves, 2010; Yang, 2009). Therefore, it is considered a valuable product, both for its nutritional properties and components of high biological value (Balbi et al., 2014; Colpo et al., 2013; Berno et al., 2010; González and Salas-Salvado, 2006). These almonds are rich in bioactive compounds, such as phenols, flavonoids and saponins, which have antioxidant, anti-inflammatory and antimicrobial activities (Costa and Jorge, 2011).

One of the aspects of swine production that most influences the final result is feeding. In pig farming, bulk grund have mainly corn (*Zea mays* Linnaeus) and soybeans (*Glycine max* (L.) Merrill) however, as *commodities*, the producer cannot control their price, which can affect overall production costs (Alves et al., 2023). Studies have shown that nutrition may account for up to 80% of animal production costs (Rostagno et al., 2017), and the quality of the ingredients that make up the feed is directly linked to the animal's nutritional performance, influencing palatability and, consequently, the animal's acceptance of the product.

Corn is considered the main energy source when feeding monogastric animals. It is also the main component of pig feed (Rostagno et al., 2017). One of the problems faced in grain production and storage units is maintaining quality while conserving these products, since several insect species use grains as a food source, causing significant losses during production (Santos, 2008).

Therefore, given the need to establish new sustainable alternatives for the control of *S. zeamais* in stored products, the objective was to investigate which compounds present in Brazil nuts are capable of reducing the lifespan of *S. zeamais* and the response when added to commercial bulk mash feed for pigs.

2. Material and Methods

Commercial mashed animal feed or early-stage pigs, purchased from a local store in the municipality of Sinop, state of Mato Grosso, Brazil, was used as a substrate for rearing *S. zeamais*. According to the manufacturer, basic feed composition includes folic and pantothenic acids, biotin, calcitic limestone, choline and sodium chlorides, ethoxyquin, rice and soybean bran, beef and bone meal, halquinol, calcium iodate, ground whole corn, manganese monoxide, niacin, zinc oxide, calcium propionate, sodium selenite, ground whole sorghum, copper and iron sulfates, vitamins A, B1, B12, B2, B6, D3, E, K3, and antioxidant additive.

Table 1. Proportions of the ingredients that composed the food substrates with adult *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae).

Treatments*	Pig feed	Nut *in natura*	Nut oil	Defatted nut	Nut mineral residue
T1	100.00%	-	-	-	-
T2	50.00%	50.00%	-	-	-
T3	62.50%	-	37.50%	-	-
T4	50.00%	-	-	50.00%	-
T5	96.70%	-	-	-	3.30%

*T1 (commercial pig feed); T2 (pig feed + nuts in natura); T3 (pig feed + 60% nut oil); T4 (pig feed + defatted nut); T5 (pig feed + fixed mineral residue from nut).

Different Brazil nut (*Bertholletia excelsa*, H.B.K.) kind were added to the feed (substrate): crushed *in natura* nuts, nut oil, defatted nut, and mineral fraction of the nut. Fractions for the treatments were determined based on the amount that each ingredient (nut oil, defatted nut, and mineral residue of the nut) naturally appear in the Brazil nut, with the exception of T2, which used 50% feed and 50% crushed raw nuts, as the study was carried out with the natural product in which *S. zeamais* showed similar survival times between the treatments in which the insects were given Brazil nuts as a food source and those without food (Pires et al., 2020).

The *in natura* nuts (shelled and dried) were purchased commercially in Sinop, Mato Grosso, and blended (Oster, Ninja) for fractionation. The samples were subjected to bromatological analysis (Silva and Queiroz, 2006), where the following fractions were observed: moisture, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein, ether extract and ash.

To extract the oil from the *in natura* nuts, 5 g of crushed nuts were placed in filter paper cartridges. The cartridges were then placed in an oil extractor (MARCONI, MA 044/8/50) with 100 mL of petroleum ether P.A. for four hours after the start of boiling. The extracted oil fraction was placed in an exhaust cabinet for six hours to evaporate the ether and then placed in a forced air circulation at 55-65 °C for 4 hours to remove residual ether. The defatted almond fraction that was removed from the filter paper cartridges and placed in oven forced air circulation at 55-65 °C for 4 hours to dry. The oil obtained was subjected to fatty acid profile analysis in a gas chromatograph coupled to a mass spectrometer (GC-MS) (Agilent Technologies, model 6890A/5975C).

The fixed mineral residue was obtained from crushed *in natura* nuts and placed in 50 mL porcelain crucibles, which were initially placed in an oven forced air circulation at 105 °C for 24 hours to remove all the water of the material. The crucibles containing the dry samples were then placed on a hot plate to burn the organic fraction. Following this, the material was placed in a muffle furnace at increasing temperatures, starting at 150 °C, with an increase of 50 °C every 30 minutes until reaching 600 °C, remaining for four hours to completely burn the material and leaving only the ashes with the mineral fraction (Silva and Queiroz, 2006).

To determine which and how many minerals are present, all Brazil nut fractions (*in natura* nuts, nut oil, fixed mineral residue of nut) and also the animal feed (from market) were analyzed through X-ray fluorescence and the results expressed in the form of oxide in Malvern Panalytical equipment, model Epsilon4.

Each Brazil nut presentation was added to the substrate (feed) to evaluate the effect of these compounds on *S. zeamais* survival. The mixtures were defined as follows, based on the percentages naturally found in the nut: nuts *in natura* 1:1 (substrate:nut); nut oil 1:0.6 (60% oil); defatted nut 1:1 and nut mineral fraction 1:0.033 (3.3% ash). Thus, five treatments were established, as follows: T1 (commercial pig feed); T2 (pig feed + nuts *in natura*); T3 (pig feed + 60% nut oil); T4 (pig feed + defatted nut); T5 (pig feed + fixed mineral residue from nut) (Table 1).

The experiment was carried out under laboratory conditions at an average room temperature of 25.7 °C. Each treatment was evaluated based on 25 replicates, mounted in transparent plastic Petri dishes with lids, 90 mm in diameter and 15 mm in height. Each dish received ± 0.2 g of each mixture (Procópio et al., 2003) and 10 unsexed, adult *S. zeamais* specimens were released, obtained from mass rearing of the "Laboratório de Pragas e Vetores da Amazônia - Cerrado (LAPVAC)" of the Federal University of Mato Grosso, Campus of Sinop.

Insect mortality was observed daily, until the final death was recorded for all treatments, with mortality being analyzed by the accumulated frequency of dead insects throughout the experimental period (Equation 1):

$$\bar{X} = \sum_{i=1}^{n} Xi.fi \tag{1}$$

where: $\bar{X}$ = mean days alive; Xi = each evaluation day; fi = number of live insects on each day of evaluation.

Aspects related to the equally of survival times between treatments were tested by the Log-rank, Wilcoxon and Tarone Ware tests, based on a chi-square distribution. The populations were compared pairwise, through their respective survival curves from the Kaplan-Meier analysis with $\alpha < 0.05$ in which the frequencies of live insects per day and survival were analyzed, expressed as means.

3. Results

The survival of the insect was different between the treatments analyzed, according to the chi-square test using three different estimators: Log-rank (V= 656.84;

α< 0.0001), Wilcoxon (V= 675.18; α< 0.0001) and Tarone-Ware (V= 688.90; α< 0.0001).

According to the Kaplan-Meier test for multiple comparisons, it was possible to observe that only T2 and T3 presented similar insect survival rates (V= 7.841; α =0.050). For all other pairwise comparisons, differences were observed for *S. zeamais* survival throughout the evaluation period.

The first report of the mortality to *S. zeamais* fed only with animal feed (T1) and feed + fixed mineral residue from nuts (T5) occurred after day five (Figure 1) with 1 and 13 dead individuals, respectively. On the other hand, mortality was observed for all other treatments already on day two of evaluation. Insects fed on animal feed + 60% nut oil (T3) presented the highest mortality, with 76 dead individuals on the second evaluation day, representing 30% mortality, with 70% of the population remaining (Figure 1). Mortality for T2 and T3 on day two was observed at 5 and 12 individuals, respectively. On day five of evaluation, insects fed on animal feed + defatted nuts (T4) and animal feed + nuts *in natura* (T2), presented 35 and 40% mortality, respectively (Figure 1).

Only insects fed onT1 and T5 presented mortality greater than 50% of the population after 10 days of evaluation, which were reached on day 11 and 10, respectively. In treatments T2 and T4, 50% of the populations had died by day 7, and in T3 this value was reached on day 3 of evaluation.

Average *S. zeamais* survival with T2, T3 and T4 did not exceed 10 days, with values of 7.74 ± 0.25, 6.48 ± 0.33 and 9.19 ± 0.29 days, respectively. Only T2 and T3 showed similar survival and these were the shortest observed (Table 2). In the control (T1) and T5 these values were 19.50 ± 0.47 and 17.56 ± 0.39 days, respectively (Table 2).

The bromatological analysis of Brazil nut and animal feed (from market) showed lipid concentrations (ether extract) of 65.43 and 1.23, respectively. The former was 53 times higher than that observed for the feed-based substrate (Table 3); however, the contribution of animal feed to the protein supply for insects is approximately twice that of the nuts (Table 3). The ash content in the almonds is lower than that observed for the animal feed (Table 3), which results in higher mineral concentrations in the animal feed. The starch or sugar content (non-fibrous carbohydrates) was lower than the detection limit of the method used for Brazil nut almonds and was over 40% in the feed.

The highest percentage of fatty acids found were unsaturated, with emphasis on linoleic (43.73%) and oleic (28.72%) acids, followed by palmitic acid (13.38%), the latter being a saturated fatty acid (Table 4).

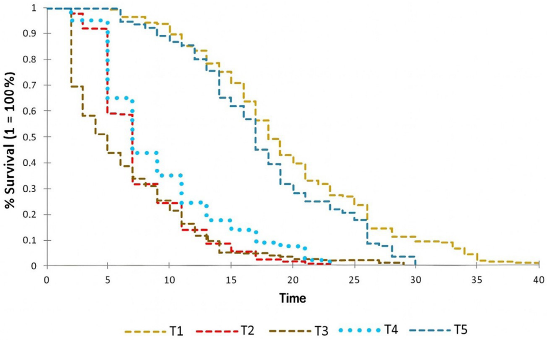

Figure 1. Survival distribution function of *Sitophilus zeamais* (Coleoptera: Curculionidae) fed with different food substrate combinations T1 (commercial pig feed); T2 (pig feed + nuts *in natura*); T3 (pig feed + 60% nut oil); T4 (pig feed + defatted nut); T5 (pig feed + fixed mineral residue from nut).

Table 2. Test of equality of distribution functions for *Sitophilus zeamais* survival (Coleoptera: Curculionidae).

Treatments*	Survival (Days) (Mean ± Standard Deviation)
T1	19.50 ± 0.47 A
T2	7.74 ± 0.25 D
T3	6.48 ± 0.33 D
T4	9.19 ± 0.29 C
T5	17.56 ± 0.39 B

*T1 (commercial pig feed); T2 (pig feed + nuts in natura); T3 (pig feed + 60% nut oil); T4 (pig feed + defatted nut); T5 (pig feed + fixed mineral residue from nut). Means followed by the same letter in the column do not differ by the analysis of multiple comparisons using the Kaplan-Meier test with α < 0.05.

Table 3. Nutritional composition of Brazil nut and animal feed.

Parameter	Brazil nut (%)	Pig feed (%)
Humidity	2.64	11.74
Fiber	12.71	4.99
Protein	15.71	31.19
Lipids (ether extract)	65.43	1.23
Ashes	3.5	10.42
Non-fibrous carbohydrate (NFC)	-	40.43

Table 4. Fatty acid profile of Brazil nut oil.

Component	Concentration (%)
Linoleic acid	43.73
Oleic acid	28.72
Palmitic acid	13.38
Stearic acid	12.33
Elaidic acid	1.15
Arachidonic acid	0.316
Palmitoleic acid	0.152
erucic acid	0.081
behenic acid	0.071
cis-11-eicosanoic acid	0.068

Table 5. Mineral composition (ppm) of animal feed, *in natura* nuts, nut oil, defatted nut and nut mineral residue of *Bertholletia excelsa* (Ericales: Lecythidaceae).

Treatments*	Animal Feed	*In natura* nut	Nut oil	Defatted nut	Nut mineral residue
MgO	5.700	4.200	-	9.060	103.700
SiO_2	4.000	-	46.20	-	-
P_2O_5	32.200	19.400	3.230	39.210	286.500
SO_4	13.000	19.000	888.70	38.130	1.300
Cl	6.100	1.600	160.10	2.880	1.100
K_2O	34.500	27.200	79.80	55.060	183.200
$CaCO_3$	109.100	26.900	1.740	52.500	134.100
Fe_2O_3	2.300	300.00	-	585.40	1.300
Br	-	1.110	-	683.50	122.60
SeO_2	-	225.40	-	130.60	-
BaO	-	3.600	-	3.190	-
CrO_3	29.40	-	-	-	-
MnO_2	397.90	-	-	-	739.50
CuO	133.50	237.70	-	281.90	748.90
ZnO	922.60	443.20	-	478.10	1.530
Rb_2O	38.00	-	-	298.90	1.580
SrO	148.40	2.650	-	1.940	11.050
SnO_2	77.30	160.90	-	-	105.80
TiO_2	162.70	-	-	-	-
NiO	-	-	-	-	54.90

*T1 (commercial pig feed); T2 (pig feed + nuts in natura); T3 (pig feed + 60% nut oil); T4 (pig feed + defatted nut); T5 (pig feed + fixed mineral residue from nut).

Elemental analyses of the animal feed, raw nuts and nuts mineral residue showed that, among the minerals found, bromine, with 1,110.00 ppm; selenium, with 225.4 ppm; and barium, with 0.365%, were the elements that stood out for being present in raw almonds. Only the animal feed did not contain bromine, which occurred in greater proportions in raw almonds with values 9.05 and 1.62 times higher than in almond ash and defatted almond, respectively (Table 5). The last two, selenium and barium, were not observed in animal feed or in almond ash (Table 5).

4. Discussion

The variable *S. zeamais* survival rates observed may be due to the substrate formulations that were offered in each treatment as a food source and their nutritional particularities. The animal feed used as a control substrate due to being a product of commercial interest that is attacked by the genus *Sitophilus* (Rodrigues et al., 2014), has high corn content. The presence of this grain, which makes up about 70% of the final product and is rich in carbohydrates, provides energy and is an important nutrient source for various animals (Rostagno et al., 2017).

Considering the treatments in which *S. zeamais* presented lower survival (T3=T2, T4), these are associated with the presence of the organic fraction found in Brazil nut, which reduced survival time. This can be explained by the presence in the nuts of essential organic compounds (Yang, 2009), which can affect insect survival times. Certain organic compounds can cause mortality in insects, through the bioactivity that occurs in plants, with these compounds being produced through primary or secondary metabolism, and may have insecticidal, repellent or antinutritional properties. Thus, when insects feed on plants, these compounds can cause toxicity, inhibit growth or reproduction, and affect the nervous system, leading to insect mortality (Rahman et al., 2023; Pinto-Zevallos et al., 2013).

Another effect of Brazil nuts in the survival of *S. zeamais* was observed when fed with this nut as the only food source, it was found that these insects could not be considered pests of this product, since no damage on the almond surface indicative of feeding was observed and the lifespan of the insects exposed to the food was similar to that of insects without food source (Pires et al., 2020).

The first reports of *S. zeamais* mortality for T1 and T5 were on days 5 and 6, respectively. This may be due to almond ash, the only ingredient presents in the substrate beyond the animal feed, having no effect on the first occurrences of insect mortality when compared to those maintained on the substrate containing only animal feed (T1). This may be due to there being only inorganic compounds in materials that were subjected to the oven process at high temperatures for extended periods of time (Silva and Queiroz, 2006).

Treatments T2, T3 and T4, which presented insect mortality on day 2, had formulations combining animal feed with another ingredient as a variable way of offering the nuts. It is known that the Brazil nut has on average, 66% lipids, 14% proteins and 12% carbohydrates in its composition (Yang, 2009) and, in these treatments, these compounds are and/or may be preserved. Proteins and carbohydrates are found in all grains according to the Brazilian food table (UNICAMP, 2011) in which *Sitophilus* sp. has also been reported. Brazil nut oil, however, presents characteristics in its lipid composition, such as the presence of bioactive substances in the oil obtained through the extraction method used (Carvalho et al., 2022; Kluczkovski et al., 2021). This may favor non-acceptance by *S. zeamais* of food substrates with traces of this oil.

The nuts *in natura* or its oil combined in the mixture, as found in T2 and T3, respectively, may have favored the death of the specimens. Treatment T4, which combined animal feed as substrate and defatted nuts, also presented insect mortality on day 2, which may suggest that there are oil traces in the mixture. This may be the result of incomplete removal of the oil from the material defatted using the organic solvent extraction method with petroleum ether in Goldfish equipment, which does not guarantee complete oil removal (Zanqui et al., 2020).

The treatment T3 presented the highest day 2 mortality rate, may be the treatment where the insect is unable to separate the feed fraction from the oil ingredient due to its being homogenized in the mixture. This may explain why 76 individuals were observed dead on day 2, in contrast to 5 and 12 for T2 and T4, respectively. This high mortality rate suggests that significant oil concentrations may produce adverse effects on insects, possibly related to the quantity of bioactive compounds present in foods, such as unsaturated fatty acids (linoleic and oleic) (Carvalho et al., 2022). However, vegetable oils contain fatty acids that are bioactive against several pests, and these phytochemicals manifest their effects on insects in several ways, such as toxicity, repellence, feeding deterrence, fumigation, growth inhibition, suppression of reproductive behavior, and reduction of fecundity and fertility (Singh et al., 2021; Rajashekar et al., 2012).

The 50% mortality indicator for the populations of treatments T1 and T5 that occurred after day 10 of evaluation and the observations of this indicator for treatments T2, T3 and T4 that were 7, 3 and 7 days, respectively, tend to confirm the hypothesis of ingredient selection by *S. zeamais* in its feeding behavior, since this beetle was unable to select only oil-free animal feed in treatment T3, which may have happened in treatments T2 and T4, which are by contrast unhomogenized (T3). The exposure time to the compounds of Brazil nuts may be a determining factor in the mortality of *S. zeamais*. In a study that evaluated the effect of inert powders on *S. zeamais*, an increase in mortality was observed in all treatments as the duration of exposure of the insects to the substrates increased (Greco et al., 2024).

The average survival times for all treatments followed the same dynamics as those observed for treatments with 50% mortality, but with increased values. This suggested that the oil, when offered in a way that the insect had greater difficulty in selecting the substrate ingredients, led to shorter survival times, different from the results observed for T1 and T5, which presented substrates without organic compounds originating from Brazil nuts in their mixture.

According to all the parameters observed through the bromatological analysis, the higher lipid concentrations present in the nuts were most significant, being 53 times greater than those observed in only animal feed. In all treatments except T1 and T5, probably there is almond oil in the mixture, which could be associated with insect mortality. It should be assumed that T1, whose substrate is solely animal feed, contains no nuts oil in its formulation, and T5, which consists of animal feed (from market) and mineral residue of nuts, also contains no oil, since the ash production process subjects the material sample to temperatures of 600 ºC for 4 hours, which is capable of eliminating all oil residues and organic compounds from the substrate (Silva and Queiroz, 2006). The other parameters evaluated and their differences observed between Brazil nuts and feed are commonly found in these proportions of their respective occurrences in grains when stored in optimal to precarious conditions, and which, in these cases, present *S. zeamais* population growth (Frazão et al., 2018; Antunes et al., 2011).

In addition to the organic compounds observed in nuts, which include high lipid concentrations, unsaturated fatty acids, proteins, fibers and minerals such as phosphorus, calcium, magnesium, potassium, zinc, manganese, copper and selenium are also found in this product (Silva et al., 2010; Yang, 2009). Elemental analysis observed the presence of bromine, selenium and barium, which are elements that can interfere with living organisms at certain quantities and can even be used as pesticides, especially the former when used via fumigation (Lima et al., 2019; Cunha and Machado, 2004; Kim et al., 2003), and their occurrence patterns agree with the phenomena observed in the survival functions of *S. zeamais*. These elements were not observed in animal feed, which was the treatment where the insects presented the longest survival time.

Bromine, in particular, is an element used in formulating many insecticides whose action relies on contact and/or ingestion, and can also have a fumigant action as in the case of methyl bromide (Fehn, 1970; Bacchi and Zink, 1962), but it can also be used in several other products such as pharmaceuticals, disinfectants, etc. (Gutiérrez-Sánchez et al., 2021; Santos, 1987). Bromine, which is a volatile element, could affect insect survival rates, since it was found in other treatments beyond just the control. In the raw nuts, this element was observed in the highest concentration compared to the mineral residue and the defatted nuts, which were approximately 9.05 and 1.62 times greater, respectively, which could lead to insect mortality. However, the fumigant action mechanism in which the bromine element could be acting does not support the hypothesis of possible mortality by volatilization of this element in modified atmosphere conditions, since total *S. zeamais* mortality for all treatments was greater than 20 days, and this phenomenon disqualifies the effect of mortality by volatilization (fumigation) of bromine.

Selenium and Barium are elements considered toxic at elevated concentrations (Lima et al., 2019; Cunha and Machado, 2004). However, in considering *S. zeamais*, they would be effective in these insects only in cases of ingestion, which could have occurred, since in both the treatment T2 (animal feed + nuts *in natura*) and the treatment T4 (animal feed + defatted nut) the insects could select their feed, so as not to ingest nut particles but rather only feed particles and therefore, survive a little longer. In the treatment T3, the possibility of avoiding ingestion of nut residues due to the ability to select what was ingested, was more difficult, given that the oil, when homogenized with animal feed (from market), adheres to solid particles which makes access by the insect to the feed alone free of oil, difficult, leading to a shorter survival time.

The absence of selenium, barium and bromine in almond oil does not support the hypothesis that mortality is conditioned mainly by the effects of these three elements, since T3 was the one that used the oil in animal feed (from market), and this treatment presented the shortest survival time, together with T2 that used feed + nuts *in natura* and, furthermore, T3 had the highest mortality on the second day of evaluation.

After analyzing the treatments and the effects of all elements on *S. zeamais* survival, the hypothesis that the elements selenium, barium and bromine would be the main cause of insect mortality can be discarded. On the other hand, almond oil, because of its higher concentration of unsaturated fatty acids, in which its presence can provide the production of bioactive compounds, such as terpenes (Costa and Jorge, 2011), which can affect insect development (Bernklau et al., 2016; Fenigstein et al., 2001), and which is present in T2, T3 and T4, being in smaller quantities in the latter, affected *S. zeamais* survival.

5. Conclusion

Organic compounds derived from Brazil nuts have insecticidal potential against *Sitophilus zeamais*, especially those present in the fat fractions. The fat-soluble components of Brazil nuts affect the survival of these insects, possibly through physical and/or chemical mechanisms. In addition, the effect observed in the defatted fraction indicates that other bioactive compounds, present in both the oil and the defatted residue, may also be involved in insect toxicity, while their absence in the ashes points to the irrelevance of certain inorganic elements such as selenium, barium, and bromine in this context.

These findings reinforce the potential of using Brazil nut by-products as a natural and sustainable alternative for pest management in stored products. Further studies are recommended to identify the active compounds responsible for the insecticidal action and evaluate their effectiveness under different storage conditions, with a view to developing less toxic and environmentally safe technologies for the control of *S. zeamais*.

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Data Availability Statement

All data are available to the journal and can be requested from the corresponding author any time.

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