

Original Article

Effect of inert dust on the mortality of *Sitophilus zeamais* (Coleoptera: Curculionidae)

Efeito de pós inertes na mortalidade de *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract

The maize weevil, *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), generally reaches pest status in stored grain. Chemical control is the most used method for population suppression, which can cause adverse impacts, thus creating a need for alternatives such as using inert powders. The present work aims to verify the effect of different concentrations of different types of inert powders on the mortality of *S. zeamais* in the laboratory. To this end, the experiments were carried out in a completely randomized design, with 13 treatments and four replications, ten adults per replication, where the effect of different inert powders (basalt powder, gypsum powder, and diatomaceous earth) was tested at concentrations of 0.025 g, 0.05 g, 0.1 g and 0.2 g/20 g of corn grains. Variance, normality, and homoscedasticity tests were applied in addition to controlling efficiency (CE%), median lethal time (TL50), and survival curves. All treatments caused mortality in *S. zeamais*, and all concentrations with diatomaceous earth were more efficient, with 100% mortality at 20 days, followed by the treatment of 0.2 g of gypsum powder/20 g of corn grains, with superior efficiency, to 95% in 20 days and 100% in 30 days. The results indicated that treatments with diatomaceous earth had the highest mortality rate and the best average survival time.

Keywords: beetle, corn, alternative control, gypsum.

Resumo

O gorgulho-do-milho, *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), geralmente atinge o status de praga em grãos armazenados. O controle químico é o método mais utilizado para sua supressão populacional, podendo causar impactos adversos, surge assim a necessidade de alternativas como o uso de pós inertes. O presente trabalho tem como objetivo verificar o efeito de diferentes concentrações de diferentes tipos de pós inertes na mortalidade de *S. zeamais* no laboratório. Para tanto, os experimentos foram realizados em delineamento inteiramente casualizado, com 13 tratamentos e quatro repetições, 10 adultos por repetição. Onde testou-se o efeito de diferentes pós inertes (pó de basalto, pó de gesso e terra diatomácea), nas concentrações de 0,025 g, 0,05 g, 0,1 g e 0,2 g/20 g de grãos de milho. Sendo aplicados testes de variância, normalidade e homocedasticidade, além de eficiência de controle (CE%), tempo letal mediano (TL50) e curvas de sobrevivência. Todos os tratamentos causaram mortalidade em *S. zeamais*, e todas as concentrações com terra diatomácea foram mais eficientes com 100% de mortalidade aos 20 dias, seguido do tratamento de 0,2 g de pó de gesso/20 g de grãos de milho, com eficiência superior a 95% aos 20 dias e 100% em 30 dias. Os resultados indicaram que os tratamentos com terra diatomácea apresentaram a maior taxa de mortalidade e a melhor média em termos de tempo de sobrevivência.

Palavras-chave: besouro, milho, controle alternativo, gesso.

1. Introduction

The production of corn grains in Brazil is forecast at 116.1 million tons in 2024 (IBGE, 2024). The good results of the agricultural output of stored grains are directly linked to the adoption of best storage, production, and protection practices, which are essential to guarantee the viability of production. This prevents losses after harvest and the product's devaluation (Lorini et al., 2015; Mussalama et al., 2023).

Among the pests of stored grains is *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), considered the main pest of stored corn grains (Trematerra et al., 2013). This insect damages grains due to its ability to penetrate deeply into its mass to feed. After ovipositing, its larvae develop inside the grain (Nwosu, 2018), causing losses in final production and having high biotic

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potential (Lorini et al., 2015). In addition to favoring the production of mycotoxins from pre-existing fungi in corn grains (Ferreira-Castro et al., 2012) and contributing to the emergence of secondary pests, increasing the rate of damage to the final product (Alencar et al., 2011). This can result in post-harvest losses of around 15% in the field, 13 to 20% during grain processing, and between 15 and 25% in storage (Manandhar et al., 2018).

Chemical control with synthetic insecticides has been the most used method to control pests such as corn weevils (Kim et al., 2019). Fumigant insecticides are the most applied in Brazil (Jagadeesan et al., 2018; Ayres et al., 2021; Ortega et al., 2021). However, several factors negatively impact its use, such as favoring the emergence of insect resistance to chemicals. Currently, weevils of the genus *Sitophilus* have been reported to exhibit moderate and robust resistance to pyrethroid-based insecticides (Singh et al., 2021).

Insect management alternatives are being studied and introduced in Brazil (Finkler, 2012). Inert powders are a method that was already used in family farming even before the emergence of synthetic insecticides (Lorini, 1998). Among the types of inert powders, diatomaceous earth stands out, as studies have shown that corn grains without diatomaceous earth control, when infested by the corn weevil, can, in two months, go from a type 1 quality classification (presenting fewer grains with defects) for a low standard classification to be sold (Antunes et al., 2013). However, diatomaceous earth generates some unwanted impacts on grains related to the interference of their physical and mechanical characteristics (Korunic et al., 2020). Therefore, the milling industry is reluctant to treat grains with diatomaceous earth due to its abrasive nature and possible damage to the machine (Losic and Korunic, 2018). Therefore, studies are needed to deepen knowledge about different types of inert powders. Thus, the present work aims to verify the effect of various concentrations of different inert powders on the mortality of *S. zeamais* in the laboratory.

2. Material and Methods

The study was conducted at the Laboratory of Insect Ecology (LAbEI) Institute of Biology of the Federal University of Pelotas, Campus Capão do Leão, RS (IB - UFPEL). Corn grains (*Zea mays*) were purchased through local trade. These were previously sieved and disinfested after being frozen for seven days at -4 °C. They were then kept in glass containers covered with Voil fabric for ten days until reaching their hygroscopic balance, thus avoiding infestations of any pre-existing pest.

To obtain insects of the same age, 20 unsexed adult insects were placed in new glass containers containing corn grains for 15 days. Then, the corn grains with possible oviposition were separated to wait for the emergence of new specimens.

The experiment was carried out in a completely randomized design with 13 treatments, four replications, and four concentrations (Table 1). Each sample unit was composed of a transparent plastic container (60 mL), the

Table 1. Treatments with basalt powder, gypsum powder, and diatomaceous earth at concentrations of 0.025 g, 0.05 g, 0.1, and 0.2 g/20 g of corn grains and a control group used in an experiment to control *Sitophilus zeamais* in the laboratory.

Treatments	Concentrations (g/t)	
Basalt	T01	250
	T02	500
	T03	1000
	T04	2000
Gypsum	T05	250
	T06	500
	T07	1000
	T08	2000
Diatomaceous earth	T09	250
	T10	500
	T11	1000
	T12	2000
Control	T13	00

lid having a circular opening sealed with Voile fabric and 20 g of corn kernels.

Each experimental unit comprised 20 g of corn kernels ($\pm 12\%$ moisture content). Each concentration of treatments was stirred for two minutes, and ten adult weevils were placed in the containers (± 14 days old) (Jairoce et al., 2016). The insects in the grain mass were kept in a B.O.D. (Biochemical oxygen demand) under temperature of 28 ± 3 °C, relative humidity of $30 \pm 10\%$, and photophase of 12h, the same conditions previously provided for the creation of weevils in the laboratory. The insect mortality analysis was carried out over 30 days every 24 hours after application. Insect mortality was quantified, which was verified when the insect showed no movement for two minutes upon stimulation with a soft, fine-tipped brush (Antunes et al., 2013).

2.1. Statistical analysis

Initially, the mortality data were submitted to the Shapiro-Wilk normality test and Bartlett's homoscedasticity of variances. Kruskal-Wallis non-parametric analysis of variance (ANOVA) with Dunn with post hoc Bonferroni correction ($P < 0.05$) was used for data that did not meet the assumptions of normality and homogeneity of variances, even after transformations, by "Easyanova" and "Dunn. test" packages of R 4.0.0 software (R Development Core Team, 2020).

The mortality data, in percentage (%), of the treatments and the control, were also used to calculate the percentage of corrected mortality (%) [or control efficiency (%)], using the mortality correction formula of Schneider-Orelli (count of dead insects; the uniform population of insects in treatments) $\{[MC\% = (\%M\text{Trat} - \%M\text{Test}) / (100 - \%M\text{Test}) * 100]$; where MC is the corrected mortality (%) as a function of the control, MTrat is the mortality (%) observed in the

treatment, and MTest is the mortality (%) observed in control} (Püntener, 1981).

In addition, to evaluate the survival over time (days) of the weevils exposed to the treatments, aiming at calculating the median lethal time (LT50), Kaplan-Meier estimators (Log-Rank method) and survival curves were used. Were compared using the Holm-Sidak test ($P < 0.05$) using the SigmaPlot 12.3 software (Systat Software, San Jose, CA, USA).

3. Results

Most treatments (basalt, diatomaceous earth, and gypsum) differed from the control group (Table 2). At ten days after application, the treatments that most differed from the control group were T9 (0.025 g of diatomaceous earth/20 g of corn), T10 (0.05 g of diatomaceous earth/20 g of corn), T11 (0.1 g of diatomaceous earth/20 g of corn) and T12 (0.2 g of diatomaceous earth/20 g of corn), with efficiencies from 81.08 to 97.30%. However, treatments T1 (0.025 g of basalt/20 g of corn), T2 (0.05 g of basalt/20 g of corn), T3 (0.1 g of basalt/20 g of corn), and T5 (0.025 g of gypsum/20 g of corn) are equal to the control group, showing low efficiency.

At 20 days after application, treatments T8 (0.2 g of gypsum/20 g of corn), T9 (0.025 g of diatomaceous earth/20 g of corn), T10 (0.05 g of diatomaceous earth/20 g of corn), T11 (0.1 g of diatomaceous earth/20 g of corn) and T12 (0.2 g of diatomaceous earth/20 g of corn) differed more from the control group, with efficiencies ranging from 97.30 to 100%. At 30 days after application, all treatments differed from the control, with efficiencies from 63.64 to 100%.

Treatments with diatomaceous earth had the highest mortality rate and the best average survival time. Among the treatments with gypsum powder, the concentration of 0.02 g/20 g of corn kernels (T8) caused the highest mortality in the shortest exposure time after the treatments related to diatomaceous earth, differing statistically from the other treatments.

There was a statistical difference between the mortality curves; all tested treatments showed significantly higher mortality than the control (Figure 1).

In ten days of exposure, a control efficiency above 50% was achieved for the treatment with 0.2 g of gypsum in 20 g of maize (T8) in maize grains and an efficiency superior to 80% for all treatments related to diatomaceous earth.

Around 20 days, it was possible to observe a control efficiency greater than 50% for all treatments related to basalt powder, except for T1 (0.025 g/20 g of corn) with the lowest dosage; for the gypsum powder, all reached a percentage greater than 50% and the treatment with the highest dosage (T8 - 0.2 g/20 g of corn) reached a percentage greater than 80%, and 100% of control efficiency for the treatments with diatomaceous earth, except for T10 (0.05 g/20 g of corn), with 97.3%, but without statistical discrepancy between the others. At the end of the evaluation with 30 days of exposure, it was possible to reach a percentage greater than 80% for all treatments, except for T1 of 0.025 g of basalt powder in 20 g of corn

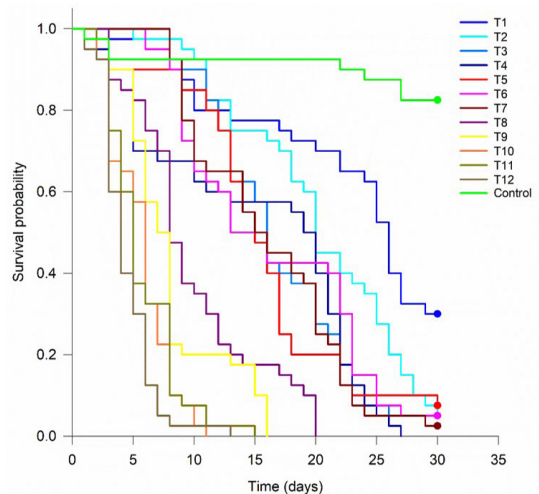


Figure 1. Survival curves of *Sitophilus zeamais* exposed to treatments with different doses of basalt powder, gypsum, and diatomaceous earth in the laboratory. Caption: T1- 0.025 g of basalt powder in 20 g of corn kernels (250 g/t); T2- 0.05 g of basalt powder in 20 g of corn kernels (500 g/t); T3- 0.1 g of basalt powder in corn kernels (1,000 g/t); T4- 0.2 g of basalt powder in 20 g of corn kernels (2,000 g/t); T5- 0.025 g of gypsum powder in 20 g of corn kernels (250 g/t); T6- 0.05 g of gypsum powder in 20 g of corn kernels (500 g/t); T7- 0.1 g of gypsum powder in 20 g of corn (1,000 g/t); T8- 0.2 g of gypsum powder in 20 g of corn kernels (2,000 g/t); T9- 0.025 g of diatomaceous earth in 20 g of corn kernels (250 g/t); T10- 0.05 g of diatomaceous earth in 20 g of corn kernels (500 g/t); T11- 0.1 g of diatomaceous earth in 20 g of corn kernels (1,000 g/t); T12- 0.2 g of diatomaceous earth in 20 g of corn kernels (2,000 g/t) and T13- Control (without added products).

grains, which reached a percentage greater than 60%, not differing statistically from the other treatments, despite not achieving a control efficiency above 80%, it is still an interesting percentage to test its influence on natural enemies in the future, leaving open as a possible alternative for use in the field to reduction of the pest insect population.

The average survival time (TL50) of weevil adults differed significantly between treatments (GL 12; X2 541.94; $P < 0.001$). Accounting for about 20 days for the treatments with basalt powder, the treatment of 0.2 g/20 g of corn (T4) reached the best average (15.07 days). The average for treatments with gypsum powder was 9 to 16 days, with the treatment of 0.2 g/20 g of corn (T8) reaching the best average, covering 9.82 days. For diatomaceous earth, the average was 4 to 8 days, with the best average related to the treatment of concentration 0.2 g/20 g of corn (T12) with 4.47 days (Table 3).

4. Discussion

All treatments tested caused mortality in *S. zeamais*. However, in relation to basalt dust, the present study differs from the report by Jairoce et al. (2016), where, after 21 days of exposure, all treatments already differed from the control group, reaching mortality rates above 80%.

Table 2. Mean mortality (m) ± standard error (SE) and efficiency of control (E%) of *Sitophilus zeamais* exposed to different dosages of basalt dust, gypsum, and diatomaceous earth in maize grains.

TREATMENTS	10 DAYS		20 DAYS		30 DAYS		
	m ± SE*	E%**	m ± SE	E%	m ± SE	E%	
Basalt	T1 (0.025 g/20 g)	2.00 ± 1.41c	13.51	3.00 ± 1.68b	24.32	7.00 ± 1.73a	63.64
	T2 (0.05 g/20 g)	0.75 ± 0.48c	0.00	5.75 ± 1.93ab	54.05	9.50 ± 0.50a	93.94
	T3 (0.1 g/20 g)	1.00 ± 0.00c	2.70	7.25 ± 2.06ab	70.27	9.50 ± 0.87a	93.94
Plaster	T4 (0.2 g/20 g)	3.75 ± 2.39bc	32.43	6.00 ± 1.83ab	56.76	10.00 ± 0.00a	100.00
	T5 (0.025 g/20 g)	1.50 ± 1.19c	8.11	8.00 ± 1.08ab	78.38	9.25 ± 0.48a	90.91
	T6 (0.05 g/20 g)	3.50 ± 1.44bc	29.73	5.75 ± 1.93ab	54.05	9.50 ± 0.29a	93.94
Diatomaceous earth	T7 (0.1 g/20 g)	3.25 ± 1.65bc	27.03	7.50 ± 1.32ab	72.97	10.00 ± 0.00a	100.00
	T8 (0.2 g/20 g)	6.50 ± 0.87ab	62.16	9.75 ± 0.25 ^a	97.30	10.00 ± 0.00a	100.00
	T9 (0.025 g/20 g)	8.25 ± 0.85 ^a	81.08	10.00 ± 0.00a	100.00	10.00 ± 0.00a	100.00
Control	T10 (0.05 g/20 g)	9.75 ± 0.25 ^a	97.30	10.00 ± 0.00a	100.00	10.00 ± 0.00a	100.00
	T11 (0.1 g/20 g)	9.25 ± 0.48 ^a	91.89	10.00 ± 0.00a	100.00	10.00 ± 0.00a	100.00
	T12 (0.2 g/20 g)	9.75 ± 0.25 ^a	97.30	10.00 ± 0.00a	100.00	10.00 ± 0.00a	100.00
	T13 - (0.00)	0.75 ± 0.25c	--	0.75 ± 0.25b	--	1.5 ± 0.63b	--
GLTrat; Error		12; 39	--	12; 39	--	12; 39	--
X2		35.39	--	35.29	--	32.07	--
P		<0.001	--	< 0.001	--	<0.013	--
CV%		48.20	--	34.92	--	13.19	--

E% = Control efficiency according to the Schneider-Orelli formula. Mean number of dead individuals ± standard error ("SE"). Kruskal-Wallis non-parametric analysis of variance with post-hoc Dunn-Bonferroni correction (P<0.05). Means (±SE) in each column followed by the same letter are not statistically significant.

Table 3. Mean mortality time (TL50, in days) of *Sitophilus zeamais* exposed to different concentrations of basalt dust, gypsum, and diatomaceous earth in corn kernels.

TREATMENTS		AVERAGE TIME (CI95%)
Basalt	T1 (0.025 g/20 g)	22.57 (20.00-25.14)b
	T2 (0.05 g/20 g)	20.15 (18.05-22.24)bc
	T3 (0.1 g/20 g)	16.87 (15.10-18.65)c
	T4 (0.2 g/20 g)	15.07 (12.47-17.68)c
Plaster	T5 (0.025 g/20 g)	15.57(13.43-17.72)c
	T6 (0.05 g/20 g)	16.32(14.06-18.58)c
	T7 (0.1 g/20 g)	16.00(14.10-17.90)c
	T8 (0.2 g/20 g)	9.82 (8.22-11.43)d
Diatomaceous Earth	T9 (0.025 g/20 g)	8.05 (6.80-9.29)de
	T10 (0.05 g/20 g)	5.57 (4.82-6.32)f
	T11 (0.1 g/20 g)	5.70 (4.83-6.56)of
Control	T12 (0.2 g/20 g)	4.47 (3.80-5.14)f
	T13 (0.0)	- (-)a
	GL	12
	X2	541.94
	P	< 0.001

The log-rank statistic for the survival curves is greater than expected by chance; there is a statistically significant difference between survival curves ($P < 0.001$). A multiple comparison procedure is used to isolate the group or groups that differ from the others. Multiple Comparisons: All pairwise multiple comparison procedures (Holm-Sidak method). Means in each column followed by the same letter are not statistically significant.

However, at a dosage of 2,000 g/t after 29 days, mortality was greater than 80%, corroborating our results for the same dosage (Jairoce et al., 2016).

The main component of basalt powder is silicon dioxide (Jairoce et al., 2016), which can break the waxy layer of insects' epicuticles (Subramanyam and Roesli, 2000). As a result, it is expected that the use of basalt powder will penetrate the superficial wax layer of the insect, breaking the layer of lipids that protects it and finally resulting in the insect's death due to the organism's loss of liquids (Jairoce et al., 2016).

The composite gypsum from gypsum rock has interesting characteristics such as rapid hardening, mechanical strength, and adherence (Aragão, 2005). Gypsum treatments showed efficiencies close to diatomaceous earth treatments, as dosages of 0.1 and 0.2 g of gypsum per 20 g of corn kernels did not differ statistically from diatomaceous earth treatments. Gypsum at low concentrations is also effective in killing bean weevil, *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae) (Carvalho, 2008), similar to the results of our study. It was observed that larvae of the Lepidoptera species *Apodemia* longer fed on plants covered with gypsum powder have a 4.8 times greater tendency to die than control larvae in a study carried out to evaluate the response of these endangered larvae; it concluded that

gypsum powder is capable of directly affecting populations of Lepidoptera larvae through mortality, as well as reducing development rates about low weight and prolongation of their development (Osborne and Longcore, 2021).

In another research, the average lethal time using gypsum powder was evaluated in *Diabrotica duodecimpunctata*, known as cucumber beetles, and thus reported that gypsum powder was more lethal when compared to bentonite clay and less lethal when compared to kaolin (Richardson and Glover, 1932). However, when they diluted gypsum and clay powder, they found it lethal for termites (Wagner and Ebeling, 1959).

Recently, a study exposed the larval and adult stages of the beetle *Callosobruchus maculatus* to gypsum dust for three days, showing a mortality rate of 8.4 to 16.9% for larvae and 38.4 to 43.7% for adults on filter paper (Sulaiman and Obaid, 2019). Previously, it was reported that sprinkling *Apodemia virgulate* Lepidoptera adults with gypsum powder from the factory adjacent to the National Wildlife Refuge at Antioch Dunes resulted in a three-day reduction in survival after capture relative to butterflies that were not exposed to gypsum powder (Clause et al., 2015).

Mortality of *S. zeamais* increases with increasing exposure time in all treatments used in this study. In addition to the increase in the applied dose, it reduces the insect's exposure time until death occurs. Corroborating studies on the control of *S. zeamais* when using other alternative products (Radünz et al., 2024). Diatomaceous earth stood out among basalt and gypsum powders, with treatments reaching 100% mortality in the first days, as observed by Jairoce et al. (2016), where the dosage of 2,000 g/t had 100% mortality on the fifth day of exposure. Despite the high mortality at diatomaceous earth concentrations with good mean lethal time (TL50) in this study, it differed from other studies. For Ribeiro et al. (2018), using the same species of weevil in their research, the average lethal time it was ranged between 1.86 and 2.18 days with concentrations of 0.1 and 0.2 g of diatomaceous earth for 20 g of corn (1,000 and 2,000 g/t), while for this study the oscillation in these concentrations was between 4.47 and 5.70 days.

Dosages of 0.1 and 0.2 g of diatomaceous earth/20 g of corn were higher than 90% after ten days of exposure and 100% after 20 days, corroborating other studies involving the same dosages with humidity of 12% (Antunes et al., 2011, 2013). The 500 g/t dosage of diatomaceous earth also drew attention, as in the study by Ceruti et al. (2008), who observed 90% mortality of *S. zeamais* with this concentration. The lowest diatomaceous earth concentration (0.025 g/20 g) provided mortality more significant than 80% after ten days of evaluation, indicating that the lowest concentration used has good efficiency (Riedo et al., 2010). Currently, other studies continue to prove the efficiency of diatomaceous earth, such as, for example, research with three different types of products based on improved diatomaceous earth, carried out in Ghana in Africa, where it was reported that the product InsectoSec® caused more mortality in adults of *S. zeamais*, followed by Fossil Shield and Diatomenerde Probe-A (Adarkwah et al., 2022).

According to Garcia (2014), it is recommended that a product has at least 80% efficiency in controlling a pest; in this sense treatments with diatomaceous earth T9 (0.025 g/20 g of corn), T10 (0.05 g/20 g of corn), T11 (0.1 g/20 g of corn) and T12 (0.2 g/20 g of corn) at ten days, adding to the previous treatments for the 20 days T8 (0.2 g of gypsum in 20 g of corn) and at thirty days, all treatments tested except T1 of 0.025 g of basalt powder in 20 g of corn (63.64%) reached this recommendation level. This can be considered a quick result, considering that, for example, female maize weevils can survive an average of 140 days, of which 104 are oviposition days. Mortality in this time frame can significantly reduce the number of new infestations, reducing the time for females to perform new oviposition (Lorini and Schneider, 1994).

The three types of dust (basalt, gypsum, and diatomaceous earth) at certain concentrations become efficient in controlling *S. zeamais*. With the passage of time and increased concentration, this efficiency tends to increase. However, the treatments with diatomaceous earth were the ones that presented the best cost-benefit ratio since its toxicity is already known, followed by the treatment with 0.2 g/20 g of gypsum powder in corn, which has a very low cost to acquire. However, its content of toxicity to non-target beings is still not completely clarified. With all this, research must continue to be carried out to improve the investigation of the application logistics so that the producer can safely practice using these substances as an alternative to control insect pests of stored grains.

5. Conclusions

The results show that the mortality of *Sitophilus zeamais* increases according to the period in which the insect is exposed to different types of inert dust. The highest mortality rates were obtained in the period of 11, 21, and 28 days with the application of diatomaceous earth (T10 - 0.05 g/20 g of corn), gypsum powder (T8 - 0.2 g/20 g of corn) and basalt powder (T4 - 0.2 g/20 g of corn), respectively. Therefore, these three treatments can be used as an effective control for *S. zeamais*.

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