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Efficacy of alternative and biological insecticides on *Sitophilus zeamais* (Coleoptera: Curculionidae) in corn grains

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ABSTRACT
The study aimed to evaluate the efficiency of alternative and biological insecticides on the population of Sitophilus zeamais in corn grains. The research was conducted in a completely randomized design, containing five treatments, diatomaceous earth (750 g/t), neem oil (10 ml/L), Beauveria bassiana (20 g/L) and smoke (10 ml/L), and nine replicates, each assay consisting of 20 adults. To evaluate the insecticidal activity, samples of corn grains were placed in plastic pots submitted to treatments. The insecticides were sprinkled and sprayed on the corn grains and later, each sample was infested with 20 adults of Sitophilus zeamais, unsexed, aged between 10 and 15 days. Insect mortality was evaluated at 48, 96, 144 and 240 h after application. The total mortality of the insects was corrected by the formula of Schneider Orelli. Afterwards, the data were submitted to the nonparametric Kruskal-Wallis test at a significance level of 5%. With significant differences between treatments, the medians were compared by Dunn's test at 5% probability of error. Only the insecticide diatomaceous earth showed a significant effect on the mortality of Sitophilus zeamais for the third day (5.56%) and the sixth day (84.21%) of exposure of the product. The treatments neem, smoke and Beauveria bassiana, did not interfere in the mortality of the insect, not differing statistically from the control in any of the evaluation dates. The diatomaceous earth product showed higher efficiency on Sitophilus zeamais, reaching a percentage of 89.77% of mortality on insects.

Keywords: Corn weevil, Integrated pest management, Stored grains.

1 INTRODUCTION

Brazil is currently considered one of the largest grain producers, with emphasis on the corn crop (Embrapa, 2022). The estimate of the 2022/2023 harvest indicated that Brazilian production is 125.8 million tons. The high production and productivity of the crop are allied to technology, seed improvement, use of pesticides and balanced fertilization (Conab, 2022).

After harvesting, the grains are destined for grain storage units, where they will be deposited in silos. During storage, this product is maintained for better conservation, since insect pests use the grain as a food source to maintain its life cycle (Alves et al., 2008; Garcia-Lara & Bergvinson, 2007; Lorini et al., 2015).

Among the main insect pests existing in silos, *Sitophilus zeamais* (Coleoptera: Curculionidae) popularly known as corn weevil or weevil, it presents significant obstacles for stored grains, especially for corn (Caneppele et al., 2010; Elijah & Oliveira, 2009; Frazão et al., 2018).

This insect is considered a primary pest because it affects whole grains internally or externally, according to the phase in which the insect presents itself. As a consequence of this, internal pests pierce the grain, feed on its interior, oviposit and after hatching survive inside the grain. In addition, they have cross-infestation and the ability to survive at great depths in the grain mass (Copatti et al., 2013; Ferrari Filho et al., 2011; Gallo et al., 2002; Pimentel et al., 2011; Norambuena et al., 2016; Procopius et al., 2015).

These insects, in high populations, cause qualitative and quantitative losses in the stored grain mass, devaluing the final product, causing partial or total loss of production (Lorini et al., 2015). The losses caused by the damage of the *Sitophilus zeamais* they reach 10% of the production already stored. However, data indicate that 10% of corn grains are already infested with the crop, intensifying the infestation during storage, increasing losses by up to 50% of the product by the insect (Antunes et al*.,* 2011; Norambuena et al., 2016; Silva et al., 2013; Suleiman et al., 2015).

For its control, the most viable method used in silos, for preventive and curative management, has been the use of chemical pesticides, with steaming insecticides and management with purges, because they have easy application and are effective, however, they present high toxicity to those who apply, in addition to contaminating the environment, eliminating natural enemies and selecting resistant insects (Lorini et al*.,* 2015; Magalhães et al*.,* 2015).

Due to these problems, there were studies on natural insecticides in the solution of post-inerts, and among the products tested, the diatomaceous earth stood out with efficiency on the insect, mainly by deteriorating the wax layer on the wing of *Sitophilus zeamais* causing death by desiccation (Jairoce et al., 2016; Korunic, 2013; Marsaro Júnior et al., 2013). In addition to this insecticide, extracts and vegetable oils such as nicotine (*Nicotiana tabacum*), neem (*Azadirachta indica*), eucalyptus (Eucalyptus *citriodora) showed significant efficacy,* since they cause a decrease in the insect population without causing human intoxication and leaving residues in the grain mass (Gomes & Favero, 2011; Ootani et al., 2011; Vedovatto et al., 2015).

These products of plant origin present bioactivity against insects, characterized as behavioral or physiological. In addition, plant products have various active compounds, scientifically proven as insecticides, having attractive action or as a repellent, acting on the nervous system, changing its development and acting in reducing the feeding of insects (Albuquerque et al., 2013; Bacci et al., 2015; Campos et al., 2015; Smith, 2017; Isman, 2017; Jesus et al., 2013; Lima-Mendonça et al., 2013). Based on this context, the present study aimed to evaluate the efficiency of alternative and biological insecticides on the mortality of *Sitophilus zeamais* in corn grains.

2 METHODOLOGY

The experiment was carried out in the Entomology laboratory of the Educational College of Medianeira – UDC Medianeira. For the breeding *of Sitophilus zeamais,* adult insects were collected in stored corn grains from the 2020/21 harvest in storage silos located in a Cooperative of the municipality of São Miguel do Iguaçu - Paraná.

As a substrate for feeding and maintenance of insects, corn grains removed from the grain mass were placed in plastic bags and exposed for 48 h in a freezer at a temperature of -10 ºC, with the purpose of eliminating any insect infestation. Subsequently, they were kept at room temperature until they returned to the temperature preceding freezing (Silva-Aquayo et al., 2006).

After the corn grains returned to the relative humidity of 13% and the stable temperature (25 ºC), they underwent a selection in order to remove grains with perforations, cuttlefish, plastered or moldy leaving only grains with type 1 classification quality according to Normative Instruction No. 60 of the Ministry of Agriculture (Mapa, 2016).

At the end of the food selection stage, 250 g of corn grains were arranged in 20 plastic containers with a capacity of 500 mL. Subsequently, 20 adult insects of *Sitophilus zeamais,* not sexed, were infested in each container, sealed with thin tissue (organza), facilitating aeration and sealed with elastic. The insects were confined in the BOD at a temperature of 25 ± 2 ºC, relative humidity of 60 ± 5% and photophase of 12 h for a period of 15 days for reproduction, forming the F1 generation.

At the end of the confinement period, the adult insects were removed from the containers and the grains were kept in BOD for 34 days, until the emergence of the insects to conduct the experimental trials (Almeida et al., 2013; Carneiro, 2019; Oak, 2019; Vilarinho, 2012; Wenneck et al., 2020).

The experiment was conducted in an air-conditioned BOD chamber in temperature of 25º ± 2ºC, relative humidity of 60 ± 5% and photophase of 12 hours, following or a completely randomized design (IHD), with six treatments (insecticides + control) (Table 1) and nine replications, totaling 54 experimental plots.

Table 1 - Treatment, common name, concentration and dosage used in the experimental trials.

Treatment	Common name	Trade name	Concentration	Dosage
Witness	-	-	-	-
Bacillariophyta	Diatomaceous earth	Keepdry®	86% m/m	750 g/t
Azadirachta indica	Before	Original Nim Fertile Base	1%	10 mL/$^{L-1}$
Beauveria bassiana	Mushroom	Boveril®	50g/kg	20 g/$^{L-1}$
Nicotiana tabacum	Smoke	Vitaplan liquid smoke	-	10 mL/$^{L-1}$

Source: Prepared by the authors (2021).

The biological and alternative insecticides were purchased in agricultural stores and cooperatives. All insecticides were applied in the experimental plots according to the dosages recommended in the product package leaflet (Table 1). To evaluate the insecticidal activity, samples of sterilized and healthy corn grains, in an amount of 50 grams, were arranged in plastic pots of 250 ml (sample units) submitted to the treatments, in their respective dosages (Table 1).

The liquid insecticides were sprayed on the corn grains with the aid of a pressurized spray bottle and the inert diatomaceous earth powder was sprinkled manually. The mixture of the grains with the products was carried out manually, in plastic bags with a capacity of two liters, which were shaken for two minutes. Subsequently, each sample was infested with 20 adults of *Sitophilus zeamais*, unsexed, aged between 10 and 15 days from rearing. At the end of the experimental trials, all containers were sealed with organza fabric and kept in the BOD (Ribeiro & Vendramim, 2019, adapted).

Insect mortality was evaluated 48, 96, 144 and 240 h after the application of the products. In each evaluation, the live insects were returned for their respective replications. The experiment was maintained without insect re-infestation. It was considered as dead, the insect that did not perform any movement for five minutes (Ribeiro & Vendramim, 2019).

The total mortality of the insects was corrected in relation to the mortality observed in the control, according to the Schneider formula Orelli (Püntener, 1981). Afterwards, the data were checked for the normality of the distribution of the residues by the Shapiro-Wilk test, and for the homogeneity of the variance of the errors by the Cochran test, both at 5% probability of error.

Due to the absence of normality of distribution of the experimental error and the homogeneity of the variances of the errors, the data were submitted to the nonparametric test of Kruskal-Wallis at the significance level of 5%. With significant differences between treatments, the medians were compared by Dunn's test at 5% probability of error, using the statistical software Past (Hammer et al., 2001).

3 RESULTS AND DISCUSSION

In the evaluation of the insecticidal activity on the corn grains, it was verified that on the third and sixth day after the application of the products, only the insecticide diatomaceous earth showed a significant effect on the mortality of *Sitophilus zeamais* (Table 2). For the same dates, the other treatments tested did not interfere in the mortality of the insect, since they would not differ statistically from the control (Table 2).

Table 2 - Mortality percentages of insects of *Sitophilus zeamais* underwent nonparametric analysis using the Kruskal-Wallis test, based on medians, and multiple Dunn comparisons.

Treatment	**Day 3 Mortality**	**Day 6 Mortality**	**Day 8 Mortality**	**Day 10 Mortality**
Witness	0 b1	0 b	0 a	0 a
Diatomaceous Earth	5,56 a	84,21a	0 a	0 a
Before	0 b	0 b	0 a	0 a
Smoke	0 b	0 b	0 a	0 a
Beauveria bassiana	0 b	0 b	0 a	0 a
H P	6,522 0,00020	23,63 0,0000001865	0,3916 0,5461	0,8863 0,4569

1Medians followed by the same letter in the column do not differ from each other by Dunn's test at 5% probability.

Source: Prepared by the authors (2021).

The chemical treatment containing *Bacillariophyta* (diatomaceous earth) presented high mortality (84.21%) for the corn weevil on the sixth day of evaluation, differing statistically from the other treatments (Table 2). This significant mortality of insects is linked to the efficiency of diatomaceous earth, since this product has silica in its composition (Lorini & Galley, 2001).

The inert dust of diatomaceous earth comes from fossils of diatomaceous algae that naturally contains silica in its composition, and its mode of action is by contact (Lorini & Galley, 2001). Dust particles are absorbed by the insect integument leading to the removal of the wax layer of the cuticle that is adsorbed by silica generating water reduction, and consequently dehydrating to death. In addition, diatomaceous earth can still have a repellent effect and influence on the mobility and locomotion of insects (Lorini et al., 2015; Santos, 2016).

The land use of diatoms has been reported in studies to control *Sitophilus zeamais*, mainly due to its non-toxicity, which makes it a sustainable product (Korunić, 2013; Lorini & Galley, 2001). Analyzing four dosages of diatomaceous earth, 250, 500, 750 and 1000 g/t, for the control of *Sitophilus zeamais*, a mortality rate of 95% was verified in 750 and 1000 g/t for the sixth and ninth day of treatment (Marsaro Junior, 2007), mortality higher than that found in the present study (Table 2). The dosages of 250 and 500 g/t obtained the same percentage of mortality of 95%, but on the 13th and 27th days of treatment, stating that mortality was directly influenced by the dosages and time of exposure of the insecticide to insects (Marsaro Junior, 2007), divergent data observed in this study (Table 2).

In order to evaluate the diatomaceous earth in the control *of Sitophilus zeamais* as an alternative method, at a dosage of 5 g/kg, an efficiency of 100% of the product was verified after 144 hours of exposure to the insect (Carvalho, 2019), corroborating the results of this research (Table 2).

In a study conducted by Embrapa corn and sorghum, with a dosage of 1.0 kg/t, diatomaceous earth caused 87% mortality *Sitophilus zeamais* after 30 days of application, causing 91.1% after 100 days of exposure of the product to the insect (Pimentel et al., 2019). With a mortality rate of 99%, the application of diatomaceous earth was also efficient in adults of *Sitophilus zeamais* in grains of wheat, barley and paddy rice (Wille et al., 2013). However, for this same study, in corn grains, the mortality recorded for the insect was 88.2% after 10 days.

After 14 days of the application of 1 and 3 g of diatomaceous earth in corn grains, for control of *Sitophilus zeamais,* mortality of 40% and 90% was recorded, respectively (Toledo, 2016). Already, at the dosage of 250 g/t diatomaceous earth recorded only 13% mortality over *Sitophilus zeamais* (Riedo et al., 2010).

When the mortality of the insects for the treatments based on neem, tobacco and the biological fungus *Beauveria bassiana was* evaluated, no difference was observed between all the treatments tested for the control in all the dates of evaluation (Table 2). Scientific studies have reported the use of neem acting in the control of the insect for different forms, such as ecdysis, control of metamorphosis, feeding deterrence, biology and oviposition (Silva, 2009). The active ingredient of neem, azadirachtin, still has a repellent effect, acts on the central nervous system of the insect, by its neurotoxic action, affecting insects by ingestion or contact (Menezes, 2005).

One of the hypotheses of neem did not have a significant effect on the mortality of *Sitophilus zeamais* for this study (Table 2) it is possibly related to the concentration of product used and the elapsed time of treatment. In this context, it was observed that neem oil, at a concentration of 2 L/t, presented 100% mortality of *Sitophilus zeamais* up to 60 days after the application of the product (Gottardi, 2014), divergent data found in this research (Table 2). The efficiency of neem for the study was attributed to the sensitivity of the product to photodegradation, since the neem molecules degraded slowly, since the product has a formulation of 99% vehicle and 1% active principle, so the volatilization that would happen in pure oil did not occur, leaving the product more conserved and acting for longer (Gottardi, 2014).

Still, diverging from the results of this study (Table 2), a study found a mortality rate of 60% for *Sitophilus zeamais*, with a dosage of 5% of neem with ashes, during the period of ten days (Barilli et al., 2014). According to the authors of the paper, The effectiveness of the treatment was due to the interference of the product in the feeding of the insect causing mortality of the insects by starvation, a characteristic that was not observed for the present research.

With the aim of testing the effectiveness of neem extract on *Sitophilus zeamais*, one study analyzed different dosages of aqueous plant extract at concentrations of 10, 20 and 30.3%, resulting in mortality of 44% at concentration of 30.3% (Borsonaro et al., 2013). Achieving a low level of efficiency, the neem oil treatment used for control of *Sitophilus zeamais* in corn grains, it accounted for 26.4% of mortality in 100 days of conducting the experiment (Pimentel et al., 2019). Although low, the mortality reported in the study was still higher than in this study (Table 2).

The tobacco-based product also did not show efficiency in the mortality of *Sitophilus zeamais* (Table 2). The active ingredient nicotine present in smoking has a mode of action on insects on contact or ingestion, presenting toxic actions in various concentrations (Santos et al., 2019). Termed as an alkaloid, nicotine can cause paralysis by affecting the nervous system, in addition to acting as an acetylcholine antagonist, binding its receptors to insect cells (Santos et al., 2019). Possibly the non-efficiency of smoking in the mortality of corn weevils, for this study (Table 2) may be related to the ability of nicotine to volatilize easily, not lasting with greater intensity, and in addition, due to the dosage of the product used (Menezes, 2005).

Aiming to evaluate the control rate of *Sitophilus zeamais* Through the aqueous and hydroalcoholic extract of tobacco, in the dosage of 1 ml for both extracts, it was verified mortality of 24.17% for the aqueous extract and 66% for the hydroalcoholic extract after 72 hours of exposure to the insect (Madaloss, 2015).

In research using the tobacco extract, in the concentration of 25%, to control *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) mortality of 4.31% (Jacomini et al., 2016). Using the tobacco extract, in rice grains it did not present significant mortality for *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Silva et al., 2015), similar results were found for this study (Table 2).

Treatment using the fungus *Beauveria bassiana* showed no insecticidal effect on *Sitophilus zeamais* (Table 2). The fungus-based product (Boveril®) also showed low efficacy on *Sitophilus zeamais* (13.8%) at dosages of 10 and 20 g/L (Pimentel et al., 2019).

Applying the product Boveril® in the dosage of 10 and 20 g / l, in order to evaluate the efficiency on adults of *Sitophilus zeamais* in corn grains, it was found that the product expressed low mortality (20%) in insects (Pimentel et al., 2019). In that same study it was also proven that the treatment based on *Beauveria bassiana* resulted in a higher percentage of grain mass loss of 4.5% after 100 days of confinement. Still, for *Sitophilus oryzae* (Coleoptera: Curculionidae), Boveril® under the dosage of 10 g caused mortality of 3.33% in the first six days and 6.67% on the ninth day of treatment, totaling 10% of mortality in twelve days of research (Agostini et al., 2015).

The fungus entomopathogenic *Beauveria bassiana* has been used in research for biological control of insect pests due to its mode of action by contact without the need for ingestion, because it penetrates directly into the cuticle of the host adhering to its spores where these will germinate forming degradation enzymes causing the rupture of the layer and consequently colonizing the fungus on the host, however, the difficulty of maintaining the efficiency of the conidia for a longer period has hindered its function, in addition, factors such as humidity, temperature and luminosity can also influence the viability since (Oliveira, 2017), factors that may be linked to the results of this research (Table 2).

Considering the results obtained in this research, with the exception of the product based on diatomaceous earth, the other alternative and biological treatments tested did not show efficacy regarding insect mortality *Sitophilus zeamais*.

4 CONCLUSION

The diatomaceous earth product showed higher efficiency over *Sitophilus zeamais* reaching 84.21% of mortality on the sixth day of confinement of the insects. Alternative treatments *Azadirachta indica*, *Nicotiana tabacum* and the biological *Beauveria bassiana* did not express significant results to the mortality levels of the *Sitophilus zeamais* for the 10 days of exposure of the insect to the products.

Due to the importance of *Sitophilus zeamais* In stored grains, it is necessary that further research be carried out, seeking to work with different alternative products, concentrations and time of exposure to the insect, in order to find promising results for the control of this insect pest.

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