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Potential of diatomaceous earth as a management tool against *Acanthoscelides obtectus* infestations

Potencial de tierra de diatomeas como una herramienta de manejo contra las infestaciones de *Acanthoscelides obtectus*

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Date Article

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Abstract

The weevil *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae) is a cosmopolitan pest that causes high losses in stored beans in small storage units and especially on-farm storages. Here, it was tested the efficacy of the inert dust diatomaceous earth (DE) as an alternative to control *A. obtectus* in common beans (i.e., *Phaseolus vulgaris* L.) on different storage temperatures and exposure intervals. Using a Completely Randomized design four doses (0.25, 0.50, 0.75 and 1.00g kg^{-1}) of diatomaceous earth on beans kept at different temperatures (25, 28, 30, 32 and 35°C). Insect mortality was evaluated after two or five days of exposure. The impacts of diatomaceous earth in the offspring of five days-exposed *A. obtectus* adults were evaluated. The offspring production (emerged adults) was evaluated 60 days after the exposure. The results revealed that *A. obtectus* mortality caused by diatomaceous earth was dose, temperature and exposure period dependent. For instance, at the lowest temperature (25°C), diatomaceous earth dose (0.25g kg^{-1} of beans) and exposure period of two days, the efficacy of diatomaceous earth was significantly reduced when compared to the other treatments. When applied at temperatures above 30°C, the diatomaceous earth treatments always resulted in *A. obtectus* mortality of at least 90%. Curiously, offspring production (F1) was reduced to over 95% in all diatomaceous earth, temperatures and exposure period treatments. Therefore, the results demonstrated that diatomaceous earth has the potential to be used as a tool to manage *A. obtectus* infestations in stored beans, considering that such control practice adequately reduced these insect infestations in several possible scenarios.

Keywords: inert dust, *Phaseolus vulgaris*, bean weevil control, biorational pest management.

Resumen

El gorgojo *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae) es una plaga cosmopolita que causa grandes pérdidas en frijoles almacenados, ya sea em pequenas unidades o en fincas. Aquí, se probó la eficacia del polvo inerte de la tierra de diatomeas (DE) como una alternativa para controlar *A. obtectus* en frijoles comunes *Phaseolus vulgaris* L., en diferentes temperaturas de almacenamiento e intervalos de exposición. Usando un diseño completamente al azar, cuatro dosis (0.25, 0.50, 0.75 y 1.00g kg^{-1}) de tierra de diatomeas en frijoles mantenidos a diferentes temperaturas (25, 28, 30, 32 y 35 °C). La mortalidad de los insectos se evaluó después de dos o cinco días de exposición. Se evaluaron los impactos de la tierra de diatomeas en la descendencia de adultos de *A. obtectus* expuestos a cinco días. La producción de descendencia (adultos emergentes) se evaluó 60 días después de la exposición. Los resultados mostraron que la mortalidad por *A. obtectus* causada por la tierra de diatomeas dependía de la dosis, la temperatura y el período de exposición. Por ejemplo, a la temperatura más baja (25 °C), la dosis de tierra de diatomeas (0.25 g kg^{-1} de frijoles) y el período de exposición de dos días, la eficacia de la tierra de diatomeas se redujo significativamente en comparación con los otros tratamientos. Cuando se aplica a temperaturas superiores a 30 °C, los tratamientos con tierra de diatomeas siempre dieron como resultado una mortalidad de $^{A.\ obtectus}$ de al menos 90%. Curiosamente, la producción de descendencia (F1) se redujo a más del 95% en todos los tratamientos de tierra de diatomeas, temperaturas y períodos de exposición. Por lo tanto, los resultados demostraron que la tierra de diatomeas tiene el potencial de ser utilizada como una herramienta para controlar las infestaciones de *A. obtectus* en frijoles almacenados, considerando que dicha práctica de control redujo adecuadamente estas infestaciones de insectos en varios escenarios posibles.

Palabras clave: polvo inerte, *Phaseolus vulgaris*, control del gorgojo del frijol, manejo bioracional de plagas.

INTRODUCTION

The bean weevil *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae) is one of the most important pests of many plants from the Fabaceae family, especially the common beans *Phaseolus vulgaris* (Lineu). Their high reproductive potential and short life cycle allow them to produce several generations within a year, especially under favorable conditions (Soares *et al*., 2015). The attack of *A. obtectus* in bean grains begins when they are still in the field and subsequently they are able to infest storage units causing losses ranging from 7 to 40%, which corresponds to 1.59 - 9.12 millions of tons each year in the world caused by bruchids (Mbogo *et al*., 2009; Jiménez *et al*., 2017). The losses caused by *A. obtectus* is a serious threat to the major protein source of hundreds of millions of people, especially those from Latin America, Asia and Africa (Zaugg *et al*., 2013; Hnatuszko-Konka *et al*., 2014; Pitura and Arntfield, 2019).

The commonly used strategies to control *A. obtectus* on small and large stored grain units rely on protectant and fumigant insecticides. However, the indiscriminate use of these products have constantly been questioned, since their use is normally associated with serious problems, such the selection of resistant populations (Pimentel *et al*., 2007; Boyer *et al*., 2012; Haddi *et al*., 2018), environmental pollution (Stehle and Schulz, 2015; Tang *et al*., 2018) and impacts on the human health (Bjørling-Poulsen *et al*., 2008; Radwan *et al*., 2015; Chen *et al*., 2016). Because of these backlashes, the investigation of new products that are less persistent in the environmental and less harmful to other organisms is of extreme importance. Among these alternatives, mechanical control, essential oils, inert dusts or combinations of them have been demonstrated to be effective to control the weevils that attack bean grains (Papachristos and Stamopoulos, 2004; Badii *et al*., 2014; Viteri Jumbo *et al*., 2018; González Armijos *et al*., 2019; Rodríguez-González *et al.,* 2019), including *A. obtectus* (Bohinc *et al*., 2013; Freitas *et al*., 2016; Viteri Jumbo *et al*., 2018).

Diatomaceous earths (DEs) are natural inert materials derived from fossilized geological deposits of siliceous; and mainly made of silicon dioxide (Korunic, 1997; Cook *et al*., 2008). Additionally, the effectiveness of diatomaceous earth as protectants of different stored beans against their major pests has been already demonstrated (Prasantha *et al*., 2003; Stathers *et al*., 2004; Bohinc *et al*., 2013; Badii *et al*., 2014). The mode of action of diatomaceous earth is by damaging the insect's tegument surface (including sensilla and pores) culminating in reduction of body water content and death through desiccation (Korunić *et al*., 2016; Subramanyam and Roesli, 2000). Besides being relatively easy to apply, diatomaceous earth toxicity to mammalians is considered low because this chemically unreactive powder is mainly composed by silica, which does not accumulate in mammals as such molecules are excreted in the urine (Korunic, 2013). Additionally, diatomaceous earth does not break down (degrade) rapidly and do not affect end-use quality of grains (Subramanyam and Roesli, 2000; Stathers *et al*., 2004; Korunic, 2013).

The insecticidal efficacy of diatomaceous earth may be affected by abiotic factors e.g., temperature and exposure period or biotic factors e.g., insect species, physiology, and behavior, especially in tropical climate conditions. Thus, this study was conducted aiming to determine whether the exposure period and temperatures levels would affect the efficacy of diatomaceous earth against *A. obtectus.*

MATERIALS AND METHODS

Insect rearing and diatomaceous earth formulation. Adults of *A. obtectus* were maintained under controlled conditions (temperature 27 ± 1ºC, relative humidity 65 ± 5% and 12 h of scotophase) on common beans (*Phaseolus vulgaris cv* Ouro Negro). Newly emerged (<48 h) unsexed adults were used in all the experiments. These insect colonies were reared under controlled conditions and in an insecticide-free environment for at least one year. We used the diatomaceous earth formulation Keepdry™ (Irrigação Dias Cruz Ltda., Santo André, SP, Brazil) which is a diatomaceous earth formulation of freshwater origin containing 86% of silicon dioxide and 14% inert ingredients. The formulation was stored in the dark for three weeks inside sealed plastic bags at 25ºC until the beginning of the experiments.

Bioassays toxicity of diatomaceous earth in different temperatures. To evaluate the susceptibility of *A. obtectus* to diatomaceous earth (DE) the experimental design was Completely Randomized. Four doses of Keepdry™ which are lower than the recommended field rate (0.25, 0.50, 0.75 and 1.00g kg-1 of beans) were tested under different temperature conditions (25, 28, 30, 32 or 35ºC) and exposure periods (two and five days). Three replicates were used for each dose, temperature, exposure period and a control treatment (grains without DE application) was used for each bioassay. In order to homogenize the DE with the bean grains, the diatomaceous earth amounts were added to a glass jar (2.0L of volume capacity) containing 150g of bean grains. After the application, the jars were rolled manually for 60 seconds. Subsequently, bean grain masses (45g) were placed inside Petri dishes (45cm diameter) and 25 newly emerged unsexed adults of *A. obtectus* were transferred into the Petri dishes. After the exposure periods, the insects were considered dead when unable of walking or flying when touched with fine brush pods.

Effects of diatomaceous earth on the offspring production of *A. obtectus*. To evaluate the impacts of DE in the offspring production of *A. obtectus,* the samples that stayed exposed for five days were maintained after mortality had been assessed. All insects (dead or alive) were removed from the bean grain masses, and the Petri dishes with grains remained in the incubators for an additional period of 60 days. After this period, the number of emerged adults (F1) was recorded and the reduction in progeny or inhibition rate (%IR) was calculated as:

$$\%IR = \frac{Cn - Tn}{Cn} * 100$$

Where Cn is the number of newly emerged insects in the untreated jar and Tn is the number of insects emerged in the treated jar (Tapondjou *et al*., 2002; Rajashekar *et al*., 2010). Three replicates were used for each treatment.

Data analysis. In order to test the effects and the interactions of temperature, dose and exposure interval in the mortality and progeny production of *A. obtectus*, the data was submitted to three-way Analysis of Variance (ANOVA). Mortality was analyzed using one-way ANOVA to determine significant differences ($P < 0.05$) among exposure times. Means were separated by Duncan's multiple range test at $P < 0.05$. The same procedure was followed to analyze for the effects of diatomaceous earth (DE) treatment on the progeny production of *A. obtectus*.

RESULTS AND DISCUSSION

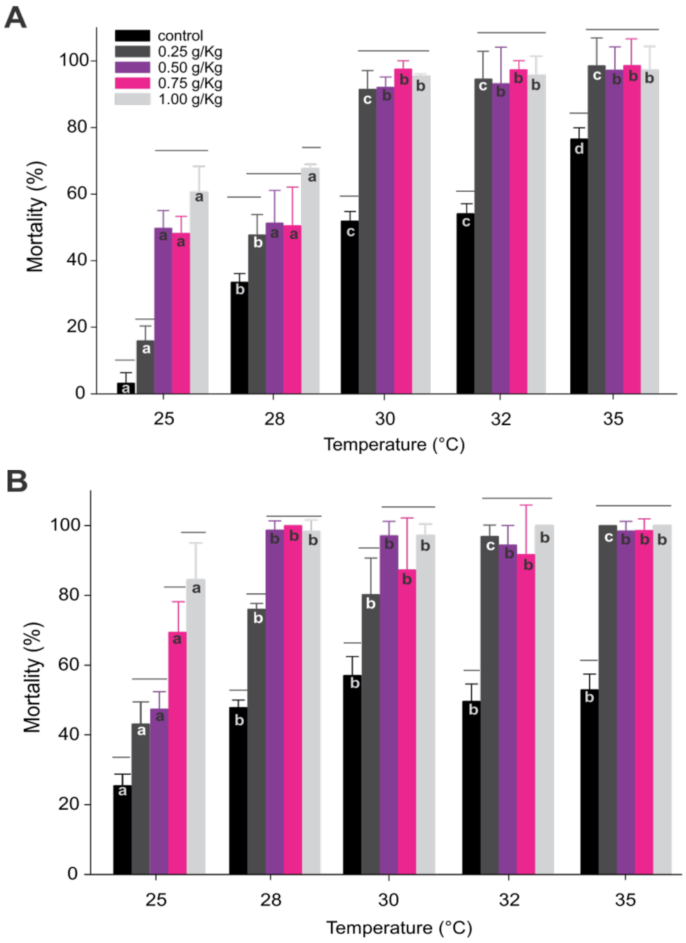
The Analysis of Variance revealed that temperature, doses, and exposure periods and the associated interactions between them were highly significant ($P < 0.0001$) for the DE efficacy against *A. obtectus* (Table 1). Similar results were obtained for the reduction of *A. obtectus* progeny in bean grain masses that were treated with DE (Table 1).

Table 1. ANOVA for main effects and interactions of mortality and progeny production of *Acanthoscelides obtectus* after being exposed to diatomaceous earth-treated beans.

Source of variation	Mortality			Progeny production		
	D.F.	***F***	***P***	***D.F.***	***F***	***P***
Model	49	63.14	<0.0001	24	207.17	<0.0001
Error	100			50		
Temperature	4	347.31	<0.0001	4	83.23	<0.0001
Dose	4	293.81	<0.0001	4	856.77	<0.0001
Exposure interval	1	95.52	<0.0001	-	-	-
Temperature × Dose	16	7.82	<0.0001	16	75.75	<0.0001
Temperature × Exposure interval	4	53.95	<0.0001	-	-	-
Dose × Exposure interval	4	6.13	<0.0001	-	-	-
Temperature × Dose × Exposure interval	16	4.27	<0.0001	-	-	-

DF= Degree of Freedom, F= test F, P= P(<0.05)

The *A. obtectus* mortality caused by DE revealed to be doses, temperature, and exposure period dependent. When the exposure period was of two days, the bean weevils kept at 25 and 28°C exhibited mortality levels of at least 50% in all treatments, excepting the mortality recorded for the treatment of 0.25g kg^{-1} at 25°C, where there was no significant difference when compared with the *A. obtectus* mortality obtained for diatomaceous earth-untreated beans. For temperature treatments above 30°C, the *A. obtectus* mortality did not differ between treatments, being always superior to 90% (Figure 1A). The mortality results obtained for the exposure period of five days showed that *A. obtectus* were very susceptible to diatomaceous earth exposure (Figure 1B). For instance, event at lower diatomaceous earth doses and temperatures, the mortality level obtained were never below 40%, resulting in significant differences when compared with the mortality recorded for *A. obtectus* reared on untreated bean masses (Figure 1B).



Bars grouped under the same horizontal line do not present significant differences (P > 0.05). Histograms of a particular color grouped by similar letters across the different temperatures do not present significant differences (Duncan's multiple range test, P > 0.05).

Figure 1. Mortality levels of *A. obtectus* when exposed for two (*A*) and five (*B*) days to different diatomaceous earth doses and temperature conditions.

Results obtained for the progeny production showed that the DE caused significant reductions in the progeny of *A. obtectus*. Even at the lowest diatomaceous earth doses tested (0.25g kg^{-1}) exposed to 25°C, the suppression of progeny production was above 90% (Table 2). Increase of temperature also affected the number of *A. obtectus* adults emerged of diatomaceous earth non-treated bean grain masses, where the average number of individuals of 96.0 ± 10.5, 106.0 ± 8.4, 67.7 ± 11.2, 30.3 ± 5.3, 10.0 ± 1.9 when exposed to 25, 28, 30, 32 and 35°C, respectively.

In the present study, it was evaluated the efficacy of diatomaceous earth to control *A. obtectus* and the contributions of dose, temperature and exposure interval on the efficacy of this inert dust. The results showed that all the doses of DE were toxic to *A. obtectus* adults and there is a positive correlation between mortality and dose, temperature and exposure period. Such increased mortality rates within exposure time increase are due to the slow-acting mode of action of DE products which are different from traditional grain protectants that may kill insects faster (Korunic, 1998).

Diatomaceous earth is an inert dust mostly composed by small silica particles that are able to abrade the insect cuticle and absorb water, which potentially leads to insect death due to dehydration (Korunic, 1998; Subramanyam and Roesli, 2000). Since adults of *A. obtectus* do not feed, it is difficult to recover the water loss caused by DE, which increases the susceptibility of these insects to diatomaceous earth exposures. Moreover, higher temperatures usually increase insect movement inside the grains, causing increased contact with the diatomaceous earth and, consequently, greater cuticular damage. Furthermore, it is important to feature that unlike synthetic insecticides, DE is inert and does not degrade with the increase of temperature, which allow farmers to use DE products combined with elevated temperatures (Dowdy, 1999; Fields and Korunic, 2000; Athanassiou *et al*., 2005; Athanassiou *et al*., 2011; Bohinc *et al*., 2013; Frederick and Subramanyam 2016).

Table 2. Progeny reductions (%) of *A. obtectus* that were subjected (during five days) to diatomaceous earth-treated bean grain masses kept at different temperatures.

Dose (g kg^{-1})	Progeny reduction (%)				
	25 °C	**28 °C**	**30 °C**	**32 °C**	**35 °C**
0.25	95.0 ± 0.2aB	99.0 ± 0.01bA	100bA	100bA	100bA
0.50	99.0 ± 0.07aA	100aA	100aA	100aA	100aA
0.75	100A	100A	100A	100A	100A
1.00	100A	100A	100A	100A	100A

Values in the same row followed by different lowercase letter are significantly different when exposed to different temperatures; Similar capital letters on the same column indicate no significant differences between treatments (Duncan's multiple range ($P < 0.05$; $df_{(4,14)}$) was 19.36 for columns and rows).

Although treatments that caused high *A. obtectus* mortality resulted in severe progeny reduction, similar reductions were observed in the exposure to lower diatomaceous earth doses, where there were considerably more insects alive. The reasons underlying the aforementioned progeny reduction may potentially be related to the accumulation of diatomaceous earth particles on the ovipositor of *A. obtectus* females, acting as a physical barrier and disturbing reproduction behaviors (Szentesi, 1976). Furthermore, such progeny reductions may also be attributed to the physiological costs of directing energy to the repairment of damaged cuticle aiming to minimize water losses (Subramanyam and Roesli, 2000; Prasantha *et al*., 2002; Prasantha *et al*., 2015). Additionally, diatomaceous earth could also have affected the egg hatching or newly emerged larvae. As *A. obtectus* females lay their eggs freely in the grain masses, the newly emerged larvae generally take few hours selecting and perforating their hosts, which may increase their potential exposure DE particles left on the bean grains (Simmonds *et al*., 1989; Thiéry *et al*., 1994).

Considering that higher doses of diatomaceous earth (> 0.3g kg^{-1}) are no longer acceptable since they cause negative effects on the grain physical proprieties (causing dusty appearance) and because of the difficulties associated to their sprayed application.(Korunic, 1998; Subramanyam and Roesli, 2000). Thus, the results showed that lower DE doses as 0.25g kg^{-1} of are sufficient to control *A. obtectus* infestation in bean grain masses in several potential scenarios, without affecting the quality of grains, which reinforce the potential of these inert dust to be used as sustainable alternatives for protecting bean grain masses during on-farm storage phases.

CONCLUSIONS

Diatomaceous earth is a potential alternative to the control of *A. obtectus*; the mortality of adult weevils was dependent of both temperature and exposure period. Additionally, diatomaceous earth significantly reduced the offspring production (F1) of *A. obtectus,* regardless of dose and temperature, which makes DE a good candidate to be integrated on management strategies of these bean weevils, especially on small storage units.

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