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# Efficacy of diatomaceous earth and temperature to control the bean weevil, *Acanthoscelides obtectus* Say, 1831 (Coleoptera: Bruchidae) in stored bean

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**Abstract**
This study was carried out to investigate the suitability of the diatomaceous earth (DE) material in biological control of the bean weevil, *Acanthoscelides obtectus* depending on the temperature. Treatment with DE is an efficient insect control technique in Integrated Pest Management (IPM) programs of stored grain pests. Its main advantages are low toxicity to environment, non-target organisms and long lasting efficacy. The objective of this application was to evaluate the efficacy doses and temperatures to control the bean weevil, *A. obtectus* in stored bean. In plastic cups with 150 g of clean and dry bean grains, 50 non-sexed 7-14 day-old adults of *A. obtectus* were submitted to the following applications in three replicates; DE (DE-CRAWL) at 350, 700 and 1000 mg $kg^{-1}$, at 20, 25 and 30 ºC. The mortality was evaluated from the 2$^{nd}$ to 30$^{th}$ day. After this period, the adults were removed and the progeny was kept until the 60$^{th}$ day when the insects were counted and the grain moisture content evaluated. The effect of temperature on *A. obtectus* mortality was significant for the three DE doses. The 700 and 1000 mg $kg^{-1}$ doses caused the highest mortality at 25 ºC and 30 ºC but with no significant difference between them. The progeny development was significantly higher in the control group compared to the treatments with DE. There was no significant difference in the number of progeny among the three DE doses at any of the temperatures studied. The results support the use of DE as an effective grain protectant against the bean weevil, *A. obtectus* in stored bean.

**Keywords:** Diatomaceous earth, stored grain protectant, temperature, the bean weevil

## Introduction

Bean weevils are originally native to Central America, however grain shipments at the end of the 19$^{th}$ century introduced the species to Europe where it subsequently spread around the globe. It is now found in Europe, Asia, North and South America, Africa, Australia and elsewhere (Pfaffenberger, 1985) [1].

The bean weevil, *Acanthoscelides obtectus* Say, 1831 is a warm-climate species, with optimal temperatures around 22-27 ºC. This pest is a small, brown beetle with a high attraction for bean plants. Once emerged from hibernation, they migrate to their first food plant, which can be vetch, pea vine, lupine, alfalfa and other leguminous cultures. They then move to a secondary host, which can range from haricot, bean, soybean and lentils. These beetles can be especially harmful in agriculture. They often migrate to fields of legumes, where they can sometimes reduce the yield by 50-60%. They harm the plant by eating through the seed shells, depleting the contents, before hibernating within the empty shell (Saqpunaru *et al*., 2006; Karakas, 2020) [2, 3].

After harvesting, insects infest the grain, causing economic loss to producers and the grain and food industry. During the past few decades application of synthetic pesticides to control agricultural stored products insect pests has been a standard practice. However, with the growing evidence regarding detrimental effects of many of the synthetic pesticides on health and environment, the grain industry wants to reduce the use of synthetic pesticides because of insecticide deregulation, resistant populations and consumer concerns over insecticide residues. Therefore, there is a pressing need for safer methods of pest management (Regnault-Roger and Hamraoui, 1995; Hamza et al., 2016) [4, 5].

Diatomaceous earth (DE) based insecticides have been finding increased use as stored product protectants because of these concerns. The main advantages of DEs are its low-toxicity to mammals and its stability (Korunić, 2013) [6].

Diatomaceous earth is a dust varying in colour from white, grey and yellow to red. Dust is formed from fossilized diatoms, single-celled algae of various shapes and sizes which are composed almost entirely of amorphous silicon dioxide (Arthur, 2003; Korunić, 2013) [7, 6].

Diatomaceous earth has great potential as a grain protectant. It is non-toxic, provides good protection when grain is stored properly, can be easily separated from the grain, and possibly recycled in storage bins. Toxicity is so low that diatomaceous earth is not counted as a foreign substance when grain is rated by the U.S. Department of Agriculture (Korunić, 1998; Desmarchelier and Allen, 2000; Korunić, 2001) [8-10].

The aim of this research was to evaluate the effectiveness of DE under different temperatures to control *A. obtectus* in stored bean. This information is important for integrated pest management (IPM) programs when combining the DE technique with artificial grain cooling or heat treatment.

## Materials and Methods

### Insect culture technique

In this study, *A. obtectus* laboratory stock maintained on dry bean seeds since 2019 under laboratory conditions (28 ± 2 ° C and 65 ± 5% RH). The newly emerged adult *A. obtectus* was transferred from the stock culture to new culture dishes and ovulation was achieved for 48 hours. After this period, adult individuals were excluded from the culture and new adults emerged and these individuals were selected for experimentation.

### Pest control material

Diatomaceous Earth Crawling Insect Killer, DE-CRAWL8, DE-CRAWL4.

Physical State: Powder; Appearance and Odour: White, free-flowing, Odourless; pH: 7.5-9.0 (1% Solution); Freezing Point: N/A; Boiling Point: N/A and Flash Point: N/A; Evaporation Rate: N/A; Flammability: N/A; Upper/Lower Explosive limits: N/A; Vapour Pressure: N/A; Vapour Density (20°C): N/A; Specific Gravity (Water=1): 2.2; Solubility: partially miscible in water/alcohol (P.F. Harris Manufacturing Company, LLC-Castersville GA).

**Bioassay**

The bean grains were previously disinfected by freezing at -20 ºC for seven days, then placed in plastic cups and mixed with different doses of DE: 350, 700 and 1000 mg kg$^{-1}$, and homogenized by vigorous hand agitation for three minutes. From each application were taken three replicates of 150 g placed in plastic cups of 500 ml capacity. Fifty non-sexed, 7 to 14 day-old adults of *A. obtectus* were placed in each flask, and covered with a screen flap. Three replications of each dosage plus a control group without DE were kept in an incubator cabins at 20, 25 and 30 ºC with 65 ± 5% relative humidity and 12 h dark / 12 h light photoperiod.

For all applications, the mortality was recorded at the 2$^{nd}$, 7$^{th}$, 12$^{th}$, 17$^{th}$, 22$^{th}$ and 30$^{th}$ days, and all the adults removed by the 30$^{th}$ day. Insects that did not move after being touched with a brush, after two or three minutes, were considered dead. On the 60$^{th}$ day after infestation the bean grain was sieved and the bean weevil adults of the second generation were counted.

The moisture content of the bean grain was determined by the oven method (Lazzari, 1997) [11], using three replicates of 15 g of bean grains placed in an oven during 72 h at 100 ± 5 ºC and weighed out on precision scale.

**Statistical evaluation**

The mean mortality and the standard error were calculated for each date of sampling for each application. The data were analysed by variance analysis and means were compared by Tukey's multiple range test at 5% probability, using the IBM SPSS Statistics 22.0.

**Results**

The average mortality of *A. obtectus* at 350 mg kg$^{-1}$ was significantly higher at 25 ºC and 30 ºC than at 20 ºC (Table 1). At 700 and 1000 mg kg$^{-1}$ there was no significant differences in mortality at the temperatures investigated.

The average mortality at 25 and 30 ºC for the three DE doses was significantly higher than that for the control group (Table 1). At 20 ºC, the doses of 700 and 1000 mg kg$^{-1}$ caused higher mortality than 350 mg kg$^{-1}$, although at this last dosage the mortality was significantly higher than the control group.

**Table 1:** Average mortality (%) ± Standard Error (SE) of *Acanthoscelides obtectus* in bean grain after 30 days of exposure to diatomaceous earth (DE) at different doses and temperatures; 65 ± 5 relative humidity and 12 h dark/12 h light photoperiod

| **Doses (mg kg$^{-1}$ DE)** | **Temperatures (ºC)** | | |
|---|---|---|---|
| | **20** | **25** | **30** |
| 350 | 78.6 ± 1.5 B b | 90.4 ± 1.1 A a | 94.2 ± 3.6 A a |
| 700 | 88.2 ± 1.4 A a | 94.8 ± 4.1 A a | 100.0 ± 0.5 A a |
| 1000 | 91.7 ± 2.4 A a | 100.0 ± 0.6 A a | 100.0 ± 0.3 A a |
| Control group | 3.66 ± 0.5 B c | 83.0 ± 4.3 A b | 0 B b |
| CV (%) | 5.06 | | |

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability. CV: Coefficient of variation at 95%.

At 20 ºC, the mortality at 700 and 1000 mg kg$^{-1}$ DE started by the 12$^{th}$ day of exposure (Table 2), and by the 22$^{th}$ day, the mortality was already 91.0% and 94.4% respectively, for two doses. The difference was not significant between the applications with 700 and 1000 mg kg$^{-1}$ of DE in the 22$^{th}$ and in the 30$^{th}$ day after exposure. Application with 350 mg kg$^{-1}$ the mortality was lower than in the other two by the 22$^{th}$ and 30$^{th}$ days after exposure, with cumulative mortality of 76.6% and 80.8%, respectively. In the control group, the cumulative mortality was only 5.4% by the 30$^{th}$ day, when the insects were removed to evaluate the number of the second generation progeny.

**Table 2:** Cumulative mortality (%) ± Standard Error (SE) of *Acanthoscelides obtectus* in bean grain after different exposure time and doses of diatomaceous earth (DE) at 20 ºC, 65 ± 5 relative humidity and 12 h dark/12 h light photoperiod

| **Exposure time (Days)** | **Mortality / DE doses (mg kg$^{-1}$)** | | | |
|---|---|---|---|---|
| | 0 | 350 | 700 | 1000 |
| 2 | 0 A a | 0 A b | 0 A c | 0 A c |
| 7 | 0 A a | 0 A b | 0 A c | 0 A c |
| 12 | 0 A a | 0 A b | 1.4 ± 0.2 A bc | 5.2 ± 0.7 A c |
| 17 | 0 C a | 5.4 ± 1.3 BC b | 10.0 ± 1.7 B b | 32.2 ± 1.3 A b |
| 22 | 0 C a | 76.6 ± 1.5 B a | 91.0 ± 1.7 A a | 94.4 ± 1.5 A a |
| 30 | 5.4 ± 1.3 C a | 80.8 ± 1.3 B a | 91.0 ± 2.1 A a | 96.5 ± 1.3 A a |
| CV (%) | 14.26 | | | |

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability. CV: Coefficient of variation at 95%.

At 25 ºC, mortality started by the 7$^{th}$ day in all applications with DE (Table 3) and there was no significant difference between 350, 700 and 1000 mg kg$^{-1}$ after the 17$^{th}$ day of application. The cumulative mortality by the 22$^{th}$ day was 94.4% for 350 mg kg$^{-1}$; 96.7% for 700 mg kg$^{-1}$ and 100.0% for 1000 mg kg$^{-1}$. The mortality in the control group was 73.8% after 30 days after application, due to a non-explained cause.

**Table 3:** Cumulative mortality (%) ± Standard Error (SE) of *Acanthoscelides obtectus* in bean grain after different exposure time and doses of diatomaceous earth (DE) at 25 ºC, 65 ± 5 relative humidity and 12 h dark/12 h light photoperiod

| Exposure time (Days) | Mortality / DE doses (mg kg$^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 350 | 700 | 1000 |
| 2 | 0 A b | 0 A c | 0 A c | 0 A d |
| 7 | 0 B b | 1.4 ± 0.6 AB c | 4.5 ± 1.2 AB c | 13.5 ± 2.2 A c |
| 12 | 0 C b | 21.1 ± 1.8 B b | 25.3 ± 2.1 B b | 42.2 ± 2.2 A b |
| 17 | 0 B b | 82.6 ± 1.1 A a | 93.3 ± 2.4 A a | 93.2 ± 2.0 A a |
| 22 | 0 B b | 94.4 ± 1.6 A a | 96.7 ± 2.0 A a | 100.0 ± 0.4 A a |
| 30 | 73.8 ± 5.0 B a | 93.5 ± 1.7 A a | 95.2 ± 2.2 A a | 100.0 ± 0.2 A a |
| CV (%) | 11.93 | | | |

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability. CV: Coefficient of variation at 95%.

At 30 ºC, mortality started by the 7th day in all applications with DE (Table 4). There was no significant difference between 350, 700 and 1000 mg kg$^{-1}$ after the 22th day of application. The cumulative mortality after the 30th day of exposure was 94.1% for 350 mg kg$^{-1}$ and 100.0% for both 700 and 1000 mg kg$^{-1}$. There was no mortality of *A. obtectus* until the 30th day in the control group at 30 ºC.

**Table 4:** Cumulative mortality (%) ± Standard Error (SE) of *Acanthoscelides obtectus* in bean grain after different exposure time and doses of diatomaceous earth (DE) at 30 ºC, 65 ± 5 relative humidity and 12 h dark/12 h light photoperiod

| Exposure time (Days) | Mortality / DE doses (mg kg$^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 350 | 700 | 1000 |
| 2 | 0 A a | 0 A c | 0 A c | 0 A c |
| 7 | 0 A a | 1.7 ± 0.2 A c | 13.6 ± 0.2 A c | 13.3 ± 0.2 A c |
| 12 | 0 B a | 13.6 ± 0.2 B c | 44.8 ± 1.2 A b | 45.6 ± 1.3 A b |
| 17 | 0 C a | 43.6 ± 0.2 B b | 86.3 ± 0.2 A a | 91.2 ± 2.2 A a |
| 22 | 0 B a | 84.4 ± 1.1 A a | 93.4 ± 1.2 A a | 96.8 ± 1.3 A a |
| 30 | 0 B a | 94.1 ± 0.2 A a | 100.0 ± 0.3 A a | 100.0 ± 0.4 A a |
| CV (%) | 18.09 | | | |

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability. CV: Coefficient of variation at 95%.

The average number of offspring produced by the 60th day is shown on Table 5. In the plastic cups with bean grains treated with 350, 700 and 1000 mg kg$^{-1}$ DE and kept either at 30 or 25 ºC, the number of *A. obtectus* from the second generation was significantly lower than in the control group. However, at 20 ºC, there was no significant difference between the 350 mg kg$^{-1}$ and the control group. At 30 ºC after 60 days of exposure, the number of descendants was significantly higher (111 insect) than at the two other temperatures for the control group, and the bean grains were severed damaged at the end. There was no significant differences in progeny production for the three DE doses in the three temperatures; however, there was a tendency of increasing of progeny with increasing temperatures and DE doses, especially at 30 ºC.

**Table 5:** Average number (%) ± Standard Error (SE) of second generation progeny of *Acanthoscelides obtectus* in bean grain after 60 days of exposure to diatomaceous earth (DE) at different doses and temperatures; 65 ± 5 relative humidity and 12 h dark/12 h light photoperiod

| Doses (mg kg$^{-1}$ DE) | Temperatures (ºC) | | |
|---|---|---|---|
| | 20 | 25 | 30 |
| 350 | 6.28 ± 1.3 A a | 10.13 ± 1.5 A ab | 30.13 ± 2.0 A b |
| 700 | 1.14 ± 0.2 A b | 2.27 ± 0.6 A b | 11.94 ± 1.3 A b |
| 1000 | 0 A b | 1.08 ± 0.2 A b | 7.34 ± 0.2 A b |
| Control group | 18.14 ± 1.3 B a | 31.46 ± 1.9 B a | 111.58 ± 1.9 A a |
| CV (%) | 65.85 | | |

Means followed by the same capital letter in the lines and the same lower letter in the column do not differ from each other by the Tukey Test at 5% probability. CV: Coefficient of variation at 95%.

## Discussion

In the last decade, there has been an increase in the use of diatomaceous earth because of its low mammalian toxicity, worker safety, low risk food residues and the occurrence of resistant insect populations associated with the use of chemical insecticides.

In order to reduce DE dosages, which have a negative effect on grain quality, DE is often mixed with other compounds such as silica gel, dry honey, inactivated yeast and sugar (Quarles and Winn, 1996; Korunić and Fields, 1998; Subramanyam and Roesli, 2000) [12-14]. However, high doses of these mixtures still have a significant negative effect on grain bulk density and flow ability (Jackson and Webley, 1994; Korunić *et al*., 1998) [15, 16]. Possible solutions for the implications of high doses of DEs include a combined use of DE and other low-risk methods, such as extreme temperatures (Fields *et al*., 1997; Dowdy 1999) [17, 18], grain cooling with surface treatment with DE (Nickson *et al*., 1994) [19], or a mixture with entomopathogenic fungi (Lord, 2001; Akbar *et al*., 2004; Kavallieratos *et al*., 2006; Vasilakos *et al*., 2006: Michalaki *et al*., 2007) [20-24], a mixture with synthetic insecticides (Korunic, 2001; Stathers, 2003; Arthur, 2004a, 2004b; Athanassiou, 2006; Chanbang *et al*., 2007; Korunić and Rozman, 2010) [10, 25-30] or a mixture with plant extracts (Korunić, 2007b, Athanassiou and Korunić, 2007) [31-32]. Experimentation with other components often revealed synergistic or enhanced effectiveness (Korunić, 2001; Lord, 2001; Korunić and Rozman, 2010; Korunić, 2007b; Athanassiou and Korunić, 2007; Stathers *et al*., 2008) [10, 20, 30-33].

## Conclusion

Although DEs have some disadvantages, there are important practical advantages in their use to control insects. Resistance to insecticides has now been one of the reasons to seek alternatives to chemical insecticides. Laboratory experiments have shown that stored product pests can achieve up to a 2-fold reduction in sensitivity when exposed to diatomaceous earth for 5-7 generations. Although there are no reports of insects developing resistance to diatomaceous earth in commercial stores, these results suggest that the use of resistance management strategies will be necessary to prevent widespread resistance to diatomaceous earth products.

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