EFFICACY OF DIATOMACEOUS EARTH AND TEMPERATURE TO CONTROL THE MAIZE WEEVIL IN STORED MAIZE

EFICÁCIA DE TERRA DE DIATOMÁCEA E TEMPERATURA PARA O CONTROLE DO GORGULHO-DO-MILHO EM MILHO ARMAZENADO

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ABSTRACT

Treatment with diatomaceous earth (DE) is an efficient insect control technique in integrated pest management programs of stored grain. Its main advantages are: low toxicity to mammals and long lasting efficacy. The objective of this research was to evaluate the efficacy of DE under different doses and temperatures to control S*itophilus zeamais* in stored maize. In vials with 100 g of clean and dry corn kernels, 30 non-sexed 7-14 day-old adults of *S. zeamais* were submitted to the following treatments in three replicates: DE (Keepdry®) at 500, 750 and 1000 mg kg^{-1}, at 15, 25 and 30 °C. The mortality was evaluated from the 1st to the 28th day. After this period, the adults were removed and the progeny was kept until the 56th day, when the insects were counted and the grain moisture content evaluated. The effect of temperature on *S. zeamais* mortality was significant for the three DE doses. The 750 and 1000 mg kg^{-1} doses caused the highest mortality at 25 °C and 30 °C, but with no significant difference between them. The progeny development was significantly higher in the control 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 *S. zeamais* in stored corn.

Key-words: inert dust; integrated pest management; *Sitophilus zeamais*; grain protectant.

RESUMO

O tratamento com Terra de Diatomácea (TD) é uma técnica eficiente para o controle de insetos em programa de manejo integrado de grãos armazenados. Suas principais vantagens são: baixa toxicidade para mamíferos e períodos de eficácia mais longos. O objetivo deste trabalho foi avaliar a eficácia de TD em diferentes doses e temperaturas para o controle de S*itophilus zeamais* em milho armazenado. Em frascos com 100 g de milho limpo e seco, foram colocados 30 adultos de *S. zeamais* não sexados de 7 a 14 dias de idade, e submetidos aos seguintes tratamentos com três repetições: TD (Keepdry®) a 500, 750 e 1000 mg kg^{-1}, mantidos a 15, 25 e 30 °C. A mortalidade foi avaliada entre o 1° e o 28° dia. Após este período, os adultos foram removidos e a progênie mantida até o 56° dia quando foi contado o número de insetos e avaliado o teor de umidade dos grãos. O efeito da temperatura na mortalidade *S. zeamais* foi significativo para as três doses de TD. As doses de 750 e 1000 mg kg^{-1} proporcionaram a maior mortalidade a 25 °C e 30 °C, mas sem diferenças significativas entre elas. O desenvolvimento da progênie foi altamente significativo no controle comparado ao grão tratado com TD; não foi constatada diferença significativa na progênie entre as três doses de TD nas temperaturas estudadas. Os resultados suportam o uso de TD como um protetor eficiente para o controle de *S. zeamais* em milho armazenado.

Palavras-chave: pó-inerte; manejo integrado de pragas; *Sitophilus zeamais*; protetor de grãos.

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INTRODUCTION

The control of stored grain pests relies mostly on insecticides of broad spectrum and fumigants; however, alternative control methods have been developed in order to reduce human exposure, development of insecticide resistance and environmental and food contamination (EBELING, 1971). The concerns towards insecticide applications and the rising pressure imposed by consumers and scientists to substitute chemical insecticides for less toxic products have lead to the development of several studies on inert dust formulations (KORUNIC, 1998).

Diatomaceous earth (DE), which is a fine powder composed by diatomaceous algae carapaces, represents one of the most efficient types of inert dusts and has been used for insect control around the world (NIKPAY, 2006; VAYIAS et al., 2006). According to MITAL and WRIGHTMAN (1989) and ARTHUR (1996), the interest on this technique has increased because the number of active ingredients for insect control in grains is restricted to four or five products, mainly due to insect resistance problems.

According to SUBRAMANYAM and ROESLI (2000), the action of DE is attributed to the desiccation caused by adsorption and the abrasive properties that breaks the epicuticular wax layer, causing loss of body water and death within hours or days. ALDRYHIM (1990 and 1993) observed that *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) are more susceptible to DE at 30 °C than at 20 °C because of increasing water loss at higher temperature. However, *Tribolium confusum* (Duv.) was more susceptible to DE at low temperatures.

When deciding between an insecticide and DE for insect control in stored grains, the advantages and disadvantages of both should be evaluated. Chemical insecticides can eliminate insects faster then DE, reducing the chances of reproduction. Some of the disadvantages of insecticides are: the development of resistance, food and feed contamination with residues that may cause human intoxication and environmental problems. DE is not toxic to humans, domestic animals, and environment and does not leave toxic residues in the grain and by products. However, its direct action on insects is slower than that of synthetic insecticides, permitting oviposition and offspring production, although in low numbers (FIELDS, 1998). Also, DE affects some physical properties of grains, particularly bulk density (KORUNIC, 1998).

The efficacy of DE to control stored product pests depend on different factors such as insect species, commodity, grain moisture, and temperature (KORUNIC, 1999; FIELDS and KORUNIC, 2000). It has been observed that insects of the same species, but of different origin, show different levels of susceptibility to DE (RIGAUX et al., 2001), as indicated by some studies, from the most to the least sensitive species: *Cryptolestes* spp. (Coleoptera: Cucujidae), *Sitophilus* spp. (Coleoptera: Curculionidae), *Oryzaephilus* spp. (Coleoptera: Silvanidae), *R. dominica*, *Tribolium* spp. (Coleoptera: Tenebrionidae), and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (SUBRAMANYAM et al., 1998; FIELDS and KORUNIC, 2000).

The results show the efficacy of DE for most insect species, under the most diverse conditions, and combined to different insect control measures. CERUTI and LAZZARI (2005) observed that treatments mixing DE with powder deltamethrin kill adult *S. zeamais* since the first day of application, whereas DE alone will start killing this insect only after the 3rd day. After the 7th day there was no statistical difference among treatments. The authors concluded that treatments using DE combined with low doses of powder deltamethrin represent an efficient control measure against *S. zeamais* in stored corn because insect mortality is faster than in treatments using DE alone and residues of active ingredients are much lower than using the insecticide in high doses.

CHANBANGA et al. (2007) determined the effectiveness of two diatomaceous earth (DE) formulations against the lesser grain borer *R. dominica*, in stored paddy rice, in laboratory conditions. The results show that although these two commercial DE formulations had previously given good control of the lesser grain borer in stored wheat, they were not as effective in paddy rice. They mention that it may be necessary to combine DE with another insecticide or control measure to give complete control of the lesser grain borer in paddy rice.

The maize weevil, *Sitophilus zeamais* Motschulsky, occurs in all warm areas around the world, and it is a primary pest of corn, wheat, rice and sorghum, and many processed grain products, such as cereals, pasta, and dehydrated cassava (DOBIE et al., 1984). Since *S. zeamais* is an active flyer, it can attack several crops in the field, particularly in areas adjacent to storage units with high infestation and from the field to the storage facilities (EVANS, 1981).

The objective of this research was to evaluate the efficacy of DE under different temperatures to control *S. zeamais* in stored corn. This information is important for integrated pest management programs, when combining the DE technique with either artificial grain chilling or heat treatment.

MATERIAL AND METHODS

To form a stock population for the experiments, specimens of *S. zeamais* were reared on corn kernels with 13% moisture content (MC) (wet basis) in an environmental chamber at 25 ± 2 ºC and 65 ± 5% of relative humidity.

The DE used was the commercial product Keepdry®, which is composed of 88 to 90% of SiO_2, particles of 10-15 mm; apparent density of 200-230 g L^{-1}; color beige, aspect of a dry loose light powder, insoluble in water and free from foreign material.

The corn kernels were previously disinfested by freezing at –25 °C for three days, then placed in brown paper bags and mixed with different doses of DE: 500, 750 and 1000 mg kg^{-1}, and homogenized by vigorous hand agitation for two minutes. From each treatment were taken three replicates of 100 g, placed in plastic vials of 500 mL capacity; 30 non-sexed, 7 to 14 day-old adults of *S. zeamais* were placed in each flask, and covered with a screen lid. Three replications of each dosage plus a control without DE were kept in chambers at 15, 25 and 30 °C, with 65 ± 5% relative humidity and 12 h photophase.

For all treatments, the mortality was recorded at the 1st, 3rd, 5th, 7th, 14th and 28th days, and all the adults removed by the 28th day. Insects that did not move after being touched with a brush, after two minutes, were considered dead. On the 56th day after infestation the grain was sieved and the adults of the second generation were counted.

The moisture content of the grain was determined by the oven method (LAZZARI, 1997), using three replicates of 10 g of corn kernels placed in an oven during 72 h at 103 °C and weighed out on precision scale.

The mean mortality and the standard error (SE) were calculated for each date of sampling for each treatment. The data were analyzed by variance analysis and means were compared by Tukey´s multiple range test at 5% probability, using the SAEG statistical analysis software application (Federal University of Viçosa - Brazil).

RESULTS AND DISCUSSION

The mean mortality of *S. zeamais* at 500 mg kg^{-1} was significantly higher at 25 °C and 30 °C than at 15 °C (Table 1). At 750 and 1000 mg kg^{-1} there was no significant differences in mortality at the temperatures investigated.

TABLE 1 - Mean mortality (%) ± Standard Error of *Sitophilus zeamais* in stored corn after 28 days of exposure to diatomaceous earth (DE), at different doses and temperatures; 65 ± 5% r.h. and 12 h of photophase.

Treatments	Temperature		
	15 ºC	25 ºC	30 ºC
Control	5.56± 0.5 B c	73.3± 6.5 A b	0 B b
500 mg kg^{-1} DE	78.8± 1.3 B b	94.4± 1.3 A a	95.5± 0.3 A a
750 mg kg^{-1} DE	92.2± 2.3 A a	98.8± 2.1 A a	100± 0.5 A a
1000 mg kg^{-1} DE	97.7± 1.9 A a	100± 0.9 A a	100± 0.6 A a
C.V.[2]		6.64	

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.
*C.V.% = coefficient of variation at 95%.

The mean mortality at 25 and 30 °C for the three DE doses was significantly higher than that for the control (Table 1). At 15 °C, the doses of 750 and 1000 mg kg^{-1} caused higher mortality than 500 mg kg^{-1}, although, at this last dosage the mortality was significantly higher than in the control vials.

At 15 °C, the mortality at 750 and 1000 mg kg^{-1} DE started by the 5th day of exposure (Table 2), and by the 14th day, the mortality was already 90% and 97%, respectively, for the two doses. The difference was not significant between the treatments with 750 and 1000 mg kg^{-1} of DE in the 14th and in the 28th day after exposure. In the treatment with 500 mg kg^{-1} the mortality was lower than in the other two by the 14th and 28th days after exposure, with accumulated mortality of 73 and 78%, respectively. In the control, the accumulated mortality was only 5,5% by the 28th day, when the insects were removed to evaluate the number of the 2nd generation progeny.

TABLE 2 - Accumulated mortality (%) ± Standard Error of *Sitophilus zeamais* in corn, after different exposure time and doses of diatomaceous earth (DE), at 15 °C, 65 ± 5% r.h. and 12 h of photophase.

Exposure Time (Day)	Mortality/DE doses			
	0 mg kg^{-1}	500 mg kg^{-1}	750 mg kg^{-1}	1000 p mg kg^{-1}pm
1	0 A a	0 A b	0 A c	0 A c
3	0 A a	0 A b	0 A c	0 A c
5	0 A a	0 A b	2.2± 0.3 A bc	6.6± 0.5 A c
7	0 C a	5.5± 0.4 BC b	10± 1.2 B b	31.1± 1.5 A b
14	0 C a	73.3± 2.5 B a	90± 2.0 A a	96.6± 0.5 A a
28	5.5± 0.5 C a	78.8± 1.3 B a	92.2± 2.3 A a	97.7± 1.9 A a
*C.V.%		14.47		

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.
*C.V.% = coefficient of variation at 95%.

At 25 °C, mortality started by the 3rd day in all treatments with DE (Table 3) and there was no significant difference between 500, 750 and 1000 mg kg^{-1} after the 7th day of application. The accumulated mortality by the 14th day was 94% for 500 mg kg^{-1}; 98% for 750 mg kg^{-1} and 100% for 1000 mg kg^{-1}. The mortality in the control was 73% after 28 days of treatment, due to a non explained cause.

At 30 °C, mortality started by the 3rd day in all treatments with DE (Table 4). There was no significant difference between 500, 750 and 1000 mg kg^{-1} after the 14th day of application. The accumulated mortality after the 28th day of exposure was 95% for 500 mg kg^{-1} and 100% for both 750 and 1000 mg kg^{-1}. There was no mortality of *S. zeamais* until the 28th day in the control at 30 ºC.

TABLE 3 - Accumulated mortality (%) ± Standard Error of *Sitophilus zeamais* in corn, after different exposure time and doses of diatomaceous earth (DE), at 25 °C, 65 ± 5% r.h. and 12 h of photophase.

Exposure Time (Day)	Mortality/DE doses			
	0 mg kg^{-1}	500 mg kg^{-1}	750 mg kg^{-1}	1000 mg kg^{-1}
1	0 A b	0 A c	0 A c	0 A d
3	0 B b	2.2± 0.3 AB c	5.5± 1.5 AB c	13.3± 1.5 A c
5	0 C b	21.1± 1.2 B b	24.4± 2.3 B b	42.2± 2.2 A b
7	0 B b	82.2± 0.5 A a	93.3± 2.3 A a	93.3± 1.4 A a
14	0 B b	94.4± 1.4 A a	98.8± 1.5 A a	100± 0.6 A a
28	73.3± 6.5 B a	94.4± 1.3 A a	98.8± 2.1 A a	100± 0.9 A a
*C.V.%	12.2			

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.
*C.V.% = coefficient of variation at 95%.

TABLE 4 - Accumulated mortality (%) ± Standard Error of *Sitophilus zeamais* in corn, after different exposure time and doses of diatomaceous earth (DE), at 30 °C, 65 ± 5% r.h. and 12 h of photophase.

Exposure Time (Day)	Mortality/DE doses			
	0 mg kg^{-1}	500 mg kg^{-1}	750 mg kg^{-1}	1000 mg kg^{-1}
1	0 A a	0 A c	0 A c	0 A c
3	0 A a	2.2± 0.4 A c	14.4± 0.6 A c	14.4± 0.5 A c
5	0 B a	14.4± 0.5 B c	44.4± 1.4 A b	46.6± 1.3 A b
7	0 C a	43.3± 1.2 B b	86.6± 0.8 A a	92.2± 2.2 A a
14	0 B a	84.4± 1.5 A a	94.4± 1,2 A a	96.6± 1.5 A a
28	0 B a	95.5± 0.3 A a	100± 0.5 A a	100± 0.6 A a
*C.V.%	17.83			

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.
*C.V.% = coefficient of variation at 95%.

Several studies using different DE doses, application methods, types of grains and species have been developed in Brazil, either under laboratory conditions or in large grain silos. The results of this research agree with those obtained by PINTO JR. (1994), who observed a correlation between the doses of DE and the time of exposure of *Sitophilus* spp. in corn, having recorded 100% mortality after 19 days of exposure to 500 and 750 mg kg^{-1}. LORINI and SCHNEIDER (1994), testing DE at 500, 750 and 1000 mg kg^{-1} to control *S. oryzae*, obtained, after seven days of treatment, mortality of 19, 87 and 100%, respectively.

The mean number of offspring produced by the 56th is shown on Table 5. In the vials with corn kernels treated with 500, 750 and 1000 mg kg^{-1} DE and kept either at 30 or 25 °C, the number of *S. zeamais* from the second generation was significantly lower than in the control. However, at 15 °C, there was no significant difference between the 500 mg kg^{-1} and the control. At 30 °C, after 56 days of exposure, the number of descendants was significantly higher (111 insects) than at the two other temperatures for the control, and the kernels were severed damaged at the end. There were 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 °C.

TABLE 5 - Mean number ± Standard Error of 2nd generation progeny of *Sitophilus zeamais* in corn, after 56 days of exposure to diatomaceous earth (DE) at different doses and temperatures; 65 ± 5% r.h. and 12 h of photophase.

Treatments	Temperature		
	15 °C	25 °C	30 °C
Control	19.34± 2.3 B a	32± 2.5 B a	111.34± 3.5 A a
500 g.t^{-1}	8.34± 1.5 A a	10.67± 1.5 A ab	29.67± 2.5 A b
750 g.t^{-1}	1.34± 0.4 A b	2± 0.8 A b	11.34± 0.8 A b
1000 g.t^{-1}	0 A b	1± 0.2 A b	7.34± 0.2 A b
*C.V.%	60.47		

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.
*C.V.% = coefficient of variation at 95%.

These results are supported by previous studies that show that DE is less effective against insects at low temperatures (ALDRYHIM, 1990 and 1993; COLLINS et al., 2001), which was probably due to reduced exposure to DE particles when insect mobility decreases. High temperatures would increase insect movement and increase contact with DE, resulting in greater cuticle damage. Increase temperature would also lead to a higher metabolic rate, which would result in increase water consumption.

Contrary to the results of this research, ATHANASSIOU et al. (2007) demonstrated that two DE formulations, Protect-it and PyriSec, were more effective at 20 °C than at 30 °C, whereas the formulation, DEA-P showed continuous efficiency at all temperatures and relative humidity examined.

PAULA (2001) observed that the number of second generation progeny of *Sitophilus* spp. in paddy rice was inversely proportional to the dose of DE. MEWIS and REICHMUTH (2000) observed similar results in a laboratory experiment with *S. granarius* exposed to treated wheat grains with DE at 25 °C and 14.5% r.h. They reported that the adults died within a few days, which was enough time to produce progeny and result in a considerable population increase after 42 days. ARTHUR and THRONE (2003) showed that, although adult weevils are killed by exposure to DE, some oviposition could still occur and progeny suppression may not be complete.

The moisture content of the grain was 11% at the beginning of the experiment in all treatments. At the end, the moisture content decreased slightly to 10.8% at 15 °C; 10.2% at 25 °C and 9.5% at 30 °C with no statistically significant difference among them, showing that DE keeps the moisture content down. In the control the MC at the end of the experiment was 11.5%, showing a slight increase without the grain protectant adhered to the corn kernels.

In stored product ecosystems, the control of stored product pests has faced two contrasting challenges the “zero insect tolerance”, imposed by some grain markets, and the absence of pesticide residues in human and animal food, required by all consumers. Thus, these results support the use of DE as an effective grain protectant against *S. zeamais* in stored corn and as an important IPM component to attend to the market and consumer pressures. The interactions of DE with several abiotic factors (temperature, relative humidity and grain moisture content), biotic factors (insect species, grain kind), and all the overall storage conditions (grain cleaning, fumigants, other insecticides) should be continuously investigated, especially under large scale storage.

CONCLUSIONS

1) DE at 750 mg kg^{-1} and 1000 mg kg^{-1} provide 100% control of *S. zeamais* by the 14th day after treatment, at 25 and 30 °C;

2) Progeny production increases with increasing temperatures at much lower rates in grain treated with DE than in unprotected lots;

3) Grain moisture content was kept at low safe range in treated and non treated kernels.

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