International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (5), pp. 001-005, May, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Insecticidal effect of diatomaceous earth against *Callosobruchus maculates* under laboratory conditions

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Accepted 23 March, 2019

Laboratory experiments were carried out in order to evaluate the insecticidal effect of diatomaceous earth against adults of *Callosobruchus maculatus* (F.) and *Sitophilus granarius* (L.), two important stored grain pests in darkness (30°C and 65% RH). Wheat and cowpea were treated with the diatomaceous earth formulation Silicosec® (Biofa, Germany), at five concentrations determined with preliminary tests and compared with untreated wheat and cowpea as control. Dead adults were counted 24, 36 and 48 h later. Results showed that increasing the concentration of Silicosec® and days after treatment (DAT) significantly increased the mortality rates of adults to above 90% in both experiments. Regarding Lc_{50} and Lc_{95} values, it was observed that *C. maculatus* adults are more susceptible to Silicosec® than S. *granarius*. The results showed that these two pests could be controlled successfully with diatomaceous earth.

Key words: *Callosobruchus maculatus*, diatomaceous earth, Silicosec®, *Sitophilus granarius*, stored grains, cowpea, wheat.

INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is a worldwide pest which infests pods in the fields as well as stored seeds. Infection rate of cowpea is very low in harvest time and may be undetectable (Huignard et al., 1985). The pest multiplies rapidly in stored condition, giving rise to a new generation every month (Ouedraogo et al., 1996). Occasionally, 100% of stored seeds are damaged with up to 60% weight losses (Káita et al., 2000). Therefore, it is necessary to reduce such losses by controlling the pest on stored grains (Tapondjou et al., 2002) by efficient methods. The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), is one of the most destructive insects of stored grains worldwide. It can infest easily the stored grains, and the immature development occurs within the kernels (Aitken, 1975).

Grain losses due to those insect pests are a serious problem throughout the world (Subramanyam and Hagstrum, 1995). Residual insecticides are the most common agents for protection of stored products against stored products pests. They have several negative properties such as mammal toxicity, residues on grain as well as increased resistance of pests (Arthur, 1996). One of the most well-studied alternatives to traditional neurotoxic grain protectants is the use of diatomaceous earths (DEs) (Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Athanassiou et al., 2005; Kavallieratos et al., 2005; El-Wakeil and Saleh, 2009).

Diatomaceous earth is composed of fossilized skeletons of freshwater or marine diatoms that kill insects by abrading the cuticle and causing water loss through desiccation (Korunic, 1998) and also reported to have very low mammalian toxicity (Athanassiou et al., 2004) and safe for natural enemies as reported by El-Wakeil and Saleh (2009). Insecticidal properties of DE mostly

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Table 1. Analysis of variance on mortality of *Callosobruchus maculatus* and *Sitophilus granarius* adults exposed to various concentrations of Silicosec® for different periods.

Variance Source	*C. maculatus* df	*C. maculatus* Mean square	*C. maculatus* F value	*S. granarius* df	*S. granarius* Mean square	*S. granarius* F value
Time	1	723.96	26.54**	2	2790.99	142.26**
Dose	5	3221.37	118.09**	5	2668.51	136.01**
Time×Dose	5	38.24	1.4**	10	154.93	7.89**
Error	24	27.27		36	19.61	
Total	35			53		

**Indicate significant difference at P≤0.01.

depend on several factors including geological origin, SiO_2 content, tapped density, oil absorbency, particle size and pH (Golob, 1997; Korunic, 1998), days after treatment, moisture and temperature (Arthur, 2001).

In this study, the activity of DE as a non-chemical insecticide against two important and destructive stored pests *C. maculatus* and *S. granarius* adults was evaluated. The main purpose of present study is introducing a safe alternative method for controlling these serious pests.

MATERIALS AND METHODS

DE formulation

Silicosec® is a formulation of diatomaceous earth including 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O. The average particle size is between 8-12 µm. A sample of dry formulation of Silicosec® was obtained from Biofa GmbH, Germany.

Test insects

The insects tested were cowpea weevil, *C. maculatus* and granary weevil, *S. granarius* adults. Insects were collected from the breeding stock of Faculty of Agriculture, Urmia University, Iran. They were reared in darkness under controlled temperature and humidity (30±1°C and 65±5% RH) on cowpea and clean wheat + cracked wheat (3:1 w/w), respectively. For obtaining 1-day-old adults of *C. maculatus*, seeds with pupa window were separated and after one day, emerged adults were collected with an aspirator. Adults of *S. granarius* were used in experiments were 1-7 days old.

Laboratory bioassay

The experimental method used was similar to that of Arthur (2000). Concentrations were determined with preliminary tests. Five concentrations of DE were used for *C. maculatus* (300, 340, 387, 439 and 500 mg kg^{-1}) and for *S. granarius* (250, 323, 426, 562 and 750 mg kg^{-1}). Plastic plates (8.8 cm in diameter, 60.7 cm^2) filled with 20 g of cowpea, 20 g of wheat for other experiments and individual doses of DE. Untreated cowpea and wheat grains were used as control. Plates were sealed and shaken manually for 1 min to distribute DE in whole grain mass. Twenty adults were placed in each plate and each treatment was repeated three times. Insects were kept in an incubator (30±1°C and 65±5% RH). Total numbers of living or dead adults were recorded after 24 and 48 h for *C. maculatus*, 24, 36 and 48 h for *S. granarius*.

Data analysis

All data were transformed into arcsine scale followed by correction of cumulative mortality percentage for the corresponding control mortality (Abbott, 1925). Analysis of variance was conducted to assess the effect of concentration, time of exposure and their interaction with the insect mortalities (Nissen, 1989). Also, concentrations required to kill 50% (Lc_{50}) and 95% (Lc_{95}) of insects were calculated using probit analysis (SPSS, 1999).

RESULTS

It was observed that exposure time of insects to different DE concentrations had significant effects (P<0.01) on mortality of *C. maculatus* and *S. granarius* adults (Table 1). The mortality percentage of *C. maculates* adults at 24 and 48 h and *S. granarius* at 24, 36 and 48 h against different Silicosec® concentrations are shown in Figures 1 and 2. Increasing of DE concentration resulted in increased insect mortality. For *C. maculatus,* to obtain 50 and 95% adult mortalities after 24 h, 351.55 and 673.80 mg kg^{-1} of Silicosec® were needed, respectively. These Lc_{50} and Lc_{95} values decreased with increased time of exposure. After 48 h exposure, the Lc_{50} and Lc_{95} values were 299.92 and 504.41 mg kg^{-1}, respectively (Table 2). Regarding *S. granarius*, the Lc_{50} and Lc_{95} values after 24 h were 1512 and 8454 mg kg^{-1}, respectively. The mortality was low in the first exposure time (24 h) and did not exceeded 30%, but after 36 h, this index was increased. The concentration required to get 50% mortality after 36 h was 533.06 mg kg^{-1}. This increasing trend was also observed after 48 h and the highest mortality rate was obtained when 750 mg kg^{-1} of Silicosec® was used. The Lc_{50} and Lc_{95} values after 48 h were 404.24 and 1050 mg kg^{-1}, respectively (Table 2). Comparison of Lc_{50} and Lc_{95} values showed that *C. maculatus* adults are more sensitive to DE formulation than *S. granarius*.

DISCUSSION

The insecticidal efficacy of DEs is highly influenced by concentration rate, time of exposure, temperature and type of DE formulation. Similar results were obtained by

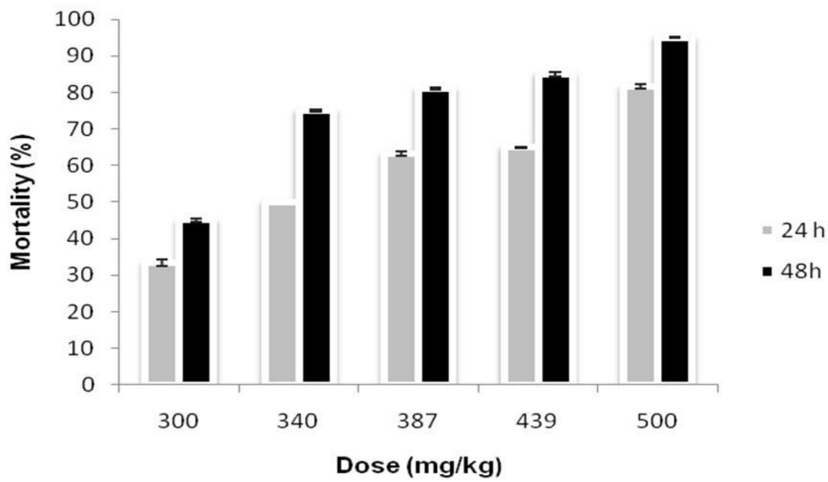

Figure 1. Mean percentage mortality (± SE) of *Callosobruchus maculatus* adults exposed to different concentrations of Silicosec® after 24 and 48 h.

Table 2. Lc_{50} and Lc_{95} (mg/kg) calculated for adult mortality within 2 days of exposure.

Insect	Exposure time(h)			95% confidence limits	χ2 (df=3)	slope± SE
C. maculatus	24	LC_{50}	351.55	323.84-373.37	0.41[NS]	5.82 ± 1.01
	24	Lc_{95}	673.80	578.14-911.41		
	48	Lc_{50}	299.92	268.12-320.87	3.32[NS]	7.28 ± 1.17
	48	Lc_{95}	504.41	460.98-592.71		
S. granarius	24	Lc_{50}	1512	990.6355551.207	0.82[NS]	2.2 ± 0.59
	24	Lc_{95}	8454	3111.94-210264.3		
	36	Lc_{50}	533.062	395.62- 1155.99	6.39	2.96 ± 0.48
	36	Lc_{95}	1916	986.43-184011.36		
	48	Lc_{50}	404.24	251.78-589.49	10.31	3.96 ± 0.51
	48	Lc_{95}	1050	670.05-19939.94		

*: Indicate significant difference at P≤0.05.
NS: No significant difference.

Korunic (1997), Arthur et al. (2001), Arnaud et al. (2005) and Vayias et al. (2006). Previous studies showed increased mortality of stored-product beetles exposed to inert dusts for increasing time intervals as mentioned by McLaughlin (1994) and Arthur (2002). Some differences among insects such as size, rate of eating, cuticular waxes, adhesion of DE to cuticle, and absorbance of water from the hind gut or tolerance to low internal water could be responsible for causing differences in susceptibility. According to present study, one key difference between two tested insects in their susceptibility is probably related to the thinner epicuticle of *C. maculatus*. As shown, the other reason that we can state is the fast rate of *C. maculatus* when eating and moving. On the other hand, longer exposure intervals may be needed to increase mortality in adults, because the longer insects walk over a treated substrate, the more dust particles are trapped by their bodies, losing more water and causing death by desiccation.

The death of insects caused by diatomaceous earth (DE) is attributed to the dehydration provoked by the abrasiveness of the small particles of this inert dust and by adsorption of oils in the body of the insect, which breaks the layer of wax on the epicuticle, exacerbating the fatal loss of water as reported by Subramanyam and Roesli (2000). Therefore, in higher concentrations, the adsorption of wax and abrasiveness caused by the product occurs faster, causing death in a shorter time

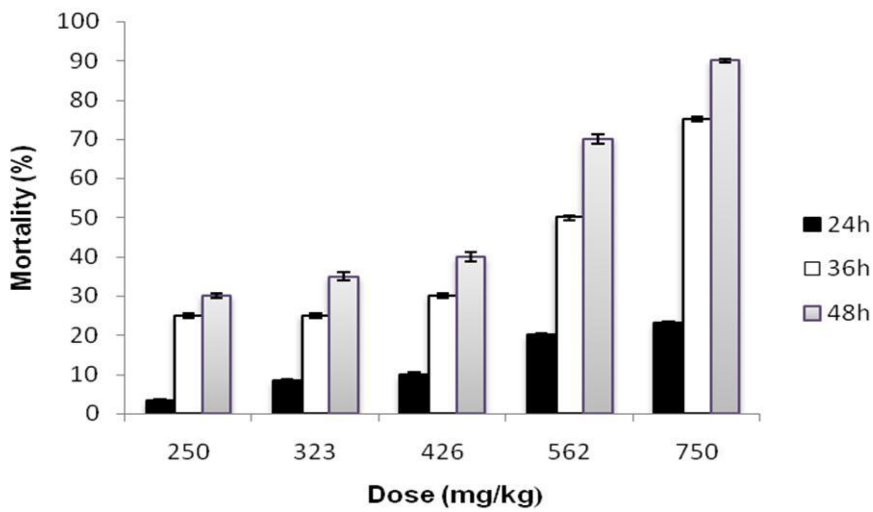

Figure 2. Mean percentage mortality (± SE) of *Sitophilus granarius* adults exposed to different concentrations of Silicosec® after 24, 36 and 48 h.

compared with low concentrations.

Diatomaceous earth showed high efficacy against *C. maculatus* and *S. granarius* adults under the conditions of this study. As the dosage of diatomaceous earth and the exposure time increased, a clear increase in mortality of insects was also observed as proposed by Aldryhim (1990). The use of DE as a safe control method against stored product pests is highly recommended. The results of present study indicate the potential of non-chemical method development with emphasis on improving high quality new formulation of DE.

ACKNOWLEDGEMENTS

We want to thank Dr. Amir Sobhanmanesh, Dr. Shahram Aramideh, Dr. Rezaee Danesh and Dr. Seyed Ali Safavi for their comments on early version of this manuscript. Thanks also to Miss Nadeali, Mr Sharifian and Abdolmaleki for their assistance in insect bioassays.

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