Effect of Diatomaceous Earth (Silicosec®) in Different Temperatures on Mortality and Progeny Production of Adult Toothed Beetle *Oryzaephilus surinamensis* L. in Laboratory Condition

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Abstract: Food grains are the key foods to human and domestic animals in the world. The quantity and quality of these products are reduced by insects during storage. Toothed beetle *Oryzaephilus surinamensis* L. is economically considered as one of the most important stored product pests. Nowadays, the use of contact synthetic insecticides such as diatoms earth (DE) is one of the common strategies to prevent pest damage to stored products. The aim of this study was to evaluate the effect of DE mortality of adult toothed weevils at different temperatures as well progeny production. The results showed that mortality of this pest was affected by temperature, concentrations and time exposure as increasing in temperature resulted in significant higher mortality. In addition, the result obtained from progeny production showed that DE could significantly reduce progeny production of this pest. In general, SilicoSec formulation of DE can preserve stored products from damaging of the pests.
[Hojjat Zarei, Reza Vafaei Shoushtari and Zahra Rafiei. **Effect of Diatomaceous Earth (Silicosec®) in Different Temperatures on Mortality and Progeny Production of Adult Toothed Beetle *Oryzaephilus surinamensis* L. in Laboratory Condition.** *Nat Sci* 2015;13(3):91-95]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 15

Key words: Diatom earth, toothed weevil, Mortality, Progeny production

Introduction:

Cereals are proteinaceous plants categorized in the first place, so they have a crucial role the human diet. The products from harvest to consumption stored in warehouses. Many efforts have been made to protect these products from pest and disease attacks in farm and stores. Despite all these activities, cereal grains are attacked by a variety of pests and diseases that have been the most effective in the role of insect pests (Hill, 1990). Given the economic importance of stored product pests and insect resistance to conventional pesticides, finding a safe, convenient, economical way to control and reduce the damage of these pests are needed. Use of fumigation because of influential participants in the stored products is the main method. In recent years, use of these methods was discarded because of insect resistance to pesticides (Weaver & Subramanyam, 2000) and environmental destroyer impact (Schlipalius et al., 2006). These days, using synthetic contact insecticides is one of the most common strategies to prevent pest feeding damage (Arthur, 1996). Among these compounds diatoms soil are used in conservation cereals, legumes and oilseeds in warehouses (Chanbang et al., 2007). These soils are effective on a wide range of pests. This product has unlimited storage life and long-term protection of stored foods, as long as the surface remains dry (Fields, 1992). The soils which were stable and unlike organophosphates, not decompose on heating and remain effective so do not leave residues on food and toxic substances in the environment (Quarles, 1992). Diatomaceous earth particles with tiny pores that are attached to the body of the insect absorb waxy cuticles layer of insects and small amounts may cause scratches on the surface of insect cuticles leading to death (Athanassiou, 2006).

Toothed beetle *Oryzaephilus surinamensis* L. (Col: Cucujidae) are economically the most important pest of stored product and it was distributed in Iran for a long time. This pest attacks to all herbal products such as wheat, rice, corn, barley, wheat flour, bran, pasta, bread, oil seeds, dried herbs, and natural history collections and creates heavy losses. This pest causes damage not only by feeding but also with his own feces-contaminated and its quality is severely reduced (Mollaie et al., 2011).

Results obtained from the effect of insecticide concentration of 10 gr/m^2 warehouse on the treated diatomaceous earth formulations SilicoSec® on toothed grain beetle *O. surinamensis* adults suggests that the ability of the insecticidal toxin increases with time (Cook et al ., 2002). Ziae (2004) carried out an investigation on *Tribolium castaneum*, *Rhyzoperta dominica* and found that high temperatures can have synergistic effects with diatomaceous earth (Ziae, 2004). The research from Sakenin and Kheshaveh (2014) also shows that the diatameous earth had long-term toxic effects on cowpea weevil. (Sakenin & Khashaveh, 2014). Research either conducted on other pests or the effect of temperature on the performance of the toxin on this pest ignored. The aim of this study was to evaluate the effect of diatomaceous earth insecticide on toothed beetle at different temperatures.

Materials and methods:

Insects

Oryzaphilus surinamensis adults was separated and collected from infected imported corn in storage located in Arak in Markazi province. After identifying the species, the mass rearing was imitated at 28 °C and 65% relative humidity, and after purification 6 generations on imported maize varieties, the adults were used in the experiments.

Bioassay of the effect of different concentrations of insecticide formulations SilicoSec® diatomaceous earth in the adult corn O. surinamensis at temperatures 22 ± 0.2, 27 ± 0.2 and 32 ± 0.2 ° C and a relative humidity of 65 ± 0.5 per cent.

Different concentrations of diatomaceous earth formulations SilicoSec® separately for each temperature was determined through preliminary tests. Concentrations of 0, 250, 500, 750, 1,000 and 1,500 ppm were selected for preliminary testing and mortality were counted 2 and 7 days after treatment. Ranges of effective concentrations were determined by a logarithmic relationship. Concentrations are determined by bioassay for the adults of *O. Surinamensis* 500, 650, 800 and 1000 ppm at 22± 0.2 ° C, 350, 500, 650 and 850 ppm at 27 ± 0.2 °C and 300, 450, 600 and 850 ppm at 32 ±0.2 °C. To assess the SilicoSec® formulation on the adult insects in each experimental unit, 60 gr of imports corn varieties were dumped in a half a liter glass. Corn seeds with given concentrations of diatomaceous earth formulations SilicoSec® were treated. The door of glass was made and was shaken for 1 minute. After 5 minutes (the time required for settling the particles), glass door open and 30 adults were released and windows were covered with mesh fabric for adequate ventilation. Concentration, temperature, and number of repeat tests was written on glasses and were transferred into the test container incubators set at temperatures of 22 ± 0.2, 27 ± 0.2 and 32 ± 0.2°C, 65 ± 5 percent relative humidity. Adult mortality was counted 1, 2 and 7 days after treatment. Three replicates for each concentration and control were considered and a total of 300 adult for every 7 to 14 days, without gender, was used.

Evaluation the ability of adult *O. Surinamensis* to produce offspring on corn pellets treated with diatomaceous earth formulations SilicoSec®

To evaluate the ability offspring production, after the last day of the counting of adult mortality (which was 7 days after treatment), all insects (dead and alive) were kept in the glass on untreated corn in the same conditions of temperature and relative humidity for 60 days. After this time and in the case of adults emerged larvae were counted.

Data analysis

Control was considered as a single concentration (zero concentration in ppm) and need not be corrected using Abbott's formula. Analysis of variance of the adult trials with factorial design (concentration the first factor and time the second factor) was applied in a completely randomized design using the software SPSS 20.0. The significance of factors of all experiments was performed using the Duncan test at 5% level of statistical comparisons with SPSS software. For homogeneity of variance, before statistical analysis, the data was standardized by Arcsin $\sqrt{x}$. In experiments on the ability to produce offspring, due to the lack of uniform progeny appeared in each iteration (block), data for each temperature were separately analyzed in randomized complete block design with 3 replications using a Software 20.0 SPSS.

The significance of factors, the data in all experiments were statistically compared using the Duncan test at 5% probability level using SPSS 20.0 software. to transform data, before statistical analysis log (X + 1) was used. Percent reduction in the production of offspring was calculated by formula (Adryhim, 1990):

$$x = \frac{\text{number of offspring in control} - \text{number of offsprings in each treatment}}{\text{number of offspring in control}} * 100$$

Results:

Analyses of statistical data showed that mortality of adult *O. surinamensis* were significantly different at different time and temperature (99%). In addition, the interactions (temperature and time) were significant (99%) (Table 1).

The results showed that the mortality of the insect was influenced by the temperature and duration of exposure to diatomaceous earth. The mortality rate increased in this situation and on the seventh day 94.44% (Table 2).

The increases in mortality at a concentration of 650 ppm at 22 ° C was also observed as the mortality rate in the first, second and seventh were 17.77, 76.66 and 96.66%, respectively. In 800 ppm concentration, mortality rate increased from 24.44% to 98.88% to 84.44% on the first, second and the seventh day, respectively.

The results showed that the ability to produce offspring in this pest at 22 and 32 °C was no significant difference between replicates but at 27 ° C there was a significant difference. While in all three temperature levels, there was significant differences at 1% level (Table 3).

Table 1. Analysis of variance of the maize grain treated with diatomaceous earth (DE) Silicosec on *O.surinamensis* adult in different concentrations and temperatures of 22, 27 and 32 ºC.

Source	df	Mean Sums of square	Sums of square	F	P
Concentration	4	6067.5	24270.1	**456.81	≤0.01
Time	2	8123.1	16246.3	**611.57	≤0.01
Concentration*time	8	510.1	4080.1	**38.39	≤0.01
Error	30	13.2	398.4		
Total	44		44995.5		
Concentration	4	9056.4	36225.8	**301.07	≤0.01
Time	2	1101.7	2203.4	**36.62	≤0.01
Concentration*time	8	66.7	534.0	**2.21	≤0.01
Error	30	30.0	902.4		
Total	44		39867.7		
Concentration	4	5401.7	21606.9	**301.07	≤0.01
Time	2	4040.9	8081.9	**36.62	≤0.01
Concentration*time	8	148.4	1187.2	**2.21	≤0.01
Error	30	37.5	1127.1		
total	44		32007.1		

Table 2. Mean ± standard error of adult mortality of toothed beetle *O. surinamensis* in corn kernels treated with diatomaceous earth (DE) formulations SilicoSec at temperatures of 22, 27 and 32 ºC

Temperature (ºC)	Time (day)	Mortality ± SE				
		Concentration (ppm)				
22		Control	500	650	800	1000
	1	0±0	0.98Cc ±12.22	3.66^{bC}±17.77	0.73^{aC}±24.44	3.03^{aC}±25.55
	2	0±0	0.75^{bB}±74.44	2.66^{bB}±76.66	0.90^{aB}±84.44	1.64^{aB}±86.66
	7	0±0	1.50^{A}±94.44	4.26^{A}±96.66	3.30^{A}±98.88	0^{A}±100
27		Control	350	500	650	850
	1	0±0	2.93^{cC}±56.66	3.10^{bC}±84.44	5.37^{aB}±86.66	6.1^{aA}±93.33
	2	0±0	4.16^{bB}±86.66	2.65^{bB}±88.88	2.30^{bA}±93.33	0^{aA}±100
	7	1.14±8.88	2.3^{A}±93.33	4.26^{A}±96.66	3.29^{A}±98.88	0^{A}±100
32		Control	100	200	300	500
	1	0±0	4.26^{dC}±3.33	2.06^{cC}±22.22	4.76^{bC}±42.22	4.52^{aB}±70.0
	2	0±0	$^{4.61cB}$±25.55	1.12^{bB}±56.66	3.70^{aB}±77.77	5.5^{aA}±84.44
	7	1.84± 10	1.19^{bA}±70.0	2.70^{aA}±87.77	4.01^{aA}±91.11	5.16^{aA}±95.55

English lowercase letters within rows indicate significant differences at a confidence level of 99% on different concentration in different day's exposure and capital letters between columns indicate significant differences between the concentration in a temperature and time exposures.

Table 3. Analysis of variance tests maize grain treated with diatomaceous earth formulations SilicoSec the progeny production of adult weevils *O. Surinamensis* in temperatures 22, 27 and 32 °C.

Temperature (°C)	Source	df	Mean Sums of square	Sums of square	F	P
22	Replicate	2	0.02	0.04	11.95	0.204
	Concentration	4	2.0	8.02	196.7	0.000
	Error	8	0.01	0.08		
	total	14		8.14		
27	Replicate	2	0.06	0.14	4.53	0.04
	Concentration	4	1.02	4.08	67.7	0.000
	Error	8	0.01	0.12		
	total	14		4.34		
32	Replicate	2	0.07	0.16	2.03	0.19
	Concentration	4	0.84	3.38	22.13	0.000
	Error	8	0.03	0.31		
	total	14		3.85		

Average progeny at 22 °C and 500 ppm concentration was 0.66 percent and the reduction was observed as 99.13 percent. While the averages in a concentration of 650, 800, 1000 ppm were zero, 0.42 and zero. 100, 99.56 and 100 % reduction in offspring were observed at 650, 800 and 1000 ppm, respectively, (Table 4).

Table 4. compares the means ± standard error of the reduction in adult progeny production of *O.surinamensis* treated with SilicoSec diatomaceous earth formulations at temperatures of (a) 22, (b) 27 and (c) 32 ° C

	Concentration (ppm)				
a	Control	500	650	800	1000
Mean of offspring		0.008ᵃ±77.33	0.1ᵇ±0.66	0ᵈ±0	0.1ᶜ±0.42
% reduction in offspring production		99.13	100	99.56	100
b	Control	350	350	650	850
Mean of offsprings	0.01ᵃ±38	0.13ᵇ±5	0.11ᶜ±2.33	0.1ᶜ±2.33	0 ᵈ ±0
% reduction in offspring production		86.84	93.85	93.85	100
c	Control	100	200	300	500
Mean of offspring	0.05ᵃ±32	0.15b±6	0.23ᵇ±2.66	0.04ᶜ±2.33	0.1ᵈ±0.33
% reduction in offspring production		81.25	91.66	92.7	98.95

Lowercase letters indicate significant differences between rows at 1% level of confidence between the progeny production in different concentrations.

The lowest mean of offspring production was observed at 27 °C in 850 ppm leading to 100% mortality. Also at 32 °C, 200 and 500 ppm, the lowest mean offspring were produced which were 91.66 98.95 respectively (Table 4).

Discussion

Analyses of statistical data showed that mortality of adult *O. surinamensis* were significantly different at different time and temperature (99%). The results showed that the mortality of the insect is influenced by the temperature and duration of exposure to diatomaceous earth.

Results showed that there were significant differences (99%) between temperature and the increases in temperature, the increases capability of killing SilicoSec® insecticide. As the temperature increases, the damage also increases due to increasing mobility of insects and diatomaceous earth particles is more contact with the body of the insect cuticle and (Fields & Korunic, 2000).

Effect of diatomaceous earth on 3 species of stored product pests treated at different levels and in empty warehouses and bulk product indicated that 20 gr/m^2 diatomaceous earth can cause 90% mortality in the toothed beetle (Scholler & Reichmuth, 2010).

Research on the efficacy of diatomaceous earth on the storage pest species including red flour beetle and toothed beetle and 65% relative humidity at 27 °C using the method of ingestion and contact bioassays were carried out. Mortality rates of contact on the first, second and third and digestive method in first, seventh and tenth was calculated. 99% of mortality in contact method was observed in the third day and the concentration of 165 ppm and as well as in digestive method in the tenth day of 8333 ppm, respectively. The results showed that mortality rate of both examined species increases with increasing time of exposure, and toothed beetle is more sensitive to the diatomaceous earth than red flour. The authors also stated that the contact way is more efficient than digestive one and using contact way in the early days will cause more deaths on toothed beetle (Mohitazar et al., 2009). The results of the present study corresponded with the findings of this research.

Effect of 0.5 mg/cm^2 of 5 formulations Dryacid, Protect-IT, SilicoSec, Insecto, Perma-Guard diatomaceous earth on *O. surinamensis*, *T. Costaneum* and *R. dominica* was investigated at 28 °C and 65% relative humidity (Ziaee & Khashaveh 2007). The results showed that the mortality rate of the first day in the formulation Dryacid, Protect-IT was 100 % and in SilicoSec, Insecto, Perma-Guard, were 73, 76 and 93 % respectively. Delays Mortality in this species in the formulations varied as the Dryacid, Protect-IT, SilicoSec formulations on the seventh day was reached to 100 % and in Insecto and Perma-Guard, were 91 and 96%, respectively (Ziaee & Khashaveh 2007). The results obtained from this study are in agreement well with that results which indicated that 100% mortality occurred on the seventh day.

Moras *et al* (2004) conducted an experiment on the effect of propionic acid mixed with diatomaceous earth on *R. dominica* and O. *surinamensis*. Two formulations of diatomaceous earth Bugram and KeepDry was used alone and in combination with the acid. Concentrations used in this experiment consisted of 0.5, 1 and 1.5 kg of Keepdry formulation and 1, 2 and 3 kg of the Bugram formulation. The results showed that both formulations, and at 2, 6, 8 and 12 months after the use of diatomaceous earth mortality rate was reached 100% (Moras et al., 2004).

Research carried out by Shafighi et al (2014) on the effect of diatomaceous earth and fungus *Metarhizium* on three species of stored product pests, showed that SilicoSec formulation could cause 78 % mortality on *O. surinamensis*. The researchers also concluded that diatomaceous earth in combination with the fungus had better effect on the mortality of this species and because diatomaceous earth cause scratches on the insect's cuticle, the fungus could rapidly be penetrated to the insect body causing early adult mortality (Shafighi et al., 2014).

Due to the lack of data compiled on an average offspring production, we could not comprise our data to them. The results of this study indicate that when concentration of SilicoSec increased, the number of adult offspring significantly reduced and reproductive ability of adult treated with SilicoSec compared with untreated maize, significantly reduced, indicating that high residual effect of SilicoSec formulation of diatomaceous earth.

In conclusion, the results of this experiment showed that diatomaceous earth (SilicoSec formulations) can be used as proper preservative of grain after storage.

Acknowledgements:

Hereby, the authors are thanking managing director of the company's support for animal husbandry Mr. Yasai and Mr. Mousavi, the manager of animal studies for their help and support.

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2/28/2015