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Efficacy of Silicosec®, a Diatomaceous Earth Formulation Against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract: Laboratory bioassays were carried out to evaluate the insecticidal efficacy of SilicoSec® against 7-14 days old adults of *Tribolium castaneum*; old and young larvae with the mean weight of 3.4±0.1 and 0.6±0.1 mg, respectively at 27°C and 55±5% r.h in the dark. Wheat treated with four dose rates of SilicoSec® with three replications. Adult's mortality was measured after 2, 7 and 14 days of exposure. After 14 days mortality count, all adults were removed and samples retained under the same conditions for a further 60 days to assess progeny production. In the case of larvae, mortality was counted after 1, 2 and 7 days. After 2 days of exposure no concentration achieved 11% mortality for adults, however; adult's mortality exceeds 89.65% when exposed for 7 days to SilicoSec®. Mortality of old and young larvae at 0.6 g kg^{-1} after 2 days were 28.88 and 22.22%, respectively and exceed to 60.71 and 69.04% at longer exposure of 7 days. Results indicated that mortality of *T. castaneum* was influenced by interval exposed to wheat treated with SilicoSec® and over this exposure; the increases in application rate of SilicoSec® had significant effect on the mortality. Young larvae of red flour beetle were more sensitive to SilicoSec® than old larvae and adults were more tolerant. Reproductive potential of adults in the treated wheat was suppressed when compared with untreated wheat. The high retention level of SilicoSec® (78.62%) was noted in wheat kernels.

Key words: Diatomaceous earth, grain protectant, Silico Sec®, *Tribolium castaneum*, wheat

INTRODUCTION

The development of resistance in many species, the demand for residue free food, the increased concerns about worker safety and the gradual withdrawal of several commonly used insecticides, lead researchers to evaluate the potential use of other reduced-risk, control methods. Recently, alternative methods are being emphasized to reduce use of insecticides to lessen the potential for human exposure and to slow the development of insecticide resistance in pests (Aldryhim, 1993). Diatomaceous Earths (DEs) are among the most promising alternatives to traditional grain protectants, because they are easy to use and combine low mammalian toxicity with natural origin, doesn't break down rapidly and dose not affect grain end-use quality (Athanassiou *et al*., 2004). DEs are almost pure silicon dioxide made up of fossilized diatoms which absorb wax from insect's cuticle resulting in water loss and death through desiccation (Korunic, 1998; Nikpay, 2006). DEs can be applied directly to the grain, without specialized equipment using much the same technology as far residual insecticides (Athanassiou *et al*., 2005). Vayias and Athanassiou (2004) exposed adults, young larvae (1-3 instars) and old larvae (4-7 instars) of *Tribolium confusum* Du Val. to SilicoSec® at the rates of 0.25, 0.5, 1 and 1.5 g kg^{-1}. High level of mortality was observed for *T. confusum* larvae after 24 h of exposure even at the lowest dose rate. Athanassiou *et al*. (2005) in the other research investigated the impact of SilicoSec® against adults of *Sitophilus oryzae* L. and *T. confusum*. The authors stated that adults of *T. confusum* were more tolerant to DE SilicoSec® than *S. oryzae*.

The red flour beetle, *Tribolium castaneum* (Herbst) is probably one of the most common and the least susceptible stored-product pests to DE, so a DE formulation able to control flour beetles should be able to control most insects occurring in stored food (Korunic, 1998; Fields and Korunic, 2000; Arnaud *et al*., 2005).

The objective of present study is to evaluate the insecticidal efficacy of SilicoSec®, a commercial DE product, against adults, young and old larvae of *T. castaneum* under laboratory conditions.

MATERIALS AND METHODS

Beetles: Beetles were reared at 28°C and 65±5% r.h (relative humidity) in continues darkness. Adults of

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T. castaneum were reared on wheat flour plus 5% brewers yeast (by weight). All adults were used in the experiment were 7-14 days old of mixed sex. Young larvae of *T. castaneum* were obtained by placing 100 unsexed adults of mixed ages on 100 g wheat flour plus 5% brewers yeast (by weight) diet in glass jars. After 7 days, young larvae were separated from the diet by using appropriate sieves with the mean±SE (n = 30) weight of 0.6±0.1 mg and old larvae separated after 20 days from the diet with the mean±SE (n = 30) weight of 3.4±0.1 mg. Beetles were obtained from cultures maintained in the Entomology laboratory of Urmia University for at least 3 years, with no history of exposure to insecticides.

DE formulation: SilicoSec® is a freshwater formulation of diatomaceous earth (Biofa GmbH, Munsingen, Germany) and is composed of 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O. The median particle size is between 8-12 µm. DE was stored in the laboratory at ambient conditions, until the beginning of the experiment (approximately for a month).

Bioassays: Experiments were conducted in the Entomology laboratory of Urmia University in 2006.

Susceptibility of adults (Test 1): Fifteen glass vials with the volume of 0.5 L were provided and poured with sixty grams of clean wheat (variety Zarin) (95% whole wheat plus 5% cracked wheat), three vials for each dose rate. The moisture content (m.c) of the wheat was measured using Dickey-John moisture meter ranged about 11.4% m.c which is equilibrium to 55% r.h (Pixton and Warburton, 1971). Wheat treated with four dose rates of SilicoSec®: 0.7, 1, 1.3 ans 1.7 g kg^{-1}. Doses were determined with a preliminary test and untreated wheat with a similar ratio of whole to cracked wheat served as a control treatment. The vials were shaken for one minute to distribute the DE in the entire product. Subsequently, 30 adults were introduced into each sample and vials were covered with muslin cloth for sufficient ventilation. The vials were then placed in incubator set at 27°C and 55±5% r.h. Adult's mortality was measured after 2, 7 and 14 days of exposure. After 14 days mortality count, all adults were removed and samples retained under the same conditions for a further 60 day to assess progeny production.

Susceptibility of larvae (Test 2): The method of this experiment was similar to that employed for adults, but in order to that the susceptibility of stages is different, therefore four doses of SilicoSec® were determined separately for each of the stages with a preliminary test. The 0.35, 0.6, 0.9, 1.2 g kg^{-1} of SilicoSec® were used for young larvae and in the case of old larvae 0.35, 0.6, 1, 1.5 g kg^{-1} of SilicoSec® was treated with 95% whole wheat + 5% cracked wheat and placed in the appropriate conditions of previous experiment after introducing individuals. Mortality of young and old larvae was counted after 1, 2 and 7 days interval.

Adherence of SilicoSec® to wheat: The method of determining DE adherence to wheat kernels was similar to that employed by Korunic (1997). First, 500 g of wheat was cleaned by sieving for a minute using a No. 10 sieve (2 mm openings, Retsch GmbH and Co KG, Germany). Subsequently, the cleaned wheat was mixed with 1 g kg^{-1} of SilicoSec® (0.5 g per 500 g) in a tightly closed glass jar and shaken for a minute. The treated grain was then sieved thoroughly using laboratory sieve No. 10 with a lid and bottom for one minute. The dust collected and weighed. The weight was subtracted from 500 mg and the value was expressed as a percentage of adherence of DE on the wheat kernels.

Data analysis: The mortality counts were corrected by using Abbott's (1925) formula. The data were analyzed using Analysis of Variance and means were separated by using the Tukey-Kramer adjustment at p = 0.05 (SAS, 2000). To equalize variances, mortality percentage of adults, young and old larvae were transformed using the square root of the arcsine and the data of adults progeny production was transformed to log (x +1) scale. The dose required to kill 50% of the insects (LC_{50}) was estimated using probit analysis (SPSS, 1999). Linear lines were drawn to define all dose-mortality relations for each exposure time. Percentage of reduction in progeny production was determined by Aldryhim (1990) is given as:

$$\frac{\text{No. progeny in control} - \text{No. progeny in treatment}}{\text{No. progeny in control}} \times 100$$

RESULTS AND DISCUSSION

The main effects for adults: Dose (F = 127.09, df = 4); exposure interval (F = 701.8, df = 2), young larvae: Dose (F = 128.1, df = 4); exposure interval (F = 276, df = 2) and old larvae: Dose (F = 201.1, df = 4); exposure interval (F = 242.05, df = 2) were all significant. In addition, all associated interactions; dose × exposure interval for adults (F = 35.8, df = 8), young larvae (F = 7.1, df = 8) and old larvae (F = 4.1, df = 8) were also significant. The mortality percentage for adults of *T. castaneum* after 2, 7 and 14 days of exposure and in the case of young and old

Table 1: Mean mortality (%) ±SE of adults, young and old larvae of *T. castaneum* exposed to SilicoSec® after 2, 7 and 14 days of exposure for adults and 1, 2 and 7 days in the case of larvae

	Dose rates (g kg^{-1})			
Exposure time (day)	0.7	1	1.3	1.7
Adults				
2	3.33±4.2g	5.55±1.51g	6.66±2.3g	10.00±3.9g
7	33.3±0.6e	51.10±2.5d	87.70±5.3c	89.65±1.8c
14	90.0±1.8c	95.50±5.1b	96.30±4.2ab	100.00±0.0a
Young larvae				
	0.35	0.60	0.90	1.20
1	11.1±3.8fg	10.00±2.1fg	15.50±1.7ef	20.00±0.0e
2	21.1±1.5e	22.20±2.0e	33.30±0d	35.50±2.7d
7	57.7±2.8c	69.04±2.5b	73.30±4.7b	91.10±2.1a
Old larvae				
	0.35	0.60	1.00	1.50
1	7.77±2.6e	18.80±2.8d	20.00±1.3d	21.10±2.9d
2	20.0±2.4d	28.80±0.6c	41.10±2.3c	42.20±1.2c
7	45.5±1.2c	60.70±1.1b	70.00±3.0ab	74.40±1.9a

Means followed by the same letter on each table are not significantly different; Tukey-Kramer at $p = 0.05$

Table 2: The LC_{50} values (g kg^{-1}) for adults, young and old larvae of *T.castaneum* exposed to wheat treated with SilicoSec® (Significant Chi-square for interaction = 5.99)

Exposure time (day)	LC_{50} (g kg^{-1})	Confidence limits (95%)	Probit/log regression line	Chi-square (df = 2)
Adult				
2	14.598	NC	-0.78+1.38x	0.63*
7	0.913	0.123-1.268	-10.40+5.2x	7.35^{NS}
14	0.267	0.025-0.446	-2.10+2.92x	1.55*
Young larvae				
1	19.535	NC	1.84+0.73x	0.65*
2	3.105	1.550-513.781	1.85+0.9x	0.94*
7	0.320	0.183-0.416	0.49+1.8x	5.03*
Old larvae				
1	11.179	3.303-1068302.924	1.64+0.83x	2.40*
2	1.929	1.240-9.379	1.96+0.92x	3.63*
7	0.446	0.252-0.590	1.61+1.26	1.08*

NC: Confidence limits could not be calculated; NS: Non Significant difference; *: Indicate significant difference at $p<0.05$

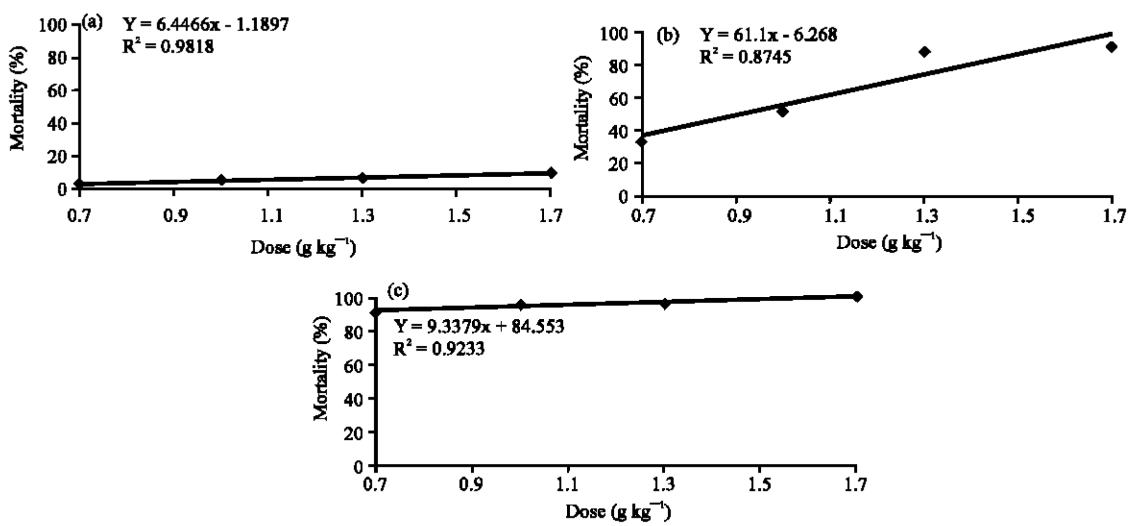

Fig. 1: Relationship between dose rates of SilicoSec® and mortality of *T. castaneum* adults for 2 (a), 7 (b) and 14 (c) days exposure time

larvae after 1, 2 and 7 days exposed to different doses of SilicoSec® has been shown on Table 1. Dose-mortality lines for each exposure time were presented on Fig. 1 for adults and Fig. 2 in the case of young and old larvae of *T. castaneum*. The determined coefficient (R^2) indicated that what percentage of variation in the mortality can be accounted by the present linear model (Fig. 1 and 2).

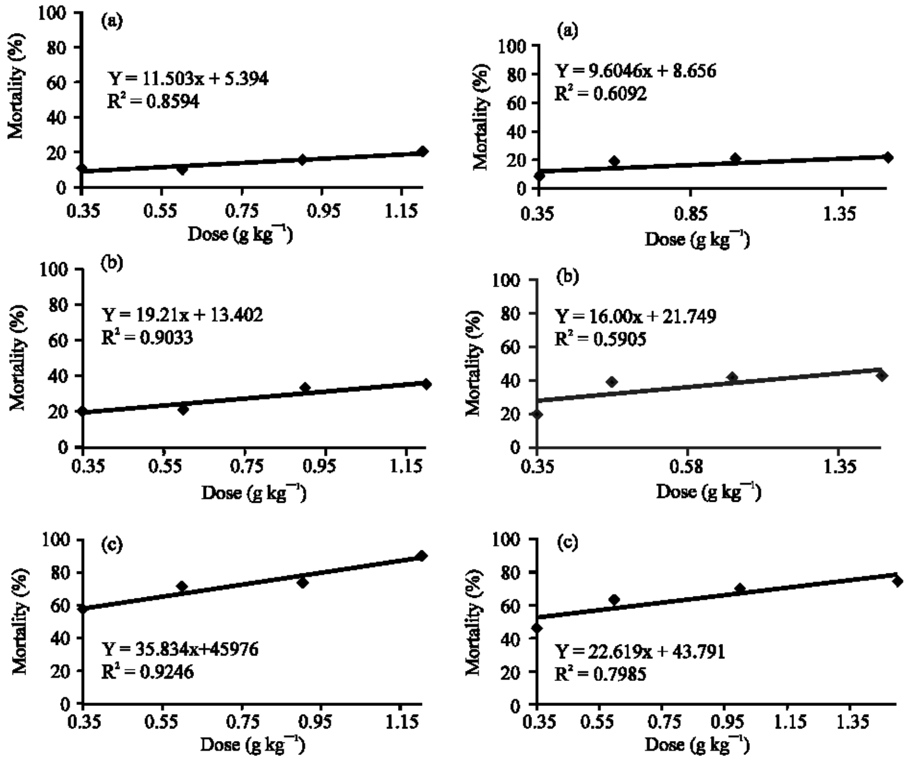

Fig. 2: Relationship between dose rates of SilicoSec® and mortality of *T. castaneum* young larvae on the left and old larvae in the right for 1 (a), 2 (b) and 7 (c) days exposure time

Table 3: The mean (number of individuals per vial±SE) and percentage of reduction in progeny production (f_1) of *T. castaneum* exposed to wheat treated with SilicoSec®

Parameters	Control	0.7 (g kg^{-1})	1 (g kg^{-1})	1.3 (g kg^{-1})	1.7 (g kg^{-1})
Mean number±SE of progeny	7.66±0.10a	0.66±0.1b	0±0b	0.33±0.1b	0±0b
Reduction in progeny production (%)	-	91.30	100	95.65	100

Means followed by the same letter are not significantly different; Tukey-Kramer at p = 0.05

The LC_{50} values decreased with increases in time of exposure. The 7 days LC_{50} for *T. castaneum* adults was 0.913 g kg^{-1}, in the case of young larvae the LC_{50} value after 7 days was 0.32 g kg^{-1}, however; 0.446 g kg^{-1} of SilicoSec® was needed to achieve 50% mortality for old larvae of *T. castaneum* after 7 days interval. Chi-square analyses of expected and observed mortalities of beetles indicated that observed mortality was the same as expected from the experiments at all testes (α = 0.05) with the exception of *T. castaneum* adults after 7 days of exposure to SilicoSec® (Table 2). The mean number±SE of progeny in the control was 7.66±0.10 individuals per sample and significant suppression in progeny production was reported even at the lowest dose rate of SilicoSec® (Table 3). The retention of SilicoSec® to wheat kernels with the 11.4% m.c was recorded 78.62%.

Present study indicates that mortality of *T. castaneum* on wheat treated with SilicoSec® increases with exposure time, this stands in accordance with previous reports by Aldryhim (1990 and 1993), Vayias and Athanassiou (2004), Athanassiou *et al.* (2005) and Ziaee *et al.* (2006).

In the present research longer exposure interval is needed to achieve 100% mortality for adults of *T. castaneum*, because the longer the insects walk over the treated substrate the more dust particles are trapped by their bodies; resulting in water loss and death through desiccation (Arthur, 2000).

Results indicated that larvae of *T. castaneum* are more sensitive to SilicoSec® than adults; however this effect is determined by the larval stages. Young larvae are significantly susceptible than older ones and this difference is apparent after 7 days of exposure. This agrees with that experiment of Vayias and Athanassiou (2004). After 24 h of exposure to DE SilicoSec®; approximately 61% of young larvae were dead, while the respective mortality for old larvae was only 26%. In young larvae the cuticle may be softer than in older ones and thus, DE may cause more rapid cuticle damage which may result in more desiccation. Also, young larvae are particularly agile; a fact which increases the contact with the dust particles, as compared to older larvae stages prior to pupation which is less active (Vayias and Athanassiou, 2004).

DE dose rate is crucial not only for efficacy but also for the physical properties of the grain. High dose rates provide a satisfactory level of protection but dramatically affect the bulk density less, but may not be sufficient for long term protection (Korunic, 1998).

In present study more than 85% of the variation in the adult and young larvae mortality can be accounted by SilicoSec® dose rate with linear lines which indicated that there is a direct relation between the mortality and DE dose rate; so the mortality increases with dose rate (Fig. 1 and 2).

The comparison of LC_{50} values after 7 days interval indicated that high amount of SilicoSec® (0.913 g kg^{-1}) was required to achieve 50% adult mortality; while, the less dose rates of 0.446 and 0.32 g kg^{-1} is sufficient for controlling 50% of old and young larvae, respectively. Therefore, adults of *T. castaneum* were more tolerant to SilicoSec® than larvae and can survive at application rates and exposure intervals that are lethal to all larval stages; so the application rate recommended for controlling adults can control different larval stages of *T. castaneum*. Results confirmed that 1.3 and 1.7 g kg^{-1} of SilicoSec ® were sufficient enough to control adults of *T. castaneum* because with 1.7 g kg^{-1} of SilicoSec®, 100% mortality and completely progeny suppression and in the case of 1.3 g kg^{-1}, 96.3% mortality after 14 days of exposure and 95.65% reduction in progeny production was recorded and these doses ranged in the same group; therefore we recommend 1.3 g kg^{-1} of SilicoSec® to control infestations of *T. castaneum*. Athanassiou *et al.* (2005) reported that 1 g kg^{-1} of SilicoSec® was equally effective with 1.5 g kg^{-1} against *S. oryzae* and *T. confusum* and this is in agreement with present results.

Stored product insect pests show a wide range of susceptibility to DE (Aldryhim, 1990, 1993). Fields and Korunic (2000) found that *T. castaneum* had noticeably less DE attached to its cuticle than other storage beetles, so *T. castaneum* appeared more tolerant stored grain species to DE and the application rate for this species can be used for protecting grain in the storage facilities.

However, not only the species of the beetles but also the strain of it may be important to its susceptibility to DEs. According to Vayias *et al.* (2006) different susceptibility to Des was observed among strains of *T. confusum*. In the same study strains of *T. confusum* from Denmark, United Kingdom and Germany were the most susceptible to Insecto, Protect-It, Protectot, PyriSec and SilicoSec whereas the strain from Portugal was the least susceptible.

Exposure to SilicoSec® suppressed reproductive potential of adults significantly. The adults probably were killed before they were able to lay eggs and therefore the SilicoSec® could provide stored wheat grain with complete protection from infestation.

One of the characteristics that determine the insecticidal efficacy of DE is the degree of adherence to the kernels.

Aldryhim (1993) reported that two factors contributed to the effectiveness of DE; (1) the degree of adhesion of DE particles to different commodities and (2) the rate that DE particles are picked up by beetles. Korunic (1997) speculated that among other characteristics of DEs, adherence to commodities correlates well with the insecticidal activity of a given DE. Kavallieratos *et al.* (2005) reported different degrees of adherence among wheat, whole barley, peeled barley, oats, rye, triticale, rice and maize for the DEs SilicoSec® and Insecto®. The retention of SilicoSec® was significantly higher than the corresponding figure for Insecto®. In the present study, the degree of SilicoSec® adherence to wheat kernels was noted 78.62% (>70%) which is marked plus (+) due to the criteria for the prediction of potential insecticidal value of diatomaceous earth recorded by Korunic (1997). The high retention level of SilicoSec® on wheat reflects the typical high adhesion rates achieved by all fine diatomaceous earth based inert-dust insecticides.

Research carried out by Desmarchelier *et al.* (1996) on Dryacide®, which has very similar chemical and physical properties and particle size distribution as SilicoSec®, clearly demonstrated that conventional cleaning in a flour mill completely removes the dust even at high dosage levels. At the end of the wheat cleaning process 98.8% of Dryacide® was removed from the highest dosage rate (1000 g t^{-1}), while, 100% was removed from the lower doses (175 and 10 g t^{-1}). End products of the highest treatment dose (1000 g t^{-1}), produced no significant dough handling or bread quality problems and there was no difference in flavor or aroma between the control and the highest treatment dose.

However, filed tests using the similar design should be done to confirm the laboratory findings.

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