

PS10-14 – 6188

Insecticidal efficacy of diatomaceous earth against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

Diatomaceous earth is a potentially useful grain protectant because it is safe to use, doesn't affect grain end-use quality and provides long term protection. Laboratory bioassays were carried out to evaluate the insecticidal efficacy of Silico-Sec[®] against 7-14 day old adults, old and young larvae of *Tribolium castaneum* (Herbst) with the mean \pm SE (n=30) weight of 3.4 ± 0.1 and 0.6 ± 0.1 mg, respectively at 27 °C and 55 ± 5 % r.h. Wheat treated with four dose rates of Silico-Sec[®] and untreated wheat served as a control with three replicates. Adult's mortality was measured after 2, 7 and 14 day of exposure. After 14 day mortality counts, 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 day. After 2 day no concentration achieved 11 % mortality for adults, however; adult's mortality exceeds 89.65 % when exposed for 7 day to Silico-Sec[®]. Mortality of old and young larvae at 600 ppm after 2 day were 28.88 and 22.22 %, respectively and exceed to 60.71 and 69.04 % at longer exposure of 7 day. Due to results mortality of *T. castaneum* was influenced by interval exposed to wheat treated with Silico-Sec[®] and over this exposure, the increases in application rate of Silico-Sec[®] had significant effect

on the mortality. Young larvae of red flour beetle were more sensitive to Silico-Sec[®] than old larvae and adults were more tolerant. The mean number \pm SE of progeny in the control was 1.66 ± 0.101 individuals per sample and there was a significant difference between treated and untreated wheat, so reproductive potential of adults in treated wheat was suppressed when compared with untreated wheat. Therefore Silico-Sec[®] can be used as a potentially useful grain protectant to control infestations of *T. castaneum*.

Key words: Silico-Sec[®], diatomaceous earth, *Tribolium castaneum*, Wheat, Grain protectant.

Introduction

Synthetic insecticides have been used extensively in controlling stored product insects 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 earth (DE) is used as an alternative to chemical insecticides and fumigants, because it has low mammalian toxicity, doesn't break down rapidly and dose not affect end-use quality (Korunic et al., 1996).

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These dusts are applied directly to the grain, without specialized equipment using much the same technology as far residual insecticides (Athanasios et al., 2005). DE particles absorb wax from the insect cuticle, causing death due to desiccation (Golob, 1997; Korunic, 1998).

The red flour beetle, *T. castaneum* is 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 our study was to evaluate the insecticidal efficacy of Silico-Sec® formulation of diatomaceous earth against adults, young and old larvae and of *Tribolium castaneum* (Herbst), red flour beetle under laboratory conditions.

Materials and methods

Insects

Adults of *T. castaneum* were reared on wheat flour plus 5 % brewers yeast (by weight) at 28 °C and 65 ± 5 % r.h. in the dark. All adults were used in the experiment were 7-14 d old of mixed sex. Young larvae of red flour beetle were obtained by placing 100 unsexed adults of mixed ages on 100 gram wheat flour plus 5 % brewers yeast (by weight) diet in glass jars. These jars were held in incubators set at 28 °C and 65 ± 5 % r.h. After 7 d, 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 d from the diet with the mean ± SE (n = 30) weight of 3.4 ± 0.1 mg.

DE formulation

Silico-Sec® is a freshwater formulation of diatomaceous earth obtained from Biofa GmbH and is composed of 92 % SiO₂, 3 % Al₂O₃, 1 % Fe₂O₃ and 1 % Na₂O. 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).

Bioassay

Susceptibility of adults (Test 1)

Twelve samples of 50 gram clean wheat plus 10 gram cracked wheat (5:1 w/w) were placed in a small glass vials. The moisture content of the grain was measured using the dielectric moisture meter ranged about 11.4 % m.c which is equilibrium to 55 % r.h (Pixton, 1971). Wheat treated with 4 dose rates of Silico-Sec®: 700, 1,000, 1,300, 1,700 ppm. Doses were determined with a preliminary test, and untreated wheat with a similar ratio of whole to cracked wheat served as the control treatment with 3 replicates (15 vials). The vials were shaken for 1 min 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 d of exposure. After 14 d mortality counts, all adults were removed and samples retained under the same conditions for a further 60 d to assess progeny production.

Susceptibility of larvae (Test 2)

The method of this experiment was similar to that of adults, but in order to that the susceptibility of stages is different, therefore 4 doses of Silico-Sec® were determined separately for each of the stages with a preliminary test. The 350, 600, 900, 1,200 ppm of Silico-Sec® were used for young larvae and in the case of old larvae 350,600,1,000,1,500 ppm of Silico-Sec® was treated with wheat + cracked wheat (5:1 w/w) 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 d interval.

Data analysis

The mortality counts were corrected by using Abbott's (1925) formula. The data were analyzed using Analysis of Variance (SAS, 2000). To equalize variances, mortality percentage of adults, young and old larvae were transformed using the square root of the arcsin 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). Percentage of reduction in progeny production was determined by the $[(\text{No. progeny in control} - \text{No. progeny in treatment}) / \text{No. progeny in control} \times 100]$ formula (Aldryhim, 1990). Means were separated by using the Duncan's multiple range test, at $P=0.05$.

Results

The main effects for adults: dose ($F = 127.09$, $df = 4$) exposure interval ($F = 701.8$, $df = 1$), young larvae: dose ($F = 128.1$, $df = 4$) exposure

interval ($F = 276$, $df = 1$) and old larvae: dose ($F = 201.1$, $df = 4$) exposure interval ($F = 242.05$, $df = 1$) were all significant. In addition, all associated interactions; dose \times 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 2,7,14 d of exposure and in the case of young and old larvae after 1, 2 and 7 d, exposed to different doses of Silico-Sec® has been shown in Figure 1 and the mortality increases as the time of exposure increased.

Insecticidal efficacy of Silico-Sec®, based on LC_{50} s is presented in Table 1. The LC_{50} values decreased with increases in time of exposure. The 7 d LC_{50} for adults was 913 ppm, in the case of young larvae the LC_{50} value after 7 d was 320 ppm, however; 446 ppm of Silico-Sec® was needed to achieve 50 % mortality for old larvae of *T. castaneum* after 7 d interval (Table 1).

The mean number \pm SE of progeny in the control was 1.66 ± 0.101 individuals per sample and the progeny production was reduced with increasing dosage (Table 2).

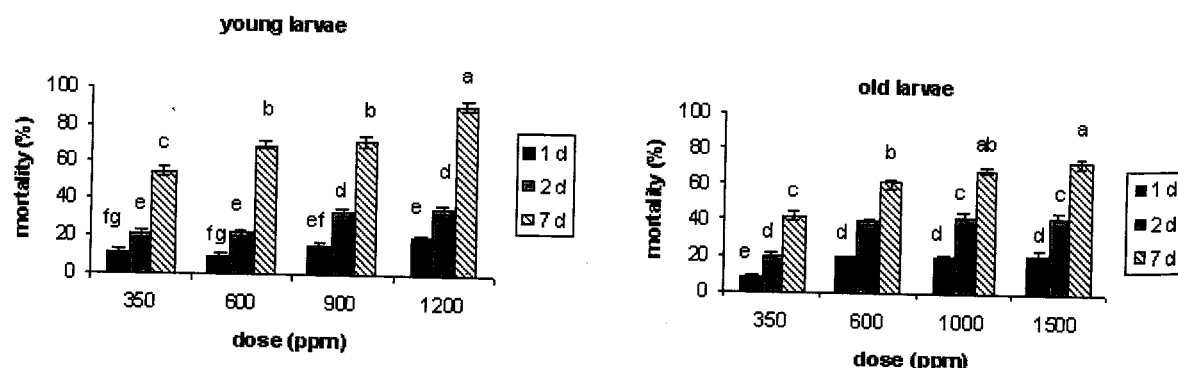


Figure 1. Mean mortality (%) \pm SE of adults, young and old larvae of *T. castaneum* exposed to different doses of Silico-Sec® after 2,7,14 d of exposure for adults and 1,2,7 d of exposure in the case of larvae.

Means followed by the same letter are not significantly different; Duncan's multiple range test at $P = 0.05$

Table 1. The LC₅₀ values (ppm) for adults, young and old larvae of *T.castaneum* exposed to wheat treated with Silico-Sec®.

	Exposure time(d)	LC ₅₀ (ppm)	Confidence limit (95%)	Probit/log regression line	x ² (df=2)
adult	2	14598	NC	-0.78 + 1.38x	0.63 *
	7	913	123 _ 1268	-10.4 + 5.2x	7.35 Ns
	14	267	25 _ 446	-2.1 + 2.92x	1.55 *
young	1	19535	NC	1.84 + 0.73x	0.65 *
larvae	2	3105	1550 _ 513781	1.85 + 0.9x	0.94 *
	7	320	183 _ 416	0.49 + 1.8x	5.03 *
old larvae	1	11179	3303 _ 1068302924	1.64 + 0.83x	2.40*
	2	1929	1240 _ 9379	1.96 + 0.92 x	3.63*
	7	446	252 _ 590	1.61 + 1.26	1.08*

NC, confidence limits could not be calculated

Ns no significant difference; * indicate significant at P < 0.05.

Table 2. The mean (number of individuals/vial ± SE) and percentage of reduction in progeny production (f₁) of *T.castaneum* exposed to wheat treated with Silico-Sec®.

	control	700 ppm	1,000 ppm	1,300 ppm	1,700 ppm
mean± SE of progeny	1.66 ± 0.05a	0.66 ± 0.1b	0 ± 0b	0.33 ± 0.1b	0 ± 0b
(%) reduction in progeny production	—	60	100	80	100

Means followed by the same letter are not significantly different; Duncan's multiple range test at P = 0.05.

Discussion

Our study indicates that mortality of the red flour beetle on wheat treated with Silico-Sec® increases with exposure time, this stands in accordance with previous reports by other researchers (Aldryhim, 1990, 1993; Vayias and Athanassiou, 2004; Athanassiou et al., 2005). Longer exposure interval is needed to achieve 100 % mortality for adults of this species, 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). The larvae of the red flour beetle are sensitive to Silico-Sec® than adults; however this effect is determined by the larval stages. Young larvae are significantly susceptible than older ones and this

difference is appeared after 7 day of exposure. This agrees with that experiment of Vayias and Athanassiou (2004), they exposed young larvae (1-3 instars) and old larvae (4-7 instars) of *T. confusum* to Silico-Sec® and stated that after 24 h of exposure to DE, approximately 61% of young larvae was 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). Our results stated that adults of *T. castaneum* were more tolerant to Silico-Sec[®] than larvae and can survive at application rates and exposure intervals that are lethal to all larval stages, therefore the application rate recommended for controlling adults can control different larval stages of the red flour beetle. Results confirm that 1,300 and 1,700 ppm of Silico-Sec[®] were effective against adults of *T. castaneum* because with 1,700 ppm 100 % mortality and completely progeny suppression and in the case of 1,300 ppm 96.3 % mortality after 14 d and 80 % reduction in progeny production were recorded and these doses ranged in the same group, therefore we recommend 1,300 ppm of Silico-Sec[®] to control infestations of *T. castaneum*. Athanassiou and coworkers (2005) found that 1000 ppm of Silico-Sec[®] was equally effective with 1,500 ppm against *Sitophilus oryzae* and *T. confusum* and this is in agreement with our results.

Stored product insects show a wide range of susceptibility to DE (Aldryhim, 1990, 1993). Fields and Korunic (2000) found *T. castaneum* had noticeably less DE attached to its cuticle than other storage beetles, so the red flour beetle appeared more tolerant stored grain species to DE and the application rate for *T. castaneum* can be used for controlling infestations of other storage beetles.

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

The main conclusion of our trial is: 1) longer exposure time increased mortality of *T. castaneum* 2) adults of *T. castaneum* are less sensitive to desiccation by DE than larval stages

3) 1,300 ppm of Silico-Sec[®] is appropriate to protect wheat from infestations of stored product beetles.

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