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Insecticidal efficacy of diatomaceous earth 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 day old adults, old and young larvae of *Tribolium castaneum* (Herbst) with the mean ± SE (n = 30) weight of 3.4 ± 0.1 and 0.6 ± 0.1 mg, respectively at 27˚C and 55 ± 5% RH. Wheat treated with four dose rates of SilicoSec® and untreated wheat served as a control with three replications. Adult's mortality was measured after 2, 7 and 14 days 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 SilicoSec®. 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 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. The mean number ± SE of progeny in the control was 1.66 ± 0.101 individuals per vial 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.

Keywords: Diatomaceous earth, Grain protectant, SilicoSec®, *Tribolium castaneum*, Wheat.

INTRODUCTION

Synthetic insecticides have been used extensively in controlling stored product insects recently; alternative methods are being emphasized to reduce use of insect-icides 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). These dusts are applied directly to the grain, without specialized equipment using much the same technology as far residual insecticides (Athanassiou *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 & Korunic, 2000; Arnaud *et al.*, 2005).The objective of our study was to evaluate the insecticidal efficacy of SilicoSec® formulation of diatomaceous earth against adults, young and old larvae and of *Tribolium castaneum* (Herbst), red flour beetle under laboratory conditions.

MATERIALS AND METHODS

Insect

Adults of *T. castaneum* were reared on wheat flour plus 5% brewers yeast (by weight) at 28˚C and 65± 5% RH 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% RH. After 7 day interval 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.

DE formulation

SilicoSec® is a freshwater formulation of diatomaceous earth obtained from Biofa GmbH 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. SilicoSec® was stored in the laboratory at ambient conditions, until the beginning of the experiment (approximately for a month).

Bioassay

1- 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 about11.4% m.c (moisture content) which is equilibrium to 55% RH (Pixton, 1971). Wheat treated with 4 dose rates of SilicoSec®: 700, 1000, 1300, 1700 ppm. Doses were determined with a preliminary test; 7 days after exposure to the DE SilicoSec® 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, 7 - 14 d old 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% RH (relative humidity). 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. After this time samples were poured to tray separately and number of the emerged insects was counted for each replicates; then the mean number of progeny and percentage of reduction in progeny production was measured for each dose rate.

2- 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 SilicoSec® were determined separately for each of the stages with a preliminary test; 4 days after exposure to the DE SilicoSec®. The 350, 600, 900, 1200 ppm of SilicoSec® were used for young larvae and in the case of old larvae 350,600,1000,1500 ppm of SilicoSec® 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 to determine the mean number of progeny per vial. 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 [(No. progeny in control - No. progeny in treatment)/ No. progeny in control × 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 ×

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 SilicoSec® has been shown in Fig.1 and the mortality increases as the time of exposure increased.

Insecticidal efficacy of SilicoSec®, 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 SilicoSec® was needed to achieve 50% mortality for old larvae of *T. castaneum* after 7 d interval (Table 1).

The mean number ±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).

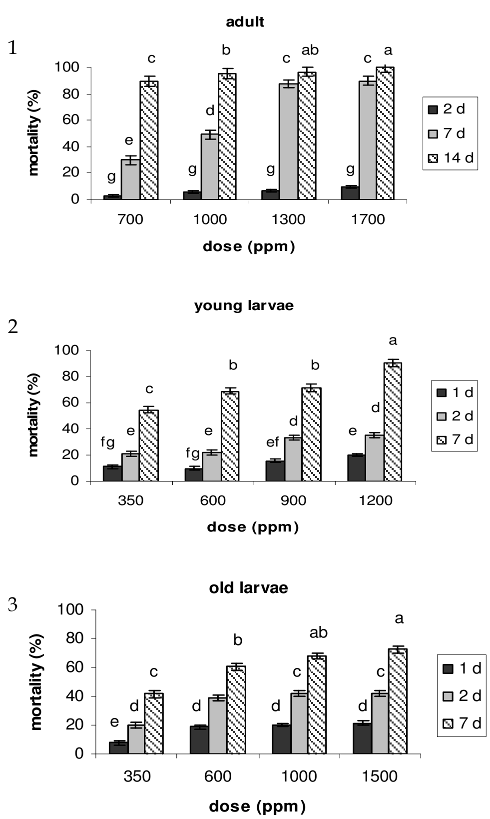

Fig 1. Mean mortality (%)±SE of adults (1), young (2) and old (3) larvae of *T. castaneum* exposed to different doses of SilicoSec® after 2, 7, 14 days of exposure for adults and 1, 2, 7 days of exposure in the case of larvae. Means followed by the same letter are not significantly different; Duncan's multiple range test at P = 0.05

DISCUSSION

Our study indicates that mortality of the red flour beetle on wheat treated with SilicoSec® increases with exposure time, this stands in accordance with previous reports by other researchers (Aldryhim, 1990, 1993; Vayias & 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 SilicoSec® 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 SilicoSec® 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 & 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 SilicoSec® 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 1300 and 1700 ppm of SilicoSec® were effective against adults of *T. castaneum* because with 1700 ppm 100% mortality and completely progeny suppression and in the case of 1300 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 1300 ppm of SilicoSec® to control infestations of *T. castaneum*. Athanassiou *et al.*, (2005) found that 1000 ppm of SilicoSec® was equally effective with 1500 ppm against *Sitophilus oryzae* L. 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 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.

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) 1300 ppm of SilicoSec® is appropriate to protect wheat from infestations of stored product beetles.

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Table 1. The LC_{50} values (ppm) for adults, young and old larvae of *T. castaneum* exposed to wheat treated with SilicoSec®.

	Exposure time(day)	LC_{50} (ppm)	Confidence limits (95%)	Slope ± SE	χ^2(df=2)
adult	2	14598	NC	1.38± 0.76	0.63 *
	7	913	123 -1268	5.2± 0.58	7.35 Ns
	14	267	25 - 446	2.92± 0.97	1.55 *
young larvae	1	19535	NC	0.73± 0.41	0.65 *
	2	3105	1550 - 513781	0.9± 0.35	0.94 *
	7	320	183 - 416	1.8± 0.38	5.03 *
old larvae	1	11179	3303 - 1068302924	0.83± 0.34	2.40*
	2	1929	1240 - 9379	0.92± 0.29	3.63*
	7	446	252 - 590	1.26± 0.30	1.08*

NC, confidence limits could not be calculated.
Ns no significant difference; * indicate significant difference at $P<0.05$.

Table 2. The mean (number of individuals/vial ± SE) and percentage of reduction in progeny production (f_1) of *T. castaneum* exposed to wheat treated with SilicoSec®.

	control	700 ppm	1000 ppm	1300 ppm	1700 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 $P = 0.05$

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