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STUDIES ON THE EFFICACY OF SILICOSEC AGAINST *ORYZAEPHILUS SURINAMENSIS* L. AND *TRIBOLIUM CASTANEUM* HERBST USING TWO BIOASSAY METHODS

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Abstract: Laboratory experiments were conducted to determine the effect of Silicosec by using contact and oral bioassay methods on survival of 1–7 days old adults of *Oryzaeohilus surinamensis* (L.) and *Tribolium castaneum* (Herbst) at 27±2°C and 65±5% RH. For contact method adult of these species were exposed to five doses of Silicosec for 3 days on plastic Petri dishes while in the case of oral bioassay technique the species were exposed to maize treated with five doses of Silicosec in small glass vials for 10 days. After exposure the initial mortality was recorded. For *O. surinamensis* in oral and contact methods 99% mortality was achieved at 8 333 ppm after 10 days and 165 ppm after 3 days, respectively, for *T. castaneum* 75% mortality achieved at 25 000 ppm after 10 days and 165 ppm after 3 days in the same order. For the oral method LC_{50} values for *O. surinamensis* and *T. castaneum* were 50 and 133 ppm, respectively after 10 days, while for the contact method these criteria were 8 and 57 ppm, respectively after 3 days. The results revealed that Silicosec with contact method could have more deleterious effect compared to the other method, and *O. surinamensis* was more susceptible to Silicosec than *T. castaneum* in either bioassay methods.

Key words: Diatomaceous earth, saw-toothed-beetle, red flour beetle, bioassay, maize

INTRODUCTION

The use of fumigants and conventional neurotoxic insecticides as grain protectants are unsuccessful in controlling store product pests because of environmental problems such as pollution and mammalian toxicity (Chintzoglu *et al*. 2008). Resistance of pests to residual insecticides and the demand for residue-free food have led researchers to evaluation of new-reduced risk insecticides to control stored product pest (Aldryhim 1993; Subramanyam and Hagstrum 1995).

One of the most promising alternatives to conventional insecticides is the use of Diatomaceous earth, DE, (Moore *et al*. 2000), which is comprised of fossils of phytoplanktons (diatoms) (Korunic 1998; Vardeman *et al*. 2007). DE causes water loss by absorbing the cuticular lipid layer of insects, leading to death by desiccation, and mode of action has been variously described as a physical disruption of the wax layer of the cuticule (Subramanyam and Roesli 2000; Chabang *et al*. 2007). DE kills all pest populations even pesticide resistance ones, and shows low mammalian toxicity and can be removed from the grain during the milling process (Korunic *et al*. 1996; Korunic 1998). Susceptibility to Silicosec can vary among insect species; generally more mobile species are more susceptible to Silicosec than less mobile ones (Rigauex *et al*. 2001).

There are very few published reports about comparing the insecticidal efficacy of Silicosec using bioassay methods. In our study we examined the insecticidal efficacy of Silicosec formulation against adult stage of saw-toothed beetle, *Oryzaephilus surinamensis* and red flour beetle, *Tribolium castaneum*, with two bioassay methods namely contact (simulating of action in empty store) and oral (simulating of action in full granary) ones.

The aims of our study were to determine: (1) Effectiveness of Silicosec on survival *O. surinamensis* and *T. castaneum.* (2) Toxicity of Silicosec tested by the contact and oral technique for beneficial use as safe and sensitive bioassay methods to determine susceptibility of saw-toothed and red flour beetles.

MATERIALS AND METHODS

The experimental trials were conducted in the laboratory of Department of Entomology, Urmia University, Iran, in 2008.

Insects

Adults of *O. surinamensis* and *T. castaneum* were collected from a laboratory culture, kept on flour maize, at 27±2°C, 65±5% RH in continuous darkness for about 3 years without exposing to insecticides. Adults used in the experiments were less than 7 days old and of mixed sex.

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DE formulation

Silicosec was a Diatomaceous earth formulation of fresh water origin and contained approximately 92% SiO_2, 3%Al_2O_3, 1%Fe_2O_3 and 1% Na_2O. The median particle size was 8–12 µm. The Silicosec sample was obtained from Biofa GmbH and was stored in the laboratory at ambient conditions, until the initiation of experiments, approximately for a month (Athanassiou *et al.* 2003).

Bioassay

Oral bioassay method

Main experiment was performed after the primary tests. Twenty four samples of 59 g clean maize plus 1 g cracked maize (for each insect) were placed in a small glass vials. Maize treated with five doses of Silicosec viz, 0.06, 0.5, 0.66, 2.5 and 8.5 g/kg for *O. surinamensis* and for *T. castaneum* 0.15, 0.5, 2, 6.6 and 25 g/kg. The vials were shaken for 1 min to distribute the DE in the entire product. Subsequently, 1–7 days old adults of each insect (30 insects per vials) were introduced into each sample and vials were covered with muslin cloth for sufficient ventilation. The vials were kept under laboratory conditions at 27±2°C, 65±5% RH. There were 3 replications plus untreated maize as a control for each test. Adult's mortality was recorded 2, 7 and 10 days post exposure.

Contact bioassay method

In this trial experimental unit were plastic petri dishes with an internal diameter of 8.8 cm and area of 121 cm^2. After primary tests, the required dose of Silicosec was placed inside each dish. The dishes were shaken to distribute the DE to all of surface of petri dishes (walls, ceiling, floor and sides of the dishes). Then petri dishes were left undistributed after shaking for 1 min (Arthur 2000a). Subsequently 30 adults of each insect species were introduced to each dish (twenty four dishes for each species). Dishes were covered with lids. Mortality was recorded after 1, 2 and 3 days after exposure. Trials were carried out in 3 replications.

Data analysis

The data were analyzed using analysis of variance (ANOVA) (SAS 1999). To equalize variance mortality, the percentage was transformed using the square-root of arcsin. The dose required to kill 50% of insects (LC_{50}) and the confidence limits was estimated using probit analysis (Gerber and Finn 2005).

RESULTS AND DISCUSSION

The mortality as the main effects for adults of *O. surinamensis* and *T. castaneum* recorded after 10 days for oral bioassay method and 3 days for contact method was significant for all treatments. In addition, all associated interactions (dose×interval exposure) were also significant (Tables 1, 2).

The mortality percentage for *O. surinamensis* and *T. castaneum* after 2, 7 and 10 days of exposure for oral bioassay and in the case of contact bioassay after 1, 2 and 3 days of exposure to different doses of Silicosec is shown in figures 1 and 2. The mortality effect was higher as the time of exposure increased.

Table 1. ANOVA parameters for main effects for initial mortality of *O. surinamensis* exposed to Silicosec using two bioassay methods

Source of variance	Contact			Oral		
	df	F	*P*	df	F	*P*
Dose of Silicosec	4	10.25*	< 0.0001	4	13.34*	< 0.0001
Exposure interval	2	13.26*	< 0.0001	4	67.61*	< 0.0001
Dosexexposure interval	8	4.10*	< 0.0021	6	177.206*	< 0.0007

*significant difference at $p < 0.05$

Table 2. ANOVA parameters for main effects for initial mortality of *T. castaneum* exposed to Silicosec using two bioassay methods

Source of variance	Contact			Oral		
	df	F	*P*	df	F	*P*
Dose of Silicosec	4	79.98*	< 0.0001	4	86.28*	< 0.0001
Exposure interval	2	346.05*	< 0.0001	4	426.93*	< 0.0001
Dose x exposure interval	8	7.75*	< 0.0001	16	190.92*	< 0.0001

*significant difference at $p < 0.05$

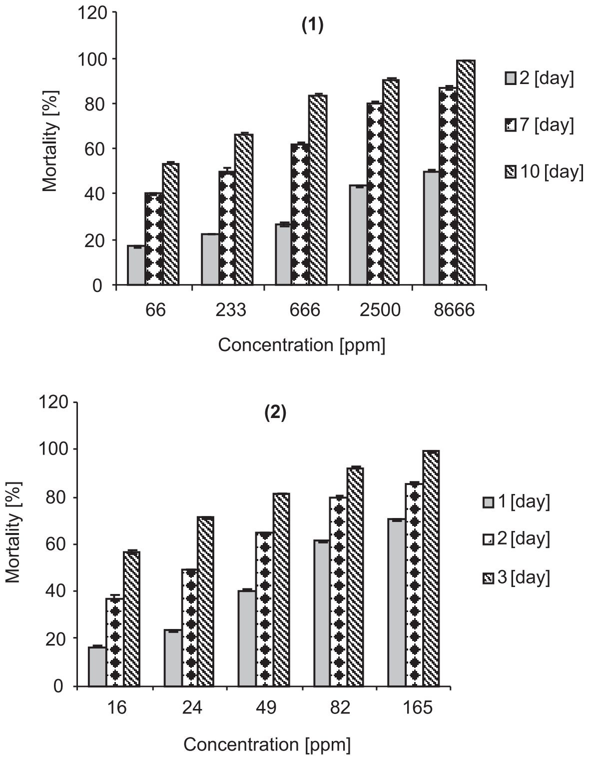

Fig. 1. Mortality±SE of *O. surinamensis* exposed to different doses of Silicosec with: (1) oral method after 2, 7 and 10 days of exposure and (2) the contact method after 1, 2 and 3 days after exposure

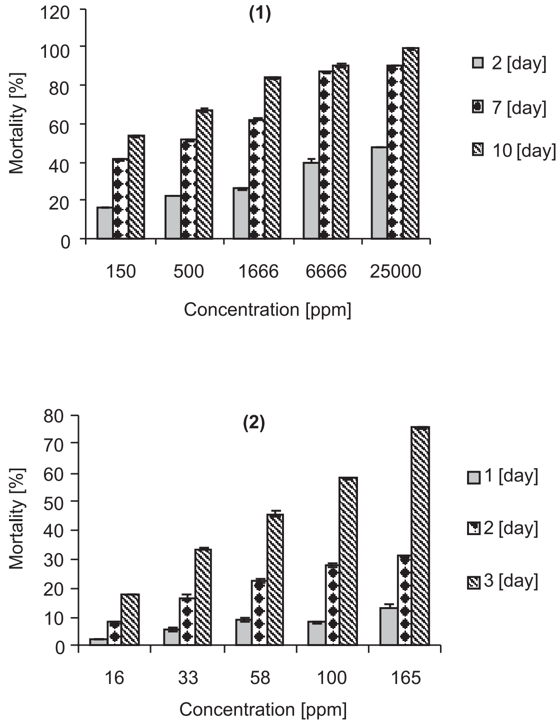

Fig. 2. Mortality±SE of *T. castaneum* exposed to different doses of Silicosec with: (1) oral method after 2, 7 and 10 days of exposure and (2) contact method after 1, 2 and 3 days after exposure

Insecticidal efficacy of Silicosec based on LC_{50} value is presented in table 3. An inverse relationship between LC_{50} values with exposure time was determined.

Results indicated that tasted LC_{50} in the oral method after 10 days for *O. surinamensis* was 50 ppm; however, for *T. castaneum* corresponding value was 1 333.3 ppm. In the contact bioassay technique after 3 days of exposure, LC_{50} value for *O. surinamensis* and *T. castaneum* were 8.26 and 57.85 ppm, respectively. Silicosec had more insecticidal efficacy on *O. surinamensis* than *T. castaneum* and red flour beetles appeared more tolerant store grain species to Silicosec. The above was in accordance with previous reports by other researchers (Korunic *et al*. 1998; Arthur 2000b; Vayias *et al*. 2006). Also *O. surinamensis* was one of the most susceptible stored product species to diatomaceous earths (Fields and Korunic 2000).

The results also indicated that mortality percentages of the red flour beetle and saw-toothed beetle were increased with exposure time. Longer exposure interval was needed to achieve 100% mortality for adults of the pest tested, especially for *T. castaneum* (Vayias and Athanassiou 2004), due to longer insect walk over the treated substrate, and more dust particles were trapped by their bodies, resulting in water loss and death by desiccation

Table 3. LC_{50} values, confidence limit (95%) and probit regression estimate for *O. surinamensis* and *T. castaneum* exposed to Silicosec for two bioassay methods

Exposure time	*O. surinamensis*			*T. castaneum*			Methods
[days]	Lc_{50} (95%CL) [ppm]	Slope (±SE)	χ^2 [df = 3]	Lc_{50} (95%CL) [ppm]	Slope (±SE)	χ^2 [df = 3]	
2	8000(3333–30000)	0.48±0.08	0.92	34000(13333–195000)	0.43±0.08	0.93	oral
7	166.66(100–300)	0.67±0.14	0.83	333.33(216.66–616.66)	0.7±0.08	4.43	
10	50(30–100)	0.89±0.11	2.30	133.33(50–216.66)	0.84±0.1	1.84	
1	66.11(56.19–83.47)	1.5±0.18	1.73	4958(743.8–15907428)	0.7±0.2	1.13	contact
2	24.79(16.52–32.23)	1.4±0.18	1.51	295(247.93–3057.85)	0.86±0.2	1.01	
3	8.26(9.09–17.35)	1.8±0.24	1.9	57.85(49.56–74.36)	1.54±0.18	0.18	

(Arthur 2000b). 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 picked up by beetles. But DE dose rate was crucial not only for efficacy but also for physical properties of the grain. High dose rate provided satisfactory level of protection but physical problem such as loss of grain weight, decreased flow rate and bulk density could occur when entire grain masses were treated (Korunic 1998; Demissie *et al*. 2008). According to Athanassiou and Korunic (2007) application of DE with reduce-risk control techniques, could led to increased use of DEs for protect of stored product in the IPM strategies. Therefore, in this research we compared toxicity of Silicosec on two important stored product insects by two bioassay methods.

Our study indicated that in contact method (surface treated) 100% mortality was achieved after 3 days of exposure to Silicosec, while the respective mortality for oral method was achieved after 10 days. Also LC_{50} value rate in the contact method was lower than LC_{50} in the oral method. So contact method is sufficiently sensitive to determine the magnitude of resistance, because it produced smaller fiducial limits. Athanassiou and Kavallieratos (2005) reported that application of DE on surface (empty store) has no harmful physical effect of dust on stored grain.

CONCLUSION

The main conclusions of our trials are: (1) the mortality of *O. surinamensis* and *T. castaneum* in two bioassay methods increased as the time of exposure and dose increased. (2) *O. surinamensis* is more susceptible to desiccation by Silicosec than *T. castaneum*. (3) Both bioassay methods were suitable for assessing magnitude of susceptibility to Silicosec of treated insects. (4) Silicosec tested by the contact bioassay method had more insecticidal effect than the oral bioassay method. (5) The contact method was superior to oral method in testing Silicosec effect and significantly demonstrated higher toxicity than the oral method. (6) The application of Silicosec with contact method did not show residue on stored grains.

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POLISH SUMMARY

BADANIA NAD EFEKTYWNOŚCIĄ PREPARATU SILICOSEC PRZECIWKO *ORYZAEPHILUS SURINAMENSIS* L. I *TRIBOLIUM CASTANEUM* HERBST DWOMA METODAMI BIOLOGICZNYMI

W celu określenia skuteczności preparatu Silicosec przeciw *Oryzaephilus surinamensis* (L.) i *Tribolium castaneum* (Herbst) przeprowadzono laboratoryjne testy biologiczne metodami kontaktową i doustną na 1–7 dniowych dorosłych osobnikach w temperaturze 27±1°C i 65±5% RH. W metodzie kontaktowej dorosłe osobniki tych gatunków wystawione były na działanie pięciu dawek preparatu Silicosec w plastikowych szalkach Petriego przez 3 dni. W metodzie doustnej były one karmione przez 10 dni kukurydzą traktowaną pięcioma dawkami preparatu. Po tym czasie badano początkową śmiertelność owadów. W przypadku *O. surinamensis* i użyciu metody doustnej oraz kontaktowej, 99% śmiertelności osiągnięto odpowiednio przy dawkach 8 333 ppm po 10 dniach i 165 ppm po 3 dniach. *T. castaneum* wykazał 75% śmiertelność przy 25 000 ppm po 10 dniach w metodzie doustnej, 165 ppm po 3 dniach w przypadku metody kontaktowej. Wartości LC_{50} dla *O. surinamensis* i *T. castaneum* w metodzie doustnej wynosiły odpowiednio 50 i 133 ppm po 10 dniach ekspozycji, a w metodzie kontaktowej odpowiednio 8 i 57 po 3 dniach. Powyższe rezultaty wykazały, że Silicosec zastosowany kontaktowo był bardziej szkodliwy niż metodą doustną. *O. surinamensis* okazał się bardziej wrażliwy na preparat niż *T. castaneum*.