INSECTICIDE POTENTIAL OF DIATOMACEOUS EARTH FROM CROATIA

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Abstract

Diatomaceous earth (DE) is a geological deposit consisting of the fossilized skeletons of numerous species of siliceous marine and fresh water one-cellular organisms, particularly diatoms and other algae. The skeletons are made of amorphous silicon dioxide. DE is probably the most effective natural inert dust used as an insecticide. DE is a low toxicity, natural insecticide. Several formulations of DE are registered and used as stored product protection insecticides around the world. The objective of the research was determination of the potential insecticide effectiveness of Croatian DE against stored products pests and the comparison of their effectiveness with the effectiveness of standard DE sample. One sample of the Croatian DE was selected and investigated. As a standard DE sample, the German fresh water DE SilicoSec was included into the experiment. SilicoSec formulation is registered as a grain protectant and belongs into a group of the most effective DEs in the world. The effectiveness of particle size fractions of the Croatian DE from most to least effective is: 0-20 microns (μm), 0-45 μm 0-150 μm, 20-45 μm and 45-150 μm. The Croatian DE sample applied at 600 mg/kg containing particle size 0 - 45 μm showed with over 90% mortality after 1 day for *Cryptolestes ferrugineus*, after 3 days for *Sitophilus oryzae*, after 7 days for *Rhyzopertha dominica* and after 25 days for *Tribolium castaneum*. SilicoSec caused 100% mortality these insects at the same or shorter time periods The effectiveness of the Croatian DE containing particles smaller than 45 μm was equally effective against the progeny if compared with the effectiveness of the standard DE.

The authors conclude that Croatia has potential sources of DEs for ecological acceptable insecticide production and it is important to find new localities with even better sources of DEs. It can be a new safe and natural insecticide product from Croatia.

Key words: Croatia, DE, grain insects, effectiveness, particle size

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Introduction

Insects infesting grain after harvest cause economic loss to producers and the grain and food industry. During the past few decades application of synthetic pesticides to control agricultural stored products insect pests has been a standard practice. However, with the growing evidence regarding detrimental effects of many of the synthetic pesticides on health and environment, the grain industry wants to reduce the use of synthetic pesticides because of insecticide deregulation, resistant populations and consumer concerns over insecticide residues. Therefore, there is a pressing need for safer methods of pest management (Fields 1999, Subramanyam and Roesli, 2000).

Diatomaceous earth (DE)-based insecticides have been finding increased use as stored product protectants because of these concerns (Korunic 1998; Subramanyam and Roesli 2000).The main advantages of DEs are its low-toxicity to mammals and its stability (Anon. 1991; Athanassiou et al. 2005). There is no effect on end use quality; baking, malting or pasta production (Desmarchelier and Dines, 1987; Aldryhim, 1990; Korunic, et al. 1996).

However, DE significantly reduces the bulk density (test weight) and flowability of grain. Because of high concentrations needed to control insect pests it is dusty to apply and it has a low efficacy against some insect species. The high grain moisture contents and high air relative humidity significantly reduce its efficacy (Fields and Korunic 2000). Currently used DEs are applied between 100 and 1000 mg/kg (Subramanyam and Roesli 2000). However, the 100 mg/kg rates are only effective against *C. ferrugineus*, the most sensitive insect against DE. Rates above 400 mg/kg cause a considerable bulk density reduction (test weight) and significant reduction in grain flowability (Jackson and Webley 1994; Korunic et al. 1998; Korunic 1998; Subramanyam and Roesli 2000). Hence, it is essential for researches to evaluate the use of novel DE formulations that are effective against insects at lower dose rates.

The physical and chemical properties of DE such as the percentage of amorphous silicon dioxide, pH value, porosity and active surface, sorption capacity, particle size distribution, adherence DE particles to kernel are the critical factors affecting their insecticidal action (Ebeling 1971, Korunic 1998). The geographical origin has a great effect on physical and chemical properties of DEs (Korunic 1997). He reported that, besides other DE characteristics, the size of DE particles may play an important role to the insecticidal value of a given DE. In the same paper, the author noted that one of the best DEs was derived from the Former Yugoslav Republic of Macedonia. Until present this is the only published paper concerning the insecticidal activity of DE from South Eastern Europe. This was a good indication that South Eastern Europe area was likely to contain other, equally or more effective natural DE deposits that should be evaluated.

Two samples of DE were collected from sources in Croatia described by Knežević (1983); Crnički and Šinkovec (1993); Croatian Geological Survey (1994). Preliminary studies showed that only one of the two DE was active and studies on the inactive DE were not continued. The investigation aimed to determine the insecticide effectiveness of this Croatian DE against major stored-product beetle species, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujiidae), the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and the red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). As a standard insecticide, SilicoSec, the German DE registered as a grain protectant was included into experiments. SilicoSec belongs into a group of the most effective DEs in the world (Athanssiou et al. 2005, Vayias and Athanassiou 2004). In this work, the effect of particle size on the insecticidal efficacy of the tested DE sample was also examined.

Materials and Methods

DE sample preparation

The DE was collected close to Zagreb (Markuševac, Zagreb Mountain) Croatia. The sample was collected directly from the ground. The moist, soft blocs were dried in a ventilated oven at 40 ^{0}C for 24h, to about 6% moisture content (m.c.). After drying, the DE sample was gently crushed into a dust with a hammer. The sample was sifted (dry sieving) through a sieve; USA standard number 100, 150 microns (µm). Particles larger than 150 µm were discarded because this fraction usually contains sands, rocks and only a few very large diatoms (Korunic 1998). Following this, the 0-150 µm fraction was sieved again to prepare an additional four fractions of 0-20 um, 20-45 µm, 45-150 µm and 0-45 μm using the appropriate sieves.

For comparative purposes, SilicoSec® (Biofa GmbH, Munchen, Germany), which is a commercially available DE formulation, was used in the experiments. SilicoSec® is a DE of fresh water origin and contains 91.2% SiO_2. SilicoSec is very light formulations with all particles smaller than 45 μm.

Insect species and commodity tested

Seven to 21 days old mixed-sex adults of *Cryptolestes ferrugineus* - rusty grain beetle, *Sitophilus oryzae* - rice weevil, *Rhyzopertha dominica* - lesser grain borer and *Tribolium castaneum* – red flour beetle were used in the experiments. *S. oryzae* and *R. dominica* were cultured on whole wheat with approximately 14% m.c. and *C. ferrugineus* on wheat with 16% m.c., while *T. castaneum* was cultured on white flour with 5% brewer's un-activated yeast. Rearing and tests were conducted at 30 $\pm 1^0$C and 70$\pm$5% RH.

Bioassays

Un-infested, clean Canadian Western Hard wheat with 13.0% moisture content was used in the experiments. The various fractions of DE were added to the grain held in 400 ml Mason jars containing 100 grams of wheat at a rate of 600 mg/kg (600 ppm). There were 3 replicates for each treatment, species were tested separately and an untreated control was run for each species. Each replication was treated separately. The jars were tightly sealed with lids and thoroughly shaken by hand for one minute, to achieve the equal distribution of the DE in the grain mass. Three jars each containing 100g of untreated grain served as controls. Jars were subsequently infested with 50 adults of either *C. ferrugineus, S. oryzae, R. dominica* or *T. castaneum.* Mortality of *C. ferrugineus* adults was assessed after 12 hours and 1 day and mortality of other 3 species was assessed after 3, 7, 18 and 25 days of exposure to treated and untreated grains. The effect of tested DEs on the progeny was assessed 55 days after the introduction of insects into jars.

Data analysis

Data were subjected to one-way analysis of variance (ANOVA) according to the GLM Procedure of SAS (1996). Means were separated using the Tukey - Kramer (HSD) test at P= 0.05 (Sokal and Rohlf 1995).

Results

The results are presented in Tables 1 to 6.

Cryptolestes ferrugineus

C. ferrugineus is one of the most sensitive stored grain insect species against DE. The efficacy of all fractions of Croatian DE after 1 day exposure period was extremely high (100%) and equally effective to SilicoSec. The only exception was Croatian DE with larger particles from 45 to 150 µm which generated mortality of 84% (Table 1).

Sitophilus oryzae

Three days post treatment of wheat with tested DEs, only SilicoSec gave 100% mortality against rice weevils. However, over the same exposure interval, the fraction of Croatian DE with the particles smaller than 45 µm gave very high mortality (94%) which is not significantly different than the mortality caused with SilicoSec.

Seven, 18 and 25 days post treatment, mortality of rice weevil caused with all fractions of Croatian DE was not significantly different than the mortality caused with SilicoSec. The only exception was the fraction of Croatian DE containing larger particles from 45 to 150 µm which generated significantly lower mortality of rice weevils (Tables 2 to 6).

Fifty five days post treatment the fraction of Croatian DE containing particles smaller than 20 µm greatly reduced the progeny of rice weevil (96.6% reduction). This reduction was not significantly different to the reduction of the progeny caused with the standard DE SilicoSec.

Rhyzopertha dominica

Three days post treatment 2 fractions of Croatian DE with particles smaller than 45 and 20 µm were equally effective against lesser grain borer as the standard DE and reached high mortalities of 82, 83 and 89%, respectively (Table 2).

Seven days post treatment the standard DE generated 100% mortality of lesser grain borer and the smaller fractions of Croatian DE (0-45 and 0-20 µm) reached 90.9 and 90.5% mortality (Table 3).

Eighteen and 25 days post treatment the effectiveness against lesser grain borer of the particles 0- 45 and 0-20 µm were not significantly different than the effectiveness of the standard DE and after 25 days reached over 97% mortality (Tables 4 and 5).

The reduction of the progeny of lesser grain borer caused with 3 fractions of Croatian DE (0-150, 0-45 and 0-20 μm) wasn't significantly different to the standard DE and was very high, from 91.5% to 95.1% (Table 6). However the fraction with the largest particles from 45 to 150 μm and 20 to 45 μm significantly less reduced the progeny of lesser grain borer (Table 6).

Tribolium castaneum

Three days post treatment the standard DE was significantly more effective against red flour beetle than Croatian DE. The most effective fraction of Croatian DE contained particles 0-45 μm (Table 2).

Seven days post treatment the standard DE reached a very high mortality of red flour beetle (97.3%). The most effective fractions of Croatian DE were fractions containing the smaller particles 0-20 μm (58.3%) 0-45 μm (47.8%), still significantly less effective than the standard DE (Table 3).

Eighteen days post treatment the effectiveness of the fraction containing the smallest particles (0-20 μm) reached a very high mortality (95.6%) which wasn't significantly different than the mortality of the standard DE (100%). The fraction of Croatian DE containing particles 0-45 μm also generated rated a high mortality (85.3%) (Table 4).

Table 1. The effectiveness of different fractions of Croatian DE against *Cryptolestes ferrugineus* after **12** and **24** hours of the exposure to treated grain with 600 ppm

Species	Mean efficacy (%) (±SD)						
	Croatian DE by particle size (μm)					SilicoSec	Control
	0-20	0-45	0-150	20-45	45-150	0-45	-
After 12 hours	38.3±3.5de	34.0±3.0d	8.0±1.0b	23.3±2.5c	0.6±1.1a	42.0±1.0e	0.0± 0.0a
After 24 hours	100.0±0.0c	100.0±0.0c	100.0±0.0c	100.0±0.0c	84.0±3.0b	100.0±0.0c	8.0±2.0a

Means in the same row followed with a different letter are significantly different, ANOVA, Tukey - Kramer (HSD) test at P= 0.05

Table 2. The effectiveness of different DE fractions after 3 days of the exposure to treated grain with 600 ppm

Species	Mortality (±SD)						
	Croatian DE by particle size (μm)					SilicoSec	Control
	0-20	0-45	0-150	20-45	45-150	0-45	-
S.oryzae	89.1 ± 4.7 d	94.0 ± 4.0 de	77.3 ± 5.0 c	77.3 ± 5.0c	34.0 ± 2.0 b	100.0 ± 0.0 e	3.3 ± 3.0 a
R. dominica	83.1 ± 3.5 d	82.0 ± 4.6 d	55.2 ± 3.6 c	46.3 ± 4.7 b	6.0 ± 2.0 a	89.6 ± 1.4 d	0.6 ± 0.5 a
T. castaneum	15.6 ± 3.5 c	21.0 ± 2.0 d	10.0 ± 2.0 b	4.0 ± 1.0 a	0.6 ± 0.5 a	45.6 ± 1.1 e	0.0 ± 0.0 a

Means in the same row followed with a different letter are significantly different, ANOVA, Tukey - Kramer (HSD) test at P= 0.05

Table 3. The effectiveness of different DE fractions after 7 days of the exposure to treated grain with 600 ppm

Species	Mortality (±SD)						
	Croatian DE by particle size (μm)					SilicoSec	Control
	0-20	0-45	0-150	20-45	45-150	0-45	-
S.oryzae	98.6 ± 1.1 c	98.6 ± 1.1 c	93.3 ± 5.0 c	92.6 ± 4.1 c	48.0 ± 6.0 b	100.0 ± 0.0 c	12.0 ± 0.0 a
R. dominica	90.5 ± 4.0 d	90.9 ± 2.5 d	79.2 ± 3.9 c	58.8 ± 3.8 b	12.0 ± 1.0 a	100.0 ± 0.0 e	5.0 ± 1.0 a
T. castaneum	58.3 ± 3.0 e	47.8 ± 1.0 d	35 ± 4.0 c	17.0 ± 3.0 b	5.0 ± 1.0 a	97.3 ± 1.1 f	2.0 ± 2.0 a

Means in the same column followed with a different letter are significantly different, ANOVA, Tukey - Kramer (HSD) test at P= 0.05

Table 4. The effectiveness of different DE fractions after 18 days of the exposure to treated grain with 600 ppm

Species	Mortality (±SD)						
	Croatian DE by particle size (μm)					SilicoSec	Control
	0-20	0-45	0-150	20-45	45-150	0-45	-
S.oryzae	100.0 ± 0.0 c	100.0 ± 0.0 c	100.0 ± 0.0 c	100.0 ± 0.0 c	60.7 ± 1.0 b	100.0 ± 0.0 c	18.0 ± 2.0 a
R. dominica	94.9 ± 3.4 de	93.6 ± 1.5 de	88.8 ± 3.8 d	68.0 ± 1.0 c	23.0 ± 3.0 b	100.0 ± 0.0 e	7.0 ± 2.0 a
T. castaneum	95.6 ± 1.4 e	85.3 ± 8.3 d	75.0 ± 1.0 c	42.8 ± 3.0 b	10.0 ± 3.0 a	100.0 ± 0.0 e	5.6 ± 1.5 a

Means in the same row followed with a different letter are significantly different, ANOVA, Tukey - Kramer (HSD) test at P= 0.05

Table 5. The effectiveness of different DE fractions after 25 days of the exposure to treated grain with 600 ppm

Species	Mortality (±SD)						
	Croatian DE by particle size (μm)					SilicoSec	Control
	0-20	0-45	0-150	20-45	45-150	0-45	-
S.oryzae	100.0 ± 0.0 c	100.0 ± 0.0 c	100.0 ± 0.0 c	100.0 ± 0.0 c	94.3 ± 0.5 b	100.0 ± 0.0 c	18.0 ± 2.0 a
R. dominica	97.3 ± 1.1 de	97.2 ± 1.1 de	93.0 ± 2.0 d	77.4 ± 2.1 c	29.3 ± 1.5 b	100.0 ± 0.0 e	17.6 ± 2.5 a
T. castaneum	100.0 ± 0.0 d	93.6 ± 1.8 c	96.0 ± 2.0 cd	56.2 ± 2.0 b	11.3 ± 1.0 a	100.0 ± 0.0 d	10.3 ± 2.0 a

Means in the same row followed with a different letter are significantly different, ANOVA, Tukey - Kramer (HSD) test at P= 0.05

Table 6. The effectiveness of different DE fractions against progeny after 55 days of the exposure to treated grain with 600 ppm

Species	Number of adults (progeny) (±SD)						
	Croatian DE by particle size (μm)					SilicoSec	Control
	0-20	0-45	0-150	20-45	45-150	0-45	-
S.oryzae	13.0 ± 3.0 cd	19.0 ± 1.0 c	22.3 ± 4.0 c	20.6± 4.0 c	68.3 ± 7.0 b	1.0 ± 1.0 d	386.7 ± 8.0 a
R. dominica	30.6 ± 5.5 d	21.6 ± 1.1 d	37.3 ± 3.5 d	170.7 ± 70.0 c	315.0 ± 3.5 b	2.0 ± 1.0 d	441.3 ± 19.1 a
T. castaneum	0.0 ± 0.0 c	2.3 ± 1.5 c	2.0 ± 2.0 c	12.0 ± 3.0 b	61.0 ± 4.5 a	0.0 ± 0.0 c	58.3 ± 6.5 a

Means in the same row followed with a different letter are significantly different, ANOVA, Tukey - Kramer (HSD) test at P= 0.05

Twenty five days post treatment the smallest particles of Croatian DE (0-20 μm) caused the mortality equal to standard DE (100%) and the fractions with particles 0-150 and 0-45 μm caused very high mortality as well (96 and 93.6%) (Table 5).

The fractions with particles from 0-20 μm, <45 μm and 0-150 μm of the Croatian DE reduced 96 to 100% the progeny of red flour beetle, equally to the standard DE. However, the fraction with the particles from 20 to 49 μm reduced the progeny for 20% only and the fraction with the largest particles generated the same results as untreated grain (Table 6).

In the present study, the significant differences between the effectiveness of the tested fractions of the Croatian DE were recorded. In all cases the fractions with particles smaller than 45 μm were significantly more effective against rice weevil and lesser grain borer and particles smaller than 20 μm were more effective against red flour beetle if compared with the effectiveness of larger particles. We do not have now a rational explanation why the fraction containing particles from 20 to 45 μm was less effective against tested insects if compared with the effectiveness generated with fractions with particles 0-45 and 0-20 μm.

Based on our findings, the standard DE SilicoSec, which is registered as a grain protectant (Athanassiou et al., 2005) applied at dose of 600 mg/kg (ppm) gave the highest mortality ratio in all of the cases that were tested here. Applied at the same dose, to reach the same level of the effectiveness, the longer exposure time is needed for the Croatian DE fractions with particles less than 45 and 20 μm. *C. ferrugineus* is one of the most sensitive insects against DE and mortality of 100% was achieved already after 1 day. After exposure to treated wheat during 25 days, SilicoSec caused 100% mortality of rice weevil, lesser grain borer and red flour beetle. The Croatian DE, fractions with particles <45 and <20 μm caused 100% mortality of rice weevil, 97% mortality of lesser grain borer and 96 to 100% mortality of red flour beetle. Also, the same fractions of the Croatian DE reduced 100% of the populations of lesser grain borer and red flour beetle and 99.5% of the rice weevil population. The reduction of the progeny caused with the fractions containing smaller particles wasn't significantly different if compared with the results generated with the standard DE SilicoSec.

4. Discussion and conclusions

In Europe DE is produced in several countries (France, Germany, Romania, Spain, Czechs Republic, Italy, Island, Denmark, Former Yugoslav Republic Macedonia, etc.) (Antonides 1997). There are only a few published papers regarding to the sources of DE in Croatia (Knezević, 1983; Crnički and Šinkovec, 1993). Two localities containing DE were described; Zagreb and island Brač. In the Report Croatian Geological Survey, (1994) the third locality close to Zagreb was described. According to Halamić (personal communication, 2009), so far DE is not used from these localities for any purpose. Due to the geographical nature of Croatia, it is a great possibility to discover other localities as well.

Geographical origin significantly effects insecticidal efficacy, with one of the most insecticidal DE coming from Former Yugoslav Republic of Macedonia (Korunic 1997). According to Vayias et al. (in press), the natural DE deposits in South Eastern Europe exist in several countries (Greece, Serbia, Slovenia) and the quality and the effectiveness of DE from some sources are pretty good and therefore, this region should be further evaluated as a source of DE.

Korunic (1977) suggested that the size of DE particles may be playing important role in the insecticidal value of a given DE and this finding was the agreement with previous results of Chiu (1939) and McLaughlin (1994). He showed that the biological activity of one DE – Celite 209 - increased significantly with reduced particle size, whereas there was no correlation between particle size and activity with another DE – from former Republic of Macedonia. Our results also show similar effect on insecticidal activity with particle size. Vayias et al. (in press) showed that the smaller DE particles were more insecticidal for several DE formulations. Authors concluded that the effect of the particle size of a given DE formulation on the effectiveness against insects should be always assessed.

The results of the effectiveness of the Croatian DE containing particles smaller than 45 μm against 4 stored grain insect species were comparable to the effectiveness of commercially available DE formulation, SilicoSec, one of the most effective DE against insects.

Conclusions

The effectiveness of the smaller particles (<45 μm) of Croatian DE is similar to the effectiveness of DE InsectoSec used as a standard insecticide in the experiment.

The effect of the particle size on the effectiveness against insects is significant; smaller particles are significantly more effective than larger particles.

There is a difference in tolerance of test insects against Croatian DE, with *S. oryzae* the most sensitive, followed by *R. dominica*. *T. castaneum* was the most tolerant species.

The present study indicates that Croatia has potential sources of DEs for the production of safe, natural and ecologically acceptable insecticide product.

Acknowledgements

We thank Dr. Paul Fields, Agriculture and Agri-Food Canada, Winnipeg, Canada, for reviewing the manuscript, and Dr. Christos Athanassiou, Athens University, Greece, for providing the sample of SilicoSec DE.

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