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The effect of diatomaceous earth of different origin, temperature and relative humidity against adults of rice weevil (*Sitophilus oryzae* [L.], Coleoptera, Curculionidae) in stored wheat

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ABSTRACT

Laboratory experiments were carried out to evaluate the impact of diatomaceous earth (DE) samples of different origin with their insecticidal properties to control one of the most important primary pest in stored grain. We tested the efficacy of three local DE, from Serbia, Greece and Slovenia, and commercial formulation SilicoSec against the rice weevil, *Sitophilus oryzae*, adults in stored wheat. The experiments were carried out at three temperatures (20, 25 and 30 °C) and two relative humidity (RH) levels (55 and 75 %). Mortality of pest was counted 7, 14 and 21 days after exposure (DAT) at the following DE dose rates: 100, 300, 500 and 900 ppm. The mortality of adults normally increased with increasing dose rates and DAT. In all samples the mortality of rice weevil adults (dose rate 900 ppm, 21 DAT) was above 90 %, except at Slovenian DE (at 20 °C and 55 % RH) and Greek DE (at 25 °C and 75 % RH), when the mortality was 85.3 and 67.6 %, respectively. With 100 % mortality (14 DAT and at 900 ppm) the most effective was SilicoSec. Slovenian DE was more effective at 55 % RH than at 75 % RH (7 DAT at all temperatures).

Key words: *Sitophilus oryzae*, diatomaceous earth, origin, stored wheat, temperature, relative humidity

IZVLEČEK

VPLIV DIATOMEJSKE ZEMLJE RAZLIČNEGA IZVORA, TEMPERATURE IN RELATIVNE VLAGE PRI ZATIRANJU ODRASLIH OSEBKOV RIŽEVEGA ŽUŽKA (*Sitophilus oryzae* [L.], Coleoptera, Curculionidae) V SKLADIŠČENI PŠENICI

V laboratorijskih poskusih smo preučevali vpliv diatomejske zemlje (DZ) različnega izvora pri zatiranju enega od najpomembnejših primarnih škodljivcev skladiščenega žita. Ugotavljali smo učinkovitost treh lokalnih tipov DZ (srbskega, grškega in slovenskega) in komercialnega pripravka SilicoSec pri zatiranju odraslih osebkov riževega žužka (*Sitophilus oryzae*) v skladiščeni pšenici. Insekticidno delovanje DZ smo preučevali pri treh temperaturah (20, 25 in 30 °C), dveh vrednostih relativne vlage (55 and 75 %) in štirih (100, 300, 500 and 900 ppm) koncentracijah vsakega vzorca DZ. Smrtnost hroščev smo ugotavljali 7., 14. in 21. dan po tretiranju (DPT) in je bila največja pri najvišji koncentraciji in 21 DPT. V vseh vzorcih žita je bila smrtnost hroščev večja od 90 % pri koncentraciji 900 ppm ter 21 DPT. Izjemi sta bili slovenski in grški vzorec, ki sta vplivali na 85,3 % smrtnost pri 25 °C in 55 % vlagi ter na 67,6 % smrtnot pri 25 °C in 75 % vlagi. Najbolj učinkovit (100 % smrtnost hroščev) je bil pripravek SilicoSec 14 DPT pri koncentraciji 900 ppm. Slovenski vzorec DE je bil bolj učinkovit pri 55 kot pri 75 % vlagi (7 DPT pri vseh temperaturah).

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Ključne besede: *Sitophilus oryzae*, diatomejska zemlja, izvor, pšenica, temperatura, relativna vlaga

1 INTRODUCTION

Diatomaceous earth (DE) is a light weight, porous sedimentary rock made up of the diatoms (Bacillariophyta), unicellular aquatic plants. Their shells walls are made of amorphous hydrated silica ($SiO_2 \times nH_2O$), therefore main constituent of DE is amorphous silica (SiO_2) with small amounts of other elements such as aluminum, iron oxide, calcium hidroxide, magnesium and sodium (Round et al., 1990). Geological deposits of DE can be hundreds of meters thick (Ross, 1981).

Residual insecticides are the most common agents for protection of stored products against stored products pests. Because of their several negative properties, such are mammal toxicity, residues on grain and increased resistance of pests on some residual insecticides (Arthur, 1996), some researchers decided to evaluate several new alternative protection methods for stored products (Athanassiou *et al*., 2005). Among them was also the use of DE, which is classified as environmental acceptable method for controlling stored products pests (Arthur, 1996). For decades, DE was intensively investigated for protection of stored products. Insecticidal properties of DE in majority depend on geological origin, SiO_2 content, tapped density, oil absorbency, particle size and pH (Golob, 1997; Korunic, 1998) and also of other factors such as days after treatment (DAT), moisture and temperature (Arthur, 2001).

Different theories about control activity of DE have been proposed: 1.) blockage of stigmata and tracheae (Webb, 1945); 2.) impairment of the digestive tract (Smith, 1969); 3) water loss from insect's body through desiccation resulting in death (DE particles absorb or/and abrasive waterproof epicuticular lipids from insect's cuticle) (Ebeling, 1971). Therefore the control activity is a physical and possibility of occurring the physiological resistance is very low (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000). DE has a several advantages above traditional grain protectants, because it has low mammalian toxicity, does not break down rapidly and does not affect end-use quality (Korunic *et al*., 1996), it can be applied to the commodity with approximately the same technology as traditional residual insecticides, it can provide long-term protection against insects pest (Stathers *et al*., 2004, Athanassiou *et al*., 2005) and it can be easily removed from grain during processing (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000).

The rice weevil, *Sitophilus oryzae* (L.), Coleoptera, Curculionidae, is a cosmopolitan pest, which is considered to be one of the most destructive and widespread species in stored grain, also in Europe (Maceljski, 1999). This pest has developed a considerable level of resistance to some insecticides and it is considered to be the most resistant to piretroids (Arthur, 1996). That's why it is impossible to suppress him by application in dose rates which is effective against most other stored-grain beetle (Arthur, 1999). Rice weevil is classified as a primary pest. That means that beside damage, caused by feeding and multiplication, rice weevil can easily infest sound seeds and enable spreading secondary pests (Maceljski, 1999).

In this work we evaluated insecticidal activity of DE of different origin (three local DEs and commercial product SilicoSec) against rice weevils adults at three temperatures and two relative humidity levels. Also the comparison of insecticidal efficiency of Slovenian local DE sample with other DEs was made. Efficacy of Slovenian and Serbian DE was evaluated for the first time.

2 MATERIALS AND METHODE

2.1 Wheat and Insect Origin

Wheat was produced in 2007 in Horjul surroundings (latitude 46° 01′N, longitude 14°31′E, 339.8 m above sea level, Slovenia). Rice weevil adults (<21 days old) which were used for the test, were taken from a population that was kept in the Laboratory for Entomology on Chair of Phytomedicine, Agricultural Engineering, Crop Production, Grassland, and Pasture Management (Biotechnical Faculty, Ljubljana, Slovenia) for several years at 25±1°C on wheat.

2.2 Diatomaceous Earth Formulations

Three local DEs and commercial DE formulation were used in the test: 1. Slovenian sample was taken in a place near the village Bela cerkev (45 52′N, 15°16′E, 190.4 m). 2. Greek sample was from surroundings of town Elassona at the foothills of the Olimp mountain (39°52′ N 22°10′ E, 308.0 m). 3. Serbian sample was from town Kolubara (44°22' N, 20°15' E, 161.0 m). 4. Commercial DE formulation SilicaSec (Biofa GmbH, Münsingen, Germany) is of freshwater origin containing approximately 92 % SiO_2 (Athanassiou *et al*., 2003). Previous studies have shown that SilicoSec is effective against several stored pests (Athanassiou *et al*., 2003;

Athanassiou *et al*., 2005) and therefore it was used for positive control. Particle sizes of all four DE were approximately 12 µm.

2.3 Laboratory Bioassay

Exposure studies were carried out at 20, 25 and 30 °C and at 55 and 75 % relative humidity (RH). Four concentrations of each DE were used (100, 300, 500 and 900 ppm). Single glass jar contained 1 kg of wheat grain and individual dose of DE. An additional jar of untreated wheat was used as control. All jars were shaken manually for approximately 5 minutes to achieve distribution of the DE in the entire grain mass (Kavallieratos *et al*., 2007). For each treating, 3 samples of 50 g each were taken from each jar and each sample was placed in a vial. Then, 30 rice weevil adults were introduced into each vial, after that vials were closed with a slender net to prevent the insects from escaping and were placed into a rearing chamber with the dark:light ratio of 24:0. Mortality of the exposed adults was measured after 7, 14 and 21 days of exposure. The entire procedure was repeated 3 times.

2.4 Data Analysis

The data obtained from the experiment were subjected to analysis of variance to determine differences in mortality rates (%) of rice weevil adults, reared under conditions of different treatments at three different temperatures (20, 25 and 30 °C) and two levels of relative humidity (55 and 75 %) (GenStat 7.1; VSN International Ltd., Hemel Hempstead, UK) and Duncan's *post hoc* test was used to determine significant differences between the treatments (Hoshmand, 2006). Before the analysis, each variable was tested for homogeneity of treatment variances. Mortality rate data were corrected for control mortality using Abbott's formula (Abbott, 1925), the arcsine square-root being transformed before analysis. The data are presented as untransformed means.

3 RESULTS

All main effects as well as their associated interactions for mortality levels were significant at the $P < 0.05$ level. Generally the most efficient was SilicoSec with 88.1 % mortality, following Serbian and Greek DE with 87.0 % and 71.9 % mortality respectively. The lowest mortality (64.0 %) was observed with Slovenian sample.

At 20 °C and after 7 DAT the highest mortality (97.9 % and above) was at SilicoSec at the highest dose rates (Figure 1). Mortality at Serbian Sample was 89.1 % and above at the highest dose rates. Greek and Slovenian samples were less efficacious with the lowest mortality (55.6 and 41.3 %). At 500 ppm only SilicoSec reached 100 % mortality and at 20 °C, 55 % RH and 30 °C, 75 % RH. At this temperature level DE samples caused in general better mortality at higher RH level.

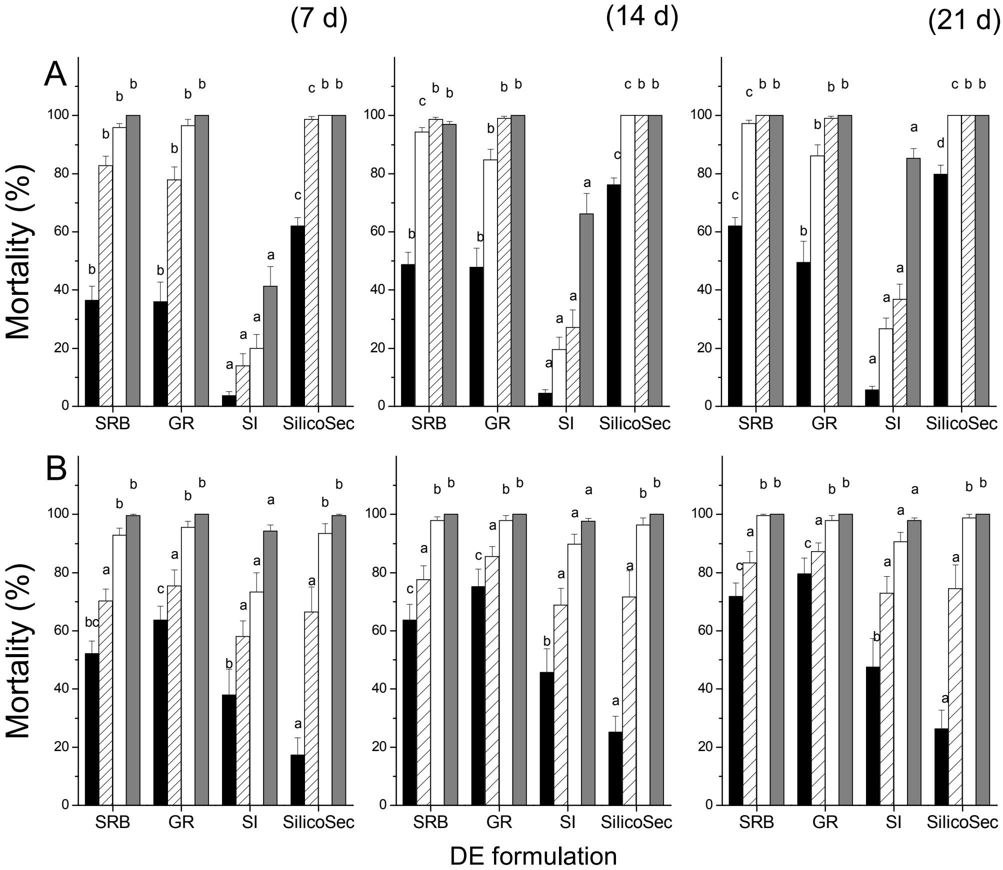

Figure 1: Mean ± SE mortality (%) of *S. oryzae* adults exposed for 7, 14 and 21 d on wheat treated with four DE formulations and at four concentrations (■ – 100, ▨ – 300, □ – 500, ■ – 900 ppm) in 55 % RH (A) and 75 % RH (B) at 20 °C (means at the same DE formulation and concentration followed by the same latter are not significantly different; Duncan test, at $P = 0.05$).

At 25 °C mortality activity of all DE samples decreased. Total mortality was reached at SilicoSec at highest dose rate and mortality of Serbian samples was above 96.9 % at the same dose rate (Figure 2). Greek sample also give a good results at the 900 ppm (mortality above 99.0 %). Slovenian sample was better at 75 % RH than at 55 % RH. At higher RH and at the 900 ppm the mortality was at least 97.6 % and at 55 % RH was under 81.7 %.

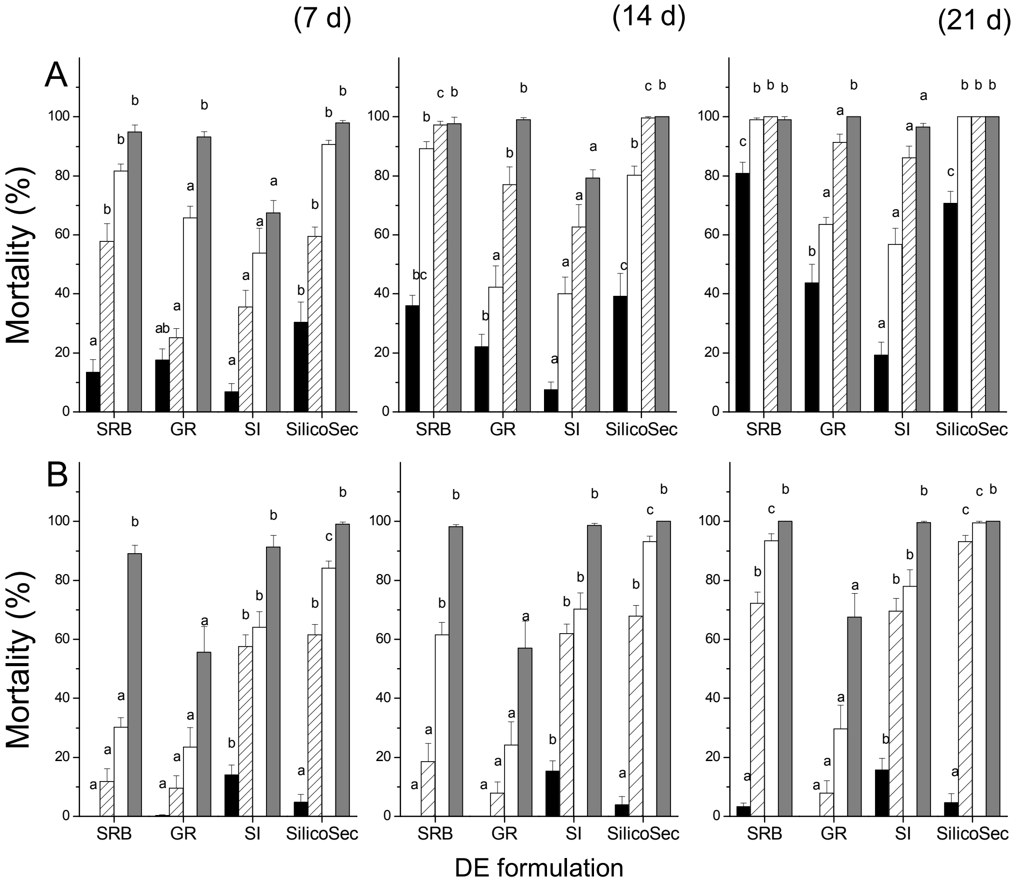

Figure 2: Mean ± SE mortality (%) of *S. oryzae* adults exposed for 7, 14 and 21 d on wheat treated with four DE formulations and at four concentrations (■ – 100, ▨ – 300, □ – 500, ■ – 900 ppm) in 55 % RH (A) and 75 % RH (B) at 25 °C (means at the same DE formulation and concentration followed by the same latter are not significantly different; Duncan test, at *P* = 0.05).

At 30 °C the mortality of rice weevils was higher than at 20 or 25 °C. At highest dose rates the mortality was at all trials above 90 % except at Slovenian sample (20 °C and 55 % RH) and Greek sample (at 25 °C and 75 % RH) where it was 85.3 and 67.6 % respectively (Figure 3). The mortality above 90 % at 500 ppm was at SilicoSec, Greek and Serbian samples, except at one treating (Greek DE, 25 °C, 75 % RH) where the mortality was only 67.6 %. Slovenian sample was the less efficient and mortality was between 36.8 and 90.6 % at the same dose rate.

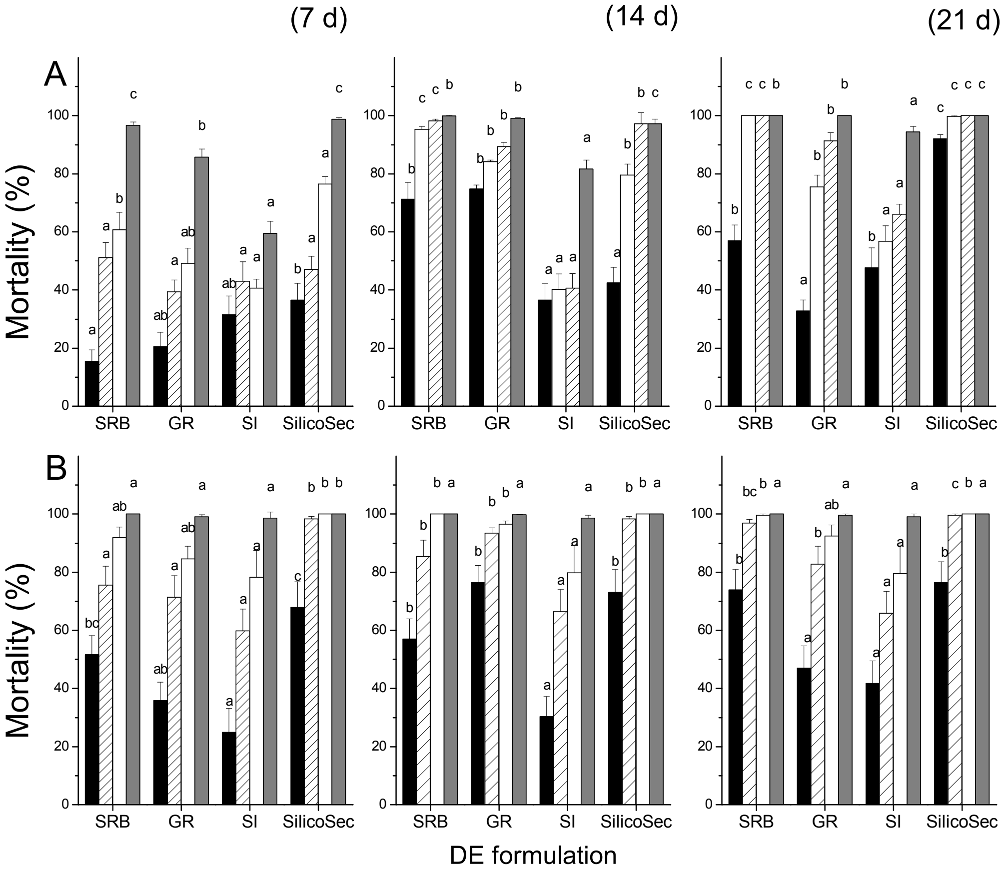

Figure 3: Mean ± SE mortality (%) of *S. oryzae* adults exposed for 7, 14 and 21 d on wheat treated with four DE formulations and at four concentrations (■ – 100, ▨ – 300, □ – 500, ■ – 900 ppm) in 55 % RH (A) and 75 % RH (B) at 30 °C (means at the same DE formulation and concentration followed by the same latter are not significantly different; Duncan test, at *P* = 0.05).

4 DISCUSSION

Insecticidal efficacy of DE is highly influenced by several factors such as temperature, type of DE formulation and dose rate (Kavallieratos *et al.* 2007). Important factor which affect the rice weevil mortality is also exposure interval. DE has physical action, since the DE particles grasp insect cuticle meanwhile weevils are moving on treated wheat (Athanassiou *et al*., 2005). Further exposure means active contact with DE particles, which damages weevil wax layer and insect dies through desiccation (Korunic, 1998). The lowest mortality between rice weevil adults were 7 days after treatment and the highest mortality was obtained after 21 days of exposure.

Some investigations showed that mortality of tested insects increased with increasing temperature (Fields and Korunic, 2000; Athanassiou *et al*., 2005). At higher temperatures the insects are more mobile, which increases contact with the DE particles and therefore greater damage of the insect cuticle. Increased water loss via spiracles due to increased respiration at higher temperatures is also observed (Arthur, 2000; Fields and Korunic, 2000). In our investigation the lowest mortality was obtained at 25 °C and not at the lowest temperature (20 °C). We believe that the rearing temperature (25 ±1 °C) of rice weevils before treatments is responsible for this result.

The results of our research showed that the relative humidity had some effects on mortality of rice weevil adults, because at 55 and 75 % relative humidity the mortality was 80.2 % and 75.4 %, respectively. This data confirm the fact that efficacy of DE decreased with increase in relative humidity (Mewis, 1998; Athanassiou *et al*., 2007). On the contrary, at Slovenian DE the mortality was with a few exceptions at 75% RH much higher as was at 55% RH.

Effective dose rate of SilicoSec for successfully rice weevil treating in wheat is between 500 and 900 ppm (Athanassiou *et al.*, 2005). In our study the mortality of rice weevil was at dose rate 900 ppm more than 97.9 % and at dose rate 500 ppm the mortality was above 90 %, except after 7 DAT at 25 °C, 75 % RH and at 30 °C, 55 % RH, where the mortality was 84.1 and 76.4 % respectively.

In our investigation SilicoSec and Serbian DE have satisfactory effect on rice weevil adults at dose rates which are acceptable to environmental, people health and it has no influence on quality of stored products (Korunic *et al.*, 1996; Korunic, 1998). Therefore it can be used for control of this pest on stored wheat. The mortality of beetle increased with increasing dose rates. Although Slovenian DE shows some insecticidal properties against rice weevils, it has to be applied at concentrations higher than 900 ppm. Concentrations higher than 1000 ppm are inappropriate for pest control, because they influence several physical and mechanical properties of grain (Korunic *et al.*, 1996; Subramanyam and Roesli, 2000). On the base of our investigation on rice weevil, species which belongs to DE sensitive pests (Fields and Korunic, 2000), we concluded that Slovenian and Greek diatomaceous sediments have no appropriated insecticidal potential for control of stored products against stored insects pests.

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