



**SEVENTH INTERNATIONAL CONFERENCE  
SUSTAINABLE POSTHARVEST AND  
FOOD TECHNOLOGIES**

**INOPTEP 2021**

**April 18<sup>th</sup> – 23<sup>rd</sup>, 2021, VRSAC, SERBIA**

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## EFFICACY OF DIATOMACEOUS EARTH IN CONTROLLING MAJOR STORE PRODUCT PESTS: *PLODIA INTERPUNCTELLA*, *TRIBOLIUM CONFUSUM* AND *ACANTHOSCLIDES OBTECTUS*

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### ABSTRACT

Diatomaceous earth (DE) is an inert dust formed from fossilized skeletal remains of diatoms. Insecticidal activity is a result of desiccation that occurs after DE particles destroy lipid layers of insect cuticle. Efficacy depends on chemical composition, particle size and geographic origin. This work assessed contact efficacy of DE originating from Kolubara open-pit mine (Serbia), in comparison to SilicoSec®, against *P. interpunctella*, *T. confusum* and *A. obtectus*. DEs were applied at rates: 5, 10, 15 and 20 mg<sup>-2</sup>. Mortality was recorded after 24, 48, 72 h and seven days. Significant mortality of *P. interpunctella* larvae was recorded after seven days of exposure at two higher rates of SilicoSec® (48.0 and 54%, respectively) and Kolubara DE (45.6 and 58.5%, respectively). Higher rates of SilicoSec® and Kolubara DE caused significant mortality of *T. confusum* after seven days (54.1, 84.3%, 49.2, 78.2%, respectively). High mortality of *A. obtectus* was recorded after 48 and 72 h in SilicoSec® (61.5, 82.1%, respectively) and in Kolubara DE (58.0, 78.5%, respectively) when applied at 20 mg<sup>-2</sup>.

**Key words:** *alumina silicates, inert dusts, contact toxicity, storage pests*

### INTRODUCTION

During storage, insects cause huge quality and quantity reduction of stored commodities and great economic losses (Puzzi, 2001). Therefore, the pest control is inevitable post-harvest measure that helps prevent damages and preserves the nutritional and commercial value of stored products. Concerns about rapid development of insecticide resistance, the environmental pollution and human health have intensified the search for alternative eco-friendly pest management strategies (Gvozdenac et al., 2018a). The use of materials like inert dusts, submicron and nanomaterials is one of the strategies that have been extensively tested as viable alternative to pesticides (Fields and Korunić, 2002) suitable for a long-term protection of stored products. Back in 1997, Golob first mentions the inert dusts as potent storage protectants in grain industry, with Diatomaceous earth (DE), being the most commonly used and evaluated (Golob, 1997).

DE consists of fossilized skeletal remains of single-celled algae-diatoms found in fresh and salt waters. Diatoms are microscopic organisms that have a fine skeleton made of amorphous silica (SiO<sub>2</sub> + n H<sub>2</sub>O). The accumulation of diatom skeletons over thousands of years produces the sedimentary rock, diatomaceous earth. The major constituent of DE is amorphous silicon dioxide (SiO<sub>2</sub>) with minor amounts other minerals (aluminium, iron oxide, calcium hydroxide, magnesium and sodium). Its insecticidal properties depend upon the geological origin, and some DEs are 20 times more effective than others. The most effective DEs have SiO<sub>2</sub> content above 80%, a pH



below 8.5 (Korunić, 1997). The insecticidal effect of DE is due to physical damage of protective wax layer of the insect's cuticle (Mewis and Ulrichs, 2001). DE particles absorb waterproof lipids from insect's cuticle, destroying epicuticular lipid layers which lead to heavy water loss and eventually desiccation of insects. DEs are effective for controlling stored product pests when applied as structural treatment on walls and floors, on the surface of grain bulks and also by admixing with grain (Arthur, 2003). However, since it was found that DE reduces the grain bulk density, affects the flow characteristics of bulk grain (flowability), and also leaves visible dust residues and causes health concerns it is recommended primarily for surface treatments (Aleen, 2001). Several DE products are commercially available and registered for stored product protection. The product SilicoSec® (Biofa GmbH) was the first approved for use in Germany in 1997 (Mewis and Ulrichs, 2001) and has a wide use. Due to differences in DE efficacy as a result of chemical composition, particle size and origin, it is necessary to evaluate every new preparation based on DE.

This work aimed to assess the contact efficacy of DE from Kolubara open-pit mine (Serbia) in comparison to SilicoSec® against: a) *P.interpunctella* larvae, as one of the most polyphagous insect species; b) *T. confusum*, the most significant secondary pest of a number commodities, that is hard to control and c) *A. obtectus*, a major pest of the common bean and other leguminous crops.

## MATERIAL AND METHOD

Laboratory studies were performed to evaluate the contact efficacy of diatomaceous earth originating from Kolobuara open-pit mine (Serbia), in comparison to SilicoSec®, a commercial preparation (uncalcinated diatomite manufactured by Biofa, Germany), as a standard control to inert dust, against *P. interpunctella* larvae, *T. confusum* and *A. obtectus* adults.

### Determination of DE particle size

The DE from Kolobuara open-pit mine was tested for physical characteristics. Particle size distribution was determined using a Malvern Mastersizer 2000 particle size analyser, capable of analysing particles between 0.01 and 2000  $\mu\text{m}$ . The Malvern Mastersizer 2000 records the light pattern scattered from a field of particles at different angles. The device uses an analytical procedure to determine the particle size distribution that created the patterns. The measurements were performed with automated dry dispersion unit Scirocco 2000. Prior to measuring several testing were performed in order to select optimal measuring condition: feed rate and nozzle pressure. Selected feed rate provide adequate obscuration in range 3.26-4.15%. Selected nozzle pressure of 2 bars gave good reproducibility of results for all samples. The sample was analysed in three replicates. The results were recorded as the particle volume percentages.

The obtained volume-based particle size distribution was broad and showed particles diameter in range from 0.3  $\mu\text{m}$  to 700  $\mu\text{m}$ . The most common value of the frequency volume based distribution (mode) was 15  $\mu\text{m}$ . The  $d(0.5)$ , values indicate 50% of the particles measured were smaller than or equal to the size stated (median) - was 16  $\mu\text{m}$ . SilicoSec® consisted of majority particle size between 2–18  $\mu\text{m}$ .

### Test insects

The insects used in this research originate from the populations reared at Institute of Field and Vegetable Crops, Novi Sad, Serbia. *P. interpunctella* culture originates from the population reared for ~50 generations on standard a laboratory diet in a thermostat chamber ( $28\pm 1^\circ\text{C}$ , r.h.  $60\pm 10\%$ , photoperiod 14:10 (L:D)). *T. confusum* experimental population is reared on substrate made of wheat and maize flour (ratio 1:1) and yeast (5%) at  $26 \pm 1^\circ\text{C}$  and relative humidity  $60 \pm 5\%$ . *A. obtectus* population is being maintained the laboratory at large size (about 1000 individuals) for

more than 20 generations, on common bean seeds (*Phaseolus vulgaris* L.) under controlled conditions (27 ±1°C, relative humidity 65 ±5%, photoperiod (L16: D8)).

### Bioassay

Two DE formulations (DE from Kolubara and SilicoSec®) were applied on glass Petri dishes (Ø 9 cm) at rates 5, 10, 15 and 20 gm<sup>-2</sup>. Dusts were dispersed over the glass surface, after which, 20 insect specimens were placed inside dishes (*P. interpunctella* larvae of L<sub>4-5</sub> stage, for beetles, unsexed adults were used). Mortality was evaluated after 24, 48, 72 h and seven days of exposure. Clean Petri dishes served as the control. After the exposure periods, the insects were considered dead when unable of walking or flying when touched with fine brush. Three replicates were used for each dose and exposure period and a control treatment.

The differences in mortality, depending on the preparation and concentrations applied, were analyzed using one-way ANOVA, Duncan's multiple range test (confidence interval 95%) in statistical software SPSS 21.

## **RESULTS AND DISCUSSION**

The effect of DEs applied at 5, 10, 15 and 20 gm<sup>-2</sup> on *P. interpunctella*, *T. confusum* and *A. obtectus* are presented on Figs. 1-3. The mortality of tested insect species increased with the increase of concentration as well as the exposure period in treatments with both DEs.

### *P. interpunctella*

Lower rates (5 and 10 gm<sup>-2</sup>) of both applied DEs (SilicoSec® and Kolubara DE) caused low mortality of *P. interpunctella* larvae, regardless on the exposure period (Fig. 1.). The mortality ranged from 0.0-5.4% after 24 h of exposure, 0.0 -12.0 % after 48 h and 5.0-26.2% after 72 h. The differences between treatments and rates within one treatment after 24 h and 48 h of exposure were not statistically significant (F=0.11NS, 7.14NS, p>0.05, respectively). In treatments with higher rates of SilicoSec® (15 and 20 gm<sup>-2</sup>) the mortality ranged from 1.4 to 26.2%, after shorter exposure (24, 48 and 72 h). However, significant mortality of *P. interpunctella* larvae was recorded after seven days of exposure to higher rates (15 and 20 gm<sup>-2</sup>), 48.0 and 54%, respectively. After seven days, the differences between mortalities among treatments and rates within one treatment were highly significant (F=111.65\*\*, p<0.01, respectively). DE from Kolubara was as effective as SilicoSec® at higher rates (15 and 20 gm<sup>-2</sup>), causing larval mortality of 45.6 and 58.5%, respectively after seven days. Lower rates (5 and 10 gm<sup>-2</sup>) and shorter exposure (24, 48 and 72 h) were low effective (0.0-26.0% mortality). These results are in accordance with previously published research of Gvozdenac et al. (2018) that indicate at high efficacy of SilicoSec®, but only after long exposure and at maximum rate (20 gm<sup>-2</sup>). Similar results were presented by Subramanyam et al. (1998) who tested the efficacy of DE product Insecto against *P. interpunctella* 5<sup>th</sup> larval instars. At the same application rates as in our study authors reported significantly lower efficacy (10-70%) on 5<sup>th</sup> larval instars compared to the efficacy on 1<sup>st</sup> larval instars (99.5-100%), indicating at higher susceptibility of younger larvae to insecticidal effects of DE products than mature larvae.

In treatments with SilicoSec® and Kolubara DE, the pupation of *P. interpunctella* occurred faster compared to the control. This was also confirmed in previous reports of Gvozdenac et al. (2018b).

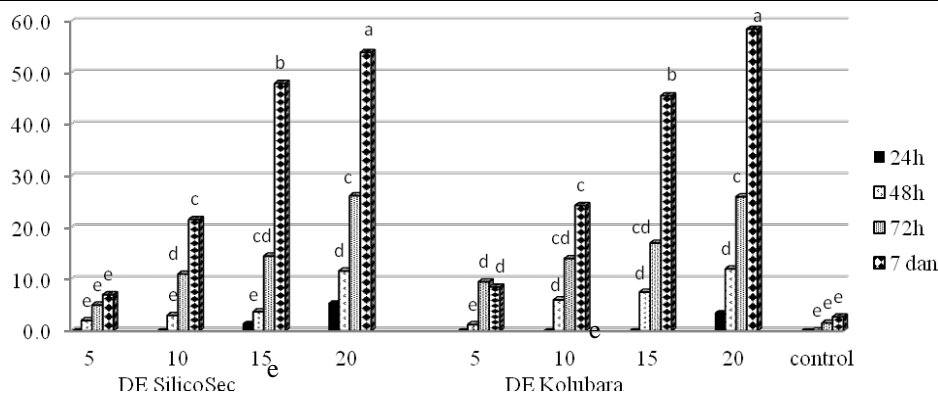


Figure 1. The effect of DE from Kolubara open-pit mine and SilicoSec® on *P. interpunctella* larvae

### *T. confusum*

The mortality of *T. confusum* was low (0.0-34.1%) after 24, 48 and 72 h at all applied rates and in both treatments. SilicoSec® caused significant mortality of confused beetles at rates 15 gm<sup>-2</sup> and 20 gm<sup>-2</sup>, only after seven days (54.1 and 84.3%, respectively). Mortalities caused by DE from Kolubara were at the same level of significance with SilicoSec®. After seven days the efficacy was 49.2% at 15 gm<sup>-2</sup> and 78.2% at 20 gm<sup>-2</sup>. Differences between mortalities after seven days at the same application rate were not significant (F=70.1NS, 101.4NS, p>0.05). The results are in accordance with reports from Collins and Cook (2006) who tested the efficacy of SilicoSec® against *T. castaneum*. It was proven to be a very effective treatment causing the mortalities from 93-100% after seven days of exposure. Similar results were obtained by Scholler and Reichmuth (2010), indicating that at different surfaces the efficacy of SilicoSec® ranged from 79% to 100%.

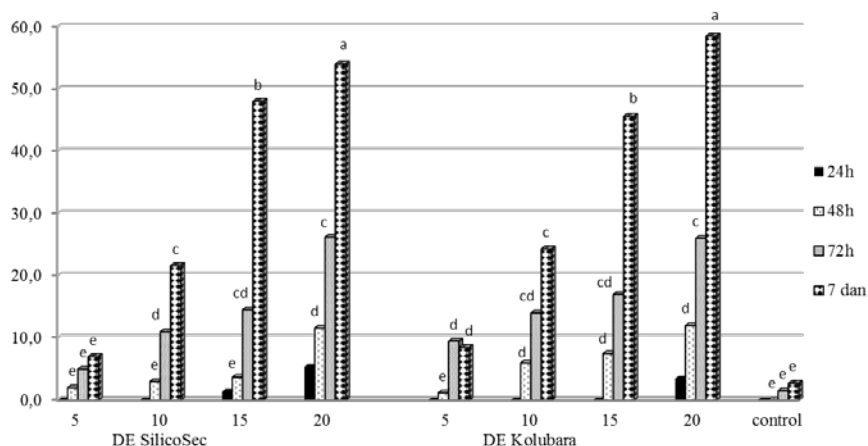


Figure 2. The effect of DE from Kolubara open-pit mine and SilicoSec® on *T. confusum* adults *A. obtectus*

SilicoSec® and DE from Kolubara did not cause significant mortalities of *A. obtectus* adults after 24 h, regardless on the applied rate (6.2-28.1%). However, after 48h SilicoSec® caused mortality of 61.5%, and DE from Kolubara 58.0% at the highest rate (20 gm<sup>-2</sup>) and the values are at the same level of significance (F=72.01NS, p>0.05). After 72 h, both DEs expressed strong insecticidal effect at higher rates (15 and 20 gm<sup>-2</sup>). SilicoSec® caused mortality of 49.4 and 82.1%, respectively, and DE from Kolubara 56.7 and 78.5%, respectively. Differences were not significant (F=1.7NS; 6.52NS respectively, p>0.05). The results of Jumbo et al. (2019) revealed that *A. obtectus* mortality

caused by DE was dose, temperature and exposure period dependent, which was also proven in this work, for dose (application rate) and exposure. The authors suggest that DEs have the potential to be used as an effective tool for managing *A. obtectus* infestations in stored beans, which can also be concluded from this research.

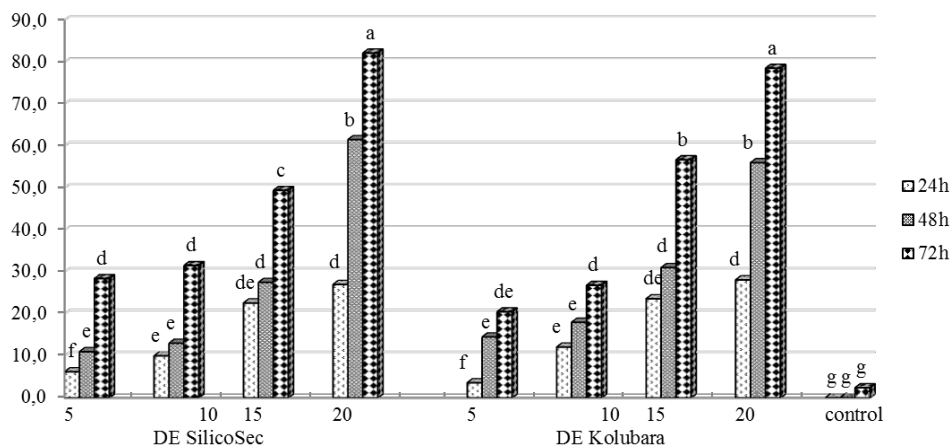


Figure 3. The effect of DE from Kolubara open-pit mine and SilicoSec® on *A. obtectus* adults

Storage insects express different susceptibility to inert dusts due to morphological, physiological and ecological characteristics of each species (Kljajić et al, 2011), as was proven in this work. Additionally, Jumbo et al. (2019) underlined that the insecticidal efficacy of DE is affected by abiotic factors (temperature, humidity, exposure period etc.), as mentioned, but also by biotic factors (insect species, physiology, behaviour, age etc.). Different insect species in our work expressed different susceptibility to DEs, regardless on their origin and concentrations.

### CONCLUSION

This work aimed to evaluate the efficacy of DE originating from Kolubara open-pit mine in comparison to commercial preparation SilicoSec®. DE from Kolubara expressed similar insecticidal activity as SilicoSec®, against all three tested insect species (*P. interpunctella*, *T. confusum*, *A. obtectus*), given that mortalities did not differ statistically. Both DEs were the most effective when applied at higher rates (15 and 20 gm<sup>-2</sup>) and the highest mortality was achieved after 72 h and seven days of exposure. The results indicate that both DEs, DE from Kolubara open-pit mine in particular, can be used as an additional surface treatment method in storages for preventing the infestation with *P. interpunctella* larvae and *T.confusum* and *A. obtectus* adults.

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