Laboratory tests on insecticidal effectiveness of disodium octaborate tetrahydrate, diatomaceous earth and amorphous silica gel against *Sitophilus oryzae* (L.) and their effect on wheat bulk density

Laboratorijska testiranja insekticidne učinkovitosti dinatrijevog oktaborat tetrahidrata, dijatomejske zemlje i amorfnoga silika gela na *Sitophilus oryzae* (L.) te njihov utjecaj na hektolitarsku masu pšenice

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# LABORATORY TESTS ON INSECTICIDAL EFFECTIVENESS OF DISODIUM OCTABORATE TETRAHYDRATE, DIATOMACEOUS EARTH AND AMORPHOUS SILICA GEL AGAINST Sitophilus oryzae (L.) AND THEIR EFFECT ON WHEAT BULK DENSITY

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#### **SUMMARY**

We examined insecticide effectiveness of three different inert dusts: disodium octaborate tetrahydrate (DOT) and diatomaceous earth (DE) Celatom® Mn 51 applied as powder and suspension, and silica gel Sipernat® 50 S applied as powder against Sitophilus oryzae (L.) and their reduction of grain bulk density. Sipernat® 50 S was the most effective dust with a very fast initial effectiveness. DE and DOT generated similar effectiveness against S. oryzae. DOT generated low initial effectiveness but after prolonged exposure time of 8 and especially after 21 days, the mortality was very high (100%), similar to the effectiveness of DE. The similar order of dusts was obtained in the reduction of wheat bulk density. Applied at dose of 200 and 500 ppm, the lowest bulk density difference in regard to bulk density of untreated wheat had DOT (-1.1 and -1.9 kg hl<sup>-1</sup>, respectively), followed by Celatom® Mn 51 (-3.5 and -4.3 kg hl<sup>-1</sup>, respectively) and Sipernat® 50 S (-5.2 and -5.5 kg hl<sup>-1</sup>, respectively). Due to the effect on wheat bulk density DOT belongs to the group with the least negative effect on bulk density and therefore, it is a promising dust to control stored grain insect pests.

Key-words: disodium octaborate tetrahydrate, diatomaceous earth, silica gel, wheat, Sitophilus oryzae (L.), bulk density

#### **INTRODUCTION**

One of the first research publications dealing with inert dust against storage pests is from 1920s (Headlee, 1924). Following this publication, there have been several reviews and research papers on the subject since (Ebeling and Wagner, 1961; Ebeling, 1961, 1971; Fields and Muir, 1996; Golob, 1997; Korunic, 1998, 2013; Quarles, 1992; Quarles and Winn, 1996; Subramanyam and Roesli, 2000; Kalinović et al., 2011; Shah and Khan, 2014; Rozman, 2015; Liška et al., 2015; Malia et al., 2016). Inert dusts are non-toxic dry powders of different origins that are chemically unreactive in nature. Inert dusts can be mixed with different stored agricultural grains to control stored grain pests and different crawling pests in storages and in different industrial objects for food production. Inert dusts do not deteriorate or break down, and therefore, provide long-term control of insect pests and are almost completely harmless to humans and mammals (Maceljski and Korunic (1972), Fields and Muir (1996), Subramanyam and Roesli (2000), Arthur and Puterka (2002), Kljajić et al. (2010) gave detailed guidelines on the key aspects for the use of inert dusts as a possible alternative to synthetic pesticides in stored products, and proposed inert dusts to be included into IPM program.

Unlike conventional contact insecticides, inert dusts function through their physical properties and therefore they generally act more slowly. Insect mortality is induced primarily as a result of water loss

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(desiccation) caused mainly by two ways of mode of actions: destruction of the cuticle with dusts (abrasion) (Alexander et al., 1944a, 1944b) and absorption of waxy layer from insect's cuticle (silica gels and diatomaceous earth containing amorphous silicon dioxide). Other possible causes are blocking the spiracles and causing insects to die from asphyxiation and ingestion of the dust particles causing the gut destruction (Ebeling, 1971; Maceljski and Korunic, 1972; Le Patourel, 1986).

There are advantages and limitations to applying inert dusts. Among the main advantages of using inert dusts, and particularly DEs is their low mammalian toxicity and stability on grain (Korunic, 1998, 2013; Subramanyam and Roesli, 2000). Among the main limitations of using inert dusts including DEs are certain regulatory issues. These issues were reviewed by Korunic (1998, 2013), Korunic et al. (1998) and Desmarchelier and Allen (2000).

Silica gel (Sipernat® 50 S) is precipitated hydrophilic silica with high absorption capacity and a large specific surface area of  $475 \text{ m}^2/\text{g}$ . It contains 97-98.5% of  $\text{SiO}_2$ , 1.4% of  $\text{Na}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$  and  $\text{SO}_3$ . It has mean particle size of 7.5 microns, and pH value (in 5% suspension) is 6.0. Silica gels are very effective insecticides and acaricides, used in controlling insects, mites and ticks in a variety of indoor and outdoor sites (St. Aubin, 1991). Silica gel is used to control target pests in stored grain crops, food handling areas, hospitals, sewage systems, and on animals/pets and their living quarters (Quarles, 1992).

During the last 20 years, DE has been the subject of several review papers as well (Quarles, 1992; Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000; Quarles and Winn, 1996; Nikpay, 2006; Korunic, 2013; Shah and Khan, 2014), with numerous references cited with each review. However, DE is not yet in wider use for direct mixing with grains because of the great obstacles and disadvantages in use (Fields, 1998; Korunić et al. 2016).

Disodium octaborate tetrahydrate (DOT), the inert dust used in the experiment, differs a little bit in the mode of action against insects. Its activity is primarily based on the toxicity and internal desiccation (damaged gut and mouth organ) and less on desiccation through epicuticle (Thurston County Health Department, 2009). These inert dusts are based on boron containing compounds, such as borax (sodium tetraborate decahydrate) and boric acid, and have been used since the early 1900s against ants (Rust, 1986).

The safety of use, stability and the effectiveness of DOT against beetles was the main reason we wanted to assess its effectiveness against stored grain beetles. The choice of safe powdered insecticides used in stored grain protection is very limited; actually, there is only one natural and safe dust in grain protection: diatomaceous earth (DE). The present study was conducted to determine the effectiveness of three chemically and physically different inert dusts to control rice weevil

Sitophilus oryzae (L.) and their effect on grain bulk density (test weight) reduction. It was expected that the most effective inert dust would have higher influence on bulk density reduction of wheat grain than less effective dusts.

#### MATERIAL AND METHODS

#### **Inert dusts**

Celatom® MN 51 is a natural diatomaceous earth from the USA (EP Minerals), containing 73.6% amorphous silica (opal A), 7.8%  $\rm Al_2O_{3,}$  5.6% CaO and the rest are other oxides. It is beige in colour, with pH of 7.5 (10% slurry) and medium particle sizes of 15.0 microns.

Disodium octaborate tetrahydrate (DOT) is odourless white powder of disodium octaborate tetrahydrate, with pH of 7.6 (in 10% solution) and extremely low toxicity to warm-blooded organisms. Among many different uses, it is used as an insecticide and fungicide (PPDB: Pesticide Properties DataBase, 2016).

Sipernat<sup>®</sup> 50 S from the USA is precipitated hydrophilic silica with high absorption capacity and a large specific surface area of 475 mg<sup>-2</sup>. It contains 97-98.5% of amorphous SiO<sub>2</sub>, 1.4% of Na<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub> and SO<sub>3</sub>. It has mean particle size of 7.5 microns, while pH value is 6.0 (in 5% suspension).

#### **Test insect**

A laboratory strain of rice weevil *Sitophilus oryzae* (L.) susceptible to insecticides was used in the experiments. Insects were reared on clean, soft, whole wheat kernels of different variety with approximately 13% moisture content (m.c.) under controlled conditions  $(28\pm2^{\circ}\text{C}, 65\pm5\% \text{ r.h.})$ , in dark). Fifty unsexed adults (7-21 days old) were used for each treatment.

#### **Bioassays**

DE and DOT samples were sieved through the wire screen with openings of 25 microns. Glass jars were filled with 100 grams of wheat grains of clean Ontario soft wheat of different variety containing approximately 13.6% m.c. All three dusts were applied as powder and also DE and DOT were applied as suspension (the same dosages of DE and DOT were mixed with water). Each tested dust was applied at 200, 300, 400 and 500 ppm. Following application of the dust and suspensions on the surface of grains, the jars were tightly closed with lid, shaken manually for 1 minute and left closed for 1 hour before the introduction of 50 adults of S. oryzae, per each replication. All treatments were set in three replications. The jars with treated grains and test insects were held in a climate chamber at 26±1°C and relative humidity (r.h.) of 55±5% during the whole testing period. The assessment of the results was performed after 6, 8 and 21 days and the progeny (F1) production of S. oryzae assessment 60 days after the introduction of insects into the replications.

#### Bulk density (test weight) reduction measurement

Five hundred grams of cleaned soft white wheat (with 13.6% m.c.) were manually mixed with 500 and 200 ppm separately (0.5 and 0.2 g kg $^{-1}$ ) of inert dusts in a tightly closed glass jar by shaking for one minute (at room temperature). Test weight was measured using the Canadian Grain Commission procedure (Official Grain Grading Guide, 2016). Measures for both, untreated and treated grain were expressed as kg per hectolitre (kg hl $^{-1}$ ). The difference in values between untreated and treated grains showed the reduction of test weight.

#### Data analysis

Experimental data were processed by statistical analysis system SAS/STAT Software 9.3. (2013- 2014). One-way analysis of variance of the tested variables was subjected to SAS Analyst module and a procedure ANOVA was used. Tukey's Studentized Range (HSD) test was used to detect differences between means at the 0.05 significance level. Schneider-Orelli formula (Püntener, 1981) was used to calculate corrected efficacy for data on the 7<sup>th</sup> and 15<sup>th</sup> day of exposure.

#### **RESULTS AND DISCUSSION**

# Insecticidal effectiveness of inert dusts against *S. oryzae*

In the treatment with DOT powder, the mortality of *S. oryzae* at the 6 days exposure interval was very low even at the highest dose of 500 ppm (36.6% only) (Table 1). This result indicates a slow initial effectiveness of formulation DOT powder. After 8 days, mortality increased threefold at 300 ppm, without significant statistical differences of mortality among higher doses. Furthermore, after 21 days of the exposure, DOT powder reached maximum mortality (100%) at 300 ppm. DOT powder greatly reduced the progeny of *S. oryzae* even at 200 ppm (86.1%) and at higher concentrations from 99.6 to 100% (Table 2).

Applied as a suspension, DOT showed lower activity overall. The mortality of *S. oryzae* after 6 and 8 days was significantly higher than the control treatment only at the 500 ppm and 400 ppm, respectively, still with mortality below 50%. However, after prolonged exposure time of insects on treated grain, the mortality reached its maximum (100%) at 300 ppm (Table 1). DOT suspension greatly reduced the progeny of *S. oryzae* even at 200 ppm (85.4%) and at higher concentrations from 99.1% to 99.8% (Table 2).

The initial effectiveness of DE Celatom®MN 51 applied as powder was higher in comparison with this effectiveness of DOT powder. At the lowest dose, the mortality of *S. oryzae* was 52.7%, 85.0% and 77.3% after 6, 8 and 21 days, respectively (Table 1). At higher doses, statistically significant higher mortality was noticed, reaching the maximum at 500 and 400 ppm after 6 and 21 days of exposure, respectively. The progeny of *S. oryzae* was not completely inhibited at even the highest concentration of 500 ppm (94.6% of inhibition) (Table 2).

The effectiveness of DE Celatom® MN 51 applied as a suspension against *S. oryzae* was similar to the results obtained with powder formulation. The only difference was the lower initial effectiveness at the exposure time of 6 days and at 200 ppm concentration after 8 days. Still, the initial effectiveness of DE Celatom® MN 51 suspension was higher in comparison with the effectiveness of DOT powder and DOT suspension (Table 1). Considering the low initial effectiveness against parents, the progeny of *S. oryzae* was not completely inhibited even at the highest concentration of 500 ppm (95.39%) (Table 2).

In the treatment with Sipernat<sup>®</sup> 50 S powder, the mortality of S. oryzae even at 200 ppm after exposure of 8 days was 100% (Table 1), and also the inhibition of the progeny of S. oryzae at all concentrations was 100% (Table 2).

Table 1. Mortality of *S. oryzae* adults (%) after 6, 8 and 21 days of exposure to treated wheat grains with inert dusts applied as powder and suspension

Tablica 1. Mortalitet odraslih S. oryzae (%) u tretiranoj pšenici s inertnim prašivima aplicirani kao prah i suspenzija pri ekspoziciji 6., 8. i 21. dana

Treatment Tretman	D	Exposition (days)* Izloženost (dani)*		
	Dose (ppm) Doza (ppm)	6 days (mean ± SD) 6 dana (prosjek ± SD)	8 days (mean ± SD) 8 dana (prosjek ± SD)	21 days (mean ± SD) 21 dan (prosjek ± SD)
DOT powder DOT prašivo	0	0.3±0.57 c	3.3±1.15 b	19.3±3.05 c
	200	$3.3 \pm 1.15 c$	16.0±3.60 b	94.0±3.46 b
	300	18.0±5.29 b	53.0±10.81 a	100.0±0.00 a
	400	20.0±6.00 b	50.6±16.01 a	100.0±0.00 a
	500	$36.6 \pm 6.42 \text{ a}$	68.3±8.08 a	100.0±0.00 a
	F P	29.88 <.0001	24.76 <.0001	886.09 <.0001

DOT   Suspension   Suspension   DOT   Suspension   DOT   Suspension   Suspension   Suspension   DOT   Suspension   Suspension   DOT   Suspension   Suspension   DOT   Suspension   Suspension   DOT   Suspension   Suspension   Suspension   Suspension   Suspension   Suspension   Suspension   Suspension   Suspension   DOT   Suspension					
DOT   Suspension   Suspension   DOT   Suspension   Suspension   DOT   Suspension   DOT   Suspension   DOT   Suspension   DOT   Suspension   DOT   Suspension   Suspension   DOT   Suspension   Suspension   DOT   Suspension   DOT   Suspension   DOT   Suspension   Suspension   DOT   Suspension   Suspension   Suspension   Suspension   DOT   Suspension   Suspension   DOT   Suspension   Suspension   DOT   Suspension		0	0.0±0.00 b	2.7±2.30 c	17.3±3.05 c
suspension DOT         400         6.7±1.15 b         22.0±6.08 b         100.0±0.00 a           suspenzija         500         22.0±7.21 a         39.0±5.19 a         100.0±0.00 a           F         12.32         29.87         1656.69           P         0.0007         <.0001         <.0001           Celatom® Mn 51         200         52.73±3.05 c         85.0±4.25 b         77.3±6.65 b           powder         300         90.0±6.00 b         97.7±3.21 a         99.7±0.57 a           Celatom® Mn 51         400         93.3±3.05 ab         99.7±0.57 a         100.0±0.00 a           F         474.61         842.01         338.31           P         <0.0001         <.0001         <.0001           P         <0.0001         <0.0001         <0.0001           F         474.61         842.01         338.31           P         <0.0001         <0.0001         <0.0001           Celatom® Mn 51         300         52.0±9.16 a         94.7±2.30 a         98.0±2.64 a           suspension         Mn 51         300         65.3±6.11 a         91.0±7.00 a         100.0±0.00 a           F         56.61         326.66         710.25           P	suspension DOT	200	5.3±2.30 b	14.7±2.08 bc	85.3±1.52 b
DOT suspenzija         400         6.7±1.15 b         22.0±6.08 b         100.0±0.00 a           suspenzija         500         22.0±7.21 a         39.0±5.19 a         100.0±0.00 a           F         12.32         29.87         1656.69           P         0.0007         <.0001		300	5.3±5.03 b	$8.7 \pm 5.03 \text{ cd}$	100.0±0.00 a
suspenzija         500         22.0±7.21 a         39.0±5.19 a         100.0±0.00 a           F         12.32         29.87         1656.69           P         0.0007         <.0001		400	6.7±1.15 b	22.0±6.08 b	100.0±0.00 a
F		500	22.0±7.21 a	39.0±5.19 a	100.0±0.00 a
Celatom® Mn 51 powder  Celatom® Mn 51 prašivo  Celatom® Mn 51 suspension Au  Celatom® Mn 51 suspension Celatom® Mn 51 suspension Celatom® Mn 51 suspension Au  Celatom® Mn 51 suspension Celatom® Mn 51 suspension Au  Celatom® Mn 51 suspen		F	12.32	29.87	1656.69
Celatom® Mn 51 powder         200         52.73±3.05 c         85.0±4.25 b         77.3±6.65 b           powder Celatom® Mn 51 powder         300         90.0±6.00 b         97.7±3.21 a         99.7±0.57 a           Celatom® Mn 51 powder         400         93.3±3.05 ab         99.7±0.57 a         100.0±0.00 a           F         474.61         842.01         338.31           P         <.0001		Р	0.0007	<. 0001	<0001
Celatom® Mn 51   P   P   P   P   P   P   P   P   P		0	0.3±0.57 d	3.3±1.15 c	19.3±3.05 c
powder Celatom® Mn 51 prašivo         300 po.be.00 b         97.7±3.21 a         99.7±0.57 a           Celatom® Mn 51 prašivo         400 po.be.00 a         100.0±0.00 a         100.0±0.00 a           500 prašivo         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a           F         474.61 prašivo         842.01 prašivo         338.31 prašivo           O         0.0±0.00 b         2.7±2.30 c         17.3±3.05 c           Celatom® Mn 51 suspension Celatom® Mn 51 suspension Celatom® Mn 51 suspenzija         300 prašivo         52.0±9.16 a         94.7±2.30 a         98.0±2.64 a           F         56.61 prašivo         326.66 prašivo         710.25 prašivo         100.0±0.00 a         100.0±0.00 a           Sipernat® 50 S prašivo         300 prašivo         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a           100 prašivo         500 prašivo         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a	Celatom® Mn 51	200	52.73±3.05 c	85.0±4.25 b	77.3±6.65 b
prašivo         500         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a           F         474.61         842.01         338.31           P         <.0001	powder	300	90.0±6.00 b	97.7±3.21 a	99.7±0.57 a
F 474.61 842.01 338.31 P < .0001		400	93.3±3.05 ab	99.7±0.57 a	100.0±0.00 a
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	prašivo	500	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		F	474.61	842.01	338.31
Celatom® Mn 51 suspension Celatom® Mn 51 suspenzija  Description  Sipernat® 50 S powder Siperat® 50 S prašivo  Description  200  17.3±6.42 b  17.3±6.42 b  48.7±3.05 b  76.3±3.21 b  76.3±3.25 b  76.3±3		P	<.0001	<.0001	<.0001
$ \begin{array}{c} \text{Celatom}^{\circledast}  \text{Mn}  51 \\ \text{suspension} \\ \text{Celatom}^{\circledast}  \text{Mn}  51 \\ \text{suspension} \\ \text{Celatom}^{\circledast}  \text{Mn}  51 \\ \text{suspenzija} \\ \end{array} \begin{array}{c} 300 \\ 400 \\ 65.3 \pm 6.11  \text{a} \\ 500 \\ 66.7 \pm 9.85  \text{a} \\ \end{array} \begin{array}{c} 91.0 \pm 7.00  \text{a} \\ 91.0 \pm 7.00  \text{a} \\ \end{array} \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ 100.0 \pm 0.00  \text{a} \\ \end{array} \\ \begin{array}{c} 500 \\ 66.7 \pm 9.85  \text{a} \\ \end{array} \begin{array}{c} 97.3 \pm 2.88  \text{a} \\ \end{array} \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ \end{array} \\ \begin{array}{c} 710.25 \\ P \\ \hline \\ \text{C}.0001 \\ \end{array} \\ \begin{array}{c} 0 \\ 0.3 \pm 0.57  \text{b} \\ \end{array} \begin{array}{c} 3.3 \pm 1.15  \text{b} \\ \end{array} \begin{array}{c} 19.3 \pm 3.05  \text{b} \\ \end{array} \\ \begin{array}{c} 300 \\ \text{Sipernat}^{\circledast}  50  \text{S} \\ \text{powder} \\ \end{array} \begin{array}{c} 200 \\ 300 \\ 300 \\ \end{array} \begin{array}{c} 96.0 \pm 4.00  \text{b} \\ 100.0 \pm 0.00  \text{a} \\ \end{array} \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ 100.0 \pm 0.00  \text{a} \\ \end{array} \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ \end{array} \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ \end{array} \\ \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ \end{array} \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ \end{array} \\ \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ \end{array} \\ \begin{array}{c} 100.0 \pm 0.00  \text{a} \\ \end{array} \begin{array}{c} 100.0 \pm 0.00 $		0	0.0±0.00 b	2.7±2.30 c	17.3±3.05 c
suspension Celatom® Mn 51 suspenzija         400         65.3 ± 6.11 a         91.0 ± 7.00 a $100.0 \pm 0.00$ a           F         500         66.7 ± 9.85 a         97.3 ± 2.88 a $100.0 \pm 0.00$ a           F         56.61         326.66         710.25           P         < .0001		200	17.3±6.42 b	48.7±3.05 b	76.3±3.21 b
Celatom® Mn 51 suspenzija         400         65.3 ± 6.11 a         91.0 ± 7.00 a         100.0 ± 0.00 a           F         500         66.7 ± 9.85 a         97.3 ± 2.88 a         100.0 ± 0.00 a           F         56.61         326.66         710.25           P         <.0001		300	52.0±9.16 a	94.7±2.30 a	98.0±2.64 a
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Celatom® Mn 51	400	65.3±6.11 a	91.0±7.00 a	100.0±0.00 a
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		500	66.7±9.85 a	97.3±2.88 a	100.0±0.00 a
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		F	56.61	326.66	710.25
Sipernat® 50 S powder         200 300 $96.0\pm4.00 \text{ b}$ $100.0\pm0.00 \text{ a}$ $100.0\pm0.00 \text{ a}$ Sipernat® 50 S powder         300 100.0±0.00 a $100.0\pm0.00 \text{ a}$ $100.0\pm0.00 \text{ a}$ Sipernat® 50 S prašivo         400 100.0±0.00 a $100.0\pm0.00 \text{ a}$ $100.0\pm0.00 \text{ a}$ 500 100.0±0.00 a $100.0\pm0.00 \text{ a}$ $100.0\pm0.00 \text{ a}$ $100.0\pm0.00 \text{ a}$		Р	<.0001	<.0001	<.0001
Sipernat <sup>®</sup> 50 S powder         300         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a           Sipernat <sup>®</sup> 50 S prašivo         400         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a           500         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a		0	0.3±0.57 b	3.3±1.15 b	19.3±3.05 b
powder         300         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a           Sipernat® 50 S         400         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a           prašivo         500         100.0±0.00 a         100.0±0.00 a         100.0±0.00 a	powder Sipernat® 50 S	200	96.0±4.00 b	100.0±0.00 a	100.0±0.00 a
<i>prašivo</i> 500 100.0±0.00 a 100.0±0.00 a 100.0±0.00 a		300	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
7 100.0 ± 0.00 a 100.0 ± 0.00 a		400	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
	prašivo	500	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
F 1790.84 21025.00 2091.57		F	1790.84	21025.00	2091.57
P <.0001 <.0001 <.0001		P	<.0001	<.0001	<.0001

<sup>\*</sup>Means in the same column within each exposition time per each treatment followed by the same letters are not significantly different (Tukey's HSD, P<0.05)

Table 2. Progeny (F1) production of *S. oryzae* 60 days after parent exposure to wheat treated with inert dusts applied as powder and suspension

Tablica 2. Potomstvo (F1) S. oryzae 60 dana nakon izlaganja roditelja tretiranoj pšenici s inertnim prašivima apliciranim kao prah i suspenzija

Treatment	Dose (ppm)	Progeny (F1) production of S. oryzae  Potomstvo (F1) S. oryzae		
Tretman	Doza (ppm)	Number of adults* (mean ± SD)  Broj imaga (prosjek ± SD)	Percentage of inhibition (%)  Postotak inhibicije (%)	
DOT	0 200	277.6±12.69 a 38.6±6.65 b	- 86.10	
powder	300	1.0±1.00 c	99.64	
DOT prašivo	400 500	0.6±0.57 c 0.0±0.00 c	99.79 100.00	
<b></b>	F P	1049.93 <.0001		
	0	338.3±40.85 a		
DOT .	200 300	49.3±24.70 b 3.0±2.00 b	85.43 99.12	
suspension DOT suspenzija	400 500	1.0±0.00 b 0.6±1.15 b	99.71 99.83	
	F P	141.35 <.0001		

<sup>\*</sup>Prosječne vrijednosti u istome stupcu pri istoj izloženosti za pojedini tretman s istim slovom nisu statistički značajno različite (Tukey's HSD, P<0,05)

	0	277.6±14.18 a	-
Celatom® Mn 51	200	80.6±4.72 b	70.97
powder	300	64.0±10.44 b	76.95
Celatom® Mn 51	400	36.3±12.09 c	86.91
prašivo	500	15.0±4.00 c	94.60
	F	336.17	
	P	<. 0001	
	0	338.3±40.85 a	-
Celatom <sup>®</sup> Mn 51	200	185.3±16.80 b	45.23
	300	83.3±10.96 c	75.38
suspension Celatom® Mn 51	400	40.6±12.66 cd	88.00
	500	15.6±5.03 d	95.39
suspenzija	F	115.70	
	P	<. 0001	
	0	277.6±14.18 a	-
Sipernat® 50 S	200	$0.0 \pm 0.00 \text{ b}$	100.00
powder	300	$0.0 \pm 0.00 \text{ b}$	100.00
Sipernat <sup>®</sup> 50 S	400	$0.0 \pm 0.00 \text{ b}$	100.00
prašivo	500	$0.0 \pm 0.00 \text{ b}$	100.00
	F	1148.82	
	P	<. 0001	

<sup>&</sup>lt;sup>a</sup>Means in the same column within each treatment followed by the same letters are not significantly different (Tukey's HSD, P<0.05)

#### Effect of inert dusts on wheat bulk density (test weight)

The results of the effect of inert dusts on bulk density reduction (test weight) indicated quite clearly that the most effective inert dusts silica gel Sipernat® 50 S reduced test weight from 5.2 (200 ppm) to 5.5 kg

hl<sup>-1</sup> (500 ppm), significantly lower than less effective dusts DE Celatom<sup>®</sup> MN 51 reducing test weight from 3.5 kg hl<sup>-1</sup> (200 ppm) to 4.3 kg hl<sup>-1</sup> (500 ppm) (Table 3). However, DOT reduced test weight for only 1.1 to 1.9 kg hl<sup>-1</sup> at 200 and 500 ppm, respectively (Table 3).

Table 3. Influence of inert dusts applied as powder on bulk density (kg hl<sup>-1</sup>) of treated wheat

Tablica 3. Utjecaj inertnih prašiva apliciranih kao prašivo na hektolitarsku masu (kg hl-1) tretirane pšenice

Treatment Tretman	Dose (ppm) Doza (ppm)	Wheat Bulk density* (kg hl-1 ± SD) Hektolitarska masa pšenice* (kg hl-1 ± SD)	Bulk density difference (kg hl-¹) Razlika hektolitarske mase (kg hl-¹)
Untreated Bez tretmana	0	76.6 ± 0.11 a	-
DOT	200	75.5 ± 0.37 b	-1.1
1001	500	74.7 ± 0.15 c	-1.9
Celatom <sup>®</sup> Mn 51	200	73.1 ± 0.25 d	-3.5
OCIALOTT IVIII 3 T	500	72.3 ± 0.40 e	-4.3
Sipernat® 50 S	200	71.1 ± 0.15 f	-5.2
olpernat 30 0	500	71.4 ± 0.36 f	-5.5
F		163.34	
P		<.0001	

<sup>\*</sup>Means in the same column followed by the same letters are not significantly different (Tukey's HSD, P<0.05)

Sipernat<sup>®</sup> 50 S had the best effectiveness against *S. oryzae*, reaching 100% mortality at 300 ppm 6 days post-treatment, followed by DE Celatom<sup>®</sup> MN 51 applied as powder, reaching maximum mortality with 500 ppm at the same exposition time, although the same rank of activity was achieved with lower doses (93.3% at 400 ppm), and at longer exposure (97.7% and 99.7%, respectively, at 300 ppm). Applied as a suspension, DE Celatom<sup>®</sup> MN 51 was slightly less effective, reaching 100% mortality at 400 ppm after 21 days post-treatment. DOT was equally effective as powder and suspension achieving 100% mortality at 300 ppm after 21 days. All tested inert dusts had significant impact on progeny

<sup>&</sup>lt;sup>a</sup>Srednje vrijednosti u istome stupcu s istim slovom nisu statistički značajno različite (Tukey's HSD, P<0,05)

<sup>\*</sup>Srednje vrijednosti u istome stupcu s istim slovom nisu značajno različite (Tukey's HSD, P<0,05)

inhibition even at the lowest dose, while Sipernat<sup>®</sup> 50 S accomplished complete progeny inhibition. In the treatment with DOT applied as a powder and suspension, the percentage of inhibition ranged from 86.1% to 100.0%, and from 85.43% to 99.83%, respectively, depending on the dose. In spite of the better initial efficacy of DE Celatom<sup>®</sup> MN 51 and efficacy of DOT after 6 and 8 days applied as powder and suspension against *S. oryzae*, an overall percentage of inhibition was lower.

Concerning the impact on quality of treated wheat with tested inert dusts, a significant effect on wheat bulk density reduction was recorded.

The active substance of silica gels, synthetic amorphous silicon dioxide, was reviewed for use in insect control (Quarles, 1992) and in Regulation (EU) n°528/2012, March 2014, Product Type Insecticides, acaricides and products to control other arthropods (PT18), considering professional uses only. This substance is acceptable and safe for application by Pest Control Operators to control insects (cockroaches). Rat  $LD_{50}$  oral is >5000 mg kg<sup>-1</sup> for various silica gels; rat  $LD_{50}^{30}$  dermal is >2000 for various silica gels as well (Assessment Report France, 2014). Liška et al. (2017) conducted experiments with silica gels Sipernat® 50 S, Aerosil® 200, several DEs and some other inert dusts in order to determine the effectiveness against S. oryzae and found out the highest effectiveness of silica gels in comparison with the effectiveness of other inert dusts in the experiment.

During the last 20 years, DE has been the subject of several review papers as well (Quarles, 1992; Golob, 1997; Korunic, 1998, 2013; Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Quarles and Winn. 1996; Nikpay, 2006; Shah and Khan, 2014; Korunić et al., 2016), with numerous references cited within each. Such comprehensive lists of hundreds of references show the great interest in the research of DE to be used as a safe grain protectant. The most extensive research with DE conducted by numerous researchers has been made in the field of the protection of stored agricultural products (Korunic, 1998, 2013; Kljajic et al., 2010). However, DE is not yet in wider use for direct mixing with grains to be placed on the market because of the great obstacles and disadvantages in use. The main obstacles are the unacceptable effect on grain flowability, the effect on bulk density or test weight shifting grains in lower grain grade, the different tolerance of insect species to DEs, the significant effect of grain moisture and temperature on the activity against insects, and the significant effect of different commodities on the effectiveness of DEs against insects (Korunic, 2016). Because of all these significant and unacceptable disadvantages in direct mixing with grains, it is quite obvious that nowadays the use of DE as a grain protectant has minimal or hardly any opportunity of being accepted by grain industry. Therefore, it is very important to continue to work on the discovery of another type of safe, effective, inert dust that has minimal

adverse effect on bulk density and grain flowability to partly replace DE in direct mixing with grains. However, DEs may have a wider application on farms to protect grains for their own use and for structural treatment in grain and the food industry (Korunic, 2016).

Disodium octaborate tetrahydrate (DOT) is a white, odourless, powdered substance containing the basic elements of boron and oxygen, and presents little or no hazard to humans. It is not flammable, combustible, or explosive and has low acute oral and dermal toxicity. Oral LD  $_{50}$  is  $\,>\,$  2000 mg kg  $^{\text{-}1}$  (rat), dermal LD  $_{50}$  is  $\,>\,$  2000 mg kg  $^{\text{-}1}$  (rat) (PPDB: Pesticide Properties DataBase, 2016). DOT possess a very good effectiveness against pests and fungus. It is most often used in pest control products that are designed to target wood destroying insects such as termites, carpenter ants, and powder post beetles. Several formulations of DOT may be found on the market: Tim-bor®, Polybor®, Bora-Care®, Borathor®, Termite Prufe® and Board Defense® usually containing about 98% of DOT. Borate compounds like DOT utilize a stop-feed mode of action. Stop-feed means that the active ingredient DOT disrupts the enzyme and digestive systems of the insect, preventing future digestion and causing death by starvation. These products are very stable and do not degrade or break down (Thurston County Health Department, 2009).

#### CONCLUSION

As a result of all these negative effects on grain quality and handling, the use of inert dusts, including DEs, is still in limited use in larger storage facilities. Based on the analysis of the significant effects on bulk density reduction and grain handling, we believe that inert dusts DE Celatom® MN 51 and Sipernat® 50 S can hardly be accepted by grain industry for mixing with grains. However, because of its acceptable effectiveness and minimal effect on bulk density reduction (test weight), inert dust DOT applied at 200 to 500 ppm is, among the three types of inert dusts, the most promising dust for grain protection when directly mixed with grains. In authors' opinion, that dust when mixed with grains may have wider use in stored grain protection.

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## LABORATORIJSKA TESTIRANJA INSEKTICIDNE UČINKOVITOSTI DINATRIJEVOG OKTABORAT TETRAHIDRATA, DIJATOMEJSKE ZEMLJE I AMORFNOGA SILIKA GELA NA *Sitophilus oryzae* (L.) TE NJIHOV UTJECAJ NA HEKTOLITARSKU MASU PŠENICE

#### SAŽETAK

Istraživali smo insekticidnu učinkovitost tri različita inertna prašiva: dinatrijev oktaborat tetrahidrat (DOT) i dijatomejsku zemlju (DZ) Celatom® Mn 51 primijenjenih kao prašivo i suspenzija i silika gel Sipernat® 50 S u obliku prašiva na Sitophilus oryzae (L.) te njihov utjecaj na smanjenje hektolitarske mase. Sipernat® 50 S bio najučinkovitije je prašivo s vrlo brzim inicijalnim djelovanjem. DZ i DOT imaju sličnu učinkovitost na S. oryzae. DOT je imao nisko inicijalno djelovanje, no nakon produženog izlaganja od 8., a osobito 21. dana, mortalitet je vrlo visok (100%), slično djelovanju DZ-a. Sličan slijed prašiva zabilježen je i kod utjecaja na smanjenje hektolitarske mase. Pri dozama od 200 i 500 ppm, najmanju razliku u hektolitarskoj masi u odnosu na hektolitarsku masu netretirane pšenice imao je DOT (-1,1 i -1,9 kg hl¹, kako slijedi), potom Celatom® MN 51 (-3,5 i -4,3 kg hl¹, kako slijedi) te Sipernat® 50 S (-5.2 i -5.5 kg hl¹, kako slijedi). Zbog takvog učinka na hektolitarsku masu pšenice, DOT pripada u skupinu s najslabijim negativnim učinkom na hektolitarsku masu i stoga je obećavajuće prašivo u suzbijanju štetnika na uskladištenoj pšenici.-

Ključne riječi: dinatrijev oktaborat tetrahidrat, dijatomejska zemlja, silika gel, pšenica, Sitophilus oryzae (L.), hektolitarska masa

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