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REGULAR ARTICLE

Efficacy of nine Croatian inert dusts against rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) on wheat

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ABSTRACT

Laboratory bioassay was carried out to study the insecticidal effect of 9 Croatian inert dusts against *Sitophilus oryzae* (L.) and to test their influence on bulk density of treated wheat. In order to compare effectiveness of Croatian inert dust samples, a standard USA diatomaceous earth (DE) Celatom® Mn 51, registered as an insecticide for stored-products protection, was used. Wheat kernels with approximately 13% moisture content were treated with inert dusts at doses 300, 400, 500 and 600 ppm and mortality of *S. oryzae* adults was assessed after 7 and 14 days and progeny after 49 days. The most effective Croatian inert dust samples were D-02B, D-01 and MA-4 with the LD_{90} values of 359.6, 447.2 and 458.7 ppm, respectively. In addition, effective dose that reduced F1 progeny in half was lower in regard to the other tested samples including the standard DE Mn 51, with the ED_{50} values 71.9, 54.6 and 137.6 ppm, respectively. According to the XRF analytical results, the highest amount of biogenic silica (BSi) was found in samples D-02B, D-01 and MA-4 (45.98, 35.09 and 21.28%, respectively). Paleontological data analysis confirmed diatoms species in only 5 samples of Croatian inert dusts (D-01, D-02B, PD-1, MA-4 and JU-1). All tested inert dusts affected reduction in bulk density of treated wheat at the LD_{90} concentrations, from 4.4 (D-02B) to 5.6 (JU-1) kg hL^{-1}. More effective inert dusts at lower doses equally reduced bulk density as less effective inert dusts at much higher doses.

Keywords: Bulk density; Diatomaceous earth; Inert dust; *Sitophilus oryzae*; Stored products

INTRODUCTION

Inert dusts include all dry powders of different origins that are chemically un-reactive in nature. Even in 1947, Watkins and Norton classified inert dusts as solvents and carriers of different substances. Eroglu et al. (2017) described these dusts as carriers of pesticides. Some inert dusts, such as clay, sand, ground phosphate, ash, diatomaceous earths were in use thousand years ago in North America and Africa. Nowadays silicates, primarily amorphous silica are in use, as well (Fields, 1999). The main use of inert dusts is in the protection of stored agricultural products and in the pest control field (Korunic, 2013). From an occupational health point of view, the USA Mine and Safety Health Administration – MSHA (Federal Mine Safety and Health Act, 2002) classified dusts by size into three primary categories: respirable dust (diameter up to 5 microns), inhalable dust (size fraction of dust which enters the body, but is trapped in the nose, throat, and upper respiratory tract) and total dust (all airborne particles, regardless of their size or composition). There are several groups of inert dusts which can be differentiated by their chemical and physical composition and with their level of activity (Banks and Fields, 1955; Maceljski and Korunic, 1972; Golob, 1997; Subramanyam and Roesli, 2000; Stadler et al., 2010). The first group are non-silica dusts which include katelsous (rock phosphate and ground sulphur), lime (calcium hydroxide), limestone (calcium carbonate) and common salt (sodium chloride). The second group; sand, kaolin, paddy husk ash, wood ash and clays constitute a group of materials which are used commonly by a small-scale farmers in the developing world as grain protectants. And the third group are diatomaceous earths (or diatomite) including zeolite, as

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well as synthetic silicates, precipitated silicas and silica gels (amorphous silica). The insecticidal effect of inert dusts against stored product insects greatly depends on their physical and chemical properties, such as an amount of amorphous silica, uniformity of particle size, oil sorption capacity, amount of clay and other impurities (Korunić, 2013), pH value (below 8.5), adherence of particles to the grain surface (Korunić, 1998). Further, if inert dusts belong to a group of diatomaceous earth (DE), besides mentioned characteristics, DE efficacy depends also on different physical and morphological properties of diatom species forming each DE (Korunić, 2013). Thus, diatoms species that have discoidal, flattened and linear body shape better cover insects cuticle in regards to diatoms of cylindrical and round shape (Korunić, 1998). Besides those characteristics whereby an insecticidal prediction could be made, there are some other conditions that greatly influence on overall inert dusts activity. The crucial factors are moisture and humidity conditions (Fields and Korunić, 2000; Korunić et al., 2016), commodity category and grain classes (Korunic, 2007; Ziaee et al., 2016), air temperature (Aldryhim, 1990; Fields and Korunić, 2000), insect species and insect stream (Wakil and Schmidt, 2015). Inert dusts, including DE have some advantages due to which they are increasingly used in stored product protection. One of the main preferences is safety of use and a low mammalian toxicity (Quarles, 1992). Nevertheless, there are some significant limitations that constrict their usage for store product protection at larger grain producers and industry. As Korunić (2013) concluded, the main limitation factor is a reduction in grain flowability and reduction in bulk density. Korunic (2016) described in details the main obstacles preventing a wider use of DEs for mixing with grain, such as health concerns, the reduction in bulk density, differences in insect species tolerance to the same DE formulation, the effects of grain moisture and temperature on the effectiveness against insects and the influence of various commodities on DE efficacy.

According to Galović et al. (2015) Croatia has several locations with DE deposits. Under the project "Development of new natural insecticide formulations based on inert dusts and botanicals to replace synthetic, conventional insecticides" financially supported by Croatian Science Foundation" we collected nine inert dusts samples from different localities in Croatia. As the main goal of the research was searching for the inert dust with acceptable effectiveness against insects with lower negative effect on grain properties, we determined the effectiveness of 9 Croatian inert dusts to control rice weevil *Sitophilus oryzae* (L.) on dusted wheat grain under controlled laboratory conditions, and to assess the effect on bulk density reduction of wheat grain.

MATERIAL AND METHODS

Inert dusts

Rock samples of nine different inert dusts were collected directly from the outcrops from three different and distant localities in Croatia; samples marked as OP-4 and OP-4A from the locality of Slavonia (Psunj), samples marked as MR-10 and MR-10B from the locality of Banovina and samples marked as D-01, D-02B, PD-1, JU-1 and MA-4 from the area of Medvednica and Žumberak. Collected samples were dried firstly at room temperature, during 10 days, and then at the temperature 40°C for 24 hours to moisture content below 5%. Grinding was carried out by the laboratory mill Retsch® Planetary Ball Mill PM 100 and sieved through a series of sieves with opening of 45μm. In order to compare effectiveness of Croatian inert dust samples, a standard USA DE Celatom® Mn 51, is used. DE Mn 51 is registered as an insecticide for stored-products protection. It belongs to a group of DE with medium to high efficacy against stored-products insects and it is used in practice in worldwide.

Physicochemical analysis

Physicochemical analyses were done by the Croatian Geological Survey. First the samples were processed (milled) in a mixer Proctor Silex 7 Blend Master and sieved through USA Standard Testing sieves No. 100 with 150 microns and No. 325 with 45 microns openings obtaining the samples with particles smaller than 45 microns. The pH values were measured in 10% slurry (ISO 10390 quality – Determination of pH). Analysis of the main elements was conducted on the sample of 15 g of each inert dust which was melted with Li2B4O7/LiBO2 (lithium tetraborate/ lithium borate) in tablets and analysed by the XRF method on the set of the main oxides.

Paleontological analysis

The standard preparation method used by the Croatian Geological Survey was applied. Approximately 1 cm^3 of the sediment was placed in a beaker and treated with 30% concentrated hydrogen peroxide (H_2O_2) to oxidize organic matter for the light microscope (LM) analyses. The sediments are then rinsed with distilled water. Standard smear slide preparation technique was used. Smear slides were examined using BH2 Olympus LM. The relative abundance of diatom species was estimated after randomly counting of 300 valves along transects under 500 x magnification (Viličić, 2003). A taxonomy of diatoms based integrating data from extant and fossil taxa developed by Hajós (1968, 1986), Hustedt (1985), Jurilj (1957), Pantocsek (1886-1905) with revised nomenclature by Galović (2009).

Test insect and commodity

A laboratory strain of *Sitophilus oryzae* (L.) susceptible to insecticides was used in experiments. Insects were reared on clean soft whole wheat kernels of different variety with approximately 13% moisture content (m.c.) under controlled conditions (28±2 °C, 65 ±5% RH, in dark). Fifty, unsexed adults (7-21 days old) were used for each treatment.

Bioassay

Glass jars of 200 mL were filled with 100 grams of clean soft wheat of different variety (approx. 13% m.c.) and determined quantity of each dust was added into each replicate. The jars were tightly closed with the lids and thoroughly shaken by hands for 30 seconds in order to have equally dusts distribution through the kernels. Jars with untreated grain served as control. After dust was settled down, 50 unsexed, 7-21 days old adults of *S. oryzae* were added into each jar. Inert dusts were tested in 3 or 4 different doses, depending on inert dust, and all treatments were conducted in 4 repetitions. Bioassay was kept under controlled conditions at 28±2 °C, 65 ±5 % RH and in dark. Mortality of adults was assessed after 7 and 14 days and progeny after 49 days.

Bulk density measurements

Inert dusts effect on the bulk density of treated wheat was valuated with the LD_{90} doses of the tested inert dusts. Bulk density, prior (served as control) and after (treatment) mixing wheat with inert dusts was measured by the GAC 2100-Agri Grain analysis computer (Dickey-john). Winter wheat, variety Maja was used, from the quality group A2 and with 13.2% m.c. All treatments and control were conducted in three replications.

Statistical analysis

Insecticidal efficiency, also bulk density 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 in 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. The LD_{50} and LD_{90} values were calculated by Probit analysis using IBM SPSS Statistics (IBM Corp. Released, 2013). The ED_{50} values related to progeny F1 reduction in half were conducted in 3 parameters logistic and 4 parameters Brain-Cousens (Brain and Cousens, 1989) models using R Core Team programme (2016).

RESULTS

Physicochemical evaluation of the Croatian inert dusts

All analysed samples of Croatian inert dusts were alkaline (Table 1). According to the measured pH values, the first group was slightly alkaline, with the pH values from 7.59 to 7.75 (MA-4, JU-1, D-01, D-02B), while the other group was moderately alkaline, with the pH values from 8.13 to 8.42 (PD-1, MR-10B, MR-10, OP-4, OP-4A). Furthermore, all samples had high amount of carbonate; from 14.45% (D-01) to 86.36% (OP-4A). Concerning tapped density, samples MA-4, D-01 and D-02B had just slightly higher values (263.1 and 250 g L^{-1}, respectively) than the standard DE Mn 51 (232.5 g L^{-1}), while other Croatian inert dusts had 2-4 times higher values of tapped density. XRF analytical results indicate that almost all samples had relatively low content of SiO_2 in regard to DE Mn 51 (Table 1). The lowest content of SiO_2 had samples OP-4A, OP-4, MR-10, MR-10B, JU-1, and PD-1. Slightly higher content hold sample MA-4, while the highest content had samples D-01 (55.58%) and D-02B (61.91%), although it was still below the content of SiO_2 at the standard DE Mn 51 (73.6%). The content of biogenic opal-A (BSi) was in the same distribution between inert dusts as the content of total silica; with the highest amount in the sample D-02B, D-01 and MA-4 (45.98, 35.09 and 21.28%, respectively). Concerning aluminium oxide, only the sample MA-4 (8.81%) had higher amount than the standard DE (7.8%). The analysed inert dusts samples contained quartz in the range from 5 (D-01 and D-02B) to

Table 1: Physical and some chemical properties of Croatian inert dusts and standard DE Mn 51

Inert dust	pH (%)[a]	Tapped density (g L^{-1})[b]	$CaCO_3$ (%)[c]	SiO_2	Al_2O_3	Opal-A[d]	Quartz
MA-4	7.59	263.1	19.82	47.71	8.81	21.28	6
JU-1	7.59	555.5	69.12	18.65	3.15	9.2	6
PD-1	8.26	476.2	53.28	29.32	5.42	13.06	6
D-01	7.63	250	14.45	55.58	6.83	35.09	5
D-02B	7.72	250	19.13	61.91	5.31	45.95	5
MR-10B	8.13	517	69.48	16.22	5.44	–0.10	6
MR-10	8.18	789	74.66	14.21	4.73	0.02	7
OP-4	8.42	681.8	86.16	7.37	2.32	0.41	9
OP-4A	8.41	833.3	86.36	3.96	1.31	0.03	9
Mn 51	7.5	232.5	-	73.6*	7.8*	50.2[e]/63-66[f]	2*

[a]pH value was measured in 10% slurry according to ISO 10390 Soil quality-Determination of pH, [b]Method by Korunić, 1997, [c]Calcium carbonate content was measured by Collins' calcimeter method, [d]calculated according to the formula Opal-A=$SiO_2 – 3×Al_2O_3$ (Boström et al., 1972), [e]according to the formula described by Boström et al., 1972, [f]according to the EP Minerals (personal communication Nyamekye, G.), *conforming to the Celatom Diatomaceous earth Functional additives, Technical data Sheet. 2010 EP Minerals. LLC. EPMO44-003 (2010)

9% (OP-4 and OP-4A). Among the other minerals, samples had on average high content of CaO (5.59-49.26%), middle content of Al_2O_3 (1.31-8.81%), and lower content of K_2O (0.09-1.43%), MgO (0.24-1.89%), Na_2O (0.16-0.48%), TiO_2 (0.07-0.41%), P_2O_5 (0.07-0.21%) and the lowest content of MnO (0.005-0.07%).

Diatoms species in Croatian inert dusts

Paleontological data analysis confirmed diatoms species in only 5 samples of Croatian inert dusts: D-01, D-02B, PD-1, MA-4 and JU-1 which were all collected from the one area Medvednica – Žumberak. The highest diversity of diatom genus and species from the order Centrales, was observed, in both D-01 and D-02B samples. The most abundant in the sample D-01 were large, rounded shapes from the *Coscinodiscus* group (65-80 microns), while in the sample D-02B, dominated its smaller forms, like *Coscinodiscus curvatulus* Grunow (25-37 microns) and also predominantly the rod shaped *Thalassionema nitzschioides* species (33-139 microns). Similar presence as in the sample D-01 and D-02B, was also observed also in the sample JU-1 with the most frequent order Pennales and *Th. nitzschioides* species. Lower diversity of diatom genus and species (order Centrales) was observed in the sample PD-1. The rod shaped *Th. nitzschioides* species (about 34 microns) were dominant with the congregation of crushed, mostly larger shapes from the *Coscinodiscus* group. The lowest diversity of diatom genus and species in sample was noted in the sample MA-4, but with some higher diversity within the order Centrales with the most prevalent species *Coscinodiscus doljensis* Pantocsek (21 – 41 microns).

Insecticidal activity of Croatian inert dusts

The efficacy of 9 Croatian inert dusts against *S. oryzae* is represented in Tables 2 and 3. Overall, the highest mortalities of *S. oryzae* adults were reached by the group of inert dusts collected from Medvednica (Table 2). Among them, samples D-01, D-02B and MA-4 achieved 100% mortality 14 days post exposure to treated wheat at 300 and 500 ppm resulting in progeny inhibition from 71% (JU-1 at 300 ppm) to 99% (D-02B at 400 ppm and PD-1 at 600 ppm) (Table 3). Inert dust samples OP-4 and OP-4A from Slavonia were less effective than the above mentioned group. The highest mortality was 98% and 97%, respectively at 600 ppm 14 days after treatment, with no statistical differences between doses. Those two samples influenced on progeny inhibition from 75% (OP-4A at 400 ppm) to 96% (OP-4 at 600 ppm) (Table 3). In general, the least effective group of inert dusts was from the locality of Banovina (samples MR-10 and MR-10B), reaching the highest mortality of *S. oryzae* adults at 600 ppm (58.7% and 95.5%, respectively), 14 days post exposure. At the same time, progeny inhibition varied from 55% (MR-10 at 400 ppm) to 78% (MR-10B at 500 ppm). In the treatment with the standard DE Mn 51, completely progeny inhibition was accomplished at 400 ppm. In addition, 100% adult mortality was reached at 600 and 400 ppm, 7 and 14 days post treatment, respectively. Based on LD_{50} and LD_{90} values and their 95% confidence limits, samples D-02B, D-01 and MA-4 were the most effective among tested Croatian inert dusts with LD_{90} values of 359.6, 447.2 and 458.7 ppm, respectively (Table 2). Although, their efficacy was lower comparing with the standard DE Mn 51 (334.1 ppm). If the effectiveness of the tested inert dusts was evaluated by the effective dose that reduces F1 progeny in half (ED_{50}) than we could claim that samples D-01, D-02B and MA-4 were more effective than Mn 51 with the ED_{50} values 54.6, 71.9 and 137.6 ppm, respectively in regard to 153.3 ppm of DE Mn 51 (Table 3).

Influence of Croatian inert dusts on bulk density

For the evaluation of the bulk density of treated wheat with inert dusts and the standard DE, a separate test was conducted with the LD_{90} values and the results were presented in the Table 4. All tested Croatian inert dusts significantly (F=133.03; df =10; p<0.0001) affected reduction in bulk density, from 4.4 (D-02B) to 5.6 (JU-1) kg hL^{-1}. When compared three the most effective Croatian inert dusts (D-02B, D-01 and MA-4) against *S. oryzae* to the standard DE Mn 51, no significant differences (F=46.03; df =10; p<0.0001) in bulk density reduction were observed, despite of higher doses of Croatian inert dusts than dose of DE Mn 51.

DISCUSSION

Considering insecticidal effect against *S. oryzae* adults, three of nine Croatian inert dusts stands out; D-02B, D-01 and MA-4 with the LD_{90} values of 359.6, 447.2 and 458.7 ppm, respectively.

In the previous research (Liška et al., 2015) sample MA-4 also showed promising results reaching mortality of 99% (14 days after wheat treatment with 500 ppm) against *Tribolium castaneum* (Herbst), one of the least sensitive species to DE (Korunic and Fields, 1995; Fields and Muir, 1996; Shah and Khan, 2014). In addition, D-02B, D-01 and MA-4 had lower effective dose that reduced F1 progeny in regard to other tested samples including the standard DE Mn 51, with the ED_{50} values 71.9, 54.6 and 137.6 ppm, respectively. That similarity in effectiveness could be partly explained with the fact that all three samples were collected from the same area (only 10 km air distance from locality of MA-4 to the locality of D-01) and that at the time of DE sedimentation the environmental conditions were very similar. However, the DE efficacy greatly depends on their physical properties (Korunić,

Table 2: Mortality of *Sitophilus oryzae* (L.) adults after 7 and 14 days of exposure to treated wheat grains with 9 Croatian inert dusts and DE Mn 51 and their LD_{50}/LD_{90} values (with 95% fiducial limits) after 7 days of exposure

Inert dust	Dose (ppm)	Mean±SD[a] mortality		Lethal doses (ppm)
		Exposition 7 days	Exposition 14 days	LD_{50}[b] LD_{90}[b]
D-01	0	0.0±0.00[c]	3.5±4.12[b]	156.5 (62.28-213.26) 447.2 (419.88-481.90)
	300	71.0±12.48[b]	100.0±0.00[a]	
	400	89.0±9.59[a]	99.5±1.00[a]	
	500	92.0±4.32[a]	100.0±0.00[a]	
	600	98.5±1.91[a]	100.0±0.00[a]	
	F	121.30	2064.08	
	P	<0.01	<0.01	
D-02B	0	0.0±0.00[c]	3.5±4.12[b]	300 ppm=82.5% 359.6 (240.55-422.56)
	300	82.5±6.19[b]	100.0±0.00[a]	
	400	93.5±2.51[a]	100.0±0.00[a]	
	500	95.5±5.25[a]	100.0±0.00[a]	
	600	98.0±2.82[a]	100.0±0.00[a]	
	F	433.61	2191.12	
	P	<0.01	<0.01	
JU-1	0	0.0±0.00[c]	3.5±4.12[c]	351.4 (324.22-373.43) 606.7 (575.90-648.08)
	300	12.5±5.74[c]	77.0±3.46[b]	
	400	75.0±16.37[b]	99.5±1.00[a]	
	500	84.0±3.65[ab]	99.5±1.00[a]	
	600	96.5±5.74[a]	99.5±1.00[a]	
	F	112.06	1080.28	
	P	<0.01	<0.01	
MA-4	0	0.0±0.00[c]	3.5±4.12[b]	232.2 (183.05-266.05) 458.7 (436.21-487.52)
	300	63.5±13.50[b]	96.5±3.00[a]	
	400	86.0±10.19[a]	99.5±1.00 [a]	
	500	97.0±6.00[a]	100.0±0.00[a]	
	600	98.5±3.00[a]	100.0±0.00[a]	
	F	101.63	1352.72	
	P	<0.01	<0.01	
MR-10	0	0.0±0.00[b]	3.5±4.12[b]	693.3 (646.91-778.77) 954.1 (847.50-1159.46)
	400	4.0±4.00[b]	63.0±13.51[a]	
	500	24.5±10.37[a]	71.0±23.12[a]	
	600	28.0±14.78[a]	85.7±6.75[a]	
	F	9.38	26.77	
	P	0.0018	<0.01	
MR-10B	0	0.0±0.00[c]	3.5±4.12[c]	588.6 (564.40-623.79) 831.1 (763.36-944.09)
	400	12.5±12.36[bc]	79.0±4.76[b]	
	500	39.5±17.23[ab]	86.5±6.60[ab]	
	600	78.5±17.07[a]	95.5±1.00[a]	
	F	11.10	339.33	
	P	0.0009	<0.01	
OP-4	0	0.0±0.00[b]	3.5±4.12[b]	421.5 (346.71-460.50) 867.7 (750.76-1161.96)
	400	49.0±6.63[a]	94.0±2.82[a]	
	500	55.7±26.00[a]	97.2±3.40[a]	
	600	71.2±3.59[a]	98.0±2.82[a]	
	F	20.67	775.68	
	P	<0.01	<0.01	
OP-4A	0	0.0±0.00[c]	3.5±4.12[b]	534.8 (517.44-555.17) 744.4 (699.14-813.40)
	400	20.5±3.41[bc]	87.0±2.002[a]	
	500	41.7±22.06[ab]	89.0±9.45[a]	

(*Contd...*)

Table 2: (*Continued*)

Inert dust	Dose (ppm)	Mean±SD[a] mortality		Lethal doses (ppm) LD_{50}[b] LD_{90}[b]
		Exposition 7 days	Exposition 14 days	
	600	65.5±13.89[b]	97.0±2.58[a]	
	F	18.3	264.3	
	P	<0.01	<0.01	
PD-1	0	0.0±0.00[d]	3.5±4.12[c]	362.8 (314.80-391.50) 726.4 (668.39-816.94)
	300	16.0±8.64[c]	84.0±9.09[b]	
	400	68.5±5.25[b]	98.5±1.91[a]	
	500	72.2±11.11[b]	98.0±2.82[a]	
	600	93.0±4.16[a]	99.5±1.00[a]	
	F	130.37	305.35	
	P	<0.01	<0.01	
Mn 51	0	0.0±0.00[c]	3.5±4.12[b]	300 ppm=83.5% 334.1 (186.64-399.00)
	300	83.5±8.06[b]	99.0±1.15[a]	
	400	94.5±6.19[a]	100.0±0.00[a]	
	500	95.0±3.82[a]	100.0±0.00[a]	
	600	100.0±0.00[a]	100.0±0.00[a]	
	F	300.92	2021.45	
	P	<0.01	<0.01	

[a]Means in the same column within each inert dust followed by the same letters are not significantly different (Tukey's HSD, $P<0.05$), [b]LD_{50} and LD_{90} expressed as parts per million (ppm), Confidence limits (CL) are given in parentheses

Table 3: Progeny (F1) production of *Sitophilus oryzae* (L.) 49 days after parent exposure to wheat treated with 9 Croatian inert dusts and DE Mn 51 and their effective dose (ED_{50})

Inert dust	Dose (ppm)	Number of adults Mean±SD[a]	Percentage of inhibition (%)	Effective dose (ppm) ED_{50}[b]±SE
D-01	0	611.5±94.50[a]	(-)	54.6±49.3
	300	44.0±18.42[b]	(93)	
	400	16.5±12.06[b]	(97)	
	500	20.7±25.70[b]	(96)	
	600	20.0±10.09[b]	(97)	
	F	135.26		
	P	<0.01		
D-02B	0	611.5±94.50[a]	(-)	71.9±62.8
	300	33.2±18.39[b]	(95)	
	400	4.0±3.82[b]	(99)	
	500	15.0±13.11[b]	(97)	
	600	32.5±11.26[b]	(95)	
	F	145.76		
	P	<0.01		
JU-1	0	611.5±94.50[a]	(-)	237.6±54.7
	300	176.2±50.36[b]	(71)	
	400	3.7±1.70[d]	(99)	
	500	33.00±32.01[cd]	(95)	
	600	114.7±18.40[bc]	(81)	
	F	94.60		
	P	<0.01		
MA-4	0	611.5±94.50[a]	(-)	137.6±22.6
	300	72.2±18.62[b]	(88)	
	400	31.0±5.29[b]	(95)	
	500	12.7±2.50[b]	(98)	
	600	26.0±11.04[b]	(96)	
	F	141.71		
	P	<0.01		
MR-10	0	611.5±94.50[a]	(-)	333.1±62

(*Contd...*)

Table 3: (*Continued*)

Inert dust	Dose (ppm)	Number of adults Mean±SD[a]	Percentage of inhibition (%)	Effective dose (ppm) ED_{50}[b]±SE
	400	274.5±81.96[b]	(55)	
	500	171.5±46.14[b]	(72)	
	600	190.0±48.33[b]	(69)	
	F	33.34		
	P	<0.01		
MR-10B	0	611.5±94.50[a]	(-)	346.2±57.6
	400	182.5±29.22[b]	(70)	
	500	131.5±37.33[b]	(78)	
	600	156.2±48.47[b]	(74)	
	F	61.62		
	P	<0.01		
OP-4	0	611.5±94.50[a]	(-)	168.6±67.5
	400	74.5±12.87[b]	(88)	
	500	67.2±44.28[b]	(89)	
	600	22.7±11.76[b]	(96)	
	F	111.59		
	P	<0.01		
OP-4A	0	611.5±94.50[a]	(-)	346.2±57.6
	400	153.0±54.57[b]	(75)	
	500	123.0±72.64[b]	(80)	
	600	65.2±36.07[b]	(89)	
	F	54.71		
	P	<0.01		
PD-1	0	611.5±94.50[a]	(-)	218.3±21.9
	300	169.2±77.12[b]	(72)	
	400	80.0±5.35[bc]	(87)	
	500	67.2±24.17[bc]	(90)	
	600	8.5±8.34[b]	(99)	
	F	76.52		
	P	<0.01		
Mn 51	0	611.5±94.50[a]	(-)	153.3±40.2
	300	35.5±14.61[b] (94%)	(94)	
	400	0.0±0.00[b] (100%)	(100)	
	500	21.0±24.04[b]	(97)	
	600	28.2±11.32[b]	(95)	
	F	141.84		
	P	<0.01		

[a]Means in the same column followed by the same letters are not significantly different (Tukey's HSD, $P<0.05$), [b]ED_{50} Effective dose (ppm) that reduces F1 progeny in half

1997, 1998). When compared the most effective Croatian inert dusts with the standard DE Mn 51 in this study, a similar physical properties (pH values, tapped density) and chemical composition (total SiO_2) was noticed. In general, DE with the lowest tapped density, 300 g L^{-1} or less are also the most efficacious (Korunić, 1997). Further, the amount of total SiO_2, one of the main factor that determinate insecticidal effect, was the highest at the most effective Croatian inert dusts (61.9, 55.5 and 47.7% at D-02B, D-01 and MA-4, respectively). The primary biogenic phase of silica is Opal-A, but its presence and amounts cannot be easily detected in X-ray diffractograms (Horvat and Mišič, 2004). It is also possible that with time amorphous opaline silica passes into crystalline quartz, depending on the temperature and the depths of sediments (Hein et al., 1985). Consequently, a rough estimation of Opal-A (BSi) content could be calculated by the equation according to the method described by Boström et al. (1972). It is based on the assumption that all silicon that surpasses the amount of aluminium by a factor of three is biogenic. That method confirmed the presence of opal in 8 tested Croatian inert dusts, although the samples OP-4, OP-4A and MR-10 had the content of only 0.41, 0.03 and 0.02%, respectively. Considering, that in those samples none of the diatomaceous species was determined by the paleontological analysis, it is assumed that low opal content was detected because of the rare appearance of some sponge spikules.

Table 4: Wheat bulk density (kg hL^{-1}) of Croatian inert dusts treated with LD_{90} values and bulk density differences in regard to bulk density of untreated wheat

Treatment	LD_{90} dose (ppm)	Wheat bulk density[a] (kg hL^{-1}±SD)	Bulk density difference (kg hL^{-1})
Untreated	0	79.6±0.15[a]	-
D-01	447	74.4±0.05[cd]	–5.2
D-02B	360	75.2±0.35[b]	–4.4
MA-4	459	74.3±0.20[cd]	–5.3
OP-4	868	74.7±0.20[bc]	–4.9
OP-4A	744	74.9±0.23[bc]	–4.7
MR-10B	831	74.5±0.17[cd]	–5.1
MR-10	954	74.6±0.20[bcd]	–5.0
PD-1	727	74.3±0.34[cd]	–5.3
JU-1	607	74.0±0.15[d]	–5.6
Mn 51	334	74.5±0.30[bcd]	–5.0

[a]Means in the same column followed by the same letters are not significantly different (Tukey's HSD, $P<0.05$)

Testing the impact of the geochemical composition of diatomaceous earth on its insecticidal activity against adults of *S. oryzae,* Rojht et al. (2010) determined that silica in the form of amorphous silica or Opal-A significantly correlated with efficacy in the bioassay against *S. oryzae*. Although, Croatian samples D-02B, D-01 and MA-4 had lower content of total SiO_2 than the standard DE Mn 51, they showed an excellent efficacy against *S. oryzae* adults (100% mortality at 300 and 500 ppm 14 days post exposure). Besides the SiO_2 content, other major mineral oxides are also relevant for a DE insecticidal value (Saez and Fuentes Mora, 2007; Rojht et al., 2010). Concerning health risk, an amount of crystalline silica should be considered. Although, DE contain 60 to 0.1% crystalline silica depending on source, DE registered as an insecticides contain moreover less than 7% crystalline silica (Fields, 1999). Our results showed that quartz content was in the range from 5 to 9% depending on the sample, where the lowest content of quartz (5 and 6%) had the most effective samples (D-02B, D-01 and MA-4, respectively). Diatoms species determined in Croatian inert dusts belong to marine diatoms with different forms and shapes. Linear diatom species with flat cell walls (like Coscinodiscus group – scattered in samples PD-1 and D-01 and abundant in samples D-02B, MA-4 and JU-1 and *Th. nitzschioides* Grunow – abundant in samples D-01 and JU-1 and dominant in samples PD-1, D-02B and MA-4), or broken skeleton better cover insect cuticula then diatom species with round or cylindrical shape (like *Paralia sulcata* (Ehrenberg) Cleve – scattered in samples D-01, D-02B, MA-4, PD-1 and JU-1), so their ability to absorb insects cuticular wax could be higher which increases water loss and accelerate insect desiccation. Furthermore, not only the body shape but also its size is highly relevant for the activity. In the sample D-02B that had the highest insect activity, the more frequently were smaller diatom species, like *C. curvatulus* (33-37 μm) and predominantly *Th. nitzschioides* (33-139 μm). While in the sample D-01, the less effective sample than previous one, the most frequent were large species from the Coscinodiscus group (65-80 μm). Presumably, the differences in insect activity between inert dust samples could be partly due to the differences in species, exactly their shapes and size. Ziaee et al. (2013) stated that each diatom species could have its own mode of action, concerning that wherever the number and size of pores and distribution of striae per unit area of species is greater, the insecticidal activity would be greater, since openings in diatom cell wall, which is composed of silicon dioxide, allowing the contact with its surrounding environment (Smol and Stoermer, 2010). Despite the fact that all 9 tested Croatian inert dusts showed efficacy against *S. oryzae* adults and interfered with progeny suppression, the main limiting factor of DE usage in stored products protection at bigger grain producers, which is bulk density reduction, should be pointed out. According to our test of LD_{90} influence on bulk reduction, we can conclude that much lower doses of effective inert dusts (D-02B, D-01, MA-4 and Mn 51) reduced bulk density as equally as much higher doses of less effective inert dusts (OP-4, OP-4A, MR-10, MR-10B, PD-1 and JU-1). Among three Croatian inert dusts that showed the highest insecticidal efficacy, only the sample D-02B caused significant lower reduction in bulk density of treated wheat (with 360 ppm). Assumingly, a higher insecticidal efficacy was induced by the higher amount of Opal-A content in the sample D-02B. That characteristic of sample D-02B is really promising since the bulk density is a broadly used grading factor and the fact that mixing DE with grain adversely affects some physical and mechanical properties of a bulk commodity (Korunić et al., 1998a). Many authors (Johnson and Kozak, 1966; Korunić, 1997; Freo et al., 2014) already described more precisely changes in wheat properties after treatment with DE: flowability and bulk density are reduced, residues of dust visible on the grain, further moisture readings taken when using a dielectric moisture metre are affected, difficulty in grain handling since there could be excessive amount of dusts, and after all changes of physical and chemical characteristics of wheat grain have also consequent reduction of the flour technological quality.

CONCLUSIONS

The highest mortality of *S. oryzae* adults was reached by the group of inert dusts collected from Medvednica, where samples D-01, D-02B and MA-4 achieved 100% mortality 14 days post exposure to treated wheat at 300 and 500 ppm. In addition, those samples had lower effective dose that reduced F1 progeny in regard to other tested samples including the standard DE Mn 51 (ED_{50} values

71.9, 54.6 and 137.6 ppm, respectively). Diatoms species determined in Croatian inert dusts belong to marine diatoms with different forms and shapes. Amount on quartz ranged from 5 to 9% depending on the sample, where the lowest content of quartz (5 and 6%) had the most effective samples (D-02B, D-01 and MA-4, respectively). Concerning influence on bulk reduction, much lower doses of effective inert dusts (D-02B, D-01, MA-4 and Mn 51) reduced bulk density as equally as much higher doses of less effective inert dusts (OP-4, OP-4A, MR-10, MR-10B, PD-1 and JU-1). Considering high insecticidal efficacy, it is evident that Croatia has promising deposits of inert dusts (samples D-01, D-02B and MA-4) which could be useful in IPM strategy of protecting stored wheat against rice weevil. Although, field testing is necessary, in order to evaluate impact of the environmental conditions on inert dust efficacy and also to test the sensitivity of other stored products species to selected inert dust samples.

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Author contribution

A. L. and P. L. conducted experiment. A. L. analysed data and wrote the manuscript. Z. K. designed trial and wrote the manuscript. V. R. edited the manuscript. J. H. and I. G. collected and analysed inert dust samples. P. L. and R. B. obtained references. All authors read and approved manuscript.

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