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The potential of Croatian diatomaceous earths as grain protectant against three stored-product insects

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Abstract: The objective of this study was to determine the potential insecticide efficacy of several Croatian diatomite samples (as inert dusts) against *Sitophilus oryzae* (Linnaeus), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (Fabricius) adults and to compare their efficacy with the efficacy of the USA standard diatomaceous earth (DE) Celatom Mn 51. DE Mn 51 belongs to a group of DE with medium to increased efficacy for stored agricultural products insects. Six Croatian samples from three different locations (MA-4, MR-10, MR-10B, OP-4, OP-4A and PD-1) were selected and prepared for testing with fractions of particles ≤ 45 µm and applied at 500 mg/kg. Insect mortality was recorded after 7 and 14 days. The highest lethal effect after 7 and 14 days showed Croatian sample MA-4 with 97% and 100% mortality, respectively, for *S. oryzae*, 61.5% and 99% mortality, respectively, for *T. castaneum* and 26.5% and 34%, respectively, for *R. dominica*. In all treatments the efficacy of MA-4 was in the same range with the efficacy of the standard DE Mn 51. The other five tested samples had significant lower efficacy regarding to MA-4, although after 14 days mortality was 85.7-98% for *S. oryzae*, 5-47% for *T. castaneum* and 2-5% for *R. dominica* depending on a different DE sample. These results show that Croatia has potential diatomite deposits with good and promising efficiency for tested insects.

Key words: Croatian diatomites, *Sitophilus oryzae*, *Tribolium castaneum*, *Rhyzopertha dominica*, efficacy

Introduction

Confronted with increasing problems of stored cereals protection with synthetic pesticides (pests resistance, harmful impact on health and environment, pesticides residues), there is a growing requisite for safer methods in stored-pests control. Application of one of the inert dusts, diatomaceous earth (DE) is one of the most promising alternatives to contact insecticides (Shah & Khan, 2014).

Diatomaceous earth is remarkable stable, without any toxic residues and does not react with substances from the environment. According to EPA (Environmental Protection Agency USA) natural DE, characterised as amorphous silicon dioxide, is classified as food additive (GRAS Generally Recognized as Safe (Anon., 1991). However, besides that, and other positive DE characteristics (minimal toxicity for mammals, physical mode of action, long-term effectiveness) (Subramanyam & Roesli, 2000; Korunić, 2014) there are many reasons why DE, or other inert dusts, are still not in use for stored products protection in practice. One of the major defect is decreasing quality of treated bulk commodity in a terms of bulk density and grain flowability reduction (Korunić *et al.*, 1998; 2011). The percentage of test weight reduction is even greater in mealy wheat grain than in high vitreous wheat grain, treated with natural zeolite, on the other hand, inert dusts have some positive effects on treated wheat; like significant improvement of rheological parameters: moisture absorption and flour (dough) energy (Bodroža-Solarov *et al*., 2012).

DE efficacy against stored-pests significantly depends, also on geological origin of DE (Korunić, 1997), wherein a huge influence has a geochemical structure of DE; among which the most relevant factor is the amount of silicon dioxide, SiO_2 (Shah & Khan, 2014). Accept geochemical composition, insecticidal efficacy depends on other DE properties, like pH value, porosity an active surface, diatoms species, tapped density (Korunić, 1997; 1998, Athanassiou *et al*., 2011). The effect of particle size is also highly relevant for the effectiveness, where smaller particles (< 45 µm) are significantly more effective than larger particles (Korunić *et al*., 2011).

In order to decrease a negative effect of DE to treated commodity, discovering DE with very high effectiveness for stored-products insects, is crucial. The best known diatomite deposits in Croatia are related to Sarmatian sediments from Medvednica Mountain. Apart from that locality, diatomite samples were collected from the Banovina and Slavonia locations.

The aim was to investigate a potential insecticidal activity of Croatian deposits against three stored-product insects rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae), lesser grain borer (*Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) and red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). In order to compare effectiveness, a standard USA DE Celatom® Mn 51, is used, which is registered as an insecticide for stored-products protection and belongs to a group of DE with medium to high efficacy against stored-products insects.

Material and methods

Samples preparation

Rock samples were collected directly from the ground deposits from three different and distant localities; 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 PD-1 and MA-4 from the area of Hrvatsko Zagorje (locality Medvednica – Žumberak). Collected samples were dried firstly at room temperature, during 10 days, following at temperature 40 °C during 24 hours.

After drying, samples were grind with laboratory mill Tube Mill control IKA and sieved through a series of sieves with opening of 45 µm. Only one sample fraction with particles smaller than 45 µm was used. In order to compare effectiveness of collected 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 some world countries. As well, a tapped density (method described by Korunić, 1997) has been determined of collected samples.

Test insects and commodity

Test insects were bred on hard wheat with approximately 13% moisture content (m.c.) (rice weevil *Sitophilus oryzae* L. and lesser grain borer *Rhyzopertha dominica* F.) and on white flour with 5% brewer's un-activated yeast (red flour beetle *Tribolium castaneum* Herbst), all under controlled circumstances, at 30 ± 2 °C and 70 ± 5% RH. Clean, un-infested wheat grain with 12.6% (m.c.), 27.6 °C and 80.2 kg/hl was used in the experiment. Whole kernel wheat for rice weevil and lesser grain borer and wheat with 1% cracked wheat for red flour beetle.

Bioassay

Glass jars of 200 ml were filled with 100 grams of wheat and determined concentrations of 500 mg/kg (500 ppm) of six Croatian dust samples were added. 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 each insect species were added into each jar. All treatments were conducted in 4 repetitions. Mortality of adults was assessed after 7 and 14 days and progeny after 49 days for *S. oryzae* and *R. dominica* and after 63 days for *T. castaneum.*

Data analysis

Insecticidal efficiency 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.

Results and discussion

After seven days of adult exposure to treated wheat with samples of dust, very good results were obtained with sample marked as MA-4 against *S. oryzae*, reaching mortality of 97% which was slightly higher than mortality in the treatment with standard Mn 51 (72.25%) (Table 1). The same rank of effectiveness, 7 days post treatment also had s sample PD-1 (72.25%).

Table 1. Efficacy of different Croatian dust samples and DE Mn 51 against *Sitophilus oryzae* adults and progeny after 7, 14 and 49 days of the exposure to treated wheat grain with 500 ppm.

Treatment	***Sitophilus oryzae* mortality* (%) ± std.dev.**		**Number of adults (progeny) ± SD**
	7 days	**14 days**	
Mn 51	94.50 ± 6.19 a	100.00 ± 0.00 a	7.00 ± 13.34 d
MA-4	97.00 ± 6.00 a	100.00 ± 0.00 a	2.75 ± 2.87 d
MR-10	24.50 ± 10.37 dc	85.75 ± 6.75 c	188.25 ± 47.38 b
MR-10B	39.50 ± 17.23 bc	86.50 ± 6.60 bc	128.00 ± 37.41 cb
OP-4	55.75 ± 26.00 bc	97.25 ± 3.40 bac	64.00 ± 43.66 cd
OP-4A	41.75 ± 22.06 bc	89.00 ± 9.45 bac	68.50 ± 41.00 cd
PD-1	72.25 ± 11.11 ba	98.00 ± 2.82 ba	58.00 ± 18.77 cd
Control	0.00 ± 0.00 d	3.50 ± 4.12 d	609.50 ± 94.29 a

* Means in the same column followed by the same letters are not significantly different $P < 0.05$

Fourteen days post treatment, the maximum efficacy was achieved with MA-4 sample as well as with standard Mn 51 also the same effectiveness ranking had samples OP-4, OP-4A and PD-1 (97.25%, 89.00%, respectively 98%). Significant reduction of the progeny of *S. oryzae* was achieved in all treatments ranging from 69.11% (MR-10) to 99.5% (MA-4).

Efficacy of Croatian dust samples against *T. castaneum,* was lower than against *S. oryzae* (Table 2), although 7 and 14 days post treatment very good results were obtained with sample MA-4 (61.50%, respectively 99%) and they were not statistical differ from standard DE Mn 51 (78%, respectively 99.5%). Remained Croatian dust samples had significantly lower efficacy even after 14 days, with the highest mortality achieved with samples OP-4A and PD-1 (47, respectively 46.5%).

Table 2. Efficacy of different Croatian dust samples and DE Mn 51 against *Tribolium castaneum* adults and progeny after 7, 14 and 63 days of the exposure to treated grain with 500 ppm.

Treatment	***Tribolium castaneum* mortality (%) ± std.dev.**		**Number of adults (progeny) ± SD**
	7 days	**14 days**	
Mn 51	78.00 ± 12.54 a	99.50 ± 1.00 a	0.00 ± 0.00 c
MA-4	61.50 ± 18.06 a	99.00 ± 2.00 a	1.25 ± 0.50 c
MR-10	4.50 ± 6.60 b	9.00 ± 10.51 c	35.50 ± 28.44 b
MR-10B	1.00 ± 1.15 b	5.00 ± 2.58 c	35.75 ± 14.40 b
OP-4	3.50 ± 3.00 b	14.00 ± 16.08 c	24.50 ± 13.17 cb
OP-4A	2.00 ± 1.63 b	47.00 ± 4.76 b	10.75 ± 3.40 cb
PD-1	3.50 ± 2.51 b	46.50 ± 22.11 b	20.50 ± 5.74 cb
Control	0.00 ± 0.00 b	0.00 ± 0.00 c	69.50 ± 14.57 a

* Means in the same column followed by the same letters are not significantly different $P < 0.05$

The lowest insecticidal efficacy of Croatian dust samples and of DE Mn 51, was recorded against *R. dominica* (Table 3). After 7 days of adult exposure to treated wheat, only the sample MA-4 and DE Mn 51 were significant different from the control without treatment, however they are not acceptable concerning to low adult mortality (26.5%, respectively 29.75%). Even after 14 days post treatment, mortality of *R. dominica* was below 50%.

Further, all samples reached significant progeny reduction and it was in the range from 22.32% (dust sample MR-10) to 93.72% (DE Mn 51), in addition, there was no statistical differences between dust sample MA-4 and DE Mn 51.

According to the results of tapped density, it is evident that there are huge differences between analysed dust samples. Only sample MA-4 has tapped density slightly higher than density of DE Mn 51 (263.1 g/l, respectively 232.5 g/l), while other Croatian samples have much more higher tapped density (PD-1 476.2 g/l; MR-10B 517 g/l; OP-4 681.8 g/l; MR-10 789 g/l; OP-4A 833.3 g/l).

Table 3. Efficacy of different Croatian dust samples and DE Mn 51 against *Rhyzopertha dominica* adults and progeny after 7, 14 and 63 days of the exposure to treated grain with 500 ppm

Treatment	***Rhyzopertha dominica* mortality (%) ± std.dev.**		**Number of adults (progeny) ± SD**
	7 days	**14 days**	
Mn 51	29.75 ± 11.52 a	42.50 ± 11.09 a	95.75 ± 31.03 d
MA-4	26.50 ± 8.38 a	34.00 ± 10.95 a	132.75 ± 28.14 d
MR-10	1.50 ± 1.91 b	2.00 ± 1.63 b	1185.50 ± 224.42 b
MR-10B	3.00 ± 2.00 b	5.00 ± 3.46 b	979.00 ± 82.04 cb
OP-4	2.00 ± 4.00 b	3.50 ± 5.74 b	1015.50 ± 97.12 cb
OP-4A	2.50 ± 2.51 b	3.50 ± 1.91 b	1179.50 ± 269.48 b
PD-1	2.00 ± 2.82 b	2.00 ± 2.82 b	838.25 ± 72.21 c
Control	0.00 ± 0.00 b	0.00 ± 0.00 b	1526.25 ± 40.01 a

[*] Means in the same column followed by the same letters are not significantly different $P < 0.05$

Among all six tested dust samples of Croatian deposits, by its insecticidal efficacy only sample MA-4 stands out, generated higher mortality rate which was not statistical different with the effectiveness of DE Mn 51. The highest MA-4 efficacy at dose of 500 ppm was achieved after 7 and 14 days against *S. oryzae* (94.5%, respectively 100%), some lower against *T. castaneum* (78%, respectively 99.5%), and the lowest efficacy against *R. dominica* (29.75%, respectively 42.5%). That efficacy level of the sample MA-4 was partly expected, due to a similar value of tapped density with standard DE Mn 51 (263.1 g/l, respectively 232.5 g/l).

Analysing 36 different DEs or formulations, Korunić (1997) determined positive correlation between tapped density and insecticidal activity, and concluded that the most efficacious DEs have, amongst other characteristics, also the lowest tapped density (300 g/l or less). A tolerance order among tested insect species in the most treatments was: *R. dominica* > *T. castaneum* > *S. oryzae.* Some other papers also confirm that *S. oryzae* is among the most susceptible species in the treatments with diatomaceous earth (Athanassiou *et al*., 2007; Korunić *et al.*, 2011; Kljajić *et al*., 2011). Variation in insect sensibility on inert dusts, in general, could be partly explained with a different amount of dust adhering to their cuticle. Thus, Fields & Korunić (2000) observed that the most sensible species *Cryptolestes ferrugineus* (Steph.) had more DE attached to its cuticle then *T. castaneum*, the least sensible species.

All results considered, it can be concluded that the most promising dust samples of Croatian deposits are sample MA-4 and partly sample PD-1, both from the Medvednica Mountain. Sarmatian deposits from that area were described as the best known diatomites in Croatia (Galović, 1997).

Meanwhile, a geochemical analysis of Croatian diatomite's are in progress in order to, determine, among others, also amorphous silica (hydrated silicon dioxide), one of the key factor of diatomaceous earths efficacy. Further it is relevant to analyse a crystalline silica amount, which should not exceed exposure limit (according to National Institute for Occupational Safety and Health, NIOSH 2002, exposure limit is 0.05 mg/m^3). Also, a paleontological analyses of collected samples are ongoing, to extract diatom assemblages, their diversity and representation of individual species, with their morphometrics. After all, we could be able to predict a quality of Croatian diatomites as a potential source of grain protection against stored-product insects.

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