Insecticidal Potential of Natural Zeolite and Diatomaceous Earth Formulations Against Rice Weevil (Coleoptera: Curculionidae) and Red Flour Beetle (Coleoptera: Tenebrionidae)

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ABSTRACT Insecticidal potential of natural zeolites and diatomaceous earths originating from Serbia against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) was evaluated. Two natural zeolite formulations (NZ and NZ Modified) were applied to wheat at rates of 0.50, 0.75, and 1.0 g/kg, while two diatomaceous earth (DE) formulations (DE S-1 and DE S-2) were applied at rates of 0.25, 0.50, 0.75, and 1.0 g/kg. A bioassay was conducted under laboratory conditions: temperature of 24 ± 1°C, relative humidity in the range 50–55%, in tests with natural zeolites, and 60–65%, in tests with DEs, and in all combinations for progeny production. Mortality was assessed after 7, 14, and 21 d of insect contact with treated wheat, and the total mortality after an additional 7-d recovery on untreated broken wheat. Progeny production was also assessed after 8 wk for *S. oryzae* and 12 wk for *T. castaneum.* The highest mortality for *S. oryzae* and *T. castaneum* was found after the longest exposure period and 7 d of recovery, on wheat treated with NZ at the highest rate and DEs at rates of 0.50–1.0 g/kg. Progeny reduction higher than 90% was achieved after 14 and 21 d of contact of both beetle pests with wheat treated with DE S-1 at 0.50–1.0 g/kg and DE S-2 at 0.75–1.0 g/kg, while the same level of reduction was achieved only for *T. castaneum* after its contact with the highest rate of NZ formulation. NZ Modified, applied even at the highest rate, revealed much lower insecticidal potential.

KEY WORDS *Sitophilus oryzae*, *Tribolium castaneum*, natural zeolite, diatomaceous earth, insecticidal potential

The rice weevil, *Sitophilus oryzae* (L.), as a primary pest, and the red flour beetle, *Tribolium castaneum* (Herbst), as a secondary pest, can cause a great damage in stored wheat (Rees 2004). The use of inert dusts in stored-product protection from insect pests is in some cases a promising alternative to the use of contact insecticides. Inert dusts have low mammalian toxicity and do not affect significantly the quality of treated products if applied at low rates (Korunić et al. 1996, 1998). The efficacy of inert dusts and especially of diatomaceous earth (DE) formulations may differ depending on insect species (Korunić 1997, Korunić et al., 1998; Fields and Korunić 2000), silicon dioxide content and structure (crystalline or amorphous), particle size distribution (Korunić 1998, Subramanyam and Roesli 2000, Vayias et al. 2009), as well as on air and substrate moisture content, temperature, and duration of insect exposure to the formulations used (Fields and Korunić 2000; Arthur 2001, 2002; Fields et al. 2003; Athanassiou et al. 2005; Kavallieratos et al. 2005; Vardeman et al. 2006). Geographical origin of DE may affect its insecticidal activity as well (Vayias et al. 2009).

Despite the use of various commercial inert dust products in current stored-product pest management programs, detection of new deposits of natural inert dusts and evaluation of their insecticidal activity have attracted significant attention. Previous studies revealed that the area of South-Eastern Europe (Slovenia, Croatia, Greece, Macedonia, and Serbia) has very rich deposits of DE, which effectiveness against stored-product pests is similar to those DE based formulations registered worldwide (Korunić 1997, Korunić et al. 2009, Vayias et al. 2009, Athanassiou et al. 2011). Contrary to DEs, composed mainly of amorphous silica (Golob 1997, Korunić 1998), zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earths (Christidis et al. 2003, Sprynskyy et al. 2005). Having physical properties similar to those of other inert dusts, zeolites, together with DEs, belong to the fourth group of dusts containing natural silica (Subramanyam and Roesli 2000). They have been widely used as highly sorptive dusts in agriculture, primarily as feed additives for domestic animal be-

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cause of effective mycotoxin absorption (Reháková et al. 2004). The data on the effectiveness of natural zeolites against stored product insect pests are scarce. Haryadi et al. (1994) have shown that natural zeolite can be effective in controlling maize weevil *Sitophilus zeamais* (Motsch), but the rates applied in this study were very high (≥50 g/kg of maize). Kljajić et al. (2010) have also found high effectiveness of natural zeolite originating from Serbia, applied however at much lower rates (0.75 g/kg) and low relative humidity (r.h.) (40–45% r.h.), against three stored-product beetle pests in treated wheat.

Because silica does not accumulate in mammals and DE dust can be removed almost completely (>99%) in the grain cleaning procedure before milling (Desmarchelier and Allen 1999), the risk to consumer health is therefore reduced to the lowest possible level. During DE application, workers' safety can be endangered by DE dust inhalation. The amount of respirable dust (particle size <7 μm) and the content of crystalline silica forms (quartz and cristobalite as the most dangerous carcinogenic) are recognized as the most important features (Desmarchelier and Allen 1999). As the application of natural zeolites in stored-product protection has not been thoroughly investigated, the same health aspects as for DE inert dusts should be considered.

The aim of this study was to assess and compare the insecticidal potentials of two natural zeolite based dusts and two DE based dusts from Serbia, applied in a wide rate range (0.25–1 g/kg), under laboratory simulated humid storage conditions (24 ± 1°C and 50–65% r.h.) and for different exposure intervals, against adult *S. oryzae* and *T. castaneum* and their progeny production.

Materials and Methods

Test Insects. The testing was carried out using laboratory populations of *S. oryzae* and *T. castaneum*, reared in an insectary, and using the procedures described by Harein and Soderstrom (1966), and Davis and Bry (1985). *S. oryzae* were reared in 2.5 liters glass jars containing whole-grain soft wheat with moisture content below 12%, and *T. castaneum* on white wheat flour with 5% of active dry yeast. Air temperature in the insectary was 26 ± 1°C, and r.h. 60 ± 5%.

Inert Dusts. The inert dusts used in the tests were raw natural materials originating from Serbia: natural zeolites (NZ and NZ Modified, location Vranje) and DEs (DE S-1 and DE S-2, location Kolubara). To obtain NZ inert dust sample, natural zeolite was processed in the following steps: separation of nonspecific impurities (soil, rocks of different kinds, and other impurities), drying, primary crushing (jaw crusher), secondary crushing (impact crusher), and grinding (Denver ball mill; Denver Industrial Machinery, Denver, CO). Further, the NZ material was modified by NH_4^+ ion to get an NZ Modified inert dust sample. Diatomite ore processing included ore breaking up (by hand) and then grinding (under laboratory conditions) performed in two steps using a turbo mill (Retch, Germany). First step yielded coarse grains and the second fine powder (at a mill-operating rate of 10,000 rev/min).

The content of Al_2O_3, CaO, Fe_2O_3, MgO, Na_2O, K_2O, and TiO_2 was determined by atomic absorption spectrometry (AAnalyst 300, PerkinElmer, USA), while the determination of SiO_2 content was done using standard gravimetric procedure. Chemical composition of NZ was as follows: 68% SiO_2, 14% Al_2O_3, 4.5% CaO, 2.5% Fe_2O_3, and up to 1.5% of MgO, Na_2O, and K_2O. After the treatment with NH_4^+ ions, the composition of NZ Modified was: 63% SiO_2, 11% Al_2O_3, 2.5% CaO, 0.8% Fe_2O_3, and up to 1.5% of MgO, Na_2O, and K_2O. Contents of SiO_2, Al_2O_3, K_2O, Na_2O, MgO, CaO, Fe_2O_3, and TiO_2 were: 78.8 and 63.2%, 9.4 and 10.3%, 0.8 and 0.9%, 0.1 and 0.1%, 0.1 and 0.3%, 0.6 and 1.0%, 1.1 and 1.7%, and 0.2 and 0.3%, in the DE S-1 and the DE S-2 sample, respectively.

Particle size distribution (inert dust samples suspended in water) in the range from 0 to lower than 63 μm (0–13, 13–18, 18–28, 28–40, 40–53, and 53–63 μm) was determined using a Cyclosizer (model M4; Weir, Tormorden, United Kingdom). The percentage of particles with diameter below 13 μm, considered as the most important size fraction with respect to the inert dust insecticidal activity, was 49.0, 51.9, 95.3, and 81.0% for NZ, NZ Modified, DE S-1, and DE S-2, respectively.

Particle size distribution (PSD) in the 0.6 nm to 6 μm range (fraction of particles below 13 μm in diameter) was measured using a Zeta-sizer Nano ZS (Malvern, UK) with a 633 He-Ne laser at 173 degree backscattering angle. Aqueous suspension (20 ml) of each sorptive dust was made using highly purified deionized water and sonicated for 5 min before each size measurement run. PSD determination, based on scattered light intensity, was performed in 10 consecutive measurements (10 × 1 ml) for each sorptive dust aqueous suspension. Based on high polydispersity index (PdI) values obtained, indicating high sorptive dust suspension polydispersity, the most intense peak in the PSD was used as relevant for particle size determination. Diameters of particles represented by the most intense peak, each obtained as the average of 10 runs, and the peak intensity values (in brackets) were 422 (77.7%), 942 (82.9%), 640 (89.6%), and 747 (83.6%) nm for NZ, NZ Modified, DE S-1, and DE S-2, respectively.

Bioassay. The wheat cultivar (Takovčanka), with grain moisture content of 11.5 ± 0.3% determined by a Dickey–John moisture meter (Dickey–John Mini GAC Dickey–John Co., USA) was used in the tests.

The testing was carried out under laboratory conditions according to modified methods (OEPP/EPPO 2004a,b) and procedures described by Collins (1990) and Chanbang et al. (2007). Zeolite based inert dusts were applied at rates 0.50, 0.75, and 1.00 g/kg of wheat, and DE dusts at rates 0.25, 0.50, 0.75, and 1.00 g/kg of wheat.

For each tested insect species and dust rate applied, 1 kg lots of whole-grain wheat were used. Lots of 1 kg of untreated wheat served as controls. To produce progeny, in tests with *T. castaneum* before applying the

Table 1. ANOVA parameters for main effects and associated interactions for initial and total mortality levels of *S. oryzae* and *T. castaneum* in wheat treated with NZs (total df = 54)

Main effects	df	*S. oryzae*				*T. castaneum*			
		Initial mortality		Total mortality		Initial mortality		Total mortality	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Dusts	1	378.6	<0.0001	307.5	<0.0001	720.7	<0.0001	655.2	<0.0001
Rates	2	71.1	<0.0001	69.6	<0.0001	103.0	<0.0001	112.8	<0.0001
Exposure	2	176.6	<0.0001	79.4	<0.0001	279.1	<0.0001	236.3	<0.0001
Dust × rates	2	3.2	0.04645	3.1	0.05473	28.2	<0.0001	21.7	<0.0001
Dusts × exposure	2	29.6	<0.0001	2.2	0.12393	103.1	<0.0001	72.9	<0.0001
Rates × exposure	4	1.9	0.13090	1.4	0.23205	9.7	<0.0001	6.6	0.00021
Dust × rates × exposure	4	1.7	0.15584	1.2	0.30886	1.8	0.13680	2.2	0.07767

dusts to wheat, 1% of broken grains were added, including control samples. After applying the dusts, the vessel contents were hand mixed and then mixed on a rotary mixer for 10 min. The same procedure was applied to control samples.

The next day, for each exposure period (7, 14, and 21 d), four samples (50 g each) of treated wheat were placed in 200 ml plastic vessels, and 25 adults of *S. oryzae* and *T. castaneum* were introduced separately into each vessel. The same procedure was applied to untreated wheat. Insect mortality was determined after 7, 14, and 21 d of contact with treated wheat. The total mortality was assessed for each exposure period after additional 7 d of recovery on untreated broken wheat grains.

The effects of dusts on insect progeny production were evaluated as follows: after counting dead adults, wheat was sieved to remove all insects (dead and alive), and then the wheat containers were covered with cotton cloth and fixed with rubber bands. Progeny production was determined by counting live insects in treated and control wheat grains sieved after 8 wk for *S. oryzae* and 12 wk for *T. castaenum* (the total number includes larvae, pupae, and adults).

The bioassay was conducted under laboratory conditions: 24 ± 1°C and 50–55% r.h., parents exposed to wheat treated with natural zeolites, or 60–65% r.h., parents exposed to wheat treated with DEs, and in all variants in the case of progeny production.

Data Analysis. The acquired mortality data are given as percentages. Only the mortality data for *S. oryzae* after its 21-d exposure to wheat treated with NZs and 7-d recovery were corrected by using Abbot's formula (Abbot 1925). The data were analyzed separately for each species and each NZ and DE formulation, using three-way analysis of variance (ANOVA) procedure with insect mortality and total mortality as response variables and dust formulation, dose rate, and exposure interval as main effects. The same procedure was followed for the analysis of progeny production data, where the mean number of individuals was the response variable while dust formulation, applied rate, and exposure interval were the main effects. The significance of mean differences was determined by Fisher least significant difference (LSD) test at $P < 0.05$ (Sokal and Rohlf 1995). Progeny production is shown as a mean number of newly hatched individuals, while the reduction in progeny number against the control is shown as a percentage (IR %) according to the formula used in a similar study by Tapondjou et al. (2002).

Results

NZ Dusts. *Insect Mortality.* All main effects and associated interactions for mortality levels of *S. oryzae* on wheat treated with NZs after exposure were significant except for interactions: rates × exposure and NZ dusts × rates × exposure, which were not significant at the $P < 0.05$ level. All main effects and associated interactions for mortality levels of *T. castaneum* on wheat treated with NZs after exposure were significant except for the interaction NZ dusts × rates x exposure, which was not significant at the $P < 0.05$ level (Table 1). After the 7-d exposure, the mortality of *S. oryzae* and *T. castaneum* adults on wheat treated with NZ and NZ Modified zeolites (Table 2) was very low, 2–35 and 0–23%, respectively. However, after 14 and 21 d of contact with the highest NZ application rates, the highest mortality of adult *S. oryzae* of 88 and 96% and *T. castaenum* of 90 and 100%, respectively, was achieved, while in the case of NZ Modified after the

Table 2. Mortality of *S. oryzae* and *T. castaneum* adults after 7, 14, and 21 d of exposure to wheat treated with natural zeolites (NZ and NZ Modified)

Natural zeolite	Rate (g/kg)	Mortality (% ± SD) after exposure		
		7 d	14 d	21 d
S. oryzae				
Control	0	0.0 ± 0.0a[a]	1.0 ± 0.5a	2.0 ± 0.6a
NZ	0.50	17.0 ± 1.5b	54.0 ± 1.3d	58.0 ± 1.9c
	0.75	31.0 ± 1.5c	79.0 ± 1.7e	84.0 ± 2.9d
	1.00	35.0 ± 2.7c	88.0 ± 1.2f	96.0 ± 1.2e
NZ Modified	0.50	2.0 ± 0.6a	17.0 ± 2.2b	26.0 ± 1.7b
	0.75	14.0 ± 0.6b	40.0 ± 3.2c	34.0 ± 1.3b
	1.00	22.0 ± 1.3b	36.0 ± 1.2c	45.0 ± 2.6bc
T. castaneum				
Control	0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
NZ	0.50	0.0 ± 0.0a	38.0 ± 3.7b	51.0 ± 2.2d
	0.75	9.0 ± 1.5b	76.0 ± 1.4c	89.0 ± 1.7e
	1.00	23.0 ± 1.3c	90.0 ± 1.4d	100f
NZ Modified	0.50	0.0 ± 0.0a	3.0 ± 0.5a	8.0 ± 0.8ab
	0.75	0.0 ± 0.0a	9.0 ± 1.5a	17.0 ± 1.3bc
	1.00	0.0 ± 0.0a	26.0 ± 3.9b	28.0 ± 1.6c

[a] For each species, means within columns followed by the same letter are not significantly different; Fisher's LSD test, $P < 0.05$.

Table 3. Mortality of *S. oryzae* and *T. castaneum* adults after 7, 14, and 21 d of exposure to wheat treated with natural zeolites (NZ and NZ Modified) and 7 d of recovery on untreated wheat

Natural zeolite	Rate (g/kg)	Mortality (% ± SD) after exposure and 7 d of recovery		
		7 d	14 d	21 d
S. oryzae				
Control	0	0.0 ± 0.0a[a]	1.0 ± 0.5a	5.0 ± 1.3a
NZ	0.50	39.0 ± 3.3c	62.0 ± 1.3d	62.1 ± 1.4d
	0.75	58.0 ± 3.1d	86.0 ± 0.6e	88.4 ± 2.4e
	1.00	66.0 ± 1.7d	92.0 ± 1.8f	100f
NZ Modified	0.50	9.0 ± 1.3ab	23.0 ± 1.7b	31.6 ± 1.3b
	0.75	20.0 ± 3.4bc	53.0 ± 2.5cd	46.3 ± 1.5c
	1.00	33.0 ± 2.9c	42.0 ± 3.0c	55.8 ± 2.4cd
T. castaneum				
Control	0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
NZ	0.50	4.0 ± 0.8a	44.0 ± 2.6c	57.0 ± 2.4d
	0.75	16.0 ± 1.6b	89.0 ± 2.2d	89.0 ± 1.9e
	1.00	36.0 ± 2.7c	97.0 ± 1.0d	100f
NZ Modified	0.50	2.0 ± 1.0a	7.0 ± 0.5a	11.0 ± 0.5ab
	0.75	2.0 ± 0.6a	15.0 ± 2.1ab	24.0 ± 0.8bc
	1.00	8.0 ± 1.4ab	35.0 ± 4.2bc	36.0 ± 0.8c

[a] For each species, means within columns followed by the same letter are not significantly different; Fisher's LSD test, $P < 0.05$.

Table 5. Mortality of *S. oryzae* and *T. castaneum* adults after 7, 14, and 21 d of exposure to wheat treated with diatomaceous earths (DE S-1 and DE S-2)

Diatomaceous earth	Rate (g/kg)	Mortality (% ± SD) after exposure		
		7 d	14 d	21 d
S. oryzae				
Control	0	0.0 ± 0.0a[a]	1.0 ± 0.5a	3.00 ± 1.0a
DE S-1	0.25	13.0 ± 1.9ab	48.0 ± 2.9b	90.0 ± 1.7c
	0.50	39.0 ± 2.7c	88.0 ± 1.4cd	99.0 ± 0.5d
	0.75	72.0 ± 1.8e	96.0 ± 1.3ef	100d
	1.00	86.0 ± 1.3f	99.0 ± 0.5f	100d
DE S-2	0.25	7.0 ± 1.3a	18.0 ± 0.6a	52.0 ± 3.6b
	0.50	21.0 ± 1.7b	76.0 ± 2.5c	95.0 ± 0.5c
	0.75	51.0 ± 2.5cd	91.0 ± 1.7de	99.0 ± 0.5d
	1.00	55.0 ± 2.7d	97.0 ± 1.0ef	98.0 ± 1.0d
T. castaneum				
Control	0	0.0 ± 0.0a	0.0 ± 0.0a	2.0 ± 0.6a
DE S-1	0.25	2.0 ± 0.6a	43.0 ± 1.5b	91.0 ± 2.2c
	0.50	16.0 ± 1.4bc	83.0 ± 3.3d	100d
	0.75	47.0 ± 2.6d	94.0 ± 1.3de	100d
	1.00	71.0 ± 1.3e	100f	100d
DE S-2	0.25	2.0 ± 0.6a	10.0 ± 1.7a	41.0 ± 3.3b
	0.50	6.0 ± 1.0ab	67.0 ± 2.7c	91.0 ± 1.7c
	0.75	22.0 ± 1.3c	89.0 ± 0.5de	100d
	1.00	39.0 ± 4.1d	96.0 ± 0.8e	100d

[a] For each species, means within columns followed by the same letter are not significantly different; Fisher's LSD test, $P < 0.05$.

same exposure intervals it was 36 and 45% for *S. oryzae* and 26 and 28% for *T. castaenum.*

Total Mortality (After Recovery). All main effects for total mortality levels of *S. oryzae* were significant, but all associated interactions were not significant at the $P < 0.05$ level. All main effects and associated interactions for total mortality levels of *T. castaneum* were significant with the exception of interaction NZ dusts × rates × exposure, which was not significant at the $P < 0.05$ level (Table 1). After 7 d of recovery on untreated wheat in all test combinations, the mortality of both exposed insect species increased (Table 3), the highest increase found after the 7-d exposure to NZ (1 g/kg) range from 35 to 66% for *S. oryzae* and from 23 to 36% for *T. castaneum*, so that the total mortality of both insect species of 100% was reached after 21 d of exposure. At the same time, the lowest total mortality of *S. oryzae* and *T. castaneum*, detected on wheat treated with NZ Modified applied at a rate of 0.5 g/kg, was 31.6 and 11.0%, respectively.

DE Dusts. *Insect Mortality.* All main effects and associated interactions for mortality levels of both insect species on wheat treated with DEs after exposure were significant except for the interaction DE dusts × exposure, which was not significant at the $P < 0.05$ level (Table 4). After 7 d of exposure to wheat treated with DEs (Table 5), 86 and 55% of *S. oryzae* and 71 and 39% of *T. castaenum* adults were dead in contact with DE S-1 and DE S-2, applied at the highest dose rates, respectively. After 14 d of exposure, the mortality of both test insect species in contact with DE S-1 (0.75 and 1 g/kg) and DE S-2 (1 g/kg) was over 95%. After 21 d of exposure, high mortality of both test species (90–100%) was recorded on wheat treated with DE S-1 at all four dose rates and DE S-2 at rates over 0.5 g/kg.

Total Mortality (After Recovery). All main effects and associated interactions for total mortality levels of both insect species were significant except for *T. castaneum* and the interaction DE dusts × exposure, which was not significant at the $P < 0.05$ level (Table 4). After the 7-d recovery, the mortality of both test insect species in all combinations increased (Table 6). The most significant increase in mortality ranging from 48 to 75% and 18–36% for *S. oryzae*, and 43–65% and 10–28% for *T. castaenum* was observed after 14-d

Table 4. ANOVA parameters for main effects and associated interactions for initial and total mortality levels of *S. oryzae* and *T. castaneum* in wheat treated with DEs (total df = 72)

Main effects	df	*S. oryzae*				*T. castaneum*			
		Initial mortality		Total mortality		Initial mortality		Total mortality	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Dusts	1	95.7	<0.0001	73.2	<0.0001	112.6	<0.0001	49.7	<0.0001
Rates	3	258.4	<0.0001	177.1	<0.0001	256.3	<0.0001	159.5	<0.0001
Exposure	2	398.5	<0.0001	387.3	<0.0001	715.6	<0.0001	371.1	<0.0001
Dust × rates	3	6.2	0.0008	5.9	0.0011	8.2	<0.0001	7.7	<0.0001
Dusts × exposure	2	2.9	0.05926	8.4	0.0005	0.2	0.7851	0.4	0.0683
Rates × exposure	6	20.5	<0.0001	26.1	<0.0001	22.2	<0.0001	20.1	<0.0001
Dust × rates × exposure	6	8.7	<0.0001	7.9	<0.0001	15.1	<0.0001	5.5	<0.0001

Table 6. Mortality of *S. oryzae* and *T. castaneum* adults after 7, 14, and 21 d of exposure to wheat treated with diatomaceous earths (DE S-1 and DE S-2) and 7 d of recovery on untreated wheat

Diatomaceous earth	Rate (g/kg)	Mortality (% ± SD) after exposure and 7 d of recovery		
		7 d	14 d	21 d
S. oryzae				
Control	0	0.0 ± 0.0a[a]	1.0 ± 0.5a	3.0 ± 1.0a
DE S-1	0.25	16.0 ± 2.7ab	75.0 ± 1.9c	95.0 ± 1.5c
	0.50	55.0 ± 2.6c	94.0 ± 0.6e	100d
	0.75	78.0 ± 0.6e	100f	100d
	1.00	90.0 ± 1.7f	100f	100d
DE S-2	0.25	11.0 ± 1.0ab	36.0 ± 1.8b	76.0 ± 3.9b
	0.50	25.0 ± 2.4b	84.0 ± 2.2d	99.0 ± 0.5cd
	0.75	56.0 ± 4.2cd	100f	100d
	1.00	71.0 ± 1.0de	100f	100d
T. castaneum				
Control	0	0.0 ± 0.0a	0.0 ± 0.0a	2.0 ± 0.6a
DE S-1	0.25	6.0 ± 1.3a	65.0 ± 1.7c	95.0 ± 0.5c
	0.50	36.0 ± 2.9b	90.0 ± 2.4de	100d
	0.75	61.0 ± 2.7c	94.0 ± 1.3de	100d
	1.00	84.0 ± 1.4d	100f	100d
DE S-2	0.25	2.0 ± 0.6a	28.0 ± 3.4b	60.0 ± 5.0b
	0.50	13.0 ± 2.6a	88.0 ± 1.6d	91.0 ± 1.7c
	0.75	47.0 ± 2.6b	92.0 ± 1.4de	100d
	1.00	69.0 ± 3.8c	98.0 ± 0.6ef	100d

[a] For each species, means within columns followed by the same letter are not significantly different; Fisher's LSD test, $P < 0.05$.

exposure of insects to wheat treated with DE S-1 and DE S-2 at 0.25 g/kg. Total mortality at the level of 100% was recorded after the recovery of *S. oryzae* and *T. castaneum*, previously exposed to wheat treated with DE S-1 at 0.5–1 g/kg, and DE S-2 at 0.75–1 g/kg for 21 d, while the lowest mortality of insects on wheat treated with DE S-2 dose rate 0.25 g/kg was 76.0% for *S. oryzae* and 60.0% for *T. castaneum*.

Progeny Production Inhibition. *NZ Dusts.* All main effects for progeny production of *S. oryzae* on wheat treated with NZs were significant at the $P < 0.05$ level, but all associated interactions were not significant at $P < 0.05$. NZ dusts and rates as main effects and associated interaction NZ dusts × exposure were significant at the $P < 0.05$ level for progeny production of *T. castaneum* on wheat treated with NZs. Exposure as the main effect and associated interactions: NZ dusts × rates, rates × exposure, and NZ dusts × rates × exposure, were not significant at $P < 0.05$ level (Table 7). In all test combinations and for both tested species, the emergence of progeny on wheat treated with natural zeolite was observed. According to the data on progeny production after the contact of both test insect species with wheat treated with natural zeolites (Table 8), the lowest mean progeny number and the highest percentage of progeny inhibition (given in brackets) are 32.7 (65.8%) and 19.3 (81.4%) and 16.0 (75.5%) and 9.0 (88.2%) for *S. oryzae* and *T. castaneum* achieved after 7- and 14-d exposure to wheat treated with NZ 1 g/kg, respectively, while 47.7 (50.1%) and 48.5 (53.3%) and 33.7 (48.1%) and 31.0 (59.4%), respectively, were found after their contact with wheat treated with NZ Modified 1 g/kg. After 21 d of contact, the lowest mean progeny number and the highest percentage of progeny inhibition for *S. oryzae* and *T. castaneum*, also achieved at the highest NZ rates applied, were 20.5 (81.8%) and 3.7 (96.5%), and for NZ Modified, 43.0 (62.0%) and 30.3 (71.3%), respectively. At the same time, the highest mean progeny number and the lowest percentage of progeny inhibition of 59.5 (47.5%) for *S. oryzae* and 53.0 (49.8%) for *T. castaneum* were recorded on wheat treated with NZ Modified 0.5 g/kg.

DE Dusts. All main effects as well as their associated interactions for progeny production of both insect species on wheat treated with DEs were significant at $P < 0.05$ with the exception of interaction DE dusts × rates × exposure (Table 9). In all test combinations for both tested insect species the emergence of offspring in wheat treated with DE was observed except for *T. castaneum* on wheat treated with DE S-1 at 0.75 and 1.00 g/kg after 14 and 21 d of parent exposure. The data on progeny production on wheat treated with DEs (Table 10) revealed that the progeny inhibition for both test species after 7-d exposure to wheat treated with DE S-1 dose rate of 1 g/kg and also after 14- and 21-d contact with 0.75 and 1 g/kg of the same dust was higher than 95%. The lowest inhibition of progeny production for *S. oryzae* and *T. castaneum*, on wheat treated with DE S-2 at 0.25 g/kg, was found to be 40.2 and 55.3%, after 7-d exposure, and 61.6 and 76.4%, after 21-d exposure, respectively. The data on progeny production on wheat treated with DEs (Table 6) revealed that lowest mean progeny number ranging from 0 to 13 and progeny inhibition higher than 95% for both test species was found after 7-d exposure to wheat treated with DE S-1 dose rate of 1 g/kg and also after 14- and 21-d contact with 0.75 and 1 g/kg of the same dust. The highest mean progeny number and the lowest percentage of progeny inhibition for *S. oryzae* and *T. castaneum*, on wheat treated with DE S-2 at 0.25 g/kg, were found to be 85.7 (40.2%) and 24.5 (55.3%), after 7-d exposure, and 142.3 (61.6%) and 21.5 (76.4%), after 21-d exposure, respectively.

Table 7. ANOVA parameters for main effects and associated interactions for progeny production of *S. oryzae* and *T. castaneum* in wheat treated with NZs (total df = 54)

Main effects	df	*S. oryzae*		*T. castaneum*	
		F	*P*	*F*	*P*
Dusts	1	93.0	<0.0001	211.5	<0.0001
Rates	2	20.7	<0.0001	28.3	<0.0001
Exposure	2	4.2	0.01959	3.1	0.05430
Dust × rates	2	0.2	0.84720	0.4	0.64071
Dusts × exposure	2	0.3	0.71440	9.0	0.00042
Rates × exposure	4	0.1	0.98593	0.5	0.71656
Dust × rates × exposure	4	0.7	0.60493	0.7	0.61461

Discussion

The results presented in this study confirm previous general conclusions that: 1) the species of the genus *Sitophilus* are more sensitive to DEs than those of *Tribolium*, especially after short-time exposures (Korunić 1997, Fields and Korunić 2000, Athanassiou et al. 2005, 2007) and also to natural zeolites (Kljajić et al. 2010); 2) the mortality of stored-product insects

Table 8. Progeny production of *S. oryzae* and *T. castaneum* after 7, 14, and 21 d of parent exposure to wheat treated with natural zeolites (NZ and NZ Modified)

Natural zeolite	Rate (g/kg)	Average no. progeny individuals (± SD) and inhibition rate (%) after exposure periods					
		7 d		14 d		21 d	
		Avg. no.	IR	Avg. no.	IR	Avg. no.	IR
S. oryzae							
Control	0	95.7 ± 6.4a[a]	—	104.0 ± 18.1a	—	113.5 ± 24.6a	—
NZ	0.50	46.0 ± 8.6c	51.9	40.7 ± 5.9cd	60.7	41.7 ± 7.8b	63.1
	0.75	35.5 ± 5.5d	62.9	30.7 ± 3.0d	70.3	23.3 ± 12.2c	79.4
	1.00	32.7 ± 6.8d	65.8	19.3 ± 9.1e	81.4	20.5 ± 10.7c	81.8
NZ Modified	0.50	66.3 ± 13.1b	30.8	62.7 ± 7.7b	39.6	59.5 ± 10.1b	47.5
	0.75	57.7 ± 8.3bc	39.7	49.5 ± 11.7bc	52.3	51.5 ± 8.7b	54.6
	1.00	47.7 ± 6.0c	50.1	48.5 ± 19.7bcd	53.3	43.0 ± 2.9b	62.0
T. castaneum							
Control	0	65.0 ± 15.6a	—	76.2 ± 16.8a	—	105.5 ± 8.4a	—
NZ	0.50	30.5 ± 7.4bc	53.1	19.3 ± 2.6d	74.8	18.7 ± 7.1c	82.2
	0.75	23.3 ± 2.5cd	64.3	11.0 ± 2.6e	85.6	7.7 ± 1.5d	92.7
	1.00	16.0 ± 5.8d	75.5	9.0 ± 2.8e	88.2	3.7 ± 2.6e	96.5
NZ Modified	0.50	43.0 ± 14.3ab	33.9	50.3 ± 2.4b	34.1	53.0 ± 5.8b	49.8
	0.75	36.7 ± 14.2bc	43.5	39.3 ± 6.7bc	48.5	41.0 ± 6.4b	61.1
	1.00	33.7 ± 6.4bc	48.1	31.0 ± 10.0c	59.4	30.3 ± 7.3bc	71.3

[a] For each species, means within columns followed by the same letter are not significantly different; Fisher's LSD test, $P < 0.05$.

increases significantly with increased duration of their exposure to wheat treated with DEs (Fields et al. 2003, Athanassiou et al. 2005); 3) the total mortality of insects exposed to wheat treated with different inert dusts, including natural zeolites, after recovery, also increases (Arthur 2001; Collins and Cook 2006a, 2006b; Kljajić et al. 2010); 4) the dose rates used are relatively high, or at least similar to those of the registered DEs; 5) the efficacy of DE based inert dusts depends primarily on the silicium dioxide content and particle size distribution, so that the highest efficacy is achieved with DE dusts of uniform particle size distribution with a mean particle size of about or below 10 μm, higher percentage of particles with diameters below 12 μm, and high amorphous SiO_2 content (Korunić 1997, 1998); and 6) DE based inert dusts, with amorphous silica structure, are more effective than natural zeolite dusts as crystalline hydrated aluminosilicates (Kljajić et al. 2010), where zeolites have some specific disadvantages because of the presence of more crystaline ingredients or smaller particles.

On closer inspection of the results, different comparisons, based on the influence of various parameters (silica structure, particle size distribution, assay conditions, insect species) on the insecticidal activity of used inert dusts, can be made. As found, the mortality of insects after 21 d of exposure to wheat treated with 0.75 g/kg of NZ, NZ Modified, DE S-1, and DE S-2, and 7 d of recovery on untreated wheat, was 88.4, 46.3, 100, and 99% for *S. oryzae*, and 89, 24, 100, and 100% for *T. castaneum*, respectively, while the total mortality of *S. oryzae* and *T. castaneum* determined by Kljajić et al. (2010) applying even 5.0 and 2.5 times lower rates of Protect-It (a commercial DE formulation) was 100%. Higher efficacy of Protect- It, compared with DE-based inert dusts used in this study, can be explained by the composition of Protect-It, containing 10% of silica gel that greatly enhanced the DE effectiveness (Korunić and Fields 1995), as well as by higher amorphous SiO_2 content present in the commercial product (83.7%) than that in DE S-1 and DE S-2 (78.8 and 63.2%). Athanassiou et al. (2011) have also found higher efficacy of SilicoSec (a commercial DE formulation) compared with DE formulations originating from Central and South-Eastern Europe. However, comparing NZ and NZ Modified with Protect- It, it can be concluded that considerably lower total mortality values obtained applying NZ and NZ Modified formulations are the consequence of their crystalline structure, in contrast to amorphous SiO_2 structure of the DE commercial product.

In addition, it can be concluded, comparing NZ and NZ Modified insecticidal activities, that the occupation of some active surface sites by ammonium ions and smaller free surface remained for sorption of insect epicuticular lipid molecules can be the reason for lower modified formulation effectiveness, as it has already been explained by Kljajić et al. (2010). Differences in efficacy, obtained for the DE-based inert dusts used, can likely be explained by different size fractions of particles with diameter up to 13 μm (95.33% for DE S-1 and 81.03% for DE S-2), considered as one of the most important size fractions with respect to the inert dust insecticidal activity. In addition, Vayias et al. (2009) have found, while testing the

Table 9. ANOVA parameters for main effects and associated interactions for progeny production of *S. oryzae* and *T. castaneum* in wheat treated with DEs (total df = 72)

Main effects	df	*S. oryzae*		*T. castaneum*	
		F	*P*	*F*	*P*
Dusts	1	138.9	<0.0001	100.8	<0.0001
Rates	3	327.5	<0.0001	100.0	<0.0001
Exposure	2	24.2	<0.0001	17.0	<0.0001
Dust × rates	3	19.0	<0.0001	4.3	0.0077
Dusts × exposure	2	7.4	0.0012	10.3	0.0001
Rates × exposure	6	5.8	<0.0001	4.9	0.0003
Dust × rates × exposure	6	0.8	0.581	1.8	0.105

Table 10. Progeny production of *S. oryzae* and *T. castaneum* after 7, 14, and 21 d of parent exposure to wheat treated with diatomaceous earths (DE S-1 and DE S-2)

Diatomaceous earth	Rate (g/kg)	Average no. of progeny individuals (± SD) and inhibition rate (%) after exposure periods					
		7 d		14 d		21 d	
		Avg. no.	IR	Avg. no.	IR	Avg. no.	IR
S. oryzae							
Control	0	143.5 ± 29.8a[a]	—	350.2 ± 51.3a	—	370.5 ± 75.7a	—
DE S-1	0.25	52.5 ± 12.4c	63.4	73.3 ± 9.4bc	78.9	78.0 ± 14.0c	79.0
	0.50	22.3 ± 3.1d	84.9	25.3 ± 9.4d	92.1	24.0 ± 4.1d	96.0
	0.75	10.3 ± 2.7ef	92.8	12.7 ± 4.1ef	96.1	8.7 ± 5.0e	97.7
	1.00	7.0 ± 2.0g	95.1	7.3 ± 1.9f	97.7	9.0 ± 4.1e	97.6
DE S-2	0.25	85.7 ± 6.1b	40.2	114.7 ± 12.9b	67.1	142.3 ± 25.7b	61.6
	0.50	42.3 ± 11.9c	70.5	57.0 ± 5.9c	83.5	55.7 ± 19.8c	85.0
	0.75	7.5 ± 1.3fg	94.8	22.0 ± 3.7d	93.5	30.0 ± 8.8d	91.9
	1.00	11.5 ± 2.6e	92.0	21.3 ± 8.3de	93.7	22.7 ± 11.6d	93.9
T. castaneum							
Control	0	54.7 ± 12.0a	—	83.7 ± 17.6a	—	91.0 ± 14.8a	—
DE S-1	0.25	18.7 ± 4.3bc	65.8	19.0 ± 4.2b	77.2	10.5 ± 2.5cd	87.4
	0.50	14.7 ± 3.1bc	73.1	5.5 ± 3.5c	95.4	6.5 ± 3.1de	92.8
	0.75	8.3 ± 2.9de	85.0	0.0 ± 0.0d	100	0.0 ± 0.0f	100
	1.00	1.8 ± 1.7f	96.9	0.0 ± 0.0d	100	0.0 ± 0.0f	100
DE S-2	0.25	24.5 ± 7.9b	55.3	40.0 ± 12.3b	52.1	21.5 ± 5.6b	76.4
	0.50	21.7 ± 5.3b	60.3	28.7 ± 3.5b	65.6	13.3 ± 3.6bc	85.4
	0.75	11.5 ± 2.4cd	79.1	8.5 ± 6.2c	89.7	7.3 ± 2.9de	92.3
	1.00	7.0 ± 4.2e	87.3	7.0 ± 2.2c	91.5	4.5 ± 4.4e	95.0

[a] For each species, means within columns followed by the same letter are not significantly different; Fisher's LSD test, $P < 0.05$.

efficacy of DE formulations from South-Eastern Europe, that smaller particles are more effective against three stored-product Coleopteras, although significant differences in efficacy between the fractions consisting of particles 0–150 μm in size and those of <45 μm were not always observed.

Additionally, higher total insect mortality achieved using DE inert dusts than that applying NZ formulations is obvious, both different SiO_2 structures and granulometric composition fractions of particles with diameter up to 13 μm (95.3 and 81.0% for DE S-1 and DE S-2; 49.0 and 51.9% for NZ and NZ Modified) can be notified as possible reasons. Furthermore, the efficacy of NZ and NZ Modified against adult *S. oryzae* and *T. castaneum* in this bioassay was significantly lower than the efficacy of similar NZ formulations, particularly after a short exposure, assessed by Kljajić et al. (2010) (e.g., the efficacy of dust applied at a rate of 0.75 g/kg against *S. oryzae* after 14 d of exposure is 1.3 and 2.3 times lower, and 1.3 and 10.3 times lower against *T. castaneum*, for NZ and NZ Modified, respectively). As the r.h. in the bioassay performed in this study was somewhat higher (50–55% r.h. during exposure of parents and 60–65% r.h. for progeny) than that (45 ± 5% r.h.) in the entire experiment run by Kljajić et al. (2010), it is therefore considered to be most likely the cause of this reduced efficacy. In addition, different NZ formulations and wheat type used in this study might be the reason of lower efficacy.

Subramanyam and Roesli (2000) have pointed out that the effects the inert dusts produce against storage-product insects, dependent not only on applied rates and exposure interval but also on the type and origin of dusts, define the insecticidal potential of the dusts. Accordingly, the experimental results obtained show that the inert dusts originating from Serbia exhibit a significant insecticidal potential, because for both DE based dusts, and also for NZ dust, after 21-d exposure of parents, the inhibition rate (IR) for both insect species studied is in the range 90–100%.

These investigations confirmed previous findings of significant insecticidal potential of inert dusts originating from the area of South-Eastern Europe, as well as that the region of Serbia is rich in inert dust deposits, which could be commercially used in future. However, compared with currently available commercial DE formulations, the effectiveness of studied DEs is lower and demands their further improvement (the way of preparation, mixing with other components with higher insecticidal potential, etc.) or finding new deposits on the same or other locations, which will be more effective when applied at lower rates.

Finally, it can be concluded that DEs applied at rates 0.75–1 g/kg and NZ applied at a rate of 1 g/kg showed high insecticidal potential against *S. oryzae* and *T. castaneum*. Further research is needed to test the efficacy of these dusts at applied rates in real, stored-product conditions as well as their influence on treated wheat quality. In addition, all parameters related to the health aspect of inert dust use should be carefully considered. As in this study particles smaller than 1 μm were detected in all tested samples, it is necessary to determine, before the use of these dusts in practical conditions, not only the crystalline silica content but also the amount of respirable dust particles. By getting the results of all these investigations only, it would be possible to acquire a complete insight on the insecticidal potential of NZ and DE formulations as inert dusts and

their role in improving current stored-product pest management programs.

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