Laboratory evaluation of insecticidal effectiveness of a natural zeolite formulation against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) in treated wheat

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Abstract

Inert dusts are increasingly becoming an integral part of programs for protection of cereal grains from stored-product insects. The intention in this study was therefore to conduct preliminary tests of insecticidal potentials of the natural zeolite formulation Minazel SP (66% SiO_2, particle size ≤ 50 µm) originating from Serbia in controlling *S. oryzae*, *R. dominica* and *T. castaneum*. Dust effectiveness was tested in the laboratory (24±1ºC and 50-55% r.h. for parents and 24±1ºC and 60±5% r.h. for F_1 progeny) by exposing insects to wheat treated with 0.50, 0.75 and 1.00 g/kg of Minazel SP. Mortality was determined after 7, 14 and 21 days of insect contact with threated wheat, and total mortality after an additional 7 days of recovery on untreated broken wheat. Progeny production in F_1 generation was also determined for each insect species after 8-12 weeks. After seven days of exposure and 7 days of recovery of all tested species, the highest efficacy of 62% was observed after the highest application rate of 1.00 g/kg against *S. oryzae*. The highest efficacy after 14 and 21 days was achieved with the same application rate against *T. castaneum* (100%), *S. oryzae* (96-98%) and *R. dominica* (70-82%). Progeny reduction (IR – inhibition rate) of all tested species depended on the duration of parents exposure to treated wheat. After 7 days of exposure progeny reduction rates were 49-67% for *S. oryzae,* 42-68% for *R. dominica* and 47-78% for *T. castaneum.* After 14 days of exposure, inhibition rates were 55-78% for *S. oryzae,* 72-81% for *R. dominica* and 53-90% for *T. castaneum,* while progeny reductions of *S. oryzae* were 51-85%, *R. dominica* 80-96% and *T. castaneum* 87-99% after 21 days of exposure.

Keywords: Wheat grain, *Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum*, Natural zeolite

1. Introduction

Modern protection of stored-products is seeking to apply some of nontoxic materials such as inert dusts which are increasingly becoming an integral part of programs for control of insect pests. Products based on diaromaceous earths (DEs) are highly effective even at low application rates, while other inert dusts require higher rates for successful control of stored-product insects (Subramanyam and Roesli, 2000; Zettler and Arthur, 2000). Environmental conditions (temperature, and relative humidity), as well as the content of silicon dioxide (SiO_2), and its particle shape and size, are known to have significant impact on the effectiveness of inert dusts against stored-product insects (Korunić, 1998; Fields and Korunić 2000; Arthur, 2001; 2002; Ferizli and Beris. 2005; Athanassiou et al., 2005; Arnaud et al., 2005; Vardeman et al., 2006).

Natural zeolite (alkaline aluminium silicates) is a sorption dust widely applicable in agriculture for remediation of polluted soils and as fodder additive due to its capacity to neutralize negative effects of mycotoxics, and together with DEs belongs to the same 4th group of inert dusts (Subramanyam and Roesli 2000; Đorđević and Dinić, 2007). However, findings in some studies have demonstrated that this material also has insecticidal potential for control of stored-product insects (Haryadi et al. 1994; Kljajić et al., 2010).

The purpose of this study was to conduct preliminary investigation of the insecticidal potential of the natural zeolite formulation Minazel SP (originating from Serbia) with 66% of SiO_2 and particle size ≤50 µm against rice weevil *Sitophilus oryzae* (L.), lesser grain borer *Rhyzopertha dominica* (F.) and red flour beetle *Tribolium castaneum* (Herbst) in treated wheat grains. Progeny production/reduction in F_1 generation was also determined for each insect species.

2. Materials and methods

2.1. Test insects and applied natural zeolite

Laboratory populations of *S. oryzae*, *R. dominica* and *T. castaneum*, reared in the insectary of the Pesticides and Environment Research Institute, Belgrade, were used in a trial which employed methods described by Harein and Soderstrom (1966), and Davis and Bry (1985). Two-to-four-week-old unsexed insects were used.

The natural zeolite dust Minazel SP used in bioassay contained: SiO_2 (65.7%), Al_2O_3 (14.1%), CaO (3.6%), Fe_2O_3 (2.4%) and up to 1.5% of MgO, Na_2O and K_2O, respectively. Particle size ≤ 50 µm was predominant.

2.2. Bioassay

A bioassay was conducted in the laboratory at 24±1ºC, and 50-55% r.h. for parents and 60±5% r.h. for F_1 progeny using a modified method of OEPP/EPPO (2004 a,b) and a method described by Collins (1990). Soft wheat (var. Takovčanka) with 11.5±0.2% grain moisture, determined by a Dickey–John moisture meter (Dickey-John Mini GAC, Dickey-John Co., USA) was used in the assay.

We chose to apply Minazel SP at the rates of 0.50, 0.75 and 1.00 g/kg wheat. The dust was applied per 500 g of whole-grain wheat (+ 1% of broken grains only in tests involving *T. castaneum*), hand mixed for 2 minutes and then left to mix on a rotary mixer for another 10 minutes. The next day, portions of 50 g of treated wheat were placed in 200 mL plastic vessels with four replicates and 25 adults of *S. oryzae*, and *T. castaneum* and 20 adults of *R. dominica* were placed separately in each vessel. The same procedure was followed on untreated wheat that was used as a control. Insect mortality was determined after 7, 14 and 21 days of contact with treated wheat, and total mortality after additional 7 days of recovery on untreated broken wheat grains.

The effect of natural zeolite on insect progeny production in F_1 generation was determined in the following manner. After recording parental mortality, wheat was seived to remove all insects, and wheat containers were then covered with cotton cloth and fixed with rubber bands. Progeny production was determined by counting live insects in treated and control wheat grains seived after a total of 8 weeks for *S. oryzae*, 10 weeks for *R. dominica* and 12 weeks for *T. castaneum*.

2.3. Data analysis

The acquired mortality data for treated insects were corrected for the mortality of control insects using Abbott's formula (1925) and they are presented as percentages with standard error. The data were statistically compared, separately for each species, using one-way ANOVA and the significance of mean differences between treatments and control was determined by Fisher's LSD test at $P > 0.05$ (Sokal and Rohlf, 1995).

3. Results

The results presented in Table 1 show that after 7 days of contact of all test insect species with all application rates of natural zeolite mortality did not exceed 40%. After 14 days of contact with the highest application rate (1.0 g/kg) mortality reached 87% for *S. oryzae* and 83% for *T. castaneum*, while mortality of *R. dominica* was notably lower, merely 61%. After 21 days of contact with the highest application rate, only the mortality of *T. castaneum* reached 100%, while mortality of *S. oryzae* and *R. dominica* was 94% and 79%, respectively.

After an additional seven-day period of recovery on untreated broken wheat grains, total mortality of all exposed insect species increased with the duration of their contact with treated grains (Table 2). After seven days of exposure and 7 days of recovery, the highest application rate (1 g/kg) of natural zeolite achieved high mortality only against *S. oryzae*, 62%, while the efficacy after 14 days of exposure and 7 days of recovery was 100% against *T. castaneum*, and 96% and 70% against *S. oryzae* and *R. dominica*, respectively. Total mortality after 21 days of exposure to the highest application rate and 7 days of recovery was again the highest against *T. castaneum* (100%), while efficacy against *S. oryzae* and *R. dominica* was 98% and 82%, respectively.

Table 1 Mortality of *S. oryzae, R. dominica* and *T. castaneum* adults after 7, 14 and 21 days of exposure to wheat treated with natural zeolite Minazel SP.

Insect	Rate (g/kg)	Mortality (% ± SE) after exposure		
		7 days	14 days	21 days
Sitophilus oryzae	1.00	40.0±1.4 d **	87.0±1.7 d	94.0±1.0 c
	0.75	28.0±0.8 c	72.0±0.8 c	67.0±2.4 b
	0.50	16.0±0.8 b	52.0±2.4 b	65.0±2.8 b
	0 *	0.0±0.0 a	1.0± 0.5 a	2.0±0.6 a
Rhyzopertha dominica	1.00	18.8±1.0 b	61.2±0.5 c	78.8±4.4 c
	0.75	11.2±2.2 a	47.5±2.1 b	61.2±1.3 bc
	0.50	3.8±1.0 a	42.5±2.1 b	56.2±4.7 b
	0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Tribolium castaneum	1.00	28.0±0.8 c	83.0±1.7 d	100.0 d
	0.75	4.0±0.8 b	66.0±1.7 c	80.0±2.3 c
	0.50	1.0±0.5 ab	25.0±1.7 b	47.0±2.4 b
	0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a

* Control; ** Means within columns (separately for each species) followed by the same letter are not significantly different (LSD test p > 0.05)

Table 2 Mortality of *S. oryzae, R. dominica* and *T. castaneum* adults after 7, 14 and 21 days of exposure to wheat treated with natural zeolite Minazel SP and 7 days of recovery on untreated broken wheat grains.

Insect	Rate (g/kg)	Mortality (% ± SE) after exposure		
		7 days	14 days	21 days
Sitophilus oryzae	1.00	62.0±1.9 c **	96.0±1.2 d	97.9±0.6 c
	0.75	53.0±1.0 b	86.0±1.5 c	74.7±2.2 b
	0.50	37.0±3.8 b	63.0±2.2 b	72.6±2.9 b
	0 *	0.0±0.0 a	1.0±0.5 a	5.0±1.3 a
Rhyzopertha dominica	1.00	21.2±2.1 c	70.0±1.4 c	82.5±3.7 c
	0.75	18.8±1.7 bc	56.2±3.0 bc	63.8±0.9 b
	0.50	8.8±0.5 ab	50.0±1.6 b	58.8±2.8 b
	0	0.0±0.0 a	5.0±0.6 a	0.0±0.0 a
Tribolium castaneum	1.00	40.0±1.2 c	100.0 d	100.0 d
	0.75	13.0±1.5 b	75.0±1.0 c	82.0±1.7 c
	0.50	6.0±3.0 ab	36.0±3.9 b	60.0±2.4 b
	0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a

* Control; ** Means within columns (separately for each species) followed by the same letter are not significantly different (LSD test p > 0.05)

The data on progeny production of stored-product beetles in F_1 generation, presented in Table 3, show that reproduction of all three species occurred in all cases examined, meaning that none of the applied natural zeolite rates caused IR 100%. According to mortality data, the highest application rate of Minazel SP achieved high inhibition of reproduction. After 7 days of exposure, progeny reduction rates were 49-67% for *S. oryzae.* 42-68% for *R. dominica* and 47-78% for *T. castaneum.* After 14 days of exposure, inhibition rates were 55-78% for *S. oryzae,* 72-81% for *R. dominica* and 53-90% for *T. castaneum,* while progeny reduction after 21 days of parental exposure was 51-85% for *S. oryzae,* 80-96% for *R. dominica* and 87-99% for *T. castaneum.*

Table 3 Progeny individuals of *S. oryzae*, *R. dominica* and *T. castaneum* per vessel in F_1 generation after 7, 14 and 21 days of parents exposure to wheat grains treated with natural zeolite Minazel SP.

Insect	Rate (g/kg)	Average number of progeny individuals (± SE) and inhibition rate (%) after exposure periods					
		7 days		14 days		21 days	
		Av. No.	IR	Av. No.	IR	Av. No.	IR
Sitophilus oryzae	1.00	31.8±2.8 d **	66.8	22.5±8.4 c	78.2	16.8±3.9 c	85.1
	0.75	44.2±4.8 c	53.8	33.5±9.6 bc	67.7	29.0±9.0 c	74.4
	0.50	55.2±6.1 b	48.3	46.5±10.0 b	55.2	55.2±7.8 b	51.3
	0 *	95.8±6.4 a	-	104.0±18.1 a	-	113.5±24.6a	-
Rhyzopertha dominica	1.00	12.2±2.5 b	68.4	10.8±2.6 b	80.8	3.2±1.8 b	96.1
	0.75	17.0±4.9 b	56.1	18.0±2.6 b	67.8	13.8±3.9 b	83.7
	0.50	22.5±6.6 b	41.9	15.5±2.5 b	72.3	16.8±5.3 b	80.2
	0	38.8±13.1 a	-	56.0±12.4 a	-	84.5±22.9 a	-
Tribolium castaneum	1.00	14.2±9.9 c	78.2	7.8±3.3 c	89.9	1.2±1.0 c	98.8
	0.75	19.8±6.7 bc	69.7	19.0±5.5 c	77.7	5.2±2.6 c	95.0
	0.50	34.2±8.9 b	47.4	36.2±5.0 b	52.5	13.5±1.9 b	87.2
	0	65.0±15.6 a	-	76.2±16.8 a	-	105.5±8.4 a	-

* - Control; ** Means within columns (separately for each species) followed by the same letter are not significantly different (LSD test $p > 0.05$)

4. Discussion

Many earlier studies have demonstrated that species of the genus *Sitophilus* are considerably more susceptible to DEs than *Tribolium* species (Korunić, 1997; Fields et al., 2003; Athanassiou et al., 2005; 2007) but such findings were not confirmed in our studies of the natural zeolite formulation Minazel SP, especially over the longest period of exposure and additional 7 days of recovering.

The data in our study show that 7 days is an insufficient exposure period for any significant effects to be achieved with natural zeolite, and confirm earlier findings that the effectiveness of inert dusts increases with the duration of exposure (Arthur, 2001; Fields et al., 2003; Athanassiou et al., 2005; Kljajić et al., 2010). Also, our data confirm reports by Collins and Cook (2006a, b) on the mortality of several stored-product insect and mite species growing significantly after contact with DEs applied to different surfaces and a subsequent period of recovery.

After 21 days exposure to wheat grains treated with 0.75 mg/kg of the natural zeolite product Minazel, and additional 7 days of recovering, Kljajić et al. (2010) observed 100% mortality of *S. oryzae* and *T. castaneum,* and 49-79% mortality of *R. dominica*, which is significantly higher effectiveness than the effectiveness of the natural zeolite product Minazel SP used in the present study. Comparing the results of progeny reduction after 21 days of parent exposure to wheat treated with 0.75 mg/kg of Minazel SP, we also found a significantly lower level of Inhibition rates for *S. oryzae* and *R. dominica* and a slightly higher inhibition rate of *T. castaneum*. Higher relative air humidity in the bioassay involving Minazel SP may explain the difference in effectiveness between the two natural zeolites, a factor that neither the large fraction of small-size particles (≤ 50 µm) in that dust nor its higher application rate (1 g/kg) were able to compensate for.

In conclusion, our experiment has confirmed the practical importance of adjusting the application rates of inert dusts chosen to provide in different environmental conditions successful control of several stored-product insect pests with different characteristics and natural levels of susceptibility. Under our laboratory conditions, the natural zeolite formulation Minazel SP showed moderate to high insecticidal effectiveness against *S. oryzae* and *T. castaneum* in treated wheat grain.

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