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Laboratory assessment of effects of a diatomaceous earth formulation on *Sitophilus oryzae* and *Tribolium castaneum* after different exposure periods

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Abstract

Lethal effects of a diatomaceous earth (DE) formulation (Protect-It) against rice weevil (*Sitophilus oryzae* L.) and red flour beetle (*Tribolium castaneum* Herbst) were investigated in wheat bioassay. Lethal effects of DE were examined indoors, at 45 % r.h. and 26 °C, after exposing adults of both insect species to treated wheat over periods of 2, 7, 14 and 21 days, and 7 days recovery on untreated wheat. DE effects on progeny reduction in both species were also investigated after each exposure period.

The mortality produced in groups with different numbers of exposed *S. oryzae* adults (25, 50 and 100 individuals per 100 g of treated wheat per replicate) after 2, 7, 14 and 21 days was 52.0-61.0 %, 73.5- 88.0 %, 98.8-100 % and 100 %, while the respective mortality of *T. castaneum* adults was 8.0-20.3 %, 77.0-87.0, 100 % and 100 %.

Reduction in the F_1 generation of *S. oryzae* adults exposed for 2, 7, 14 and 21 days, depending on the initial number of exposed individuals, ranged 7.14-47.06 %, 2.77- 13.11 %, 2.46-5.00 % and 1.45-4.00 % for. A corresponding reduction in *T. castaneum* fecundity over the same exposure periods ranged 0.00-25.00 %, 1.51-3.33 %, 0.00 % and 0.00 %. The reduction in F_2 generation ranged 1.75-4.60 %, 0.00- 0.83 %, 0.00-0.50 % and 0.00-0.37 % for *S. oryzae* exposed for 2, 7, 14 and 21 days, and 0.00-3.84 %, 0.00-1.92 %, 0.00 % and 0.00 % for *T. castaneum*.

Key words: *Sitophilus oryzae*; *Tribolium castaneum*; Diatomaceous earth (Protect-It); Adult mortality; Progeny reduction.

Introduction

Diatomaceous earth is the most important of inert powder products from several chemical groups that are used as natural insecticides. It has low toxicity to mammals, insignificant influence on the quality of cereals and their products, slower activity than contact insecticides, and mortality of stored-products insects is caused by respiratory arrest, loss of water and suffocation (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000).

Data reported by several authors so far show that stored product insects carry different susceptibilities to diatomaceous earth products, so that coleopter species of the *Cryptolestes* order are most susceptible, *Sitophilus* species slightly less susceptible, while *Oryzaephilus* species, *R.*

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dominica and *Tribolium* species are more tolerant (Golob, 1997; Korunic, 1998; Arthur, 2001 and 2002; Arnaud et al., 2005). Effectiveness of treatment has been reported either to decrease significantly with an increase in air humidity and substrate moisture or to increase with rising temperature, with an exception of *T. castaneum* and *T. confusum*, which have been found mostly unaffected.

The topical issue of resistance of stored product insects to contact insecticides and fumigants worldwide, as well as in Serbia (Subramanyam and Hagstrum, 1996; Collins et al., 2003; Kljajic and Peric, 2005) has shifted efforts in the direction of utilisation of alternative means and methods of stored products protection (Subramanyam and Roesli, 2000).

No product based on diatomaceous earth has yet been registered in Serbia for the protection of plant products from insect pests. This research therefore focused on testing the effectivenees of the diatomaceous earth product Protect-It under laboratory conditions against two important insect pests of stored products. Conducting the experiment under low relative air humidity and using wheat with low moisture content, we tested the product on different numbers of adults of rice weevil, *Sitophilus oryzae* (L.), and red flour beetle, *Tribolium castaneum* (Herbst), after different exposure periods, and determined their progeny reduction in the F_1 and F_2 generations.

Materials and methods

Test insects

Laboratory populations of *S. oryzae* and *T. castaneum*, reared in an insectarium according to methods proposed by Harein and Soderstrom (1966) and Davis and Bry (1985) were used in the experiment. Rice weevils were reared in 2.5 L glass jars on whole grain soft wheat with moisture content below 12 %, and red flour beetle in 2.5 L glass jars on a substrate consisting of a mixture of wheat flour, maize flour and yeast. Air temperature in the insectarium was set to 26 ± 1 ºC, and relative air humidity to 60 ± 5 %.

Insect adults aged two to four weeks with indiscriminate sex ratio were used in the experiment.

Bioassay procedure

Effects of a diatomaceous earth product (Protect-It^{tm}) against rice weevil and red flour beetle adults were investigated in the laboratory using modified EPPO methods (1997) (Standards PP 1/203 and PP 1/204) and a method described by Collins (1990). The dustable powder Protect-It^{tm}, manufactured by Hedley Technologies Inc., Vancouver, Canada, contained 90 % thermally untreated diatomaceous earth and 10 % amorphous silica gel.

As moisture content of wheat was below 12 %, doses recommended for existing conditions were used in all treatments, so that 0.15g/kg wheat was used in the *S. oryzae* bioassay, and 0.30 g/kg in *T. castaneum* bioassay. Effects of diatomaceous earth on different initial numbers of test insects were investigated in three variants with four replicates. Twenty-five *S. oryzae* adults were inserted in 100 g wheat in the first variant, 50 adults in the second variant and 100 in the third, and the *T. castaneum* bioassay followed the same pattern. Mortality of the exposed adults of both species was determined after 2, 7, 14 and 21 days of contact with treated wheat, and seven days of recovery on untreated wheat. Air temperature in the room in which bioassays were set up was 27 ± 1 ºC, and relative air humidity 45 ± 5 %. Control wheat was left untreated and was handled the same way and under identical conditions.

To determine progeny production, *S. oryzae* and *T. castaneum* adults were sieved out of wheat substrate after each exposure period. The treated wheat was put back into plastic vessels covered with cotton cloth and fixed with rubber bands. Those were stored under conditions of 27 ± 1 ºC air temperature and 60 ± 5 % r.h. *S. oryzae* progeny in the F_1 generation was determined after six weeks, and in the F_2 generation after 10 weeks from the beginning of contact with treated wheat. *T. castaneum* progeny was determined after 10

weeks for the F_1 and 16 weeks for the F_2 generation.

Adult mortality data in treatments and controls were calculated as percentages and corrected for control mortality using Abbott's formula (1925), including standard deviation. F_1 and F_2 generation fecundity was expressed as an average number of live individuals with calculated standard deviation, and progeny reduction as percentages showing the relationship between surviving insects in treatments compared to control.

Results

Experimental data show that mortality was growing with the duration of exposure of adults of both species, especially red flour beetle *T. castaneum* (Table 1).

After 2 days of contact of *S. oryzae* adults with treated wheat, mortality was at its highest, 61 %, in the variant of 100 g wheat containing 100 adults, while it was slightly above 50 % in the variants with 25 and 50 adults, in which mutual difference was not statistically significant. After 7 days of exposure, however, mortality was lowest in the variant with the top number of insects, while full mortality of rice weevil adults was achieved after 14 and 21 days in all variants.

Mortality of *T. castaneum* was also highest, 20.25 %, after 2 days of exposure in the variant of 100 adults/100 g of wheat treated with diatomaceous earth, and lowest in the variant with the fewest insects. After 7 days of exposure, the highest mortality, 87 %, was achieved in the variant of 50 adults/100 g wheat, and again lowest in the variant with the fewest insects. Mortality of red flour beetle after 14 and 21 days was 100 %, the same as in the rice weevil bioassay.

Data on rice weevil and red flour beetle progenies in the F_1 generation (Table 2) show a significantly higher number of rice weevil offspring individuals after parent exposure for 14 and 21 days, compared to 2-day exposure, only in the variant of 100 individuals/100 g wheat. However, fecundity differences were not high after 2 and 7 days, and after 14 and 21 days of exposure in any of the variants with different initial numbers of weevils. As for the red flour beetle, differences were not high between any fecundity values.

After 2 days of contact of *S. oryzae* parents with diatomaceous earth-treated wheat, the lowest abundance F_1 generation progeny was recorded in the variant of initial 100 adults/100 g wheat (0.75 adults on the average), with progeny reduction being 7.14 % against control, while the highest reduction was recorded in the variant of 25 adults/100 g wheat - 47.06 %. Such trend continued also after 7 days of parent

Table 1. Mortality of *Sitophilus oryzae* and *Tribolium castaneum* adults after 2, 7, 14 and 21 days of contact with wheat treated with Protect-It and 7 days recovery on untreated wheat.

Protect-It dose (g/kg)	No. of exposed adults/100 g treated wheat	Mortality (% ± SE) after exposure			
		2 days	7 days	14 days	21 days
Sitophilus oryzae					
0.15	25	54.00 ± 1.29	88.00 ± 2.16	100	100
	50	52.00 ± 2.16	88.00 ± 2.71	100	100
	100	61.00 ± 1.41	73.50 ± 6.03	98.75 ± 1.50	100
Tribolium castaneum					
0.30	25	8.00 ± 0.81	77.00 ± 1.89	100	100
	50	14.00 ± 1.82	87.00 ± 1.73	100	100
	100	20.25 ± 2.63	81.25 ± 1.71	100	100

exposure to treated wheat, only within a narrower range (2.77-13.11 %), while the percentage of progeny reduction was decreasing with the duration of parent exposure to treated wheat (14 and 21 dan), being up to 5.00 % and 4.00 % in the variant with the lowest number of insects.

After 2 days of contact of *T. castaneum* parent insects with treated wheat, the highest progeny reduction in the F_1 generation, 25.00 %, was found in the variant of 25 adults/100 g wheat. The situation was similar after 7 days of parent exposure, only the range narrowed (1.51-3.33 %), while no progeny was found in the treated wheat after 14 and 21 days of parent exposure.

Data on progeny production of rice weevil and red flour beetle in the F_2 generation (Table 3) disclosed no evident deviation in any variant. The highest progeny reduction, 4.60 %, was found in the variant of 100 adults/100 g wheat after 2 days

Table 2. F_1 generation progeny of *Sitophilus oryzae* and *Tribolium castaneum* after 2, 7, 14 and 21 days of contact with wheat treated with Protect-It.

No. of exposed adults /100 g treated wheat	Average number of progeny individuals (± SE) after exposure periods							
	2 days		7 days		14 days		21 days	
	No.	S* (%)	No.	S* (%)	No.	S* (%)	No.	S* (%)
Sitophilus oryzae								
25	2.00±1.41	47.06	2.00±1.41	13.11	1.25±0.50	5.00	1.75±1.26	4.00
50	1.00±0.82	12.50	1.25±0.96	3.76	1.50±1.29	2.46	1.50±1.00	1.45
100	0.75±0.96	7.14	2.25±0.96	2.77	4.75±2.22	2.89	4.25±2.22	1.98
Tribolium castaneum								
25	0.50±0.58	25.00	0.25±0.50	3.33	0.00±0.00	0.00	0.00±0.00	0.00
50	0.00±0.00	0.00	0.25±0.50	1.51	0.00±0.00	0.00	0.00±0.00	0.00
100	0.25±0.50	4.34	1.00±0.81	3.12	0.00±0.00	0.00	0.00±0.00	0.00

* - progeny reduction in treatments, compared to control

Table 3. F_2 generation progeny of *Sitophilus oryzae* and *Tribolium castaneum* after 2, 7, 14 and 21 days of contact with wheat treated with Protect-It

No. of exposed adults /100 g treated wheat	Average number of progeny individuals (± SE) after exposure							
	2 days		7 days		14 days		21 days	
	No.	S* (%)	No.	S* (%)	No.	S* (%)	No.	S* (%)
Sitophilus oryzae								
25	0.25±0.50	3.85	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00	0.00
50	0.25±0.50	1.75	0.25±0.50	0.38	0.75±1.50	0.50	0.75±0.95	0.37
100	1.00±1.41	4.60	1.00±1.15	0.83	0.75±0.96	0.29	0.75±0.96	0.14
Tribolium castaneum								
25	0.25±0.50	3.84	0.25±0.50	1.92	0.00±0.00	0.00	0.00±0.00	0.00
50	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00	0.00
100	0.25±0.50	3.33	0.25±0.50	0.38	0.00±0.00	0.00	0.00±0.00	0.00

* - progeny reduction in treatments, compared to control.

of contact of *S. oryzae* parents with wheat treated with diatomaceous earth. Similar results were recorded after 7 days of parent exposure, only with a 5.5 x lower percentage of progeny reduction (0.83 %). However, no progeny was found after 7, 14 and 21 days of parent exposure in the variant with the lowest number of insects (25 adults/100 g wheat).

The highest progeny reduction in the F_2 generation after 2 and 7 days of contact of *T. castaneum* parents with diatomaceous earth-treated wheat was found in the variant of 25 adults/100 g wheat - 3.33 and 1.92 %. After 14 and 21 days of parent exposure, however, no progeny was found.

Discussion

The data acquired in this investigation show that diatomaceous earth and the product Protect-It, applied at recommended dose (0.15 g/kg against *S. oryzae* and 0.30 g/kg against *T. castaneum*) to wheat with low moisture content and under low relative air humidity, had high effectiveness in controlling the stored product insects investigated.

After two days of exposure, rice weevil adult mortality was 3.0-6.5 x higher than that of red flour beetle, while no significant mortality difference was recorded between the two species after 7, 14 and 21 days of exposure, not counting the double dose applied to *T. castaneum*.

Comparing fecundities in the F_1 generation, the rice weevil progeny was found to be significantly higher in numbers (average range 0.75-4.75) than red flour beetle progeny (average range 0.00-1.00). Furthermore, regardless of the initial number of adults exposed to treated wheat (25, 50 or 100/100g), progeny reduction in treatment, compared to control, was higher for *S. oryzae* than for *T. castaneum* in all exposure periods (2, 7, 14 and 21 days).

Fecundity in the F_2 generation (average range 0.00-1.00 for rice weevil and 0.00-0.25 for red flour beetle) was similar for both species of stored products insects. Red flour beetle progeny was found only after 2 and 7 days of parent exposure to treated wheat in the variants of 25 and 100 adults/100 g wheat. Progeny of rice weevil was not found only after 7, 14 and 21 days of exposure in the variant with the lowest initial number of parent adults.

Analysing the applicability of diatomaceous earths of different origins, Korunic (1997) had found that the LC_{50} values for rice weevil after 5 days of exposure to treated wheat ranged 270- 5000 ppm, and 417-5000 ppm or more for red flour beetle after 14 days of exposure. Arthur (2002) tested the product Protect-It in a 71-300 ppm range applied to wheat and found no surviving *S. oryzae* adults after 2 and 3 weeks of exposure under 57 % r.h., while survival under 75 % r.h. was null under concentrations of 214 and 300 ppm. However, after exposure for one week under 75 % r.h. and 27 °C, and with a population density of 30 individuals/35 g wheat, around 25 % weevils were found to survive, while parent exposure for seven days produced the lowest F_1 generation progeny under lower air humidity (40 % r.h.). Arnaud et al.(2005) found that the efficacy of diatomaceous earth in controlling *T. castaneum* often varied significantly depending on population properties and that Protect-It was most efficient after 21 days of adult exposure to 400 ppm dose of treated wheat.

The results of our experiment appear to be more favourable than those reported by Athanassiou et al. (2005) after testing the efficacy and persistance of three products based on diatomaceous earth (Insecto, SilicoSec and PyriSec). The mortality of *S. oryzae* adults reported by these authors over 270 days exceeded 90 %, and fecundity was below one adult per sample, regardless of the application dose (0.75, 1.0 or 1.5 g/kg). Ferizli and Beris (2005) found, for instance, that an application dose of Protect-It of 1 g/kg wheat was sufficient to achieve high effectiveness against *R. dominica*, regardless of exposure duration (one, two or three weeks). Arthur (2003) reported a 100 % mortality after 48 h exposure of *T. castaneum* adults to treated filter paper in Petri dishes at 0.5 mg/cm^2 dose under experimental conditions similar to those in our own investigation.

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