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PERSISTENCE AND EFFICACY OF DIATOMACEOUS EARTH FROM SERBIA AGAINST *RHYZOPERTHA DOMINICA* F. ON WHEAT

Abstract

The persistence and efficacy of diatomaceous earth from Serbia (DE S-1 and DE S-2) on *Rhyzopertha dominica* F. in stored wheat was examined. Commercial DE formulation, Protect-It, was used as a standard. Three months after treatment with DEs at rates of 1 mg kg^{-1}, grains were infested with adults of *R. dominica* F. Insect mortality was determined after 7, 14 and 21 days. The results show that the efficacy of DEs increased with the duration of exposure. After the 21st day the higher mortality was detected in grains treated with Protect-It (99.5%), while there were no differences between DE S-1 and DE S-2 (96.5% and 95.5%, respectively). The progeny/progeny reduction then weak after exposure were 6/99.2% Protect-It, 54.8/93.1% DE S-1 and 193/75.84% DE S-2.

Keywords: *Rhyzopertha. dominica, diatomaceous earth, insecticidal activity, persistence, wheat*

INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae) is a primary pest of stored grain, with the great economic importance in the Republic of Serbia and many regions of the world. *R. dominica* spends most of its life inside kernels, feeding on its endosperm, which causes damage and changes in grain physicochemical properties. Modern methods of protection of stored grains and

other plant products from insect pests strive towards optimizing the use of different techniques and methods within integrated pest management (IPM) programs. Diatomaceous earths (DEs) have been identified as natural materials and promising alternatives to stored-grain protectants, such as residual insecticides (Athanassiou et al., 2014; Andrić et al., 2012; Kavallieratos et al., 2005, 2010; Korunić, 2013). They are leaves no toxic residues on the product and according to the US EPA (Environmental Protection Agency) they are classified in the category of GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA, 1995). Moreover, they are non-toxic to mammals (rat oral LD50 > 5000 mg/kg of body weight) and as inert materials, no interaction with the environment occurs.

Available data show that stored-product insects are variably susceptible to inert dust, resulting from their different morphological, physiological and ecological characteristics. The DEs mined currently vary remarkably in their insecticidal activity, depending upon the geological and geographical origin as well as certain characteristics, such as SiO_2 content, pH, tapped density and adherence to kernels (Korunić, 2013). Several DEs, based on natural deposits, are now commercially available. However, the search for newer, naturally occurring DEs that are more effective for insect control is still in progress. Korunić (2013) and Athanassiou et al. (2011) found that local DEs from the former Yugoslavia were very effective, and could be used with success against stored-grain pests.

Also, as inert materials, DE particles remain unaffected and can persist on the product, providing long-term protection (Athanassiou et al., 2005; Vayias et al., 2006). DEs persist in the treated substrate, providing long-term protection against stored products insect pests that are not possible with the use of residual insecticides.

In the present study, we evaluated the potential of long-term protection of wheat against *R. dominica* through the use of DEs from Serbia and compared with a commercialized DE product, Protect-It. In addition to parental mortality, we also evaluated progeny production on the treated grains.

MATERIAL AND METHODS

The trial was carried out on laboratory populations of *R. dominica.* Insects were reared in 2.5 L glass jars containing soft wheat grain with moisture degree below 12%. The temperature in the insectary was 26±1 ºC, and relative humidity – r.h. 60±5%. Unsexed 2-4 week old adults were used in all trial variants.

Two DE samples from Serbia, DE-S1 and DE-S2, were tested, and a commercial DE formulation, Protect-It (Hedley Technologies Inc., Canada), was used as a standard for comparing the effectiveness of the Serbian DEs.

Table 1. Chemical composition of diatomaceous earth

DE samples	*Chemical composition*							
	SiO_2	Al_2O_3	Fe_2O_3	K_2O	Na_2O	MgO	CaO	TiO_2
DEs from Serbia								
DE S-1	79,7	9,41	1,11	0,79	0,08	0,14	0,63	0,21
DE S-2	63,15	10,31	1,67	0,91	0,08	0,31	1,01	0,31
Standard								
DZ Protect-It™	83,7%*	3%	1%	<1	<1	<1	<1	–

* +10% silica aerogel

The wheat cultivar Vizija, grain variety originating from the Center for Small Grains Kragujevac, Serbia, was used. The tested grain was infestation-, pesticide-, and dockage-free.

Three 0.5 kg lots of grain were prepared. The application rates were 1.0 g kg^{-1} (1000 ppm) for all three DEs. The lots were placed in glass jars of 1000 mL volume. In order to secure equal distribution of the DEs, the grain was shaken manually for 2-3 min and then mixed on a rotary mixer for 10 min. Treated and untreated grain was stored under ambient conditions (21.0 ± 1.0°C, 40-60% r.h.) in 2 L plastic containers with vented bottoms and lids. After three months, four samples of 50 g were taken from each DE and placed into a 200 mL plastic vessel. The quantity of 50 g was weighed on an analytical balance. Subsequently, 25 adults of *R. dominica* were released into each vessel and the vessel was topped with cotton cloth and fixed with a rubber band. The same procedure was applied to untreated grain samples which served as a Control. So, Control is infested with 25 adults of *R. dominica*, but pesticide-, and dockage-free. All vessels were placed in an incubator set to 26±1°C temperature and 60±5% r.h. Insect mortality was determined after 7, 14 and 21 days of contact with treated or untreated grain types. After the last mortality count, both dead and living adults were removed and all vessels were returned to the incubator for 7 additional weeks under the same (described) conditions. Progeny emergence/suppression was determined by counting insects in treated and control grains.

The whole procedure was repeated twice.

Data analysis

Before analysis, the percentage of mortality was transformed using *arcsine,* progeny number was transformed by *log(x+1)*. Untransformed means for mortality and progeny emergence with standard errors are shown in the tables. All data were submitted to a one-way ANOVA and the means were separated by Fisher (LSD) test at *P*=0.05. Insect progeny reduction in wheat was determined by using the formula PR (%) = (K-T) x 100/K where K – number of progeny in the untreated control group, and T – number of progeny in treatment groups. All data were processed on StatSoft version 7.1 (StatSoft Inc., Tulsa, Oklahoma).

RESULTS

Results obtained for *R. dominica* mortality after 7, 14 and 21 days of exposure in wheat treated with DEs are presented in Table 2. After 7 days of exposure, the highest insect's mortality of 87.25% was recorded for Protect-It, while DEs from Serbia caused the mortality from 53.25% (DE S-2) to 62.0% (DE S-1).

After 14 days of exposure, all three tested DEs caused significantly higher mortality of *R. dominica* than after the initial 7-day exposure period. High adult mortality, 98.5%, was found in wheat treated with Protect-It. After 14 days of exposure, the lowest *R. dominica* mortality was found in grains treated with DE S-2 (89.0%), but there were no statistical differences between DEs from Serbia.

Over the interval of 21.day, high mortality, 95.5%-99.5%, was observed. Generally, the higher mortality were detected in grains treated with Protect-It (99.5%), while there were no differences between DE S-1 and DE S-2 (96.5% and 95.5%, respectively).

Table 2. Mean percentage mortality (±SE) of Rhyzopertha dominica adults exposed in wheat treated with DEs from Serbia three months before exposure

Formulation	Mortality (%±SE) after exposure in grains		
	After 7 days of exposure	After 14 days of exposure	After 21 days of exposure
DE S-1	62.00±3.15^{b}	92.00±1.19^{b}	96.50±1.19^{b}
DE S-2	53.25±4.6.^{c}	89.00±1.6^{b}	95.50±1.61^{b}
Protect-It	87.25±2.6^{a}	98.50±1.06^{a}	99.50±0.5^{a}
Control	1.00±0.66^{d}	2.50±0.74^{c}	6.50±1.51^{c}
F	50.40	84.73	170.34

* For each exposure period separately, means within columns followed by the same letter are not significantly different. Fisher (LSD) test at $P>0.05$; in all cases df=7,32; $P<0.05$.

High levels of progeny reduction of 99.2% were found in wheat treated with Protect-It as well as in grains treated with DE S-1 (93.2%). The least progeny reduction of 75.84% was found in samples treated with DE S-2 (Table 3.).

Table 3. Progeny emergence (adults/val) and reduction of R. dominica in wheat treated with DEs from Serbia at dose of 1000 ppm three months before exposure

Formulation	Mean number of progeny (adults/val ± SE)	Progeny reduction (%)
DE S-1	54.8±3.17^{b}	93.10±
DE S-2	193.0±4.98^{c}	75.84±
Protect-It	6.37±0.75^{a}	99.20±
Control	799.0±20,02^{d}	/
F	20.78	/

* Means within columns followed by the same letter are not significantly different. Fisher (LSD) test at $P>0.05$; in all cases df=7,32; $P<0.05$.

DISCUSSION

Available data show that stored-product insects are variably susceptible to inert dust, resulting from their different morphological, physiological and ecological characteristics. The dust DE S-1 and DE S-2, originating from Serbia, are less effective against *R. dominica* than against rice weevil *Sitophilus oryzae* (L.) or red flour beetle *Tribolium castaneum* (Herbst) (Andrić et al., 2012). Despite this, Perišić et al. (2018), after 21 days of exposure of Protect-It, DE S-1 and DE S-2 reported high mortality of *R. dominica* in wheat (93.0-96.5 %). The high mortality of *R. dominica* after application of different commercially available DEs has been reported by many authors (Athanassiou et al., 2005, 20011, 2014; Korunić, 2013; Kavallieratos et al., 2005, 2010).

Previous studies indicated the high efficacy of DEs, but these studies do not indicate the possibility of long-term protection of stored wheat. Protection of wheat for extended time periods of storage is a basic purpose of any grain management program. Although, Athanassiou et al. (2005) reported residual efficacy of DEs – Insecto, SilicoSec, and PyriSec against *S. oryzae* L. on wheat and barley for up to 450 days, Vayias et al. (2006) conducted the first study of the residual effect of three DEs on wheat and maize against *T. confusum* and indicate good protection over 360 days. Similar results have also been reported by Stathers et al. (2008) for the DE Protect-It on farm-stored maize in the field in Tanzania. The authors showed that Protect-It maintained its insecticidal efficacy for 20 months.

The present study provides clear evidence that the tested DEs were effective and could offer long-term protection to the treated wheat against *R. dominica*. DEs from Serbia achieved efficacy of 95.5-96.5% against *R. dominica* exposed for 21 days. The results are similar as results of studies were adults of *R. dominica* were exposed immediately after treatment of different DEs (Kavallieratos et al., 2005; 2010; Athanassiou et al., 2011; Perišić et al., 2018).

The results show that the efficacy of DEs from Serbia against *R. dominica* increased with the duration of exposure. This is consistent with findings reported by many other studies (Athanassiou et al., 2005, 2011, 2014; Kavallieratos et al., 2010; Andrić et al., 2012; Korunić, 2013; Perišić et al., 2018). In the cited studies, insects were also exposed immediately after wheat treatment with diatomaceous earth. Vayias et al. (2006) treated the wheat and maize with DEs – Insecto, PyriSec and SilicoSec. Samples were taken on the day of storage and every 30 days until completion of a 360 day period of storage and bioassayed with *T. confusum* adults. Thus a DE treatment of 1 g kg^{-1} was shown to provide long-term protection of wheat against *T. confusum.*

Although DE S-1 and DE S-2 were not formulated or silica gel was added to them, as is the case with Protect-It (+10% of silico gel), these DEs showed good efficacy even after three months of wheat treatment.

The effect of inert dust on progeny emergence of stored-product insect pests is a very important parameter, which reveals the potential for long-term protection of stored grains. Athanassiou et al. (2014) recorded that even when parents' mortality was 100%, progeny emergence was inevitable with *R. dominica*. In the present study, Protect-It achieved 99.5% mortality after 21 days of exposure but a small number of offspring did emerge in some of the examined grain varieties, i.e. 6.37 insects. Significant dif-

ferences in progeny reduction of DE S-1 and DE S-2 were found. In DEs treated grains, offspring counts were the highest in wheat treated with DE S-2 (193.0). This finding is consistent with the finding of Perišić et al. (2018) who examined the efficacy of DE from Serbia immediately after treatment.

These results imply that DE S-1 has better efficiency than DE S-2. Vayias et al. (2006) and Korunić (2013) have identified SiO_2 content and particle size as the characteristics of DEs that primarily determine the efficacy of DEs. Similar conclusions could be inferred from present findings as DE S-1 contains 79.8% SiO_2, while DE S-2 contains 63.2% SiO_2. The particle fraction < 13 μm is the prevailing fraction in the DE S-1, 95.3% somewhat lower in DE S-2, 81.0% (Andrić et al., 2012). Based on the above mentioned, in this study, the differences in the content of SiO_2 and particle size between DE S-1 and DE S-2 were recorded.

Athanassiou et al. (2011) and Korunić (2013) in an extensive screening of DEs from several parts of the world found that local DEs from the former Yugoslavia were very effective, and could be used with success against stored-grain pests. Andrić et al. (2012) and Perišić et al. (2018) showed the good insecticidal potential of DEs from Serbia in stored insects control in grains such as wheat, barley, rye, oats and triticale. The presented results confirm the high persistence of DEs as the good insecticidal potential of DEs from Serbia. The use of a natural, non-toxic insecticide that persists in the grain could be a very promising alternative to the residual pesticides that currently dominate stored grain protection.

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PERZISTENTNOST I EFIKASNOST DIATOMEJSKE ZEMLJE POREKLOM IZ SRBIJE U SUZBIJANJU *RHYZOPERTHA DOMINICA* F. U PŠENICI

Abstract

Ispitivana je perzistentnost i efikasnost diatomejske zemlje poreklom iz Srbije (DZ S-1 i DZ S-2) u suzbijanju *Rhyzopertha dominica* F. Kao standard korišćen je preparat na bazi DZ Protect-It. Uskladištena pšenica tretirana je DZ u količini od 1000 ppm (1 g DZ kg^{-1} žita). Tri meseca nakon tretiranja pšenica je infestirana adultima *R. dominica.* Smrtnost izlaganih jedinki utvrđivana je posle 7, 14 i 21. dana izlaganja. Na procenat smrtnosti značajno je uticao period izlaganja. Najveću smrtnost posle 21.dana izlaganja prouzrokovao je Protect-It (99,5%). Nije ustanovljena razlika u efikasnosti DZ S-1(96,5%) i DZ S-2 (95,5%). Prosečan broj potomaka (±SG) / redukcija potomstva (%) deset nedelja posle izlaganja bio je 6/99.2% Protect-It, 54.8/93.1% DZ S-1 i 193/75.84% DZ S-2.

Ključne reči: *Rhyzopertha dominica, diatomejska zemlja, insekticidna efikasnost, perzistentnost, pšenica*