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Laboratory evaluation of diatomaceous earth deposits mined from several locations in central and southeastern Europe as potential protectants against coleopteran grain pests

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ABSTRACT

Diatomaceous earth (DE) deposits from regions of central and southeastern Europe were evaluated for their insecticidal efficacy against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) in comparison with the commercially available DE formulation SilicoSec. The effects of temperature, RH, grain commodity (wheat, barley, maize, rice), application method (spraying vs. dusting) were evaluated. FYROM, a DE from the Former Yugoslavian Republic of Macedonia, was the most effective of the DE deposits for grain treatment, whereas the least effective was from Greece (named Crete). However, for surface treatment, Slovenia was the most effective followed by Elassona 1 and Begora. Increase of temperature increased DE efficacy, while the reverse was noted with the increase of RH. Furthermore, the DEs were more effective in barley or wheat than in maize or rice. Neither the mined DEs nor SilicoSec were able to suppress progeny production of the tested species after previous exposure on the treated commodities. Generally, dust application of DEs was more efficacious than spraying against *S. oryzae* and *T. confusum*. However, spraying of wheat significantly reduced the bulk density (test weight) compared to dusting. For surface treatment, after 1 d of exposure, Slovenia was the most effective of the mined DEs followed by Elassona 1 and Begora, whereas after 6 d of exposure the mortality was almost complete (>99%) with all three DEs. More than 6 d of exposure were required for an effective control of *T. confusum* adults with the remainder of the mined DEs.

1. Introduction

Diatomaceous earths (DEs) are composed by the fossil skeletons of phytoplankton, also known as diatoms, which occur in fresh and salt water since the Eocene period and produce a soft sedimentary rock, which is composed mainly by amorphous silicon dioxide (Korunic, 1998). They are natural inert dusts that are efficacious when used as insecticides and are considered among the most promising alternatives to the use of traditional pesticides in stored grain (Korunic, 1998; Subramanyam and Roesli, 2000). DEs act on the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss as long as DE particles are trapped and adhered to insects' body (Ebeling, 1971). They are non-toxic to mammals (rat oral $LD_{50} > 5000$ mg/kg of body weight), leaves no toxic residues on the product and according to the US EPA (Environmental Protection Agency) they are classified in the category of

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GRAS (Generally Recognized As Safe) since they are used as food or feed additives (FDA, 1995). Moreover, they are used in filters, fillers, detergents and deodorizers (Korunic, 1998). DEs can be applied with the same application technology as traditional grain protectants, which means that no specialized equipment is required (Athanassiou et al., 2005a). Moreover, since they are inert materials, no interaction with the environment occurs. DEs persist in the treated substrate, providing long-term protection against stored-products insect pests that is not possible with the use of residual insecticides (Athanassiou et al., 2005b; Vayias et al., 2006b). 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 (Korunic, 1997). Several DEs, based on natural deposits, are now commercially available, and have been proved very effective against stored-grain pests (Subramanyam and Roesli, 2000).

However, the search for newer, naturally occurring DEs that are more effective for insect control is still in progress, especially in areas rich in silicaceous rocks. Korunic (1997, 1998), 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. Similar results have been reported by Inđić et al. (1999) for certain DEs from the former Yugoslavia. Despite their advantages, the use of DEs in stored-product protection remains rather limited, due to their main drawback: DE application reduces grain bulk density (volume/weight ratio). For a satisfactory level of efficacy, the commercially available DE formulations should be applied at doses between 400 and 1000 ppm (Fields and Korunic, 2000). Many researchers underline the need for using new DEs, which are effective at low dose rates (Arthur, 2003; Athanassiou et al., 2006b, 2007). Some newer DE formulations, combined with low doses of insecticides, have already been evaluated with promising results (Athanassiou and Kavallieratos, 2005; Vayias et al., 2006a, 2006b). For instance, Athanassiou et al. (2006b) noted that two new enhanced DEs were very effective at dose rates as low as 75 ppm.

Considering the increasing global focus on the safety of pesticides on human health and the environment, the development and assessment of suitable replacements for traditional contact insecticides, is of great importance. The development of Integrated Pest Management (IPM) programs based on natural-resources in which new effective DEs are involved, would help the adoption of a judicious control protocol in stored grains. However, the newer DEs besides their effectiveness against stored-product pests, should also be tested as regards their impact on grain properties. In our series of experiments the efficacy of several DEs originating from different geographical locations of central and southeastern Europe was assessed against serious stored-product insect pests such as the primary colonizers: *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and the secondary colonizer, *Tribolium confusum* Jacquelin du Val (Coleoptera: Curculionidae). Furthermore, the progeny production of the above species in substrates treated with the DEs was also evaluated. Factors that affect DE efficacy, such as exposure interval, dose, temperature, RH and grain commodity were also examined. A new alternative application method to dusting was evaluated. The DEs were also assessed for surface treatment.

2. Materials and methods

2.1. Effect of temperature and RH on the efficacy of DEs

Ten DEs, of fresh water origin, which were mined during autumn 2007, originating from Greece (named Crete, Elassona 1, Elassona 2), from Serbia (named Begora, Kolubara, Vranje, Vranje 311207), from Slovenia (named Slovenia), from the Former Yugoslavian Republic of Macedonia (named FYROM), from Germany (named Germany) and one commercially available formulation of fresh water origin, SilicoSec (BioFa GmbH, Munchigen, Germany), were assessed for their effectiveness on hard wheat (var. Mexa), against adults of *S. oryzae*, *R. dominica* and *T. confusum*. The tested wheat was infestation and pesticide free. For all species, unsexed, <21 d old adults were used in the tests. At the beginning of the tests, all DE samples were dried at 40 °C for 24 h to about 6% moisture content (Korunic, 1997). The wheat that was used for experimentation was untreated and had very little dockage (<0.8%). Prior to tests, the grain was kept at 25 °C and 60% RH for 2 weeks to equilibrate with its moisture content. Before experimentation, the moisture content of the grain was determined by a Dickey-John moisture meter (Dickey-John Multigrain CAC II, Dickey-John Co, Lawrence, KS, USA) and ranged between 11.1 and 12.0%. Insects used for the bioassays were obtained from laboratory cultures on hard wheat at 25 °C and 65% RH in the case of *S. oryzae* and *R. dominica* and on hard-wheat flour plus 5% brewers yeast at 27 °C and 65% RH in the case of *T. confusum*.

Eleven 1 kg lots of grain were prepared and each of them was treated with 900 ppm of each of the DE samples. Furthermore, an additional 1 kg lot of grain was left untreated and served as control. The treated wheat lots were shaken manually for approximately 15 min to achieve equal distribution of the DE particles inside the grain mass (Subramanyam and Roesli, 2000). Three 60 g samples were taken from each treated lot as well as from the control and placed separately in 4 glass vials (7 cm in diameter, 12 cm in height). The lid of the vials had a 15 mm hole in the middle, which was covered by gauze, to permit sufficient aeration inside the vial. Subsequently, 30 adults of the tested species were added into separate glass vials and all vials were placed in incubators set at the desired combination of temperature and RH each time. Three temperature levels 20 °C, 25 °C and 30 °C and two RH levels 45% and 65% were tested. Mortality was assessed after 7 d and 14 d of exposure of the individuals on the treated wheat samples or control. The above procedure was carried out separately for each species and was repeated three times, by preparing new lots of wheat each time ($3 \times 3 = 9$ replications per species).

Control mortality was generally low and did not exceed 5% in all of the tested cases. Nevertheless, insect mortality was corrected according to Abbott's formula (Abbott, 1925). Data were analyzed separately for each species according to the Repeated Measures Analysis of SAS (SAS Institute, 2000). The repeated factor was exposure interval, while mortality was the response variable and DE, temperature and RH were the main effects. Also the 2-way associated interactions of the main effects were incorporated in the analysis. To overall assess the efficacy of the tested DEs data were pooled for each exposure interval. Means for mortality counts were separated using Tukey–Kramer (HSD) test at $P = 0.05$ (Sokal and Rohlf, 1995).

2.2. Effectiveness of DEs in different grain commodities

The DEs used in this series of experiments are described in Section 2.1. Before the beginning of experiments all the tested samples were dried out to about 6% moisture content as described in Section 2.1. The DEs were evaluated regarding their effectiveness on four grain commodities against adults of *S. oryzae*, *R. dominica* and *T. confusum* obtained from laboratory cultures as described in Section 2.1. Untreated clean wheat (var. Mexa), maize (var. Dias), barley (var. Persephone) and rice (var. Thaibonnet) were used in the tests. Prior to tests, the grain was kept at 25 °C and 60% RH for 2 weeks to equilibrate with its moisture content. Before the beginning of the experiments, the moisture content of each grain was determined by a Dickey-John moisture meter (Dickey-John Multigrain CAC II, Dickey-John Co, Lawrence, KS, USA) and ranged 10.8–12.2%.

Lots of 1 kg of each grain were treated with 500 and 1000 ppm of each DE. Furthermore, an additional 1 kg lot of each grain remained untreated and served as control. Three 60 g samples from each treated (and untreated) lot of grains were taken and put inside glass vials, as described in Section 2.1. Then, 30 individuals of each species were added into each glass vial and all vials were placed in incubators set at 25 °C and 65% RH. Mortality was assessed after 7 d and 14 d of exposure of the individuals on the treated or untreated grains. At the end of the 14 d exposure interval all dead or alive individuals were removed from the vials and the vials were left in the incubators for an additional period of 50 d. Finally, the vials were opened and the number of alive progeny per treated commodity was counted. The above procedure was conducted separately for each species and was repeated three times. Each time, new grain lots were prepared ($3 \times 3 = 9$ replications per species).

Data were analyzed separately for each species according to the Repeated Measures Analysis of SAS (SAS Institute, 2000) with exposure interval as repeated factor. The response variable was insect mortality while DE, dose rate and commodity were the main effects. Also the 2-way associated interactions of the main effects were incorporated in the analysis. Means for mortality counts were separated using Tukey–Kramer (HSD) test at $P = 0.05$ (Sokal and Rohlf, 1995). Dunett's test was also used to compare progeny production between treated and untreated commodities, in the case of progeny production data. No mortality correction was considered necessary because of the very low (<2% for *S. oryzae* and *T. confusum* and <2.8% for *R. dominica*) mortality levels in the control groups.

Table 1
MANOVA parameters for main effects and associated interactions for mortality levels of *R. dominica*, *S. oryzae* and *T. confusum* adults between or within exposure intervals (for all species total df = 593).

Species	Source	df	*F*	*P*
Between exposure intervals				
R. dominica	DE	10	70.7	<0.01
	RH	1	92.1	<0.01
	Temperature	2	3044.5	<0.01
	DE × RH	10	6.9	<0.01
	DE × temperature	20	32.8	<0.01
	RH × temperature	2	14.2	<0.01
S. oryzae	DE	10	35.9	<0.01
	RH	1	340.8	<0.01
	Temperature	2	895.5	<0.01
	DE × RH	10	10.7	<0.01
	DE × temperature	20	9.7	<0.01
	RH × temperature	2	95.1	<0.01
T. confusum	DE	10	420.7	<0.01
	RH	1	161.5	<0.01
	Temperature	2	9959.5	<0.01
	DE × RH	10	1.3	0.22
	DE × temperature	20	100.9	<0.01
	RH × temperature	2	15.2	<0.01
Within exposure intervals				
R. dominica	Exposure × DE	10	14.8	<0.01
	Exposure × RH	1	372.3	<0.01
	Exposure × temperature	2	1253.5	<0.01
	Exposure × DE × RH	10	12.4	<0.01
	Exposure × DE × temperature	20	11.3	<0.01
	Exposure × RH × temperature	2	184.7	<0.01
S. oryzae	Exposure × DE	10	50.3	<0.01
	Exposure × RH	1	56.0	<0.01
	Exposure × temperature	2	2907.1	<0.01
	Exposure × DE × RH	10	5.0	<0.01
	Exposure × DE × temperature	20	28.4	<0.01
	Exposure × RH × temperature	2	3.8	0.02
T. confusum	Exposure × DE	10	115.8	<0.01
	Exposure × RH	1	8.3	<0.01
	Exposure × temperature	2	1562.3	<0.01
	Exposure × DE × RH	10	3.4	<0.01
	Exposure × DE × temperature	20	38.5	<0.01
	Exposure × RH × temperature	2	29.1	< 0.01

2.3. Effectiveness of DE application and impact on bulk density of wheat

The DEs tested here were SilicoSec, FYROM and Slovenia. The tested DEs were applied on hard wheat (var. Mexa) by two different methods: dusting and spraying. The application rates for both methods were 300 ppm and 500 ppm. Spraying of DEs was achieved by preparing aqueous suspensions of 30 mg or 50 mg of each DE made up with distilled water up to 1.5 ml of volume. Each DE suspension, corresponding to each of the doses, was applied to a 1 kg lot of commodity (six 1 kg commodity lots in total). Spraying was carried out using an AG-4 airbrush (Mecafer, Valence, France). Additionally, six 1 kg lots of commodity were separately dusted with 300 ppm and 500 ppm of the tested DEs. Also, a 1 kg lot of each wheat variety was left untreated to serve as a control. Five samples of 100 g were taken from each lot and put into glass vials as described in the Section 2.1. Next, 25 adults of *S. oryzae* and *T. confusum* (obtained from laboratory cultures as described in Section 2.1) were added separately into each vial and all vials were placed inside incubators set at 25 °C and 55% RH. Mortality was assessed after 7 d and 14 d of exposure of the tested species on the sprayed or dusted commodity.

In order to determine the impact of the above DE application methods on the bulk density (test weight) of grain, two 1 kg lots of hard red wheat were sprayed separately with 10 mg (100 ppm) or 30 mg (300 ppm) of each of the above tested DEs in a 1.5 ml aqueous suspension. Furthermore, two 1 kg lots of grain were dusted with 100 ppm and 300 ppm of DE respectively. Additionally, two 1 kg lots of wheat were prepared to serve as controls; the first was left untreated and the second was sprayed with 1.5 ml of distilled water. Each treated and untreated lot was subdivided into 4 samples (of 250 g) and all samples were held at 25 °C and 55% RH for 25 d. The test weight (kg/hl) of each of the samples was determined 30 min, 6 d and 25 d post treatment. After 6 d and 25 d of treatment, the samples were shaken for 30 sec to initiate the unloading and loading of the grain.

Control mortality was very low (<2.5%) for both species and therefore, no correction for mortality was considered necessary. Data were analyzed separately for each species, according to the Repeated Measures Analysis of SAS (SAS Institute, 2000) with exposure interval as the repeated factor. The response variable was insect mortality while DE, dose and application method were the main effects. Also, the 2-way associated interactions of the main effects were incorporated in the analysis. The results for the impact of the DEs on bulk density were subjected to one-way ANOVA for each post treatment period. Means were separated using the Tukey–Kramer (HSD) test at $P = 0.05$ (Sokal and Rohlf, 1995).

2.4. Effectiveness of DEs at surface treatment

In this series of experiments, the tested DEs were those described in Section 2.1 except for FYROM. The tested DEs were added in glass Petri dishes (5.5 cm in diameter) at three different application rates; 5, 10 and 20 g/m^2 equal to 0.001187, 0.002375 and 0.00475 g per Petri dish respectively. Five Petri dishes were treated with each dose of each DE, whereas five Petri dishes were left untreated and served as controls. Then, 10 adults of *T. confusum* (obtained from laboratory cultures as described in Section 2.1) were added into each of the Petri dishes. To prevent insects from

Table 2
Mean mortality (%) of *S. oryzae, R. dominica* and *T. confusum* adults after 7 d and 14 d on wheat treated with ten local DEs and one commercially available DE at 900 ppm. Values in brackets are standard errors (SE). For each exposure interval and insect species, means followed by the same letter do not differ significantly. In all cases df = 10, 593; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	*S. oryzae*		*R. dominica*		*T. confusum*	
7 d	SilicoSec	89.6 (1.7) a	SilicoSec	96.1 (0.7) a	SilicoSec	81.2 (2.2) a
	FYROM	88.3 (1.7) a	FYROM	84.6 (2.8) ab	FYROM	57.1 (4.1) b
	Elassona 1	86.0 (2.1) a	Kolubara	83.3 (2.9) abc	Vranje	48.9 (4.7) bc
	Germany	84.5 (2.3) ab	Vranje 3112107	80.7 (3.5) abc	Kolubara	44.4 (5.3) cd
	Elassona 2	83.5 (2.2) ab	Germany	80.6 (3.3) abc	Elassona 2	44.0 (4.5) cd
	Kolubara	82.0 (2.6) ab	Elassona 1	79.8 (3.5) bc	Vranje 3112107	40.0 (5.6) cde
	Vranje	80.3 (2.8) ab	Elassona 2	78.9 (3.3) bc	Slovenia	38.3 (5.8) cde
	Begora	80.2 (2.3) ab	Crete	75.3 (3.9) bc	Elassona 1	37.2 (6.0) cde
	Slovenia	79.3 (3.0) ab	Vranje	74.4 (3.8) bc	Germany	34.9 (6.2) de
	Vranje 311207	79.2 (2.7) ab	Begora	71.6 (3.6) bc	Begora	32.1 (5.9) de
	Crete	73.0 (2.9) b	Slovenia	68.6 (4.4) c	Kriti	30.4 (5.6) e
14 d	SilicoSec	100.0 (0.0) a	SilicoSec	100.0 (0.0) a	SilicoSec	93.3 (3.5) a
	FYROM	100.0 (0.0) a	FYROM	100.0 (0.0) a	FYROM	91.1 (1.4) a
	Elassona 1	100.0 (0.0) a	Kolubara	100.0 (0.0) a	Kolubara	86.3 (3.8) ab
	Germany	99.6 (0.3) a	Vranje 3112107	100.0 (0.0) a	Vranje	76.9 (3.1) bc
	Elassona 2	99.3 (0.2) a	Germany	100.0 (0.0) a	Elassona 2	69.1 (2.9) bcd
	Kolubara	99.2 (0.3) a	Elassona 1	99.8 (0.1) ab	Vranje 3112107	61.6 (3.3) cd
	Vranje	98.9 (0.3) ab	Elassona 2	99.8 (0.1) ab	Slovenia	60.5 (4.3) cd
	Begora	98.8 (0.4) ab	Vranje	98.2 (0.3) abc	Elassona 1	59.6 (4.1) cd
	Slovenia	97.6 (0.9) ab	Slovenia	97.6 (0.2) abc	Germany	56.9 (4.7) cde
	Vranje 311207	95.3 (2.3) b	Crete	97.3 (0.3) c	Begora	54.3 (4.7) de
	Crete	87.9 (2.6) c	Begora	93.3 (0.2) d	Kriti	40.7 (5.8) e

escaping, the dishes were covered with an empty Petri dish with a 15 mm hole in the middle, which was covered by gauze, to permit sufficient aeration inside the vial. Mortality of the exposed adults, was measured on a daily basis for a total period of 19 d at 25 °C and 65% RH.

To examine variations in mortality of *T. confusum* adults among the exposure periods, data were initially subjected to Repeated Measures Analysis (SAS Institute, 2000) with exposure interval as the repeated factor. The response variable was insect mortality while the main effects were DE and dose. Also, the associated interaction of the main effects were incorporated in the analysis. Means were separated using the Tukey–Kramer (HSD) test at $P = 0.05$ (Sokal and Rohlf, 1995).

3. Results

3.1. Effect of temperature and RH on the efficacy of DEs

Mortality of the tested species was significantly affected by the exposure interval (*S. oryzae*: $F_{1,593} = 4017.8$; $P < 0.01$; *R. dominica*: $F_{1,593} = 4879.8$; $P < 0.01$; *T. confusum*: $F_{1,593} = 6956.1$; $P < 0.01$). Between exposure intervals, all main effects and their associated interactions were significant for mortality levels of *S. oryzae* at $P = 0.05$ (Table 1). In the case of *T. confusum* all main effects and associated interactions for mortality levels between exposure intervals were significant, with the sole exception of RH × DE which was not significant at $P = 0.05$ (Table 1). In addition, all main effects

Table 3
Mean mortality (%) of *S. oryzae, R. dominica* and *T. confusum* adults after 7 d and 14 d on wheat treated with ten local DEs and one commercially available DE at 900 ppm and two RH levels. Values in brackets are standard errors (SE). For each exposure interval and insect species, means followed by the same letter do not differ significantly. In all cases df = 1, 53; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	*S. oryzae*		*R. dominica*		*T. confusum*	
		55%	65%	55%	65%	55%	65%
7 d	Begora	83.3 (3.3) a	77.3 (3.1) a	74.0 (2.1) a	61.1 (2.3) b	34.9 (8.1) a	29.2 (7.6) b
	Elassona 1	89.4 (2.9) a	82.5 (2.9) a	82.0 (2.3) a	71.6 (4.2) b	38.6 (8.1) a	25.1 (3.2) b
	Elassona 2	87.5 (2.9) a	79.6 (3.0) a	80.1 (4.3) a	67.8 (4.5) b	46.3 (3.4) a	41.7 (3.7) b
	FYROM	92.4 (2.1) a	84.2 (2.3) b	85.4 (3.9) a	83.7 (4.1) a	59.7 (5.1) a	54.5 (5.9) a
	Germany	89.6 (2.9) a	79.5 (3.3) b	81.3 (4.5) a	70.3 (4.1) b	35.6 (8.7) a	24.2 (7.1) b
	Kolubara	89.1 (3.0) a	74.8 (3.1) b	86.3 (3.3) a	73.3 (2.2) b	46.4 (7.3) a	43.2 (7.6) b
	Crete	81.4 (2.5) a	64.5 (4.3) b	81.6 (4.1) a	69.7 (6.1) b	33.1 (8.6) a	22.8 (7.1) b
	Slovenia	88.6 (3.1) a	69.9 (4.3) b	70.6 (6.5) a	61.6 (5.5) b	39.7 (8.2) a	36.9 (8.2) a
	Vranje	88.2 (3.0) a	72.3 (3.5) b	74.2 (5.1) a	64.1 (4.1) b	42.4 (8.1) a	37.5 (8.5) a
	Vranje 311207	88.7 (2.7) a	69.8 (3.9) b	88.4 (2.6) a	72.4 (5.1) b	51.6 (6.1) a	46.1 (6.1) b
	SilicoSec	93.1 (1.9) a	86.1 (2.6) b	97.9 (0.8) a	94.2 (1.1) a	86.4 (2.4) a	76.1 (3.5) b
14 d	Begora	95.9 (1.2) a	94.7 (1.5) a	94.5 (1.1) a	92.0 (1.4) a	41.6 (8.1) a	39.7 (7.1) a
	Elassona 1	100.0 (0.0) a	99.6 (0.3) a	100.0 (0.0) a	99.5 (0.3) a	65.4 (4.1) a	57.5 (3.4) b
	Elassona 2	99.1 (0.4) a	98.7 (0.4) a	99.1 (0.3) a	98.6 (0.4) a	64.3 (4.3) a	51.5 (3.3) b
	FYROM	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a	92.9 (1.7) a	90.3 (2.9) a
	Germany	99.1 (0.5) a	98.4 (0.6) a	99.1 (0.5) a	98.3 (0.6) a	58.2 (6.3) a	50.4 (6.1) a
	Kolubara	100.0 (0.0) a	98.2 (0.5) a	100.0 (0.0) a	99.3 (0.4) a	89.3 (2.1) a	83.2 (2.8) a
	Crete	96.7 (0.9) a	79.1 (4.3) b	98.1 (0.6) a	96.4 (1.1) a	61.9 (5.6) a	58.5 (5.8) b
	Slovenia	98.2 (0.7) a	97.1 (1.1) a	98.2 (0.7) a	97.1 (1.1) a	73.3 (4.4) a	65.8 (5.1) b
	Vranje	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a	61.2 (6.3) a	52.5 (6.1) a
	Vranje 311207	99.5 (0.2) a	96.9 (1.2) a	99.5 (0.2) a	96.6 (1.2) a	75.8 (4.1) a	58.2 (4.9) b
	SilicoSec	100.0 (0.0) a	98.4 (0.6) a	100.0 (0.0) a	100.0 (0.0) a	97.6 (0.6) a	89.4 (2.9) b

Table 4
Mean mortality (%) of *S. oryzae* adults after 7 d and 14 d on wheat treated with ten local DEs and one commercially available DE at 900 ppm and three temperature levels. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 2, 53; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Temperature		
		20 °C	25 °C	30 °C
7 d	Begora	58.8 (1.3) c	85.1 (1.2) b	96.8 (1.1) a
	Elassona 1	67.9 (1.4) c	90.0 (2.9) b	100.0 (0.0) a
	Elassona 2	65.9 (1.2) c	84.7 (2.9) b	100.0 (0.0) a
	FYROM	75.4 (0.8) c	89.4 (2.8) b	100.0 (0.0) a
	Germany	66.9 (1.5) c	88.7 (3.8) b	100.0 (0.0) a
	Kolubara	65.6 (1.2) c	80.3 (5.2) b	100.0 (0.0) a
	Crete	52.3 (4.4) c	75.5 (2.9) b	93.2 (1.1) a
	Slovenia	56.7 (2.3) c	81.1 (4.5) b	100.0 (0.0) a
	Vranje	60.6 (2.3) c	80.3 (4.2) b	100.0 (0.0) a
	Vranje 311207	60.3 (2.7) c	79.2 (4.4) b	98.1 (1.1) a
	SilicoSec	74.2 (1.8) c	94.7 (1.5) b	100.0 (0.0) a
14 d	Begora	86.0 (1.1) b	100.0 (0.0) a	100.0 (0.0) a
	Elassona 1	99.3 (0.4) b	100.0 (0.0) a	100.0 (0.0) a
	Elassona 2	96.7 (0.4) b	100.0 (0.0) a	100.0 (0.0) a
	FYROM	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a
	Germany	96.3 (1.1) b	100.0 (0.0) a	100.0 (0.0) a
	Kolubara	98.9 (0.6) b	98.4 (0.5) ab	100.0 (0.0) a
	Crete	71.2 (6.1) c	92.4 (1.4) b	100.0 (0.0) a
	Slovenia	97.3 (1.1) ab	96.0 (1.5) b	100.0 (0.0) a
	Vranje	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a
	Vranje 311207	99.2 (0.3) a	95.4 (1.7) b	100.0 (0.0) a
	SilicoSec	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a

Table 5
Mean mortality (%) of *R. dominica* adults after 7 d and 14 d on wheat treated with ten local DEs and one commercially available DE at 900 ppm and three temperature levels. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 2, 53; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Temperature		
		20 °C	25 °C	30 °C
7 d	Begora	32.5 (1.2) c	86.0 (1.0) b	93.3 (1.9) a
	Elassona 1	44.5 (1.2) c	94.8 (1.1) b	100.0 (0.0) a
	Elassona 2	45.9 (1.1) c	90.9 (1.0) b	100.0 (0.0) a
	FYROM	55.3 (0.9) b	98.8 (0.4) a	100.0 (0.0) a
	Germany	47.4 (1.5) c	94.4 (1.1) b	100.0 (0.0) a
	Kolubara	56.2 (3.1) b	93.8 (1.6) a	100.0 (0.0) a
	Crete	36.3 (2.8) b	91.7 (1.6) a	97.8 (0.9) a
	Slovenia	28.9 (0.9) c	77.9 (5.1) b	99.0 (0.6) a
	Vranje	35.6 (0.9) c	87.9 (1.1) b	100.0 (0.0) a
	Vranje 311207	49.2 (4.9) b	93.8 (1.2) a	99.1 (0.7) a
	SilicoSec	92.0 (1.1) c	96.2 (1.3) b	100.0 (0.0) a
14 d	Begora	86.0 (1.1) c	93.8 (0.9) b	100.0 (0.0) a
	Elassona 1	99.3 (0.4) b	100.0 (0.0) a	100.0 (0.0) a
	Elassona 2	96.7 (0.4) b	100.0 (0.0) a	100.0 (0.0) a
	FYROM	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a
	Germany	96.3 (1.1) b	100.0 (0.0) a	100.0 (0.0) a
	Kolubara	98.9 (0.6) b	100.0 (0.0) a	100.0 (0.0) a
	Crete	91.9 (1.1) b	100.0 (0.0) a	100.0 (0.0) a
	Slovenia	92.9 (1.4) b	100.0 (0.0) a	100.0 (0.0) a
	Vranje	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a
	Vranje 311207	94.7 (1.7) b	100.0 (0.0) a	100.0 (0.0) a
	SilicoSec	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a

and associated interactions were significant within exposure intervals at $P = 0.05$ and this was the case for all of the tested species (Table 1).

After 7 d of exposure, mortality ranged from 73.0% to 88.3% for *S. oryzae*, 68.6%–84.6% for *R. dominica* and 30.4%–57.1% for *T. confusum* on wheat treated with the mined DEs. In the case of SilicoSec, mortality of all tested species was higher than with the mined DEs (Table 2). After 14 d of exposure, mortality of all species increased further on wheat treated with the DEs and in several cases approached 100%. Adults of *S. oryzae* were very susceptible to all of the DEs tested. However, the mortality of *S. oryzae* adults was significantly higher on wheat treated with FYROM than with Crete after 7 d and 14 d of exposure (Table 2). FYROM, was ranked among the most effective DEs against all the tested species while Crete was the least effective DE. The mortality of *R. dominica* adults was significantly higher on wheat treated with FYROM than those treated with Slovenia and Crete or Begora after 7 d and 14 d of exposure respectively (Table 2). Adults of *T. confusum* were the least susceptible of the tested species to the mined DEs and SilicoSec. Like in the case of *S. oryzae*, the mortality of *T. confusum* adults was significantly higher on wheat treated with FYROM than with Crete after 7 d and 14 d of exposure.

After 7 d of exposure, mortality of adults of the three tested species on wheat treated with all DEs decreased significantly with the increase of RH with the exception of Begora, Elassona 1 and Elassona 2 in the case of *S. oryzae*, FYROM and Silcosec in the case of *R. dominica*, FYROM and Slovenia in the case of *T. confusum* where the decrease was not significant (Table 3). After 14 d of exposure, mortality of the three tested species on wheat treated with all DEs was increased at both levels of RH.

The mortality of the tested species on wheat treated with all DEs increased, with the increase of temperature except for *S. oryzae* treated with Kolubara and Slovenia at 20 °C–25 °C (Tables 4–6). At 20 °C, after 7 d of exposure on wheat treated with all DEs, the mortality of *S. oryzae* adults ranged from 52.3% (Crete) to 75.4% (FYROM) while it was >93% at 30 °C for all DEs (Table 4). After 14 d of exposure, the mortality of *S. oryzae* adults on wheat treated with all DEs >71% regardless of temperature (Table 4). At 20 °C, after 7 d of exposure in wheat treated with all mined DEs the mortality of *R. dominica* adults, ranged from 28.9% (Slovenia) to 56.2% (Kolubara) but it did not fall below 77% at 25 °C for all DEs (Table 5). At the same exposure interval, more than 93% of the treated *R. dominica* adults died at 30 °C. After 14 d of exposure on wheat treated with all DEs, the mortality ranged from 86.0% to 100% for *R. dominica* adults as temperature increased from 20 to 30 °C (Table 5). At 20 °C, after 7 d of exposure on wheat treated with the mined DEs the mortality of *T. confusum* adults didn't exceed 36% (Table 5). For this species also, the

Table 6
Mean mortality (%) of *T. confusum* adults after 7 d and 14 d on wheat treated with ten local DEs and one commercially available DE at 900 ppm and three temperature levels. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 2, 53; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Temperature		
		20 °C	25 °C	30 °C
7 d	Begora	0.7 (0.2) b	3.0 (0.6) b	92.7 (1.9) c
	Elassona 1	3.0 (0.3) c	9.0 (0.9) b	99.4 (0.3) a
	Elassona 2	9.3 (1.1) c	23.7 (3.2) b	99.0 (0.4) a
	FYROM	35.6 (1.3) b	35.9 (1.8) b	99.0 (0.3) a
	Germany	1.1 (0.3) c	4.1 (0.7) b	99.6 (0.2) a
	Kolubara	14.7 (0.9) c	20.3 (1.2) b	99.3 (0.3) a
	Crete	0.4 (0.2) c	3.1 (0.6) b	87.7 (2.1) a
	Slovenia	4.8 (0.6) c	11.6 (0.4) b	99.6 (0.4) a
	Vranje	3.0 (0.5) c	17.2 (2.2) b	99.7 (0.2) a
	Vranje 311207	18.9 (1.2) c	30.7 (1.3) b	97.1 (0.9) a
	SilicoSec	65.9 (2.2) c	78.2 (0.3) b	99.6 (0.3) a
14 d	Begora	6.1 (0.6) c	15.9 (2.3) b	100.0 (0.0) a
	Elassona 1	24.2 (2.4) c	60.5 (2.7) b	100.0 (0.0) a
	Elassona 2	28.3 (1.6) c	53.1 (4.2) b	100.0 (0.0) a
	FYROM	79.7 (1.7) c	95.2 (1.1) b	100.0 (0.0) a
	Germany	23.1 (1.7) c	39.9 (4.3) b	100.0 (0.0) a
	Kolubara	71.3 (2.1) c	87.4 (1.4) b	100.0 (0.0) a
	Crete	35.3 (1.7) c	45.3 (3.1) b	100.0 (0.0) a
	Slovenia	43.9 (2.1) c	64.8 (1.9) b	100.0 (0.0) a
	Vranje	21.2 (1.3) c	49.3 (4.0) b	100.0 (0.0) a
	Vranje 311207	43.3 (2.1) c	72.8 (1.8) b	100.0 (0.0) a
	SilicoSec	81.7 (2.9) c	98.3 (0.5) b	100.0 (0.0) a

Table 7
MANOVA parameters for main effects and associated interactions for mortality levels of *R. dominica*, *S. oryzae* and *T. confusum* adults between or within exposure intervals (for all species total df = 791).

Species	Source	df	*F*	*P*
Between exposure intervals				
R. dominica	Commodity	3	4427.8	<0.01
	DE	10	291.5	<0.01
	Dose	1	6742.3	<0.01
	Commodity × DE	30	42.2	<0.01
	Commodity × dose	3	68.1	<0.01
	DE × dose	10	14.9	<0.01
S. oryzae	Commodity	3	2341.1	<0.01
	DE	10	132.6	<0.01
	Dose	1	6694.1	<0.01
	Commodity × DE	30	17.4	<0.01
	Commodity × dose	3	36.5	<0.01
	DE × dose	10	12.9	<0.01
T. confusum	Commodity	3	622.4	<0.01
	DE	10	692.7	<0.01
	Dose	1	970.0	<0.01
	Commodity × DE	30	9.3	<0.01
	Commodity × dose	3	6.2	<0.01
	DE × dose	10	39.2	<0.01
Within exposure intervals				
R. dominica	Exposure × commodity	3	205.0	<0.01
	Exposure × DE	10	16.2	<0.01
	Exposure × dose	1	32.2	<0.01
	Exposure × commodity × DE	30	15.2	<0.01
	Exposure × commodity × dose	3	150.4	<0.01
	Exposure × DE × dose	10	21.3	<0.01
S. oryzae	Exposure × commodity	3	283.5	<0.01
	Exposure × DE	10	12.9	<0.01
	Exposure × dose	1	83.9	<0.01
	Exposure × commodity × DE	30	6.5	<0.01
	Exposure × commodity × dose	3	73.5	<0.01
	Exposure × DE × dose	10	16.2	<0.01
T. confusum	Exposure × commodity	3	506.9	<0.01
	Exposure × DE	10	249.0	<0.01
	Exposure × dose	1	3.3	0.07
	Exposure × commodity × DE	30	12.0	<0.01
	Exposure × commodity × dose	3	1.7	0.16
	Exposure × DE × dose	10	7.5	<0.01

mortality increased with the increase of temperature. Although mortality at 25 °C after 14 d exposure of adults on wheat treated with the mined DEs, ranged from 15.9% (Begora) to 95.2% (FYROM) it was consistently 100% at 30 °C (Table 6).

3.2. Effectiveness of DEs in different grain commodities

Mortality of the tested species was significantly affected by the exposure interval (*S. oryzae*: $F_{1,791} = 5706.3$; $P < 0.01$; *R. dominica*: $F_{1,791} = 6336.9$; $P < 0.01$; *T. confusum*: $F_{1,791} = 13{,}234.3$; df = 1, 791; $P < 0.01$). All main effects and associated interactions for mortality levels of *R. dominica* and *S. oryzae* were significant either between or within exposure intervals at $P = 0.05$ (Table 7). All main effects and associated interactions for mortality levels of *T. confusum* were significant between exposure intervals at $P = 0.05$ (Table 7). All main effects and associated interactions for mortality levels of *T. confusum* were significant within exposure intervals with the exception of exposure × dose and exposure × dose × commodity, which were not significant, all the remainder interactions were significant at $P = 0.05$ (Table 7).

In the case of *S. oryzae*, more adults were dead on treated barley than on treated wheat, maize or rice except in the case of Crete at 7 d of exposure where more adults were dead in wheat than in the other commodities (Table 8). When *S. oryzae* adults were exposed for 7 d in grains treated with 500 ppm of mined DEs, mortality ranged from 7.3% (Crete) to 28.9% (Elassona 1) while after 14 d of exposure it ranged from 25.8% (Crete) to 58.7% (Elassona 2). In contrast, after 7 d or 14 d of exposure, mortality of *S. oryzae* adults in rice treated with 500 ppm of mined DEs didn't exceed 47.1% (FYROM) and 79.3% (Elassona 1), respectively (Table 8). Increasing exposure and dose rate produced higher mortality to *S. oryzae* adults in all of the treated grains. After 14 d of exposure, mortality of *S. oryzae* adults in barley or wheat treated with 1000 ppm of all DEs was 100%, except in the case of Crete where mortality did not exceed 95% and 96%, respectively (Table 9).

Table 8
Mean mortality (%) of *S. oryzae* adults after 7 d and 14 d on four commodities treated with ten local DEs and one commercially available DE at 500 ppm. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 3, 35; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Commodity			
		Barley	Wheat	Maize	Rice
7 d	Begora	60.4 (1.8) a	56.4 (1.6) a	18.7 (1.7) c	32.9 (1.9) b
	Elassona 1	61.3 (0.3) a	59.3 (3.9) a	28.9 (2.1) c	40.9 (2.1) b
	Elassona 2	67.6 (1.5) a	60.2 (2.6) b	26.9 (1.7) d	38.4 (1.8) c
	FYROM	73.1 (0.5) a	59.3 (2.3) b	25.8 (1.4) d	47.1 (2.2) c
	Germany	72.7 (0.6) a	55.3 (3.1) b	27.1 (1.9) d	40.7 (2.4) c
	Kolubara	72.7 (1.7) a	61.2 (2.6) b	25.8 (1.3) d	39.3 (2.0) c
	Crete	54.8 (3.1) a	50.0 (2.4) a	7.3 (2.9) c	39.1 (2.2) b
	Slovenia	71.0 (2.3) a	44.2 (1.8) b	9.6 (1.1) d	37.8 (1.6) c
	Vranje	59.8 (3.2) a	48.9 (2.5) b	16.7 (4.6) d	38.4 (1.6) c
	Vranje 311207	68.9 (0.6) a	55.8 (2.8) b	12.2 (2.3) d	41.1 (2.2) c
	SilicoSec	78.6 (0.9) a	69.8 (1.1) b	31.1 (1.9) d	51.3 (2.0) c
14 d	Begora	78.2 (1.7) a	81.3 (1.7) a	56.2 (1.4) c	69.8 (1.8) b
	Elassona 1	89.8 (2.2) a	82.7 (1.1) b	50.7 (0.7) d	79.3 (2.0) c
	Elassona 2	87.8 (2.2) a	80.4 (1.5) b	58.7 (2.8) d	72.3 (0.7) c
	FYROM	89.3 (2.9) a	78.2 (1.2) b	53.6 (2.3) d	70.0 (2.0) c
	Germany	90.9 (2.6) a	82.0 (1.9) b	67.1 (1.6) d	76.6 (1.6) c
	Kolubara	87.8 (2.4) a	79.8 (2.2) b	55.1 (3.0) c	55.6 (1.3) c
	Crete	72.2 (2.0) a	65.8 (3.0) a	25.8 (2.8) c	52.2 (1.0) b
	Slovenia	90.2 (2.2) a	66.9 (1.4) b	24.7 (2.2) d	55.6 (1.3) c
	Vranje	83.6 (3.1) a	68.4 (1.0) b	35.8 (1.4) d	50.4 (1.4) c
	Vranje 311207	79.6 (2.8) a	73.6 (1.9) a	48.4 (1.9) b	59.8 (1.7) b
	SilicoSec	100.0 (0.0) a	100.0 (0.0) a	69.1 (1.7) c	83.8 (1.2) b

Table 9
Mean mortality (%) of *S. oryzae* adults after 7 d and 14 d on four commodities treated with ten local DEs and one commercially available DE at 1000 ppm. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 3, 35; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Commodity			
		Barley	Wheat	Maize	Rice
7 d	Begora	88.4 (1.8) a	84.0 (1.2) b	46.7 (1.7) d	60.9 (1.9) c
	Elassona 1	99.3 (0.3) a	92.7 (1.6) b	56.9 (2.1) d	68.9 (2.1) c
	Elassona 2	95.6 (1.5) a	88.4 (2.2) b	54.9 (1.7) d	66.9 (1.9) c
	FYROM	100.0 (0.0) a	97.3 (1.3) a	63.8 (1.4) c	77.1 (0.5) b
	Germany	100.0 (0.0) a	93.6 (1.3) b	55.1 (1.9) d	68.7 (2.4) c
	Kolubara	100.0 (0.0) a	89.3 (2.0) b	53.8 (1.3) d	67.3 (2.0) c
	Crete	83.3 (3.4) a	86.4 (2.1) a	27.5 (5.2) c	67.1 (2.2) b
	Slovenia	100.0 (0.0) a	73.1 (1.2) a	37.6 (1.1) c	65.8 (1.6) b
	Vranje	98.2 (0.6) a	86.7 (3.0) b	44.7 (4.6) d	66.4 (1.6) c
	Vranje 311207	96.9 (0.6) a	91.3 (2.0) a	41.6 (3.0) c	69.1 (2.2) b
	SilicoSec	100.0 (0.0) a	100.0 (0.0) a	59.1 (1.9) c	89.3 (2.0) b
14 d	Begora	100.0 (0.0) a	100.0 (0.0) a	74.2 (1.4) c	87.8 (1.8) b
	Elassona 1	100.0 (0.0) a	100.0 (0.0) a	88.7 (0.7) b	100.0 (0.0) a
	Elassona 2	100.0 (0.0) a	100.0 (0.0) a	86.0 (0.5) c	97.3 (0.7) b
	FYROM	100.0 (0.0) a	100.0 (0.0) a	90.0 (1.5) b	100.0 (0.0) a
	Germany	100.0 (0.0) a	100.0 (0.0) a	85.1 (1.6) b	97.6 (1.6) a
	Kolubara	100.0 (0.0) a	100.0 (0.0) a	86.9 (1.3) b	97.8 (1.3) a
	Crete	94.4 (2.1) a	95.6 (1.6) a	38.1 (5.5) c	89.3 (2.0) b
	Slovenia	100.0 (0.0) a	100.0 (0.0) a	81.1 (2.4) b	100.0 (0.0) a
	Vranje	100.0 (0.0) a	100.0 (0.0) a	90.3 (1.3) b	100.0 (0.0) a
	Vranje 311207	100.0 (0.0) a	100.0 (0.0) a	80.9 (2.2) b	100.0 (0.0) a
	SilicoSec	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a

Table 10
Mean mortality (%) of *R. dominica* adults after 7 d and 14 d on four commodities treated with ten local DEs and one commercially available DE at 500 ppm. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 3, 35; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Commodity			
		Barley	Wheat	Maize	Rice
7 d	Begora	60.0 (1.4) a	53.8 (1.8) b	8.7 (1.7) c	6.2 (1.7) c
	Elassona 1	69.1 (0.5) a	52.4 (1.8) b	2.2 (2.9) d	20.9 (2.1) c
	Elassona 2	65.3 (0.8) a	45.6 (2.2) b	6.9 (1.7) d	18.9 (1.9) c
	FYROM	71.3 (0.3) a	50.7 (1.7) b	15.8 (1.4) d	28.9 (0.6) c
	Germany	67.3 (1.6) a	45.1 (1.8) b	18.2 (1.8) c	20.7 (2.4) c
	Kolubara	70.4 (0.8) a	32.4 (2.9) b	13.8 (4.7) c	32.7 (4.2) b
	Crete	68.7 (0.7) a	35.6 (2.2) b	7.6 (1.2) d	19.1 (2.2) c
	Slovenia	56.2 (7.3) a	32.9 (1.8) b	4.4 (0.6) c	6.9 (0.6) c
	Vranje	61.1 (1.6) a	38.4 (2.6) b	9.6 (0.9) c	5.6 (1.6) c
	Vranje 311207	68.0 (1.2) a	33.6 (1.4) b	11.3 (2.6) c	31.1 (2.2) b
	SilicoSec	76.2 (2.6) a	61.3 (1.7) b	48.2 (1.2) c	55.8 (1.9) b
14 d	Begora	77.8 (1.1) a	73.8 (1.4) ab	56.2 (1.4) c	69.8 (1.8) b
	Elassona 1	89.3 (2.0) a	80.0 (1.5) b	34.2 (1.4) c	37.1 (2.5) c
	Elassona 2	78.4 (2.5) a	80.9 (2.5) a	28.9 (1.8) c	44.4 (2.8) b
	FYROM	89.3 (2.2) a	85.6 (1.5) a	45.6 (2.3) c	60.2 (1.5) b
	Germany	88.4 (2.2) a	82.4 (1.8) a	23.6 (1.4) b	48.2 (2.0) c
	Kolubara	92.2 (2.1) a	84.2 (2.0) b	36.2 (2.0) d	65.3 (2.7) c
	Crete	84.4 (1.4) a	77.8 (2.1) a	12.4 (1.3) c	35.1 (2.2) b
	Slovenia	77.1 (2.4) a	66.4 (2.6) b	9.6 (2.0) c	17.6 (2.7) b
	Vranje	80.0 (1.6) a	73.8 (2.5) a	12.4 (2.0) c	20.7 (2.0) b
	Vranje 311207	94.2 (1.7) a	80.4 (2.1) b	29.3 (2.7) d	67.3 (1.8) c
	SilicoSec	100.0 (0.0) a	97.6 (1.1) a	70.4 (2.0) c	90.7 (2.9) b

In the case of *R. dominica*, more adults were dead in treated barley than in treated wheat, maize or rice (Table 10). When *R. dominica* adults were exposed for 7 d in grains treated with 500 ppm of the mined DEs, adult mortality didn't exceed 71.3% (FYROM), 53.8% (Begora), 18.2% (Germany), and 32.7% (Kolubara) in the case of treated barley, wheat, maize and rice, respectively (Table 10). After 14 d of exposure, mortality of *R. dominica* adults in all commodities treated with 1000 ppm of all DEs increased further and reached 100% in the case of treated barley or wheat, with the exception of Begora (Table 11).

Table 11
Mean mortality (%) of *R. dominica* adults after 7 d and 14 d on four commodities treated with ten local DEs and one commercially available DE at 1000 ppm. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 3, 35; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Commodity			
		Barley	Wheat	Maize	Rice
7 d	Begora	88.0 (1.4) a	81.6 (2.0) a	26.7 (1.7) c	34.2 (1.7) b
	Elassona 1	97.1 (0.5) a	80.7 (1.8) b	30.2 (2.9) c	48.9 (2.1) d
	Elassona 2	93.3 (0.8) a	83.8 (2.8) b	34.9 (1.7) d	46.9 (1.9) c
	FYROM	99.3 (0.3) a	88.9 (2.3) b	43.8 (1.4) d	56.9 (1.6) c
	Germany	95.3 (1.6) a	83.3 (2.5) b	36.2 (1.8) d	48.7 (2.4) c
	Kolubara	98.4 (0.6) a	80.7 (1.8) b	41.8 (4.7) d	60.7 (4.2) c
	Crete	96.7 (0.7) a	73.9 (2.1) b	15.6 (1.2) d	47.1 (2.2) c
	Slovenia	92.9 (1.7) a	62.2 (2.3) b	18.9 (1.4) d	26.9 (2.1) c
	Vranje	89.1 (1.6) a	82.7 (1.9) b	27.6 (0.9) d	33.6 (1.6) c
	Vranje 311207	96.0 (1.2) a	79.8 (2.2) b	19.3 (2.6) d	69.1 (2.2) c
	SilicoSec	100.0 (0.0) a	100.0 (0.0) a	80.2 (1.4) b	93.8 (1.9) b
14 d	Begora	95.8 (1.1) a	91.8 (1.4) a	66.7 (1.2) c	84.2 (1.4) b
	Elassona 1	100.0 (0.0) a	100.0 (0.0) a	78.0 (2.1) c	90.9 (2.2) b
	Elassona 2	100.0 (0.0) a	100.0 (0.0) a	76.4 (2.8) b	96.7 (0.9) a
	FYROM	100.0 (0.0) a	100.0 (0.0) a	80.9 (2.4) b	100.0 (0.0) a
	Germany	100.0 (0.0) a	100.0 (0.0) a	70.4 (1.9) b	100.0 (0.0) a
	Kolubara	100.0 (0.0) a	100.0 (0.0) a	65.3 (2.9) b	100.0 (0.0) a
	Crete	100.0 (0.0) a	100.0 (0.0) a	39.3 (1.7) c	68.2 (2.0) b
	Slovenia	100.0 (0.0) a	100.0 (0.0) a	43.3 (2.5) c	70.7 (1.8) b
	Vranje	100.0 (0.0) a	100.0 (0.0) a	59.1 (2.9) c	78.9 (2.9) b
	Vranje 311207	100.0 (0.0) a	100.0 (0.0) a	60.4 (2.2) c	95.1 (1.4) b
	SilicoSec	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a	100.0 (0.0) a

Table 12
Mean mortality (%) of *T. confusum* adults after 7 d and 14 d on four commodities treated with ten local DEs and one commercially available DE at 500 ppm. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 3, 35; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Commodity			
		Barley	Wheat	Maize	Rice
7 d	Begora	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a
	Elassona 1	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a
	Elassona 2	12.0 (5.0) a	1.8 (0.7) b	0.0 (0.0) b	0.0 (0.0) b
	FYROM	22.0 (2.1) a	3.8 (1.5) b	6.0 (1.5) b	1.1 (0.4) b
	Germany	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a
	Kolubara	5.3 (1.8) a	0.9 (0.7) b	0.0 (0.0) b	0.0 (0.0) b
	Crete	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a
	Slovenia	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a	0.0 (0.0) a
	Vranje	8.0 (2.0) a	0.0 (0.0) b	0.0 (0.0) b	0.0 (0.0) b
	Vranje 311207	10.0 (1.2) a	7.3 (1.9) ab	1.6 (0.6) c	3.6 (0.3) bc
	SilicoSec	57.6 (3.4) a	42.7 (3.2) b	30.2 (1.5) c	35.6 (2.3) bc
14 d	Begora	12.0 (2.9) a	10.7 (1.5) ab	3.6 (0.6) c	5.3 (0.7) bc
	Elassona 1	51.3 (3.2) a	42.2 (2.9) b	5.1 (1.7) d	22.0 (3.3) c
	Elassona 2	44.7 (6.1) a	29.1 (4.1) b	5.3 (0.6) d	19.7 (1.1) c
	FYROM	78.2 (2.0) a	76.4 (1.2) a	28.4 (2.2) c	34.9 (2.1) b
	Germany	38.7 (4.7) a	24.4 (2.8) b	6.7 (1.2) c	21.1 (2.3) b
	Kolubara	74.7 (1.5) a	65.3 (1.2) b	41.3 (1.5) d	58.9 (1.7) c
	Crete	39.3 (3.8) a	34.4 (3.4) ab	17.6 (2.0) c	31.7 (2.0) b
	Slovenia	56.6 (4.2) a	47.6 (1.8) a	18.9 (1.1) d	40.4 (1.6) c
	Vranje	43.3 (5.0) a	26.4 (3.5) b	3.6 (1.1) d	17.8 (1.5) c
	Vranje 311207	61.3 (1.5) a	48.2 (1.3) b	12.4 (3.2) d	30.2 (1.7) c
	SilicoSec	82.0 (1.8) a	78.7 (0.6) a	42.4 (2.7) c	74.9 (2.2) b

Similarly, the grain type influenced the efficacy of the tested DEs against adults of *T. confusum* as well. When *T. confusum* adults were exposed for 7 d or 14 d in grains treated with 500 ppm or 1000 ppm of all DEs, adult mortality was higher in barley than in the other commodities (Tables 12, 13). However, this species appeared to be less susceptible to DEs than *S. oryzae* and *R. dominica*. Thus, after 7 d or 14 d of exposure in grains treated with 500 ppm of the mined DEs, mortality did not exceed 22.0% (FYROM) or 74.7% (Kolubara) (Tables 12, 13). The respective mortality values in the case of 1000 ppm of the mined DEs were 40.0% or 96.0% (FYROM). In all

Table 13
Mean mortality (%) of *T. confusum* adults after 7 d and 14 d on four commodities treated with ten local DEs and one commercially available DE at 1000 ppm. Values in brackets are standard errors (SE). For each exposure interval and DE, means followed by the same letter do not differ significantly. In all cases df = 3, 35; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Commodity			
		Barley	Wheat	Maize	Rice
7 d	Begora	4.0 (1.0) a	2.0 (0.3) ab	0.4 (0.3) b	0.9 (0.5) b
	Elassona 1	12.0 (1.0) a	6.0 (0.7) b	2.2 (0.5) c	3.8 (0.2) bc
	Elassona 2	26.7 (6.2) a	20.7 (2.1) a	6.4 (0.4) c	12.2 (1.6) b
	FYROM	40.0 (2.1) a	31.8 (2.3) b	24.0 (1.5) c	39.1 (1.8) ab
	Germany	5.3 (1.2) a	2.9 (0.4) ab	0.7 (0.5) b	1.6 (0.3) b
	Kolubara	22.7 (2.0) a	18.0 (1.9) ab	12.9 (1.5) b	16.4 (1.6) b
	Crete	3.3 (1.2) a	2.9 (0.4) ab	0.7 (0.3) b	0.2 (0.2) b
	Slovenia	12.7 (0.7) a	10.4 (0.3) ab	3.1 (0.7) b	6.4 (0.7) ab
	Vranje	22.7 (3.7) a	11.8 (1.0) b	1.3 (0.7) d	4.7 (0.3) c
	Vranje 311207	34.0 (1.2) a	27.3 (1.9) b	16.9 (1.8) c	20.9 (1.6) c
	SilicoSec	85.6 (3.4) a	73.6 (2.3) b	58.2 (1.5) c	70.3 (3.2) b
14 d	Begora	17.3 (4.2) a	14.4 (2.4) b	4.7 (0.3) c	7.6 (0.9) c
	Elassona 1	67.3 (3.5) a	53.6 (3.0) b	9.6 (2.4) d	28.9 (4.0) c
	Elassona 2	60.0 (7.4) a	46.2 (3.5) b	13.6 (0.9) d	33.1 (2.2) c
	FYROM	96.0 (2.0) a	94.4 (1.2) a	66.4 (2.2) c	82.9 (2.5) b
	Germany	48.7 (3.4) a	31.1 (5.0) b	10.2 (1.5) c	26.0 (3.0) b
	Kolubara	92.0 (1.5) a	82.9 (1.0) b	56.7 (3.1) c	76.0 (2.0) b
	Crete	48.0 (5.5) a	42.7 (3.4) ab	22.9 (2.4) c	37.8 (2.5) b
	Slovenia	68.0 (2.9) a	61.6 (2.4) a	26.0 (1.4) c	51.8 (1.7) b
	Vranje	58.7 (6.3) a	40.0 (3.2) b	7.6 (0.8) d	24.9 (2.0) c
	Vranje 311207	79.3 (1.5) a	66.2 (1.3) b	28.7 (3.4) d	48.0 (1.6) c
	SilicoSec	100.0 (0.0) a	96.7 (0.6) ab	60.4 (2.7) c	92.9 (0.7) b

Table 14
Mean number of *S. oryzae* per vial on four commodities treated with ten local DEs and one commercially available DE at three doses (inclusive of 0 ppm) 50 d after the removal of the parental adults. Values in brackets are standard errors (SE). Within each commodity and dose, means followed by an asterisk do not differ significantly from the mean of control; Dunnet's test at $P = 0.05$.

Dose (ppm)	DE	Commodity			
		Barley	Wheat	Maize	Rice
0	Control	9.1 (1.1)	18.2 (4.4)	16.0 (3.9)	10.5 (0.8)
500	Begora	4.7 (1.3)	8.2 (1.3)	8.6 (0.8)	6.4 (1.0)
	Elassona 1	3.3 (0.3)	4.1 (0.6)	6.4 (1.1)	6.6 (1.2)
	Elassona 2	4.7 (0.6)	10.2 (1.6)	10.1 (0.8)	7.1 (0.9)
	FYROM	3.2 (0.5)	7.0 (1.3)	7.8 (0.5)	6.4 (0.9)
	Germany	3.4 (0.3)	11.3 (1.6)	11.0 (1.1)	5.6 (0.7)
	Kolubara	3.4 (0.3)	6.1 (1.1)	7.1 (1.4)	5.4 (1.3)
	Crete	8.0 (1.4)*	11.2 (1.0)	14.4 (0.8)*	10.3 (0.7)*
	Slovenia	3.6 (0.3)	9.6 (1.1)	8.9 (0.6)	8.8 (1.0)
	Vranje	4.6 (0.6)	8.3 (1.2)	7.3 (1.3)	10.4 (0.8)*
	Vranje 311207	3.8 (0.4)	14.4 (0.7)*	11.4 (0.7)	8.3 (1.2)
	SilicoSec	2.8 (0.4)	2.2 (0.3)	1.9 (2.2)	2.2 (1.2)
1000	Begora	2.7 (0.2)	6.2 (1.3)	6.6 (0.6)	4.4 (0.9)
	Elassona 1	1.3 (0.2)	2.1 (0.6)	4.4 (1.0)	4.4 (1.0)
	Elassona 2	2.7 (0.4)	8.2 (1.6)	8.1 (0.9)	5.1 (0.7)
	FYROM	1.2 (0.3)	5.0 (1.3)	5.8 (0.5)	4.4 (0.8)
	Germany	1.4 (0.2)	9.3 (1.5)	9.0 (1.0)	3.6 (0.6)
	Kolubara	1.4 (0.3)	4.1 (0.9)	5.1 (1.4)	3.4 (1.2)
	Crete	6.0 (1.5)	9.2 (0.9)	12.4 (0.8)*	13.3 (0.7)
	Slovenia	1.6 (0.2)	7.6 (1.0)	6.9 (0.4)	6.8 (0.8)
	Vranje	2.6 (0.4)	6.3 (1.1)	5.3 (1.1)	8.4 (0.7)
	Vranje 311207	1.8 (0.2)	12.4 (0.7)*	9.4 (0.7)	6.3 (1.1)
	SilicoSec	0.8 (0.3)	0.5 (0.4)	0.4 (0.1)	0.6 (0.2)

cases however, more *T. confusum* adults were dead in grains treated with SilicoSec than with the mined DEs (Tables 12, 13).

DEs and SilicoSec didn't suppress progeny production of *S. oryzae* or *R. dominica* in the treated commodities (Tables 14, 15). However, in the majority of cases, significantly more F1 adults were counted in the treated substrates than in controls. In the case of *T. confusum* very few progeny were found either in treated or untreated commodities, and thus, only in a very few cases progeny production in treated substrates varied significantly from that recorded in the controls (Table 16).

Table 15
Mean number of *R. dominica* per vial on four commodities treated with ten local DEs and one commercially available DE at three doses 50 d after the removal of the parental adults. Values in brackets are standard errors (SE). Within each commodity and dose, means followed by an asterisk do not differ significantly from the mean of control; Dunnet's test at $P = 0.05$.

Dose (ppm)	DE	Commodity			
		Barley	Wheat	Maize	Rice
0	Control	18.4 (1.0)	24.4 (1.1)	19.1 (1.2)	21.2 (2.2)
500	Begora	16.4 (0.6)*	18.3 (0.7)	18.7 (0.9)*	17.6 (1.1)
	Elassona 1	10.2 (0.4)	10.2 (0.6)	10.9 (0.3)	10.7 (0.3)
	Elassona 2	14.1 (1.1)	15.1 (0.8)	15.2 (0.9)	14.8 (1.0)
	FYROM	11.0 (0.9)	11.7 (0.6)	11.4 (0.8)	11.1 (0.8)
	Germany	10.7 (1.2)	10.9 (0.3)	12.0 (0.8)	11.3 (1.0)
	Kolubara	4.3 (1.0)	6.9 (1.5)	7.3 (1.3)	5.3 (0.9)
	Crete	15.1 (0.8)	16.1 (0.8)	19.9 (1.7)*	17.6 (1.1)
	Slovenia	17.4 (0.7)	19.3 (1.2)	17.4 (0.7)	17.1 (0.5)
	Vranje	14.3 (0.7)	15.1 (0.7)	15.1 (0.5)	14.7 (0.6)
	Vranje 311207	16.2 (1.4)*	19.6 (1.7)	19.0 (1.2)*	17.9 (1.3)
	SilicoSec	3.4 (0.4)	2.1 (0.3)	2.0 (0.5)	2.2 (0.6)
1000	Begora	14.4 (0.6)	16.3 (0.7)	16.7 (1.0)	15.6 (1.2)
	Elassona 1	8.2 (0.3)	8.1 (0.5)	8.4 (0.4)	8.7 (0.4)
	Elassona 2	12.1 (0.9)	13.1 (0.7)	13.2 (0.8)	6.6 (0.5)
	FYROM	9.0 (0.9)	9.7 (0.7)	9.4 (0.9)	9.1 (0.5)
	Germany	8.7 (1.2)	8.4 (2.1)	10.0 (0.7)	9.3 (1.0)
	Kolubara	2.3 (0.8)	4.9 (1.3)	5.3 (1.2)	3.3 (0.6)
	Crete	13.1 (0.8)	14.1 (0.9)	17.9 (1.5)	15.6 (1.0)
	Slovenia	11.1 (2.1)	12.4 (0.8)	15.4 (0.6)	15.4 (0.6)
	Vranje	12.3 (0.6)	13.1 (0.7)	13.1 (0.5)	12.7 (0.5)
	Vranje 311207	16.2 (1.3)*	17.6 (1.6)	17.0 (1.2)*	15.9 (1.2)
	SilicoSec	1.4 (0.3)	2.0 (1.1)	4.4 (0.5)	2.2 (0.5)

Table 16
Mean number of *T. confusum* per vial on four commodities treated with ten local DEs and one commercially available DE at three doses 50 d after the removal of the parental adults. Values in brackets are standard errors (SE). Within each commodity and dose, means followed by an asterisk do not differ significantly from the mean of control; Dunnet's test at $P = 0.05$.

Dose (ppm)	DE	Commodity			
		Barley	Wheat	Maize	Rice
0	Control	1.8 (0.4)	2.4 (0.3)	1.2 (0.4)	1.6 (0.9)
500	Begora	0.7 (0.2)*	0.2 (0.1)	1.6 (0.2)*	1.9 (0.4)*
	Elassona 1	0.8 (0.1)*	1.0 (0.3)*	1.4 (0.4)*	0.7 (0.2)*
	Elassona 2	1.1 (0.3)*	1.2 (0.2)*	0.4 (0.2)*	1.0 (0.2)*
	FYROM	1.6 (0.2)*	0.7 (0.1)	0.7 (0.2)*	1.6 (0.2)*
	Germany	1.0 (0.3)*	1.2 (0.3)*	1.2 (0.3)*	1.7 (0.2)*
	Kolubara	1.1 (0.3)*	1.7 (0.4)*	1.8 (0.5)*	1.3 (0.2)*
	Crete	0.7 (0.3)*	0.8 (0.3)	0.6 (0.1)*	1.2 (0.1)*
	Slovenia	1.1 (0.5)*	1.8 (0.6)	0.6 (0.3)*	1.0 (0.3)*
	Vranje	1.9 (0.3)*	0.9 (0.4)	1.6 (0.5)*	1.4 (0.2)*
	Vranje 311207	1.7 (0.2)*	1.4 (0.2)*	1.2 (0.7)*	1.4 (0.2)*
	SilicoSec	0.7 (0.5)*	1.0 (0.4)*	0.6 (0.2)*	1.2 (0.5)*
1000	Begora	0.7 (0.2)*	0.2 (0.1)	1.6 (0.2)*	0.9 (0.4)*
	Elassona 1	1.0 (0.3)*	0.7 (0.2)	1.1 (0.4)*	0.7 (0.4)*
	Elassona 2	1.1 (0.2)*	1.2 (0.3)	0.4 (0.2)*	1.0 (0.4)*
	FYROM	1.6 (0.2)*	0.7 (0.2)	0.6 (0.1)*	1.6 (0.4)*
	Germany	1.0 (0.3)*	1.2 (0.3)	1.7 (0.2)*	1.1 (0.6)*
	Kolubara	1.1 (0.3)*	1.7 (0.4)*	1.8 (0.6)*	1.3 (0.2)*
	Crete	0.7 (0.3)*	0.8 (0.3)	0.9 (0.3)*	1.2 (0.1)*
	Slovenia	1.2 (0.3)*	1.5 (0.4)*	1.6 (0.2)*	1.2 (0.3)*
	Vranje	1.4 (0.1)*	0.6 (0.1)	1.2 (0.3)*	1.6 (0.4)*
	Vranje 311207	0.9 (0.5)*	1.8 (0.5)*	1.6 (0.4)*	1.7 (0.8)*
	SilicoSec	0.7 (0.5)*	1.0 (0.4)*	0.3 (0.2)*	1.2 (0.8)*

3.3. Effectiveness of DE application and impact on bulk density of wheat

Mortality of the tested species was significantly affected by the exposure interval (*S. oryzae*: $F_{1,119} = 155.3$; $P < 0.01$, *T. confusum*:

Table 17
MANOVA parameters for main effects and associated interactions for mortality levels of *S. oryzae* and *T. confusum* adults between or within exposure intervals (for all species total df = 119).

Between exposure intervals				
Species	Source	df	*F*	*P*
S. oryzae	Application method	1	45.6	<0.01
	DE	2	305.2	<0.01
	Dose	1	56.1	<0.01
	Application method × DE	2	3.7	0.03
	Application method × dose	1	1.9	0.15
	DE × dose	2	0.0	0.96
T. confusum	Application method	1	324.6	<0.01
	DE	2	138.1	<0.01
	Dose	1	320.0	<0.01
	Application method × DE	2	75.5	<0.01
	Application method × dose	1	27.9	<0.01
	DE × dose	2	2.3	0.06
Within exposure intervals				
S. oryzae	Exposure × application method	1	8.8	<0.01
	Exposure × DE	2	21.4	<0.01
	Exposure × dose	1	2.9	0.09
	Exposure × application method × DE	2	0.9	0.82
	Exposure × application method × dose	1	0.2	0.65
	Exposure × DE × dose	1	6.4	<0.01
T. confusum	Exposure × application method	1	61.2	<0.01
	Exposure × DE	2	0.7	0.50
	Exposure × dose	1	165.5	<0.01
	Exposure × application method × DE	2	0.2	0.80
	Exposure × application method × dose	1	10.2	<0.01
	Exposure × DE × dose	1	10.3	<0.01

Table 18
Mean mortality (%) of *S. oryzae* and *T. confusum* on wheat treated with two local DEs and one commercially available DE with two application methods at two exposure intervals. Values in brackets are standard errors (SE). Within each exposure interval, DE, dose and insect species means followed by the same letter do not differ significantly. In all cases df = 1, 9; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Dose (ppm)	Insect species			
			S. oryzae		*T. confusum*	
			Dusting	Spraying	Dusting	Spraying
7 d	SilicoSec	300	91.2 (2.7) a	67.2 (5.8) b	26.4 (2.7) a	0.0 (0.0) b
		500	100.0 (0.0) a	100.0 (0.0) a	41.6 (2.4) a	11.2 (2.3) b
	FYROM	300	28.8 (5.1) a	5.6 (3.6) b	0.0 (0.0) a	0.0 (0.0) a
		500	60.0 (4.7) a	27.2 (5.9) b	8.0 (1.8) a	0.0 (0.0) b
	Slovenia	300	17.6 (6.1) a	8.0 (4.9) b	0.0 (0.0) a	0.0 (0.0) a
		500	37.6 (2.7) a	11.2 (3.2) b	2.4 (1.6) a	0.0 (0.0) a
14 d	SilicoSec	300	100.0 (0.0) a	100.0 (0.0) a	47.2 (4.6) a	5.6 (1.0) b
		500	100.0 (0.0) a	100.0 (0.0) a	77.6 (4.1) a	28.0 (8.0) b
	FYROM	300	49.2 (9.0) a	33.6 (3.5) a	17.2 (5.2) a	1.6 (1.0) b
		500	73.8 (5.4) a	66.0 (7.6) a	58.4 (4.1) a	27.2 (2.6) b
	Slovenia	300	23.2 (3.4) a	19.2 (5.4) a	8.0 (2.5) a	1.6 (1.0) b
		500	46.4 (3.2) a	32.0 (7.7) a	46.4 (4.7) a	21.6 (5.4) b

$F_{1,119} = 419.8$; $P < 0.01$). All main effects and the associated interactions for mortality levels of *S. oryzae* between exposure intervals were significant with the exception of application method × dose and DE × dose which were not significant at $P = 0.05$ (Table 17). All main effects for mortality levels of *S. oryzae* within exposure intervals were significant with the exception of exposure × dose which was not significant at $P = 0.05$ (Table 17). For mortality levels of *S. oryzae* the associated interactions exposure × application method × DE and exposure × application method × dose were not significant whereas the interaction exposure × DE × dose was significant at $P = 0.05$ (Table 17). In the case of *T. confusum*, all main effects and associated interactions were significant between exposure intervals except of the interaction DE × dose which was not significant at $P = 0.05$ (Table 17). All main effects and associated interactions were significant within exposure intervals with the exception of exposure × DE and exposure × application method × DE which were not significant at $P = 0.05$ (Table 17).

Table 19
Mean test weight of hard wheat sprayed or dusted with two local DEs and one commercially available DE at three post treatment periods. Values in brackets are standard errors (SE). Within each post treatment period and DE, means followed by the same lowercase letter do not differ significantly, in all cases df = 5, 23; within each post treatment period and application method, means followed by the same uppercase letter do not differ significantly. In all cases df = 2, 11; Tukey–Kramer (HSD) test at $P = 0.05$.

Post treatment period	Application method	Test weight in kg/Hl		
		FYROM	Slovenia	SilicoSec
		79.2 (0.2) Aa	79.1 (0.3) Aa	79.3 (0.2) Aa
	2 ml of water/1 kg of wheat	78.0 (0.2) Ab	78.3 (0.2) Ab	78.4 (0.2) Ab
	sprayed-100 ppm	70.1 (0.2) Ad	73.9 (0.1) Bc	75.9 (0.1) Cc
	dusted-100 ppm	68.5 (0.5) Ac	72.0 (0.1) Be	74.0 (0.1) Ce
	sprayed-300 ppm	68.5 (0.1) Ac	72.9 (0.2) Bd	74.9 (0.3) Cd
	dusted-300 ppm	67.8 (0.2) Ae	71.0 (0.1) Bf	73.0 (0.1) Cf
6 d	Untreated	79.2 (0.2) Aa	79.1 (0.3) Aa	79.3 (0.2) Aa
	2 ml of water/1 kg of wheat	78.0 (0.2) Ab	78.3 (0.2) Ab	78.4 (0.2) Ab
	sprayed-100 ppm	70.2 (0.2) Ac	73.3 (0.1) Bc	75.4 (0.1) Cc
	dusted-100 ppm	69.2 (0.4) Ad	72.1 (0.1) Bd	74.2 (0.1) Cd
	sprayed-300 ppm	69.0 (0.2) Ad	71.6 (0.3) Be	73.6 (0.1) Ce
	dusted-300 ppm	68.0 (0.1) Ae	70.5 (0.5) Bf	72.9 (0.2) Cf
25 d	Untreated	79.2 (0.2) Aa	79.1 (0.3) Aa	79.3 (0.2) Aa
	2 ml of water/1 kg of wheat	78.0 (0.2) Ab	78.3 (0.2) Ab	78.4 (0.2) Ab
	sprayed-100 ppm	74.2 (0.3) Ab	75.1 (0.2) Bb	75.3 (0.2) Bb
	dusted-100 ppm	72.9 (0.1) Ac	74.2 (0.2) Bc	74.3 (0.2) Bc
	sprayed-300 ppm	72.1 (0.2) Ad	73.6 (0.1) Bd	73.7 (0.1) Bd
	dusted-300 ppm	71.3 (0.1) Ae	72.9 (0.2) Be	73.0 (0.2) Be

When Slovenia and FYROM were applied in wheat, the mortality of *S. oryzae* adults after 7 d of exposure was significantly higher with dusting compared to spraying. However, in the case of the highest of the tested doses with SilicoSec, the mortality of *S. oryzae* adults was 100% irrespectively of the application method at the same exposure interval. After 14 d of exposure, although dusting appeared to be more effective than spraying, differences in mortality of *S. oryzae* adults were not significant (Table 18).

In the case of *T. confusum*, low (≤8%) mortality levels were recorded either with spraying or with dusting wheat with both FYROM and Slovenia after 7 d of exposure. In the case of SilicoSec the mortality of *T. confusum* adults ranged from 26.4% to 41.6% in dusted wheat and it was significantly higher than that in sprayed wheat (Table 15). After 14 d of exposure, mortality of *T. confusum* adults became higher with both sprayed and dusted with FYROM and Sovenian DEs wheat. However significantly higher mortality was recorded when DEs were dusted than when they were sprayed in wheat (Table 18).

Sprayed or dusted wheat with all DEs reduced significantly the bulk density of wheat in comparison with untreated wheat (Table 19). However, the former method reduced the bulk density significantly less than the latter and this occurred for all post treatment periods. Furthermore, in the case of FYROM, the bulk density of wheat was reduced significantly compared to Slovenia or SilicoSec and this was noted in all the tests (Table 19).

3.4. Effectiveness of DEs as surface treatments

Mortality of *T. confusum* was significantly affected by the exposure interval ($F_{18,405} = 1462.6$; $P < 0.01$). All main effects and their associated interaction for mortality levels of this species were

Table 20
MANOVA parameters for main effects and associated interactions for mortality levels of *T. confusum* adults between or within exposure intervals (for all species total df = 405).

Between exposure intervals			
Source	df	*F*	*P*
DE	8	522.6	<0.01
Dose	2	5.4	<0.01
DE × dose	16	2.5	<0.01
Within exposure intervals			
Exposure × DE	144	16.2	<0.01
Exposure × dose	36	32.2	<0.01
Exposure × DE × dose	288	21.3	<0.01

Table 21
Range of means mortality (%) of *T. confusum* adults exposed for 19 d at three different rates of nine local DEs and one commercially available DE. Values in brackets are standard errors (SE). Within each exposure range and DE, means followed by the same letter do not differ significantly, lowercase letters show dose comparisons at 1 d, 7 d and 13 d of exposures, uppercase letters show dose comparisons at 6 d, 12 d and 19 d of exposures. In all cases df = 2, 44; Tukey–Kramer (HSD) test at $P = 0.05$.

Exposure	DE	Application rate		
		5 g/m^2	10 g/m^2	20 g/m^2
1 d–6 d	Kolubara	0.0 (0.0) a–14.7 (3.8) A	0.0 (0.0) a–16.7 (4.5) A	0.0 (0.0) a–22.0 (4.4) A
	Vranje 311207	0.0 (0.0) a–22.0 (5.3) A	0.0 (0.0) a–36.0 (8.2) A	0.0 (0.0) a–38.0 (6.3) A
	Vranje	0.0 (0.0) a–57.3 (5.8) A	4.0 (1.6) b–84.7 (4.1) B	0.0 (0.0) a–92.0 (2.0) B
	Germany	0.0 (0.0) a–0.7 (0.7) A	0.0 (0.0) a–0.7 (0.7) A	1.3 (1.3) a–6.7 (6.7) A
	Slovenia	17.3 (4.2) a–100.0 (0.0) A	27.3 (6.5) a–100.0 (0.0) A	15.3 (3.6) a–100.0 (0.0) A
	Begora	5.3 (2.4) a–99.3 (0.7) A	6.7 (2.1) a–100.0 (0.0) A	4.7 (2.4) a–100.0 (0.0) A
	Elassona 1	6.0 (2.2) ab–100.0(0.0)A	1.3 (0.9) a–100.0 (0.0) A	8.0 (1.8) ab–100.0(0.0)A
	Crete	0.0 (0.0) a–63.3 (4.7) A	1.3 (0.9) a–68.0 (5.0) A	1.3 (1.3) a–70.7 (4.7) A
	SilicoSec	46.0 (5.3) a–100.0 (0.0) A	50.0 (4.5) a–100.0 (0.0) A	26.0 (7.3) b–94.7 (5.3) A
7 d–12 d	Kolubara	30.7 (5.7) a–96.0 (2.1) A	34.7 (5.7) a–96.7 (1.2) A	42.7 (5.2) a–100.0 (0.0) A
	Vranje 311207	43.3 (6.3) a–99.3 (0.7) A	50.0 (8.2) ab–99.3(0.7)A	70.0 (5.9) b–100.0 (0.0) A
	Vranje	84.0 (4.2) a–100.0 (0.0) A	96.7 (1.6) b–100.0 (0.0) A	97.3 (1.5) b–100.0 (0.0) A
	Germany	0.7 (0.7) a–40.0 (6.2) A	0.7 (0.7) a–32.7 (7.3) A	6.7 (6.7) a–52.0 (4.7) A
	Slovenia	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	Begora	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	Elassona 1	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	Crete	84.7 (2.9) a–100.0 (0.0) A	88.7 (3.4) a–100.0 (0.0) A	94.0 (1.6) a–100.0 (0.0) A
	SilicoSec	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
13 d–19 d	Kolubara	98.7 (0.9) a–100.0 (0.0) A	98.0 (1.1) a–100.0 (0.0) A	100.0 (0.0) aA
	Vranje 311207	100.0 (0.0) aA	99.3 (0.7) a–100.0 (0.0) A	100.0 (0.0) aA
	Vranje	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	Germany	56.7 (7.3) a–98.7 (0.9) A	47.3 (6.7) a–94.7 (2.3) A	67.3 (4.2) a–100.0 (0.0) A
	Slovenia	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	Begora	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	Elassona 1	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	Crete	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA
	SilicoSec	100.0 (0.0) aA	100.0 (0.0) aA	100.0 (0.0) aA

significant either between or within exposure intervals at $P = 0.05$ (Table 20).

After 1 d of exposure, more adults were dead after exposure to SilicoSec than to the other DE treatments (Table 21). Of the mined DEs, after 1 d of exposure, the most effective was Slovenia followed by Elassona 1 and Begora whereas after 6 d of exposure the mortality was almost complete (>99%) for all three DEs (Table 20). More than 6 d of exposure was required for effective control of *T. confusum* adults with the other mined DEs. Thus, in the case of Vranje and Crete, ≥84% of the treated individuals were dead after 7 d of exposure. The least effective of the mined DEs was Germany where more than 13 d was required for satisfactory control of *T. confusum* (Table 21).

4. Discussion

In light of our findings, FYROM was the most effective DE for grain treatment. In contrast, Begora, Crete and Slovenia were shown to be the least effective among the tested DEs and therefore their use should not be recommended for grain treatment. Nevertheless, this was not noted for treatment of surfaces as Slovenia, Begora and Elassona 1 were the most effective compared to the other DEs. Although these contradictory findings need further evaluation, generalizations on the efficacy of the mined DEs should be avoided as, in light of our results, some DEs are more suitable for surface treatment than grain treatment and vice versa. According to Korunic (1997, 1998), DEs originating from different parts of the world may vary as regards their efficacy against stored-product insects and our results support this claim. Furthermore, DE originating from southeastern Europe has been used in previous studies for applications against stored-products insects (Korunic, 1998; Fields and Korunic, 2000). In our studies however, the DE samples originating from the central or southeastern Europe deposits, were less effective than the commercially available formulation SilicoSec. Except for drying to 6%, no additional process was carried out on the DE samples before testing. This suggests that besides drying, additional process is required for improving the efficacy of the raw DEs to make them suitable for commercial use.

Factors such as temperature, relative humidity and grain type, which affect DE efficacy, also affected the efficacy of the mined DEs (Korunic, 1998; Arthur, 2000; Fields and Korunic, 2000; Athanassiou et al., 2003, 2004, 2005a, 2007; Vayias and Athanassiou, 2004; Kavallieratos et al., 2005, 2007). The fact that increasing temperatures were positively related to the efficacy of the tested DEs is very interesting given that heat and the mined DEs can be successfully combined in an IPM strategy (Dowdy and Fields, 2002). It is not always feasible to involve heat in an IPM strategy since it can impair the performance of other control methods, such as chemical control. For instance, temperature may negatively affect the efficacy of some pyrethroids although the reverse has been noted for organophosphates (Thaung and Collins, 1986; Arthur, 1999). In the present study, the increase of RH decreased the efficacy of all DEs. At high relative humidity levels, insects moderate water loss and therefore their survival is increased after exposure in a DE-treated substrate (Korunic, 1998; Nielsen, 1998; Subramanyam and Roesli, 2000; Fields and Korunic, 2000; Mewis and Ulrichs, 2001). Similar results are reported for several commercially available DE formulations such as SilicoSec tested against larvae and adults of *T. confusum* (Vayias and Athanassiou, 2004) or larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Athanassiou et al., 2006a), and for Protect-It and PyriSec against adults of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) (Athanassiou et al., 2007). However, further research is required into the spraying of the tested DE samples in order to standardize an optimum water/DE ratio in an effective slurry formulation for grain protection.

The fact that performance of the mined DEs varied among different grains should be taken into account when a DE-based control strategy is applied. Efficacy of DEs is influenced by the type or the variety of grain to which the dust is applied due to differences in physical or chemical characteristics of the grain, variations in insect behavior after contact with the treated kernels or combination of these factors (Athanassiou et al., 2003, 2004; Kavallieratos et al., 2005, 2010).

Besides dusting, DE can be applied successfully as spray mainly for surface treatment in empty storage facilities (Subramanyam and Roesli, 2000). In our experiments, although spraying was less effective than dusting for *S. oryzae* and *T. confusum*, no significant differences in the efficacy of these methods were recorded for *S. oryzae*14 d after exposure. Nevertheless, in the case of *T. confusum*, which is more tolerant than *S. oryzae* to DE treatments (Athanassiou et al., 2005a), dusting was still more effective than spraying. This fact suggests that although the majority of water from the spray should have evaporated in a short time after application, a proportion of water still remains absorbed by the DE particles and as a result, DE looses its absorbent capacity and becomes inactive (Subramanyam and Roesli, 2000).

Another parameter that was tested here was the effect of spraying on the physical properties of grain. According to our results, bulk density (test weight) of grain was reduced significantly less with spraying in comparison to dusting. This is an important finding, since the main drawback of the use of DEs is their negative impact on the physical properties of the grain (Korunic, 1998, 1997; Subramanyam and Roesli, 2000). Applying DEs as sprays has some significant advantages over dusting such as reduced friction among the kernels (because of lower effect on test weight), better flow ability of grain and less airborne dust during transportation (Subramanyam and Roesli, 2000). Although encouraging, these results correspond to specific conditions, doses, formulations and grain. Before adopting such a method, spray application of DE on grain commodities needs further evaluation in terms of efficacy, application apparatus and impact on physical properties of grain and thus, generalizations should be avoided.

According to our results, when the DEs Slovenia, Elassona 1 and Begora were applied as surface treatments, they caused almost complete mortality to adults of *T. confusum*, one of the most tolerant species to DEs after 6 d of exposure. It is concluded that the insects pick up DE particles more easily as long as DE is equally applied onto the surface of the Petridished and not adhered on the grain kernels. In the latter case, DE particles are also likely to also loose effectiveness by absorbing lipids from the pericarp of the kernels (Subramanyam and Roesli, 2000). Based on our results, 5 g of the mined DEs is an adequate quantity to treat 1 m^2 of surface. However, the above application rate should also be confirmed in field trials and under different surfaces.

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References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.
Arthur, F.H., 1999. Effect of temperature on residual toxicity of cyfluthrin wettable powder. J. Econ. Entomol. 92, 695–699.
Arthur, F.H., 2000. Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. J. Econ. Entomol. 93, 526–532.
Arthur, F.H., 2003. Optimization of inert dusts used as grain protectants and residual surface treatments. In: Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M., Highley, E. (Eds.), Proceedings of the Eighth International Conference on Stored-Product Protection, 22-26 July 2002, York. CAB International, Wallingford, Oxon, pp. 629–634.
Athanassiou, C.G., Kavallieratos, N.G., Tsaganou, F.C., Vayias, B.J., Dimizas, C.B., Buchelos, C.Th., 2003. Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Prot. 22, 1141–1147.
Athanassiou, C.G., Kavallieratos, N.G., Andris, N.S., 2004. Insecticidal effect of three diatomaceous earth formulations against adults of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) on oat, rye and triticale. J. Econ. Entomol. 97, 2160–2167.
Athanassiou, C.G., Kavallieratos, N.G., 2005. Insecticidal effect and adherence of PyriSec in different grain commodities. Crop Prot. 24, 703–710.
Athanassiou, C.G., Vayias, B.J., Dimizas, C.B., Kavallieratos, N.G., Papagregoriou, A.S., Buchelos, C.Th., 2005a. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. J. Stored Prod. Res. 41, 47–55.
Athanassiou, C.G., Kavallieratos, N.G., Economou, L.P., Dimizas, C.B., Vayias, B.J., Tomanović, S., Milutinović, M., 2005b. Persistence and efficacy of three diatomaceous earth formulations against *Sitophilus oryzae* (Coleoptera: Curculionidae) on wheat and barley. J. Econ. Entomol. 98, 1404–1412.
Athanassiou, C.G., Kavallieratos, N.G., Tsakiri, J.B., Xyrafidis, S.N., Vayias, B.J., 2006a. Effect of temperature and humidity on the insecticidal effect of SilicoSec® against *Ephestia kuehniella* (Lepidoptera: Pyralidae) larvae. J. Econ. Entomol. 99, 1520–1524.
Athanassiou, C.G., Korunic, Z., Kavallieratos, N.G., Peteinatos, G.G., Boukouvala, M.C., Mikeli, N.H., 2006b. New trends in the use of diatomaceous earth against stored-grain insects. In: Lorini, I., Bacaltchuk, B., Beckel, H., Deckers, E., Sundfeld, E., dos Santos, J.P., Biagi, J.D., Celaro, J.C., Faroni, L.R.D.'A., Bortolini, L.O.F.de, Sartori, M.R., Elias, M.C., Guedes, R.N.C., da Fonseca, R.G., Scussel, V.M. (Eds.), Proceedings of the Ninth International Conference on Stored-Product Protection, 15-18, October, Campinas, Brazil. ABRAPOS, Rodovia, pp. 730–740.
Athanassiou, C.G., Kavallieratos, N.G., Peteinatos, G.G., Petrou, S.E., Boukouvala, M.C., Tomanović, Ž, 2007. Influence of temperature and humidity on insecticidal effect of three diatomaceous earth formulations against larger grain borer (Coleoptera: Bostrychidae). J. Econ. Entomol. 100, 599–603.
Dowdy, A.K., Fields, P.G., 2002. Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill. J. Stored Prod. Res. 38, 11–22.
Ebeling, W., 1971. Sorptive dust for pest control. Annu. Rev. Entomol. 16l, 123–158.
FDA (Food and Drug Administration), 1995. Specifications for diatomaceous earths as a maximum 2 % animal feed additive. 21 CFR Section 573. 340.
Fields, P., Korunic, Z., 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. J. Stored Prod. Res. 36, 1–13.
Inđić, D., Almaši, R., Klokocar-Smit, Z., Jovanović, S., Vajović, M., 1999. Effect of nopesticide products on insects in storage. In: Perić, I., Ivanović, M. (Eds.), Proceedings of the International Symposium on Integrated Protection of Field Crops, 21-26 September 1998, Vrnjačka Banja, pp. 219–227.
Kavallieratos, N.G., Athanassiou, C.G., Pashalidou, F.G., Andris, N.S., Tomanović, Ž., 2005. Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Manag. Sci. 61, 660–666.
Kavallieratos, N.G., Athanassiou, C.G., Vayias, B.J., Maistrou, S.N., 2007. Influence of temperature on susceptibility of *Tribolium confusum* (Coleoptera: Tenebrionidae) populations to three modified diatomaceous earth formulations. Fla. Entomol. 90, 616–625.
Kavallieratos, N.G., Athanassiou, C.G., Vayias, B.J., Kotzamanidis, S., Synodis, S.D., 2010. Efficacy and adherence ratio of diatomaceous earth and spinosad in three wheat varieties against three stored-product insect pests. J. Stored Prod. Res. 46, 73–80.
Korunic, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. J. Stored Prod. Res. 33, 219–229.
Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. J. Stored Prod. Res. 34, 87–97.
Mewis, I., Ulrichs, Ch., 2001. Action of amorphous diatomaceous earth against different stages of the stored product pests *Tribolium confusum, Tenebrio molitor, Sitophilus granarius* and *Plodia interpunctella*. J. Stored Prod. Res. 37, 153–164.
Nielsen, P.S., 1998. The effect of a diatomaceous earth formulation on the larvae of*Ephestia kuehniella* Zeller. J. Stored Prod. Res. 34, 113–121.
SAS Institute, 2000. The SAS System Version 7 for Windows. SAS Institute, Gary, North Carolina.
Sokal, R.R., Rohlf, F.J., 1995. Biometry, third ed. Freedman & Company, New York.
Subramanyam, Bh., Roesli, R., 2000. Inert dusts. In: Subramanyam, Bh., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Dordrecht, pp. 321–380.
Thaung, M., Collins, P.J., 1986. Joint effects of temperature and insecticides on mortality and fecundity of *Sitophilus oryz*ae (Coleoptera: Curculionidae) in wheat and maize. J. Econ. Entomol. 79, 909–914.
Vayias, B.J., Athanassiou, C.G., 2004. Factors affecting efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused beetle *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae). Crop Prot. 23, 565–573.
Vayias, B.J., Athanassiou, C.G., Buchelos, C.Th., 2006a. Evaluation of three diatomaceous earth and one natural pyrethrum formulations against pupae of *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) on wheat and flour. Crop Prot. 25, 766–772.
Vayias, B.J., Athanassiou, C.G., Kavallieratos, N.G., Tsesmeli, C.D., Buchelos, C.Th., 2006b. Persistence and efficacy of two diatomaceous earth formulations and a mixture of diatomaceous earth with natural pyrethrum against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) on wheat and maize. Pest Manag. Sci. 62, 456–464.