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Insecticidal efficacy of natural diatomaceous earth deposits from Greece and Romania against four stored grain beetles: the effect of temperature and relative humidity

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Abstract

In two separate experimental bioassays, the efficacy of diatomaceous earth (DE) deposits from Greece and Romania, as well as two DE commercial formulations were evaluated against four stored-product beetle species. In the 1st experiment, four doses (100, 300, 500 and 900 ppm) of two Greek (Elassona 1 and Elassona 2), two Romanian (PatRom and AdRom) DEs and two commercial DEs (SilicoSec and PyriSec) were tested against adults of *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera Silvanidae) in wheat and barley. In the 2nd experiment, the effect of temperature (20, 25 and 30 °C) and relative humidity (RH) (55 and 75%) on the efficacy of DEs from Greece (Elassona 1), Romania (PatRom) and the commercial DE SilicoSec at three doses (300, 600 and 900 ppm) in wheat was investigated against adults of *Sitophilus oryzae* (L.) (Coleoptera Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera Bostrychidae). Mortality of the exposed adults in both experiments was assessed after 7, 14 and 21 days of exposure. In the 1st experiment, the most effective DE was PyriSec which caused complete mortality of *O. surinamensis* adults at 300 ppm after 7 and 14 days of exposure in wheat and barley respectively. In both commodities, the Greek (Elassona 1) and Romanian (PatRom) DEs had the same performance with SilicoSec at 500 ppm. Complete mortality of *T. castaneum* adults was recorded after 21 days at the highest dose in both commodities treated with PyriSec. Greek and Romanian DEs caused low mortality (< 25%) even after 21 days of exposure at the highest dose. *T. castaneum* was less susceptible than *O. surinamensis*, especially to Greek and Romanian DEs. Results from the 2nd experiment indicated that *S. oryzae* adults were more susceptible than *R. dominica* adults to the tested DEs. Mortality levels for both species were high, but only at 900 ppm. The increase of temperature, increased the efficacy of the tested DEs against adults of both species. Higher mortality levels were recorded in the low RH for both species. The Romanian DE PatRom was more effective compared with SilicoSec and the Greek DE Elassona 1 against *S. oryzae* and *R. dominica* adults at 900 ppm and 25 °C.

Key words: diatomaceous earths, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum*, *Oryzaephilus surinamensis*, temperature, relative humidity.

Introduction

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera Curculionidae), the lesser grain borer, *Rhyzopertha dominica* (Coleoptera Bostrychidae), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae), and the saw toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera Silvanidae) are destructive cosmopolitan pests of a wide variety of stored products (Hill, 1990). Several chemical insecticides are used for their control, but the future of some of them remains uncertain, due to both health and environmental concerns (Arthur, 2012). Diatomaceous earths (DEs) are used as alternatives to chemical insecticides and fumigants due to certain advantages; they exhibit low mammalian toxicity, their origin is natural (fossils of phytoplanktons) and they have non-chemical mode of action by absorbing the waterproof epicuticular lipids from insects' cuticle which die because of desiccation (Ebeling, 1971; Korunic, 1998). Several previous studies have demonstrated that DEs are very effective against stored-product insects (Fields and Korunic, 2000; Arthur, 2000a; 2000b; Subramanyam and Roesli, 2000; Arthur, 2002; Arthur and Throne, 2003; Athanassiou *et al*., 2003; 2004; 2005; 2011, 2014; Andrić *et al*., 2012). The efficacy of DEs against stored grain pests is influenced by various factors, i.e., insect species, strain or stage, geographical origin, commodity, temperature and relative humidity (RH) (Le Patourel, 1986; Desmarchelier and Dines, 1987; Aldryhim, 1993; Golob, 1997; Arthur, 2000b; Stathers *et al*., 2004; Kavallieratos *et al*., 2007; Athanassiou *et al*. 2011; 2014; Baldassari and Martini, 2014). The investigation for the discovery of novel DEs with insecticidal properties enhances the available tools for the Integrated Pest Management of stored-product insects (Athanassiou *et al*., 2011; Nwaubani *et al*., 2014). Although previous reports have indicated that DE deposits from south-eastern Europe are potentially effective for the control of stored-product insect pests (Vayias *et al*., 2009; Rojht *et al*., 2010; Athanassiou *et al*., 2011; Andrić *et al*., 2012), very little information is available on the insecticidal efficacy of mined DEs in Romania. The only available studies on this issue by Chiriloaie *et al*. (2013; 2014) have pointed out that DEs originating from Romania can satisfactorily control *S. oryzae* in stored wheat. Therefore, the objective of the present study was to evaluated the insecticidal efficacy of DEs from Romania and compare them with commercially available DE formulations and DEs from Greece against *S. oryzae*, *R. dominica*, *T. castaneum* and *O. surinamensis*. Furthermore, the influence of temperature, RH and type of commodity in their insecticidal efficacy was also investigated. For this purpose two separate experimental tests were conducted in Romania and Greece.

Materials and methods

Insects

Adults of mixed sex, with age < 21 days, reared at 25 °C, 60% RH and continuous darkness were used in the tests. For the 1st experiment, adults of *O. surinamensis* and *T. castaneum* were used from laboratory populations that are kept at the Research Development Institute for Plant Protection Bucharest. The *O. surinamensis* individuals were cultured in whole wheat. The *T. castaneum* individuals were cultured in mixture of maize and wheat flour, with 5% brewer yeast. For the 2nd experiment, adults of *S. oryzae* and *R. dominica* were used from laboratory cultures that are kept in the Laboratory of Entomology and Agricultural Zoology, University of Thessaly in Greece. Individuals of both species were reared in whole wheat.

DEs processing and commodity application

The amorphous DE samples from two local DE deposits from Romania, Buzău Valey-Pătârlagele (namely PatRom) and South of Dobroudja-Adamclisi (namely AdRom), and two from Greece (Elassona, Thessaly, namely Elassona 1 and Elassona 2) were processed at the University of Thessaly in Greece. DE samples were dried in an oven to 4.5% moisture content at 40 °C (Arnaud *et al.*, 2005). After drying, small pieces were grounded in a laboratory mill at full speed for 10 sec. Then, each sample powder was sieved through a standard sieve of 200 mesh (75 µm, Advantech Manufacturing Inc., New Berlin, WI, USA) and separately kept for 24 h in airproof jars. The commercially available DE formulations SilicoSec (Biofa GmbH, Münsingen, Germany) and PyriSec (Agrinova GmbH, Obrigheim/Mühleim, Germany) were also used. SilicoSec contains 92% SiO_2 while PyriSec contains 1.2% natural pyrethrum (25%), 3.1% piperonyl butoxide and 95.7% of SilicoSec (Vayias *et al.*, 2006).

In the 1st experiment untreated whole hard wheat (*Triticum durum* L., var. Miranda) and barley (*Hordeum vulgare* L., var. Laverda) was used in the tests. All DEs were applied in 1 kg of grain, at different amounts, creating lots with dose rates of 100, 300, 500 and 900 ppm (mg DE/kg of grain). The lots were placed in glass jars and shaken manually for 5 min to achieve equal distribution of the DE dust to the entire grain mass. For the 2nd experiment only one Greek (Elassona 1), one Romanian (PatRom) and one commercial DE (SilicoSec) where used. DEs were applied to whole hard wheat (var. Simeto), as previously, at doses of 300, 600 and 900 ppm. Additional 1 kg lots of wheat were sprayed with distilled water and used as control, for both experiments.

Bioassays

In the 1st experiment, three samples of 50 g each, were taken from each lot, and placed in small cylindrical glass vials (9 cm in diameter, 15 cm in height) with a different scoop that was inside each jar. The vials were covered with fine mesh for ventilation, Then, 30 adults were placed in each vial. The vials were then placed in controlled rooms set at 25 °C, 60% RH and continuous darkness. For the 2nd experiment, three samples of 20 g each, were taken from each lot, and placed in small plastic cylindrical vials (3 cm in diameter, 8 cm in height) with a different scoop that was inside each jar and 20 adults of each species were separately placed into each vial. The vials were covered with fine mesh as previously. The vials were then placed to incubators set at the combinations of three temperatures (20, 25 and 30 °C) and two RH levels (55 and 75%) in continuous darkness. Therefore, there were six temperature × RH combinations. In both experiments, the internal "necks" of the vials were covered by Fluon (Northern Products Inc., Woonsocket, USA), to prevent insects from escaping. Mortality of the exposed individuals in both experiments was assessed after 7, 14 and 21 days of exposure. All bioassays were carried out in three replicates, by preparing new lots.

Data analysis

Adult control mortality was low (< 5%), therefore no correction was considered necessary (Abbott, 1925). Adult mortality was analyzed separately for each species and grain by using the Multivariate Analysis of Variance (MANOVA) Repeated Measures and Wilks' Lambda estimate, by using the JMP software (Sall *et al.*, 2001). In the 1st experiment, dose, DE and commodity were the main effects, while dose, DE, temperature and relative humidity were the main effects in the 2nd experiment. The associated interactions of the main effects were also included in the analysis. In both experiments, the repeated factor was exposure interval, while mortality was the response variable. Means were separated by the Tukey-Kramer honestly significant difference (HSD) test at 0.05 significance level (Sokal and Rohlf, 1995).

Results

1st experiment

For *O. surinamensis* and *T. castaneum*, the main effects and the associate interactions between and within exposure intervals are presented in table 1. Mortality of *O. surinamensis* adults was 100% after 7 days of exposure at doses > 300 and 500 ppm of PyriSec treated wheat and barley respectively, while at the lowest dose mortality was 67% in treated wheat and 95.5% in treated barley (figure 1). For SilicoSec, after 21 days, mortality was 95 and 97% at the highest dose in barley and wheat respectively. In both commodities, the Greek (Elassona 1) and the Romanian (PatRom) DEs had the same performance with SilicoSec at 500 ppm. This was also evident at the lowest dose for all Greek and Romanian DEs but mortality did not exceed 42%. Generally, the tested DEs performed better in barley than in wheat.

In the case of *T. castaneum*, all adults were dead at 900 ppm in barley and wheat treated with PyriSec after 21 days of exposure. Generally, *T. castaneum* was less

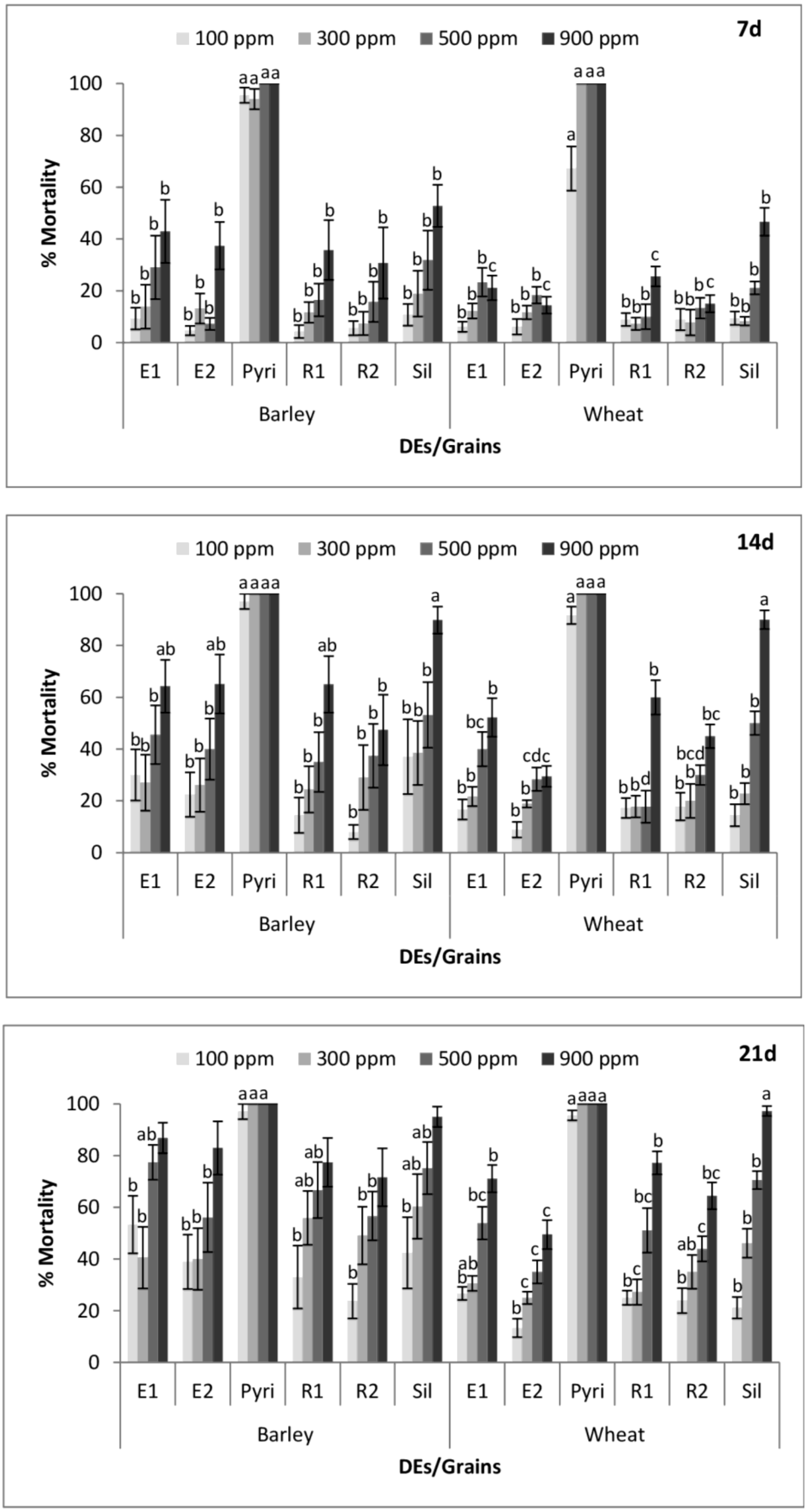

Figure 1. Percent mortality (mean ± SE) of *O. surinamensis* adults exposed to barley and wheat treated with six DEs (E1 = Elassona 1, E2 = Elassona 2, Pyri = PyriSec, R1 = PatRom, R2 = AdRom, Sil = SilicoSec) for 7, 14 and 21 days at four doses. Within each exposure, commodity and dose, means followed by the same letter are not significantly different. In all cases d.f. = 5, 53; Tukey-Kramer (HSD) test at $p = 0.05$. Where no letters exist, no significant differences were noted.

Table 1. Repeated-measures MANOVA parameters for main effects and associated interactions for mortality levels of *O. surinamensis* and *T. castaneum* adults between and within exposure intervals (for both species error d.f. = 384).

		O. surinamensis		*T. castaneun*	
	d.f.	F	p	F	p
Between exposure intervals					
Intercept	1	2595.9	< 0.01	981.7	< 0.01
DE	5	145.9	< 0.01	375.2	< 0.01
Commodity	1	22.7	< 0.01	44.0	< 0.01
Dose	3	66.3	< 0.01	117.4	< 0.01
DE × commodity	5	1.1	0.38	3.2	< 0.01
DE × dose	15	2.9	< 0.01	43.8	< 0.01
Commodity × dose	3	0.1	0.94	7.2	< 0.01
DE × commodity × dose	15	0.8	0.71	1.2	0.26
Within exposure intervals					
Exposure × DE	10	14.2	< 0.01	83.2	< 0.01
Exposure × commodity	2	6.9	< 0.01	21.1	< 0.01
Exposure × dose	6	9.3	< 0.01	28.9	< 0.01
Exposure × DE × commodity	10	2.2	< 0.01	5.0	< 0.01
Exposure × DE × dose	30	2.8	< 0.01	21.9	< 0.01
Exposure × commodity × dose	6	1.7	0.13	4.9	< 0.01
Exposure × DE × commodity × dose	30	1.2	0.19	2.9	< 0.01

Table 2. Repeated-measures MANOVA parameters for main effects and associated interactions for mortality levels of *S. oryzae* and *R. dominica* adults between and within exposure intervals (for both species error d.f. = 432).

		S. oryzae		*R. dominica*	
	d.f.	F	p	F	p
Between exposure intervals					
Intercept	1	11637.6	< 0.01	9222.2	< 0.01
DE	2	5.2	< 0.01	1.5	0.22
Dose	2	4216.5	< 0.01	4908.6	< 0.01
Temperature	2	539.3	< 0.01	510.0	< 0.01
RH	1	27.4	< 0.01	15.7	< 0.01
DE × dose	4	10.4	< 0.01	9.0	< 0.01
DE × temperature	4	1.5	0.21	1.7	0.14
DE × RH	2	0.2	0.83	0.1	0.94
Dose × temperature	4	211.7	< 0.01	281.6	< 0.01
Dose × RH	2	1.3	0.27	6.4	< 0.01
Temperature × RH	2	0.3	0.77	2.1	0.13
DE × dose × temperature	8	2.0	0.05	1.1	0.36
DE × dose × RH	4	0.1	0.99	0.1	0.97
DE × temperature × RH	4	0.1	0.99	0.1	0.98
Dose × temperature × RH	4	0.3	0.90	0.1	1.00
DE × dose × temperature × RH	8	0.1	1.00	0.3	0.97
Within exposure intervals					
Exposure × DE	4	8.2	< 0.01	6.5	< 0.01
Exposure × dose	4	120.6	< 0.01	202.0	< 0.01
Exposure × temperature	4	13.4	< 0.01	16.9	< 0.01
Exposure × RH	2	10.3	< 0.01	2.0	0.14
Exposure × DE × dose	8	2.1	0.04	1.4	0.18
Exposure × DE × temperature	8	3.5	< 0.01	3.2	< 0.01
Exposure × DE × RH	4	0.1	0.97	0.4	0.83
Exposure × dose × temperature	8	27.3	< 0.01	15.2	< 0.01
Exposure × dose × RH	4	0.3	0.89	0.4	0.78
Exposure × temperature × RH	4	0.2	0.95	0.3	0.89
Exposure × DE × dose × temperature	16	3.7	< 0.01	3.6	< 0.01
Exposure × DE × dose × RH	8	0.1	1.00	0.2	0.99
Exposure × DE × temperature × RH	8	0.1	1.00	0.2	0.99
Exposure × dose × temperature × RH	8	0.4	0.94	0.2	0.99
Exposure × DE × dose × temperature × RH	16	0.1	1.00	0.1	1.00

susceptible than *O. surinamensis* to the tested DEs. For SilicoSec adult mortality reached 74% only after 21 days at 900 ppm in wheat, while for the same exposure and dose in barley, mortality was 36%. Greek and Romanian DEs caused low mortality (< 25%) even after 21 days of exposure at the highest dose (figure 2). The local DEs and SilicoSec performed equally but for doses < 900 ppm. Higher mortality levels were recorded in wheat treated kernels compared with barley.

2nd experiment

For *S. oryzae* and *R. dominica*, the main effects and the associate interactions between and within exposure intervals are presented in table 2. Generally, *S. oryzae* adults were more susceptible than *R. dominica* adults to the tested DEs (figures 3, 4). Mortality levels for both species were high, but only at 900 ppm. After 21 days of exposure, mortality of *S. oryzae* and *R. dominica* adults reached 98% and 88% in the combination of 30 °C and 55% RH respectively. The increase of temperature, increased the efficacy of the tested DEs against adults of both species. This was more obvious at 900 ppm at both RH levels. For example, the Greek DE caused 58, 89 and 94% mortality to *S. oryzae* adults or 48, 79 and 85% to *R. dominica* adults at 20, 25 and 30 °C respectively at 55% RH after 21 days of exposure. The two RH levels influenced the insecticidal efficacy of DEs. Thus, higher mortality levels were recorded in the low RH for both species. Among the tested DEs there were no differences in their efficacy, with few exceptions. SilicoSec was more effective at 300 and 600 ppm at 20 °C compared to Greek and Romanian DEs, while at 900 ppm the Romanian DE was more effective compared with SilicoSec and Greek DE at 25 °C.

Discussion

In the light of our findings, among DEs tested here, the most effective was PyriSec. The high mortality levels of *O. surinamensis* and *T. castaneum* adults recorded in the present study could be explained by the existence of natural pyrethrum and piperonyl butoxide in PyriSec through a possible synergism (Athanassiou and Kavallieratos, 2005). Previous studies have also demonstrated the superiority of this DE upon other DEs. For example, Athanassiou *et al*. (2004) reported that PyriSec caused significantly higher mortality of *S. oryzae* adults than the DEs Insecto and SilicoSec after 48 h of exposure in the treated substrate.

Of the insect species tested here, *O. surinamensis* was the most susceptible while *T. castaneum* the most tolerant to DEs, with the exception of PyriSec. *S. oryzae* and *R. dominica* were susceptible to the highest dose of all tested DEs but *R. dominica* was more tolerant than *S. oryzae*. Arthur (1991) reported that *O. surinamensis* adults were susceptible in wheat treated with the DE formulation Protect-It, given that both immediate and delay mortality were high. The susceptibility of this species to DEs could be attributed to the fact that it is mobile (Arthur, 1991) and thus, the probability of picking up DE particles is high. The mobility of an insect species increases the susceptibility to DE (Rigaux *et al*., 2001). Furthermore, *O. surinamensis* is a small insect, i.e., it can be dehydrated easily due to the DE activity (Korunic, 1998). Similarly, adults of *S. oryzae* are agile (Hill, 1990) while adults of *R. dominica* are moving slowly (Flinn and Hagstrum, 2011). *Tribolium* spp. are considered as the most tolerant stored-grain beetle species to DEs at the adult stage (Korunic, 1998; Fields and Korunic, 2000; Vayias and Athanassiou, 2004; Athanassiou *et al*., 2011). This could be attributed to the fact that the DE particles are not easily attached on the body of *T. castaneum* (Fields and Korunic, 2000), given that this species is not hairy. Practically, the local DEs were ineffective against *T. castaneum* suggesting that longer exposure intervals and higher doses are required for the control of this species.

Temperature was associated positively with *R. dominica* and *S. oryzae* adult mortality and this was more obvious at the highest dose of all tested DEs. The increase of temperature increases insect mobility and the absorption of DE particles on the body surface. High temperatures also lead to increased respiration and water loss (Arthur, 2000b; Subramanyam and Roesli, 2000). However, the overall mortality of *R. dominica* adults did not exceed 49 or 44% at 20 °C and 55 or 75% RH respectively after 21 days of exposure. Similarly, the respective mortality values for *S. oryzae* were 59 and 54%. Thus, the tested DEs were not effective at 20 °C in both RH levels. This finding has been previously reported for a different DE (Athanassiou *et al*., 2014). The Romanian DE PatRom was highly efficacious mostly against *S. oryzae*, and in a lesser degree against *R. dominica*, at 900 ppm at 25 or 30 °C and 55% RH after 21 days of exposure. This finding is important because at 29.1 °C *S. oryzae* develops quickly (25 days) from egg to adult (Sharifi and Mills, 1971) and PatRom could offer a good level of protection to wheat against this pest. Furthermore, novel DEs with high insecticidal value in relatively low doses could be further improved with the addition of other substances, i.e., botanicals or insecticides which exhibit low mammalian toxicity, and consequently reduce the application dose (Athanassiou and Korunic, 2007; Athanassiou *et al*., 2007; 2008; 2009; Vayias and Stephou, 2009). However, further processing and experimentation is needed to clarify this hypothesis.

Another important issue was the influence of RH to the Romanian DE PatRom. The high insecticidal efficacy of this DE against *S. oryzae* was slightly reduced with the increase of RH. When relative humidity is high, insects regulate water loss and thus their survival is increased in DE-treated substrate. Furthermore, at high air RH or grain moisture content levels, the DE particles become partially ineffective due the absorption of moisture (Subramanyam and Roesli, 2000; Mewis and Ulrichs, 2001). Newer studies have clearly demonstrated that even a small change of the level of relative humidity is able to influence the efficacy of DEs. For example, Athanassiou *et al*. (2011) reported that mortalities of *R. dominica*, *S. oryzae* and *T. confusum* adults in wheat that had been previously treated with various DEs were lower at 65% than at 55%. Thus, the finding

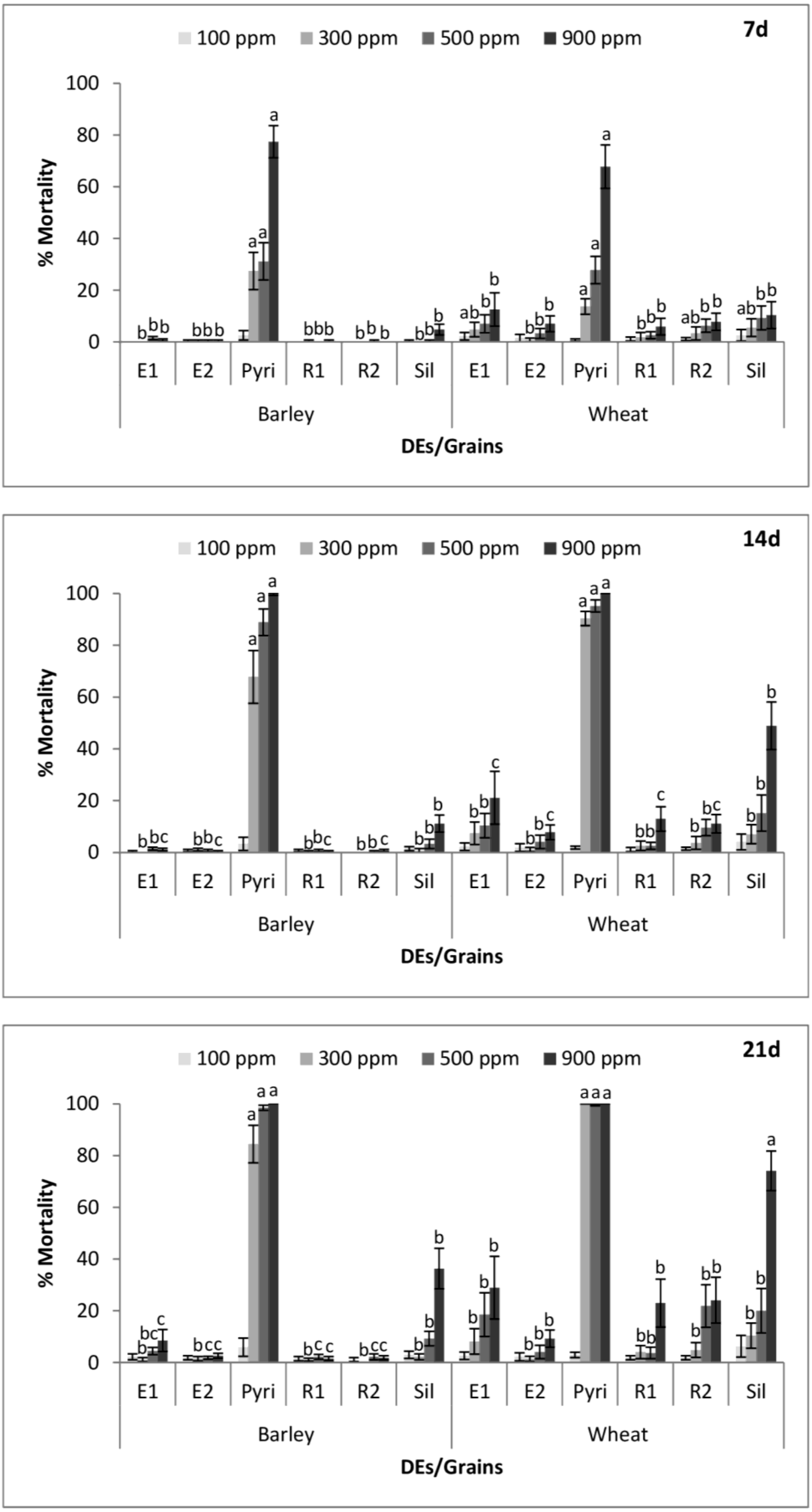

Figure 2. Percent mortality (mean ± SE) of *T. castaneum* adults exposed to barley and wheat treated with different DEs (E1 = Elassona 1, E2 = Elassona 2, Pyri = PyriSec, R1 = PatRom, R2 = AdRom, Sil = SilicoSec) for 7, 14 and 21 days at four doses. Within each exposure, commodity and dose, means followed by the same letter are not significantly different. In all cases d.f. = 5, 53; Tukey-Kramer (HSD) test at p = 0.05. Where no letters exist, no significant differences were noted.

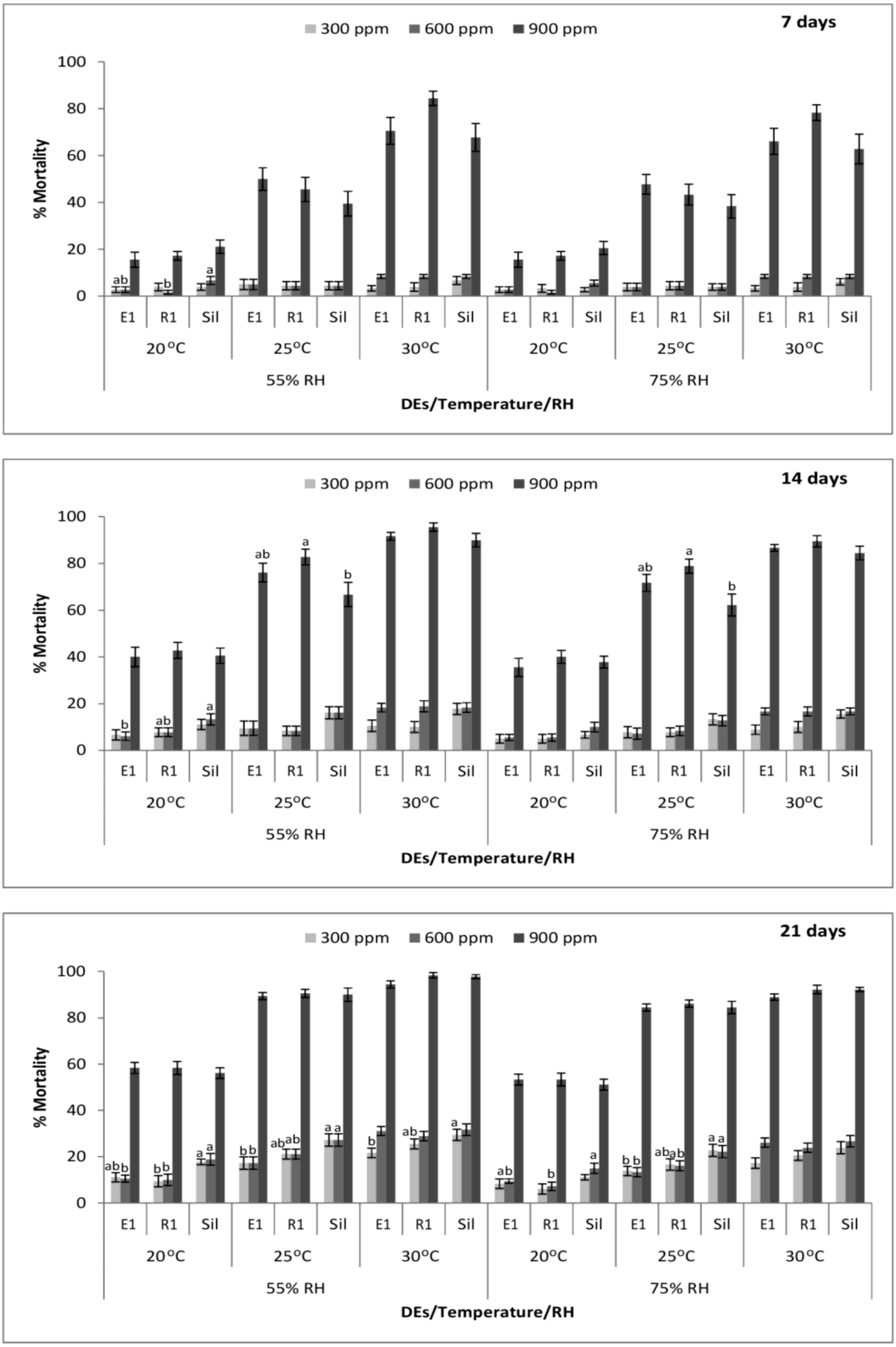

Figure 3. Percent mortality (mean ± SE) of *S. oryzae* adults exposed to wheat treated with three DEs (E1 = Elassona 1, R1 = PatRom, Sil = SilicoSec) for 7, 14 and 21 days of under three temperatures and two RH levels. Within each RH, temperature and dose, means followed by the same letter are not significantly different. In all cases d.f. = 2, 26; Tukey-Kramer (HSD) test at p = 0.05. Where no letters exist, no significant differences were noted.

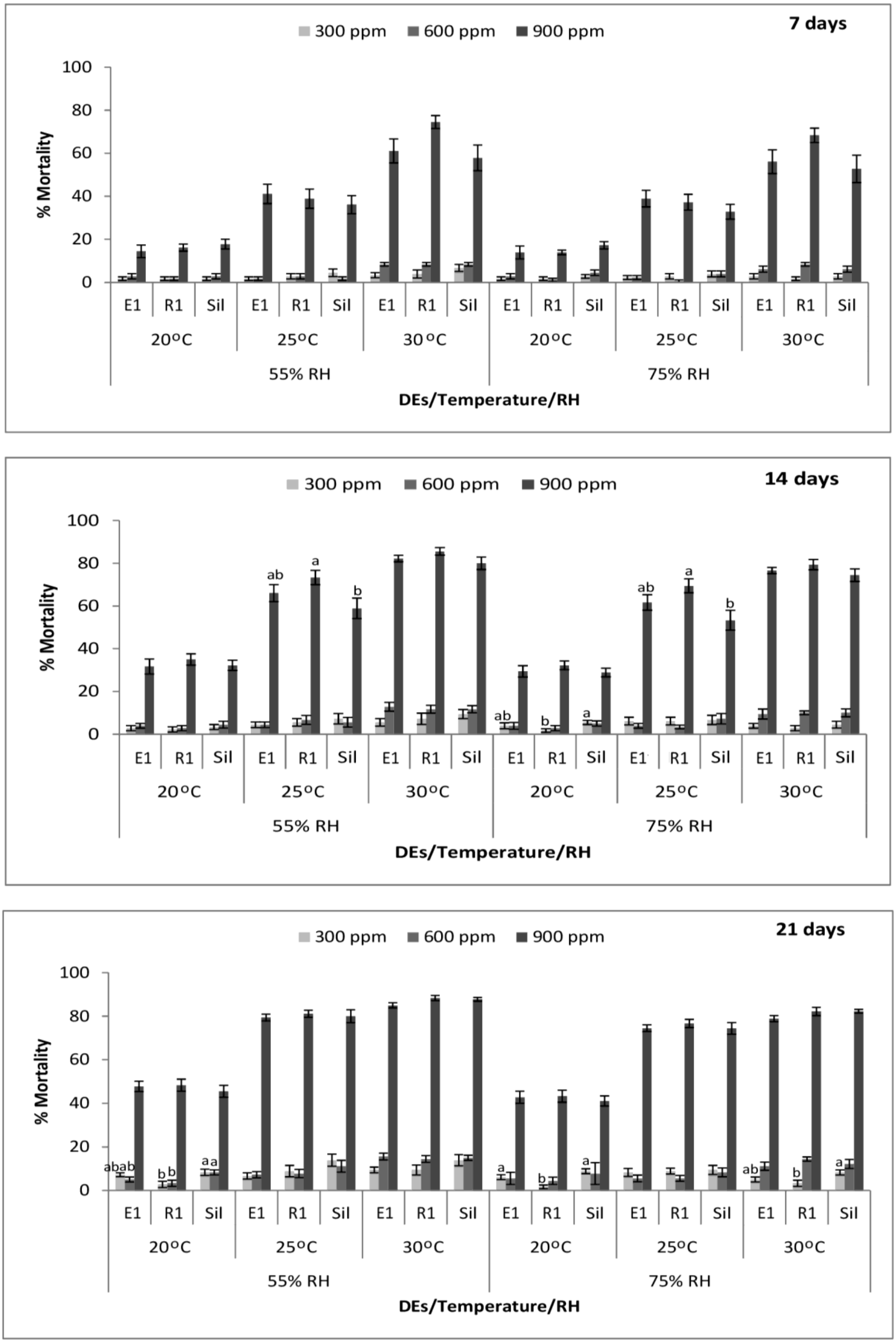

Figure 4. Percent mortality (mean ± SE) of *R. dominica* adults exposed to wheat treated with three DEs (E1 = Elassona 1, R1 = PatRom, Sil = SilicoSec) for 7, 14 and 21 days of under three temperatures and two RH levels. Within each RH, temperature and dose, means followed by the same letter are not significantly different. In all cases d.f. = 2, 26; Tukey-Kramer (HSD) test at p = 0.05. Where no letters exist, no significant differences were noted.

of a DE which efficacy is stable to the increase of RH is particularly interesting, given that *S. oryzae* oviposition and survival of larvae are favored by elevated moisture content of the commodities (Singh *et al.*, 1974).

In the present study we found that the mortality levels of *O. surinamensis* and *T. castaneum* were different between barley and wheat. The fact that the type of grain determines the efficiency of DEs has been previously documented (Athanassiou *et al.*, 2003; 2004, Kavallieratos *et al.*, 2005; 2010). Chiriloaie *et al.* (2014) also found that the efficacy of two Romanian and one Greek mined DEs was different among barley, maize, rice and wheat against *S. oryzae* adults. Our results are in agreement with these studies. Thus, for potential maximization of the efficiency of the management strategies, which are based on mined DEs, the type of commodity that is going to be treated and the target species are necessary to be known.

In summary, our results indicate that the Romanian DE PatRom tested here was found effective and compatible to the commercially available DEs, against *S. oryzae* adults mostly but also against *R. dominica* adults. However, further experimentation is needed on this DE against more stored-product insect species including studies on its influence against insects' progeny production in order to make a decision about the industrialization of this material.

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