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Original article (Orijinal araştırma)

Effect of temperature on insecticidal efficiency of local diatomaceous earth against stored-grain insects[1]

Yerel diatomit topraklarının depolanmış tahıl zararlılarına karşı insektisidal etkinliği üzerine sıcaklığın etkisi

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Abstract

A study was conducted in 2017 in Entomology Laboratory of Kahramanmaraş Sütçü İmam University to determine effect of temperature on insecticidal efficacy of local diatomaceous earth (DE), collected from Turkey, against the rice weevil, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), the confused flour beetle, *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae), and the lesser grain borer, *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae). Bioassays were performed at three temperatures (20, 25 and 30ºC) and 55% RH on wheat treated with 0, 100, 300, 500, 900 and 1500 ppm (mg DE/kg grain) concentrations of local DE. Temperature had significant effect on insecticidal efficacy of local DE against the tested stored-grain insects. The effect of temperature on the insecticidal efficacy of local DE varied with insect species and concentration. Mortality of *S. oryzae* and *T. confusum* adults generally increased with increasing temperature and mortality at 30ºC was significantly higher than at 20 and 25ºC. However, for *R. dominica* adults treated with local DE, mortality at 20ºC was significantly higher than at 25 and 30ºC. The results indicated that complete mortality of *T. confusum* and *S. oryzae* can be achieved at lower concentrations ranging from 500 to 900 ppm. In conclusion, local DE formulation (ACN-1) has potential to be used for control of stored-grain insects.

Keywords: Local diatomaceous earth, *Rhyzopertha dominica*, *Sitophilus oryzae*, temperature, *Tribolium confusum*

Öz

Bu çalışma, 2017 yılında Kahramanmaraş Sütçü İmam Üniversitesi'nin Entomoloji Laboratuvarı'nda Türkiye'nin farklı bölgelerinden elde edilen yerel diatom toprağının depolanmış tahıl zararlısı, Pirinç biti, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), Kırma un biti, *Tribolium confusum* Du Val., 1863 (Coleoptera: Tenebrionidae) ve Ekin kambur biti *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae)'ne karşı insektisidal etkinliği üzerine sıcaklığın etkisini belirlemek için yürütülmüştür. Bu amaçla buğday üzerinde üç farklı sıcaklıklarda (20, 25 ve 30ºC) ve %55 nispi nem ortamında buğday üzerinde yerel diatom toprağının 0, 100, 300, 500, 900 ile 1500 ppm (mg/kg) konsantrasyonlarında biyolojik testler yürütülmüştür. Mevcut çalışma sıcaklığın test edilen yerel diatom toprağın depolanmış tahıl zararlılarının karşı etkinliği üzerine önemli etkiye sahip olduğunu göstermiştir. Çalışmada sıcaklığın yerel diatom toprağının etkinliğine etkisi test edilen böcek türüne ve diatom konsantrasyonuna göre değişiklik gösterdiği görülmüştür. Yerel diatom toprağı uygulamalarında sıcaklık artışıyla *S. oryzae* ve *T. confusum* erginlerinin ölüm oranlarında artış saptanırken 30ºC'deki ölüm oranlarının 20ºC ve 25ºC'deki ölüm oranlarından önemli derecede daha yüksek olduğu görülmüştür. Ancak, yerel diatom toprağı uygulanan *R. dominica* erginlerinde ise 20ºC sıcaklıkta elde edilen *R. dominica* erginlerinin ölüm oranlarının 25ºC ve 30ºC sıcaklıktan elde edilen ölüm oranlarından daha yüksek olduğu bulunmuştur. Bu çalışmanın sonuçları, 500 ppm ila 900 ppm arasında değişen düşük konsantrasyonlarda *T. confusum* ve *S. oryzae* erginlerinde %100 ölüm oranının elde edildiğini göstermiştir. Sonuç olarak, bu çalışmada yürütülen biyolojik test sonuçlarına göre, yerel diatom toprak formülasyonun (ACN-1), buğday üzerinde depolanmış tahıl böceklerinin kontrolünde kullanılma potansiyeline sahip olabileceği sonucuna varılmıştır.

Anahtar sözcükler: Yerel diatom toprağı, *Rhyzopertha dominica*, *Sitophilus oryzae*, sıcaklık, *Tribolium confusum*

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Introduction

Stored grains and their byproducts are the most important durable food category for human nutrition. During grain storage, damage can be caused by a numerous pest, particularly insect species, known as stored product pests, which cause very serious quantitative loss and qualitative degradation (Hill, 2002; Rees, 2004). In grain commodities, residual contact insecticides, primarily pyrethroid and organophosphorus compounds, applied directly to grain when it is loaded into storage facilities have been the main components of grain management programs to maintain grain quality. The residues from single application of these insecticides protects the grains from the insects in stored grain. However, intensive research has focused on alternative control methods that can replace insecticides in stored grain due to the development of resistance to commonly used grain protectant insecticides (Subramanyam & Hagstrum, 1995), an increasing attention on testing and evaluating nontoxic and environmentally-friendly control methods (Arthur, 1996), and a possible loss of organophosphorus grain protectants in Europe and the USA due to regulatory action.

The use of diatomaceous earth (DE) is one of the most promising alternatives to insecticides in stored grains. The insecticidal activity of DE currently mined varies depending upon diatom species composition, geological and geographical origin as well as certain physical and chemical characteristics, such as particle size, silicon dioxide content, pH and density (Korunic, 1997). DEs are natural resource-based dry substances that can be used as insecticides (Korunic, 1998). They act on the insect cuticle by absorbing the lipids or cuticular abrasion, resulting in insect death through rapid desiccation. DEs are non-toxic to mammals (acute oral toxicity for the rats; LD_{50} > 5 g/kg of body weight), leave no toxic residues on the commodity and according to the US Environmental Protection Agency. Since DEs are classified in the category of GRAS (generally recognized as safe) they are used as food or feed additives (FDA, 1995). Regarding their insecticidal use, there is no specialized equipment required for DE treatment since they can be applied with the same application technology as other grain protectants (Athanassiou et al., 2005). They persist in the treated commodity, providing a long-term protection against insect pests, which is currently a major problem for the use of synthetic pesticides. Eventually, DEs are also completely compatible with organic food production (Subramanyam & Roesli, 2000).

Several DE formulations, based on natural deposits, are now commercially available, and have proven to be very effective against stored-grain pests (Subramanyam & Roesli, 2000; Athanassiou et al., 2011). However, the investigation to discover new naturally-occurring DEs that are more effective in insect control is still in progress, especially in areas rich in siliceous rocks. Based on the initial evidence and preliminary results, Turkey is considered to have rich natural DE deposits, and there is clear evidence for the existence of large DE deposits in different regions of Turkey (Özbey & Atamer, 1987; Mete, 1988; Sıvacı & Dere, 2006; Çetin & Taş, 2012). Diatomite reserves of Turkey are about 125 Gt. Hırka (Kayseri) in Turkey has the largest diatomite reserve (106 Gt) (Çetin & Taş, 2012). However, there are only a few published reports on the potential use of local DEs against stored-product insect pests. Doğanay (2013) reported that 750 and 1000 ppm of a Turkish DE formulation (Turco 1) applied to wheat and paddy rice resulted in high mortalities of *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae) (lesser grain borer) adults and significantly reduced progeny production, while 500 ppm and lower concentrations of Turco 1 had low efficacy against both tested insects and did not prevent reproduction. Akçalı et al. (2018) investigated efficacy of nine local DEs collected from different regions of Turkey against stored-grain insects, *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae) (rice weevil), *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) (confused flour beetle) and *R. dominica* and reported that CB2N-1 and BGN-1 local DE had high efficacy against *S. oryzae*, *T. confusum* and *R. dominica* adults and thus have potential to be successfully used for controlling stored-grain insect pests as a grain protectant. Sağlam et al. (2017) also evaluated some physical properties of local DEs collected from different locations of Turkey.

Different factors such as insect species, commodity, grain moisture and temperature are important for the efficiency of DEs in controlling stored-grain insects (Korunic, 1999; Fields & Korunic, 2000), and can be somewhat limiting for effective use of DEs in grain protection. Temperature is a crucial factor for the efficiency of DEs against stored-grain insects. Generally, increasing temperature increases the insecticidal efficiency of DE formulations against stored-grain insects such as *R. dominica* and *S. oryzae* (Fields & Korunic, 2000; Athanassiou et al., 2005). At higher temperatures, water loss of the insects is faster since insects are more mobile and more DE particles are adsorbed onto the cuticle (Fields & Korunic, 2000; Subramanyam & Roesli, 2000). Recent research has shown that DEs from different geographical locations can have different insecticidal activity, diatom species, pH, density, particle size distribution, internal surface area, lipid adsorption capability and effects on grain bulk density (Korunic, 1997). A few reports have indicated that DE deposits from Turkey are potentially effective for the control of stored-product insect pests (Doğanay, 2013; Sağlam et al., 2017; Akçalı et al., 2018). However, there is still inadequate information about the effectiveness of local DE mined from different locations of Turkey against stored-grain insects and the effect of abiotic factors (temperature and relative humidity) on the efficacy of these local DEs against stored-grain insects. The lack of information on toxic effects of these local DEs on stored-grain insects under different abiotic conditions justifies the study of these DE deposits for control of these insect pests. The objective of this study was to evaluate the efficiency of local DE formulation against *S. oryzae*, *R. dominica* and *T. confusum* adults in wheat at various temperatures.

Materials and Methods

Test insects

Sitophilus oryzae, T. confusum and *R. dominica* adults used in the bioassays were from stock cultures maintained in Entomology Laboratory of Plant Protection Department, Kahramanmaraş Sütçü İmam University. *Sitophilus oryzae* and *R. dominica* were reared on whole soft wheat with11% moisture content at 26±1ºC, 65±5% RH and 30±1ºC, 65±5% RH, respectively, and *T. confusum* were reared at 26±1ºC and 65±5% RH on a diet of wheat flour mixed with yeast (17:1, w/w) using standard culture techniques. Seven to 10 d-old adults of *S. oryzae*, *T. confusum* and *R. dominica* were exposed to DE treatments in bioassays.

Wheat cultivar

Untreated, clean, low admixture (0.8%) and infestation-free soft wheat (*Triticum aestivum* L., cv. Elbistan Yazlığı) was used in bioassays. One g of wheat corresponded to 21.3 individual grains. The moisture content of wheat used in bioassays, as determined by a Dickey-John moisture meter (Dickey-John Multigrain CACII, DICKEY-John Co., Lawrence, KS), ranged between 11.0 and 11.4%.

Local diatomaceous earth formulation

A local DE formulation (ACN-1) was used in bioassays. The DE was collected from diatomite reserves in Ankara Province located in Central Anatolia, Turkey. With the DE samples were crashed coarsely with a laboratory type knife hammer mill (LB 160, Mertest, Eskişehir, Turkey), they were oven dried oven (UF260 Memmert, Germany) at 100ºC for 48 h to give 3-4% moisture content. These samples were ground to powder using the same laboratory mill. With the DE powder was sieved by using metal sieve with 140 µm diameter (Retsch, Germany), natural grade local DE was obtained and used in bioassays. Some physical and chemical properties of the DE formulation (ACN-1) used in bioassays are given in Table 1 & 2.

Table 1. Some physical properties of local diatomaceous earth formulation (ACN-1)

DE formulation	Diatom type	Median particle Diameter (d (0.5)) (µm)[1]	Adherence of DE on wheat kernels (%)[2]	Percentage of crystalline silica (%)[3]	Colour
ACN-1	Freshwater	14.2	91.9	<3	Yellowish-white

[1] Median particle diameter value that corresponds to 50% of the total particle volume in the volumetric cumulative particle size distribution. Particle size analysis was conducted using laser light diffraction technique by Accredited Mineralogy and Petrography Laboratory of General Directorate of Mineral, Research and Exploration of Turkey.
[2] Tests for adherence rate of DE on wheat kernels were performed using method of Korunic (1997) in Stored Product Insects Laboratory of Kahramanmaraş Sütçü Imam University.
[3] Mineral/phase analysis of diatomaceous earth sample was performed using analytical Emperian X-Ray Diffraction device by the chemical-analytical laboratory of Advance Technologies Center of Kütahya Dumlupınar University.

Table 2. Total quantitative chemical analysis of local diatomaceous earth formulation (ACN-1)[1]

DE formulation	Loss on ignition (%)	Al_2O_3 (%)	CaO (%)	Fe_2O_3 (%)	K_2O (%)	MgO (%)	Na_2O (%)	SO_3 (%)	SiO_2 (%)	TiO_2 (%)
ACN-1	6.25	11.75	1.80	2.40	0.90	0.80	1.30	0.07	73.80	0.55

[1] Quantitative chemical analysis was conducted by Accredited Mineralogy and Petrography Laboratory of General Directorate of Mineral, Research and Exploration of Turkey. Atomic absorption spectroscopy device was also used in the analysis of the elements following melting and acid removal processes.

Bioassays

Bioassays were performed according to randomized parcel design with 3 x 5 factorial layout and with three replicates at three temperatures (20, 25 and 30±1ºC), 55±3% RH and five concentrations of local DE formulation (ACN-1): 100, 300, 500, 900 and 1500 ppm (mg DE/kg wheat). The desired relative humidity was maintained using saturated salt solution of magnesium nitrate as recommend by Greenspan (1977). For each experiment (temperature-DE concentration combination), five samples of 50 g wheat were taken. Each grain sample was placed in a small cylindrical self-standing centrifuge tube that was closed, apart from a hole 1.5 cm in diameter (at the top of the tube), and that was covered with muslin cloth to allow sufficient ventilation. The grain samples were treated individually with the respective DE concentration and then shaken manually for 5 min to achieve equal distribution of DE dust in the entire grain quantity. Three additional tubes containing untreated wheat served as control in each case. Subsequently, 30 1-wk-old adults of *S. oryzae* were introduced into each tube. The same procedure was followed with *T. confusum* and *R. dominica* adults. All tubes containing DE-treated wheat were placed in the lockable 80 l (26 × 36.5 × 15 cm) plastic container which contained saturated salt solution of magnesium nitrate under the plastic. The plastic containers were then placed in the incubator (IPP55 Plus, Memmert, Germany) set at the desired temperature. Three replicates were used for each trial (temperature-DE concentration combination). Dead adults of three species were counted 7 and 14 d after DE exposure. Temperature and relative humidity during bioassays were monitored by using HOBO digital recorders (HOBO H8, Onset Computers, Bourne, MA, USA).

Data analysis

Generally, for three species, the control mortality ranging from 0 to 7.7% was very low and therefore, no corrections were used. All mortality data for each species were normalized using arcsine transformation and then subjected to two-way ANOVA with main factors, temperature and DE concentration by using the GLM Procedure of SAS/STAT® 12.1 (SAS Institute, 2012). Mean mortality percentages for each species were separated by using the Duncan’s multiple range test at 5% significance level.

Results

The ANOVA analysis for mortality of *S. oryzae* indicated significant differences for the main effects, DE concentration (7-d exposure $F_{4,30}$ = 126, P<0.0001; 14-d exposure $F_{4,30}$ = 109, P < 0.0001), temperature (for 7-d exposure $F_{2,30}$ = 37.8, P < 0.0001; 14-d exposure $F_{2,30}$ = 26.5, P < 0.0001) and DE concentration x temperature interaction (7-d exposure $F_{8,30}$ = 11.4, P < 0.0001; 14-d exposure $F_{8,30}$ = 13.7, P < 0.0001). With *S. oryzae* at each temperature, increasing DE concentration from 100 to 300 ppm and from 300 to 500 ppm at 20 and 25°C resulted in significant increases in mortality with 7-d and 14-d exposure, and increasing DE from 500 to 900 ppm and 1000 ppm at the three temperatures did not produce any significant increase in mortality with 7-d and 14-d exposure (Table 3). With 7-d exposure, complete mortality was obtained at 1500, 900 and 500 ppm DE at 20, 25 and 30°C, respectively. However, with 14-d exposure, compete mortality was obtained at 900, 500 and 500 ppm DE at 20, 25 and 30°C, respectively. With *S. oryzae,* temperature had no significant effect on mortality at 500, 900 and 1500 ppm DE with 7-d and 14-d exposure (Table 3). At 100 ppm DE, there was no significant difference in mortality of *S. oryzae* at 20 and 25°C, while mortality at 30°C was significantly higher than at 20 and 25°C. At 300 ppm DE, mortality at 30°C was significantly higher than at 25°C, while there was no significant difference between mortality at 20 and 30°C.

Table 3. Mean mortality (%) of *Sitophilus oryzae* adults exposed to wheat-treated with local diatomaceous earth at five concentrations and three temperatures with 7-d and 14-d exposure

Concentration (ppm)	Mean mortality rate (%)±S.E.			F and P value	Mean mortality rate (%)±S.E.			F and P value
	7.day				14. day			
	20ºC	25ºC	30ºC		20ºC	25ºC	30ºC	
100 ppm	16.6±1.9 Bc*	33.3±3.0 Bc	88.3±4.6 Ab	$F_{2,6}$=83.15 P<0.0001	22.1±3.0 Bc	46.5±4.1 Bc	90.3±6.3 Aa	$F_{2,6}$=23.70 P=0.0019
300 ppm	76.6±6.6 BAb	67.8±5.0 Bb	95.3±3.0 Aba	$F_{2,6}$=8.40 P=0.0182	93.1±0.0 BAb	81.4±7.0 Bb	98.7±1.2 Aa	$F_{2,6}$=7.81 P=0.0214
500 ppm	95.5±2.2 Aa	98.8±1.1 Aa	100.0±0.0 Aa	$F_{2,6}$=2.04 P=0.2111	98.8±1.1 Aa	100.0±0.0 Aa	100.0±0.0 Aa	$F_{2,6}$=1.00 P=0.4219
900 ppm	97.7±2.2 Aa	100.0±0.0 Aa	100.0±0.0 Aa	$F_{2,6}$=1.00 P=0.4219	100.0±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-
1500 ppm	100.0±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-	100.0±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-
Control	0.0±0.0	3.3±1.9	4.4±1.1		4.4±1.1	4.4±1.1	7.7±1.1	
F and P value	$F_{4,10}$=50.77 P<0.0001	$F_{4,10}$=120 P<0.0001	$F_{4,10}$=8.20 P=0.0034		$F_{4,10}$=198.38 P<0.0001	$F_{4,10}$=63.71 P<0.0001	$F_{4,10}$=2.51 P=10.810	

* Two-way ANOVA was applied to the data. Means within a row with the same upper-case letter and a column with the same lowercase letter are not significantly different (Duncan test at 5% level).

With *T. confusum*, the analysis for mortality indicated significant differences for the main effects, DE concentration (7-d exposure $F_{4,30}$ = 527, P < 0.0001; 14-d exposure $F_{4,30}$ = 712, P < 0.0001), temperature (7-d exposure $F_{2,30}$ = 64.5, P < 0.0001; 14-d exposure $F_{2,30}$ = 20.8, P < 0.0001) and DE concentration x temperature interaction (7-d exposure $F_{8,30}$ = 12.4, P < 0.0001; for 14-d exposure $F_{8,30}$ = 5.37, P = 0.0003). At 20°C, mortality significantly increased with increasing DE concentration with 7-d exposure. At 25°C, there was no significant difference between mortality at 100 and 300 ppm DE, while increasing DE from 300 to 500, 900 and 1500 ppm resulted in significant increases in mortality. At 30°C, DE from 100 to 300 ppm and from 500 to 900 and 1500 ppm resulted in significant increase in mortality with 7-d exposure, while increasing DE from 900 to 1500 ppm did not produce significant increase in mortality. With 14-d exposure, mortality of *T. confusum* at 100 ppm DE was significantly lower than at 300 ppm DE at all temperatures, while there were not significant differences between mortality at 500, 900 and 1500 ppm DE. With 14-d exposure, complete mortality of *T. confusum* was obtained with 1500, 900 and 500 ppm DE at 20, 25 and 30°C, respectively (Table 4). For *T. confusum* at 100 and 300 ppm DE, there was no significant temperature effect on mortality at 20 and 25°C, while mortality at 30°C was significantly higher than at 20 and 25°C with 7-d and 14-d exposure. At 900 and 1500 ppm DE, there was no significant difference in mortality at 25 and 30°C, while mortality at 25 and 30°C was significantly higher than at 20°C with 7-d exposure. With 14-d exposure, at 900 and 1500 ppm DE, there was no significant difference between mortality between the temperatures.

Table 4. Mean mortality (%) of *Tribolium confusum* adults exposed to wheat-treated with local diatomaceous earth at five concentrations and three temperatures with 7-d and 14-d exposure

Concentration (ppm)	Mean mortality rate (%)±S.E. 7.day			F and P value	Mean mortality rate (%)±S.E. 14. day			F and P value
	20ºC	25ºC	30ºC		20ºC	25ºC	30ºC	
100 ppm	0.0±0.0 Bc*	1.1±1.1 Bd	8.8±2.2 Ad	$F_{2,6}$=14.49 P=0.0050	13.3±3.3 Bc	14.4±4.1 Bc	55.5±4.8 Ab	$F_{2,6}$=43.96 P=0.0003
300 ppm	3.3±1.9 Bc	1.1±1.1 Bd	46.6±3.3 Ac	$F_{2,6}$=39.10 P=0.0004	58.8±2.9 Bb	62.2±4.8 Bb	94.4±2.9 Aa	$F_{2,6}$=17.89 P=0.0030
500 ppm	23.3±3.3 Cb	48.8±2.9 Bc	85.5±6.1 Ab	$F_{2,6}$=37.00 P=0.0004	90±3.8 Ba	97.7±1.1 ABa	100.0±0.0 Aa	$F_{2,6}$=8.56 P=0.0175
900 ppm	28.8±2.2 Bb	90±1.9 Ab	97.7±2.2 Aa	$F_{2,6}$=73.89 P<0.0001	93.3±5.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	$F_{2,6}$=2.74 P=0.1430
1500 ppm	87.7±5.5 Ba	100±0.0 Aa	100±0.0 Aa	$F_{2,6}$=17.85 P=0.0030	100±0.0 Aa	100.0±0.0 Aa	100.0±0.0 Aa	-
Control	0.0±0.0	0.0±0.0	0.0±0.0		0.0±0.0	0.0±0.0	0.0±0.0	
F and P value	$F_{4,10}$=77.58 P<0.0001	$F_{4,10}$=248.83 P<0.0001	$F_{4,10}$=81.32 P<0.0001		$F_{4,10}$=49.90 P<0.0001	$F_{4,10}$=207.08 P<0.0001	F_{410}=41.10 P<0.0001	

* Two-way ANOVA was applied to the data. Means within a row with the same upper-case letter and a column with the same lower-caseletter are not significantly different (Duncan test at 5% level).

With *R. dominica*, the analysis for mortality indicated significant differences for the main effects, DE concentration (7-d exposure $F_{4,30}$ = 339, P<0.0001; 14-d exposure $F_{4,30}$ = 191, P<0.0001), temperature (7-d exposure $F_{2,30}$ = 87.3, P<0.0001; 14-d exposure $F_{2,30}$ = 33.9, P<0.0001) and DE concentration x temperature interaction (7-d exposure $F_{8,30}$ = 10.7, P<0.0001; 14-d exposure $F_{8,30}$ = 3.80, P = 0.0035). In most cases, the increasing DE concentration increased mortality of *R. dominica* (Table 5). With 7-d exposure at 20ºC, and with 14-d exposure at 20 and 30ºC, increasing DE concentration from 100 to 300 ppm resulted in a significant increase in mortality. With 7-d and 14-d exposure at 30ºC, mortalities at 900 and 1500 ppm were significantly higher than at all other DE concentrations, and at 25ºC, mortality at 1500 ppm was significantly higher than at all other concentrations. Complete mortality of *R. dominica* was only recorded at 20ºC at 1500 ppm DE with 14-d exposure (Table 5). For *R. dominica* at 100 ppm, there was no significant effect of temperature with 7-d and 14-d exposure. At the concentrations above 100 ppm, mortality at 20ºC was significantly higher than at 25ºC with 7-d and 14-d exposure. At all concentrations, 20 and 30ºC gave similar mortality with 7-d and 14-d exposure, other than at 300 and 500 ppm with 7-d exposure.

Table 5. Mean mortality (%) of *Rhyzopertha dominica* adults exposed to wheat-treated with local diatomaceous earth at five concentrations and three temperatures with 7-d and 14-d exposure

Concentration (ppm)	Mean mortality rate (%)±S.E 7. day			F and P value	Mean mortality rate (%)±S.E 14. day			F and P value
	20ºC	25ºC	30ºC		20ºC	25ºC	30ºC	
100 ppm	3.3±1.9 Ad*	0.0±0.0 Ad	0.0±0.0 Ac	$F_{2,6}$=3.67 P=0.0912	5.9±3.1 Ad	1.8±1.8 Ad	0.0±0.0 Ad	$F_{2,6}$=3.40 P=0.1032
300 ppm	20.0±5.0 Ac	3.3±1.9 Bdc	0.0±0.0 Bc	$F_{2,6}$=16.5 P=0.0036	30.6±4.2 Ac	8.9±3.8 Bdc	18.4±2.2 BAc	$F_{2,6}$=7.57 P=0.0228
500 ppm	77.7±2.9 Ab	6.6±1.9 Cc	43.2±6.2 Bb	$F_{2,6}$=74.84 P<0.0001	87±4.7 Ab	20.2±2.9 Bc	66.6±6.9 Ab	$F_{2,6}$=32.06 P=0.0006
900 ppm	87.7±2.2 Aba	54.4±2.2 Bb	83.1±3.3 Aa	$F_{2,6}$=36.69 P=0.0004	95.2±3.1 Aba	71.9±2.2 Bb	90.8±3.0 BAa	$F_{2,6}$=8.75 P=0.0166
1500 ppm	95.5±2.2 Aa	75.5±2.9 Ba	87.6±1.1 BAa	$F_{2,6}$=9.71 P=0.0132	100.0±0.0 Aa	89.8±3.3 Aa	94.2±4.1 Aa	$F_{2,6}$=1.67 P=0.2654
Control	0.0±0.0	0.0±0.0	1.1±1.1		5.5±1.1	1.1±1.1	3.3±0.0	
F and P value	$F_{4,10}$=71.34 P<0.0001	$F_{4,10}$=113.76 P<0.0001	$F_{4,10}$=284.7 P<0.0001		$F_{4,10}$=71.17 P<0.0001	$F_{4,10}$=53.29 P<0.0001	$F_{4,10}$=75.96 P<0.0001	

* Two-way ANOVA was applied to the data. Means within a row with the same upper-case letter and a column with the same lowercase letter are not significantly different (Duncan test at 5% level).

Discussion

Insecticidal efficacy of DE is highly influenced by several factors including temperature and DE formulation and concentration (Kavallieratos et al., 2007). The present study indicated that temperature had a significant effect on the insecticidal efficacy of local DE when tested on stored-grain insects. The temperature effects on insecticidal efficacy varied with insect species and DE concentration. Mortality of *S. oryzae* and *T. confusum* adults generally increased with increasing temperature and mortality at 30ºC was significantly higher than at 20ºC and 25ºC. Studies of the influence of temperature on efficiency of some commercial DEs against *S. oryzae* and *T. confusum* adults indicated that increasing temperature generally resulted in increasing insecticidal efficiency against *S. oryzae* adults (Fields & Korunic, 2000; Arthur, 2002; Athanassiou et al., 2005; Vassilakos et al., 2006; Rojht et al., 2010). Arthur (2000) reported that *T. castaneum* and *T. confusum* exposed directly to the DE formulation, Protec-It, at controlled temperatures showed a progressive increase in mortality as temperature increased from 22 to 27 and 32°C. Similarly, Vassilakos et al. (2006) reported that insecticidal efficacy of the commercial DE formulation, Silisosec®, against *S. oryzae* adults increased with increasing temperature. These results parallel those obtained in the present study of local DE. This could be attributed to the fact that at higher temperatures insects are usually more mobile and the possibility of picking up dust particles is increased (Arthur, 2000, 2001; Fields & Korunic, 2000; Rigaux et al., 2001). In addition, water loss is likely to be increased at higher temperatures (Arthur, 2000; Fields & Korunic, 2000). Also, the rate of cuticular transpiration rises only slightly with temperature until the transition temperature, which for most insects is above 30°C (Wigglesworth, 1972). However, increased temperature would also increase feeding and therefore moisture replacement through the food and production of metabolic water. The synthesis of cuticular waxes may be faster at higher temperatures because of temperature effects on the biochemical pathways. This positive increase in toxicity with temperature is also similar to data reported for exposure studies with organophosphate insecticides (Turnbull & Harris, 1986).

In the present study, inconsistent results for *R. dominica* were obtained regarding the effect of temperature on the insecticidal efficacy of the local DE. The mortality at 20ºC was significantly higher than at 25ºC and 30ºC. It is clear that there is not a positive correlation between temperature and mortality of *R. dominica* adults exposed to the local DE. Inconsistent results have been reports for the effect of temperature on the insecticidal efficacy of DEs against *R. dominica* adults. Several studies regarding efficiency of some commercial DEs against *R. dominica* adults indicated that increasing temperature generally resulted in increasing mortality (Fields & Korunic, 2000; Athanassiou et al., 2005; Vassilakos, 2006). These results do not parallel those obtained in present study of local DE. However, while using the DE formulation, Protec-It, Vardeman et al. (2006) noted that temperature did not significantly affect mortality of *R. dominica* adults. These results parallel those obtained in present study.

Studies of several commercially available DEs demonstrated that a satisfactory level of grain protection is achieved with application rates that are much lower than 1000 ppm (Arthur, 2000; Fields & Korunic, 2000; Arthur & Throne, 2003; Athanassiou et al., 2003, 2005; Ceruti et al., 2008; Kljajic et al., 2010; Athanassiou et al., 2014; Baldassari & Martini, 2014; Nesvorna & Hubert, 2014). Athanassiou et al. (2011) noted that DEs mined from several parts of Europe were effective at 900 ppm. In the present study, complete mortality was obtained against adults of *S. oryzae* and *T. confusum* with concentrations ranging from 500 to 900 ppm with 14-d DE exposure. However, complete mortality of *R. dominica* was obtained only at 1500 ppm DE at 20°C. Generally, lower efficacy of local DE against *R. dominica* adults was observed at all tested temperatures compared with those reported for commercial DE formulations (Silicosec, Insecto and others). This difference can be attributed to physical, morphological and chemical characters of the local DE formulation, and internal characteristics or physical properties of the wheat cultivar used. Other studies have shown that *S. oryzae* is the most DE-susceptible, followed by *R. dominica* and *T. confusum* (Korunic, 1998; Arthur, 2000; Subramanyam & Roesli, 2000; Fields & Korunic, 2000; Kavallieratos et al., 2005; Athanassiou et al., 2014). In the present study, *S. oryzae* was the most DE-susceptible, followed by *T. confusum* and *R. dominica*. It seems that *R. dominica* was the most tolerant species to the local DE. *Rhyzopertha dominica* adults are not very agile (Flinn & Hagstrum, 2011), so the possibility of picking up DE particles is decreased and this might be why this species is among the most DE-tolerant species (Korunic, 1998; Fields & Korunic, 2000). Kavallieratos et al. (2005) also reported that 750 ppm DEs (SilicoSec and Insecto) were needed to obtain high mortality of *R. dominica* adults in wheat and maize.

The results of the present study indicated that complete mortality of *T. confusum* achieved at lower concentrations, ranging from 500 to 900 ppm. This is particularly important, since other studies with other DEs suggest that the same mortality can be achieved with 1000 ppm or higher (Aldryhim, 1990; Athanassiou et al., 2004, 2005; Vayias & Athanassiou, 2004). In conclusion, the present study indicated that temperature had significant effect on the insecticidal efficacy of a local DE against stored-grain insects. Generally, higher temperature increased the efficacy of the local DE with an exception of species of *R. dominica*, which showed greater tolerance at 30°C than that at 20°C. Therefore, temperature effects on insecticidal efficacy of the DE tested varied with insect species and concentration of DE. Moreover, based on the results of the bioassays in this study, the local DE (ACN-1) has potential to be used for control of stored-grain insects in wheat. Additional studies are required to determine the effects of biotic and abiotic factors on efficacy of the local DE deposit against other stored-grain insects and then to evaluate its insecticidal performance under field conditions.

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