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Original article (Orijinal araştırma)

Insecticidal and repellency effects of a Turkish diatomaceous earth formulation (Detech) on adults of three important pests of stored grain

Yeni bir Türk diatom toprağı formülasyonunun (Detech) üç ana depolanmış tahıl zararlıların erginlerine karşı insektisidal ve kaçırıcı etkisi

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Abstract

In this study, laboratory experiments were conducted in order to assess the insecticidal and repellency effects of a novel Turkish diatomaceous earth (DE) formulation (Detech) on adults of 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). For the insecticidal activity, bioassays were conducted with soft wheat treated with 0, 600 and 900 ppm of Detech at 25 ± 1°C and 65 ± 5% RH and progeny production was assessed after 45 days. For the repellency tests, two-choice tests on mono-layer wheat were conducted at 1,000 ppm of Detech at 25 ± 1°C and 65 ± 5% RH. Overall, Detech was effective against *T. confusum* and *S. oryzae* adults in wheat, causing 82% to 100% mortality at 600 and 900 ppm after 7 and 14 days exposure. The complete, or almost complete, progeny inhibition of *S. oryzae* were found at both concentrations, whereas the highest reduction in *R. dominica* progeny (84%) was obtained at 900 ppm. Detech was highly and moderately repellent to *T. confusum* and *S. oryzae* adults, respectively, whereas it had no or low repellency effect on *R. dominica* adults. In conclusion, Detech, which consists of a mixture of three DE deposits with different diatom frustules has potential for use against stored-grain insect pests as a promising grain protectant. Experiments were conducted in 2020-2021 in Entomology Laboratory of Tekirdağ Namık Kemal University and Kahramanmaraş Sütçü İmam University.

Keywords: Natural insecticides, repellency, *Rhyzopertha dominica, Sitophilus oryzae, Tribolium confusum,* wheat

Öz

Bu çalışmada, yeni bir Türk diyatom toprağı formülasyonunun (Detech) pirinç biti *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), kırma biti, *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) ve ekin kambur biti, *Rhyzopertha dominica* (F., 1792) erginlerine karşı insektisidal ve kaçırıcı etkisini değerlendirmek için laboratuvar denemeleri yürütülmüştür. İnsektisidal aktivite için, Detech'in 0, 600 ve 900 ppm konsantasyonu uygulanan ekmeklik yumuşak buğday üzerinde 25 ± 1°C sıcaklık ve %65 ± 5 nispi nemde biyolojik testler yürütülmüş ve 45 gün sonra yeni nesil ergin sayıları değerlendirilmiştir. Kaçırıcı etki için, 25 ± 1°C sıcaklık ve %65 ± 5 nispi nemde ve Detech'nin 1000 ppm konsantrasyonu uygulanan tek katmanlı buğday üzerinde iki seçenekli testler gerçekleştirilmiştir. Genel olarak, Detech buğday üzerinde *T. confusum* ve *S. oryzae* erginlerine karşı çok etkili olurken 7 ve 14 gün maruz bırakma süresi sonunda 600 ve 900 ppm'de *T. confusum* ve *S. oryzae* erginlerin %82-100 ölüme neden olmuştur. Her iki konsantrasyonda da *S. oryzae*'nın yeni nesil ergin çıkışı tamamen veya tamama yayın engellenirken, *R. dominica* da ise yeni nesil ergin çıkışındaki en yüksek azalma (%84) 900 ppm'de elde edilmiştir. Detech, *T. confusum* ve *S. oryzae* erginlerinde karşı oldukça yüksek düzeyde, *R. dominica* erginlerinde düşük kaçırıcı etki göstermiştir. Sonuç olarak, Detech'in, tahıllarda umut verici bir koruyucu insektisit olarak depolanmış tahıl zararlılarına karşı etkin bir şekilde kullanım potansiyeline sahip olduğunu göstermektedir. Denemeler 2020-2021 yılında Tekirdağ Namık Kemal ve Kahramanmaraş Sütçü İmam Üniversitesi Entomoloji laboratuvarlarında yürütülmüştür.

Anahtar sözcükler: Doğal insektisitler, kaçırıcı etki, *Rhyzopertha dominica*, *Sitophilus oryzae, Tribolium confusum,* buğday

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Introduction

One of the most promising alternatives to the use of insecticides, including synthetic, petroleum-based contact organophosphate and pyrethroid compounds, in durable stored products is the use of diatomaceous earths (DEs). DEs are composed by the fossil skeletons of phytoplankton, also known as diatoms, which occur in fresh and salt water (Quarles, 1992; Losic & Korunic, 2018). Differences in physical properties of the diatoms include shape, bulk density, surface area, oil and water absorption capabilities, average pore size and pore size distribution (Losic & Korunic, 2018). Therefore, the DEs currently mined vary markably in their insecticidal activity, depending upon species composition, geological and geographical origin as well as certain chemical characteristics, such as SiO_2 content, pH and tapped density (Korunic, 1997). DEs are probably the most efficacious natural resource-based dry materials that can be used as insecticides (Korunic, 1998). The mode of action of DEs has been described indicatively in many studies (Ebeling, 1971; La Hue, 1978; Golob, 1997; Korunic, 1998; Korunic & Fields, 2016). In general, DEs abrade the insect cuticle, absorb lipids from the epicuticle, and cause death by desiccation and water loss (Ebeling, 1971; Golob, 1997; Korunic, 1998; Subramanyam & Roesli, 2000). DEs are also repellent to insects and this repellence depends on the dose (Quarles, 1992; White et al., 1966; Rigaux et al., 2001; Mohan & Fields, 2002). Since DEs are inert (silicaceous) materials, no interaction with the environment occurs. Thus, DEs persist in the treated substrate, providing a long-term protection against insect pests.

There are several (>30) DE formulations, based on natural deposits, which are now commercially available throughout the world (Subramanyam & Roesli, 2000; Fields & Korunic, 2000; Kavallieratos et al., 2005; Losic & Korunic, 2018). Many studies have shown that they can be quite effective against a wide range of stored-grain insect species (Subramanyam et al., 1994; Korunic, 1998; Subramanyam & Roesli, 2000; Wakil et al., 2006; Athanassiou et al., 2007, 2011; Kostyukovsky et al., 2010). However, the use of diatomaceous earth was limited because the required dose rates of 1.0 to 3.5 g per kg of grain (1,000 to 3,500 ppm) for most DE products significantly reduced the grain bulk density and flowability, and left visible dust residues, which are not widely accepted by quality standards in many countries (Subramanyam et al., 1994; Golob, 1997). Considerable research has been conducted to address these problems and develop new DE formulations with enhanced performance and reduced DE dosage that have no adverse effect on grain quality (Arnaud, 2005; Nikpay, 2006; Athanassiou & Korunic, 2007; Korunic, 2013).

Based on initial evidence and preliminary samples, it seems that Turkey is a rich source of natural DE deposits, and there is clear evidence for the existence of large DE deposits in the Central and West Anatolia Regions of Turkey (Özbey & Atamer, 1987; Mete, 1988; Sıvacı & Dere, 2006; Çetin & Taş, 2012). Diatomite reserves of Turkey are around 125 Mt. However, there are few published data on the potential use of some local DEs against stored-grain insect pests. Doğanay (2013), Işıkber et al. (2016) and Akçalı et al. (2018) evaluated some local DEs collected from different regions of Turkey against stored-grain insects with promising results. Akçalı et al. (2018) investigated efficacy of nine local DEs collected from different regions of Turkey against stored-grain insects, 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) and reported that CB2N-1 and BGN-1 local DEs had high efficacy against these stored-grain insects and thus, could be potential to be successfully used for controlling stored-grain insect pests as a grain protectant.

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. Thus, as result of extensive screening tests of DEs from several regions of Turkey, a novel Turkish DE deposit, Detech, which consists of a mixture of three freshwater DEs collected from different DE reserves located in different regions of Turkey, has been commercially formulated by Entoteam R&D Food Agriculture Co. This novel DE formulation, Detech, is consisted of two different diatom frustules, which are completely different from the other commercial DE formulations with single diatom frustules. However, there is inadequate or limited information about the effectiveness of Detech against stored-grain insects. The lack of information on insecticidal and repellency effects of Detech on stored-grain insects justifies the need of the present study. The objective of our study was to evaluate the insecticidal and repellency effects of novel Turkish diatomaceous earth formulation, Detech against adults of *S. oryzae*, *T. confusum*, and *R. dominica* on wheat.

Materials and Methods

Experiments were conducted in 2020-2021 at Entomology Laboratory of Tekirdağ Namık Kemal University and Kahramanmaraş Sütçü İmam University.

Test insects

For both the insecticidal and repellency tests, *S. oryzae, T. confusum* and *R. dominica* adults were reared in the Entomology Laboratory of Plant Protection Department, Tekirdağ Namık Kemal University and Kahramanmaraş Sütçü İmam University, Turkey. *Sitophilus oryzae* and *R. dominica* individuals were reared on whole soft wheat with 11% moisture content at 26 ± 1ºC, 65 ± 5% RH and 30 ± 1ºC, 65 ± 5% RH, respectively, whereas *T. confusum* was reared at 26 ± 1°C and 65 ± 5% RH in mixture of wheat flour with yeast (17:1, w/w). Seven- to 10-day-old adults of all the above species were used for all bioassays.

DE formulation

A Turkish diatomaceous earth formulation (Detech) was used in the bioassays and repellency tests. Detech is a mixture of freshwater diatomaceous earth deposits collected from there different DE reserves located in Central Anatolia of Turkey. It is consisted of naturally occurring amorphous silica and has white-gray color and is formulated by Entoteam R&D Food Agriculture Co. to use for insect pests control as an insecticide (pest control grade). According to total quantitative chemical analysis of Detech by atomic absorption spectroscopy, it contains 80.6% SiO_2, 4.75% CaO, 4.7% Al_2O_3, 1.5% Fe_2O_3, 0.85% MgO, 0.5% K_2O, 0.4% Na_2O, and less than 0.01% TiO_2. Some physical properties of Detech used in bioassays are given in Table 1. Scanning electron microscopy images of diatom frustules present in Turkish diatomaceous earth formulation (Detech) is presented in Figure 1. Strong uptake and attachment were observed when analyzing the images of Detech, it consists of a large number of cylindrical- and rod-shape diatom skeletons.

Table 1. Some physical properties of a proprietary Turkish diatomaceous earth (DE) formulation, Detech

DE formulation	Diatom type	Median particle diameter (µm)[1]	Adherence of DE to wheat kernels (%) mean ± SE (n = 3)[2]	Tapped bulk density (g/L) mean ± SE (n = 3)[3]	pH, mean ± SE (n = 3)	Color
Detech	Freshwater	14.1	84.0 ± 1.7	300.0 ± 2.9	8.25 ± 0.01	Yellowish-white

[1] The 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, Turkey.

[2] Tests for adherence rate of DE on wheat kernels were performed using method described by Korunic (1997) in Stored Product Insects Laboratory of Kahramanmaraş Sütçü Imam University.

[3] Tests for bulk density of DE on wheat kernels were performed using method described by Korunic (1997) in Stored Product Insects Laboratory of Kahramanmaraş Sütçü Imam University.

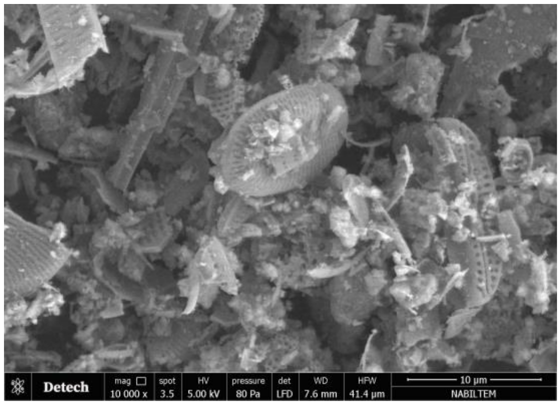

Figure 1. Scanning electron microscopy image of diatom frustules present in Turkish DE formulation (Detech) (10,000x magnification), (NABILTEM, Tekirdağ Namık Kemal University).

Commodity

Untreated, clean, with low dockage (0.8%) and infestation-free soft wheat (*Triticum aestivum* L., cv. Elbistan Yazlığı) was used for the bioassays. The moisture content of wheat cultivar, as determined by a Dickey-John moisture meter (Dickey-John Multigrain CAC II, DICKEY-John Co., Lawrence, KS), ranged between 11.0 and 11.4%.

Experimental arena used for the repellency tests

For two-choice test on mono-layer wheat, experimental test arena consisted plastic rectangular containers (25 × 13 × 6 cm LWH; Akyüz Plastic Co., Istanbul, Turkey). Dry concrete mix (Daracon C30/37, Draco Co., Istanbul, Turkey) was mixed with three parts water and poured into plastic rectangular container to a depth of 2 cm. After the concrete surface was completely dry, it was divided by 2 mm metal sieve into three different areas given below: 1. The area containing DE-treated wheat (10 × 13 cm), 2. Empty area between DE-treated and non-treated wheat (transition area) (5 × 13 cm), 3. The area containing non-DE-treated wheat (10 × 13 cm).

Insecticidal bioassays

Bioassays were conducted according to randomized block design with 3 x 2 factorial layout and with three replicates for all tested insect species and two concentrations 600 and 900 ppm (mg DE/kg wheat), which were selected according to according preliminary tests. Bioassays were conducted in the lockable 80 l (26 cm × 36.5 cm × 15 cm) plastic container; Akyüz Plastic Co., Istanbul, Turkey) in the incubator (IPP55 Plus, Memmert, Germany) at 25 ± 1°C temperature, 65 ± 5% RH and continuous darkness. The desired RH was maintained by using saturated salt solution of magnesium nitrate as recommend by Greenspan (1977). One kg lots of grain were prepared for each replicate and each was treated with 600 and 900 ppm of Detech. Additional 1 kg lots of wheat grain were 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 & Roesli, 2000). For each trial (insect species-DE concentration combination), three sub-samples of 50 g wheat were taken from Detech-treated lots, and placed in 50 ml small cylindrical self-standing centrifuge tube (Isolab, Labor Teknik Co., Istanbul, Turkey) 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 for sufficient ventilation. Three additional tubes containing untreated wheat served as control in each treatment. Then, 30 one-week-old adults of each species were introduced into each tube. All tubes were placed in the lockable plastic container which contained saturated salt solution of magnesium nitrate under the plastic and set at the desired temperature level. Three replicates were used for each trial (tested insect species-DE concentration combination). Adult mortality was counted after 7 and 14 days exposure.

After 14 days individuals of *S. oryzae* and *R. dominica* were removed from the vials and the grain were left for an additional period of 45 days. Then, the vials were opened and the number of total progenies per treated commodity was counted. Since *T. confusum* is secondary pest that is not able to feed on the whole wheat grain, its progeny production was not assessed in present study. Temperature and RH during all the bioassays were monitored by using HOBO digital recorders (HOBO H8, Onset Computers, Bourne, MA, USA).

Repellency bioassays

Repellency bioassays were conducted according to randomized parcel design with five replicates at 1,000 ppm concentration of DE (mg DE/kg wheat) using the two-way choice method to assess the likelihood of the insects avoiding contact with DE-treated grain. Three kg lots of grain were prepared and each of them was treated with 1,000 ppm of Detech. 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 & Roesli, 2000). For each treatment, 25 g of Detech-treated and untreated (control) wheat grain were placed in a single layer on each area of plastic rectangular respectively. Fluon (polytetrafluoroethylene, Sigma Aldrich, St. Louis, MO, USA) was applied to the edges of the containers to prevent insects from climbing and escaping from the experimental arena. Thirty adults of mixed sex and >14 days old were placed in empty area without wheat grain and then experimental arena was closed with a plexiglass with small holes. The number of insects found within DE-treated or untreated wheat was counted after 0.5, 1, 3, 5 and 7 days. No dead insects were observed for each observation time. The repellency tests were conducted under laboratory conditions at 25 ± 1ºC, 60 ± 5% RH and complete darkness.

Data analysis

Mortality counts were corrected by Abbott's formula (Abbott, 1925). All mortality data after 7 and 14 days for each species were normalized using arcsine transformation and then subjected to two-way ANOVA with main factors, insect species and DE concentrations by using the GLM Procedure of SAS/STAT 12.1 (SAS, 2012). Mean mortality percentages for each species and DE concentrations were separated by using the Tukey's HSD (honestly significant difference) test and a two-sided *t*-test (comparing treatment groups) at 5% significant level respectively. The differences in number of adult emergences of the new generation from the control were tested using Dunnett's test (P = 0.005). Adult progeny in each insect species-DE concentration combination was compared with adult progeny in the control vials using Dunnett's test at 5% significant level. The formula was used to determine the percentage reduction in the number of adults in new generation: ((Nc-Nt) / Nc) x 100, where, Nc was the number of adult progenies in the control and Nt the number of adult progenies in DE treatments. The means of percentage reduction in the number of adults of new generation for each insect species were separated by using a two-sided *t*-test (comparing treatment groups) at 5% significant level.

For the repellency tests, adults of *S. oryzae*, *T. confusum* and *R. dominica* adults, the proportion of the insects present in arenas with Detech-treated and untreated wheat for each observation time was analyzed using a two-sample Chi-square test for equality of proportions with continuity correction to see whether mean proportions of the insects in arenas with DE-treated and untreated wheat differ significantly from an equal distribution of 50%. The percentage repellence (PR) of DE treatment for each insect species was also calculated after each observation time using the formula: ((Nc-Nt) / (Nc+Nt)) × 100, where, Nc is the number of insects present in untreated wheat arena and Nt is the number of insects present in DE-treated wheat arena. The averages were then assigned to different classes (0 to V) using the scale given in Table 2 (Juliana & Su, 1983).

Table 2. Repellency classes assigned according to the percentage repellency ranges

Class	Repellency (%)	Class	Repellency (%)*
0	0.01-0.10	III	40.1-60.0
I	0.11-20.0	IV	60.1-80.0
II	20.1-40.0	V	80.1-100

***** All percentage repellency data for each species were normalized using arcsine transformation and then subjected to two-way ANOVA with main factors, insect species and observation intervals by using the GLM Procedure of SAS/STAT 12.1 (SAS, 2012). Mean percentage repellency for each species and observation interval was separated by using the Tukey's HSD (honestly significant difference) test at 5% significant level.

Results

Mortality

The two-way ANOVA analysis for insect mortality indicated significant differences for the main effects, tested insect species, DE concentration, and tested insect species x DE concentration interaction after 7 and 14 days exposure (Table 3). After 7 and 14 days exposure, no significant differences were obtained between mortality of *S. oryzae* and *T. confusum* at both concentrations, whereas the mortality of both *S. oryzae* and *T. confusum* were significantly higher than those of *R. dominica* (Table 4). After 7 days exposure, the highest mortality (93-97%) was observed in *T. confusum* at both concentrations, followed by *S. oryzae* (82-92%) whereas the lowest mortality (24-61%) was recorded in *R. dominica* adults. Total mortality was observed for *T. confusum* after 14 days at both concentrations, followed by *S. oryzae* (97-100%) whereas the lowest mortality (28-67%) was recorded in *R. dominica*. No complete mortality (100%) was observed for *R. dominica* after 7 and 14 days exposure whereas mortality ranged between 82-100% for *S. oryzae* and *T. confusum*.

Table 3. ANOVA parameters for main effects and associated interactions for mortality of *Sitophilus oryzae, Tribolium confusum* and *Rhyzopertha dominica* after two exposure intervals (total df = 12)

Exposure interval	Source	df	F	P
7 days	Insect species	2	101.0	<0.0001
	DE concentration	1	27.0	<0.0001
	Insect species*DE concentration	2	4.01	<0.046
14 days	Insect species	2	296.0	<0.0001
	DE concentration	1	36.9	<0.0001
	Insect species*DE concentration	2	15.0	<0.0001

DE concentration effects on adult mortality of the tested insect species are shown in Table 4. Increasing the concentration from 600 to 900 ppm resulted in significant increase of mortality of *R. dominica* after 7 and 14 days exposure whereas increasing the concentration from 600 to 900 ppm did not result in a significant increase of mortality of *S. oryzae* and *T. confusum* after 7 and 14 days exposure. Mortality of *R. dominica* was significantly higher at 900 ppm than that at 600 ppm after 7 and 14 days exposure, whereas there were not significant differences in the mortality of *S. oryzae* and *T. confusum* at 600 and 900 ppm for both exposure time.

Table 4. Mean mortality (mean ± SE) of *Sitophilus oryzae, Tribolium confusum* and *Rhyzopertha dominica* adults exposed for 7 and 14 days on wheat treated with at 600 and 900 ppm concentration of Detech in wheat

Exposure time	Concentration (ppm)	Insect species						F and P* value
		S. oryzae		*T. confusum*		*R. dominica*		
7 days	600	82.1 ± 4.0	A	93.3 ± 1.9	A	23.6 ± 4.0	Bb	$F_{2,6}$ = 82.3 P < 0.0001*
	900	92.2 ± 4.4	A	96.6 ± 0	A	60.7 ± 2.9	Ba	$F_{2,6}$ = 29.4 P = 0.0008*
	Control	0 ± 0		0 ± 0		1.1 ± 1.1		
	t and P value	*t* (3) = 1.77 P = 0.220		*t* (3) = 1.80 P = 0.214		*t* (3) = 7.49 P = 0.017**		
14 days	600	96.6 ± 1.9	A	100 ± 0	A	28.4 ± 3.4	Bb	$F_{2,6}$ = 82.25 P < 0.0001*
	900	100 ± 0	A	100 ± 0	A	67.1 ± 2.3	Ba	$F_{2,6}$ = 29.42 P = 0.0008*
	Control	1.1 ± 1.1		1.1 ± 1.1		2.2 ± 1.1		
	t and P* value	*t* (3) = 1.91 P = 0.196		- -		*t* (3) = 7.29 P = 0.018**		

* One-way ANOVA was applied to the data for insect species in the row for each exposure time. Means within rows followed by the same uppercase letter are not significantly different (Tukey's HSD test at 5% level).

** Student's t-test was applied to the data for DE concentration in the column for each exposure time and means within columns followed by the same lowercase letter are not significantly different (at 5% level).

Progeny production

Dunnett's test showed that progeny production of *S. oryzae*, *T. confusum* and *R. dominica* in control groups was significantly higher than that recorded in Detech-treated wheat (Table 5). All main effects were significant for progeny production in Detech-treated wheat. High progeny suppression was recorded for *S. oryzae* and *T. confusum* individuals (94-100%) whereas lower suppression was recorded for *R. dominica* (60-85%). When Detech at both concentrations were applied, significantly more progeny was suppressed for *S. oryzae* and *T. confusum* than for *R. dominica*. DE concentration also had a significant effect on the suppression of progeny production. Detech treatment at 900 ppm caused significantly more progeny suppression of *S. oryzae* and *R. dominica* than that at 600 ppm. However, in the case of *T. confusum* DE concentration was not significantly associated with the progeny suppression.

Table 5. Number of adult emergence (mean ± SE) and suppression rate of progeny production for *Sitophilus oryzae* and *Rhyzopertha dominica* adults exposed to 600 and 900 ppm Detech 45 d after the removal of their parental adults from the treated wheat

Insect species	Concentration (ppm)	Adult emergences (no.), mean ± SE	P value***	Reduction in progeny production (%), mean ± SE
Sitophilus oryzae	600	6.3 ± 1.4 (114 ± 3.6)**	0.016	94.0 ± 0.7 B
	900	1.6 ± 0.6 (114 ± 3.6)	0.013	98.3 ± 0.5 A
	t and P value*	-	-	*t* (3) = 4.25 P = 0.042*
Rhyzopertha dominica	600	13.3 ± 2.9 (33 ± 2)**	0.001	60.3 ± 6.4 B
	900	4.6 ± 1.4 (33 ± 2)	0.000	85.2 ± 5.0 A
	t and P value*	-	-	*t* (3) = 4.38 P = 0.039*

* Means within columns followed by the same letter are not significantly different (Student's t test at 5% level).

** Values in brackets are the average number of new generation adults obtained from the control treatment.

*** Dunnett's test was used to compare the number of new generation adults obtained from DE treatments with that in control treatment.

Repellency

Mean proportions of *S. oryzae, T. confusum* and *R. dominica* adults present in arenas with Detech-treated and untreated mono-layer wheat at each observation for the repellency tests in plastic container are given Figure 2.

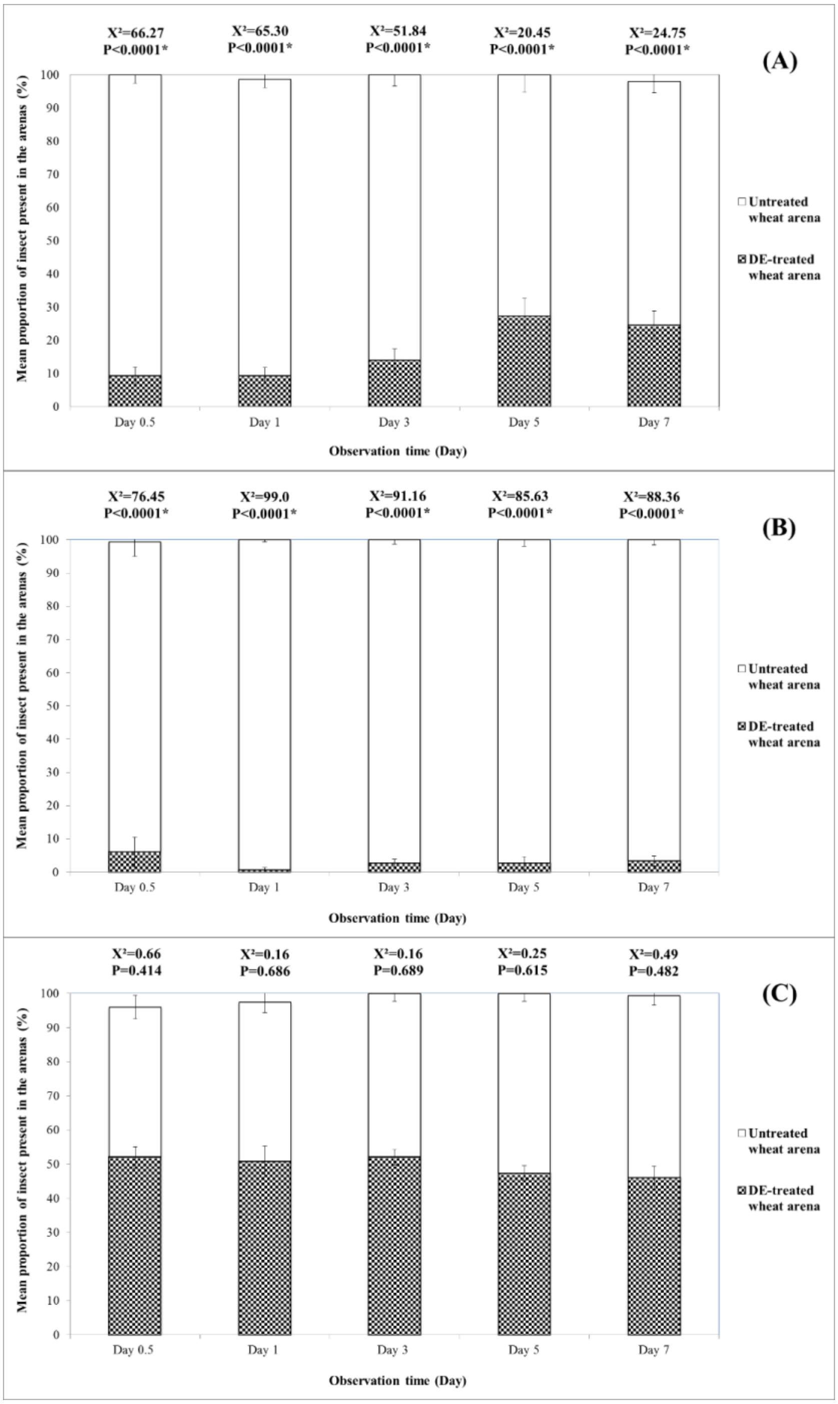

Figure 2. Mean proportion of *Sitophilus oryzae* (A), *Tribolium confusum* (B) and *Rhyzopertha dominica* (C) adults present in arenas with Detech-treated (1,000 ppm) and untreated mono-layer wheat at each observation for the repellency tests.

In the repellency tests conducted on single-layer wheat, there were significant differences between mean proportions of *S. oryzae* and *T. confusum* adults present in arenas with 1,000 ppm Detech-treated and untreated mono-layer wheat at all observation intervals (*S. oryzae* for 0.5, 1, 3, 5 and 7 days respectively; χ^2 = 66.3, χ^2 = 65.3, χ^2 = 51.8, χ^2 = 20.5 and χ^2 = 24.8 P < 0.001; *T. confusum* for 0.5, 1, 3, 5 and 7 days, respectively; χ^2 = 76.5, χ^2 = 99.0, χ^2 = 91.2, χ^2 = 85.6 and χ^2 = 88.4 P < 0.001). However, there were not significant differences between mean proportions of *S. oryzae* and *T. confusum* adults present in arenas with 1,000 ppm Detech-treated and untreated mono-layer wheat at all observation intervals (chi-square test; 0.5, 1, 3, 5, and 7 day respectively; χ^2 = 0.66 P = 0.414, χ^2 = 0.16 P = 0.686, χ^2 = 0.16 P = 0.686, χ^2 = 0.25 P = 0.615 and χ^2 = 0.49 P = 0.482). In the repellency test, the highest proportions of *S. oryzae* and *T. confusum* adults were found in arena with untreated wheat at each observation time. However, the numbers of the insects for *R. dominica* were about the same in arena with Detech-treated and untreated wheat, and therefore, there was no preference for arena with Detech-treated and untreated wheat.

All main effects were significant for the percentage repellency in Detech-treated wheat (for tested insect species, $F_{2,60}$ = 167, P < 0.0001; for observation intervals, $F_{4,60}$ = 2.52, P = 0.05), whereas their interaction was not significant (for insect species x observation *intervals;* $F_{8,60}$ = 1.74, P = 0.107). Repellency of *S. oryzae, T. confusum* and *R. dominica* in arenas with Detech-treated and untreated mono-layer wheat for each observation for the repellency tests are given Table 6. For all observation intervals, the percentages of repellency for *T. confusum* were significantly higher than those for *S. oryzae* and *R. dominica* with exception of the day 0.5). The highest percentage of repellency (88.0-98.8%) was observed in *T. confusum* at all observation intervals, followed by *S. oryzae* adults (45.3-81.3%) whereas the lowest percentage of repellency (1.3-8.9%) was recorded in *R. dominica*. The repellency tests indicated that Detech at 1,000 ppm was highly repellency to *T. confusum* with repellency class V and moderately repellency to *S. oryzae* with repellency class III-V whereas it has no or low repellency to *R. dominica*.

Table 6. Repellency percentage (mean ± SE) of *Sitophilus oryzae, Tribolium confusum* and *Rhyzopertha dominica* adults in arenas with Detech-treated and untreated mono-layer wheat at each observation for the repellency tests

Observation interval (day)	Repellency (%)						F and P value
	S. oryzae		*T. confusum*		*R. dominica*		
Day 0.5	81.3 ± 5.3 (V)**	Aa	88.0 ± 9.1 (V)**	A	2.1 ± 1.3 (I)**	B	$F_{2,12}$ = 61.3 P < 0.0001*
Day 1	81.1 ± 4.9 (V)	Ba	98.7 ± 1.3 (V)	A	3.6 ± 2.3 (I)	C	$F_{2,12}$ = 243 P < 0.0001*
Day 3	72.0 ± 6.8 (IV)	Bab	94.7 ± 2.5 (V)	A	1.3 ± 1.3 (I)	C	$F_{2,12}$ = 131 P < 0.0001*
Day 5	45.3 ± 10.6 (III)	Bb	94.7 ± 3.9 (V)	A	6.7 ± 3.7 (I)	C	$F_{2,12}$ = 41.3 P < 0.0001*
Day 7	49.9 ± 8.1 (III)	Bb	93.3 ± 3.0 (V)	A	8.9 ± 5.7 (I)	C	$F_{2,12}$ = 50.3 P < 0.0001*
F and P value	$F_{4,20}$ = 5.35 P = 0.004*		$F_{4,20}$ = 0.65 P = 0.635		$F_{4,20}$ = 0.93 P = 0.467		

* Means within rows followed by the same uppercase letter or in columns followed by the same lowercase letter are not significantly different (Tukey's HSD test at 5% level).

** The number in the brackets refers to repellency classes.

Considering observation interval effect on the repellency to tested insect species, increasing observation period did not result in significant increase in repellency to *T. confusum* and *R. dominica* (Table 6). However, increasing observation period resulted in significant reduction in repellency to *S. oryza*. In early observation period (0.5 and 1 day), repellency to *S. oryzae* was significantly higher than those in longer observation period (5 and 7 days).

Discussion

Commercially-available DEs (Insecto, USA; Dryacide, Australia; Protect-It, Canada) have been tested against a wide range of stored-product insect species (Korunic, 1998; Subramanyam et al., 1994; Subramanyam & Roesli, 2000; Wakil et al., 2006; Athanassiou et al., 2007, 2011; Kostyukovsky et al., 2010). They vary markably in their efficacy on different stored-product insect species (Fields & Korunic, 2000; Subramanyam & Roesli, 2000; Fields et al., 2003; Athanassiou et al., 2004). Generally, the recommended dose rate which is effective against stored-product insects (under certain temperature and moisture conditions) is ≥1,000 ppm. Many researchers underline the need for using new DEs, which are effective at low dose rates (≤1,000 ppm) (Arthur & Throne, 2003; Athanassiou et al., 2006, 2007). The search for new, naturally occurring DEs that are more effective for insect control is still in progress, especially in areas rich in silicaceous rocks. In present study, novel Turkish DE deposit, Detech, which consists of a mixture of three freshwater DEs collected from different DE reserves located in different regions of Turkey was tested for its efficacy against and repellency to *S. oryzae*, *T. confusum* and *R. dominica* adults.

Our bioassays indicated that Detech is highly effective against *T. confusum* on soft wheat, causing 93% and 97% mortality rate at 600 and 900 ppm, respectively, after 7 days exposure. Also, it is confirmed that *T. confusum* is one of the most tolerant stored-grain insects to DE (Korunic, 1998; Arthur, 2000). For example, Kavallieratos et al. (2007) evaluated the efficacy of three commercially available modified DE formulations (PyriSec, Insecto and Protect-It) against adults of *T. confusum* originating from different European geographical locations in laboratory tests. In their study, PyriSec, Insecto and Protect-It at 500 ppm with 7 days exposure resulted in the mortality rates ranging from 13-36%, 5-25% and 18-39%, respectively, depending on the tested *T. confusum* population. According to these results, Detech appears more effective against *T. confusum* adults than PyriSec, Insecto and Protect-It. In the case of *S. oryzae*, Detech was also highly effective against *S. oryzae* adults in wheat, causing 82 and 92% mortality rates at 600 and 900 ppm, respectively, after 7 days exposure. Athanassiou et al. (2007) reported that PyriSec, Insecto and Protect-It on wheat at 500 ppm caused 99.9, 96.5 and 99.7% mortality, respectively, after 7 days exposure. Based on these mortality results it appears that Detech is slightly less effective against *S. oryzae* than PyriSec, Insecto and Protect-It. Also, Detech was less effective against *R. dominica* on soft wheat, causing 24 and 61% mortality at 600 and 900 ppm, respectively, after 7 days exposure.

The type of the commodity affected the efficacy of DEs (Korunic & Mackay, 2000; Athanassiou et al. 2003, 2007; Kavallieratos et al., 2005). For example, Athanassiou et al. (2007) tested PyriSec, Insecto and Protect-It treatments on hard wheat at 500 ppm with 7 days exposure causing 97.4, 92.0 and 98.5% mortality, against *R. dominica*. In contrast, in the current study, Detech was less effective against *R. dominica* adults than PyriSec, Insecto and Protect-It. The large difference between mortality results of *R. dominica* can be attributed to the wheat cultivar (hard or soft) used in the bioassays. Also, Ferizli & Beris (2005) found that Protect-It gave a low mortality rate (20%) of *R. dominica* on a soft wheat cultivar at 500 ppm after 14 days exposure. These findings suggested that DEs are more effective on hard wheat than on soft wheat. We assume that this is likely to be due to the fact that *R. dominica* adults rapidly penetrate soft wheat kernels even with DE treatment and therefore these insects that stay inside wheat kernels have less exposure to DE dust particles from the surface of grain than other more active insect species which feed predominantly outside the kernel. Similar observations were also reported by Ferizli & Beris (2005).

DEs vary markably in their insecticidal effect on different stored-grain insects (Desmarchelier & Dines, 1987; White & Loschiavo, 1989; Korunic, 1998; Arthur, 2000; Fields & Korunic, 2000; Athanassiou et al., 2005; Kavallieratos et al., 2005). Though there are different rankings among these studies, overall the rankings (most to least susceptible) are: *Cryptolestes* spp., *Oryzaephilus* spp., *Sitophilus* spp., *R. dominica* and *Tribolium* spp. Of the species tested in our study, *T. confusum* is considered the most DE-susceptible, followed by *S. oryzae* and *R. dominica*. This is interesting finding because the most of previous studies reported that *T. confusum* was considered the most DE-tolerant. This difference in DE-susceptibility of *T. confusum* can be attributed to the physical and chemical properties of Detech, which consists of a mixture of three freshwater DEs collected from different DE reserves located in different regions of Turkey. Arnaud et al. (2005) proposed as a possible solution the use of a DE mixture, in which more than one DE is present. In the same study, the mixture of the tested DEs was more effective than the application of each DE alone. Generally, a mixture can combine all the positive characteristics of different DEs, such as the use of low insecticidal rates and the presence of food additives. Athanassiou et al. (2007) found that a mixture of Protect-It, PyriSec and Insecto was more effective than a single DE application on both wheat and maize, against adults of *T. confusum*. There are also only a few studies that have examined the reasons for different susceptibilities of pest species. Nair (1957) using magnesite dust and White and Loschiavo (1989) using silica aerogel found that susceptible insects had more dust adhering to the cuticle. Fields & Korunic (2000) reported that general resistance to desiccation, either through better water retention, better water acquisition, or greater tolerance of desiccation could also be responsible for these differences in susceptibility.

Subramanyam & Roesli (2000) noted that it is often more important in practical conditions of cereal storage to prevent progeny formation than to concentrate on obtaining direct lethal effects of DE against parent insects. In the present study, *S. oryzae* showed complete, or almost complete, progeny inhibition, 94 and 98%, at 600 and 900 ppm, whereas the highest progeny reduction (84%) was observed in *R. dominica* at 900 ppm. Ferizli & Beris (2005) found that Protect-It resulted in reduced F_1 adult progeny of *R. dominica* according to application dosage and almost complete suppression (99%) of progeny production was achieved at 1,000 ppm on soft wheat. Desmarchelier & Dines (1987) also indicated that at least 1,000 ppm Dryacide applied to wheat with 28 days exposure was necessary to achieve 100% mortality and progeny control of *R. dominica*. These findings are almost in accordance with the present study. In the case of *S. oryzae,* similar results for suppression of progeny production are reported for several commercially available DE formulations, such as SilicoSec, Insecto and Protect-It (Subramanyam & Roesli, 2000; Athanassiou et al., 2003, 2005, 2008).

The repellence phenomena of inert dust to stored-grain insects has been described by several authors (White et al., 1966; Quarles, 1992; Rigaux et al., 2001). Our experiments were conducted to determine whether there was any evidence for avoidance of DE deposit by the insects when given a choice between DE-treated and untreated wheat surfaces. In our study, the repellency tests on mono-layer wheat indicated that there were significant differences in repellency of DE to tested insect species. *Sitophilus oryzae* and *T. confusum* adults preferred untreated wheat. Detech at 1,000 ppm was highly repellent to *T. confusum* adults with repellency class V and moderately repellent to *S. oryzae* adults with repellency class III-V. Similar high repellency observations have been reported in studies with several DE deposits against *S. oryzae* (White et al., 1966; Mohan & Fields, 2002; Nwaubani et al., 2014). Nwaubani et al. (2014) reported that adults of *S. oryzae* avoid contact with wheat treated with two DEs obtained from Nigeria, and therefore, there were significantly fewer adults of both insect species in DE-treated wheat, whereas there were more adults in the untreated wheat. However, in our study the numbers of the insects with *R. dominica* had approximately equal distribution in arena with DE-treated and untreated wheat, and therefore, there was no preference for arena with DE-treated and untreated wheat. Hence, Detech on soft wheat cultivar has no or limited repellency to *R. dominica* adults. Similar to the results obtained for *R. dominica* adults in the present study, Vardeman et al. (2007) reported that although adult deaths were directly related to DE concentration, the movement of *R. dominica* in the product did not diminish with the presence of DE and therefore, DE did not show a clear repellency to *R. dominica* adults. In contrast to these results for *R. dominica*, Nwaubani et al. (2014) reported that adults of *S. oryzae* avoid contact with wheat treated with two DEs obtained from Nigeria. This difference could be due to different physical and chemical properties of the tested DEs, wheat cultivars or repellency test methods. Mohan & Fields (2002) reported that one positive effect of DE repellency would be that there could be reduced insect immigration into a grain mass and greater insect emigration out of a grain mass treated with DEs, hence, reducing insect populations. In present study, it appeared that high repellency to *S. oryzae* and *T. confusum* could be substantially correlated with their high mortality rates. This could be due to the fact that DE application could potentially increase the rate of *S. oryzae* and *T. confusum* movement in grain mass, which therefore, could cause picking up significantly more DE dust particles from the surface of grain.

In conclusion, the present study indicates that novel Turkish DE formulation (Detech) which consists of a mixture of three DEs from different DE reserves in different regions of Turkey, has potential as a stored grain protectant given its toxicity and repellency to the insect species studied here. Additional studies are required to determine the effects of biotic and abiotic factors on efficacy of Detech against other stored grain insects and then to evaluate its insecticidal performance under commercial storage conditions.

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