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Original article (Orijinal araştırma)

Combined and individual effects of diatomaceous earth and methyl eugenol against stored products insect pests

Metil öjenol ve diyatom toprağının depolanmış ürün zararlısı böceklere karşı tek başına ve birlikte etkisi

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Abstract

The insecticidal activity of a proprietary diatomaceous earth, Detech, and methyl eugenol was tested with *Sitophilus granarius* (L., 1758), *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrychidae), *Tribolium confusum* Jaquelin Du Val, 1868 (Coleoptera: Tenebrionidae) adults at Stored Products Pest Laboratory of Plant Protection Central Research Institute (Ankara, Turkey) in 2020. A range of doses of Detech (100, 250 and 500 mg kg^{-1}) and methyl eugenol (5, 10 and 15 mg kg^{-1}) were used. To determine insect mortality, at a range of times after treatment, and half-doses of both products were also studied in order to examine simultaneous use for synergistic effect. The greatest effect of Detech on *R. dominica*, *S. zeamais, S. granarius* and *T. confusum* was observed with 500 mg kg^{-1} giving 81, 29, 26 and 18% mortality 28 d after treatment (DAT), respectively. Methyl eugenol at 15 mg kg^{-1} gave complete mortality with *R. dominica* adults 3 DAT. Simultaneous application of both products did not give complete mortality in test insects. The data show that Detech and methyl eugenol are promising treatments for the control of insect pests of stored products.

Keywords: Essential oils, mortality, physical control, stored products insects, synergism

Öz

Tescilli bir diyatom toprağı Detech ve metil öjenolün *Sitophilus granarius* (L., 1758), *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrychidae), *Tribolium confusum* Jaquelin Du Val, 1868 (Coleoptera: Tenebrionidae) erginlerine karşı Zirai Mücadele Merkez Araştırma Enstitüsü, Depolanmış Ürün Zararlıları Laboratuvarı (Ankara, Türkiye)'nda 2020 yılında test edilmiştir. Bu amaçla farklı dozlarda Detech (100, 250 ve 500 mg kg^{-1}) ve metil öjenol (5, 10 ve 15 mg kg^{-1}) kullanılmıştır. Böceklerin ölüm oranını belirlemek için farklı maruz kalma süreleri kullanılmıştır. Sinerjistik etkiyi belirlemek amacıyla, her iki ürünün de yarı dozları böcekler üzerinde çalışılmıştır. Diyatom toprağı Detech, *R. dominica, S. zeamais, S. granarius* ve *T. confusum* için en yüksek etkisi 500 mg kg-1 dozda, 28 günlük maruziyet süresinin sonunda sırasıyla %81, %29, %26 ve %18 ölüm oranı şeklinde gerçekleşmiştir. 15 mg kg^{-1}'lık metil öjenol dozu, *R. dominica* için günlük maruziyetten sonra %100 ölüm oranı sağlamıştır. Her iki ürünün aynı anda kullanılması, üzerinde çalışılan böceklerde tam bir ölüm oranına ulaşmamıştır. Elde edilen veriler, Detech ve metil öjenol kullanımının depolanmış ürün zararlısı böcekler ile mücadelede ümit var sonuçlar verdiğini ortaya koymuştur.

Anahtar sözcükler: Uçucu yağlar, ölüm oranı, fiziksel kontrol, depolanmış ürün zararlısı böcekler, sinerjizm

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Introduction

The confused flour beetle, *Tribolium confusum* Jaquelin Du Val, 1868 (Coleoptera: Tenebrionidae), the maize weevil, *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), the lesser grain borer, *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrychidae), and the granary weevil, *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae), are some of the most destructive insect pests of the stored grain. The damage of these insects is important in terms of both quality and quantity loss of the product. *Sitophilus granarius*, *S. zeamais* and *R. dominica* are known as primary pests because they can easily break the seeds with their mandibles. *Tribolium confusum* is known as a secondary pest; it can easily infest damaged grain kernels and feed on different commodities (Aitken, 1975; Mewis & Ulrichs, 2001; Gałęcki et al., 2019). They not only consume the grain, but also contaminate the commodity with their body parts, feces, and the excretions, the latter of which may cause a bad smell in the commodity (Houssou et al., 2009). Additionally, by altering the microflora, they may cause mycotoxin formation on the products, which are dangerous especially for warm-blooded living organisms (Dawson et al., 2004; Richard et al., 2007). The inability to consume the harvested grain immediately, the demands of the food industry, mills, government and nongovernment organizations are important in determining storage period. Loss of grain and related commodities seriously threat global food security and availability (Oliveira, et al., 2014). It is predicted that the human population will reach 9.1 billion by the year 2050, with the concomitant increased needs for food production (FAO, 2009).

Currently, synthetic residual insecticides and fumigants are most commonly used to control stored products pests worldwide. However, several negative effects, such as the environmental contamination and toxicity to warm-blooded organisms, pesticides residues and pest resistance have arisen from their continuous use. Therefore, research has focused on developing alternative control measures (Halliday et al., 1988; Pacheco et al., 1990; Arthur, 1996; Ribeiro et al., 2003; Boulogne et al., 2012; Boyer et al., 2012; Barres et al., 2016; Hubert et al., 2018).

Inert dusts and botanical derivatives are among of the most promising alternatives to replace synthetic insecticides (Ferizli et al., 2012; Islam & Rahman, 2016; Ertürk et al., 2017; Alkan et al., 2019a, b, c, 2020a, b; Ertürk et al., 2020; Korunic & Fields, 2020; Zhanda et al., 2020). Diatomaceous earth (DE) is a kind of inert dust that is composed mostly of amorphous silica and several oxides of Al, Ca, Mg, Fe, K, Na and Ti. DE originated from single-cell phytoplankton that lived in salty or freshwater sources (Vayias & Athanassiou, 2004; Ashraf et al., 2016; Adarkwah et al., 2017; Baliota & Athanassiou, 2020). The fossils of these tiny creatures turned into diatom rocks and DE is obtained from these rocks by fine grinding. Unlike synthetic chemicals, the mode of action of DE is non-chemical. The main cause of insect mortality occurs by desiccation due to water loss from the entire body. DE particles absorb the epicuticular lipids from insect cuticles, which is a waterproof layer of the integument, consequently, insect mortality is caused by desiccation (Zacher & Kunike, 1931; Ebeling, 1971; Korunic, 1998; Prasantha et al., 2015). However, the use of DE has been limited due to the decrease in the hectoliter weight of the product, non-target effects on workers, and damage to machinery and equipment (Merget et al., 2002).

As an alternative to synthetic pesticides, plant derivatives are recognized as safer and it has been known for a long time that plant metabolites have biological activity against insects (Obeng-Ofori & Reichmuth, 1997; Chang et al., 2009; Regnault-Roger et al., 2012; Dwivedy et al., 2015; Jankowska et al., 2017). Pesticides obtained from essential oils of botanical origin are a promising control option for stored product pests (Upadhyay et al., 2018). To ensure the successful sustainability of pest control, bioactive products from plants can be used in integrated pest management programs (Regnault-Roger et al., 2012; Mossa, 2016). Plants have many secondary metabolites that are synthesized for defense against harmful organisms. Methyl eugenol (ME) is a phenylpropanoid derived from eugenol and is found in more than 450 species across 80 plant families (Herrmann & Weaver, 1999; Tan & Nishida, 2012). ME causes death by inhibiting acetylcholine esterase, an essential enzyme in insect nervous systems (Lee et al., 2001).

DE and ME have mostly been studied separately, so there is a gap in the literature regarding their combined effect on stored product pests. Therefore, this study was conducted to investigate the synergistic effect of these agents against *T. confusum, S. granarius*, *R. dominica* and *S. zeamais,* as well as to reduce the potential risks arising from their individual use.

Materials and Methods

Insects

Adults of *S. granarius* and *S. zeamais* were reared on whole wheat and maize, respectively. Rearing of *T. confusum* and *R. dominica* was performed on cracked wheat with 5% brewer's yeast (by weight) (Athanassiou et al., 2016). In the experiments mixed-sex insects between 7 and 28 d old were used. All insects were incubated in climate cabinet at 25 ± 1ºC and 60 ± 5% RH in the dark (Nüve ID 501, Ankara, Turkey). The laboratory experiments were conducted in the stored products pest laboratory at Plant Protection Central Research Institute, Ankara, Turkey in 2020.

Diatomaceous earth and methyl eugenol

A proprietary DE product, Detech, was used in this study. This product comes from the Central Anatolia Region in Turkey from light-gray soil and consists of Fe_2O_3 1.50%, Al_2O_3 4.70%, CaO 4.75%, SiO_2 80.6%, MgO 0.85%, K_2O 0.50%, Na_2O 0.40% and TiO_2 <0.01% (Bayram et al., 2020) with an average granule size of 14 mm (Sağlam et al., 2020). ME (≥98% purity, CAS Number: 93-15-2) was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Contact activity bioassays

The insecticidal activity of Detech and ME against adults of four stored product insects was evaluated by direct application to the grain. For this purpose, white soft winter wheat, *Triticum aestivum* L. (Poaceae), and maize, *Zea mays* L. (Poaceae), were weighed into 2-L plastic bags. Detech was applied as a dust at 100, 250 and 500 mg kg^{-1} of grain and ME was used in liquid form at 5, 10 and 15 ml kg^{-1} grain. The Detech and ME were added to the plastic bags separately. The plastic bags were closed and shaken to achieve equal distribution (Ertürk et al., 2017). To determine the synergistic effect of half doses of both agents, Detech and ME were applied to wheat and maize according to the method described by Khorrami et al., (2018). Also, the label rate of K-Obiol EC25 (25 g/L deltamethrin plus 250 g/L piperonyl butoxide, Bayer AG, Leverkusen, Germany) and untreated grain were prepared (Table 1). For each observation, separate PVC vials were used. Twenty g of treated grain was added to into each PVC vials (7 x 5 cm) with 20 adult insects and then the vials were incubated at 25ºC and 60 ± 5% RH. Mortality of insects was assessed 1, 2, 3, 7, 14, 21 and 28 d after of treatment (DAT). The experiments were included three replicates per treatment, and the experiments were repeated three times.

Table 1. Treatments used in the bioassays

Treatment	Dose (mg kg^{-1})
Detech	100
	250
	500
Methyl Eugenol	5
	10
	15
Detech+Methyl Eugenol	DE 50+ ME 2.5
	DE 125 + ME 5
	DE 250 + ME 7.5
K-Obiol EC25	Label Rate
Control (untreated grain)	-

Statistical analysis

Data from the dose screening tests were first converted to percentage mortality and then arcsine transformation (Zar, 1999; Warton & Hui, 2011). Tukey's multiple comparison test was used to reveal the differences between the treatments at 5% significance level. To determine the relationship between treatments, MINITAB Release 18 was used with a general linear model (Mckenzie & Goldman, 2004).

Results

The ANOVA (treatment × dosage × observation time × insect) revealed that interaction between the experimental factors tested including different rates of Detech and ME against the insect species were significant (Table 2).

For Detech, the greatest effect on *S. granarius* was obtained at 500 mg kg^{-1} with a 25.9% mortality 28 DAT, whereas the lowest rate of 100 mg kg^{-1} did not cause any mortality 1 DAT ($F_{10,88} = 34.5$, $P < 0.05$). The doses of 250 and 500 mg kg^{-1} Detech gave similar mortality results (Table 3). The highest insecticidal efficacy of Detech applied at 100, 250 and 500 mg kg^{-1} on *S. zeamais* were found at 28 DAT, with the highest mortalities being 12, 19 and 29%, respectively ($F_{10,88} = 101$, $P < 0.05$). Mortality caused by 100 mg kg^{-1} 1 DAT was negligible ($F_{10,88} = 26.4$, $P < 0.05$) (Table 4). For *T. confusum,* the highest adult mortality with 100 mg kg^{-1} Detech 28 DAT was <1% ($F_{10,88} = 146$, $P < 0.05$). Mortality did not exceed 18% for the three doses tested (Table 5). Unlike other insect species, the highest mortality for *R. dominica* was at 28 DAT for all doses. The highest mortalities for 100, 250 and 500 mg kg^{-1} Detech 28 DAT were 58, 74 and 81%, respectively. Two DATl, there was no mortality, but by 3 DAT, the lowest mortality <1% at 500 mg kg^{-1} was recorded (Table 6).

Table 2. ANOVA for different applications of Detech and methyl eugenol against *Sitophilus oryzae, Sitophilus granarius, Tribolium confusum* and *Rhyzopertha dominica*

Source	DF	F- Value	P-Value
Treatment	2	8746.1	<0.05
Dosage	2	175.5	<0.05
Insect	3	532.8	<0.05
Observation time	7	631.9	<0.05
Treatment * Dosage	4	44.6	<0.05
Treatment * Insect	6	131.1	<0.05
Treatment * Observation time	14	114.7	<0.05
Dosage * Insect	6	46.0	<0.05
Dosage * Observation time	14	2.1	<0.05
Insect * Observation time	21	24.8	<0.05
Treatment * Dosage * Insect	12	16.9	<0.05
Treatment * Dosage * Observation time	28	2.7	<0.05
Treatment * Insect * Observation time	42	30.3	<0.05
Dosage * Insect * Observation time	42	2.4	<0.05
Treatment * Dosage * Insect * Observation time	84	1.5	<0.05
Error	2304		
Total	2591		

Table 3. Mortality (%, mean ± SE) of *Sitophilus granarius* exposed to wheat grain treated with Detech and methyl eugenol 1 to 28 d after treatment

Treatment		1 DAT	2 DAT	3 DAT	5 DAT	7 DAT	14 DAT	21 DAT	28 DAT
DE (mg kg^{-1})	100	0.0±0.0 c*B♦	1.1±0.4 deAB	3.7±1.2 deAB	5.9±1.2 cdA	6.7±1.4 cdeA	6.7±1.4 cdA	9.5±1.2 cdeA	11.4±1.2 cdA
	250	0.0±0.0 cC	1.1±0.9 eBC	6.1±0.7 deABC	6.7±1.4 cdABC	7.7±1.0 cdABC	8.6±1.1 cdAB	15.3±1.3 bcdeA	21.1±3.7 cdA
	500	0.1±0.0 cD	3.2±1.0 deCD	6.6±1.5 deBC	17.1±0.4 bcAB	22.5±0.6 bcA	24.9±0.5 bcA	24.9±0.5 bcdA	25.9±0.6 bcA
ME (mg kg^{-1})	5	0.1±0.0 bcC	13.1±1.6 dB	31.5±0.6 cAB	36.9±1.1 bA	42.4±1.2 bA	44.6±1.4 bA	44.6±1.4 bA	53.6±1.3 bA
	10	0.5±0.3 cD	37.4±1.5 cC	67.4±1.6 bB	93.4±1.5 aA	93.4±1.5 aA	93.4±1.5 aA	94.2±1.3 aA	96.3±1.2 aA
	15	5.9±1.2 bC	82.8±2.6 bB	98.1±1.5 aA	98.5±1.2 aA	98.9±0.9 aA	99.9±0.4 aA	99.9±0.4 aA	99.9±0.4 aA
DE+ME (mg kg^{-1})	50+2.5	0.0±0.0 cA	0.1±0.0 eA	0.8±0.4 eA	1.5±0.8 dA	3.5±1.8 deA	4.8±3.1 dA	4.8±3.1 deA	7.6±3.1 dA
	125+5	0.1±0.0 cC	0.8±.0.3 eBC	5.8±1.3 deABC	7.4±1.6 cdAB	10.3±1.4 cdA	10.3±1.4 cdA	10.3±1.4 cdeA	15.2±1.4 cdA
	250+7.5	0.8±0.1 bcC	4.9±1.7 deBC	16.1±1.5 cdAB	19.0±1.9 bcAB	23.9±1.4 bcAB	26.4±1.2 bcA	27.4±1.3 bcA	27.4±1.3 bcA
Control		0.0±0.0 cB	0.0±0.0 eB	0.0±0.0 eB	0.0±0.0 dB	0.0±0.0 eB	1.1±0.9 dAB	2.2±1.4 eA	2.2±1.4 dA
Insecticide		47.9±0.8 aB	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	100.0±0.0 aA

* Means followed by the same lowercase letter within columns are not significantly different at P < 0.05.
♦ Means followed by the same uppercase letter within rows are not significantly different at P < 0.05.
DAT, d after treatment; DE, Detech, ME: methyl eugenol, Insecticide, K-Obiol EC 25.

Table 4. Mortality (%, mean ± SE) of *Sitophilus zeamais* exposed to maize grain treated with Detech and methyl eugenol 1 to 28 d after treatment

Treatment		1 DAT	2 DAT	3 DAT	5 DAT	7 DAT	14 DAT	21 DAT	28 DAT
DE (mg kg^{-1})	100	0.1±0.0 c*B♦	0.5±0.2 cAB	1.1±0.9 bAB	1.1±0.9 bAB	2.0±1.0 bAB	4.4±1.4 bAB	8.0±1.9 bA	12.4±2.5bA bA
	250	0.5±0.2 cB	1.1±0.9 cB	1.1±0.9 bB	2.0±1.0 bB	3.0±1.5 bAB	9.7±1.8 b bAB	16.0±1.6 bA	19.4±3.8 bA
	500	1.1±0.8 cB	3.3±1.9 cB	3.3±1.9 bAB	3.3±1.9 bAB	3.7±2.4 bAB	10.3±1.4 bAB	16.7±0.8 bAB	28.7±1.6 bA
ME (mg kg^{-1})	5	21.5±1.7 bC	79.6±1.6 bB	96.5±1.8 aA	97.1±1.6 aA	99.5±0.7 aA	99.5±0.7 aA	99.5±0.7 aA	100.0±0.0 aA
	10	22.1±3.6 bB	96.9±2.6 aA	99.5±0.7 aA	99.9±0.4 aA	99.9±0.4 aA	100.0±0.0 aA	100.0±0.0 aA	100.0±0.0 aA
	15	30.0±1.2 bB	98.0±1.0 aA	99.9±0.4 aA	99.9±0.4 aA	100.0±0.0 aA	100.0±0.0 aA	100.0±0.0 aA	100.0±0.0 aA
DE+ME (mg kg^{-1})	50+2.5	0.0±0.0 cB	0.1±0.0 cB	0.1±0.0 bB	0.1±0.0 bB	0.1±0.0 bB	0.5±0.2 bB	2.2±2.0 bA	16.7±1.4 bA
	125+5	0.0±0.0 cA	0.8±0.1 cA	0.8±0.1 bA	0.8±0.1 bA	0.8±0.1 bA	1.0±0.4 bA	7.4±1.6 bA	18.7±1.3 bA
	250+7.5	0.5±0.2 cB	1.5±1.2 cB	1.5±1.2 bB	1.5±1.2 bB	1.5±1.2 bB	4.9±1.7 bAB	8.2±1.8 bAB	20.5±0.4 bA
Control		0.3±0.1 cB	0.4±0.1 cB	0.4±0.1 bB	0.4±0.1 bB	0.4±0.1 bB	1.8±1.0 bAB	2.4±1.2 bAB	2.4±1.2 bA
Insecticide		65.9±2.7 aB	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	100.0±0.0 aA

* Means followed by the same lowercase letter within columns are not significantly different at P < 0.05.
♦ Means followed by the same uppercase letter within rows are not significantly different at P < 0.05.
DAT, d after treatment; DE, Detech, ME: methyl eugenol, Insecticide, K-Obiol EC 25.

Table 5. Mortality (%, mean ±SE) of *Tribolium confusum* exposed to wheat grain treated with Detech and methyl eugenol 1 to 28 d after treatment

Treatment		1 DAT	2 DAT	3 DAT	5 DAT	7 DAT	14 DAT	21 DAT	28 DAT
DE (mg kg^{-1})	100	0.0±0.0 b*A♦	0.0±0.0 cA	0.0±0.0 eA	0.0±0.0eA eA	0.0±0.0 dA	0.0±0.0dA dA	0.0±0.0dA dA	0.5±0.7 eA
	250	0.0±0.0 bB	0.0±0.0 bB	0.0±0.0 eB	0.0±0.0 eB	0.0±0.0 dB	0.1±0.4 dB	0.1±0.4 dB	3.2±1.0 dA
	500	0.0±0.0 bB	0.1±0.0 cB	0.1±0.0 eB	0.5±0.1 eB	0.5±0.1 cdB	0.5±0.1 cdB	0.5±0.1 cdB	18.3±1.5 bA
ME (mg kg^{-1})	5	0.0±0.0 bC	1.6±1.1 bcC	9.1±3.0 cdB	29.2±0.7 cB	46.5±0.4 bB	87.6±2.4 bA	95.3±2.2 bA	98.9±1.4 aA
	10	0.0±0.0 bD	1.9±1.5 bcD	18.7±2.5 bcD	31.6±1.8 cC	63.1±1.1 bB	98.9±1.4 bA	100.0±0.0 aA	100.0±0.0 aA
	15	0.5±0.3 bE	9.6±2.2 bD	39.3±1.2 bC	63.6±1.8 bB	97.7±1.8 aA	100.0±0.0 aA	100.0±0.0 aA	100.0±0.0 aA
DE+ME (mg kg^{-1})	50+2.5	0.0±0.0 bA	0.0±0.0 cA	0.0±0.0 eA	0.1±0.0 eA	0.1±0.0 dA	0.1±0.1 dA	0.5±0.2 cdA	0.5±0.2 deA
	125+5	0.0±0.0 bA	0.5±0.1 cA	0.5±0.1 dA	0.5±0.1 deA	0.5±0.1 cdA	0.5±0.1 cdA	0.8±0.2 cdA	1.8±0.2 dA
	250+7.5	0.1±0.0 bA	0.5±0.2 cA	0.5±0.2 deA	1.1±0.9 dA	3.2±1.0 cA	3.2±1.0 cA	3.2±1.0 cA	7.2±1.0 cA
Control		0.0±0.0 bA	0.0±0.0 cA	0.0±0.0 eA	0.0±0.0 eA	0.0±0.0 eA	0.1±0.0 dA	0.1±0.0 dA	1.1±0.9 eA
Insecticide		45.3±0.6 aB	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	100.0±0.0 aA

* Means followed by the same lowercase letter within columns are not significantly different at P < 0.05.
♦ Means followed by the same uppercase letter within rows are not significantly different at P < 0.05.
DAT, d after treatment; DE, Detech, ME: methyl eugenol, Insecticide, K-Obiol EC 25.

Table 6. Mortality (%, mean ± SE) of *Rhyzopertha dominica* exposed to wheat grain treated with Detech and methyl eugenol 1 to 28 d after treatment

Treatment		1 DAT	2 DAT	3 DAT	5 DAT	7 DAT	14 DAT	21 DAT	28 DAT
DE (mg kg^{-1})	100	0.0±0.0 c*C♦	0.0±0.0 cC	0.0±0.0 bC	0.5±0.2 cC	3.5±1.8 cdC	28.1±2.0 cB	54.0±1.2 bA	57.6±1.6 bcA
	250	0.0±0.0 cC	0.0±0.0cC cC	0.0±0.0 bC	0.8±0.3 bcC	12.7±0.8 bcB	23.7±1.7 cdB	56.8±0.5 bA	73.6±0.2 bcA
	500	0.0±0.0 cE	0.0±0.0 cE	0.5±0.2 bDE	9.5±1.2 bD	31.6±0.5 bC	53.4±0.6 bBC	67.0±1.5 bAB	80.8±2.9 bA
ME (mg kg^{-1})	5	4.4±2.5 bcC	82.7±2.8 bB	98.7±1.8 aA	99.7±0.8 aA	99.7±0.8 aA	100.0±0.0 aA	100.0±0.0 aA	100.0±0.0 aA
	10	10.7±1.9 bC	88.9±1.5 aB	99.5±0.7 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
	15	16.5±2.4 bB	99.7±0.8 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	100.0±0.0 aA
DE+ME (mg kg^{-1})	50+2.5	0.0±0.0 cD	0.0±0.0 cD	0.0±0.0 bD	1.1±0.9 bcCD	3.0±1.5 bcC	17.1±0.4 bcB	29.2±0.7 bcAB	50.0±0.6 bcA
	125+5	0.0±0.0 cD	0.0±0.0 cD	0.1±0.0 bD	3.0±1.5bcCD bcCD	15.3±1.3 cdBC	20.0±1.9 cdB	30.2±0.9 cdAB	66.4±1.8 bcA
	250+7.5	0.0±0.0 cC	0.0±0.0 cC	0.5±0.1 bC	4.4±1.4 bcC	16.5±2.4 bcC	38.5±0.6 cdB	48.8±0.6 cdB	79.6±1.7 bA
Control		0.0±0.0 cB	0.0±0.0 cB	0.1±0.0 bB	0.5±0.7 cAB	0.5±0.7 dAB	0.5±0.7 dAB	0.5±0.7 dAB	1.0±0.3 dA
Insecticide		64.8±2.8 aB	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	100.0±0.0 aA

* Means followed by the same lowercase letter within columns are not significantly different at P < 0.05.
♦ Means followed by the same uppercase letter within rows are not significantly different at P < 0.05.
DAT, d after treatment; DE, Detech, ME: methyl eugenol, Insecticide, K-Obiol EC 25.

The efficacy of ME on test species was revealed by differences in mortality. The greatest effect on *S. granarius* was nearly complete mortality with 15 ml kg^{-1} 28y DAT ($F_{10,88} = 69.7$, $P < 0.05$). One DAT, complete mortality was not seen in *S. granarius,* the minimum mortality was <14% at 5 ml kg^{-1} ($F_{10,88} = 34.5$, $P < 0.05$)

(Table 3). For *S. zeamais,* complete mortality was recorded 7 DAT at 15 ml kg^{-1} ($F_{10,88}$ = 175, P < 0.05). The minimum insecticidal effect was with 5 ml kg^{-1} gave 227% mortality 1 DAT ($F_{10,88}$ = 26.4, P < 0.05) (Table 4). For *T. confusum,* complete mortality was observed in 14 DAT with 15 ml kg^{-1} ($F_{10,88}$ = 227, P < 0.05). At the same dose, the mortality was <1% 1 DAT ($F_{10,88}$ = 99.5, P < 0.05) (Table 5). With *R. dominica,* the lowest mortality of 4% was observed 1 DAT at 5 ml kg^{-1} ($F_{10,88}$ = 29.6, P < 0.05). Complete mortality with all the three doses was observed from 14 DAT (Table 6).

No mortality was observed at the lowest combined dose of the agents (50 mg kg^{-1} Detech plus 2.5 ml kg^{-1} ME) 1 DAT ($F_{10,88}$ = 34.5, P < 0.05) with *S. granarius*. The greatest effect of the combined products (250 mg kg^{-1} Detech plus 7.5 ml kg^{-1} ME) on *S. granarius* was 27% mortality 28 DAT ($F_{10,88}$ = 69.7, P < 0.05) (Table 3). The combined reduced doses had a different effect on *S. zeamais*. The greatest mortality (250 mg kg^{-1} Detech plus 7.5 ml kg^{-1} ME) of 29% was observed 28 DAT ($F_{10,88}$ = 101, P < 0.05). Increasing dose and time resulted in increased insect mortality (Table 4). With *T. confusum*, mortality with combined products ranged from 0 to 7% (Table 5). There was no mortality observed for *R. dominica* 2 DAT. The lowest mortality 3 DAT was <1% with 125 mg kg^{-1} Detech plus 5 ml kg^{-1} ME. The highest morality of 80% was observed 28 DAT with 250 mg kg^{-1} Detech plus 7.5 ml kg^{-1} ME (Table 6). The mortality with K-Obiol EC 25 varied from 45 to 66% 1 DAT. However, complete mortality was observed from 2 DAT onwards. The mortality in the control did not exceed ~2%.

Discussion

The present study showed that different doses of Detech and ME had different efficacy against *T. confusum, R. dominica, S. granarius* and *S. zeamais*. The highest mortality of 81% for *R. dominica* was with Detech at 500 mg kg^{-1} 28 DAT. In a study conducted with diatomaceous earth products, Insecto and SilicoSec, mortality of *R. dominica* adults in different types of grain treated with 750 mg kg^{-1} varied between 63 and 97% 14 DAT (Kavallieratos et al., 2005). Similarly, Protector, another commercial DE product, did not give complete mortality in *R. dominica* adults in wheat treated with 0.5 g kg^{-1} 14 DAT (Baldassari et al., 2008). However, in the present study not all of the tested doses of Detech gave complete mortality of *S. zeamais, R. dominica, T. confusum* and *S. granarius*. Doumbia et al. (2014) shown that a complete mortality was achieved with *S. zeamais* adults 2 DAT in maize treated with DE at 3 g kg^{-1}. The mode of action of diatomaceous earth is known as abrasion of the insect cuticle and death occurs due to dehydration (Carlson & Ball, 1962; Ebeling, 1971; Krzyzowski et al., 2019). So, this effect may be due to the use of relatively low dose in the present study. Unlike other insects treated with Detech, *T. confusum* had the minimum mortality under similar conditions. Some studies showed that in order to achieve the desired level of mortality of *T. confusum*, DE doses equal to 500 mg kg^{-1} or above need to be used (Athanassiou et al., 2004; Athanassiou & Kavallieratos, 2005). The maximum Detech dose used in the present study was 500 mg kg^{-1}. The results obtained for *T. confusum* are similar to those in the literature. In the experiments with Detech, the desired mortality rates could not be obtained even after the longest time and the highest dose applications. Ashraf et al. (2016) stated that although DE, Inert-PMS, was effective against test insects, shorter periods gave lower mortality and mortality increased with time. The factors include the structure of the epicuticule, insect behavior or mobility, body shape and size of the insects and tolerance for water loss (Ebeling, 1971; Rigaux et al., 2001; Losic & Korunić, 2017, Korunić et al., 2020). Therefore, the tolerance of different insect species to DE products varies. A recent study revealed that *Cryptolestes pusillus* (Schönherr, 1817) (Coleoptera: Laemophloeidae) was by far more susceptible to DE compared to *R. dominica, T. confusum, Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae), *S. oryzae* and *S. granarius* (Baliota & Athanassiou, 2020). In the present study, as in the previous studies with different DEs, *T. confusum* was as most tolerant species to Detech. It is also known that increased RH reduces the efficacy of the DEs (Mewis & Ulrichs, 2001; Ertürk, 2020). The present study was conducted at a constant temperature and RH, therefore, no comparison could be made in the efficacy at different temperature and RH.

The present results indicate that ME is a potential and effective plant-derived synthetic chemical insecticide to minimize pests damage in stored products, such as wheat and maize. In present study, *S. zeamais* was the most susceptible insect to ME 1 DAT. However, from 2 DAT onwards, the highest mortality was obtained with *R. dominica*, the most susceptible species to ME. ME is a benzene derivative and has a high insecticidal activity. The fact that ME has more methoxy groups is a feature that increases its knockdown effect (Smith et al., 2002). The presence of a double bond in the side chain of the aromatic ring in ME, an analog of eugenol, and the substitution of the methoxy group is positively correlated with toxicity (Mossa, 2016; Imai & Masuda, 2017). Norambuena et al. (2016) reported that the main essential oil components of *Laureliopsis philippiana* (Looser) Schodde (Atherospermataceae) were ME (61%) and safrole (17%) and the *a*essential oil toxicity ranged from low to high as *S. granarius* < *S. zeamais* < *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). In the present study, similar to the previous study, *S. zeamais* was found to be more sensitive to ME than *S. granarius*. The insecticidal activity of ME essential oil increased with increasing dose and time. Previous studies showed that mortality of insect species even in the same genus could be different (Ertürk et al., 2014; Liang et al., 2017; Guo et al., 2017; Alkan 2020a, b). In the present study, *T. confusum* was the most tolerant species to ME and this was similar to other studies (Shojaei et al., 2017). However, the differences are thought to be due to the physiological properties of insects. With the expectation of an increased efficacy of DE and ME together, both agents were also investigated in combination at half doses. Based on the results, it is considered that a Detech and ME combination is feasible against *R. dominica* but it was not effective against *T. confusum, S granarius* and *S. zeamais*. In a previous study, Form N, a mixture of SilicoSec and other ingredients was reported to provide higher mortality of *S. oryzae, R. dominica* and *T. castaneum* than DE alone, especially in barley (Paponja et al., 2020). Korunic & Fields (2020) revealed that the simultaneous usage of diatomaceous earth with dill essential oil, silica gel, pyrethrin, disodium octaborate tetrahydrate and yeast were more effective than DE alone. The combined application of DE and ME in maize and wheat, in the present study did not affect the test insects at the desired level. It is thought that the low effect obtained by using Detech and ME together may be due to the low concentrations used. Therefore, it was assumed that insects were not sufficiently in contact with the DE and ME. One of the most important factors limiting the use of DE in the protection of stored grain is the necessity for high concentrations for successful insect control. Therefore, reducing the amount of DE while maintaining its effectiveness is an important challenge.

The result of the present study indicates a possible synergistic effect of the combination of DE and ME on *R. dominica, T. confusum, S granarius* and *S. zeamais.* This is particularly important, as such a combination is expected to enhance a DE treatment so it can cause rapid mortality without adverse effects on the physicochemical characteristics of the grain. At the same time, such a formulation can be registered more easily than combinations with synthetic insecticides, as ME is a natural substance. Additional work is needed to clarify the data that would be needed for regulatory approval.

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