

## Original article (Orijinal araştırma)

# Determination of lambda-cyhalothrin and imidacloprid resistance and synergistic activity of diatomaceous earth in *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) populations<sup>1</sup>

Leptinotarsa decemlineata Say, 1824 (Coleoptera: Chrysomelidae) popülasyonlarında lambdacyhalothrin ve imidacloprid direncinin ve diatom toprağının sinerjistik etkinliğinin belirlenmesi

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### Abstract

In this study conducted in 2020, the resistance of *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) populations collected from Karapınar, Çumra, Seydişehir, Güneysınır and Doğanhisar Districts of Konya Province, Turkey to lambda-cyhalothrin and imidacloprid was investigated. Insecticides were applied topically to fourth instar larvae. In addition, the synergistic efficiency of diatomaceous earth (DE) was investigated by applying imidacloprid plus DE (2% w/v) with the same methodology in the Karapınar population. As a result of the research, the resistance rate of all populations was found to be higher in imidacloprid than in lambda-cyhalothrin. The highest LD<sub>50</sub> was found in the Çumra (0.212 µg/larvae) and Karapınar (0.456 µg/larvae) population for lambda-cyhalothrin and imidacloprid respectively. The highest synergism ratio was determined as 1.93 after 48 hours in imidacloprid plus DE application. As a result of the research, it is considered that the use of DE will make a great contribution to the control and resistance management of the imidacloprid resistant potato beetle.

Keywords: Diatomaceous earth, insecticide, Leptinotarsa decemlineata, resistance, synergism

## Öz

Bu çalışma 2020 yılında yürütülmüş olup, Konya'nın Karapınar, Çumra, Seydişehir, Güneysınır ve Doğanhisar ilçelerinden toplanan patates böceği, *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) popülasyonların lambda-cyhalothrin ve imidacloprid etken maddelerine direnci araştırılmıştır. İnsektisit uygulamaları 4. dönem larvalara topikal olarak gerçekleştirilmiştir. Ayrıca Karapınar popülasyonunda aynı metodoloji ile imidacloprid ve diatom (%2 w/v) uygulanarak, diatom toprağının sinerjistik etkinliği araştırılmıştır. Araştırma sonucunda tüm popülasyonların direnç oranı imidacloprid aktif maddesinde, lambda-cyhalothrine nazaran daha yüksek bulunmuştur. En yüksek LD<sub>50</sub> değeri lambda-cyhalothrin ve imidacloprid için sırasıyla Çumra (0.212 µg larva<sup>-1</sup>) ve Karapınar (0.456 µg larva<sup>-1</sup>) popülasyonunda tespit edilmiştir. İmidacloprid ve diatom uygulamasında en yüksek sinerji oranı uygulamadan 48 saat sonra 1.93 olarak belirlenmiştir. Araştırma sonucunda diatom toprağı kullanımının imidacloprid etken maddesine direnç geliştirmiş olan patates böceğinin mücadelesinde ve direnç yönetiminde büyük katkı sağlayacağı değerlendirilmektedir.

Anahtar sözcükler: Diatom toprağı, insektisit, Leptinotarsa decemlineata, direnç, sinerjizm

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### Introduction

Colorado potato beetle, Leptinotarsa decemlineata Say, 1824 (Coleoptera: Chrysomelidae), the main pest of potato crops, feeds voraciously in the larval and adult stages and causes a 70-80% decrease in yield depending on population density (Oerke et al., 1994). In addition to the direct damage from its herbivory, it is a vector for pathogens such as brown rot bacterium, and ringspot and spindle tuber viruses (Yüceer, 2011). Currently, control methods other than chemical control and rotation are not used by growers as they are not easily applied nor effective. Presently, 262 plant protection products with 20 different active substances are registered for Colorado potato beetle in Turkey (Anonymous, 2021a). It is one of the species that develop dangerous resistance as a result of intense insecticide pressure, its high reproduction rate (Bishop & Grafius, 1996), and the development of physiological capacity for detoxification (Ferro, 1993) as a result of the glycoalkaloids found in the solanaceous plants, which are its normal hosts (Weisz et al., 1994). The first resistance was observed in 1952 to DDT (Quinton, 1955), resistance was detected to permethrin after 2 years and imidacloprid after 5 years from their first registration (Huseth et al., 2014). Ioannidis et al. (1991) report that resistance is 1000-fold to azinphos-methyl and 2000-fold to carbofuran. It has been reported that resistance develops 26 and 130-fold, respectively, for thiamethoxam and imidacloprid, which are neonicotinoid group insecticides that have been used extensively in the recent past (Szendrei et al., 2011), and resistance can reach up to 300-fold to imidacloprid (Mota-Sanchez et al., 2006). Since the pest develops cross resistance to insecticides, rotation among insecticides with the same mode of action is ineffective in delaying resistance.

In Colorado potato beetle, although it varies in different populations, reducing insecticide penetration, increasing secretions enhance detoxification (Clark et al., 2001), desensitization of target tissue and conversion of active substance to low-toxicity metabolites (Mota-Sanchez, 2003) by increasing esterase, carboxylesterase, monooxygenase enzyme secretions are known to be significant resistance mechanisms. Recently, studies on the use of some compounds with known synergistic effects with insecticides in resistance management have attracted attention. For example, it has been reported that the susceptibility to permethrin (Silcox et al., 1985), azinphos-methyl (Ahammad-Sahib et al., 1994), abamectin (Yoon et al., 2002) of insect pests increases with the use of piperonyl butoxide (PBO), an oxygenase enzyme inhibitor. Zamojska et al. (2011) reported that DEF (S-S-S-tributyl phosphorotrithioate), DEM (diethyl malonate) inhibitors have synergism rates of 15.3 and 3.63, respectively, when used with deltamethrin in resistant populations.

Another substance with the potential for synergistic benefit is diatomaceous earth (DE), which is a siliceous sedimentary material consisting of fossilized diatoms that is applied to many stored-product insect pests (Başkaya, 2020). DE can act by adsorption or abrasion. In adsorption, the powders can adhere to the cuticle and lead to the disruption of the lipid layer. This disruption causes the loss of water by dehydration and, death (Golob,1997). Başkaya (2020) reported that DE is also effective in different pests such as *Callosobruchus maculatus* Fabricius, 1775 (Coleoptera: Bruchidae) and *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae).

In this study, the resistance to imidacloprid and lambda-cyhalothrin in fourth instar larvae of Colorado potato beetle collected from different districts of Konya was investigated. In addition, the synergistic effect of DE was studied.

### **Materials and Methods**

#### Insect populations

In preliminary experiments conducted in 2018, it was observed that the susceptibility to imidacloprid has decreased in Colorado potato beetle populations from Karapınar District and LD<sub>50</sub> were determined. In 2020, the fourth instar larvae of Colorado potato beetle were collected from potato production areas in Çumra, Doğanhisar, Güneysınır, Karapınar, Meram and Seydişehir Districts of Konya Province, Turkey,

placed in plastic containers and kept in refrigerated boxes until transferred to the laboratory. In the laboratory, they were fed on insecticide-free potato leaves for 1 day at  $25 \pm 1^{\circ}$ C with 16:8 h L:D photoperiod and 60  $\pm$  10% RH, thus, it was ensured that the impacts of collection and transportation were eliminated (Huseth & Groves, 2013). The population with the lowest LD<sub>50</sub> came from an organic farm that has been operating for 6 years in Meram District was considered as a susceptible population. The food of larvae (insecticide-free potato leaves) was changed daily.

#### Insecticides application

In the study, imidacloprid (Confidor SC 350 Bayer Crop Science) and lambda-cyhalothrin (Petra 5 EC, Agrobest), which are commonly used insecticides for Colorado potato beetle control, were used. In the preliminary experiments, a dose range for 10-90% mortality was determined for imidacloprid and lambda-cyhalothrin using 8 and 7 dose range series and control, respectively. The dose range series was created by diluting 50% at each step. The insecticides were applied with 3 replicates for each dose to 20 larvae, and as a control, distilled water was applied. Two  $\mu$ l of insecticide solution was dripped to the dorsal surface of larvae with a hand microapplicator using a microsyringe (Burkard Scientific, Uxbridge, UK). The larvae were then placed in 90-mm plastic Petri dishes with sufficient potato leaves in the incubator (Nüve EN500). After 24 h, the larvae that did not react when touched their body gently with a soft brush were considered to be dead (Alkan et al., 2017). Resistance ratio (RR) was classified according to Lee et al., (1999): RR < 2, no resistance or very low resistance; RR = 2-5, low level resistance; RR=5-10, moderate level resistance; and RR > 10, high level resistance.

#### Diatomaceous earth and its synergistic activity

A 10 µm diameter powder of Turkish DE product (Turco 010) was obtained from Beg Tuğ Mineral Company (Istanbul, Turkey) was used. Turco 010 has a pH of 5.1 and contains 83.4% SiO<sub>2</sub> and 1.3% CaO (Anonymous, 2021b). The synergistic effect of DE was investigated in the Karapınar population, with the highest LD<sub>50</sub>, by using it together with imidacloprid. For this purpose, separate treatment for each day were created and imidacloprid and imidacloprid plus 2% (w/v) DE mixture were applied by the same method as described above. Distilled water plus 2% (w/v) DE was used as a control. Mortality and LD<sub>50</sub> for imidacloprid alone and its mixture with DE were determined after 24, 48, 72, and 96 h of exposure. Corrected mortality data were calculated by using Abbott's formula (Abbott, 1925). The synergy rate (SR) was calculated with the following formula (Zamojska et al., 2011): SR = LD of active substance alone/LD of active substance with a synergist, SR < 1, antagonist; SR = 1, the lack of synergism or antagonism; and SR > 1, synergism.

#### Data analysis

The probit analysis for the data of mortality vs dose was conducted using the POLO computer package program (LeOra, 2002) to estimate  $LD_{50}$  and  $LD_{90}$ . Susceptibility ratios were determined by dividing the  $LD_{50}$  of tested field population by the  $LD_{50}$  of the susceptible population. Percentage mortality in imidacloprid alone and imidacloprid plus DE 2% (w/v) treatments were evaluated variance with LSD in the JUMP statistical program.

### **Result and Discussion**

#### Resistance to lambda-cyhalothrin

The highest LD<sub>50</sub> of 0.212 (0.164-0.263)  $\mu$ g ai larvae<sup>-1</sup> for lambda-cyhalothrin was found in Çumra population (Table 1). The resistance rate based on LD<sub>50</sub> was determined as 2.98, 2.63 and 2.40 in the Çumra, Karapınar and Seydişehir populations, respectively. It is considered that the relatively low resistance rate in the Güneysınır and Doğanhisar populations is most probably because that the crop production in these areas is in the form of small family businesses and less insecticide is used in the control of Colorado potato beetles.

Determination of lambda-cyhalothrin and imidacloprid resistance and synergistic activity of diatomaceous earth in *Leptinotarsa* decemlineata Say, 1824 (Coleoptera: Chrysomelidae) populations

Population	n	LD₅₀ (µg ai larvae <sup>-1</sup> ) (95% CL)	LD <sub>90</sub> (μg ai larvae <sup>-1</sup> ) (95% CL)	Slope ± SEM	$\lambda^2$ (df)	Н	RR
Susceptible	420	0.071 (0.052-0.090)	0.317 (0.232-0.518)	1.97 ± 0.278	3.47 (4)	0.867	-
Çumra	420	0.212 (0.164-0.263)	0.892 (0.636-1.571)	2.05 ± 0.305	2.75 (4)	0.687	2.98
Karapınar	420	0.187 (0.147-0.234)	0.843 (0.603-1.419)	1.96 ± 0.258	2.30 (4)	0.574	2.63
Seydişehir	420	0.171 (0.127-0.214)	0.672 (0.497-1.103)	2.16 ± 0.327	2.54 (4)	0.635	2.40
Güneysınır	420	0.117 (0.086-0.162)	0.577 (0.364-1.248)	1.85 ± 0.172	5.25 (4)	1.312	1.64
Doğanhisar	420	0.092 (0.071-0.116)	0.407 (0.307-0.602)	1.99 ± 0.226	2.95 (4)	0.737	1.29

Table 1. Contact toxicity of lambda-cyhalothrin to fourth instar larvae of Leptinotarsa decemlineata after 24 h

n, number of larvae tested; SEM, standard error of the mean;  $\lambda^2$ , chi-square; H, heterogeneity; RR, resistance ratio (resistant population LD<sub>50</sub>/susceptible population LD<sub>50</sub>).

It has been reported in similar studies that the Colorado potato beetle develops resistance against lambda-cyhalothrin and other pyrethroids (Cutler et al., 2005; Zamojska et al. 2011). For example, Keskin & Yorulmaz Salman (2020) found that different populations collected from Afyonkarahisar developed resistance to deltamethrin in the range of 9.41-77.2 times on third instar larvae. Slađan et al. (2012) observed that adults developed up to 60 times resistance to cypermethrin in Romania. In another study using adults, resistance rates for lambda-cyhalothrin in different populations were found to be in the range of 14.3 to 617 times, and it was determined that the resistance rates for cypermethrin and deltamethrin increased up to 41 and 2,325 times, respectively (Jiang et al., 2010). This difference in resistance may be due to differences in life stages and insecticide pressure.

#### **Resistance to imidacloprid**

Contact toxicity of imidacloprid in different populations of *L. decemlineata* is given in Table 2. Although, the LD<sub>50</sub> of the susceptible population was 0.065 (0.048-0.087) µg ai larvae<sup>-1</sup>, the highest LD<sub>50</sub> was 0.456 (0.337-0.599) µg ai larvae<sup>-1</sup> in the Karapınar population. The lowest LD<sub>50</sub> was found in the Doğanhisar population at 0.159 (0.101-0.220) µg ai larvae<sup>-1</sup>. Resistance rates were found in the range of 2.44 to 7.01 times. Imidacloprid resistance level was found to be moderate in the Karapınar population and low in the populations from other districts. Similar studies demonstrate that Colorado potato beetle develops resistance to imidacloprid at varying rates. Olson et al. (2000) found that the resistance rate in larvae was 30 times, Baker et al. (2014) stated that it varies between 7.6 to 71 times in different populations. However, Slađan et al. (2012) showed the resistance rate in adults 1.56 to 82.9 times, Mota-Sanchez et al. (2006) determined it 310 times. Keskin & Yorulmaz Salman (2020) determined that the populations of Afyonkarahisar districts gained resistance to imidacloprid in the range of 3.96 to 27.3 times.

Crassley et al. (2018) found that the LD<sub>50</sub> of different populations were in the range of 0.0005-0.16  $\mu$ g larvae<sup>-1</sup> for second instar larvae and 0.051-2.401  $\mu$ g adults<sup>-1</sup>. According to the results of the same study, it is determined that resistance develops up to 320 times in larvae and up to 47 times in adults.

In the preliminary study conducted in 2018, it was determined that the LD<sub>50</sub> of the population collected from the same field in Karapınar District was 0.342  $\mu$ g larvae<sup>-1</sup> but had increased to 0.456  $\mu$ g larvae<sup>-1</sup> by 2020 (Table 3). In a similar study, it was observed that LD<sub>50</sub> of four populations increased 1.8-3.75 times in the third year in Poland (Wegorek, 2005). This situation is considered to be a result of intensive production in the region and spraying tubers and foliage with imidacloprid and other neonicotinoid group insecticides. Jeschke & Nauen (2008) reported that cross resistance developed even when imidacloprid was not used. Resistance development is detected as a decrease in insecticide effectiveness and a shortening of the control period under field conditions (Stewart et al., 1997).

Population	n	LD <sub>50</sub> (µg ai larvae <sup>-1</sup> ) (95% CL)	LD <sub>90</sub> (µg ai larvae <sup>-1</sup> ) (95% CL)	Slope ± SEM	$\lambda^2$ (df)	н	RR
Susceptible	540	0.065 (0.048-0.087)	0.67 (0.45-1.15)	1.27 ± 0.123	2.65 (5)	0.441	-
Karapınar	480	0.456 (0.337-0.599)	3.68 (2.47-6.46)	1.41 ± 0.152	2.59 (5)	0.518	7.01
Çumra	300	0.301 (0.198-0.416)	2.22 (1.44-4.41)	1.48 ± 0.216	2.60 (4)	0.651	4.63
Seydişehir	540	0.271 (0.185-0.364)	1.75 (1.26-2.73)	1.58 ± 0.184	4.48 (5)	0.746	4.16
Güneysınır	540	0.185 (0.132-0.245)	1.22 (0.89-1.84)	1.57 ± 0.163	5.37 (5)	0.895	2.84
Doğanhisar	540	0.159 (0.101-0.220)	0.85 (0.63-1.26)	1.76 ± 0.231	5.30 (5)	0.883	2.44

Table 2. Contact toxicity of imidacloprid to fourth instar larvae of Leptinotarsa decemlineata after 24-h treatment

n, number of larvae tested; SEM, standard error of the mean;  $\lambda^2$ , chi-square; H, heterogeneity; RR, resistance ratio (resistant population LD<sub>50</sub>/susceptible population LD<sub>50</sub>).

Table 3. Contact toxicity of imidacloprid to fourth instar larvae of Leptinotarsa decemlineata in different years

Population	n	LD₅₀ (µg ai larvae⁻¹) (95% CL)	LD <sub>90</sub> (μg ai larvae <sup>-1</sup> ) (95% CL)	Slope ± SEM	$\lambda^2$ (df)	Н
Karapınar (2018)	480	0.342 (0.242-0.457)	2.77 (1.91-4.66)	1.41 ± 0.256	2.58 (6)	0.441
Karapınar (2020)	480	0.456 (0.337-0.599)	3.68 (2.47-6.46)	1.41 ± 0.152	2.59 (5)	0.518

n, number of larvae tested; SEM, standard error of the mean;  $\lambda^2$ , chi-square; H, heterogeneity.

#### Synergistic effect of diatomaceous earth application

For imidacloprid, the LD<sub>50</sub> was in the range of 0.387-0.456  $\mu$ g larvae<sup>-1</sup> according to exposure times (Table 4). The LD<sub>50</sub> was 0.354, 0.231, 0.223 and 0.214  $\mu$ g larvae<sup>-1</sup> after 24, 48, 72 and 96 h exposure respectively, when imidacloprid plus DE mixtures were applied. According to the exposure times, the maximum synergy rate was 1.93 for 48 h exposure.

Mortality rates in imidacloprid alone and imidacloprid plus DE mixtures treatments were close to each other for 24 h exposure and were found to be 48.5 and 53.9%, respectively (Table 5, 6). For the increase of exposure time from 24 to 48 h of exposure, the mean mortality rate was 48.5% in imidacloprid alone treatment whereas it was 59.0% with a 10% increase in imdacloprid plus DE treatment.

In the studies using synergism in the control of Colorado potato beetle, inhibitors of enzymes that are involved in resistance mechanisms were tested. For example, Sharif et al. (2007), found synergism rates of 3.5 and 2.3, respectively, for DEF (S-S-S tributyl phosphorotrithioate) and PBO (Piperonyl butoxide) with endosulfan in fourth instar larvae. In another study, it was determined that the synergism rates increased up to 48.2, 15.2 and 3.6 levels as a result of the use of deltamethrin and PBO, DEF and DEM (diethyl malonate), respectively (Zamojska et al., 2011). However, Jiang et al. (2010) reported that when carbofuran and PBO, DEM, triphenyl phosphate (TPP) were used in adults from different populations, the maximum synergism rates were 5.7, 2.9, and 2.6, respectively. Zhao et al. (2000), found that when they used DEF together with imidacloprid, there was no DEF synergistic or antagonistic effect in the susceptible larvae, but antagonistic effect in susceptible adults. In the same study, synergism was observed with the use of imidacloprid plus PBO at a rate of 1.1 and 1.3 in susceptible adults and larvae, respectively.

Determination of lambda-cyhalothrin and imidacloprid resistance and synergistic activity of diatomaceous earth in *Leptinotarsa* decemlineata Say, 1824 (Coleoptera: Chrysomelidae) populations

Table 4. Contact toxicity of imidacloprid and imidacloprid plus diatomaceous earth (DE) 2% (w/v) against fourth instar larvae of Leptinotarsa decemlineata in the Karapınar population

Application	Exposure (h)	n	LD <sub>50</sub> (µg ai larvae <sup>-1</sup> ) (95% CL)	LD <sub>90</sub> (µg ai larvae <sup>-1</sup> ) (95% CL)	Slope ± SEM	$\lambda^2$ (df)	Н	SR
Imidacloprid	24	480	0.456 (0.337-0.599)	3.68 (2.47-6.46)	1.41 ± 0.152	2.59 (5)	0.518	-
	48	480	0.447 (0.321-0.596)	3.62 (2.42-6.42)	1.41 ± 0.159	2.45 (5)	0.490	-
	72	480	0.401 (0.284-0.540)	3.50 (2.32-6.31)	1.36 ± 0.155	3.64 (5)	0.728	-
	96	480	0.387 (0.263-0.550)	4.15 (2.34-10.70)	1.24 ± 0.179	0.39 (5)	0.162	-
Imidacloprid + DE	24	480	0.354 (0.260-0.464)	2.65 (1.84-4.38)	1.47 ± 0.155	1.04 (5)	0.208	1.28
	48	480	0.231 (0.151-0.322)	1.85 (1.27-3.12)	1.42 ± 0.166	3.37 (5)	0.673	1.93
	72	480	0.223 (0.139-0.318)	1.65 (1.13-2.76)	1.48 ± 0.184	1.48 (5)	0.296	1.79
	96	480	0.214 (0.123-0.317)	1.49 (1.01-2.52)	1.52 ± 0.209	3.14 (5)	0.628	1.80

n, number of larvae tested; SEM, standard error of the mean; λ<sup>2</sup>, chi-square; H, heterogeneity; SR, synergy ratio (LD<sub>50</sub> of imidacloprid alone/LD<sub>50</sub> of imidacloprid plus diatomaceaus earth).

Table 5. Mortality (%) with imidacloprid application in fourth instar larvae of Leptinotarsa decemlineata at different doses and times

Dose	Hours after treatment									
(µg imidacloprid ai larvae <sup>-1</sup> )	24 <sup>1</sup>		48		72		96		Mean <sup>1</sup>	
0.052	6.9 ± 1.66	е	7.1 ± 1.66	f	10.6 ± 2.88	g	12.8 ± 1.85	е	9.36 ± 1.66	G
0.105	18.9 ± 7.31	е	19.7 ± 2.18	е	21.4 ± 3.21	f	23.7 ± 1.85	de	20.9 ± 1.88	F
0.210	35.2 ± 2.40	d	37.3 ± 5.78	d	37.4 ± 5.03	е	36.4 ± 4.91	d	36.6 ± 2.02	Е
0.420	50.7 ± 3.28	с	46.2 ± 6.96	d	53.5 ± 2.08	d	56.2 ± 6.02	с	51.7 ± 2.41	D
0.840	61.6 ± 6.64	bc	62.4 ± 3.78	с	64.2 ± 2.08	с	63.6 ± 4.91	с	63.0 ± 2.01	С
1.680	73.6 ± 5.45	b	75.0 ± 1.85	b	73.1 ± 3.48	b	78.4 ± 5.78	b	75.0 ± 1.98	В
3.360	92.9 ± 2.18	а	92.8 ± 3.84	а	94.5 ± 3.46	а	$100.0 \pm 0.00$	а	95.1 ± 1.53	А
Mean <sup>2</sup>	48.5 ± 6.45	в	48.5 ± 6.41	в	50.7 ± 6.36	AB	53.0 ± 6.55	А		

<sup>1</sup> Means followed by same letters within columns are not statistical different at P<0.05; <sup>2</sup> Means same letters within the row and not statistical different at P<0.05.

In previous studies, it was shown that the synergistic activity of DE can be used for storage pests. For example, Başkaya (2020), when using DE (0.4% w/v) with cypermethrin for *Callosobruchus maculatus* (Coleoptera: Bruchidae) found synergism of 2.6, 4.0, 3.3 after 48, 72 and 96 h of the treatment, respectively. The effectiveness of DE varies according to the physical and chemical properties of the material, dose, type of insect, life stage, application method and temperature (Losic & Korunic, 2018). However, it is also known that DE has a repellent effect as well as an insecticidal effect (Bayram, 2018).

Dose	Hours after treatment									
(µg imidacloprid ai larvae <sup>-1</sup> )	24 <sup>1</sup>		48		72		96		Mean <sup>1</sup>	
0.052	12.2 ± 1.52	g	14.7 ± 7.62	е	14.5 ± 4.58	е	15.3 ± 2.51	е	14.16 ± 2.02	G
0.105	22.7 ± 3.17	f	32.8 ± 2.40	de	35.4 ± 9.16	d	39.2 ± 7.83	d	32.52 ± 3.26	F
0.210	36.8 ± 2.72	е	47.7 ± 6.48	cd	49.4 ± 7.37	d	49.9 ± 3.33	d	45.95 ± 2.77	Е
0.420	59.6 ± 3.84	d	66.6 ± 6.42	bc	67.3 ± 5.20	с	71.9 ± 5.23	С	66.36 ± 2.60	D
0.840	70.4 ± 3.60	с	69.6 ± 10.10	b	77.9 ± 4.66	bc	78.2 ± 2.51	bc	74.03 ± 2.83	С
1.680	80.9 ± 4.33	b	83.0 ± 6.11	ab	87.5 ± 4.05	ab	89.3 ± 4.00	ab	85.15 ± 2.26	В
3.360	94.6 ± 3.17	а	98.2 ± 2.00	а	97.8 ± 2.33	а	$100.0 \pm 0.00$	а	97.66 ± 1.12	А
Mean <sup>2</sup>	$53.9 \pm 6.44$	В	59.0 ± 6.35	А	61.4 ± 6.45	А	63.4 ± 6.37	А		

Table 6. Mortality (%) with imidacloprid plus diatomaceous earth (2% w/v) application in fourth instar larvae of *Leptinotarsa* decemlineata at different doses and exposure times

<sup>1</sup> Means followed by same letters within columns are not statistical different at P<0.05; <sup>2</sup> Means same letters within the row and not statistical different at P<0.05.

In the current study, it was determined that the pest has developed resistance to both imidacloprid and lambda-cyhalothrin in different populations. It has been determined that relatively high rates of resistance had developed in the Karapınar and Çumra populations from districts where crop production is intensive and the both active substances are intensively applied. It is considered that the higher rate of imidacloprid resistance compared to lambda-cyhalothrin is due to imidacloprid being used in both tuber and spray applications. It is observed that other neonicotinoid class insecticides, such as acetamiprid, are also used in spray applications, and it is thought that this may increase imidacloprid resistance due to the development of cross resistance. Although lambda-cyhalothrin is older than imidacloprid in Colorado potato beetle control, there has been an increase in spray applications of lambda-cyhalothrin, due to the decrease in imidacloprid efficacy. Since the use of insecticides is the most common and effective method in the control of Colorado potato beetle, delaying and management of resistance against insecticides gains great importance. In the present study, it was shown that DE can be used as an effective synergist for insecticide treatments against Colorado potato beetle larvae. In future studies, the synergistic effect of DE against adults and its effectiveness in field conditions should be investigated.

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