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Original article (Orijinal araştırma)

Influence of different grain storage types on Khapra beetle, *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae), infestation in southeastern Anatolia (Turkey) and its resistance to malathion and deltamethrin[1]

Güneydoğu Anadolu Bölgesi (Türkiye)'nde farklı depo tiplerinin Khapra böceğinin, *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae), bulaşıklığına olan etkisi ve zararlının malathion ve deltametrine olan direnci

Çetin MUTLU[2*] **Ayhan ÖĞRETEN[3]** **Cahit KAYA[3]** **Mehmet MAMAY[2]**

Abstract

This study was conducted to determine the effect of different storage types on the infestation of Khapra beetle, *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae), and its resistance to malathion and deltamethrin in southeastern Anatolia, Turkey. A total 355 various grain storage facilities (metal silo, concrete wall and basic plasterless) were surveyed in five provinces, (Diyarbakır, Mardin, Şanlıurfa, Adıyaman and Batman) during April-December in 2014-2016 and wheat grain samples were collected. Also, 24 populations of Khapra beetle were collected for bioassay studies. The provinces and storage types significantly influenced the infestation rate of Khapra beetle. The highest infestation of the beetle was recorded in Mardin (77.5%), followed by Şanlıurfa (67.5%). Whereas the lowest infestation observed was in Diyarbakır (43.4%) and Adıyaman (44.1%). For storage types, the highest infestation was observed in basic plasterless storage type (80.0%), while the lowest (27.1%) was noted for storage type of metal silos. Bioassay studies indicated that Khapra beetle has evolved low resistance to deltamethrin, whereas it was tolerant to malathion. Resistance ratios of the populations exposed to deltamethrin were 4-10.7 times, while the ratios for malathion were 1.32-1.92 times. It is concluded that the higher resistance ratios for deltamethrin were linked to its frequent use compared to malathion.

Keywords: Infestation, insecticide resistance, storage types, survey, *Trogoderma granarium*

Öz

Bu çalışma Güneydoğu Anadolu Bölgesi'nde farklı depo tiplerinin Khapra böceğinin, *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae), bulaşıklığına olan etkisi ve zararlının malathion ve deltametrine olan direncini belirlemek amacıyla yapılmıştır. Bölgedeki beş ilden (Diyarbakır, Mardin, Şanlıurfa, Adıyaman ve Batman) farklı tahıl depo tiplerinden (metal silo, betonarme ve basit sıvasız) 2014-2016 yıllarında nisan-aralık ayları arasında sürveyler yapılmış ve toplam 355 depodan buğday örnekleri alınmıştır. Ayrıca, direnç çalışmaları için farklı depolardan 24 Khapra böceği popülasyonu toplanmıştır. Çalışma sonucunda, iller ve depo tipleri Khapra böceği bulaşıklığına önemli derecede etki etmiştir. En yüksek bulaşıklık Mardin ilinde (%77.5), bunu takiben Şanlıurfa ilinde (%67.5) kayıt edilmiştir. Buna karşın en düşük Khapra böceği bulaşıklığı Diyarbakır (%43.4) ve Adıyaman (%44.1) ilinde kayıt edilmiştir. Depo tiplerine göre en yüksek bulaşıklık basit sıvasız depolarda (%80) belirlenmiş iken, en düşük bulaşıklık metal silolarda (%27.1) bulunmuştur. Direnç çalışmaları, Kapra böceğinin deltametrine karşı düşük düzeyde direnç geliştirdiği, buna karşın malathiona toleranslı olduğu göstermiştir. Deltametrin uygulanmış popülasyonların 4-10.7 kat direnç geliştirmiş olduğu, ancak malathion için bu rakamın 1.32-1.92 kat olduğu kayıt edilmiştir. Deltametrin için belirlenen yüksek direnç oranları, malathiona kıyasla bu insektisitin daha sık kullanımını ile ilgili olduğu düşünülmektedir.

Anahtar sözcükler: Bulaşıklık, insektisit direnci, depo tipleri, sürvey, *Trogoderma granarium*

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[2] Harran University, Faculty of Agriculture, Department of Plant Protection, 63000, Şanlıurfa, Turkey
[3] Republic of Turkey Ministry of Agriculture and Forestry, Diyarbakir Plant Protection Research Institute, Silvan Highway 7. Km. 21110, Diyarbakır, Turkey
* Corresponding author (Sorumlu yazar) e-mail: cetinmutlu21@hotmail.com
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Introduction

Southeastern Anatolia is one of the important production areas for cereals in Turkey. The region produces ~15% of the national wheat production of the country (TUIK, 2017). Since cereals are harvested at a certain time and used throughout the year for human and animal feed, their safe storage is an important issue. Stored-grain pests, such as, *Sitophilus* spp. Schoenherr, 1838 (Coleoptera: Curculionidae) *Tribolium* spp. MacLeay, 1825 (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrychidae), *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) and *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae), are responsible for weight, germination and quality losses during storage of wheat in Turkey (Ergül et al., 1972; Erakay, 1974; Özar & Yücel, 1982, 1988; Özer et al., 1989; Işıkber et al., 2005; Anonymous, 2008).

Khapra beetle (*T. granarium*) is one of the most important pests of stored wheat, and is subject to quarantine restrictions (Banks, 1977; USDA, 1983; Lowe et al., 2004; EPPO, 2005, 2007; French & Venette, 2005; Hasan et al., 2006; CABI, 2018). The beetle is included in the list of 100 worst invasive species worldwide (Lowe et al., 2004). Khapra beetle is very common in granaries, bins, silos as well as farmhouses in southeastern Anatolia due to suitable climatic and storage conditions. Wheat produced in the region is used commercially (i.e., stored and then processed); therefore, Khapra beetle should be carefully monitored in stored grains to avoid economic losses. Stored-grain pests are known to cause ~10% losses during storage of grain in Turkey (Emekçi & Ferizli, 2000).

The use of synthetic insecticides is the most common method of controlling agricultural insect pests around the world (Matthews, 1993; Sathyan et al., 2016; Wojciechowska et al., 2016); however, their excessive and unconscious use leads to the evolution of insecticide resistance (Fragoso et al., 2003; Ribeiro et al., 2003; Hasan et al., 2006; Wojciechowska et al., 2016). Aluminum phosphide fumigation is the most prevalent method used to control stored-grain pests in Turkey. However, stored-grain pests have developed resistance to phosphine used for the fumigation of grain storage facilities (Zettler & Keever, 1994; Benhalima et al., 2004; Pimentel et al., 2010). In addition, malathion has been used as a protective insecticide against stored-grain pests for a long time in Turkey. A synthetic pyrethroid (deltamethrin) was used excessively in grain storage facilities before the use of fumigation to control Khapra beetle and other stored-grain pests in the world as well as Turkey. Consequently, numerous researchers have reported that Khapra beetle has developed resistance against deltamethrin (Irshad & Iqbal, 1994; Tarakanov et al., 1994; Saxena & Sinha, 1995; Kumar et al., 2010; Hafiz et al., 2018). Also, malathion is more widely used in empty storage facilities than other registered insecticides in the country. During 1998, 297 t of pesticide were used against stored-product pests in Turkey (Emekçi & Ferizli, 2000). The use of same insecticide, for example malathion, for extended periods leads to the evolution of insecticide resistance in stored-grain pests (Champ & Dyte, 1976; Navarro et al., 1986). The frequent use of malathion has led to the evolution of resistance in *Tribolium castaneum* (Herbst) and *Sitophilus* spp. (Dyte & Blackman, 1970).

Khapra beetle usually has four to five generations per year and it can have 12 generations under suitable conditions. The female can lay 50-100 eggs, which are loosely scattered on host material (Harris, 2006; Szito, 2007). Larvae are able to hide in cracks and crevices of shipping containers, bulk cargo holds and packing material. Khapra beetle can stay in diapause for up to 6 years, until the onset of suitable conditions for development (Burges, 1962; Pasek, 1998; Stibick, 2007). Khapra beetle damages stored wheat by reducing weight and grade of the grain. The damage caused by Khapra beetle to stored wheat grain may reach to 73% (Rahman et al., 1945; Kalkan, 1963; Prasad et al., 1977). The control and eradication of Khapra beetle is difficult, which may reduce its susceptibility to some control methods (Ahmad, 1994). Phosphine gas fumigation, deltamethrin and malathion are widely used to control Khapra beetle in Turkey.

There are no studies reporting the distribution, infestation and resistance of Khapra beetle to certain insecticides in southeastern Anatolia region of Turkey. The only study related to the distribution and infestation of Khapra beetle in southeastern Anatolia dates back to 1982 (Özar & Yücel, 1982). A recent study in 2005 investigated the stored-grain pest species in Kahramanmaraş and Adıyaman Provinces, and found a lower infestation of Khapra beetle in Adıyaman (Işıkber et al., 2005). The precautionary measures based on the knowledge on the distribution of pest, resistance level to insecticides used could effectively control Khapra beetle. However, no such data are available for southeastern Anatolia. This study was therefore conducted to determine the distribution, infestation and resistance level of Khapra beetle to malathion and deltamethrin insecticides in southeastern Anatolia. The results of the study will help to devise effective precautionary measures for reducing Khapra beetle infestation and subsequently damage to stored wheat grain in the region.

Material and Method

Field study

Infestation level of the Khapra beetle

Different storage types in five provinces (Adıyaman, Batman, Diyarbakır, Mardin and Şanlıurfa) in southeastern Anatolia were surveyed to investigate the infestation of Khapra beetle in the region. Various kinds of storage are used to store wheat grain in Turkey; however, the most prevalent storage types in southeastern Anatolia are metal silos, basic plasterless and reinforced concrete. Grain samples were collected from 355 different storage types (metal silos, basic plasterless and reinforced concrete) through April to December following Işıkber et al. (2005). Five different grain samples (~800 g each) were collected from five different points and depths of stored-grain using a 2-m long probe. The collected samples were pooled, which made a composite sample of 4 kg from each storage facility. The stored grain close to the walls and corners were also inspected for Khapra infestation. Samples were placed in plastic containers and brought to the laboratory. The presence of Khapra beetle (either larvae or adults) in the samples was visually determined in the laboratory. The samples were regarded as infested when presence of the beetle was confirmed. Whereas, where beetle presence was not observed in the samples, were regarded as uninfested. The percentage infestation was calculated as follows:

$$\text{Infestation (\%)} = 100 \times n / N$$

where, n is the number of infested samples and N is total number of samples.

Laboratory study

Collection of test populations

Test populations for resistance studies were collected from different storage types where malathion and deltamethrin have been extensively used. The grain samples were collected from the same five provinces of southeastern Anatolia where field study was conducted. A total of 24 putative resistant populations were collected for laboratory study. Also, a susceptible population was obtained from a on-farm storage where insecticides have never been used.

Insect cultures

Khapra beetle larvae were obtained from the collected grain samples from different types of storage. The insects were reared in glass jars (3 L) covered with muslin cloth. The jars were incubated in continuous darkness at 32±1°C and 60% RH (Hasan et al., 2006). The larvae were fed with bread wheat cv. Pehlivan. The cultured insects were reared for two generations until adequate number of insects was obtained for experiments. The reared insects were kept at 10±1°C and 60% RH until used in the experiments.

Selection of Khapra beetle populations for bioassay studies

The recommended dose (discriminate dose) of both insecticides (malathion and deltamethrin) was applied to the collected populations to select the populations for bioassay studies. However, 100% mortality was noted in all populations 24 h after the application of the insecticides, indicating absence of resistance in these populations. Several pretests were conducted on the available susceptible population, which indicated a higher sensitivity of the susceptible populations compared to the other susceptible populations used in the literature (Singh & Yadav, 1994). LC_{50} (lethal $\text{concentration}_{50}$) value of the available susceptible population was determined and two times of the LC_{50} was considered as discriminatory dose for the selection of populations for bioassay studies. Six populations were selected for use in bioassay studies. Adult individuals from the selected six populations were kept on sterilized grain of wheat cv. Pehlivan to oviposit for 2 d, after which the adults were removed. In this way, larvae with the same age (fourth instar) were obtained and used in the bioassay studies.

Preparation of pesticide solutions

The pure active ingredients of malathion and deltamethrin, produced by Sigma-Aldrich under the trademark Pestanal®, were used for the preparation of solutions in desired concentration. The active substances were first dissolved in pure acetone (Merck) and 100% stock solution was prepared. The target concentrations were then obtained by making dilutions of this solution with distilled water containing 0.02% Triton X-100 (Immaraju et al., 1989).

Bioassay studies

Film residue method

The method devised by Busvine (1971) was followed to test larval mortality in the film residue studies. Five different doses of malathion and deltamethrin were included in bioassay studies. Deltamethrin doses were 0.1, 0.2, 0.4, 0.8 and 1.6 ppm, while 10, 15, 20, 30 and 40 ppm doses were used for malathion. Bioassay experiments were conducted in Petri dishes (10 cm diameter). One ml solution of each dose was dropped by automatic pipette in each Petri dish. The Petri dishes were carefully shaken for the homogenous distribution of the solution. After drying at room temperature for up to 2 h, 25 larvae were released in each Petri dish. For control treatment, 0.02% Triton X-100 was used instead of insecticide. Larval mortality was recorded 72 h after treatment. The experiments were conducted in completely randomized design with four replicates of each dose of each insecticide and there was one Petri dish in each replicate.

Topical application method

Five different doses of both insecticides were used in the topical application experiment. Deltamethrin doses were 0.5, 1, 1.5, 2 and 2.5 ppm, whereas 50, 60, 70, 80 and 90 ppm doses were used for malathion. The fourth instar larvae were released in Petri dishes (25 larvae per dish) and 0.1 µl of the prepared solutions were applied to each larva by using Eppendorf micropipette following IRAC method 029 (www.irac-online.org/methods/euschistus-heros-adults-2). For control treatment, 0.1 µl of 0.02% Triton X-100 was applied to each larva instead of insecticides. The experiment was conducted in completely randomized design with four replicates. The Petri dishes were placed in an electronically controlled climate chamber at 25±1°C and 60% RH. Mortality was observed 72 h after the application of insecticides.

Statistical analyses

Analysis of variance (ANOVA) was used to test the differences among surveyed provinces and storage types using infestation percentage data. The normality in the data was tested prior to ANOVA by Shapiro-Wilk test, which indicated non-normal distribution. Therefor data was normalized by arcsine transformation method and two-way ANOVA was conducted on transformed data. Least significant difference test at 5% probability was applied where ANOVA indicated significant differences. The mortality percentage data of the bioassay studies was corrected using Abbott's formula (Abbott, 1925). The corrected mortality data was subjected to probit analysis (Busvine, 1971; Finney, 1971) and LC_{50}/LD_{50} values were calculated using the POLO Plus-PC software (LeOra, 1987). Resistance ratio was calculated by dividing the LC_{50}/LD_{50} of each population by the LC_{50} value of susceptible population. Confidence intervals were generated by POLO Plus-PC software as described by Robertson et al. (2007).

Results

Field study

Infestation level of the Khapra beetle

A total of 355 grain samples were collected from three different storage types in five provinces. Different provinces and storage types significantly influenced Khapra beetle infestation rate, whereas their interaction was non-significant (Table 1).

Table 1. Analysis pf variance of different provinces, storage types and their interactions on infestation rate of Khapra beetle in southeastern Anatolia, Turkey

Source of Variation	DF	Sum of squares	Mean squares	F Value	P Value
Province (P)	4	5531.88	1382.97	9.64	0.0001*
Storage Type (S)	2	17088.45	8544.23	59.53	0.0001*
P × S	8	1491.48	186.43	1.30	0.2968^{NS}

DF = Degree of freedom, * = significant at P ≤ 0.05, NS = non-significant.

Khapra infestation rate varied from 43.5 to 77.5% in the surveyed provinces (Table 2). Similarly, high variation was noted for storage types surveyed within different provinces. The number of metal silos in the surveyed provinces ranged from eight to 48, whereas the infestation rate varied from 15.6 to 50%. The number of concrete wall storage types varied from three to 57, whereas the infestation rate was between 44.4 and 84.6%.

Table 2. Infestation of Khapra beetle in wheat grain storage facilities in different provinces of southeastern Anatolia, Turkey

Sampled provinces	No. of facilities sampled	No. of infested facilities	Infestation (%)	No. of sampled populations
Adıyaman	34	15	44.1	2
Batman	25	13	52.0	2
Diyarbakır	145	63	43.5	9
Mardin	40	31	77.5	3
Şanlıurfa	111	75	67.6	8
Total	355	197	Average 56.92	Total 24

Similarly, the number of basic plasterless storage ranged from seven to 43 with an infestation range of 71.4-90.9% (Table 3). Overall, the highest infestation of Khapra beetle was noted for Mardin Province, followed by Şanlıurfa Province (Figure 1). Similarly, the lowest infestation of the beetle was noted for Adıyaman and Diyarbakır Provinces (Figure 1).

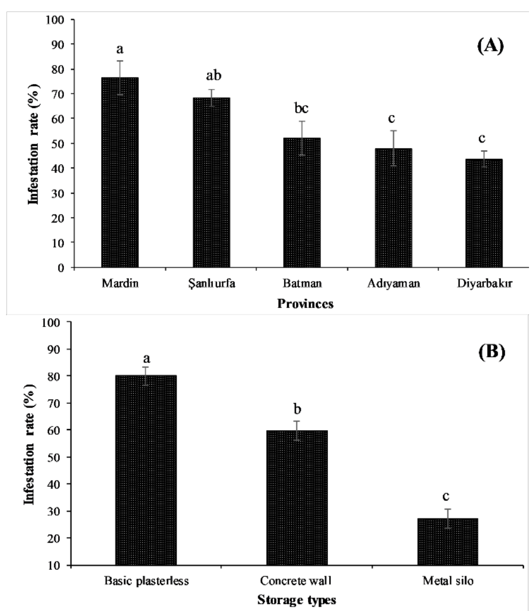

Figure 1. The influence of different provinces (A) and storage types (B) on Khapra beetle infestation in southeastern Anatolia region, Turkey.

For storage type, the highest Khapra infestation was recorded for basic plasterless storage, whereas the lowest infestation was noted for metal silos (Figure 1). The province by storage type interaction was not significant for Khapra infestation.

Table 3. Infestation level of Khapra beetle in different storage types of southeastern Anatolia, Turkey

Provinces	Collected Samples	Infested Samples	Infestation (%)
Metal Silos			
Adıyaman	10	2	20.0
Batman	8	2	25.0
Diyarbakır	45	7	15.6
Mardin	12	6	50.0
Şanlıurfa	48	19	39.6
Total	123	36	Mean 30.0
Concrete Wall			
Adıyaman	13	6	46.2
Batman	10	6	60.0
Diyarbakır	57	23	40.4
Mardin	17	15	88.2
Şanlıurfa	39	33	84.6
Total	136	83	Mean 63.9
Basic Plasterless			
Adıyaman	9	7	77.8
Batman	7	5	71.4
Diyarbakır	43	33	76.7
Mardin	11	10	90.9
Şanlıurfa	26	23	88.4
Total	96	78	Mean 81.0

Bioassay studies

Film residue method

A high variation was observed in LC_{50} values and resistance ratio of susceptible control population and the test populations for deltamethrin, whereas little variation was recorded for malathion (Table 4). The highest LC_{50} values were observed for Mardin, Şanlıurfa and Batman populations exposed to deltamethrin, while the lowest LC_{50} value was detected in susceptible populations. The resistance ratios of Mardin, Şanlıurfa and Batman populations were 10.7, 8.09 and 7.43 times, respectively (Table 4). The resistance ratio of the test populations exposed to malathion ranged from 1.47 to 1.92 times with the highest resistance ratio in Şanlıurfa population. Overall the resistance ratios of the populations exposed to deltamethrin were higher than malathion exposure (Table 4).

Table 4. LC_{50} values of different Khapra beetle populations treated with different doses of malathion and delthamethrin in film residue method

	Deltamethrin				Malathion			
Population	LC_{50}	CI 95%	Slope	RR	LC_{50}	CI 95%	Slope	RR
Susceptible	0.03	0.02-0.04	0.89±0.13	Ref.	19.45	14.1-25.7	3.44±0.31	Ref.
Batman	0.26	0.15-0.82	0.66±0.14	7.43	28.63	21.6-51.8	2.41±0.29	1.47
Diyarbakır-1	0.13	0.08-0.25	0.67±0.13	3.71	31.36	23.3-67.8	2.46±0.30	1.61
Diyarbakır-2	0.14	0.09-0.31	0.64±0.13	4.11	32.79	24.8-64.5	2.35±0.30	1.69
Diyarbakır-3	0.12	0.08-0.32	0.66±0.13	3.69	35.37	30.7-43.4	2.33±0.30	1.82
Mardin	0.37	0.20-1.54	0.64±0.14	10.7	33.74	24.9-81.8	2.44±0.30	1.73
Şanlıurfa	0.28	0.15-1.22	0.57±0.13	8.09	37.26	27.6-91.8	2.50±0.32	1.92

CI = confidence interval; RR = resistance ratio.

Topical application method

Slight variation was noted in LD_{50} values and resistance ratio of susceptible and test populations exposed to deltamethrin and malathion (Table 5). Overall the LD_{50} values and resistance ratios were higher for the populations exposed to deltamethrin than those for malathion (Table 5). The highest LD_{50} values were observed for Mardin, Şanlıurfa and Batman populations exposed to deltamethrin as noted in film application method. The resistance ratios of Mardin, Batman and Şanlıurfa populations were 4.00, 3.27 and 3.22 times, respectively (Table 5). The resistance ratio of the test populations exposed to malathion ranged from 1.18 to 1.29 times with the highest resistance ratio in Diyarbakır-3 population (Table 5).

Table 5. LD_{50} values of different Khapra beetle populations treated with different doses of malathion and delthamethrin in topical application method

	Deltamethrin				Malathion			
Populations	LD_{50}	CI 95%	Slope	RR	LD_{50}	CI 95%	Slope	RR
Susceptible	0.102	0.05-0.14	1.44±0.23	Ref.	68.78	64.3-73.4	8.14±0.73	Ref.
Batman	0.334	0.23-0.72	1.04±0.24	3.27	80.85	74.2-93.5	5.89±0.72	1.18
Diyarbakır-1	0.222	0.17-0.34	1.19±0.24	2.18	84.01	75.6-106.2	5.81±0.73	1.22
Diyarbakır-2	0.246	0.18-0.42	1.09±0.24	2.41	86.15	81.2-93.9	5.54±0.73	1.25
Diyarbakır-3	0.282	0.21-0.49	1.20±0.24	2.76	88.63	83.3-97.3	5.63±0.75	1.29
Mardin	0.408	0.27-1.01	1.13±0.25	4.00	86.33	77.8-109.2	6.00±0.76	1.26
Şanlıurfa	0.328	0.22-0.80	0.99±0.24	3.22	90.66	82.04-112.27	5.99±0.73	1.32

CI = confidence interval; RR = resistance ratio.

Discussion

The field study indicated that different provinces and storage types significantly influenced the Khapra beetle infestation in southeastern Anatolia. The most infested provinces were Mardin and Şanlıurfa, and the lowest were Diyarbakır and Batman Provinces. Although the highest number of storage facilities was surveyed in Diyarbakır Province, the beetle infestation was the lowest in that province. The interprovincial differences may arise due to management options used to control Khapra beetle, storage types and conditions, product circulation, storage sanitation and farmer awareness. A study conducted in 1982 in southeastern Anatolia revealed no significant effect of storage types on the infestation rate of

Khapra beetle (Özar & Yücel, 1982). However, the current study indicated significant effect of the provinces and storage types. These differences could be explained by the improvements in storage types over the last two decades in the region. Metal silos, which were not prevalent in 1982, have significantly increased with rising awareness of the private companies storing wheat grain.

Moreover, wheat-dependent industrial zones of Mardin and Şanlıurfa Provinces have large number of flour, bulgur and feed factories, and cereals are purchased from different provinces in southeastern Anatolia. The grain is stored to ensure the continuous availability for processing. The main storage type in the region is reinforced concrete, which is owned by most of the wheat grain traders. Metal silos are not widely used because of their high investment cost. Therefore, surveyed storage types in the study were mostly reinforced concrete. Whereas, the storage owned by wheat farmers are generally simple plasterless type with briquette. Cracks and crevices were frequently observed in reinforced concrete and basic plasterless storage during the survey.

Khapra beetle has a refuge seeking behavior (Bell & Wilson, 1995; EPPO, 2005; French & Venette, 2005; Harris, 2006; Anonymous, 2008); therefore, cracks and crevices found in reinforced concrete storage provide shelter to the beetle. Grain in factory storage is used in the manufacturing process within a short time, and the grain is continually stored without frequent use of insecticides to control Khapra beetle and other pests. For this reason, a significant increase in infestation level and distribution of various pests could easily occur in these storage situations. The highest infestation level was noted for basic plasterless storage, which was followed by reinforced concrete storage. The main reason of high infestation rate in the region is unsatisfactory control of Khapra beetle due to unsuitable storage conditions and incorrect application of insecticides (unpublished field observations).

The cracks and crevices formed in the walls made of bricks, mud, concrete or stone provide highly suitable shelter for Khapra beetle, making control of the beetle almost impossible. Furthermore, the leftover grain (when the storage facilities are emptied) remains permanently in the cracks and crevices of these facilities, providing a long-term food source for the beetle (EPPO, 2005; French & Venette, 2005; Harris, 2006; Anonymous, 2008). Since Khapra beetle individuals settled in the crevices and cracks do not come in contact with the insecticides applied, sustainable control and eradication becomes impractical and expensive (Lindgren & Vincent, 1959; Ahmad, 1994; Harris, 2006; Saidana et al., 2010; Singh et al., 2017). Moreover, ensuring sufficient gas tightness in reinforced concrete and basic plasterless storage facilities is difficult; the desired level of fumigation success cannot be achieved in such storage types. However, the beetle infestation was the lowest in the metal siloes. The gas tightness, adjustable temperature and relative humidity, controlled environmental conditions (Fidan & Satuk, 2011; Pekmez, 2016), relatively less crevices and cracks, easy cleaning and subsequent high sanitation, high awareness and trained workers in these storage types are the reasons linked with the low infestation of Khapra beetle. Low temperature forces the pest to enter diapause and metal silos are usually equipped with aeration system that reduces the temperature of the grain bulk. Therefore, lower temperature of the metal silos is another reason of reduced infestation of the pest. Under the subtropical climate of Israel Khapra beetle was suppressed in the grain bulks after the introduction of aeration system (Navarro et al., 1969).

The management of stored-grain pests is as important field as grain production (Ahmad, 1994). The insecticides are generally applied in March-April in empty storage facilities for controlling Khapra beetle in the southeastern Anatolia. The insecticide application time coincides the inactive period of the beetle in concrete wall and basic plasterless storage types; thus, leading to poor control. Whereas, the metal silos heat up earlier than the other storage types and insecticide application timing coincides with the active period of the beetle, giving better control. The reduced infestation in metal silos compared to other storage types could also be explained by better control of the beetle compared to the other storage types. Khapra larvae enter diapause when temperatures fall below 25°C or when populations are very dense (Anonymous, 1978). Therefore, insecticides should be applied in the storage facility when temperature is >25°C for effective control of Khapra beetle.

Complete mortality was recorded in all populations in preliminary tests to select the populations for bioassay studies. These results indicated that the selected populations have not become resistance to deltamethrin and malathion insecticides. Similar to the results of current study, Dörtbudak et al., (1987) also reported that Khapra beetle has not developed resistance to malathion. However, several researchers have indicated that excessive use of deltamethrin and malathion led to resistance development in Khapra beetle and other stored-grain pests (Dyte & Blackman, 1970; Champ & Dyte, 1976; Irshad & Iqbal, 1994; Tarakanov et al., 1994; Saxena & Sinha, 1995; Kumar et al., 2010; Hafiz et al., 2017, 2018). The absence of insecticide resistance in our studies could be explained by the lower use of these pesticides compared to other countries where resistance has been confirmed. Unfortunately, no data on the use of deltamethrin and malathion insecticides is available for southeastern Anatolia to strengthen our argument. Therefore, a detailed survey study is needed to obtain the data on insecticide use and link it with the absence of resistance in the region.

Although preliminary studies conducted with recommended dose indicated absence of resistance, using double the LC_{50}/LD_{50} of susceptible population suggested some resistance in six populations. The bioassay studies indicated that the highest resistance ratio for the populations exposed to deltamethrin was 10.6 for the Mardin population (Table 4), while the highest resistance ratio for the populations exposed to malathion was recorded for Şanlıurfa (Table 4). Testing resistance with two different methods yielded almost similar results for resistance ratios and LC_{50}/LD_{50} values (Tables 4 & 5). Nonetheless, deltamethrin is preferred over malathion due to its weaker smell in empty storage facilities and easier availability in the market (unpublished field observation). Therefore, deltamethrin is more frequently used than malathion, which resulted in higher resistance ratio for deltamethrin compared to malathion. The results of Singh & Yadav (1994) support our finding that Khapra beetle was more resistant to deltamethrin than malathion. Overall, the results of the current study indicate that the beetle has not developed resistance to the frequently used pesticide; however, slight resistance was observed compared with the susceptible populations. These results suggest that intensive use without rotating the insecticides with different mode of action/active ingredients could lead to the development insecticide resistance in future. Therefore, the use of these insecticides should be carefully monitored and farmers should be warned of the possible negative outcomes of over using these insecticides.

The successful management of stored-grain pests requires sound knowledge of their distribution, infestation ratio, biology of pests and storage conditions (such as, sanitation, temperature, relative humidity, prevention of new infestations, and avoiding use of old and new products in the same storage). The current study has provided useful information on the infestation and storage conditions, which provide valuable insights for effective control of Khapra beetle in the region. In conclusion, use of metal silos should be encouraged in the region and use of insecticides and other management options should be improved based on the biology of insect, product circulation and environmental conditions.

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