

Original article (Orijinal araştırma)

Determination of malathion resistance in *Sitophilus oryzae* L., 1763 and *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae) populations in Türkiye¹

Türkiye'deki *Sitophilus oryzae* L., 1763 ve *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae) popülasyonlarında malathion direncinin belirlenmesi

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Abstract

This study was conducted to determine the malathion resistance levels of two important stored grain pest beetles, *Sitophilus oryzae* L., 1763 and *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae), collected from different provinces of Türkiye in the years 2017-2018. To control these pests, chemical insecticides have been widely used for a long time as a grain protectant in farmer warehouses, flour mills, or silos in many countries, including Türkiye. In the current study, insects were exposed to malathion for 24 hours to determine resistance rates. The highest resistance ratio to malathion in *S. oryzae* was found in the Adana-Kartepe population with 5.73-fold, and the lowest resistance was found in the İstanbul-Büyükçekmece population with 1.57-fold. While the Konya-Alibeyhöyüğü population of *S. granarius* had the highest resistance ratio of 6-fold, the lowest resistance rate of 2.54-fold was found in the population obtained from the same location but from a different warehouse. According to this study, we found that slight resistance developed in the populations of *S. oryzae* and *S. granarius* in Türkiye. In order to prevent the occurrence of resistance due to synthetic insecticides used against stored product pests, it is thought that various insecticide groups with different mechanisms of action should be used.

Keywords: Malathion, resistance, *Sitophilus* spp., Türkiye, warehouse

Öz

Bu çalışma, 2017-2018 yılları arasında Türkiye'nin farklı illerinden toplanan iki önemli depolanmış ürün zararlısı *Sitophilus oryzae* L., 1763 ve *Sitophilus granarius* L., 1758 (Coleoptera: Curculionidae)'un malathion direnç düzeylerini belirlemek amacıyla yapılmıştır. Bu zararlıları kontrol altına almak için insektisitler, Türkiye dahil birçok ülkede çiftçi depolarında, un değirmenlerinde veya silolarda tahıl koruyucu olarak uzun zamandan beri yaygın olarak kullanılmaktadır. Direnç oranlarını belirlemek için böcekler 24 saat boyunca malathion'a maruz bırakılmıştır. Malathion'a karşı en yüksek direnç oranı *Sitophilus oryzae*'de 5.73 kat ile Adana-Çukurtepe (K4) popülasyonunda, en düşük direnç ise 1.57 kat ile İstanbul-Büyükçekmece (R1) popülasyonunda bulunmuştur. *Sitophilus granarius*'un Konya-Alibeyhöyüğü (E3) popülasyonunda en yüksek direnç oranı malathion'a karşı 6 kat iken, en düşük direnç oranı 2.54 kat ile *S. granarius*'un aynı lokasyonun farklı bir deposundan alınan popülasyonunda (E4) bulunmuştur. Bu çalışmaya göre Türkiye'deki *S. oryzae* ve *S. granarius* popülasyonlarında hafif bir direnç geliştiği tespit edilmiştir. Depolanmış ürün zararlısı böceklerle karşı kullanılan sentetik insektisitler nedeniyle oluşabilecek direnç vakalarının önlenmesi amacıyla, farklı etki mekanizmalarına sahip çeşitli insektisit gruplarının kullanılması gerektiği düşünülmektedir.

Anahtar sözcükler: Malathion, direnç, *Sitophilus* spp., Türkiye, depo

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Introduction

Wheat, *Triticum aestivum* L. (Poales: Poaceae) ranks first in Türkiye in terms of cultivation area and production, and is considered a strategic product in human nutrition (Aydın, 2022). Notably, in the last two decades, wheat cultivation areas have changed between 6.8-9.4 million hectares and production has changed between 17.2-22.6 million tons in Türkiye (TMO, 2020; Aydın, 2022). Wheat is also the raw material of food products such as bread, bulgur, semolina, and biscuits. It holds strategic importance due to high production amounts, its prolonged storage capacity, and its significance in special situations such as natural disasters, and war.

The granary weevil, *Sitophilus granarius* L., 1758 and the rice weevil, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae), are important primary pest insects of stored grain that cause economic losses by feeding internally and externally on stored grains such as wheat, rice, corn, barley, and etc. These insects have been detected in almost all provinces in Türkiye (Özer, 1957; Coşkuncu, 2004; Işıkber et al., 2005, 2016; Bağcı et al., 2014; Atabay et al., 2018; Koçak et al., 2018; Özder & Toğantimur, 2019; Zengin & Karaca, 2019; Ertürk et al., 2020; Yetkin & Atakan, 2022). Besides, Atabay et al. (2013) reported that *Sitophilus* spp. had the highest incidence rate of 70.4% in stored paddy, rice, and bran in Balıkesir province. It has been reported that in intense Liposcelididae contamination, wheat loses its germination power and insect excrement and wastes cause allergic reactions (Obr, 1978; Kucerova, 2002). *Sitophilus granarius* had the highest density in the wheat warehouses of Kütahya province with a rate of 52.6%, followed by *O. surinamensis* with a rate of 40.2% (Zengin & Karaca, 2020). In addition, it has been reported that these two species are widespread throughout the world (Mason & McDonough, 2012; Correa et al., 2013; Keszthelyi et al., 2021; Nietupski et al., 2021). Grain damaged by these insects decreases in nutritional and market value, as well as in germination percentage and weight. In heavy infestation, molding, heating, odor, and decay occur in the product (Erakay, 1974; Arthur, 1996; Magan et al., 2003; Yiğit et al., 2022). Moreover, high humidity of the grain and poor storage conditions allow insects to enter stored wheat. It has also been reported that if no measures are taken against these pests, the damage caused by insects can reach up to 50% of the harvested crop. Besides, this causes a loss of more than 100 billion USD annually on a global scale (Boxall, 2002; Mebarkia et al., 2010; Asrar et al., 2016).

To minimize the economic loss originating from stored product pests, synthetic insecticides have been used for decades. Malathion, an organophosphate (OP) insecticide, is a widely used insecticide in post-harvest grains in many countries to protect stored products from insect pests (Boyer et al., 2012). Organophosphates are responsible for the inhibition of acetylcholinesterase in the insect nervous system. It causes accumulation of acetylcholine in the synaptic gap due to the inhibited enzyme, impaired neurotransmission, and ultimately death occurs (Attia et al., 2020). In addition, malathion, which was first registered in Türkiye in 1964, is still used today as a residual insecticide against insect pests (Oden & Sahin, 1964; Alagöz & Sağlam, 2022). Factors such as long-term and repeated applications of insecticides, intensive and high-dose applications, and changes in insect populations increase the frequency of insecticide-resistant individuals in insect populations and lead to failure in pesticide applications (Ferizli & Berisli, 2005; Baliota et al., 2022; Hoobdel et al., 2022). In addition, it was determined that stored insect pests showed differences in terms of sensitivity to malathion. (Navarro et al., 1986; Guedes et al., 1996; Mendoza, 1999; Yesir & Koçak, 2017; Baliota et al., 2022). Furthermore, failures in application due to insecticide resistance can lead to control failures, and cause economic losses of several billion dollars worldwide each year (Dennehy, 1987; Elzen & Hardee, 2003). The rapid spread of stored product insect populations through national and international trade, evaluation of used insecticides for resistance, and suggestions for corrective actions are the basic needs. Although long-term storage of grain in local warehouses and silos is common practice in Türkiye, data on the occurrence of resistant populations are scarce. Therefore, the aim of this study was to determine and quantify the resistance status of different field populations of *S. granarius* and *S. oryzae* against malathion, which is used as registered in Türkiye.

Materials and Methods

Field survey, insect populations and rearing condition

For this purpose, adults of *S. oryzae* and *S. granarius* were collected from granaries and mills in Adana, Ankara, Burdur, Isparta, İstanbul, Konya, and Manisa provinces between 2017 and 2018. Besides that, approximately four kg samples were taken from five different points and different depths of the wheat mass in each warehouse using a two-meter probe and the collected samples were brought to the laboratory. Samples were transferred to one-liter glass jars and the jar lids were covered with a fine mesh cloth to allow ventilation. To obtain adults of *S. oryzae* and *S. granarius* individuals, wheat samples were kept in climate cabinets (Nüve ID 501, Türkiye) for 60 days at 25°C temperature and 65±5% relative humidity condition (Işıkber, 2005; Ertürk, 2021). Grain samples were sieved (mesh size 0.85 mm) for isolation and insect species were identified (Freeman, 1980). The insects reared on sterilized whole wheat under the same conditions. The progeny of the insects (F1) was used for bioassays.

Insecticide bioassay

Malathion was selected among widely used insecticides used for chemical control of insect pests in storage facilities. For the determination of median lethal concentration (LC₅₀), a series of at least five different concentrations (3-250 ml/L) of malathion (650 g/L, EC) were prepared with distilled water for *S. granarius* and *S. oryzae*. Glass Petri dishes with a diameter of 9 cm were used for toxicity tests. Filter papers (Whatman No.1) were placed into Petri dishes and sprayed (Spray Tower, Burkard Scientific-BS00281, England) with 2.0 ml of insecticide solutions. Petri dishes sprayed with malathion were kept under a hood to dry for one hour before being used for testing. In the control group, only distilled water was used. Afterward, twenty-five, 1-3-week-old unsexed adult individuals were left in each Petri dish and exposed to the insecticide for 24 hours. To prevent insect escape from the Petri dishes, they were covered with parafilm. After 24 hours, dead and live insects were counted and recorded. Experiments were set up with 3 replications. The test method used in the study was according to IRAC 006 (IRAC, 2009), but no plastic ring was used, and this method was revised and applied in this way.

Statistical analyses

The trial results were initially converted into mortality percentages, followed by analysis using the Polo-PC probit package program (LeOra, 1994). LC₅₀ values and 95% confidence intervals were then calculated at a significance level of 5% (Robertson et al., 2003). Resistance ratios (RRs) between populations for all assays were calculated for malathion by dividing LC₅₀ values for resistant strains by the LC₅₀ value of the most susceptible strain as there was no susceptible strain to assess the resistance factor for these insects (Perez-Mendoza, 1999; Yalçın et al., 2015; Lv et al., 2021).

Results

Sitophilus oryzae and *S. granarius* species samples collected from warehouses in Adana, Ankara, Burdur, Isparta, İstanbul, Konya and Manisa, and the species obtained from wheat samples are presented in Table 1. Different concentrations of malathion were applied to *S. oryzae* populations and their resistance levels are displayed in Table 2.

Within the populations of *S. oryzae*, the LC₅₀ value of the Konya/Alibeyhöyük (E5) population was calculated as 8.26 ppm and was considered as the most sensitive population to malathion. The LC₅₀ values of other populations were calculated as İstanbul/Küçükçekmece (R1) 13.05 ppm, Ankara/Haymana (B2) 19.97 ppm, Manisa/Saruhanlılar (A2) 26.34 ppm, and Adana/Çukurtepe (K4) 47.33 ppm, respectively. When the toxic effects of different concentrations of the malathion were evaluated according to the E5 population, the R1 population were found 1.57-fold more resistant, while the resistance levels were reported as 2.41-fold for the B2 population, 3.18-fold for the A2 population, and 5.73-fold for the K4 population. Different doses of malathion were applied to *S. granarius* populations collected from the study areas and the resistance levels found are presented in Table 3.

Table 1. Provinces, districts and facilities where *Sitophilus oryzae* and *Sitophilus granarius* individuals were collected

Code	Species	Collection of places	Facilities	Latitude	Longitude
E5	<i>S. oryzae</i>	Konya/ Alibeyhöyüğü	Farmer Warehouse	N37°31'52.234"	E 32°39' 44.2"
A2	<i>S. oryzae</i>	Manisa/ Saruhanlılar	Farmer Warehouse	N38°40' 18.781"	E27°37' 24.143"
B2	<i>S. oryzae</i>	Ankara/ Haymana	Ankara University Faculty of Agriculture Farm	N39°37'1.031"	E32°41'30.703"
K4	<i>S. oryzae</i>	Adana/ Çukurtepe	Turkish Grain Board	N37°26'42.616"	E 35°49'7.028"
R1	<i>S. oryzae</i>	İstanbul/ Büyükçekmece	Eriş Flour Mill	N41°4'13.427"	E 28°19'9.085"
H1	<i>S. granarius</i>	Burdur/ Merkez	Farmer Warehouse	N37°43'32.586"	E30°17'26.387"
D2	<i>S. granarius</i>	Elazığ	Hasbek Flour Mill	N38°40'29.338"	E39°13'21.054"
E3	<i>S. granarius</i>	Konya/ Alibeyhöyüğü	Farmer Warehouse	N37°31'47.496"	E32°39'37.191"
E4	<i>S. granarius</i>	Konya/ Alibeyhöyüğü	Farmer Warehouse	N37°31'57.148"	E32°39'42.659"
G2	<i>S. granarius</i>	Isparta/ Merkez	Farmer Warehouse	N37°45'50.941"	E30°34'23.914"
H3	<i>S. granarius</i>	Burdur/ Merkez	Farmer Warehouse	N37°43'31.305"	E30°17'26.022"

Table 2. Lethal concentrations of malathion applied to *Sitophilus oryzae* populations

Population Codes	n	Slope±SE	χ^2	LC ₅₀ (ppm) (95% CL)	RR ₅₀	LC ₉₀ (ppm) (95% CL)	H	p values	Duration of exposure (hour)
E5	450	1.49±0.20	8.65	8.26 (5.88±10.46)	1.00	56.02 (41.96-86.81)	0.72	<0.05	
A2	450	3.57±0.32	23.99	26.34 (22.11±31.49)	3.18	58.83 (47.26-81.86)	1.99	<0.05	
B2	360	4.38±0.43	11.19	19.97 (17.41±22.62)	2.41	39.07 (33.59-48.42)	1.24	<0.05	24
K4	450	2.39±0.22	3.56	47.33 (41.17±54.67)	5.73	161.15 (129.90-214.54)	0.28	<0.05	
R1	450	3.05±0.29	15.38	13.05 (11.20±15.21)	1.57	34.39 (27.47-47.92)	1.28	<0.05	

n= The number of individuals used; CL, Confidence limit; RR₅₀, Resistance Ratio; H, Heterogeneity; SE, Standard Error.

Table 3. Lethal concentrations of malathion applied to *Sitophilus granarius* populations

Population Codes	n	Slope±SE	χ^2	LC ₅₀ (ppm) (95% CL)	RR ₅₀	LC ₉₀ (ppm) (95% CL)	H	p values	Duration of exposure (hour)
H1	450	1.94±0.18	13.02	7.17 (5.84±8.65)	1.00	30.74 (23.44-44.99)	1.09	<0.05	
D2	540	2.36±0.18	26.33	41.79 (33.70±50.77)	5.82	141.70 (111.01-197.72)	1.76	<0.05	
E3	360	3.10±0.27	20.63	43.07 (33.67±54.94)	6.00	113.58 (86.63-169.73)	2.29	<0.05	24
E4	540	1.71±0.14	20.49	18.28 (14.77±22.87)	2.54	92.18 (65.31-149.87)	1.37	<0.05	
G2	450	3.53±0.27	6.11	39.32 (35.40±43.64)	5.48	88.97 (77.55-105.54)	0.51	<0.05	
H3	540	3.06±0.25	16.96	32.60 (28.77±36.61)	4.54	85.63 (72.46-107.12)	1.13	<0.05	

n= The number of individuals used; CL, Confidence limit; RR₅₀, Resistance Ratio; H, Heterogeneity; SE, Standard Error.

Among the *S. granarius* populations, the LC₅₀ value of the Burdur/Merkez (H1) population was calculated as 7.17 ppm and was considered as the most sensitive population to malathion. The LC₅₀ values of the other populations were calculated as Konya/Alibeyhöyük (E4) 18.28 ppm, Burdur/Center (H3) 32.60 ppm, Isparta/Center (G2) 39.32 ppm, Elazığ (D2) 41.79 ppm and Konya/Alibeyhöyük (E3) 43.03 ppm. It was calculated that the E4 population was 2.54-fold, the H3 population was 4.54-fold, G2 population was 5.48-fold, the D2 population was 5.82-fold and the E3 population was 6-fold more resistant than the H1 population.

Discussion

In this study, we collected grain samples from 11 different localities and warehouses in 2017 and 2018 years. Hill (2002) and Esin (1971) were used to identify the insect species collected from these warehouses and these species were identified as *S. oryzae* and *S. granarius*. The rice weevil and the granary weevil are among the primary pests in wheat warehouses in our country. The genus of *Sitophilus*, which has a cosmopolitan distribution, is common in almost all provinces of Türkiye (Obr, 1978; Kucerova, 2002; Atabay et al., 2013; Zengin & Karaca, 2020). In addition, the distribution of this genus is considered to be related to the domestic grain trade. The fact that these insects are so widespread in the country, as well as the use of insecticides to protect against insect damage in grain trading areas, has raised suspicions about the presence of resistance.

Synthetic insecticides applications are mostly preferred by warehouse enterprises for the control of stored product pests (Yesir & Koçak, 2017). It is also known that the excessive and frequent use of chemical insecticides causes insecticide residues, the death of non-target species and the development of resistance in insecticide applied populations (Isman, 2006). The level of resistance of the populations can be classified into three groups; as high resistance ($RR > 10$), moderate resistance ($5 < RR < 10$), and low resistance ($3 < RR < 5$) (Mazzarri & Georghiou, 1995). In this study, the H1 population of *S. granarius* was found to be the most sensitive population, with an LC_{50} value of 7.17 ppm. Considering the resistance rates among these populations, the RR was highest in the E3 population with 6-fold increment. According to the results of this study, moderate malathion resistance was determined in *S. granarius* D2, G2, and E3 populations. Moreover, low levels of resistance were recorded in E4 and H3 populations. Furthermore, the slope values determined from probit analysis can provide clues about the homogeneity or heterogeneity of the target insect populations of insecticides in terms of resistance. High slope values (>2) indicate that the population is relatively homogeneous, while low slope values (<1) indicate that the population is relatively heterogeneous (Georghiou & Metcalf, 1961; Yu, 2008; Rodrigues et al., 2020). It is noteworthy that the B2 population of *S. oryzae* has the highest slope values suggesting that the B2 populations were homogenous in their response to the malathion. In contrast, the E5 population has the lowest LC_{50} value and can be considered a heterogeneous population. Similarly, the E4 population of *S. granarius* has the lowest slope value, and this population can be considered heterogeneous. On the other hand, the E3 population of granary weevil has the highest slope value and can be considered homogeneous.

Similar to our findings, Kljajić & Perić (2007) reported that the resistance rates obtained for *S. granarius* in the Belgrade Harbor population after 24 hours of malathion exposure were 4.3-fold higher than in the susceptible population. It was also reported that the Apatin population developed a 3.2-fold resistance to malathion in granary weevil populations from different regions of Yugoslavia (Kljajić & Perić, 2006). The lowest LC_{50} value of *S. oryzae* against malathion was obtained from the population of E5. While the R1 population had the lowest resistance rate with 1.57-fold, the K4 population had the highest resistance rate with 5.73-fold. In parallel with this study, Yesir & Koçak (2017) reported that the resistance of *S. oryzae* populations collected from Konya, Manisa, İzmir, and Samsun provinces varied between 1.8 and 8.4-fold in individuals exposed to malathion for 24 hours. Furthermore, Attia et al. (2020) found that the Egyptian populations of the rice weevil *S. oryzae* were 199.6 times resistant to 24 hours of malathion exposure. Karaağaç & Konuş (2015) determined the mortality rates at the dose of 1.5 mg malathion/disc of *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) populations collected from Kırıkkale and Samsun as 72.2 and 92.2%, respectively. They reported that cytochrome P450 monooxygenase and glutathione S-Transferase enzymes may have a role in the resistance against malathion. In another study, while the Ankara population of *S. zeamais* showed resistance to malathion, no resistance was detected in the Samsun population. They evaluated that cytochrome P450 monooxygenases play an active role in malathion resistance in the Ankara population of *S. zeamais* (Konuş, 2015). Karaağaç (2015) found that there was no resistance against malathion except for Ankara and Karaman strains of *S. zeamais*. Mutlu et al. (2019), revealed that because of deltamethrin is more frequently used than malathion, the Khapra beetle *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) has higher resistance ratio for deltamethrin (4-10.7 RR) compared to malathion (1.32-1.92).

The first studies conducted in Türkiye on malathion resistance showed that *Sitophilus* spp., *Tribolium confusum* Jaquelin Du Val, 1868 and *Tribolium castaneum* Herbst, 1797 (Coleoptera: Tenebrionidae) did not develop resistance to malathion (Kalkan et al., 1977; Keyder et al., 1979; Dörtbudak et al., 1987). Similarly, Ribeiro et al. (2003) reported that no resistance cases were observed in *S. zeamais* populations against organophosphate insecticides such as malathion and pirimiphos-methyl, which were widely and frequently used in Brazil in the 1970s and 1980s. However, they reported that malathion was banned due to its use in powder formulation and control failures in *S. zeamais* populations (Santos et al., 1986; Guedes et al., 1994, 1995, 1996). Malathion is actively used in the control of stored product pests in Türkiye, and its use has not been banned yet. However, when the data obtained are evaluated, it is concluded that low and moderate resistance begins to occur in the sampled populations. In addition to these, the lack of knowledge of the insecticide applications previously used in the sample warehouses may be effective in this variation in resistance rates.

As a result, slowing down or preventing the development of resistance in pests or maintaining the effect of pesticides on harmful organisms are very important in terms of managing resistance. Initial toxicity studies of insecticides, determining the presence and level of resistance, comparing them with future resistance studies, and establishing appropriate control methods and resistance distribution maps for the management of pests will contribute to insect resistance management studies.

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