Türk. entomol. derg., 2023, 47 (4): 387-399
DOI: http: //dx.doi.org/10.16970/entoted.1344982

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Insecticide resistance of *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) in cotton fields in Çukurova Region (Türkiye)[1]

Çukurova Bölgesi (Türkiye) pamuk alanlarında *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)'nin insektisit direncinin belirlenmesi

Selçuk ULUSOY[2*] **Doğancan KAHYA[3]**

Mustafa Gökhan BİLGİN[2] **Okan ÖZGÜR[4]**

Abstract

This study aimed to reveal resistance levels of *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) populations against dimethoate, λ-cyhalothrin and chlorpyrifos-ethyl used in cotton fields in Adana province in Çukurova Region (Türkiye). Bioassay, biochemical and molecular methods were used to determine resistance on the populations collected from 16 locations between 2020 and 2021. Six populations were resistant according to the susceptible Toktamış population with leaf dip discriminating dose bioassays. Compared to the susceptible population, four populations were found at decreased susceptibility (DS) resistance levels to dimethoate and one population to chlorpyrifos-ethyl. Only two populations resistance ratio were detected in MR (Moderate resistance) category to chlorpyrifos-ethyl. Resistance levels of other populations were observed as S (susceptible) category. Resistant populations had higher acetylcholinesterase, glutathione-S transferase and cytochrome P450 monooxygenase enzyme activities in biochemical analysis. The carboxylesterase gene transcription levels were higher in resistant populations. S431F and Kdr (knockdown) mutation were determined by the PCR-RLFP method, which is effective in organophosphate and pyrethroid insecticides resistance and 17% and 100% recessive alleles were detected in populations. The biochemical and mutation-induced resistance to dimethoate and chlorpyrifos-ethyl was detected. These results will contribute to developing strategies for resistance management of *A. gossypii.*

Keywords: *Aphis gossypii*, insecticide, resistance, Türkiye, organophosphate, pyrethroid

Öz

Bu çalışma ile, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) popülasyonlarının Çukurova bölgesinde (Türkiye) Adana ilinde pamuk alanlarında kullanılan dimethoate, λ-cyhalothrin ve chlorpyrifos-ethyl'e karşı direnç seviyelerinin ortaya konması amaçlanmıştır. 2020 ile 2021 yılları arasında 16 koordinattan elde edilen popülasyonlar üzerinde bioassay, biyokimyasal ve moleküler yöntemler uygulanmıştır. Yaprak daldırma metodu ile ayırıcı doz çalışmaları yapılarak 16 popülasyon incelenmiş ve Toktamış popülasyonu hassas popülasyon olarak belirlenmiştir. Hassas popülasyona göre, dört popülasyonun dimethoate'a ve bir popülasyon chlorpyrifos-ethyl'e karşı direnci düşük seviye (DD) kategorisindedir. Yalnızca iki popülasyonun chlorpyrifos-ethyl'e karşı direnci orta seviye (OD) direnç kategorisindedir. Diğer popülasyonların direnç oranlarının hassas (D) seviye kategorisinde kaldığı tespit edilmiştir. Dirençli popülasyonların, biyokimyasal analizlerde daha yüksek asetilkolinesteraz, glutatyon-S transferaz ve sitokrom P450 monooksigenaz enzim aktivitelerine sahip oldukları gözlenmiştir. Karboksilesteraz gen ifadesi seviyeleri dirençli popülasyonlarda daha yüksektir. S431F ve Kdr (knockdown) mutasyonları, organofosforlu ve piretroit insektisitlere karşı etkili olan PCR-RLFP yöntemi ile belirlenmiş olup, popülasyonlarda sırasıyla %17 ve %100 oranında baskın alellere rastlanmıştır. Bu çalışmada dimethoate ve chlorpyrifos-ethyl'e karşı biyokimyasal ve mutasyon kaynaklı direnç olduğu düşünülmektedir. Bu sonuçlar, bölgede *A. gossypii*'ye karşı direnç yönetimi stratejilerinin geliştirilmesinde katkı sağlayacaktır.

Anahtar sözcükler: *Aphis gossypii*, direnç, insektisit, Türkiye, organikfosfatlılar, piretroit

[1] This study was supported by Republic of Türkiye Ministry of Agriculture & Forestry, Project No: TAGEM/BSAD/B/20/A2/P2/1537. Part of this work was presented at the 3rd International Congress of Agriculture, Environment and Health, 12-14 November 2020, Aydın, Türkiye.
[2] Biological Control Research Institute, 01321, Yüreğir, Adana, Türkiye
[3] Eskisehir Osmangazi University Agriculture Faculty, Plant Protection Department, 26040, Eskisehir, Türkiye
[4] Bayer Turkish Chemical Industry Trade Limited Company, 01200, Adana, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: selcukby@gmail.com
Received (Alınış): 17.08.2023 Accepted (Kabul ediliş): 30.10.2023 Published Online (Çevrimiçi Yayın Tarihi): 31.10.2023

Introduction

Türkiye is one of the vital cotton-growing countries with high yield, considering its ecological characteristics, soil fertility and irrigation capacity (IRAC, 2023). *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is a cotton pest and known as polyphagous species with a broad-spectrum host plant (Cheng et al., 2023). Chemical pesticides, including the commonly used organophosphorus, pyrethroid and carbamates, have been used intensely against various pests, and the resistance problem has been revealed in recent years (Alpkent et al., 2020; Erdoğan et al., 2023). It has been reported that *A. gossypii* developed resistance to more than 50 active ingredients in Türkiye and around the World (Anonymous, 2023). Aphids have rapid reproduction capability, and it is called parthenogenetic telescopic generation (Moran, 1992). This phenomenon caused millions of aphids from one aphid in one season (Moran, 1992; Kersting et al., 1999). This reproduction system has an important effect on resistance development; therefore, rapidly growing generations are exposed to more insecticides and develop resistance fast (Roush & McKenzie, 1987). Carbamate, organophosphate (OP's), organochlorine, and pyrethroid are the most used insecticide groups worldwide. Carbamate and OP's groups are known to inhibit acetylcholinesterase (AChE's) enzymes. These groups impact the nervous system and cause the organism's death (Yorulmaz & Ay, 2010). Pyrethroids affect target site proteins and cause negative activities on sodium ion channels (voltage-gated sodium channel, VGSC) which are active for signal transmission in the nervous system. This channel provides the displacement of sodium ions in the nerve cell membrane, causing action potential and starting neural transmission. However, pyrethroids inhibit channel activation and cause continuous discharge of nerve cells. This situation results with the paralysation and death of the organism (Amad et al., 2003; Marshall et al., 2012). Although new insecticides have been developed for pest control, OP's and pyrethroid have been used widely and intensively. The intensive and uncontrolled use of insecticides against aphids has been causing failure, yield loss, and high resistance development (Pan et al., 2009, 2010). Although chlorpyrifos is banned in cotton-growing areas in Türkiye, dimethoate among the OP's and synthetic pyrethroids (SP's) have been used for 40 years against aphids by farmers (Wang et al., 2021). In resistance management, determining the level of resistance against different insecticides over time is important for updating the integrated pest management (IPM) strategy. The introduction of new active ingredients into the market in the last 30 years, especially the intensive use of neonicotinoid group insecticides, has led to a decrease in the use of old actives. The level of resistance allele and susceptibility of the harmful organism in agricultural areas is an important fact that must be followed in order to guide resistance management strategy. For this purpose, in Çukurova region, determining the resistance status against OP's and SP's insecticides, which have been used extensively, will make positive contributions to insecticide resistance management (IRM) and IPM. According to the results of the previous studies, aphids develop resistance against OP's and SP's rapidly, and this resistance occurs owing to various mechanisms (O'Brien & Graves, 1992; Shang et al., 2012). Depending on increasing metabolic activity, carboxylesterases (CarE's), glutathione S-transferases (GST's) and cytochrome P450 enzymes may cause insecticide resistance in *A. gossypii* (Pan et al., 2009; Carletto et al., 2010; Shang et al., 2012). In addition, it has been reported that point mutations carrying AChE's may be the reason for resistance (Li & Han, 2004; Pan et al., 2010). Generally, CarE's combines with OP's and hydrolyzes the insecticide esters into non-toxic products. Thus, preventing OP's from reaching the target zone AChE's (Pan et al., 2009). It has been reported that higher CarE's levels (E4 and FE4) owing to gene duplications and amplifications cause increasing detoxification of OP's, carbamates, and SP's on *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) (Field & Devonshire, 1998; Field, 2000). Similarly, it was reported that the increase in gene amplification or transcription level in CarE's played a role in OP's resistance in *A. gossypii* (Devonshire & Moores 1982; Cao et al. 2008a; Pan et al., 2009, 2010; Lokeshwari et al., 2016). In addition, the structural changes (mutations) in the CarE's gene have also been reported to be associated with OP's resistance (Sun et al., 2005; Pan et al., 2009). GST's provide the excretion of metabolism, detoxification, and many pesticide and plant toxins by catalyzing the conjugation of a diverse array of electrophilic compounds with glutathione,

unlike CarE's (Liu et al., 2006). The occurring products are less toxic, more soluble in water, and excreted quickly from cells compared to non-GSH conjugated substrates. GST's and CarE's play essential roles in the OP's resistance to various insecticides (Abel et al., 2004). Monooxygenases, which are catalysed by cytochrome P450, are multifunctional and the most effective enzyme system for the detoxification of insecticides (Pan et al., 2009). Due to the broad substrate spectrum, it can affect insecticide classes and cause resistance (Scott, 1999). There was some report about resistance connected with monooxygenase activity for *Culex pipiens* L., 1758 (Diptera: Culicidae) (Shen et al., 2003), *Plutella xylostella* (L. 1767) (Lepidoptera: Plutellidae) (Bautista et al., 2009), *Bemisia tabaci* (Gennadius, 1889) (Homoptera: Aleyrodidae) (Karunker et al., 2008) and *M. persicae* (Puinean et al. 2010). The resistance against SP's and OP's associated with increasing monooxygenase activity in *A. gossypii*, has been reported (Shang et al., 2012). Another insecticide resistance mechanism for *A. gossypii* is target site mutation that occurs at the S431F (known as ACE1 resistance) position on acetylcholinesterase. It causes specific and cross-resistance in Carbamates (pirimicarb), OP's (omethoate and dimethoate) (Andrews et al., 2004; Toda et al., 2004). S431F mutation on the ACE1 gene causes resistance by using OP's insecticide against *A. gossypii* in Australian Cotton vegetation areas (Herron & Rophail, 2001; Herron et al., 2003; McLoon & Herron, 2009). Similarly, Knockdown Resistance (Kdr) was first revealed against houseflies (*Musca domestica*) in 1951 as a resistance mechanism based on target site insensitivity to pyrethroids (Busvine, 1951). Two point mutations (Leu1014 to Phe and Met918 to Thr) on the Voltage-Gated Sodium Channel (VGSC) gene reduce the binding of the insecticide to its target site, resulting in the emergence of the Kdr phenotype in some insect species (Williamson et al., 1996; Lee et al., 1999). Marshall et al. (2012) reported the occurrence of Kdr mutations with PCR-RFLP methods in *A. gossypii*. *Aphis gossypii* populations resistance situations from different coordinates in the Çukurova region of Adana province was studied against dimethoate (OP's), chlorpyrifos-Ethyl (OP's) and λ-cyhalothrin (SP's).

The objective of this study was to determine the resistance levels and mechanisms in *A. gossypii* cotton populations. For this purpose, bioassay studies with discriminant dose, metabolic enzyme assays, the occurrence of S431F and KDR mutations, and some CarE's gene expression levels were determined.

Materials and Methods

Collecting *Aphis gossypii* populations

Aphis gossypii populations were collected from 16 different *Gossypium hirsutum* L. (Malvaceae) vegetation coordinates in Çukurova Region in Adana, Mersin (Türkiye) (Figure 1, Table 1). Cotton leaf samples were plucked from cotton plants and brought with in paper bag to Adana Biological Control Research Institute climate rooms. Populations were reared and cultured on cotton plants in cages with insect net separately at 22±1°C, % 65±5 RH, 16:8 LD in climate rooms. *A. gossypii* were identified and classified with morphological methods by Dr. Işıl Özdemir in Kocaeli University Agriculture Faculty, Plant Protection Department, Kocaeli (Türkiye).

Insecticides and bioassays

Commercial products of dimethoate (400 g/l:EC), chlorpyrifos-ethyl (480 g/l:EC) and λ-cyhalothrin (50 g/l:EC) were used during this study. Insecticide Resistance Action Committee (IRAC) No: 019 leaf dipped method used in bioassay studies (IRAC, 2023). Discriminating dose bioassays were applied to distinguish resistant and susceptible *A. gossypii* populations. For this purpose, the most susceptible population against insecticides were accepted as a reference during the first-year surveys. After that, a discriminating dose study was performed based on the LC_{90} (lethal concentration to kill 90 % of the test population) values of the reference population for resistant/susceptible distinction of all the collected populations. The LC_{90} dose obtained for each insecticide from the reference population was applied to all other populations. If the mortality rate was >90%, it was considered susceptible, if the mortality rate was

<90%, it was regarded as a resistant population. Bioassay experiments were performed on all populations considered resistant. In insecticide bioassay studies, leaves from cotton plants were cut into 4 cm diameter discs. These discs were dipped in the insecticide solution for 10 seconds and then placed in petri dishes containing agar after drying. Twenty wingless individuals of aphid from the rearing colonies were taken, placed into Petri dishes with a fine brush and then transferred into climate rooms. Bioassay studies were done according to randomized parcel experimental design, and six different doses with three replications were used for each insecticide in this study. Each experiment included at least three control petri; if the mortality rate of control petri aphids was <20%, experiments were repeated. After 72 hours of application, counting was done by touching the specimens with a fine brush under the stereo microscope to determine if they are dead or alive. Dead /alive individuals were recorded. LC_{50} and LC_{90} values for populations were calculated via probit analysis using the POLO Plus software (Leora Software, 1987).

Acetylcholinesterase (AChE) enzyme activity

According to Kranthi (2005), 10 individuals of wingless aphids were homogenized within 300 µL 0.05 M, %0.1 Triton X-100 phosphate buffer (pH: 7.0) by a homogenizer. Sample tubes were centrifuged at 10000 g +4°C for 20 minutes, and the supernatant was used as an enzyme source. 2.86 ml sodium phosphate buffer (0.1 M pH 8.0), 10 µL 0.1 M DTNB (0.01 M, in pH 8.0 sodium phosphate buffer) and 30 µL of 0.1 M acetylcholine iodide (0.1 M, pH 8.0, in sodium phosphate buffer) were added into the supernatant (100 µL) and the reaction was initiated. Under the same conditions, the enzyme-free blank sample was prepared, 250 µL samples were transferred to microplates, and the alteration in absorbance was recorded at 412 nm wavelength at room temperature for 30 minutes in Thermofisher MultiscanGo spectrophotometer device.

Glutathione S-Transferase (GST) enzyme activity

Thirty wingless adult aphids were homogenized in 300 µl 0.1 M pH 6.5 sodium phosphate buffer, centrifuged at 10000 g +4°C for 20 minutes, and the supernatant was used as an enzyme source. 50 µl 50 mM CDNB, 150 µl 50 mM reduced glutathione (0.1 M, pH 6.5 in phosphate buffer) were added to the 30 µl enzyme sample. The enzyme mixture was diluted in 2.77 ml of phosphate buffer (0.1 M pH 6.5), and 250 µl of the diluted mixture was transferred to microplates for spectrophotometer readings. The enzyme-free blank sample was measured as a control without homogenate. The change in absorbance was recorded by reading at 340 nm wavelength for 10 minutes in Thermofisher MultiscanGo spectrophotometer device. (Habig et al., 1974; Kranthi, 2005).

Cytochrome P450 monooxygenase enzyme activity

Hansen & Hodgson (1971) method was used to determine P450 enzyme activity. Two hundred wingless adult aphids were homogenized in 300 µl 0.1 M pH 6.5 sodium phosphate buffer, centrifuged at 10000 g +4°C for 20 minutes, and the supernatant was used as an enzyme source. According to this method, 90 µl enzyme source and 100 µl 2mM p-nitroanisole (PNOD) as a substrate were mixed into each microplate cell. The reaction was initiated by adding 10 µl 9.6 mM nicotinamide adenine dinucleotide phosphate (NADPH) after incubating at 27°C for 2 minutes. The changes in enzyme activity were read at 27°C and 405 nm wavelength for 30 minutes with a microplate reader in Thermofisher MultiscanGo spectrophotometer device. All protein readings and quantifications after the determination of activity were performed according to Bradford (1976).

Detection of the presence of S431F mutation on the AChE enzyme

To detect the presence of S431F mutation on the AChE enzyme gene in *A. gossypi* populations, DNA isolation was performed with a kit (Thermo Scientific) and five wingless adult aphids were used DNA extraction. S431F mutation, which is one of the effective resistances to the CarE gene in populations, was determined by the PCR-RLFP method. As stated in the method, 36 replications and 180 individuals were

done from all resistant populations. Isolated DNA samples and ACE-F - (5'-CAA GCC ATC ATG GAA TCA GG-3'), ACE-R- (5'-TCA TCA CCA TGC ATC ACA CC-3') primers were performed to a polymerase chain reaction (PCR) (Chen et al., 2013). The conditions of PCR were followed by denaturation at 94°C, 5 min 1 cycle, 40 cycles at 94°C for 30 seconds, 53°C for 30 seconds, 72°C for 45 seconds and at 72°C for 10 minutes extension. After the PCR products were visualized by agarose gel electrophoresis (1.5%), 20 µL of PCR product was cut by incubating with four units of SspI restriction endonuclease enzyme at 37ºC for 3 hours and the condition of the bands was observed in agarose gel electrophoresis (1.5%) (Figure 4).

Detection of the presence of knockdown resistance (Kdr) mutation

According to Marshall et al. (2012) the PCR-RFLP method was applied to detect the presence of Kdr mutation, which is effective in the resistance of pyrethroid insecticides in *A. gossypii* individuals. DNA isolation was performed with the help of a kit (Thermo Scientific) with at least six replications, each including five wingless aphid individuals. Isolated DNA samples and Kdr-DP1 (5'- TCTTGGCCCACACTTAATCTTT-3'), Kdr-DP4 (5'-CTCGCCGTTTGCATCTTATT-3') primers were performed to a polymerase chain reaction (PCR) (Marshall et al., 2012). PCR conditions were followed by denaturation at 94°C, 2 min one cycle, 35 cycles at 94°C for 30 seconds, 48°C for 1 minute, 72°C for 90 seconds, and final elongation at 72°C for 5 minutes. After the PCR products were visualized by agarose gel electrophoresis (1.5%), 40 µL of PCR product was cut by incubating with five units of BstEII cutting enzyme at 60ºC for 6 hours and the condition of the bands was observed in agarose gel electrophoresis (1.5%) (Figure 4).

Expression profiles of carboxylesterase (CarE) enzyme gene

At least eight replications and 50 wingless adult aphid individuals for each population were used to perform the Quantitative Real-time PCR study. Fresh aphid samples were frozen at -80°C, homogenized with the help of buffer and their total RNA was extracted according to the Thermo Scientific RNA purification kit. After extraction, the RNA amounts of the populations were measured by nano-drop and diluted with TE buffer to obtain equal concentrations of 50 ng/µl for each of them. In the Quantitative real-time PCR study, the expression profile of the carboxylesterase gene was examined based on the relative activity of the CarE gene (Accession No. AB016720). P1-F-5'-CATACCCTACGCTCAACCAC-3', P2-R-5'-GCAATCTTCACTTCCAACGA-3' primers were used specifically for the CarE gene (Cao et al., 2008b). Primers of *β-actin* gene Forward 5'-AGCTCTATTCCAACCTTCCTTCT-3', Reverse 5'-TGTATGTAGTCTCGTGGATACCG-3' were used as the housekeeping gene. PCR temperature table 50°C 15 min 1 cycle, 95°C 15 min 1 cycle, 40 cycles at 95°C for 20 seconds at 59°C for 30 seconds and at 72°C for 2 minutes and final elongation 72°C with one cycle of 10 min was followed. The Quantitative Real-time PCR was performed with the help of Thermo Fisher Scientific, One-Step qRT-PCR kit, USA. The average of Obtained Ct values recorded, and ΔΔCt calculations were done according to susceptible populations to determine the relative activity levels of the CarE gene (Livak & Schmittgen, 2001).

Statistical analysis

Dose-response regressions were calculated with the Polo-plus software. LC_{50} values of resistant populations were rated with LC_{50} values of susceptible populations to determine the resistance rate. Other statistical analyses were done with SPSS 23 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to determine the differences between means and Duncan's multiple comparison tests were applied to compare groups between aphid populations collected from Adana during this study ($p<0.05$). The classification was done according to the World Health Organization (WHO, 1980) standards. Susceptible (RR<3 times) (S), Decreased Susceptible (DS) (RR=3 to 5 times), Lower Resistance (LR) (RR=5 to 10 times), Medium Resistance (MR) (RR=10 to 40 times), Higher Resistance (HR) (RR=40 to 160 times) and Extremely High Resistance (RR>160) are classified as resistance levels. The percentage of sensitive and resistant alles rate was calculated from total PCR-RFLP analysis samples.

Results

Aphis gossypii populations were collected from 16 different cotton fields between 2020 and 2021 in this study (Table 1, Figure 1).

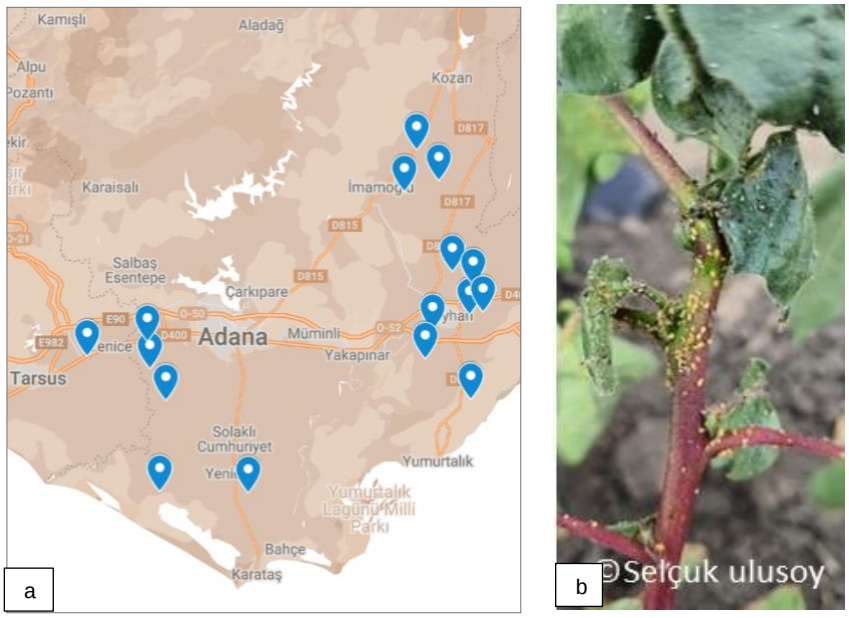

Figure 1. a) Locations of *Aphis gossypii* populations from cotton fields in Adana, Türkiye; b) Cotton plant infested with *Aphis gossypii* individuals.

Table 1. *Aphis gossypii* populations collected from cotton growing fields

Population	Location	Coordinate	Date
1	Çiftlikler, Ceyhan	37°01'49.9"N 35°51'24.2"E	June, 2020
2	Hamzalı, Yumurtalık	36°52'44.2"N 35°51'35.0"E	May, 2020
3	Helvacı, Karataş	36°42'46.4"N 35°21'40.7"E	May, 2020
4	Karagöçer, Karataş	36°42'50.3"N 35°09'49.4"E	May, 2020
5	Karayusuflu, Seyhan	36°52'39.7"N 35°10'39.4"E	June, 2020
6	Küçükçıldırım, Seyhan	36°56'03.8"N 35°08'29.0"E	May, 2020
7	Toktamış, Ceyhan	37°00'11.2"N 35°46'27.9"E	June,2020
8	Yeniköynazımbey, Ceyhan	36°57'10.8"N 35°45'29.4"E	June, 2020
9	Değirmendere, Ceyhan	37°02'09.2"N 35°53'15.4"E	May, 2021
10	Gokçeler, Seyhan	36°58'59.9"N 35°08'14.8"E	June, 2021
11	Hamitbeybucağı, Ceyhan	37°06'27.2"N 35°49'04.6"E	June, 2021
12	İncetarla, Ceyhan/Adana	37°04'59.7"N 35°51'50.1"E	June, 2021
13	Konaklar, Tarsus	36°57'25.5" 35°00'09.4"E	June, 2021
14	Sokutaş, İmamoğlu	37°16'13.8"N 35°47'11.9"E	June, 2021
15	Üçdutyeşilova, Ceyhan	37°15'03.9"N 35°42'38.7"E	June, 2021
16	Ufacıkören, İmamoğlu	37°19'28.0"N 35°44'19.8"E	May, 2021

Bioassay analysis

Dimethoate, chlorpyrifos-ethyl and λ-cyhalothrin resistant aphid populations were distinguished with discriminating dose bioassay. LC_{50} and LC_{90} values of all resistant populations were determined (Table 2). All populations' resistance levels were classified according to WHO (1980) scale. With discriminating dose bioassay, except for the Toktamış population, six *A. gossypii* populations were identified as resistant. Compared to the susceptible population, four populations showed DS level resistance to dimethoate, and one population to chlorpyrifos-ethyl insecticides. Only two populations showed MR levels to chlorpyrifos-ethyl. Other populations were observed as S levels within this study (Table 2, Figure 2). LC_{50}: 11.4 ppm for Dimethoate, LC_{50}: 165.3 ppm for λ-cyhalothrin and LC_{50}: 108.7 ppm for chlorpyrifos-ethyl were found in the Toktamış population. Çiftlikler, Helvacı, Karagöçer, Hamzalı, Hamitbeybucağı, İncetarla, Değirmendere, Üçdutyeşilova, Gökçeler and Konaklar populations were detected as susceptible and eliminated according to the discriminating dose. Yeniköynazımbey, Küçükçıldırım, Karayusuflu, Sokutaş and Ufacıkören populations were detected as resistant populations. Yeniköynazımbey and Küçükçıldırım populations were the most resistant to dimethoate and chlorpyrifos-ethyl, RR_{50} was found 5.81 and 10.9 times, respectively. Sokutaş population was observed as the most resistant (RR_{50}: 3.7) population for λ-cyhalothrin (Table 2).

Table 2. Bioassay of Dimethoate, λ-cyalothrin, Chlorpyrifos in test populations of *Aphis gossypii*

Insecticide	Population	n	LC_{50} (mg/L) (CL)	LC_{90} (mg/L) (CL)	Slope (±SE)	X^2	RR_{50}	Resistance level
Dimethoate	Toktamış*	360	11.4 (8-14.7)	32.1 (23.6- 56.9)	2.86±0.5	0.55	-	-
	Yeniköynazımbey	380	66.5 (47.1-107)	352.3 (178.5-2397.1)	1.77±0.4	1.08	5.8	LR
	Küçükçıldırım	365	51.2 (33.2-83.2)	293.9 (150-1523.5)	1.68±0.3	4.13	4.4	DS
	Karayusuflu	360	46.1 (26.5-81)	568.2 (237.2-4270.6)	1.17±0.2	2.19	4.0	DS
	Sokutaş	370	42.2 (32.3-55.4)	106.7 (73.8- 290.6)	3.18±0.8	1.22	3.7	DS
	Ufacıkören	360	47.6 (36.3-66.6)	128.5 (83.9-439.7)	2.97±0.7	1.75	4.1	DS
λ-Cyhalothrin	Toktamış*	360	165.3 (122.3- 203.1)	337 (263.5- 578.9)	4.14±0.9	2.64	-	-
	Yeniköynazımbey	360	370.7 (240.7-508.7)	1594.7 (1013.7-4363.4)	2.02±0.4	2.54	2.2	S
	Küçükçıldırım	365	437.0 (345.5-528.3)	797.7 (631.2-1443.1)	4.90±1.2	2.20	2.6	S
	Karayusuflu	380	387.7 (259.8-536.8)	1717.9 (1066.5-4902.8)	1.98±0.4	1.88	2.3	S
	Sokutaş	360	370.6 (276.1-439.7)	646 (511.9-1896.8)	5.31±1.8	3.77	2.2	S
	Ufacıkören	360	306.5 (201.8-413)	938 (598.8-5383.1)	2.63±0.8	2.54	1.8	S
Chlorpyrifos	Toktamış*	360	108.7 (73.9- 140.2)	335.2 (243.3-638.6)	2.62±0.5	1.37	-	-
	Yeniköynazımbey	380	1130.4 (770-1301.9)	2624.3 (1901.4-5454.5)	3.14±0.7	2.11	10.3	MR
	Küçükçıldırım	365	1184.8 (780.3-1454.1)	3451.6 (2268.8-8863.6)	2.52±0.5	0.36	10.8	MR
	Karayusuflu	360	525.3 (311-746.9)	2005.6 (1299.5-5132.7)	2.20±0.5	3.12	4.8	DS

CL: Confidence Limits; *: Susceptible population; RR_{50}: Resistance Rate R_{50} ; n: Individual number

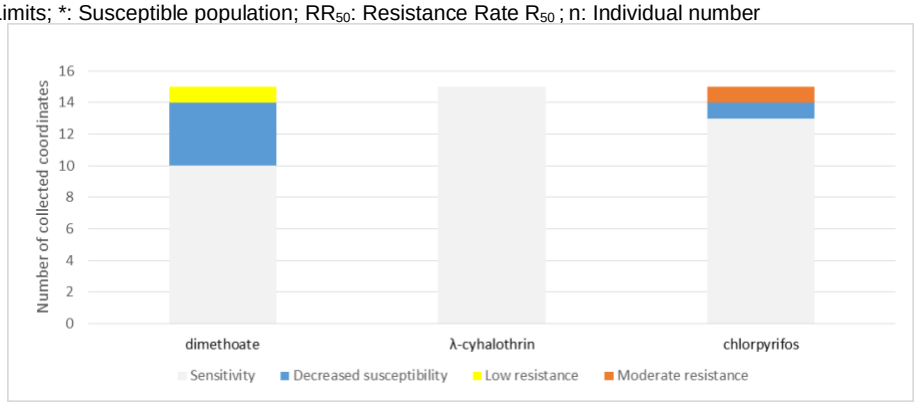

Figure 2. The distribution of Resistance levels for *Aphis gossypii* to dimethoate λ-cyhalothrin and chlorpyrifos-ethyl from the 16 different locations in Adana, Türkiye.

The enzyme and gene expression analysis

According to the enzyme analysis, enzyme activity values were relatively lower in the Toktamış population than other resistant populations. Activities were higher and at different rates according to the enzyme type in other populations (Table 3). When gene expression level of the CarE gene was examined, all populations were more regulated than the susceptible population. 1.25 times more gene regulation was seen in the Sokutaş population (Figure 3).

Table 3. Acetylcholinesterase, glutathione S-transferase (GST), cytochrome P450 monooxygenase (P450) enzyme activities of resistant and susceptible *Aphis gossypii* populations

Populations	Specific Enzyme activities U.mg^{-1}.min					
	Acetylcholinesterase		GST		P450	
Toktamış*	19.08±3.0	a	6.45±1.44	a	0.02±0.01	a
Yeniköynazımbey	52.21±6.40	bc	17.02±3.42	ab	0.10±0.03	ab
Küçükçıldırım	76.81±7.84	d	27.69±7.72	b	0.05±0.02	ab
Karayusuflu	32.82±6.16	ab	23.39±4.40	b	0.05±0.01	ab
Sokutaş	65.70±7.47	cd	17.60±2.79	ab	0.13±0.05	ab
Ufacıkören	66.89±9.67	cd	67.31±1.90	c	0.10±0.01	b

* Susceptible population, the difference between average number of specific enzyme activity of *Aphis gossypii* were statistically significant ($p<0.05$), Duncan multiple comparison tests were applied; ±: standart error

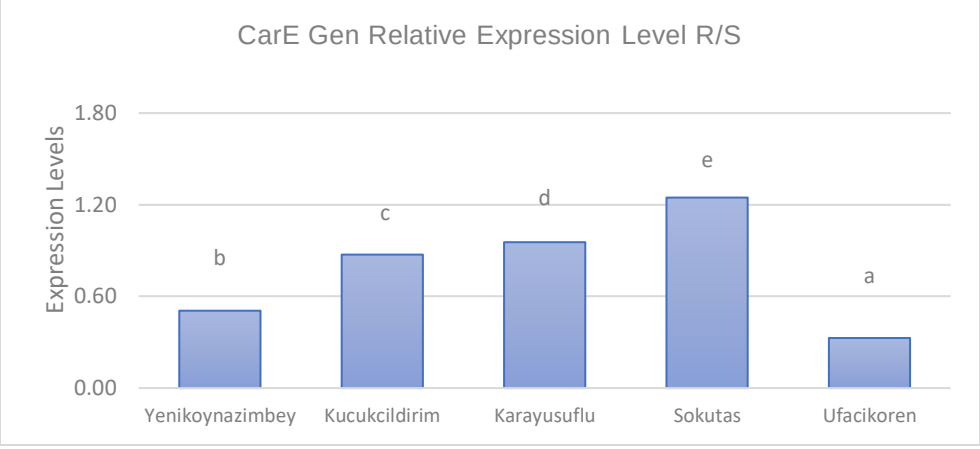

Figure 3 Relative expression levels (R/S) of Carboxylesterase gen (CarE) in *Aphis gossypii* populations (R/S: Resistant population/susceptible population).

The difference between average number of CarE gen relative expression levels of *Aphis gossypii* were statistically significant ($p<0.05$).

Mutation screening analysis

The recessive allele was found only in the Toktamış population (Figure 4). Approximately 17% of recessive alleles were detected for S431F mutation. A total of 36 replications was scanned from all populations for Kdr mutation (Table 4). It was seen that enzyme cutting occurred in all populations by PCR-RLFP method (Figure 4). All populations were found to have 100% recessive alleles for Kdr mutation. All populations were detected as susceptible in terms of Kdr mutation.

Table 4. S431F and KDR mutation frequency *in Aphis gossypii* populations

Mutation	Recessive alleles %	Resistant alleles %
S431F mutation	17	83
KDR mutation	100	-

Figure 4. *Aphis gossypii* PCR-RFLP agarose gel electrophrosesis photograph. M: 100 bp ladder, 1: S431F PCR product (667 bp), 2: S431F PCR cut by SspI RLFP product (Resistant allel: 667 bp, uncut by SspI); 3: S431F PCR cut by SspI RLFP product (Susceptible allel: 335 bp, cut by SspI); 1a: Knockdown resistance (Kdr), Negative control PCR product 500 bp; 2a: Kdr PCR cut by BstEII RLFP product (Susceptible allel: 325 bp, cut by BstEII).

Discussion

Resistance management is a key factor to prevent the development of resistance in pests and to maintain insecticide activity against the harmful organism (Alpkent et al., 2023). Insecticide resistance is an important problem for *A. gossypii* (Ulusoy et al., 2018; Ullah et al., 2020; Erdogan et al., 2023; Cheng et al., 2023). The resistance levels of 16 populations in the Çukurova region were observed in this study; four populations showed DS level resistance against dimethoate, and one population against chlorpyrifos-ethyl. Only two populations against chlorpyrifos-ethyl showed MR-level resistance. Other populations were found susceptible. Considering this aspect, moderate and low levels of resistance was observed in the region and it was not detected at high and extremely high resistance levels (Figure 2). The reference population is of significant importance in determining resistance levels. In this study, susceptible aphids that were cultured in the laboratory environment for many years could not be obtained for various reasons. Instead, the susceptible population was obtained from cotton growing areas. This may have caused resistance levels to be lower. In addition, the fact that the Çukurova region is suitable for polyculture agriculture and extensive agricultural practices throughout the year may bring about higher resistance level expectations. However, the widespread use of neonicotinoids or new active groups in the last 30 years may have reduced the use of organophosphate and pyrethroid insecticides. The reducing excessive insecticide consumption and using different insecticide groups is an important factor that reduces resistance. Before, Ulusoy et al. (2018) reported high-level resistance against neonicotinoid for *A. gossypii* owing to exposure to more insecticides in the same region. According to the results of enzyme analysis, all activities were found to be relatively lower in the Toktamış population compared with other populations. Activities in other populations were observed as higher and different rates. As observed in the previous studies, AChE's and GST enzyme activities were found at high rates in organophosphate, carbamate and pyrethroid resistance populations (Devonshire & Moores, 1982; Lokeshwari et al., 2016; Ulusoy et al., 2018). As in this study, monooxygenase P450 enzyme activity, which is responsible for many metabolic activities, was observed at high levels in each resistant population (Shang et al., 2012; Seyedebrahimi et al., 2015). There was a general increase in metabolic enzyme activities compared to the susceptible population with the evaluation of all enzyme analyses. Moreover, the relative expression activity of the CarE gene increased compared to the susceptible population in this study. In parallel with this study, it has been reported that the transcription

level increased in populations of *A. gossypii,* which showed a resistant organophosphate population (Hawkes, 2002; Cao et al., 2008a; Wang et al., 2021). CarE's have a significant role in the detoxification of exogenous harmful ingredients in arthropods (Ma et al., 2018). Also, the overexpression of carboxylesterase gene activity in resistant *A. gossypii* populations against λ-cyhalothrin was reported (Sial et al., 2018; Wang et al., 2021). Regarding the presence of S431F mutation, a high percentage of resistant alleles were found between populations. Parallel to these findings, it was seen that dimethoate and chlorpyrifos-ethyl resistant populations resistance levels were higher than λ-cyhalothrin resistant populations. Similar studies have reported the mutation relationship between the AChE gene with organophosphate and pirimicarb-resistant populations (Benting & Nauen, 2004; Chen et al., 2013; Hlaoui et al., 2022; Nam et al., 2022). In this study, recessive alleles were found in all populations in terms of Kdr mutation. The low levels of resistance to λ-cyhalothrin in all populations support this case. Similar studies have reported a relationship between Kdr mutation and resistance to synthetic pyrethroids in *A. gossypii* and different aphid species (Marshall et al., 2012; Wang et al., 2021; Valmorbida et al., 2022; Fontaine et al., 2023). It is possible that the presence of lower resistance levels of λ-cyhalothrin can be associated with metabolic resistance. The higher enzyme activities in biochemical analysis support this phenomenon (Lokeshwari et al., 2016). In conclusion, moderate and low resistance rates against dimethoate and chlorpyrifos-ethyl insecticides in *A. gossypii* were observed in sixteen different populations in the Çukurova region of Adana provinces. Recessive Kdr mutation in aphid populations overlaps with low λ-cyhalothrin resistance.

It has been concluded that the S431F mutation, overexpression of the CarE gene and high biochemical metabolic activities are effective in the occurrence of higher rates of resistance levels to dimethoate and chlorpyrifos-ethyl. These results should be taken into account in resistance management. In chemical control, the same group of insecticides should be reduced and selection of aphid populations with resistant alleles should be prevented. In Türkiye, resistance monitoring studies should be accelerated and chemical control programs should be carried out regionally and temporally.

Acknowledgements

We wish to express my gratitude to Dr. Işıl Özdemir for morphologically identifying aphid species. In addition, we would like to thank Dilek Ulusoy and İkbal Burcu Özgür for their significant contributions during this study.

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