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Neonicotinoid resistance in populations of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) in cotton plantation areas of Türkiye

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Abstract: The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is a polyphagous pest that could cause economic crop losses in various crops. Cotton production areas are under insecticide application pressure, and the possibility of insecticide resistance development is higher than in other crops. Chemical insecticides, especially neonicotinoids, are the most common instruments of Integrated Pest Management (IPM) strategies against *A. gossypii*. In this study, the resistance status of *A. gossypii* populations from the largest cotton plantation areas of Türkiye was analyzed. Nine field-collected aphid populations and a susceptible strain were examined in leaf-dip bioassays with three neonicotinoid insecticides. The resistance ratios of bioassays ranged from 22.6 to 82.6 for acetamiprid, 23.5 to 67.3 for imidacloprid, and 1.1 to 20.8 for thiamethoxam. Comparative sequence analysis between susceptible and resistant strains was analyzed to identify known mutations to confer resistance to neonicotinoids. The mean enzyme activity in some populations was significantly higher than in the susceptible strain. The enzyme activity ratios ranged from 1.9 to 3.9 for *CarE* and 1.5 to 3.1 for *GST*. The bioassay data revealed moderate to high resistance levels in acetamiprid and imidacloprid and low to medium levels in thiamethoxam. A partial sequence of the β1 subunit of the *nAChR* in specimens of the populations examined did not reveal any of the *V62I*, *L80S,* and *R81T* and point mutations. The lack of any correlation between the carboxylesterase or glutathione-S-transferase activity and the LC_{50} values of three insecticides suggested that these two detoxification enzymes were not involved in the resistance levels observed. However, the resistance levels observed in the present study could be attributed to metabolic resistance mechanisms. Another important point is the cross-resistance observed between the neonicotinoids in the present study. Their extensive use, especially in cotton, might select aphid genotypes resistant to more than one neonicotinoid.

Key words: *Aphis gossypii*, resistance, cotton, neonicotinoids, nicotinic acetylcholine receptors

1. Introduction

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is one of the most important aphids, that feeds on various host plants in agricultural and nonagricultural areas worldwide. *A. gossypii* is highly polyphagous and can infest over 900 plant species worldwide (Satar et al., 1999; Blackman and Eastop 2000; Begum et al., 2018). The wide host range of *A. gossypii* includes various field and glasshouse crops, especially cotton [*Gossypium hirsutum* L. (Malvaceae)], cucurbits (Cucurbitaceae), citrus (Rutaceae), cocoa [*Theobroma cacao* L. (Malvaceae)], aubergine [*Solanum melongena* L. (Solanaceae)], pepper [*Capsicum annum* L. (Solanaceae)], potato [*Solanum tuberosum* L.(Solanaceae)], okra [*Abelmoschus esculentus* L. (Malvaceae)], and many ornamental plants. Moreover, it also causes indirect damage by transmitting more than 50 plant viruses (Blackman and Eastop, 2000). *A. gossypii* reproduces parthenogenetically all year round (anholocyclic life-cycle category) in the warmer parts of the world (Blackman and Eastop, 2017). In many countries throughout the world, the aphid causes severe losses in cotton production together with significant qualitative degradation (sticky lint). Due to its economic importance, *A. gossypii* is a target of intense chemical control programs

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worldwide.

The cotton aphid is also a key pest in the cotton production areas of Türkiye (Anonymous, 2017). Many active ingredients have been used for the control of *A. gossypii* in Türkiye, such as organophosphates, pyrethroids, and neonicotinoids (Anonymous, 2022a[1]). Insecticides have been a valuable tool for pest control for many decades; however, as with other classes, their extensive and widespread use has led to cases of resistance in many pest species, which can compromise successful control (Bass et al., 2015).

The first case of insecticide resistance in *A. gossypii* was reported against the organophosphate compound Demeton (Ghong et al., 1964). Later resistance to carbamate (Furk et al., 1980) and pyrethroid insecticides (Zil'bermints and Zhuravela, 1984) has been documented. Recent studies showed that the resistance of *A. gossypii* to various insecticides was quite common worldwide (Gubran et al., 1992; Nauen and Elbert, 2003; Wang et al., 2007; Carletto et al., 2010; Herron and Wilson, 2011; Foster et al., 2017). Especially many cases of resistance to neonicotinoids have been well documented in *A. gossypii* clones/samples from various regions, such as the Far East, USA, Australia, and Iran which focused on the underlying resistance mechanisms (Herron and Wilson, 2011; Gore et al., 2013; Koo et al., 2014; Matsuura and Nakamura, 2014; Seyedebrahimi et al., 2015; Hirata et al., 2017; Chen et al., 2020; Wang et al., 2021). Furthermore, target site neonicotinoid resistance in *A. gossypii* and three mutations in β1 subunit of nAChR (L80S, R81T, V62I and K264E) have been found to compromise neonicotinoid efficacy (Koo et al., 2014; Kim et al., 2015; Chen et al., 2017). Specifically, a higher resistance to nitro-substituted neonicotinoids have been shown than to cyano-substituted neonicotinoids in *A. gossypii* (Hirata et al., 2015; 2017). Metabolic resistance has also been reported in some *A. gossypii* genotypes and particularly the involvement of P450 monooxygenases and carboxylesterases (Chen et al., 2015; Wei et al., 2017). Moreover, transcriptome data and RNAi experiments showed an up-regulation in UDP-glucuronosyl transferases might be associated with thiamethoxam tolerance in a resistant strain of *A. gossypii* from China (Pan et al., 2015; 2018).

Cotton production areas in Türkiye are about 432,279 ha, and the production amount is about 2.250.000 t. The Southeastern Anatolian Region is one of the most important cotton production areas in Türkiye, consisting of 58.2% production areas and 56.5% production amount (Anonymous, 2022b[2,3]). The control of *A. gossypii* is based on chemical insecticides, particularly neonicotinoids (Ulusoy et al., 2018). Farmers and pesticide dealers stated that neonicotinoid insecticides were the most common insecticide group used in cotton production against *A. gossypii* in Southeastern Anatolian Region, and insensitivity has developed over time against this group (personal interviews). Therefore, the present study aimed to examine resistance levels of the neonicotinoids including acetamiprid, imidacloprid, and thiamethoxam against *A. gossypii* field-collected samples in dose-response bioassays, as well as to determine the presence and frequency of known resistance mechanisms. The data obtained from this study will provide scientific evidence for improving the current Integrated Pest Management (IPM)/Insecticide Resistance Management (IRM) strategies.

2. Material and methods

2.1. Aphid material

A susceptible clone maintained during 20 years under in vitro conditions was obtained from Bayer (Leverkusen, Germany). Nine samples of *A. gossypii* were collected from cotton fields in various localities of Southeastern Anatolia in Türkiye, during the summer of 2017 (Table 1 and Figure 1). The aphid samples and the susceptible strain were reared on cotton plants [*Gossypium hirsutum* L. (Progen BA 119)] in cages in the laboratory at 23 ± 1 °C, 65% ± 5% relative humidity, and 16: 8 h L: D photoperiod. Field samples were reared for 2–3 generations under laboratory conditions until bioassays.

Adult apterous females of approximately the same age were used in dose-response bioassays and biochemical and molecular analyses. To obtain equal-aged aphid individuals, 50 apterous adult individuals of females were transferred to each leaf of cotton plants at the stage of 5–8 permanent leaves. The plants were kept in plexiglass cages (60 × 60 × 80 cm dimensions) with two ventilation holes covered with fine muslin at three sidewalls of each cage. All adults were removed after 48 h, and the nymphs were reared until adulthood. The newly emerged adults were used either in dose-response bioassays or stored at –80 °C until biochemical and molecular analyses.

2.2. Insecticides

Commercial formulations of acetamiprid (Mospilan, SP formulation, %20 Active ingredient, Sumi Agro Türkiye), imidacloprid (Confidor, SC formulation, 350 g/L Active ingredient, Bayer CropScience AG), and thiamethoxam (Actara, SC formulation, 240g/L Active ingredient, Syngenta Crop Protection AG) were used in bioassays.

1 https://bku.tarimorman.gov.tr/Kullanim/TavsiyeArama

2 https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr

Table 1. The field samples of *Aphis gossypii* collection sites and map coordinates in Southeastern Anatolia, Türkiye.

Sample name	Sampling date	Sample location
Susceptible	1998	Germany
Babahaki	02.06.2017	37°51'49.3"N 40°45'16.7"E
Şekerören	06.06.2017	37°44'21.4"N 40°26'05.4"E
Yukarıkılıçtaşı	31.05.2017	37°56'28.9"N 40°15'20.7"E
Develi	12.06.2017	37°15'03.8"N 40°03'11.9"E
Altıntoprak	26.05.2017	37°06'05.3"N 40°35'31.7"E
Çamlıca	14.06.2017	37°10'02.9"N 40°42'20.6"E
Çatalhurma	30.06.2017	36°50'59.6"N 38°56'02.5"E
Yazılıkavak	20.06.2017	37°04'14.4"N 39°04'30.7"E
Yarımsu	03.07.2017	37°08'30.5"N 39°04'20.6"E

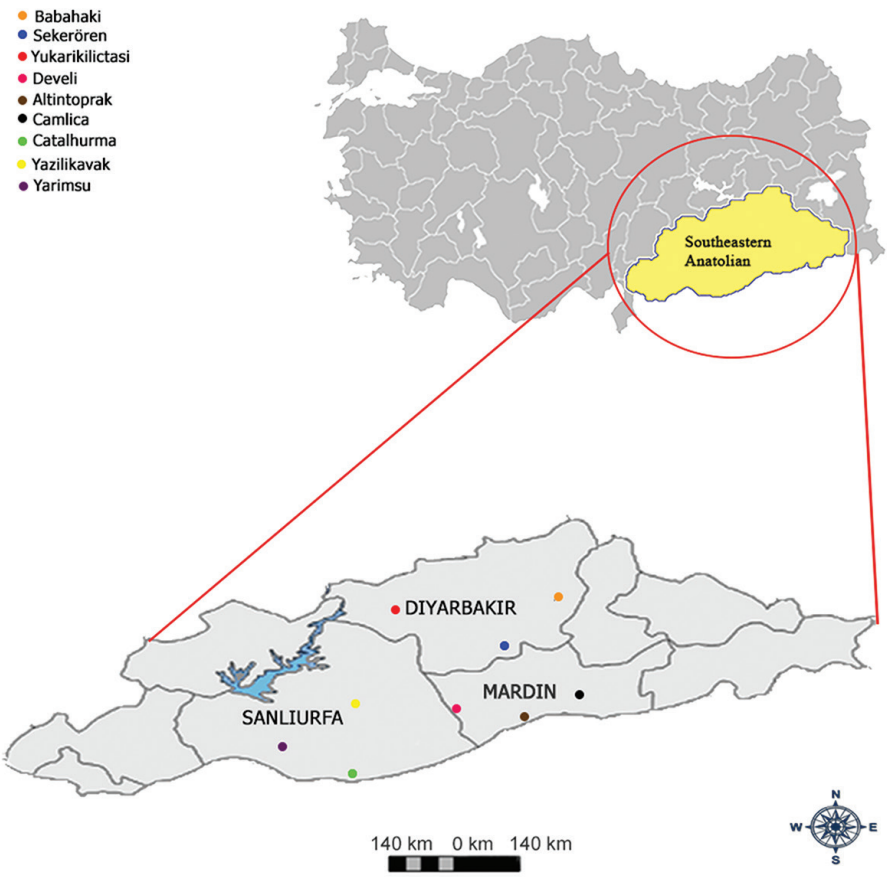

Figure 1. Sampling sites of *Aphis gossypii* from Southeastern Anatolia, Türkiye.

2.3. Dose-response bioassays

The bioassay method IRAC No 019 (IRAC, 2015) was used after modifications. A 4-cm diameter cotton leaf disc was dipped into at least eight different concentrations of water dispersions (including 0.2%, Triton X-100) with insecticides for 5 s. The control solution consisted of ddH_2O and 0.2% Triton X-100. Leaf discs were placed upside down in plastic dishes (4-cm diameter) containing 1% agar. The lid had a 2.4 cm diameter hole covered with fine muslin to allow ventilation. A total of 20 adult females were carefully brushed onto the leaf disc. Treated aphids were kept under constant conditions (20 °C, 60 ± 5% RH, and 16:8 h L: D photoperiod). Mortality was assessed after 72 h according to the IRAC (IRAC, 2015) test method. *A.*

gossypii individuals were turned down with a soft brush, and the individuals who could not change their position were considered as dead. The field-collected populations were tested against 1–250 ppm of the acetamiprid and imidacloprid, and 1–400 ppm of the thiamethoxam, while the susceptible population was tested against 0.1–20 ppm of the insecticides. The bioassays were performed with four replicates for each dose. About 720 apterous *A. gossypii* individuals were used in acetamiprid and imidacloprid bioassays and about 800 apterous individuals were used in thiamethoxam bioassays.

2.4. Metabolic enzyme activities

The method described by Stumpf and Nauen (2002) was used with slight modifications to determine carboxylesterase (CarE) activity. Ten apterous adult females per sample were homogenized in 100 µL sodium phosphate buffer (0.1 M, pH: 7.5). The homogenate was centrifuged at 10,000 rpm and 4 °C and the supernatant was used as enzyme source. Each well of plate was filled with 25 µL of 0.2 M pH: 6.0 sodium phosphate buffer, 25 µL of the enzyme source and 200 µL of substrate solution. Enzyme activity was read at 23 °C for 10 min at 450 nm wavelength with the Versamax kinetic microplate reader spectrophotometer using SoftMAX PRO (Molecular Devices, CA, United States). Three replicates per sample were performed, and 25 µL of 0.1% Triton X-100 containing 0.1 M pH: 7.5 sodium phosphate buffer was used as control.

Glutathione-S-transferase (GST) activity was determined using modified version of the method described by Stumpf and Nauen (2002). Thirty apterous adult females were homogenized in 300 µL Tris-HCL buffer (0.05 M, pH 7.5). Homogenate was centrifuged at 10,000 rpm and 4 °C and the supernatant was used as an enzyme source. Each well of plate was filled with 100 µL of the enzyme source, 100 µL of 0.4 mM CDNB and 100 µL of 4 mM GSH. The changes in absorbance were read at 25 °C for 5 min at 340 nm wavelength by using a Versamax kinetic microplate. The enzyme activity was analyzed with SoftMAX PRO software. Trials were replicated three times and nonenzymatic reaction of CDNB and GSH was quantified without homogenate as control.

The Bradford (Bradford, 1976) method was used to calculate the total proteins of each sample using bovine serum albumin (BSA) to build the standard curve. CarE activity was expressed as µmol naphthol/min/mg protein and GST activity as µmol conjugated glutathione/min/mg protein.

2.5. RNA extraction, cDNA synthesis, nAChR β1 subunit sequencing

RNA was extracted from pools of adult *A. gossypii* individuals using the GeneJET RNA isolation kit (Thermo Fisher Scientific, USA) and quantified using NanoDrop® 1000 (Thermo Scientific, USA). One µg RNA was used for cDNA synthesis using SuperScript III RT (Thermo Scientific, USA) and oligo-dT (Thermo Scientific, USA). PCR reactions (25 µL total volume) contained Platinum II Taq DNA Polymerase (5 U/µL; Thermo Scientific, USA), 1 µL of cDNA, and 10 pmol of each primer pair. Amplification conditions were as follows: 95 °C for 3 min; 35 cycle of 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 10 min; and a final step at 72 °C for 5 min. A negative control was included in each run with nuclease free water. The primers used for the amplification of partial nAChR β1 subunits that encompass R81T and V62I point mutations were nAChR β1-F (5'-CAA CAA ACT AAT CAG ACC TGT CC-3') and nAChR β1-R (5'-GGC AAG TAG AAC ACT AGC ACG C-3') (Hirata et al., 2015). PCR products were visualized on 1.5% TAE agarose gel electrophoresis. All PCR products were sequenced in both directions with same primers using the Sanger method. Sequencing results were analyzed using Bioedit version 7.2.5 (Hall, 1999). Multiple sequence alignment results were converted to the proteins with MegaX software (Kumar et al., 2018).

2.6. Statistical analyses

The data from dose-response bioassays were analyzed by a probit analysis in order to calculate LC_{50} values and the 95% fiducial limits using the software Polo-Plus 2.0 (LeOra Software, Berkeley, CA, USA). Resistance Factors (RF) were calculated by dividing the LC_{50} value of each field sample to LC_{50} of the susceptible population. In order to determine differences between field samples GST and EST enzyme test, the data were analyzed using a one-way analysis of variance and, the Tukey HSD was used to test differences among sample means for significance. The data did not show any departure from normal distribution (Shapiro-Wilk test, $p > 0.05$), and the criterion of equality of variance was also met (Levene's test, $p > 0.05$). Pearson's correlation analysis was applied to determine any relation between LC_{50} values and enzyme activities. The analyses were performed using IBM SPSS Statistics Version 19.0 (IBM SPSS Statistics for Windows, IBM, Armonk, NY, USA).

3. Results

3.1. Dose-response bioassays

The most of the bioassay data fit the probit model well ($p < 0.05$). The significant heterogeneity observed in some cases could be attributed to the aphid samples consisting of a mixture of genotypes of different resistance levels.

The dose-response bioassays with acetamiprid and imidacloprid revealed medium to high RF values, ranging from 22.6–82.6 (median: 55.2) and 23.5–67.3 (median: 30.6), respectively. Five of the nine aphid samples examined with acetamiprid and three with imidacloprid showed relatively high RF values (>50). By contrast, six out of the nine samples examined with thiamethoxam showed

relatively low RF values (<7.7), and in three samples, the RF values ranged from 11.3 to 20.8 (median: 7.7). The median and the range of the RF values showed that the resistance level was higher in acetamiprid, followed by imidacloprid and thiamethoxam, respectively. Interestingly, the sample from Yarımsu displayed the highest RF value for imidacloprid (RF = 67.3) and thiamethoxam (RF = 20.8) and the second highest value for acetamiprid (RF = 68.8). The most resistant sample to acetamiprid was detected from Şekerören (RF = 82.6) (Table 2, Figure 2).

A positive correlation for the LC_{50} values among three insecticides was observed. However, Pearson's correlation coefficient values were moderate (0.544 for acetamiprid-imidacloprid, 0.643 for acetamiprid-thiamethoxam, and 0.600 for imidacloprid-thiamethoxam), and the correlation was significant only between acetamiprid and thiamethoxam ($r = 0.643$, $N = 10$, $p = 0.045$).

3.2. Metabolic enzyme activities

The field samples showed higher CarE (1.9 to 3.9-fold) and GST activity (1.8 to 3.1-fold) than the susceptible strain. Significant differences among the field samples were observed in CaRb ($F = 9.769$, $df = 9$, $p < 0.001$) and GST ($F = 21.827$, $df = 9$, $p < 0.001$) mean activities, and most of the samples showed significant higher activities than the susceptible strain (Tukey Test, $p < 0.05$). However, no significant correlation has been detected ($p > 0.378$) between CarE or GST activity and LC_{50} values of three neonicotinoids examined, with Pearson's correlation coefficient values being low (–0.026 to 0.313) (Table 3).

3.3. Screening for target site resistance

None of the L80S, R81T, and V62I mutations that confer resistance to neonicotinoids has been detected in partial sequences of the nAChR β1 subunit in *A. gossypii* samples and the susceptible strain (Figure 3).

4. Discussion

Due to the extensive use of the neonicotinoid insecticides for controlling cotton aphids in the field, we presented detailed data to understand resistance mechanisms against neonicotinoids of *A. gossypii* samples collected from important cotton growing regions of Türkiye. The bioassay data revealed different resistance ratios; while higher resistance ratio levels were observed for acetamiprid and imidacloprid, lower resistance ratio levels were observed for thiamethoxam. The development of resistance was more evident in acetamiprid than in imidacloprid as suggested by the median RF values (55.2 vs. 30.7) (Table 2). This might be due to the higher selection pressure by acetamiprid since it has a broad target spectrum in cotton, which is often used against *A. gossypii* and other cotton pests in this region (Anonymous, 2018).

In general, the RF values observed in the present study were lower than those in *A. gossypii* resistant strains/samples with the R81T mutation (Koo et al., 2014; Hirata et al., 2015; Kim et al., 2015; Chen et al., 2017), however, they were in the same order with the values reported in studies where metabolic resistance has been documented (Chen et al., 2015; Seyedebrahimi et al., 2015; Wei et al., 2017; Ulusoy et al., 2018). This is in accordance with the lack of R81T (or V62I) mutations in the examined samples. The resistance levels observed in the present study could be attributed to metabolic resistance mechanisms. Published studies which reported metabolic resistance in *A. gossypii* strains/samples (including from Türkiye), showed the involvement of cytochrome P450 monooxygenases and CarE in neonicotinoid resistance (Seyedebrahimi et al., 2015; Chen et al., 2017; Wei et al., 2017; Ulusoy et al., 2018). In our study, no significant correlation has been detected between CarE and GST activity and LC_{50} values of three neonicotinoids suggested that these enzymes families are not involved in the resistance observed. A possible resistance mechanism might be attributed to cytochrome P450 monooxygenases, which should be investigated in future studies. Furthermore, recent transcriptome studies presented the importance of phase II enzymes such as Uridine diphosphate (UDP) and glycosyltransferases (UGTs) in resistance of *A. gossypii* to the neonicotinoid insecticides (Pan et al., 2018), which should be addressed, as well.

Another point of discussion is the cross-resistance observed between the neonicotinoids in the present study, as revealed by the positive Pearson's correlation coefficients (0.543–0.643; significant between acetamiprid and thiamethoxam) and a few samples showed the highest RF values in two or three insecticides. Cross resistance among neonicotinoids in *A. gossypii* has been observed in various studies even in populations from Türkiye (Chen et al., 2015; Ulusoy et al., 2018). In Türkiye, there are many registered neonicotinoid insecticides for the control of insect pests in various crops (Anonymous, 2018). Their extensive use in Türkiye, especially in cotton and vegetable crops (hosts of *A. gossypii*), might select aphid genotypes resistant to more than one neonicotinoid which makes the control of cotton aphid difficult.

Interestingly, the sample from Yarımsu locality showed the highest RF value for imidacloprid and thiamethoxam and the second highest value for acetamiprid, which might be due to genetic variation in *A. gossypii* populations (Brévault et al., 2008; Carletto et al., 2009). The selection, under intense chemical control scenarios, and eventually the proliferation of asexual clones with resistant traits such as that observed Yarımsu locality might pose a threat for the chemical control programs in Southeastern Anatolia Region. Since widespread and predominant *A. gossypii* clones (known as super-clones), resistant to insecticides, have been detected in various countries (e.g., Chen et al., 2013).

Table 2. Response of *Aphis gossypii* clones to acetamiprid, imidacloprid, and thiamethoxam in leaf-dip bioassays.

Sample	Acetamiprid				Imidacloprid				Thiamethoxam			
	LC_{50} (95% FL)		Slope±SE	RF	LC_{50} (95% FL)		Slope±SE	RF	LC_{50} (95% FL)		Slope±SE	RF
Susceptible	0.261	(0.20–0.34)	1.060 ± 0.067	–	0.482	(0.38–0.61)	1.076 ± 0.068	–	4.187	(3.43–5.10)	1.301 ± 0.084	-
Babahaki	15.692	(12.69–19.21)	1.388 ± 0.097	60.1	14.776	(11.50–18.75)	1.152 ± 0.095	30.7	61.516	(48.30–79.81)	1.089 ± 0.084	14.7
Şekerören	21.550	(17.31–26.79)	1.306 ± 0.103	82.6	11.336	(8.98–14.09)	1.322 ± 0.101	23.5	25.370	(19.29–33.12)	0.946 ± 0.076	6.1
Yukarıkılıçtaşı	5.910	(4.38–7.67)	1.160 ± 0.110	22.6	22.680	(17.23–30.73)	1.427 ± 0.127	47.1	4.522	(2.90–6.46)	0834 ± 0.076	1.1
Altıntoprak	7.313	(5.70–9.13)	1.381 ± 0.108	28.1	12.259	(9.71–15.23)	1.296 ± 0.091	25.4	30.940	(24.63–38.86)	1.167 ± 0.084	7.4
Çamlıca	7.849	(6.15–9.77)	1.395 ± 0.108	30.1	14.555	(11.39–18.36)	1.192 ± 0.097	30.2	31.848	(24.43–40.40)	1.246 ± 0.102	7.6
Develi	14.406	(11.58–17.77)	1.484 ± 0.107	55.2	26.171	(21.69–31.43)	1.572 ± 0.103	54.3	32.056	(25.02–41.60)	1.213 ± 0.096	7.7
Çatalhurma	7.461	(5.87–9.31)	1.393 ± 0.118	28.6	13.920	(10.96–17.34)	1.403 ± 0.117	28.9	32.094	(25.80–40.41)	1.294 ± 0.104	7.7
Yarımsu	17.945	(14.87–21.72)	1.694 ± 0.141	68.8	32.449	(25.72–40.95)	1.136 ± 0.083	67.3	87.251	(69.69–111.69)	1.366 ± 0.102	20.8
Yazılıkavak	17.376	(14.44–20.76)	1.721 ± 0.127	66.6	25.661	(21.01–31.13)	1.454 ± 0.099	53.2	47.132	(36.74–61.25)	1.031 ± 0.080	11.3

LC_{50} in ppm; FL, fiducial limits; RF, lethal dose ratios (resistance factors).

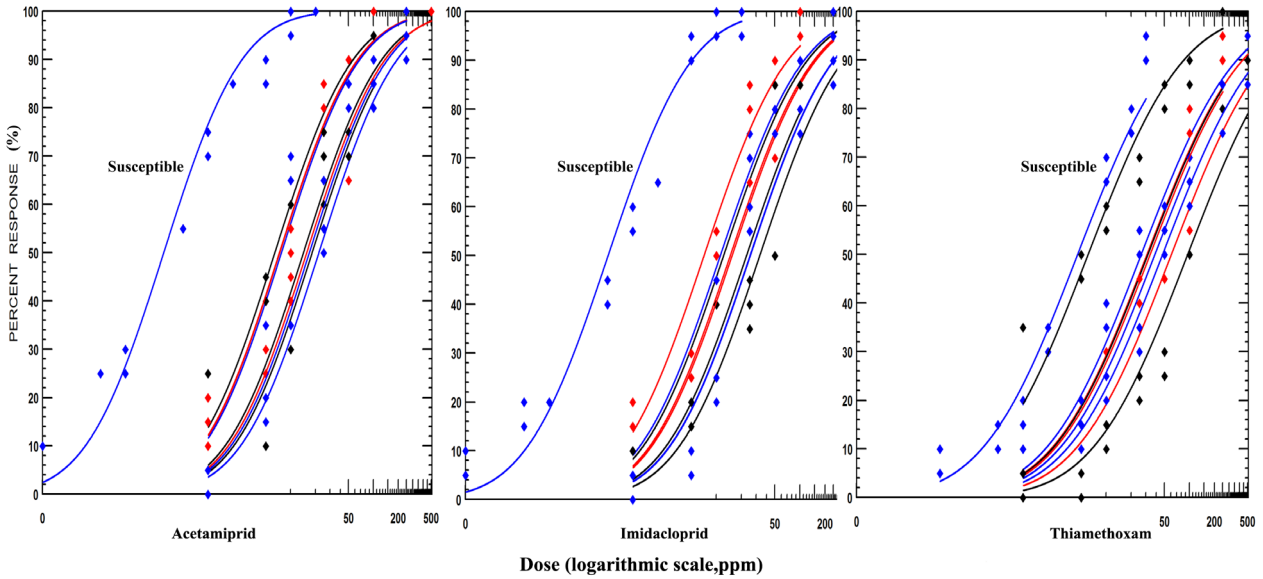

Figure 2. Logarithmic dose probit lines.

Table 3. Carboxylesterase (in µmol naphtol/min/mg protein) and glutathione-S-transferase (in µmol conjugated glutathione/min/mg protein) activities in the field samples and the susceptible strain.

Sample	CarE activity[1]		CarE ratio	GST activity[1]		GST ratio
Susceptible	4.570 ± 0.349	d	1.0	1.795 ± 0.022	c	1.0
Develi	10.616 ± 0.746	c	2.3	5.505 ± 0.111	a	3.1
Altıntoprak	9.553 ± 0.498	cd	2.1	4.813 ± 0.384	a	2.7
Çamlıca	17.053 ± 2.264	ab	3.7	2.830 ± 0.164	bc	1.6
Yukarıkılıçtaşı	9.160 ± 0.699	cd	2.0	2.670 ± 0.140	bc	1.5
Şekerören	8.605 ± 1.076	cd	1.9	3.475 ± 0.384	b	1.9
Babahaki	8.560 ± 1.923	cd	1.9	3.521 ± 0.356	b	2.0
Çatalhurma	17.788 ± 1.521	a	3.9	5.015 ± 0.142	a	2.8
Yarımsu	11.155 ±0.770	bc	2.4	3.572 ± 0.316	b	2.0
Yazılıkavak	9.068 ± 1.426	cd	2.0	3.159 ± 0.106	b	1.8
***Correlations*[2]**						
Acetamiprid	r = –0.026, p = 0.942			r = 0.267, p = 0.456		
Imidacloprid	r = 0.209, p = 0.563			r = 0.313, p = 0.378		
Thiamethoxam	r = 0.188, p = 0.604			r = 0.246, p = 0.493		

[1]Means followed by a different letter differ significantly (Tukey test, $p < 0.05$).
[2]Pearson's correlation coefficients between enzyme activities and LC_{50} values of the examined neonicotinoids.

Our study demonstrated the development of neonicotinoid resistance in *A. gossypii* in Türkiye, which was also confirmed by Ulusoy et al., (2018). Scientists and farmers likewise should be alert and close monitoring of the progress of resistance in the aphid populations is guaranteed. Surveys for the early detection of invaded genotypes carrying the R81T mutation, which will further deteriorate the situation, are also needed. Measures also should be taken to alleviate the selection pressure from neonicotinoids. Insecticide Resistance Management based on rotation approaches, which use a 'window' or block strategy frequently defined by pest life cycle and

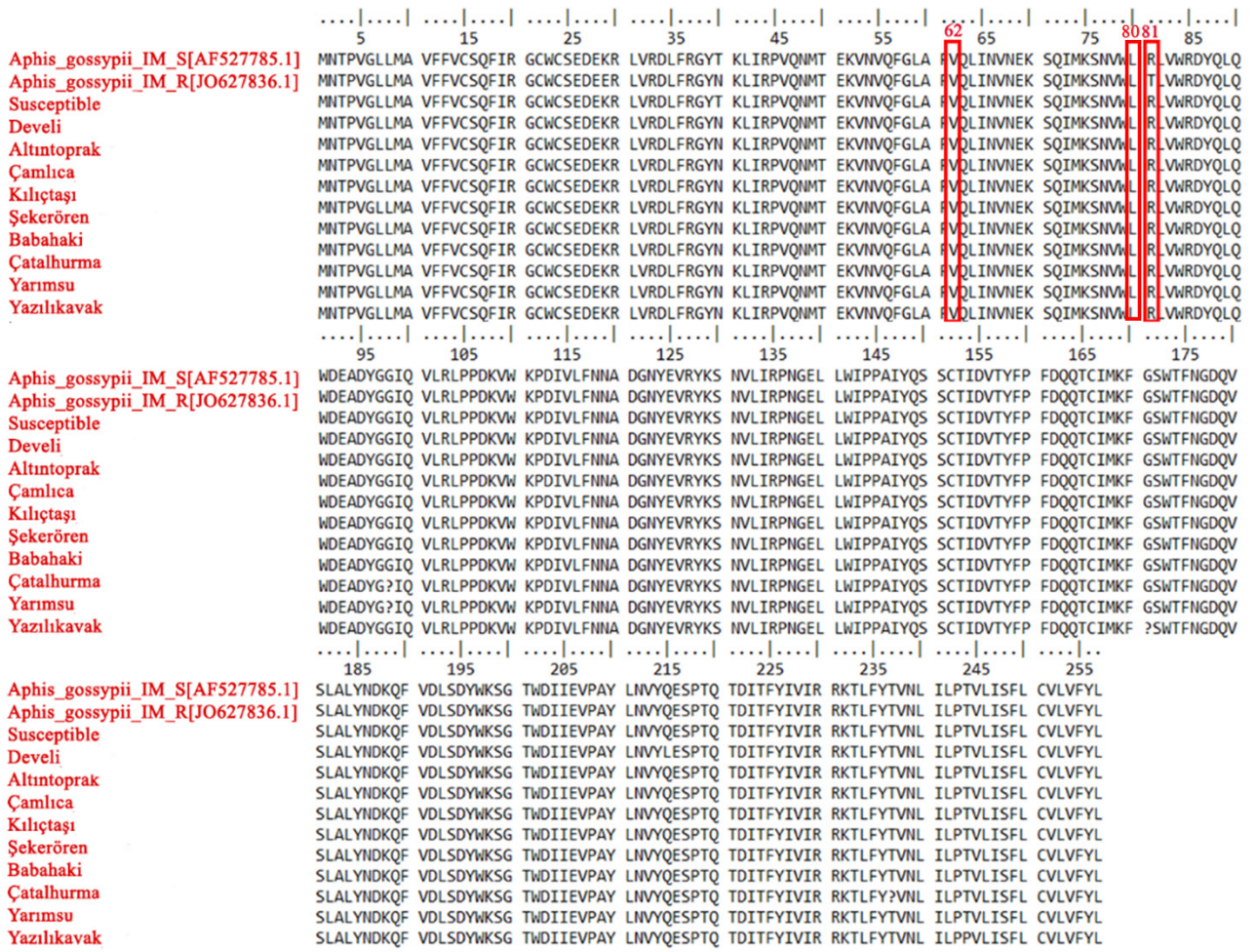

Figure 3. The results of sequence analyze of β1 subunit of *Aphis gossypii* nAChrs.

the crop growth stages should be widespread adopted in cotton grown areas of Türkiye. These strategies involve alternation of insecticides with different mode of actions, population monitoring and the timing of insecticide application according to pest biology and elimination of alternative host plants. Furthermore, natural enemies should be protected and supported, which is one of the most important instruments of IPM and IRM programs.

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Conflict of interest

The authors declare no conflict of interest.

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