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Original article (Orijinal araştırma)

Resistance status in *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) populations against single and mixture of neonicotinoid and synthetic pyrethroid insecticides[1]

Myzus persicae (Sulzer, 1776) (Hemiptera: Aphididae) popülasyonlarında neonikotinoid ve sentetik piretroid insektisitlerin tekli ve karışımlarına karşı direnç durumu

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Abstract

The peach potato aphid, *Myzus persicae (*Sulzer, 1776) (Hemiptera: Aphididae), a vector of many plant virus diseases, causes damage to its wide range of hosts by direct feeding. Chemical control has been the primary method to control this species, and the intensive use of insecticides has led to the development of resistance. In this study, conducted between the years 2017-2019, firstly resistance ratio of five *M. persicae* populations from Antalya, Türkiye were determined by leaf-dip bioassay method. The field populations showed significant resistance to thiamethoxam (between 201-332 fold) and lambda-cyhalothrin (between 50-103 fold) when compared to susceptible population. To identify whether resistance mediated by mutations in sodium channel and nicotinic acetylcholine receptor, DNA regions that encompass “mutation hot-spot” were sequenced. This revealed no population contained R81T mutation that has been previously linked with neonicotinoid resistance. As to synthetic pyrethroid resistance, the L1014F *kdr* mutation was fixed in all field populations. This study is the first description of *kdr* mutation in *M. persicae* populations from Türkiye. Bioassay results also indicated that the toxicity of thiamethoxam and lambda-cyhalothrin mixture was higher than that of lambda-cyhalothrin alone. Our findings can make significant contributions to *M. persicae* resistance management.

Keywords: Bioassay, insecticides, *kdr* mutation, *Myzus persicae*, resistance management

Öz

Birçok bitki virüs hastalığının vektörü olan yeşil şeftali yaprakbiti, *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), doğrudan beslenerek çok sayıda konukçusuna zarar vermektedir. Kimyasal kontrol, bu tür ile mücadelede birincil yol olması ve yoğun kimyasal kullanımı insektisit direnci gelişimine yol açmaktadır. 2017-2019 yılları arasında yapılan bu çalışmada ilk olarak, Antalya, Türkiye'den beş *M. persicae* popülasyonunun direnç oranları yaprak daldırma biyoassay yöntemi ile belirlenmiştir. Örtüaltı popülasyonları, hassas popülasyona kıyasla, thiamethoxam için (201-332 kat aralığında) ve lambda-cyhalothrin için (50-103 kat aralığında) önemli direnç göstermiştir. Direncin sodyum kanalı ve nikotinik asetilkolin reseptöründeki mutasyonlardan kaynaklı olup olmadığını belirlemek için, “mutasyon sıcak nokta”larını kapsayan DNA bölgeleri dizilenmiştir. Popülasyonların neonikotinoid direncine neden olan R81T mutasyonunu içermediği belirlenmiştir. Sentetik piretroid direncinde, L1014F *kdr* mutasyonu tüm arazi popülasyonlarında tespit edilmiştir. Bu çalışma ile Türkiye'deki yeşil şeftali yaprakbiti popülasyonlarında *kdr* mutasyonu ilk defa gösterilmiştir. Biyoassay sonuçları ayrıca, thiamethoxam ve lambda-cyhalothrin karışım toksisitesinin, tek başına, lambda-cyhalothrin toksisitesinden daha yüksek olduğunu göstermiştir. Bulgularımız *M. persicae* direnç yönetimi için önemli katkılar sağlayabilir.

Anahtar sözcükler: Biyoassay, insektisitler, *kdr* mutasyonu, *Myzus persicae*, direnç yönetimi

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Introduction

The peach potato aphid, *Myzus persicae (*Sulzer, 1776) (Hemiptera: Aphididae), occurs worldwide in all vegetable-producing areas. *Myzus persicae* directly absorbs plant sap, causing discoloration and curling of young seedlings and leaves, so the plant growth slows down. In addition, *M. persicae* is the vector of over 100 plant virus diseases (Stevens & Lacomme, 2017). Since it is harmful all year long, especially in greenhouses, growers frequently apply insecticides to control this pest. As a result of this extensive use, resistance to several classes of insecticides including synthetic pyrethroids and neonicotinoids has been documented (IRAC, 2022).

Pyrethroids are neurotoxic insecticides widely used to control many insect pests due to their rapid action, high insecticidal activity and low toxicity on mammals (Rinkevich et al., 2013). The target of pyrethroids are the voltage-gated sodium channels and binding pyrethroids lead to incapacitation of the insect, known as 'knock-down' (Williamson et al., 1993). The resistance to pyrethroids is mainly caused by a point mutation in the S6 segment of domain II of the channel, termed knock-down resistance (*kdr*), resulting in a leucine to phenylalanine (L1014F) replacement. Secondary mutations such as M918L/T and L932F on the segment IIS5 have been associated with an enhanced form of resistance, termed super-*kdr*, in a range of insect species (Davies et al., 2007).

Neonicotinoid insecticides are used against numerous sucking and certain chewing insect pests because of their high efficacy. As selective agonists, neonicotinoids act by binding on insect nicotinic acetylcholine receptors (nAChR) (Jeschke et al., 2011). Previous studies have identified both metabolic and target-site mechanisms underlying resistance to neonicotinoids (Bass et al., 2015). For metabolism-based mechanism, a single P450 gene, *CYP6CY3*, plays a primary role in resistance by metabolizing the nicotine to fewer toxic metabolites (Puinean et al., 2010; Bass et al., 2013). Work on target-site mechanism in *M. persicae* has demonstrated the importance of the arginine to threonine substitution (R81T) in the loop D region of the β1 subunit (Bass et al., 2014).

Due to the failure in control as a result of resistance development, overdose and frequent spraying is carried out, which increases the cost of the product. It causes residues in freshly consumed vegetables, threatens human health and causes problems in exports. It also causes many problems such as environmental pollution and affecting pollinators. Therefore, insecticide resistance management (IRM) is needed for preventing or slowing the development of resistance to insecticides. Monitoring insecticide resistance status is a prerequisite for effective interventions. Distinguishing the underlying mechanism of resistance and cross-resistance spectrum are of key importance to determine effective insecticides for the management of the problem (Vontas & Mavridis, 2019). Application of the different IRM strategies such as rotations and mixtures of two or more insecticides having different modes of action could help to slow the rate of resistance evolution (Georghiou et al., 1986).

In a previous study (Velioğlu et al., 2008) revealing the insecticide susceptibility of *M. persicae* populations collected from vegetable growing fields and greenhouses in Ankara, Antalya and Mersin the concurrent occurrence of high levels of resistance to imidacloprid and thiamethoxam over 4,000 ppm have been recorded. However, the current resistance status and molecular basis of resistance to insecticides in Turkish *M. persicae* populations remains unknown.

In recent years, there has been an increase in the applications of plant protection products in mixtures from different groups in Türkiye. It is stated that the reason for making mixtures is the development of resistance in target pests and that mixture insecticides solve this problem. Therefore, there is a need to clarify the status of active ingredients such as thiamethoxam and lambda-cyhalothrin, which are known to develop resistance alone, in mixture insecticides.

In the present study, insecticide bioassays were conducted to measure the resistance level of *M. persicae* populations against neonicotinoid and synthetic pyrethroid insecticides. The mutations responsible for the resistance were screened across populations. We also tested the efficacy of binary mixtures of neonicotinoid and synthetic pyrethroid insecticides as a resistance management strategy. According to the results of this study, it will be possible to restrict the use of ineffective insecticides due to resistance. In this way, excessive and unnecessary use of insecticides will be prevented, protecting human and environmental health while contributing to the national economy.

Materials and Methods

Aphid populations

The *M. persicae* populations were collected from vegetable greenhouses in Antalya, Türkiye where insecticides were intensively applied in 2017 (Table 1). Susceptible population collected from the unsprayed home garden over years in Çubuk district located at Ankara province in June 2017. Bioassay studies were completed in 2017-2018 and molecular studies were conducted in 2019. Using a modified version of Velioğlu & Toros (2002) approach, the populations were reared in plexiglass cabinets in insect rearing rooms with 27±2°C temperature, 50-60% relative humidity, and 16 hours light and 8 hours dark conditions. In addition, eggplant (*Solanum melongena* L., Solanaceae) plants grown in a temperature-controlled greenhouse were used for maintaining the populations.

Table 1. Sample areas of *M. persicae* populations collected from greenhouses

Population name	Location	Date	Host
Kocaahmetler	Aksu	May 2017	Eggplant
Bahtılı	Konyaaltı	May 2017	Eggplant
Göçmenler	Konyaaltı	May 2017	Pepper
Topallı	Aksu	May 2017	Courgette
Kurşunlu	Kurşunlu	May 2017	Tomato

Insecticides and bioassay studies

Synthetic pyrethroid, lambda-cyhalothrin 50 g/l (EC) and neonicotinoid insecticides, thiamethoxam 240 g/l (SC) and their mixtures, thiamethoxam+lambda-cyhalothrin 141g/l+106 g/l (SC) were used in bioassay studies. Dose-response bioassays were based on the standard leaf-dip method (Cahill et al.,1995). Briefly, eggplant leaf discs (3.7 cm in diameter) were immersed in serial dilutions of insecticide for 20 seconds. Leaf discs were placed in bioassay containers poured with 1% agar and left to dry under a fume hood for 2 hours. 20 wingless adults *M. persicae* were then transferred onto the dried leaf discs. Control treatment consisted of only 0.02% Triton X-100. Mortality was assessed after 48 hours in a climate cabinet with 25 ±1°C temperature and 16 hours of light and 8 hours of darkness. Trials were carried out between 30-400 ppm and 3 replications for each insecticide.

Detection of target-site resistance mutations

Sanger sequencing were used to screen the populations for the *kdr* and *super-kdr* resistance (M918L/T, L932F, L1014F) for synthetic pyrethroid resistance; R81T for neonicotinoid resistance. DNA extraction was performed using “HP PCR product purification (Roche)” kit following the manufacturer’s instructions (Roche Diagnostics, Meylan, France). The quality and quantity of DNA samples were evaluated by spectrophotometer (Nanodrop). A partial gene fragments containing aforementioned mutations were amplified by PCR from 50 ng aliquots of DNA with the primers in Table 2.

Table 2**.** Primer sequences used in molecular studies

Primer name	Sequence 5′-3′	Fragment size	Reference
kdr-F1	TCGTGGCCCACACTGAATCT	578 bp	Cassanelli et al., 2005
kdr-R4	GTTCATGTAAGATACATGAATTC		
MpB1TMF	TAGTTCTAACTTATTGCCTGCAGCTAT	225 bp	Puinean et al., 2011
MpB1TMR	GCGGTCAGGAAGTCTAATACGTTA		

The PCR reactions consisted of 4 µl of 5xPCR Buffer Solution (FirePol, Solis BioDyne, Tartu, Estonia), 0.5 µl of each primer (10 mM), 1 µl gDNA, 14 µl distilled water in a final volume of 20 µl. Thermal cycling amplification consisted of an initial denaturation phase of 3 min at 95°C and then 30 cycles (95°C for 30 s, 60°C for 30 s, 72°C for 1 min) with a final extension at 72°C for 7 min. Electrophoresis on 1.2% agarose gel in 1X TBE buffer for 1 h at 100 V was performed to verify the size of the PCR products.

Data analysis

LC_{50} values and 95% confidence intervals (CI) were calculated by probit analysis using PoloPC (LeOra Software,1994 Company, Petaluma, USA). Resistance ratios were calculated as the LC_{50} value of field populations with respect to the LC_{50} calculated for the susceptible population. LC_{50} values were considered statistically significant if their respective 95% confidence limits (CL) did not overlap (Robertson et al., 2007).

The presence/absence of insecticide resistance mutations were determined by visual examination of sequencing chromatograms. Obtained chromatograms were analyzed using the Geneious 11.1.4 software (http: //www.geneious.com, Kearse et al., 2012) and compared with the sequences deposited in NCBI database (GenBank accession: AM711603 for sodium channel, GenBank accession: AJ251838 for nAChR).

Results

Toxicity of single insecticides

Results of the *M. persicae* bioassays with insecticides alone are shown in Table 3. Between 10-90% mortality has been observed among populations. A mortality rate below 10% was observed in the control group. The response of thiamethoxam and lambda-cyhalothrin were different from susceptible population (non-overlapping of 95% CI) in all field populations**.** The LC_{50} value of the susceptible population for thiamethoxam and lambda-cyhalothrin were found to be 0.172 and 0.971 ppm. The LC_{50} values of field populations were in the range of 34.578 to 57.256 pmm and 48.758 to 100.066 ppm for thiamethoxam and lambda-cyhalothrin, respectively. While Göçmenler recorded lowest resistance ratio of 201.03 and 50.214 fold, Kocaahmetler was showing highest resistance ratio of 332.88 and 103.05 fold to thiamethoxam and lambda-cyhalothrin, respectively in comparison to susceptible population.

Table 3. Toxicity of thiamethoxam and lambda-cyhalothrin alone to *M. persicae*

Insecticide	Populations	N	LC_{50} (CI 95%)		Slope+SE	X^2 (df)	*P* value	RR_{50}
Thiamethoxam	Susceptible	400	0.17 (0.028-0.295)	a[1]	1.02±0.29	2.11 (14)	0.99	-
	Bahtılı	400	46.43 (36.662-57.365)	bc	2.24±0.33	12.55 (15)	0.64	269.94
	Göçmenler	400	34.57 (26.358-43.500)	b	2.11±0.24	16.37 (15)	0.36	201.03
	Kocaahmetler	420	57.25 (45.573-70.432)	c	1.70±0.16	12.65 (16)	0.70	332.88
	Kurşunlu	400	49.86 (38.386-63.078)	bc	1.77±0.17	16.50 (15)	0.35	289.91
	Topallı	400	48.07 (38.103-58.618)	bc	2.19±0.30	9.46 (15)	0.85	279.48
Lambda-cyhalothrin	Susceptible	400	0.97 (0.761-1.039)	a[1]	1.78±0.22	8.41 (14)	0.86	-
	Bahtılı	420	94.81 (80.045-111.042)	d	2.18±0.20	12.62 (16)	0.70	97.64
	Göçmenler	400	48.75 (39.494-57.921)	b	2.35±0.25	12.30 (15)	0.66	50.21
	Kocaahmetler	420	100.06 (84.846-116.642)	d	2.34±0.22	9.02 (16)	0.91	103.05
	Kurşunlu	400	69.12 (59.279-79.607)	c	2.75±-0.25	6.34 (15)	0.97	71.18
	Topallı	400	93.26 (77.854-110.237)	cd	2.14±0.22	9.76 (15)	0.83	96.04

[1] Values followed by the same letter within a row are not statistically different.
N: number of individuals; *SE*: Standard Error; *df*: degrees of freedom; X^2: chi-squared test; *CI*: confidence interval; *RR*: resistance ratio.

Toxicity of binary mixture

Results of the *M. persicae* bioassays with insecticides mixture are shown in Table 4. The response of thiamethoxam and lambda-cyhalothrin binary mixture were different from susceptible population (non-overlapping of 95% CI) in all field populations (Robertson et al., 2007). The LC_{50} values were ranging from 30.758 to 76.114 ppm in field populations resulting in 39.841 to 98.593 fold resistance compared to susceptible population. Interestingly, the mixture of thiamethoxam and lambda-cyhalothrin exhibited significantly higher toxicity to Bahtılı, Kurşunlu and Topallı ($p < 0.05$, non-overlapping 95% CI) and marginally significant toxicity in Göçmenler and Kocaahmentler than the toxicity of lambda-cyhalothrin alone Table 3-4). Thiamethoxam and lambda-cyhalothrin together have been found to be slightly more effective than thiamethoxam taken alone.

Table 4. Toxicity of thiamethoxam and lambda-cyhalothrin in combination to *M. persicae*

Insecticide	Populations	N	LC_{50} (CI 95%)		Slope+SE	X^2 (df)	*P* value	RR
Thiamethoxam + Lambda-cyhalothrin	Susceptible	340	0.77 (0.131-1.261)	a[1]	1.76±0.21	5.44 (12)	0.94	-
	Bahtılı	340	57.88 (46.798-68.822)	c	2.54±0.29	4.55 (12)	0.97	74.97
	Göçmenler	340	30.75 (25.904-40.424)	b	3.50±0.54	3.56 (12)	0.99	39.84
	Kocaahmetler	340	76.11 (62.983-89.833)	c	2.45±0.26	6.67 (12)	0.88	98.59
	Kurşunlu	340	44.83 (36.639-54.390)	bc	2.59±0.33	5.66 (12)	0.93	58.07
	Topallı	340	55.36 (44.406-66.075)	bc	2.52±0.29	4.56 (12)	0.97	71.72

[1] Values followed by the same letter within a row are not statistically different.
N: number of individuals; *SE*: Standard Error; *df*: degrees of freedom; X^2: chi-squared test; *CI*: confidence interval; *RR*: resistance ratio.

Detection of target-site resistance mutations

The ~ 578 and 225 bp fragments of the partial (IIS4-IIS6) sodium channel and nAChR β1 subunit were PCR amplified and sequenced. The sequences were aligned against known sequences of *M. persicae* (GenBank accession: AM711603 for sodium channel, GenBank accession: AJ251838 for nAChR) and depicted in Figure 1.

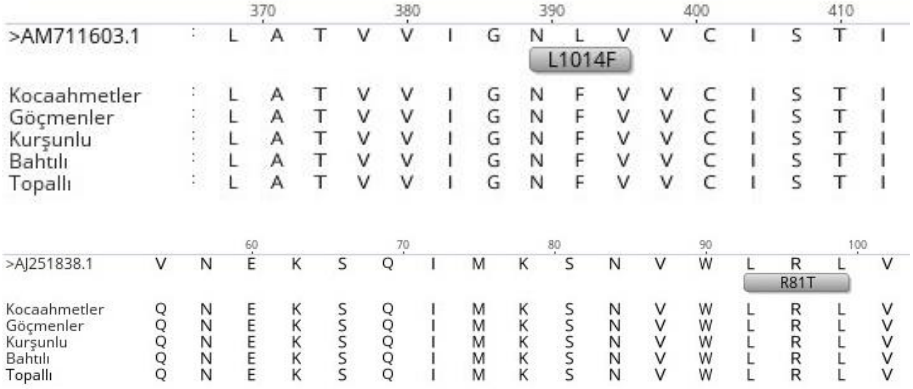

Figure 1. Detection of *kdr* (L1014F) mutation on sodium channel and R81T mutation in nAChR.

The comparisons revealed a *kdr* mutation (L1014F mutation, numbering based on the housefly sequence GenBank accession: X96668) was fixed in all field populations of *M. persicae*. The secondary mutations, M918T\L and L932F were absent. In the case of neonicotinoids, the R81T mutation could not be verified by direct sequencing (Figure 1). Thus, the involvement of R81T mutation in neonicotinoid resistance seems unlikely. Whether other target-site mechanisms and/or metabolic mechanisms play a role in the resistance warrants further investigation.

Discussion

Resistance to insecticides in *M. persicae* in previous studies is widespread worldwide. Insecticide resistance of *M. persicae* has been detected in Europe, Japan, North America and Australia (Soderlund, 2008). Velioğlu&Toros (2002) stated that more than 600-fold resistance was observed against pirimicarb in three different *M. persicae* populations collected from the province of İçel. Additionally, Velioğlu et al. (2008) investigated the resistance of *M. persicae* populations collected from vegetable growing fields and greenhouses in Ankara, Antalya and Mersin. They found the highest LC_{50} value for imidacloprid as 7,624 ppm and for thiamethoxam as 4,098 ppm for the populations collected from Antalya. In this study, different levels of LC_{50} values against insecticides from different groups were found in *M. persicae* populations. Different levels of resistance could be influenced by operational factors such as application frequency and insecticide coverage (Rosenheim & Tabashnik, 1990). According to the results there was a significant level of resistance to the insecticides tested in five different populations (Kocaahmetler, Topallı, Kurşunlu, Bahtılı, Göçmenler) collected from greenhouse vegetable fields in Antalya province. Overall, it has been confirmed that *M. persicae* has gained serious resistance to insecticides in and around Antalya province where pest control depends intensely on insecticide use.

To explore the molecular basis of lambda-cyhalothrin resistance, DNA regions of voltage-gated sodium channel that encompass a 'mutation hot-spot' was amplified and sequenced. This revealed a *kdr* mutation was fixed in all field populations of *M. persicae*. No populations were observed that carry either M918L/T and L932F. The current study is the first report of L1014F mutation in pyrethroid-resistant aphid populations from Türkiye. This mutation has been associated with pyrethroid resistance in over 20 different arthropod species including houseflies, cockroaches, mosquitoes, and aphids (Miyazaki et al., 1996; Martinez-Torres et al., 1997; Martinez-Torres et al., 1999). It was shown that the presence of kdr both reduced the action of insecticide and made the sodium channel less likely to open by changing the gating properties (Davies & Williamson, 2009; Du et al., 2010).

To investigate whether neonicotinoid resistance was mediated by R81T mutation in *M. persicae* we PCR amplified and sequenced the β1 subunit of the nAChR. The R81T mutation could not be verified by direct sequencing in this study. This mutation was first reported in a neonicotinoid-resistant population of *M. persicae* from France (Bass et al., 2011; Slater et al., 2011). Studies using enzyme inhibitors and microarray analysis revealed that P450-mediated detoxification, with the overexpression of *CYP6CY3*, may contribute to neonicotinoid resistance (Puinean et al., 2010; Bass et al., 2013). Indeed, further study confirmed that functionally expressed *CYP6CY3* is highly efficient at metabolizing nicotine to fewer toxic metabolites in vitro (Bass et al., 2013). Therefore, further investigations are needed to identify whether overexpression of *CYP6CY3* confer resistance to neonicotinoids in aphids populations from Türkiye.

The high selection pressure to *M. persicae* is seems to be the main cause about insecticide resistance in Antalya. To delay the onset of resistance development, it is essential to use IRM strategies which include use of insecticide in mixture. In theory, a binary mixture could ensure that insects that survive exposed to one compound will be killed by the other compound (Shi et al., 2012). In the current study, bioassay results indicated that the toxicity of thiamethoxam and lambda-cyhalothrin mixture was higher than that of lambda-cyhalothrin alone. Carbamates and organophosphates have been shown to synergize pyrethroids in *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae), *Anopheles gambiae* Giles, 1900 (Diptera: Culicidae), *Leptinotarsa decemlineata (*Say, 1824) (Coleoptera: Chrysomelidae) and *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) (Guillet et al., 2001; Martin et al., 2003; Bielza et al., 2007; Jiang et al., 2011). Thus, the high rates of resistance to insecticides in insects could be reduced by the use of insecticide mixtures, making it a good option for an anti-resistance strategy. However, it is important to bear in mind that it should be used carefully since it may accelerate the development of multiple resistance (Sayyed et al., 2004).

In conclusion, our research findings suggest that over use of these insecticides to control *M. persicae* is likely to contribute to the development of resistance to thiamethoxam and lambda-cyhalothrin. In the study, all field populations were found to be resistant to thiamethoxam and lambda-cyhalothrin. This is supported by the presence of L1014F, a *kdr* mutation in the sodium channel. Further study is required to identify whether P450-based detoxification confer resistance to neonicotinoids in aphid populations from Türkiye. The present data show the application of thiamethoxam and lambda-cyhalothrin mixture could be considered to slow the development of resistance to lambda-cyhalothrin. Our findings may have considerable implications for *M. persicae* resistance management.

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References

Ahmad, M., M. Arif & I. Denholm, 2003. High Resistance of Field Populations of the Cotton Aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) to Pyrethroid Insecticides in Pakistan. Journal of Economic Entomology, 96 (3): 875-878.

Bass, C., A. M. Puinean, C. T. Zimmer, I. Denholm, L. M. Field, S. P. Foster, O. Gutbrod, R. Nauen, R. Slater & M. S. Williamson, 2014. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. Insect Biochemistry and Molecular Biology, 51: 41-51.

Bass, C., A. M. Puinean, M. Andrews, P. Cutler, M. Daniels, J. Elias, V. L. Paul, A. J. Crossthwaite, I. Denholm, L. M. Field, S. P. Foster, R. Lind, M. S. Williamson & R. Slater, 2011. Mutation of a nicotinic acetylcholine receptor b subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. BMC Neuroscience, 12: 1-11.

Bass, C., C. T. Zimmer, J. M. Riveron, C. S. Wilding, C. S. Wondji, M. Kaussmann, L. M. Field, M. S. Williamson & R. Nauen, 2013. Gene amplification and microsatellite polymorphism underlie a recent insect host shift. Proceedings of the National Academy of Sciences of the United States of America, 110 (48): 19460-19465.

Bass, C., I. Denholm, M. S. Williamson & R. Nauen 2015. The global status of insect resistance to neonicotinoid insecticides. Pesticide Biochemistry and Physiology, 121: 78-87.

Bielza, P., P. J. Espinosa, V. Quinto, J. Abellan & J. Contreras, 2007. Synergism studies with binary mixtures of pyrethroid, carbamate, and organophosphate insecticides on *Frankliniella occidentalis* (Pergande). Pest Management Science, 63 (1): 84-89.

Cahill, M., F. J. Byrne, K. Gorman, I. Denholm & A. L. Devonshire, 1995. Pyretroid and organophosphate resistance in the tobacco whitefly *Bemisia tabaci* (Homoptera : Aleyrodidae). Bulletin of Entomological Research, 85 (2): 181-187.

Cassanelli, S., B. Cerchiari, S. Giannini, D. Bizzaro, E. Mazzoni & G. C. Manicardi, 2005. Use of the RFLP-PCR diagnostic test for characterizing MACE and kdr insecticide resistance in the peach potato aphid *Myzus persicae*. Pest Management Science, 61 (1): 91-96.

Davies, T. G. & M. S. Williamson, 2009. Interactions of pyrethroids with the voltagegated sodium channel. Bayer CropScience Journal, 62 (2): 159-178.

Davies, T. G., L. M. Field, P. N. Usherwood & M. S. Williamson, 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. IUBMB Life, 59 (3): 151-62.

Du, Y., W. Song, J. R. Groome, Y. Nomura, N. Luo & K. Dong, 2010. A negative charge in transmembrane segment 1 of domain II of the cockroach sodium channel is critical for channel gating and action of pyrethroid insecticides. Toxicology and Applied Pharmacology, 247 (1): 53-59.

Georghiou, G.P. & C.E. Tayler, 1986. “Factors Influencing the Evolution of Resistance, 157-169”. In: Pesticide Resistance: Strategies and Tactics for Management. National Academy of Sciences, Washington, DC, 277 pp.

Guillet, P., R. N’Guessan, F. Darriet, M. Traorelamizana, F. Chandre & P. Carnevale, 2001. Combined pyrethroid and carbamate ‘twoin-one’ treated mosquito nets: field efficacy against pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus*. Medical and Veterinary Entomology, 15 (1): 105-112.

Herron, G.A., K. Powis & J. Rophail, 2001. Insecticide resistance in *Aphis gossypii* Glover (Hemiptera: Aphididae), a serious threat to Australian cotton. Australian Journal of Entomology, 40 (1): 85-89.

IRAC, 2022. Insecticide Resistance Action Committee. IRAC Susceptibility Test Methods Series. Method No: 019 (Web page: https:// irac-online.org) (Date accessed 10.11.2022).

Jeschke, P., R. Nauen, M. Schindler & A. Elbert, 2011. Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry, 59 (7): 2897-2908.

Jiang, W-H., W-C. Guo, W-P. Lu, X-Q. Shi, M-H. Xiong, Z-T. Wang & G-Q. Li, 2011. Target site insensitivity mutations in the ache and ldvssc1 confer resistance to pyrethroids and carbamates in *Leptinotarsa decemlineata* in northern xinjiang uygur autonomous region. Pesticide Biochemistry and Physiology. 100 (1): 74-81.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes & A. Drummond, 2012. Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28 (12): 1647-1649.

Leora Software, 1994. Polo-Pc: A User’s Guide to Probit or Logit Analysis. LeOra Software, Berkeley, CA., 28 pp.

Martin T., O. G. Ochoi, M. Vaissayre & D. Fournier, 2003. Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from West Africa. Journal of Economic Entomology, 96 (2): 468-474.

Martinez-Torres, D., A. L. Devonshire & M.S. Williamson, 1997. Molecular studies of knockdown resistance to pyrethroids: cloning of domain II sodium channel gene sequences from insects. Pesticide Science, 51 (3): 265-270.

Martinez-Torres, D., S. P. Foster, L. M. Field, A. L. Devonshire & M. S. Williamson, 1999. A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Insect Molecular Biology, 8 (3): 339-346.

Miyazaki, M., K. Ohyama, D. Y. Dunlap & F. Matsumura, 1996. Cloning and sequencing of the para-type sodium channel gene from susceptible and kdr-resistant German cockroaches (*Blattella germanica*) and housefly (*Musca domestica*) Molecular Genetics and Genomics, 252: 61-68.

Puinean A. M., B. Janelias, R. B. Slater, A. Warren, L. M. Field, A. Williamson, S. A Martin & C. Bass 2011. A development of a high-through put real-time PCR assay for the detection of the R81T mutationin the nicotinic acetylcholine receptor of neonicotinoid-resistant *Myzus persicae*. Pest Management Science, 96 (2): 80-85.

Puinean, A. M., S. P. Foster, L. Oliphant, I. Denholm, L. M. Field, N. S. Millar, M. S. Williamson & C. Bass, 2010. Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. Plos Genetics, 6 (6): 1000999.

Rinkevich, F. D., Y. Du & K. Dong, 2013. Diversity and Convergence of Sodium Channel Mutations Involved in Resistance to Pyrethroids. Pesticide Biochemistry and Physiology, 106 (3): 93-100.

Robertson, J. L., R. M. Russell, H. K. Preisler & N. E. Savin, 2007. Pesticide Bioassays with Arthropods, Second ed. CRC Press, Boca Raton, FL, U.S.A. 2nd edition, xxii + 199 pp. Hardcover, ISBN-10: 0-8493-2331-2, Florida Entomologist, 91 (3): 510-511.

Rosenheim, J. A. & B. E. Tabashnik, 1990. Evolution of pesticide resistance: interactions between generation time and genetic, ecological, and operational factors. Journal of Economic Entomology, 83 (4): 1184-1193.

Sayyed, A. H., D. Omar & D. J. Wright, 2004. Genetics of spinosad resistance in a multiresistant field-selected population of *Plutella xylostella*. Pest Management Science, 60 (8): 827-832.

Shi, X., M. Xiong, W. Jiang, Z. Wang, W. Guo, Z. Xia, W. Fu & G. Li, 2012. Efficacy of endosulfan and fipronil and joint toxic action of endosulfan mixtures against *Leptinotarsa decemlineata* (Say). Journal of Pest Science, 85: 519-526.

Slater, R., V. L. Paul, M. Andrews, M. Garbay & P. Camblin, 2011. Identifying the presence of neonicotinoid resistant peach-potato aphid (*Myzus persicae*) in the peach growing regions of southern France and northern Spain. Pest Management Science, 68 (4): 634-638.

Soderlund, D. M., 2008. Pyrethroids, knockdown resistance and sodium channels. Pest management science, 64 (6): 610-616.

Stevens, M.& C. Lacomme, 2017. "Transmission of Plant Viruses, 323-361". In: Aphids as Crop Pests (Eds. H. F. van Emden & R. Harrington) CABI, Wallingford, 686 pp.

Velioğlu, A. S. & S. Toros, 2002. Değişik bölgelerden toplanan *Myzus persicae* (Sulz.) (Hom.: Aphididae) popülasyonlarının bazı insektisitlere karşı dayanıklılık düzeylerinin araştırılması. Bitki Koruma Bülteni, 42 (1-4): 67-79 (in Turkish with abstract in English).

Velioğlu, A. S., C. Erdoğan, M. O. Gürkan & G.D. Moores, 2008. Sebzelerde Zarar Yapan *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) Popülasyonlarının İnsektisitlere Direnci ile Biyokimyasal Mekanizmalarının İncelenmesi, (TÜBITAK TOVAG 105 O 576'nolu Proje Sonuç Raporu), Türkiye Bilimsel ve Teknolojik Araştırma Kurumu, 110 s (in Turkish with abstract in English).

Vontas, J. & K. Mavridis, 2019. Vector population monitoring tools for insecticide resistance management: Myth or fact? Pesticide Biochemistry and Physiology, 161: 54-60.

Williamson, M. S., I. Denholm, C. A. Bell & A. L. Devonshire, 1993. Knockdown resistance (*kdr*) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). Molecular and General Genetics, 240: 17-22.