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Original article (Orijinal araştırma)

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

Çukurova Bölgesi (Türkiye) pamuk alanlarında *Aphis gossypii* (Glover) (Hemiptera: Aphididae) neonikotinoid direnci

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Abstract

Cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), is a major pest in cotton fields. Neonicotinoids are important and highly prevalent insecticides currently used against *A. gossypii* and other herbivorous insect pests in the Mediterranean Region of Turkey. However, some insecticide applications against *A. gossypii* in the Çukurova Region have failed despite using high rates. Therefore, bioassays and enzyme analyses were conducted to determine resistance to imidacloprid and thiamethoxam in *A. gossypii* populations collected in 2015-2016 from cotton fields in this region. Resistance factors (RF) were 54.6 to 206.5 fold for imidacloprid and 5.7 to 65.7 fold for thiamethoxam. Populations from Kürkçüler (RF 206.5) had the highest LD_{50} for imidacloprid and from Körkuyu (RF 65.7) for thiamethoxam. Enzyme analysis revealed statistically higher metabolic resistance. Maximum enzyme activities were 17.8, 142.3 and 3.8 nM/min/mg protein for carboxylesterase for in Körkuyu, for glutathione S-transferase in Bahçe and for cytochrome P450 monooxygenase in Körkuyu, respectively. This study revealed the development of resistance in *A.gossypii* to neonicotinoid insecticides in Turkey and the need for new management strategies to break this resistance.

Keywords: *Aphis gossypii*, biyoassay, cotton, neonicotinoid, resistance

Öz

Pamuk yaprakbiti, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), pamuk tarım alanlarında ana zararlılardandır. Neonicotinoidler, Akdeniz Bölgesi'nde *A. gossypii* ve diğer herbivor böceklerin mücadelesinde oldukça yaygın kullanılan önemli bir insektisit grubudur. Ancak, Çukurova Bölgesi'nde *A. gossypii*'ye karşı bazı insektisit uygulamaları yüksek oranlarda kullanılmasına rağmen başarısız olmuştur. Bu nedenle, bu bölgedeki pamuk alanlardan 2015-2016 toplanan *A. gossypii* popülasyonlarında imidacloprid ve thiamethoxam dayanıklılık düzeyi belirlemek amacıyla biyoassay ve enzim analizleri yapılmıştır. Analizler sonucunda imidacloprid için 54.6-206.5 (dirençlilik faktörü: RF) arasında, thiamethoxam için 5.7-65.7 arasında LD_{50} dayanıklılık katsayıları bulunmuştur. Kürkçüler (RF 206.55) popülasyonu imidacloprid için, Körkuyu (RF 65.72) popülasyonu da thiamethoxam için en yüksek LD_{50} değerine sahiptir. Enzim analizi istatistiki anlamda yüksek metabolik direnci ortaya çıkarmıştır. Her iki insektisit içinde en yüksek enzim akitviteleri, karboksil esteraz enzimi Körkuyu popülasyonunda 17,8 nM/dk/mg protein, glutatyon S-transferaz (GSTs) enzimi Bahçe popülasyonunda 142,3 nM/dk/mg protein ve Körkuyu popülasyonunda 3,8 nM/dk/mg protein ile en yüksek monooksigenaz P450 enzim aktivitesi bulunmuştur. Bu çalışma, Türkiye'de neonicotinoidlere karşı *A. gosyypii*'de direnç gelişmesini ve bu direncin kırılması için yeni yönetim stratejilerine ihtiyaç olduğunu ortaya koymuştur.

Anahtar sözcükler: *Aphis gossypii,* biyoassay, pamuk, neonicotinoid, dirençlilik

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Introduction

The cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is one of the most important pest of cotton plants. It has a polyphagous habit and has a wide host range (Blackman & Eastop, 2000; Tomizawa & Casida, 2005). As the main pest of cotton, *A. gossypii* produces honeydew on the plant that supports the development of sooty molds. It causes direct damage through sucking leaves and indirectly by transmitting some viral pathogens to its host (Kim et al., 1986). One of the most effective control methods being used worldwide for this pest is the application of insecticides. However, the development of resistance to insecticides (organophosphate, carbamate, pyrethroid and neonicotinoids) has been reported in many countries (Ahmad et al., 2003; Wang et al., 2007).

Neonicotinoids are highly effective chemical insecticides that control many important pests; providing good market value (Nauen et al., 2008; Jeschke et al., 2011). They have been used effectively against the coleopteran, dipteran and lepidopteran insects by foliar, soil and seed treatments in more than 120 countries for 25 years (Nauen et al., 2008; Bass et al., 2015). The first neonicotinoid, imidacloprid, was discovered in 1990 and it affects the central nervous system of insects (Elbert et al., 2008; Bass et al., 2015).

Recently, an increasing number of studies associated with neonicotinoid resistance in cotton aphids have been published (Herron et al., 2011; Bass et al., 2015). Thiamethoxam and imidacloprid, which belong to the neonicotinoid group, are the most commonly used insecticides (Herron et al., 2011). These insecticides bind irreversibly to insect nerve cell nicotinic acetylcholine receptors (nAChR), resulting in impaired nerve function in insects (Herron et al., 2011; Jeschke et al., 2011). More than 500 peer-reviewed papers have been published on neonicotinoid resistance issues in different pest insects in which a substantial proportion of these refer specifically on imidacloprid resistance (Bass et al., 2015). Neonicotinoides have been widely used in the management of aphids since 1990. This has led to the development of resistance to imidacloprid and thiamethoxam in the aphids (Herron et al., 2011; Gore et al., 2013). The different mechanisms of insecticide resistance have been a focus of many studies. Particularly, in *A. gossypii* a number of enzymes having been reported to be involved in detoxification of these insecticides.

Metabolic resistance due to overproduction of total esterase causes detoxification of organophosphates, carbamates and pyrethroids in Hemiptera and Diptera (Field et al., 1999; Bass & Field, 2011). In addition, it has been reported that acetylcholinesterase and alpha-naphthyl acetate (α-NA) esterases levels were higher in the imidacloprid resistant *A. gossypii* populations (Wang et al., 2002). High levels of glutathione S-transferases (GST) activity has been widely observed in organophosphate, organochlorine, dichlorodiphenyltrichloroethane (DDT), and pyrethroid insecticide chemical classes in the formation of individual resistance (Ranson & Hemingway, 2005; Li et al., 2007).

Cytochrome P450 occurs widely in nature and is involved in many biological processes, such as hormone synthesis and the metabolism of xenobiotics (Scott & Wen, 2001). In insects, cytochrome P450 monooxygenases (P450) is implicated in resistance to insecticides through the degradation of these foreign compounds to more soluble and less toxic forms (Scott & Wen, 2001; Rauch & Nauen, 2003). In addition, high levels of P450 enzyme activity were found in neonicotinoid insecticide resistant *A. gossypii* populations (Shang et al., 2012; Seyedebrahimi et al., 2015).

Pesticide use in Adana, Mersin and Antalya Provinces accounts for about 40% of Turkey's annual pesticide consumption. While the rate of pesticide usage in Adana is 11%, this rate is 16% in Icel. It has been estimated that about 40% of total pesticide use is on cotton and cereals and this is mostly insecticides (Dağ et al., 2000; Ulusoy et al., 2017a,b). As of 2018, in Turkey there were more than 250 licensed insecticide products for use against *A. gossypii* in cotton fields (Anonymous, 2018). Half of these insecticide are neonicotinoids (40% imidacloprid, 46% acetamiprid, 12% thiamethoxam, 2% clothianidin), 39% organophosphates, <8% pyrethroids and <5% pyridines (Anonymous, 2018). So clearly the highest consumption is of neonictinoid group insecticides. Velioğlu et al. (2008) reported that insecticide resistance resulted in insensitivity to acetylcholinesterase in *A. gossypii* populations in Çukurova cotton fields. Ulusoy et al. (2017a,b) reported varying levels of insecticide resistance in *A. gossypii* populations for clothianidin and acetamiprid in cotton fields of Adana Province. The problem of cotton pest has been rapidly increasing worldwide, especially in irrigated fields. The increase in pest populations leads

extensive chemical applications and occurrence of the resistance problems (Tomlin, 1997). The most intensive insecticide applications are in the Mediterranean Region of Turkey (Velioğlu et al., 2008). Intensive use of chemicals disrupts the existing natural balance in agroecosystems and leads to development of insecticide resistance. Similar to other countries, the cotton agroecosystems in Turkey has been challenged by the insecticide resistance problems (Tomlin, 1997).

The Mediterranean Region of Turkey is one of the most important agricultural areas where polyculture is frequently practiced. For this reason, pest management systems are highly dependent on the use of insecticides. Thus, the objective of this study is to determine the neonicotinoid resistance status in the *A. gossypii* populations collected from the different cotton growing areas of in Adana Province located in Çukurova Region of Turkey.

Material and Methods

Aphid populations

Aphis gossypii populations was sampled in 2015 in the Çukurova Region where intensive insecticide applications have been used (Table 1). A susceptible clone which has been maintained for 20 years under in vitro conditions was obtained from Bayer (Leverkusen, Germany). All populations were routinely reared on *Gossypium hirsutum* grown in net-covered cages, (70×50×40 cm), under greenhouse conditions at 22°C, 65±5% RH and 16:8 h L:D photoperiod. The plants in the cages were replaced every 2 weeks with new ones in order to keep colonies alive. The test population was collected from the cotton fields located in the different areas of Çukurova Region (namely, Körkuyu, Durhasandede, Bebeli, Kürkçüler, Bahçe, Çukurova, Yumurtalık) in Adana Province.

Table 1. *Aphis gossypii* populations from cotton fields and the susceptible population tested in this study

Population	Coordinates	Collection date
Körkuyu	35º57’09.3" N, 35º47’36.9" E	May 2015
Durhasandede	36º56’47.5" N, 35º45’25.6" E	May 2015
Bebeli	36º38'15.2" N, 35º27'08.0" E	June 2015
Bahçe	36º37'26.8" N, 35º25'47.3" E	June 2015
Çukurova	37º01'16.1" N, 35º21'17.6" E	May 2015
Kürkçüler	37º02'16.1" N, 35º33'32.6" E	May 2015
Yumurtalık	36º48'29.0" N, 35º43'10.2" E	June 2015
Susceptible	Germany	1998

Insecticides and bioassays

In the experiments, imidacloprid (350 g/L soluble concentrate) and thiamethoxam (400 g/L soluble concentrate) commercial formulations were used. Aphid samples were taken for bioassay experiments after one to two generations of greenhouse culture conditions. The Insecticide Resistance Action Committee 019 bioassay method was used to determine the resistance status of the aphids to the insecticides (IRAC, 2015). Leaf samples taken from cotton plants were cut into 4 cm diameter discs. The leaves were dipped in the insecticide solutions for 10 s, dried and then placed in Petri dishes containing 1.5% agar. About six different doses, excluding a control, were tested in three replicates. The field-collected populations were tested against 1-100 ppm for imidacloprid and 1-200 ppm for thiamethoxam, and the susceptible population against 0.1-30 ppm for both insecticides. Distilled water containing 0.2% Triton-X (0.2 g/L) was used as the control. About 30 adult aphids were transferred to each Petri dish. After the Petri dishes were covered with Parafilm, they were placed in a controlled environment at 22±1ºC, 70% RH and 16:8 h L:D photoperiod. Mortality was assessed after 72 h.

Biochemical assays

Populations from cotton fields were collected into ice boxes and kept at -80°C until used within two weeks for enzyme analysis.

Determination of carboxylesterase activity

Twenty individual aphids were homogenized in 100 µl sodium phosphate buffer (0.1 M, pH 7.5) (containing 0.1% Triton X-100). This homogenate was used as an enzyme source after centrifugation at 10,000 g, at 4ºC for 5 min. The supernatant used as an enzyme source was diluted 10 times. Supernatant of 25 µl was combined with 25 µl of phosphate buffer (0.2 M, pH 6) in a microplate. The reaction was initiated by the addition of 200 µl substrate solution to the wells. The substrate solution was prepared by dissolving 30 mg fast blue RR salt in 50 ml of 0.2 M sodium phosphate buffer and adding 500 µl of 100 mM α-napthyl acetate to this mixture. Enzyme activity was read at 23ºC with a Multiskan GO Microplate Spectrophotometer for 10 min at 450 nm. Blank cells were read without homogenization. Enzyme readings were made three times (Stumpf & Nauen, 2002). Mean levels of carboxylesterase **(**CE) activity were based on protein content and α- naphthol standard curves. Protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Determination of glutathione S-transferase activity

The method developed by Stumpf & Nauen (2002) and was used to determine GST activity. About 30 individuals were homogenized in 300 µl Tris HCL buffer (0.05 M, pH 7.5). The supernatant was centrifuged at 10,000 g at 4ºC for 5 min. One hundred 100 µl supernatant, 100 µl 1-chloro-2,4-dinitrobenzene (CDNB) and 100 µl reduced glutathione (GSH) were added to a microplate. CDNB was prepared in 0.1% ethanol and 0.4 mM CDNB was added to the microplate wells at final concentration. GSH was prepared in Tris HCL buffer and 4 mM GSH was added to the wells at final concentration. The change in absorbance was read at 340 nm at 25ºC for 5 min. Enzyme readings were made at three-time replicates. Changes in absorbance per minute were converted into nM CDNB conjugated/min/mg protein using the extinction coefficient of the resulting 2,4-dinitrophenyl-glutatione (Habig et al., 1974).

Determination of cytochrome P450 monooxygenase activity

The method of Hansen and Hodgson (1971) was used to determine the P450 enzyme activity. Accordingly, 90 µl of the enzyme from stock solutions and 100 µl 2 mM p-nitroanisole (substrate) were added to each of the microplate wells. After incubating for 2 min at 27ºC, 10 µl of 9.6 mM nicotinamide adenine dinucleotide phosphate (NADPH) was added to initiate the reaction. Measurement of P450 enzyme activity was made in a microplate reader at 405 nm at 27ºC for 10 min at intervals of 10 s, three-time replicates. Protein quantities were calculated according to Bradford (1976) with OD values determined. The enzyme activity was determined using the extinction coefficient of p-nitrophenol (Kranthi, 2005).

Statistical analysis

Dose-response regressions were computed using Polo-Plus computer program (LeOra Software, Berkeley, CA, USA). In order to estimate the LD_{50} (lethal dose to kill 50% of the test population), resistance factors were calculated by dividing the LD_{50} of the field collected population by the LD_{50} of the susceptible population.

Results

Bioassay

Imidacloprid resistance factors (RF) ranged from 54 to 206 fold. The most sensitive population was from Bebeli, whereas the most resistant was from Kürkçüler. For thiamethoxam the RF ranged from 5 to 65 fold. The most susceptible population was from Yumurtalık and the most resistant from Körkuyu (Table 2).

Table 2. Bioassay of imidacloprid and thiamethoxam in test and susceptible populations of *Aphis gossypii*

	Imidacloprid					Thiamethoxam				
Population	n	Slope±SE	LD_{50} µl/ml confidence	X^2	RF	n	Slope±SE	LD_{50} µl/ml confidence	X^2	RF
Körkuyu	540	1.49±0.457	12.001 (2.909-20.826)	0.986	139,54	540	1.20±1.650	85.111 (45.672-106.868)	0.602	65.72
Durhasandede	540	1.93±0.602	9.768 (3.008-14.669)	0.296	113,58	540	1.26±0.402	33.152 (11.347-76.001)	0.602	25.60
Bebeli	540	1.77±0.391	4.698 (1.758-7.775)	0.958	54,62	540	1.37±0.337	16.284 (7.163-25.954)	0.602	12.57
Bahçe	540	1.38±0.299	5.465 (2.289-8.870)	0.863	63,54	540	2.45±0.661	17.387 (7.674-25.704)	0.602	4.22
Çukurova	540	1.52±0.353	12.360 (3.506-23.590)	0.991	143,72	540	1.48±0.38	68.214 (34.754-109.595)	0.602	52.67
Kürkçüler	540	2.24±0.743	17.764 (9.454-24.930)	0.475	206,55	540	1.75±0.202	18.570 (8.190-28.706)	0.602	14.34
Yumurtalık	540	1.36±0.422	7.741 (1.839-13.006)	0.819	90,01	540	0.90±0.252	7.496 (1.415-15.782)	0.602	5.78
Susceptibe	540	0.50±0.164	0.086 (0.001-0.376)	0.387	-	540	0.745±0.238	1.295 (0.158-3.859)	0.857	-

RF, resistant factor; $X^{2,}$ lower than ($p \leq 0.05$) indicates a significant fit between the observed and expected regression lines.

Enzyme analyses

Maximum enzyme activity and ratios relative to susceptible population were 17.8 nM/min/mg protein (6.4 fold) in Körkuyu for carboxylesterase activity, 142.3 nM/min/mg protein (3.32 fold) in Bahçe for GST activity and 3.8 nM/min/mg protein (75 fold) in Körkuyu for P450 (Table 3). Bebeli, Durhasandede, Körkuyu and Bahçe populations were statistically different from other populations and their CE activity levels were higher than the susceptible populations. Çukurova, Yumurtalık and Kürkçüler populations were in the same statistical group with lower CE activity than the other populations. GST activity ranged from 10 to 140 M/min/mg protein. The Bahçe population had the highest enzyme activity relative to susceptible population. For GST activity, all populations were statistically different from each other. For P450 activity, the Körkuyu population had highest level enzyme activity (3.803 nM/min/mg protein) and was statistically different from the other populations. Metabolic resistance levels were found to be higher in the enzyme analyses.

Table 3. Carboxylesterase (CE), glutathione S-transferase (GTS), cytochrome P450 monooxygenase (P450) enzyme activities of resistant and susceptible populations

Population	n	CE (M/min/mg protein)	CE ratio (rest.pop./sus.pop.)	GSTs (M/min/mg protein)	GSTs ratio (rest.pop./sus.pop.)	P450 (M/min/mg protein)	P450 ratio (rest.pop./sus.pop.)
Körkuyu	3	17.85±0.57 a	6.46	75.17±1.154 c	1.75	3.803±0.57 a	74.57
Durhasandede	3	16.45±1.15 a	5.96	58.93±0.570 e	1.37	1.521±0.57 bcd	29.83
Bebeli	3	16.50±0.57 a	5.97	54.58±0,570 f	1.27	0.338±0.02 ab	6.63
Bahçe	3	17.21±0.57 a	6.23	142.23±1,670 a	3.31	1.876±0.57 cd	36.79
Çukurova	3	9.36±0.57 b	3.39	64.78±0,570 d	1.51	0.761±0.06 ab	14.91
Kürkçüler	3	8.85±0.57 b	3.20	88.02±0,570 b	2.05	2.789±0.57 de	54.68
Yumurtalık	3	8.61±1.15 b	3.11	90.66±0,570 b	2.11	0.507±0.04 ab	9.94
Susceptible (Control)	3	2.76±0.57 c	-	42.87±1,154 g	-	0.051±0.05 e	-

Discussion

Varying resistance levels were observed to imidacloprid and thiamethoxam, neonicotinoid insecticides, in seven populations collected from cotton fields. The Kürkçüler, Körkuyu and Durhasandede populations had the highest LD_{50} values compared to the other populations, while the susceptible populations changed with each insecticide application rate (Table 2). Resistance to imidacloprid was found to be the highest. Imidacloprid is an active ingredient in a large number of licensed insecticide products applied to cotton in Turkey (Anonymous, 2018). Its wide spectrum, very widespread and intensive use will have contributed to the high level of resistance of *A. gossypii* to this agent (Bass et al., 2015). Imidacloprid and thiamethoxam are also being used against major cotton pests, for example, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae). In addition, these insecticides have also been extensively used on vegetables and other field crops in Çukurova. We consider that the resistance of *A. gossypii* is likely to remain at high levels as long as neonicotinoid insecticides are used for managing aphids, whiteflies and other piercing-sucking insect pests of cotton and vegetables in the study area (Dağ et al., 2000; Ulusoy et al., 2017a). The presence of piercing-sucking pests such as the polyphagous *A. gossypii* and *B. tabacii* in the same region, can lead to exposure to multiple insecticide applications in both cotton and vegetable fields. Intensive applications may create ideal condition for the selection of resistant populations. Furthermore, higher LD_{50} values were obtained in Kürkçüler and Körkuyu populations sprayed with the thiamethoxam and imidacloprid. There is a possibility of cross resistance due to overuse of insecticides of the same group (Marshall et al., 2012). In addition, studies have also reported cross resistance to imidacloprid and thiamethoxam insecticides (mode of action 4A neonicotinoid) (Shi et al., 2011; Wang et al., 2007).

Aphis gossypii exhibits different levels of resistance to insecticides of the neonicotinoid group worldwide. For instance, in China there was 1200 fold resistance to imidacloprid (Chen et al., 2017), but in Australia 85 fold to thiamethoxam was observed (Marshall et al., 2014). Resistance of 66.5 fold to imidacloprid in some cotton fields in the Asian continent (Shi et al., 2011). Furthermore, 6.4, 10 and 22 fold resistance has been recorded to acetamiprid, clothianidin and thiamethoxam, respectively (Herron & Wilson, 2011). Additionally, the resistance of 17 fold to imidacloprid and thiamethoxam were found in Shandong, China (Wang et al., 2007). Similarly, 74 fold-resistances in Körkuyu population was observed in the current study.

In the enzyme analysis, Yumurtalık population had minimal CE activity, whereas, Körkuyu population had the highest CE activity among the seven populations tested, followed by the susceptible population (Table 3). Bahçe population had the highest GST activity. Both these CE and GST activities are consistent with the bioassay results. It was observed that the increase in metabolic enzyme activities responsible for resistance paralleled the measured increase in resistance.

Previous studies have indicated that there was a strong positive correlation among the organophosphate, carbamate, pyrethroid chlorinated hydrocarbon group insecticide resistance levels and general esterase and GST activities in aphids and many insect species (Devonshire & Moores, 1982; Hemmingway & Georghiou, 1984; Rauch & Nauen, 2003). In vivo, high esterase enzyme activity representing up to 3% the amount of total protein was observed in highly resistant aphid populations (Devonshire & Sawicki, 1979; Devonshire, 1989).

Even though organophosphate insecticide resistance was not assayed in the current study, it is predicted that resistance to organophosphate insecticides could be higher because more than 40% of licensed insecticides used in cotton and vegetable fields in this region contain organophosphates (Anonymous, 2018). In a study of Velioğlu et al. (2008), it was reported that insecticide resistance in *A. gossypii* resulted from higher acetylcholinesterase activity in a population from a Çukurova cotton field. It has also been reported that intensive insecticide application causes increased production of detoxifying enzymes, such as acetylcholinesterase, carboxylesterase and P450 group enzymes, in insects (Wang et al., 2002; Field et al., 1999; Bass & Field, 2011). Furthermore, acetylcholinesterase and alpha-naphthyl acetate (α-NA) esterase levels were found to be higher in imidacloprid-resistant *A. gossypii* populations (Wang et al., 2002).

In the current study, P450 analysis of the Körkuyu population of *A. gossypii* population showed a 74-fold increase in resistance (Table 2). P450 is an effective enzyme to give resistance to neonicotinoid insecticides in insects (Wang et al., 2007). P450 activities in Körkuyu and Çukurova populations were also higher than the susceptible populations. The P450 activities also parallel the bioassay results.

Some studies have reported that the resistant aphid populations have higher P450 activity compared to susceptible aphid populations (Shang et al., 2012; Seyedebrahimi et al., 2015). According to some studies, the neonicotinoid resistance mechanism is associated with mutations in the nAChR gene, but is usually directly associated with xenobiotic detoxification enzyme, 7-ethoxycoumarin O-deethylase, which is catalyzed by cytochrome P450 (Karunker et al., 2008; Nauen et al., 2008; Wang et al., 2009).

A. gossypii resistance to imidacloprid and thiamethoxam were determined by the bioassay and biochemical methods in Çukurova Province. Also, increasing resistance to neonicotinoids has been observed in this cotton fields. The suitable climatic conditions along with rich soils enable the establishment of polyculture in Çukurova Region. This region comprises intertwined cotton, vegetable and other agricultural fields. Proximity of vegetable and cotton growing areas to each other in the region facilitates movement of *A. gossypii* between different crops. Furthermore, extensive applications of neonicotinoid insecticides, such as imidacloprid and thiamethoxam, may lead to resistance development. The continuous use of pesticides with the same mode of action in the management of aphids may lead to the selection of resistant *A. gossypii* populations and the elimination of susceptible populations. It may contribute to the development of cross resistance. The results of this study suggest that new management methods and strategies should be developed and implemented for management of insecticide resistant *A. gossypii* in cotton growing areas of the region.

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