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Imidacloprid Resistance Status and Role of Detoxification Enzymes in *Bemisia tabaci* (Hemiptera: Aleyrodidae) Populations from Iran

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ABSTRACT

Neonicotinoid pesticides such as imidacloprid and thiacloprid are agonists of nicotinic Acetylcholine Receptors (nAChRs). This chemical group is commonly used in controlling sucking pests such as cotton whitefly, *B. tabaci*, one of the most serious and destructive pests of agricultural crops worldwide. Bioassays were performed using a leaf dip method and Ahvaz population with the lowest LC_{50} value (24.40 mg ai L^{-1}) was considered as the susceptible population. LC_{50} values of Karaj, Isfahan, Kashan, Gorgan and Minab populations were estimated as 189.81, 136.91, 106.95, 141.09, and 68.31 mg ai L^{-1}, respectively. Low Resistance Ratios (RR) to imidacloprid were observed in the tested populations (RR values< 10). The piperonylbutoxide (PBO) and TriPhenyl Phosphate (TPP) showed the highest synergistic ratios of 1.99 and 2.42 in the population of Kashan, respectively, but DiEthyl Maleate (DEM) did not show a high synergistic ratio. The activity of cytochrome P450-dependent monooxygenase (P450s), CarboxylEsterase (CarEs) and Dlutathione S-Transferases (GST) were measured. There was an increase in the activity of P450s up to 3-fold in the Gorgan population and CarE activity in Kashan population up to 2-fold in comparison to the susceptible population. Based on the results, P450s and CarEs are possibly the enzyme systems responsible for imidacloprid resistance in the tested populations of *B. tabaci*.

Keywords: Bioassays, Cotton whitefly, Monooxygenases, Neonicotinoid, Synergism.

INTRODUCTION

Neonicotinoid pesticides are important in pest management because of their high effectiveness against a wide range of pests in the world and have been registered in more than 120 countries (Bass *et al*., 2015). Their discovery has been considered as a turning point in research for new insecticides (Nauen and Denholm, 2005). The rates of application and toxicity of neonicotinoids are lower than those of previous insecticides (Tomizawa and Casida, 2003). The ionic channels of nicotinic Acetylcholine Receptors (nAChRs) that are placed in the central nervous system of insects are the target of neonicotinoids (Casida, 2010; Crossthwaite *et al*., 2017). The first neonicotinoid insecticide released was imidacloprid, in 1991 (Karunker *et al*., 2008). Imidacloprid was first registered in Iran for controlling tobacco aphids in 1996 (Meschi, 2007). Resistance development is a major risk to the agricultural efficiency of

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commercial insecticides (Rauch and Nauen, 2003). Most reported cases of neonicotinoid resistance by 2016 belong to *Bemisia tabaci* (Gennadius), *Nilaparvata lugens* (Stål), *Myzus persicae* (Sulzer), and *Aphis gossypii* (Glover), and imidacloprid has the greatest share to the resistance of neonicotinoids (APRD, 2016; Bass *et al*., 2015).

The cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is an important polyphagous pest which has developed resistance to various insecticides worldwide (Gravalos *et al*., 2015; Oliveira *et al*., 2001). This pest damages more than 500 host plant species of 60 families (Mound and Halsey, 1978). The cotton whitefly directly damages host plants by feeding and indirectly by transmission of viruses and excretion of honeydew (Brown *et al*., 1995; Byrne and Bellows Jr, 1991). *B. tabaci* is a species complex with at least 34 biotypes that based on the molecular criteria are distinct (Cuthbertson and Vanninen, 2015). The biotypes B (Middle East–Asia Minor1, MEAM1) and Q (Mediterranean, MED) have worldwide distribution and have been studied more extensively (Barbosa *et al*., 2015; Horowitz *et al*., 2005).

B. tabaci has developed resistance to different groups of insecticides including carbamates, organophosphates, pyrethroids, Insect Growth Regulators (IGRs), Ketoenols (Bielza *et al*., 2018), and neonicotinoids (Byrne *et al*., 2003; Houndete *et al*., 2010; Nauen *et al*., 2015; Roditakis *et al*., 2009). The first case of neonicotinoid resistance was reported against imidacloprid in 1996 in Spanish populations of *B. tabaci*. Imidacloprid resistance in *B. tabaci* has been suggested to be associated with increased detoxification by cytochrome P450 monooxygenases (Karunker *et al*., 2008; Rauch and Nauen, 2003).

Few studies have investigated neonicotinoid resistance in Iranian pest populations. Nazemi and Khajehali (2016) assessed imidacloprid resistance in *Thrips tabaci* populations collected from Iranian onion fields and found different levels of resistance in most populations. Basij *et al*. (2017) reported development of resistance to neonicotinoids (imidacloprid and acetamiprid) in *B. tabaci* populations from Iran.

Iranian growers have been facing many problems in controlling *B. tabaci*. The aim of this study was to evaluate the resistance status to imidacloprid in several cotton whitefly populations. Additionally, to study the metabolic mechanism of resistance, we aimed to investigate the activity of cytochrome P450-dependent monooxygenase (P450s), CarboxylEsterase (CarEs) and Glutathione S-Transferases (GST) *in vitro* as well as *in vivo* using different synergists.

MATERIALS AND METHODS

Insects

The various *B. tabaci* populations were collected from Isfahan, Karaj, Kashan, Ahvaz, Minab, and Gorgan in 2017-2018 (Figure 1). The populations had been exposed to frequent application of insecticides such as neonicotinoids, organophosphates, pyrethroids, and Insect Growth Regulators (IGRs) in protected or open field crops, except for the population of Ahvaz that was reared in the laboratory without selection pressure for two years. The collected populations were transferred to the cotton landrace, *Gossypium hirsutum*, inside separate net-covered cages, and reared under greenhouse conditions: photoperiod (16:8 h), temperature (27±2°C) and humidity (65±2%). To keep whitefly populations, the plants in the cages were replaced every 3 weeks with new ones.

Insecticides and Synergists

Imidacloprid SC 35% (Golsam, Gorgan, Iran), Piperonyl Butoxide (PBO, Sigma-Aldrich, Bornem, Belgium), TriPhenyl Phosphate (TPP, Merck, Darmstadt, Germany) and DiEthyl Maleate (DEM,

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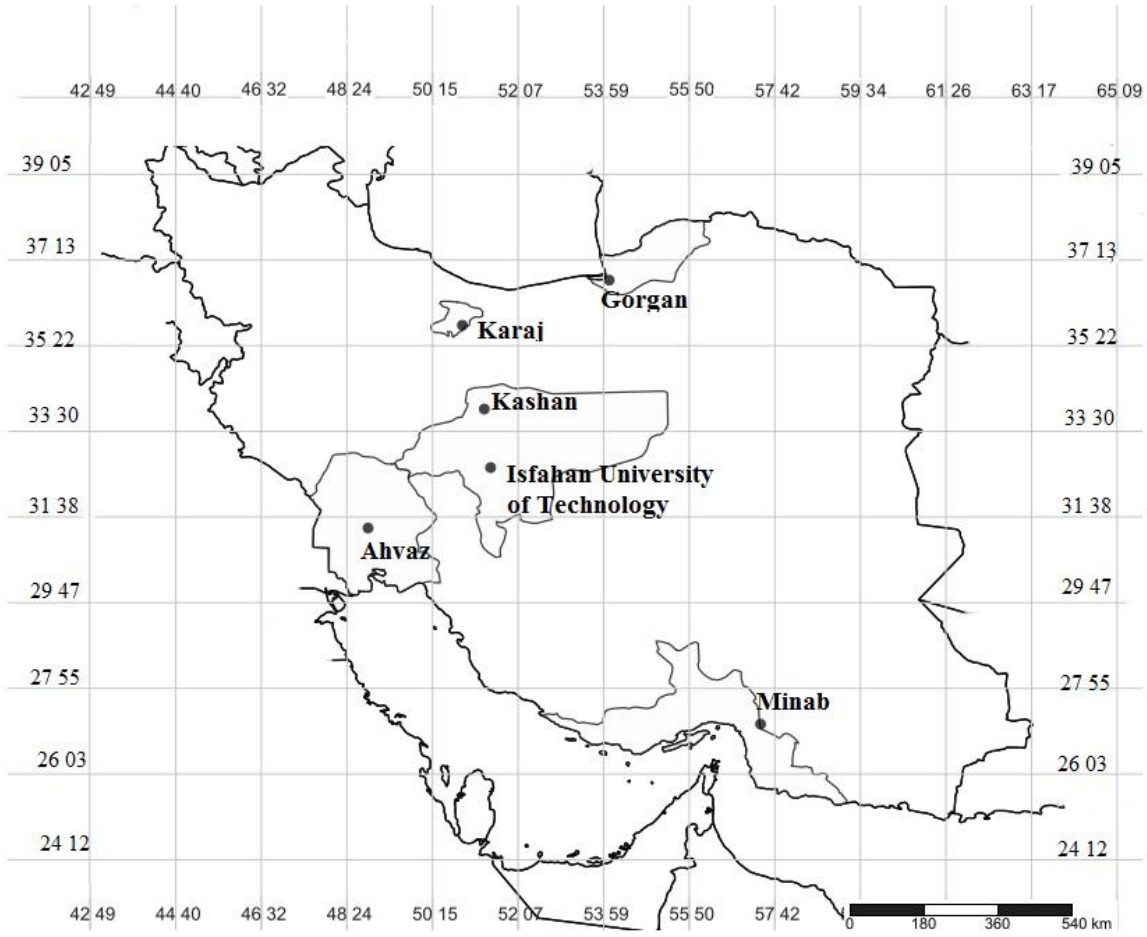

Figure 1. Map of collection regions of *B. tabaci* populations in Iran.

Sigma-Aldrich, Bornem, Belgium) were used.

Bioassays

Bioassays were performed using the leaf-dip method (Feng *et al*., 2010; Rauch and Nauen, 2003). Leaf discs of 3-4 cm in diameter were made from cotton leaves. The discs were dipped in various concentrations of imidacloprid (1 to 1,000 mg active ingredient per Liter) for 10 seconds and dried on air. Leaf discs were put with their adaxial surface downwards onto a bed of agar (10 g L^{-1}) in Petri dishes. Each treatment had four replicates. Distilled water was considered as the control treatment. The mixed (male and female) adult whiteflies (2 days old) were collected from the rearing cages by aspirator and, after CO_2 anesthesia, 20 adults were transferred to each Petri dish. Each Petri dish was sealed with parafilm. The dishes were kept at 27±2°C, 65±2% RH under 16:8 (Light: Dark) photoperiod. The mortality of the insects was recorded after 48 hours.

Effect of Synergists on the Insecticide Resistance

To measure the effect of inhibition of detoxifying enzymes on imidacloprid resistance, three synergists PBO, TPP, and DEM were used in combination with the

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insecticide. Three populations of Ahvaz (as a reference population), Kashan and Minab with different levels of imidacloprid resistance were tested. Stock solutions (10,000 mg L^{-1}) of PBO, TPP, and DEM were prepared in acetone and subsequently diluted with water. To determine the highest concentration with less than 10% mortality, different concentrations of synergists were tested. The toxicity test method was similar to the leaf-dip bioassay described above. After pre-exposure for five hours to 200 mg L^{-1} of either PBO, TPP or DEM, adults were transferred to imidacloprid treated leaf discs.

Total Carboxylesterase Activity

Total CarEs activity was measured by using 1-naphthyl acetate (Sigma-Aldrich, USA) as substrate (Van Asperen, 1962). From each population, 50 adults were homogenized in 300 µL of ice-cold sodium phosphate buffer (0.2 M, pH 7, containing 0.1% of Triton X-100). The homogenates were centrifuged at 12,000×g and 4°C for 15 minutes. The supernatant was used as the source of the enzyme extract. Thirty µL of supernatant was added to 200 µL phosphate buffer (0.1M, pH 7.0) and 200 µL of the substrate (64 mM in acetone). Then, 120 µL fast blue RR (1.6 mg mL^{-1} in distilled water) was added to the reaction mixture, and the released naphthol was continuously measured using spectrophotometer (UNICO, Dayton, USA) at 450 nm for the presence of 1-naphthyl acetate, for 20 minutes. Experiments were performed in three replications. Using different concentrations of naphthol as the reaction product, the standard curve of absorbance was obtained.

Glutathione *S*-Transferase (GST) Activity

GST was measured by using 1-Chloro-2,4-DiNitroBenzene (CDNB) (Sigma-Aldrich, USA) and Reduced Glutathione (GSH) (Sigma-Aldrich, USA) as substrate (Habig *et al*., 1974). About 50 adults were homogenized in 300 µL of ice-cold sodium phosphate buffer (0.2M, pH 7). Total volume of reaction included, 30 µL supernatant (12,000×g, 15 min), 200 µL of CDNB (1.2 mM) and 200 µL of GSH (3 mg mL^{-1} in distilled water). The change in absorbance was determined continuously for 5 minutes at 340 nm using the spectrophotometer (UNICO, Dayton, USA) with at least four replicates for each population. The absorbance changes in minutes were calculated using an absorption coefficient of 9.6 mM cm^{-1}. Results were reported in terms of the amount of CDNB congestion per minute per mg protein.

Cytochrome P450-Dependent Monooxygenase Activity

Cytochrome P450 activity was estimated by measuring heme peroxidase activity similarly to Brogdon (1997). The total reaction volume per vial was 650 µL, consisting of 40 µL of enzyme solution, 160 µL of 0.625 M potassium phosphate buffer (pH 7.2), 400 µL of TMBZ (Sigma-Aldrich, USA) solution and 50 µL of hydrogen peroxide (3%). Vials were incubated at room temperature for 2 hours before reading at 450 nm as the endpoint in the spectrophotometer (UNICO, Dayton, USA). A standard curve for heme peroxidase activity was provided using different concentrations of cytochrome C from horse heart (Merck, Germany). Monooxygenase levels were expressed as equivalent units of cytochrome P450 mg^{-1} protein using the standard curve of cytochrome C.

In the enzymes assay, the total protein content of the enzyme samples was measured according to the Bradford (1976). The standard curve was plotted using the BSA (Merck, Germany) solution.

Data Analysis

LC_{50} values and their 95% confidence limits, resistance ratios, and synergistic

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ratios were estimated by Polo Plus software (Robertson *et al*., 2017; Software, 2002). The log-dose and probit-mortality lines were plotted for all whitefly populations. The enzyme activity results were analyzed by ANOVA and the mean of the populations was compared with the Tukey test in SAS 9.4 (SAS Institute, 2015).

RESULTS

Toxicity and Resistance

Imidacloprid bioassay results for the different populations are shown in Table 1. In this study, using the leaf-dip method, the population of Ahvaz with an LC_{50} value of 24.40 mg active ingredient per Liter (mg ai L^{-1}) and the Karaj population with an LC_{50} value of 189.81 mg ai L^{-1} presented the lowest and highest LC_{50} values, respectively. The highest and the lowest Resistance Ratios (RRs) obtained were 7.79- and 2.80-fold for the populations of Karaj and Minab, respectively. The steepest slope of probit line was observed in response to imidacloprid in the population of Karaj. The RR values in Gorgan, Isfahan, and Kashan populations were 5.79, 5.61, and 4.39, respectively.

Effect of Synergists

The results of the effect of three different synergists in combination with imidacloprid in Ahvaz population as a susceptible strain showed no significant decrease in LC_{50} (Table 2). The oxidase inhibitor PBO and the esterase inhibitor TPP, in Kashan population showed 1.99- and 2.42-fold synergism, respectively. Synergism ratio was not significantly higher in Minab population than in Ahvaz population based on their confidence limits.

Enzyme Activity Assays

The comparison of the average activity of CarEs showed a significant difference between the populations of Gorgan, Kashan, and Minab ($F_{2,8}$= 571.31; P= 0.0001). Kashan population had the highest activity (1,366.52 nmol mg^{-1} min^{-1}) of CarEs. There was also a significant difference in P450s ($F_{3,11}$= 11.72; P= 0.0027) between all tested populations. The populations of Gorgan and Ahvaz showed the highest and lowest P450 activity, respectively.

The average of the GST activity in Minab and Kashan populations was almost twice the population of Gorgan. However, Gorgan, Kashan, and Minab populations did not show a significant difference in the GST activity assay ($F_{2,8}$= 4.35; P= 0.0680). Correlations between the LC_{50} values of the tested populations and the activity of detoxification enzymes are shown in Figure 2. A positive correlation was observed only between P450 monoxygenase activity and LC_{50} values.

Table 1. Log-dose probit-mortality data for populations of *B. tabaci* in response to imidacloprid.[a]

Population	n	LC_{50} mg ai L^{-1} (95% CI)	LC_{90} mg ai L^{-1} (95% CI)	Slope±SE	χ^2 (df)	RR (95% CI)
Ahvaz	145	24.40 (15.94-34.34)	180.02 (101.94-584.12)	1.48±0.29	1.08(2)	1
Isfahan	381	136.91 (108.51-189.28)	743.89 (439.10-1866.13)	1.74±0.25	1.10(4)	5.61
Karaj	310	189.81 (151.32-251.96)	952.47 (583.24-2351.64)	1.83±0.29	1.25(3)	7.79
Kashan	447	106.95 (87.27-129.44)	597.82 (432.20-954.91)	1.72±0.18	0.83(4)	4.39
Gorgan	242	141.09 (81.25-212.07)	1119.82 (586.55-4041.15)	1.13±0.20	1.32(3)	5.79
Minab	357	68.31 (43.91-92.49)	1901.94 (995.24-6526.95)	1.06±0.19	0.42(4)	2.80

[a] n: Number of *B. tabaci* tested; Resistance Ratio (RR)= LC_{50} of any population/LC_{50} of Ahvaz, 95% CI: Confidence limits.

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Table 2. Synergistic effect of PBO, TPP, and DEM on imidacloprid resistance in *B. tabaci* populations.

Population	Synergist	LC_{50} mg ai L^{-1} (95% CI)	Slope±SE	SR[a] (95% CI)
Ahvaz	Imidacloprid	24.40 (15.94-34.34)	1.48±0.29	1
	+PBO	22.98 (12.97- 34.56)	1.46±0.38	1.06 (0.58-1.93)
	+TPP	24.77 (15.22- 36.15)	1.58±0.42	0.98 (0.56-1.74)
	+DEM	27.96 (17.99- 43.38)	1.56±0.42	0.87 (0.49-1.55)
Kashan	Imidacloprid	106.95 (87.27-129.44)	1.72±0.18	1
	+PBO	53.71 (31.70-74.24)	1.66±0.31	1.99 (1.29-3.09)
	+TPP	44.16 (33.80-54.32)	3.25±0.58	2.42 (1.80-3.27)
	+DEM	83.13 (59.07-135.73)	2.33±0.64	1.29 (0.86-1.92)
Minab	Imidacloprid	68.31 (43.91-92.49)	1.06±0.19	1
	+PBO	55.38 (26.82-78.66)	1.67±0.38	1.23 (0.69-2.20)
	+TPP	52.67 (15.81-93.53)	0.92±0.27	1.30 (0.6-2.83)
	+DEM	58.32 (23.80-102.87)	0.97±0.27	1.17 (.58-2.37)

[a] Synergistic Ratio (SR)= LC_{50} of pretreatment population/LC_{50} of population without pretreatment.

Table 3. Enzyme activities (mean±SE) in different populations of *B. tabaci*.

Population	CarE activity (nmol mg^{-1} min^{-1})	Ratio[A]	GST activity (nmol mg^{-1} min^{-1})	Ratio[A]	P450s activity (Unit mg^{-1})	Ratio[B]
Ahvaz	-		-		3.67±0.25 c	1
Minab	514.75±10.05 b[4]	1	534.44±115.76 a	1	5.98±1.23 bc	1.63
Kashan	1366.50±40.10 a	2.65	521.95±24.48 a	0.98	8.99±0.91 ab	2.45
Gorgan	274.40±4.56 c	0.53	242.37±69.37 a	0.45	11.21±1.15 a	3.05

[a-b] The same letters indicate no significant difference using Tukey test, P≤ 0.05.
[A] Ratio= Activity of any population/Minab activity, [B] Ratio= Activity of any population/ Ahvaz activity.

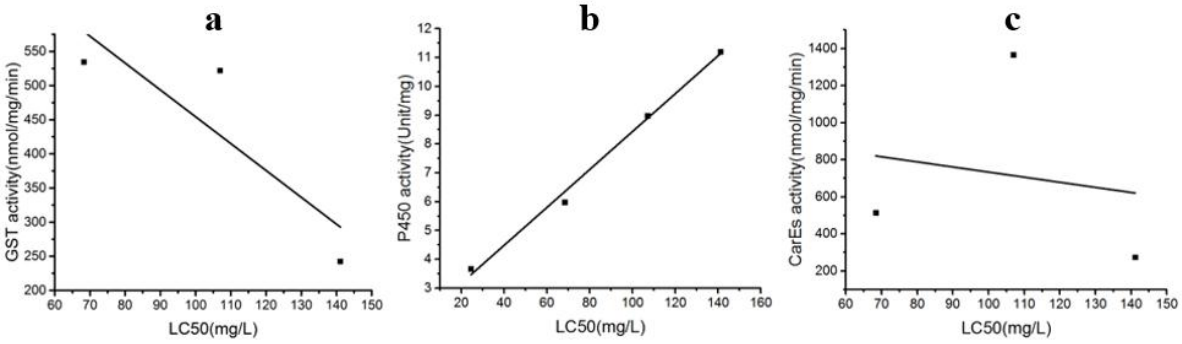

Figure 2. Correlation between the LC_{50} values of the populations and activity of detoxification enzymes. Pearson's r: -0.87 (a), 0.99 (b) and -0.17 (c).

DISCUSSION

Over-use of insecticides often leads to the fast development of pest resistance to a wide range of pesticides, especially in developing countries. On the other hand, the most dangerous pesticides are used in these countries (Wilson and Otsuki, 2002) and in case of pest resistance, sometimes higher doses of pesticides are applied. Thus, it is important to determine which chemical compounds can be effective against local populations of key pests. In recent years, neonicotinoid insecticides have been considered to protect a wide range of pests on different plants. Diversity in the

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formulations and application methods seems to have resulted in increasing neonicotinoid applications (Jeschke *et al*., 2010; Takacs *et al*., 2017).

In this study, resistance to imidacloprid was evaluated in several *B. tabaci* populations. Ahvaz population was considered as the reference population with the lowest LC_{50} value. Other collected populations had frequently been sprayed by farmers, often more than four times a year, but the Ahvaz population had not been treated with insecticides for 2 years. Based on the results, none of the tested whitefly populations showed a high resistance ratio to imidacloprid. Probably, the reference population of this research had been exposed to insecticide treatments and was not fully susceptible to imidacloprid. In most studies, a susceptible laboratory strain has been used in RR calculation. In this study, the LC_{50} of reference population was relatively high (24.40 mg ai L^{-1}) compared with that of susceptible populations used in other studies and tested with similar bioassays (Gorman *et al*., 2010; Jones *et al*., 2011; Nauen *et al*., 2002). A study in Greece showed that the LC_{50} values of a reference population of *Trialeurodes vaporariorum* against imidacloprid and thiacloprid were 94.4 and 100.6 mg L^{-1}, respectively. Also, high resistance levels to imidacloprid were not found and RR values ranged from 1.5 to 4.4, which was very close to our results (Pappas *et al*., 2013). The results of a study in Turkey indicated moderate to high levels of resistance to neonicotinoids in field populations of *B. tabaci.* The resistance ratios exhibited a different range when different susceptible populations were considered as the reference population (up to 30-fold based on Lab1, and 300-fold based on Lab2) (Sahin and Ikten, 2017).

Previous studies on the resistance dynamic had shown that imidacloprid resistance was not stable and declined with decreasing selection pressure (Wen *et al*., 2009). Considering the highest slope of probit-mortality line in Karaj population, it can be concluded that this population has relatively high homogeneity.

The LC_{50} of Ahvaz population did not significantly change after pre-treatment with PBO, TPP, and DEM synergists. This population was the population most susceptible to imidacloprid compared to other populations. In the Kashan population, the LC_{50} value was significantly decreased after application of either PBO or TPP synergists, based on non-overlapping 95% confidence limits of LC_{50} values. Many studies have proved the role of cytochrome P450-dependent monooxygenases in reducing the toxicity of neonicotinoids through synergistic tests (Feng *et al*., 2010; Rauch and Nauen, 2003) and increasing the expression of involved genes (Yang *et al*., 2013). Carboxylesterases also play an important role in the detoxification of organophosphates, carbamates, and pyrethroid insecticides (Sogorb and Vilanova, 2002; Wheelock *et al*., 2005). In the population of Kashan, TPP had a higher synergistic effect, probably reflecting a more prominent role of CarEs. Previous studies on neonicotinoids resistance in *B. tabaci* populations from Cyprus showed a moderate correlation between imidacloprid resistance and carboxylesterase activity (Vassiliou *et al*., 2011). Based on *in vivo* assays, the involvement of several enzymes to detoxify imidacloprid in Kashan population is possibly associated with application of neonicotinoids along with other insecticides in controlling *B. tabaci* during the growing season of the host plant. In this population, the synergistic ratio of DEM was 1.29-fold, with no significant difference compared to Ahvaz population. Thus, possibly GSTs do not play a significant role in decreasing the toxicity of neonicotinoids in this population. The effect of PBO, TPP, and DEM synergists in reducing the LC_{50} value in Minab population was not significant. This population was relatively susceptible to imidacloprid with a resistance ratio of 2.80-fold.

Measurement of *in vitro* activity of detoxifying enzymes showed significant

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differences in all tested populations. According to the results, it can be stated that the increase in P450 activity was positively correlated with the increase in LC_{50} values (Pearson's r: 0.99). A strong correlation between increased resistance and cytochrome P450 level has been reported in *B. tabaci* populations from India (Vivek *et al*., 2018). In addition, the highest monooxygenases activity has been observed in *B. tabaci* populations with the highest resistance ratio (RR= 2060) for the neonicotinoids (Satar *et al*., 2018). Rauch and Nauen (2003) emphasize that in *B. tabaci* populations, the metabolism of imidacloprid is under oxidative degradation. The activity of CarEs in the populations of Kashan, Minab, and Gorgan also showed a significant difference. The Kashan population had the highest CarE activity, which was consistent with the results of pre-treatment with TPP synergist. As shown in Table 2, the highest decrease in the LC_{50} value occurred after inhibiting the CarEs. The level of CarE activity in the Minab and Gorgan populations were much lower than that of Kashan. Activity of CarEs and GSTs were not measured in Ahvaz population, because of sudden decrease of the populations. Activity of GSTs was not significantly different between Kashan and Minab populations. The estimated *in vitro* activity of the CarEs and GSTs did not show any correlation with the LC_{50} values.

In this study, the tested populations showed relatively low levels of resistance to imidacloprid. The synergistic tests and measurements of the activity of detoxification enzymes indicated the role of P450s and CarEs in the development of *B. tabaci* resistance to imidacloprid. However, further works are needed to provide more solid evidence to demonstrate the role of these enzymes in resistance to imidacloprid. With suitable management actions, the resistance ratio can be kept to a minimum and prevent or delay the development of higher levels of imidacloprid resistance.

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وضعیت مقاومت به ایمیداکلوپرید و نقش آنزیم‌های سم‌زدا در جمعیت‌های عسلک پنبه *Bemisia tabaci* (Hemiptera: Aleyrodidae) از ایران

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چکیده

حشره‌کش‌های نئونیکوتینوئید همانند ایمیداکلوپرید و تیاکلوپرید آگونیست گیرنده‌های نیکوتینیک استیل کولین (nAChR) می‌باشند. این گروه شیمیایی اغلب جهت کنترل آفات مکنده استفاده می شود، نظیر عسلک پنبه، *B. tabaci* ، که یکی از آفات جدی و مخرب محصولات کشاورزی در سراسر جهان می‌باشد. آزمایش‌های زیست‌سنجی به روش غوطه‌وری برگ انجام شد و جمعیت اهواز با کمترین مقدار LC_{50} (۲۴/۴۰ میلی‌گرم بر لیتر) به عنوان جمعیت حساس در نظر گرفته شد. در جمعیت‌های کرج، اصفهان، کاشان، گرگان و میناب مقادیرLC_{50} به ترتیب ،۱۸۹/۸۱، ۱۳۶/۹۱، ۱۰۶/۹۵، ۱۴۱/۰۹ و ۶۸/۳۱ میلی‌گرم بر لیتر محاسبه شد. نسبت مقاومت پایینی به حشره‌کش ایمیداکلوپرید در جمعیت‌های مورد ارزیابی عسلک پنبه مشاهده شد (نسبت‌های مقاومت< ۱۰ برابر). سینرژیست‌های پیپرونیل بوتوکساید (PBO) و تری فنیل فسفات (TPP) بیشترین نسبت سینرژیستی را در جمعیت کاشان به ترتیب ۱/۹۹ و ۲/۴۲ برابر نشان دادند، اما دی‌اتیل‌مالئات (DEM) نسبت سینرژیستی بالایی را نشان نداد. جهت تعیین نقش آنزیم‌های سم‌زدا، فعالیت آنزیمی مونواکسیژنازهای وابسته به سیتوکروم P450، کربوکسیل‌استرازها و گلوتاتیون‌اس‌ترانسفرازها اندازه‌گیری شد. نسبت به جمعیت حساس، افزایش ۳ برابری فعالیت سیتوکروم P450 در جمعیت گرگان و افزایش ۲ برابری فعالیت کربوکسیل‌استرازها در جمعیت کاشان وجود داشت. بر اساس نتایج به نظر می رسد مونواکسیژنازهای وابسته به سیتوکروم P450 و کربوکسیل‌استرازها، آنزیم‌های مسئول در ایجاد مقاومت به نئونیکوتینوئیدها در جمعیت‌های *B. tabaci* آزمایش شده دراین تحقیق می‌باشند.

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