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*Orijinal araştırma (Original article)*

# Characterization of imidacloprid resistance in *Aphis gossypii* (Glover) (Hemiptera: Aphididae) in Southern Iran[1]

Güney İran'da *Aphis gossypii* (Glover) (Hemiptera: Aphididae)'de imidacloprid direnç karakterizasyonu

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## Summary

The cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae), is a key pest of cucurbits in Fars Province, Southern Iran and is managed with repetitive applications of insecticides such as imidacloprid. Recently, reports of insecticide control failures have increased, particularly with imidacloprid. In present work susceptibility of two *A. gossypii* populations to imidacloprid and effects of possible synergist, Triphenyl phosphate (TPP), Diethyl maleate (DEM) and Piperonyl butoxide (PBO) were checked using micro-applicator bioassay. The resistant population was collected from cucumber host plant in greenhouse and susceptible population had been reared under greenhouse conditions on *Cucumis sativus* L. cv. Negin (Cucurbitaceae) for two years. A resistance to imidacloprid with resistance ratio of 11.24 was found in resistant population compare with the susceptible population. Combination of *in vivo* differential synergism studies and biochemical assays (esterase, GSTs, cytochrome $P_{450}$ monooxygenase and heme peroxidase assay) suggest that the enhanced cytochrome $P_{450}$ activity is the primary mechanism of increased resistance in these populations.

**Keywords:** Cotton aphid, bioassay, esterase, GSTs, $P_{450}$ activity, heme peroxidase assay

## Özet

Pamuk yaprak biti *Aphis gossypii* (Glover) (Hemiptera: Aphididae), güney İran'ın Fars bölgesinde cucurbitlerin anahtar zararlılarından biridir ve imidaclorpid gibi tekrarlanarak kullanılan insektisitler ile kontrol edilmektedir. Son yıllarda insektisitlerin özellikle de imidaclorpid uygulamalarının başarısız olduğu kayıt edilmektedir. Bu çalışmada *A. gossypii*'nin iki duyarlı popülasyonunun imidaclorpidlere duyarlılığının ve Triphenyl phosphate (TPP), Diethyl maleate (DEM) ve Piperonyl butoxide (PBO) gibi bazı sinerjist etkili kimyasalların imidaclorpit duyarlılığına etkisi mikro aplikatör kullanılan denemeler ile test edilmiştir. Yaprakbitinin dayanıklı popülasyonu seralardan kabakgiller üzerinden toplanmış, duyarlı popülasyon ise *Cucumis sativus* L. cv. Negin (Cucurbitaceae) üzerinde iki yıl süre ile yetiştirilmiştir. Imidoclorpid dayanıklılığı duyarlı popülasyona oranla 11.24 kat daha fazla bulunmuştur. Farklı sinerjik etkili maddelerin (esteraz, GSTs, cytochrome $P_{450}$ monooxygenaz ve heme peroxidaz) kombinasyonlarının test edildiği biyokimyasal çalışmalar sonucunda cytochrome $P_{450}$ mekanizmasının dayanıklılığı arttıran birincil mekanizma olduğu tespit edilmiştir.

**Anahtar sözcükler:** Pamuk yaprakbiti, esteraz, GSTs, $P_{450}$ activitesi, heme peroksidaz

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## Introduction

The cotton aphid, *A. gossypii* (Glover) (Hemiptera: Aphididae), is a cosmopolitan insect pest on cotton and many field crops and vegetables (Kim et al., 1986). In Iran in addition to cotton, it is the major pest of Cucurbitaceae, especially on cucumber, *Cucumis sativus* L. (Khanjani, 2005). *A. gossypii* causes direct damage through sucking nutrients from the plant and indirect damage through contamination with honeydew and by vectoring viral pathogens (Ebert & Cartwright, 1997). The cotton aphid has progressed towards high resistance to many commonly used insecticides in many agricultural areas, including organophosphates, carbamates, pyrethroids and neonicotinoids (Ahmad et al., 2003; El-Kady, 2007; Furk & Hines, 1993; Gubran et al., 1992; Herron & Wilson, 2011; Hollingsworth et al., 1994; Martin & Workman, 1997; Wang et al., 2002; Wang et al., 2007).

Neonicotinoids are an important group of insecticides and functions as a competitive inhibitor on nicotinic acetylcholine receptors in the central nerve system. Because of distinctive action mechanism, neonicotinoids have strong insecticidal activity, especially against hemipteran pests, also low levels of toxicity to mammals and relatively little toxicity to non-target insects are reported (Nauen & Denholm, 2005). In spite of slow development of resistance to neonicotinoids, several insect pests including *A. gossypii* have been shown to have the ability of resistance development (Naun & Denholm, 2005; Shi et al., 2011).

In *A. gossypii* different types of enzymes have been reported to involve in detoxification of insecticides. Several studies have found a relation between esterase activity and resistance to organophosphates (Hama et al., 1995; O'Brien & Graves, 1992). Carbamate resistance has a relationship with enhanced mixed function oxidase (MFO) activity (Saito et al., 1995). It is reported that carboxylesterase imparted resistance to cypermethrin in *A. gossypii* (Jhansi & Subbaratnam, 2004). Alteration in the target site for the toxicant is another resistance mechanism in insects (Talebi Jahromi, 2011). Han et al*.,* (1998) concluded that carbamate primicarb resistance in *A. gossypii* clones is because of alteration in the target site, acetylcholinesterase (AChE).

The results of biochemical assays using model substrates for determination of responsible relationship between enzyme activities and insecticide resistance may be ambiguous and must be interpreted carefully. Isozymes that metabolize model substrates may not necessarily be those that detoxify insecticides (Huang, 2002). Studies of possible synergistic effects are important for detection of detoxification mechanisms. Use of enzyme inhibitors, Triphenyl phosphate (TPP, inhibitor of various esterases), Diethyl maleate (DEM, Glutathion-S-transferase inhibitor) and Piperonyl butoxide (PBO, inhibitor of cytochrome p450-dependent polysubstrate monooxygenases) may be useful in cases where an increased enzymatic detoxification contribute to resistance.

In Iran, control of *A. gossypii* relies heavily on insecticides, especially in greenhouses. It is done mostly by imidacloprid, the most extensively used neonicotinoid that was introduced in 1991 (Karunker et al., 2009). Thereforethe purpose of this study was to compare two populations of this pest against imidacloprid in Fars province, Iran, and to compare the activity of three well-known enzymes that detoxify xenobiotics in resistant and susceptible populations.

## Materials and Methods

### Insects

The susceptible population (Shiraz) had been routinely reared in net-covered cages, 70* 50* 40 cm, under greenhouse conditions at 28: 18 °C, 65 ± 5 RH and 16:8 (L:D) photoperiod on cucumber plants, *Cucumis sativus* L. cv. Negin (gynoecious) (Cucurbitaceae) since 2012. The plants in the cages were replaced every 2 weeks with new ones in order to keep colonies alive. The resistant population was collected from cucumber host plant in a greenhouse located in Sadra town near Shiraz in Fars province, Iran in 2014. This population had a history of previous exposure to different insecticides such as pirimicarb, oxydemeton-methyl, malathion in the last year, but in the last 6 months imidacloprid was sprayed every two weeks by the grower in greenhouse. The population was assessed after 3 weeks of rearing in mentioned greenhouse conditions. Synchronized five days old aphids were used in all experiments.

**Chemicals**

Imidacloprid (95% technical grade) used in all bioassays was obtained from Moshkfam Fars Co., Iran. Triton X-100, 1-chloro-2,4-dinitrobenzene (CDNB), the synergist Triphenyl phosphate (TPP, 99% purity), Diethyl maleate (DEM) and Piperonyl butoxide (PBO) were purchased from Merck company (Germany), 3,3‘,5,5‘ tetramethyl benzidine (TMBZ), Hydrogen peroxide, Cytochrome C, Bovine serum albumin, 1-Naphthyl acetate (1-NA), 2-naphthyl acetate (2-NA), 1-naphthol, 2-naphthol, Reduced glutathione (GSH), 7-ethoxycoumarin (7-EC) were obtained from Sigma Chemical Co. and Fast Blue RR salt (o-dianisidine, tetrazolized zinc chloridecomplex) was obtained from Fluka (Buchs, Switzerland).

**Micro-applicator bioassay and calculation of synergistic effects**

The solvent in all bioassays was 50% acetone in aqueous Triton X-100 (0.5 g litre$^{-1}$). Susceptible and resistant populations were compared by microapplicator bioassay and then effects of synergists were evaluated on these two populations by this method. Microapplicator bioassay was done using the method described by Immaraju et al. (1990). 5 days old apterous aphids were placed on filter paper. Using a microapplicator (the Hamilton Company. INC. Whittier, California) a 0.5 µl of each insecticide solution was topically applied to individual insects. Controls were treated with the solvent alone. Treated insects were quickly transferred on cucumber leaf discs (55mm diameter) which had been placed upside down on an agar bed (0.9 g litre$^{-1}$) in 55 mm Petri-dishes. Petri-dishes were ventilated by a 15 mm hole covered with a fine-mesh net. Petri-dishes containing aphids were kept in incubator at 25±1ºC, 65% RH and 16:8 (L:D) photoperiod. Mortality was assessed after 48 h.

To confirm the results of enzyme activity measurement, the synergisms of TPP, PBO, and DEM on susceptible and resistant populations were determined. To choose the appropriate dose of synergists, a preliminary microapplicator bioassay was done using different doses of them. For each chemical the highest dose that caused comparable mortality with the control after 48 h was selected. So the synergists PBO, DEM and TPP were prepared at concentrations of 20, 20 and 10µg ml$^{-1}$ respectively. The above-mentioned synergists solutions were used as solvent to prepare stock solution and serial dilutions (five concentrations) of imidacloprid for studying of synergistic effects as described by Nauen et al. (1998).

**Determination of esterase activity**

Fifty, 5 days old apterous aphids of *A. gossypii* from resistant and susceptible populations were homogenized in 600 µl of ice-cold sodium phosphate buffer (100 mM, pH 7, containing 0.1% of Triton X-100) and centrifuged at 10,000g at 4°C for 10 min. The resulting supernatants were used for biochemical analyses.

Total esterase activities against the substrates, 1-NA and 2-NA were measured following the method of Van Asperen (1962) with some modifications, including changes in ratio of materials and using fast blue RR instead of diazoblue laurylsulphate solution. The reaction was initiated with addition of 20 µl enzyme samples to 200 µl of substrates (1 part of substrate 30mM in acetone + 99 parts 0.02M phosphate buffer pH 7.0). 50 microliter fast blue RR (3mg mL$^{-1}$ in distilled water) was then added to the reaction mixture and the released product was continuously measured at 450nm for 1-NA and at 540nm with 2-NA at 30 sec intervals for 15 min using a microplate reader (ELX808 Bio-Tek). Reactions without enzyme source served as control. At least three replicates were performed for each population. The specific activity was expressed as µmoles of product min$^{-1}$ mg protein$^{-1}$. Protein content of the enzyme samples was determined following the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard. Standard curves of absorbance versus concentrations of 1-naphthol and 2-naphthol were constructed to enable calculation of the amount of product produced during the esterase assay.

**Determination of GSTs activity**

Enzyme source was prepared from fifty,5 days old apterous aphids from resistant and susceptible populations in the same way as esterase (buffer without Triton X-100). GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates with the method used by Habig et al.(1976) with slight modifications to conduct the experiment in 96-well microplate, including:

two hundred microliter reaction mixtures (250 µl CDNB 63mM in methanol and 5ml GSH 10 mM in 0.1M sodium phosphate buffer, pH 6.5) were placed in a well containing 60µl of the enzyme sample. Reagents were prepared fresh prior to use. The change in absorbance was measured continuously at 30 sec intervals for 11 min at 340 nm. The rate of product glutathione conjugated (S-(2,4-dinitrophenyl)glutathione) formation, that indicates the enzyme activity was calculated by using molar extinction coefficient of product as 9.6 $mM^{-1}$ $cm^{-1}$ at 340 nm and 25°C (Lizuka et al., 1989). Reactions without enzyme source served as control. At least three replicates were performed for each population. The specific activity was expressed as µ moles of product $min^{-1}$ mg protein-$^{-1}$. Protein content of the enzyme samples was determined following the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard.

**Determination of cytochrome $P_{450}$ monooxygenase activity**

$P_{450}$s activity was determined using the 7-ethoxycoumarine-*O*-deethylase activity based on the method of de Sousa et al. (1995) as described about aphids by Castaneda et al. (2009, 2010) and Cabrera-Brandt et al. (2010) with modifications, described here. Fifty, 5 days old apterous aphids of *A. gossypii* from resistant and susceptible populations were homogenized in 400 µl of ice-cold sodium phosphate buffer (50 mM, pH 7.2) and centrifuged at 1000g at 4°C for 10 min. The resulting supernatants were used as the enzyme source. Fifty µl enzyme sample was added to each well of a black 96-wells microplate containing 100 µl of substrate solution (0.4 mM 7-ethoxycoumarin in 50 mM pH 7.2 phosphate buffer). The reaction was incubated for 4 h in darkness at 30 ºC and then stopped by the addition of 100 µl of buffer glycine/ethanol (50% v/v). For the controls, the same volume of buffer glycine/ethanol (50% v/v) was added before the incubation to avoid the reaction. Fluorescence was read at 390 nm for excitation and 460 nm for emission, using infinite M200 microplate reader (Tecan ®). The assay contained 3 replicates of each population. Protein content was measured according to Bradford (Bradford, 1976). The specific activity was expressed as changes in fluorescence units $min^{-1}$ mg protein-$^{-1}$.

**Heme peroxidase assay**

Twenty µl homogenate (fifty,5 days old apterous aphids were homogenized in 600 µl of 0.1 M phosphate buffer, pH 7.0) was mixed with 80 µl of 0.625 M potassium phosphate buffer pH 7.2, 200 µl of TMBZ solution (0.01 g TMBZ dissolved in 5 ml methanol and then 15 ml of 0.25 M sodium acetate buffer pH 5.0) and 25 µl of 3% hydrogen peroxide in a microplate well. After 2 h incubation at room temperature in darkness, the plate was read at 450 nm as an end point assay. By using a standard curve of pure cytochrome C, which contains one bound heme per molecule, an estimate of the amount of monooxygenases present was obtained and expressed as equivalent units of cytochrome $P_{450}$ (Damayanthi & Karunaratne, 2005). The assay contained 3 replicates for each population.

**Statistical analysis**

Resistance Ratio (RR) was calculated by dividing the $LC_{50}$ of resistant population (Sadra) by the $LC_{50}$ of susceptible population (Shiraz). Data were analyzed by employing ANOVA and means were compared by Duncan multiple range test ($p < 0.05$) using SPSS (version 21). $LC_{50}$ was determined using probit analysis with the PC-software Polo-Plus (LeOra Software, Berkeley, CA). Dose responses were considered significantly different if the confidence interval calculated from their ratio did not overlap 1.

## Results

**Microapplicator bioassay and calculation of synergistic effects**

The results revealed that the resistance ratio (RR) in resistant population was 11.24 (Table 1). There was a significant difference between $LC_{50}$ values of PBO-imidacloprid-treated and imidacloprid-treated in resistant population. Imidacloprid was approximately six times more toxic in the presence of PBO than in the absence of PBO (synergism ratio= 5.82). Therefore, PBO had an obvious synergism to imidacloprid on resistant population. However, PBO had no significant synergism to imidacloprid in susceptible population. Also DEM and TPP had no significant synergism to imidacloprid in both populations. The tests confirmed that enhanced MFO activity is responsible for the observed imidacloprid resistance.

Table 1. Synergism of PBO, DEM and TPP on imidacloprid in resistant and susceptible populations of *Aphis gossypii*

| Population[A] | Treatment | N-d[B] | N-i[C] | LC50 µg ml-1 (LCL–UCL)*[D] | Slope ± SE | $X^2$ (df)[E] | RR[F] | SR[G] |
|---|---|---|---|---|---|---|---|---|
| resistant | Imidacloprid | 5 | 297 | 673.04[a] (542.76- 810.6) | 2.58± 0.29 | 11.23 (18) | 11.24 | ----- |
| | Imidacloprid+ PBO | 5 | 305 | 115.01[b] (92.42- 142.79) | 2.01± 0.26 | 7.82 (18) | 1.92 | 5.82 |
| | Imidacloprid+ DEM | 5 | 308 | 570.35[a] (471.18- 681.27) | 2.31± 0.23 | 13.41 (18) | 9.53 | 1.18 |
| | Imidacloprid+TPP | 5 | 262 | 709.35[a] (577.81- 856.06) | 2.55± 0.30 | 16.36 (18) | 11.85 | 0.95 |
| susceptible | Imidacloprid | 5 | 278 | 59.86[b] (49.016- 72.02) | 2.30± 0.253 | 14.41 (18) | ------ | ----- |
| | Imidacloprid+ PBO | 5 | 249 | 67.44[b] (54.29- 81.91) | 2.45± 0.30 | 12.56 (18) | ------ | 0.89 |
| | Imidacloprid+ DEM | 5 | 377 | 49.41[b] (38.98- 59.96) | 2.39± 0.29 | 14.57 (18) | ------ | 1.21 |
| | Imidacloprid+TPP | 5 | 311 | 63.40[b] (51.09- 77.35) | 1.78± 0.20 | 7.56 (18) | ------ | 0.94 |

[A] Shiraz: susceptible, Sadra 1: resistant
[B] Number of doses
[C] Number of insects tested without controls.
[D] The $LC_{50}$ values are expressed as µg $ml^{-1}$, LCL: lower confidence limit at 95%; UCL: upper confidence limit at 95%.
* Means within the same rank followed by different letters are significantly different at $p < 0.05$.
[E] Values of $X^2$, lower than ($p \leq 0.05$) indicate a significant fit between the observed and expected regression lines.
[F] RR: Resistance ratio: $LC_{50}$ of different treatments of resistant population/ $LC_{50}$ of susceptible population.
[G] SR: Synergist ratio = $LC_{50}$ of imidacloprid alone/$LC_{50}$ of imidacloprid + synergist.

**Esterase activity**

Results of esterase assay showed that there were significant differences in esterase activities between the susceptible and resistant populations (Fig. 1). Quantitative analysis of general esterase activity with 1-NA as substrates revealed that activity of esterase in susceptible population is 2.4-fold higher than resistant population. The same situation was observed when esterase activity against the substrate 2-NA was compared. Susceptible population showed more esterase activity (2.2-fold) than resistant population.

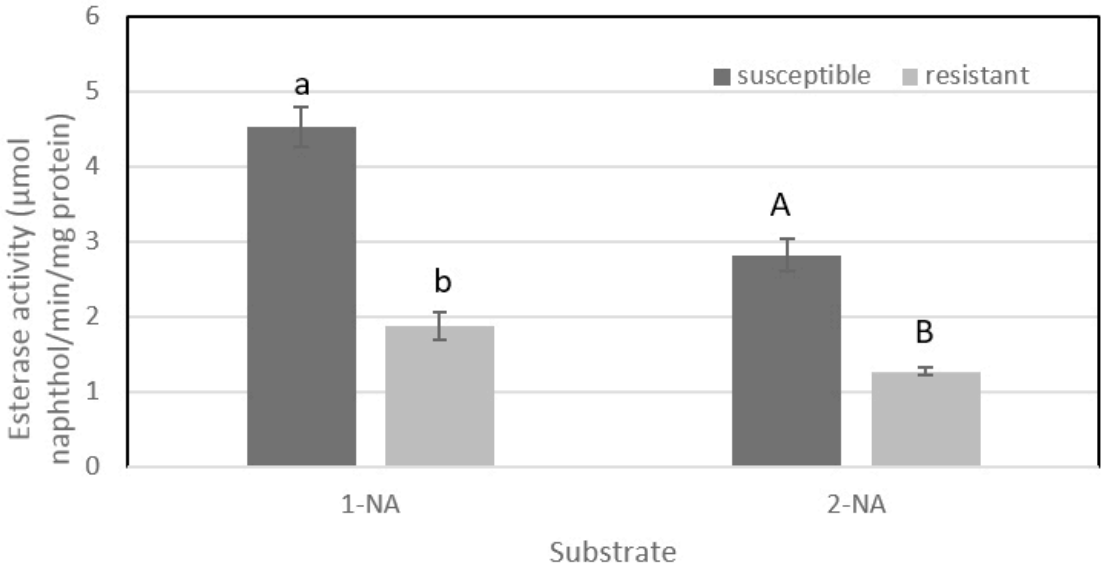


Fig. 1. Esterase activities in susceptible and resistant populations of *Aphis gossypii* from Fars province, Iran, using 1-NA and 2- NA substrates. Data are means ± SE. The means followed by different letters are significantly different at $p < 0.05$.

Results of this study demonstrated that TPP had no effect on imidacloprid toxicity in resistant population (synergism ratio was 0.95) (Table 1). This indicated that, in this population, esterase doesn't play a role in imidacloprid detoxification. Although due to structure of imidacloprid, hydrolysis of that is not possible (Nauen et al., 1998), esterase activity sometimes has been reported as an important mechanism for imidacloprid resistance, for example on *Aphis craccivora* C.L. Koch (Hemiptera: Aphididae) (Mokbel & Mohamed, 2009) and *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Choi et al., 2001), even in some reports in *A. gossypii* esterase has been known as the reason of resistance to imidacloprid (Li & Han, 2007). In contrast it has been shown that the esterase does not confer resistance to imidacloprid in *Myzus* spp. (Nauen et al., 1996; Philippou et al., 2010). Also esterase could have a little effect in super-high resistant strain of brown plant hopper (Wen et al., 2009).

Metabolic enzyme activity analysis showed that the esterases specific activity is significantly higher in susceptible than resistant population (Fig. 1). The difference may be due to the different origins and genetic variation of these two populations. Another possibility is that insects must cope with the toxicity of insecticides and it requires energy and resource allocation for adaptation and survival. Several mechanisms which are used by insects for resistance against insecticide may affect reproduction, impair dispersal ability and have several other effects on the insect's fitness (Kliot & Ghanim, 2012). Reduction of a detoxificant enzyme because of increase in the other detoxificant enzyme has not been reported yet, but possibly it is a reason for low amount of esterase in resistant population.

**GST activity**

Comparison of the GST activity of susceptible and resistant populations showed 1.4-fold higher activity in resistant population (Fig. 2). Specific activity of GSTs was significantly more in resistant than susceptible population.

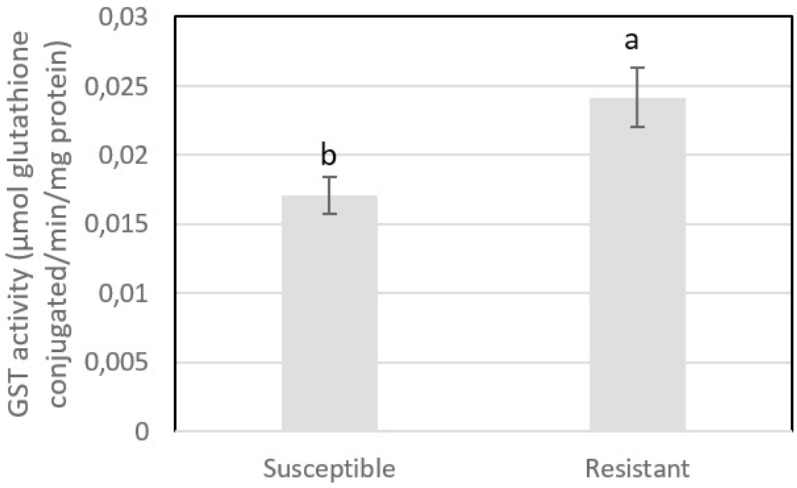


Fig. 2. GST activities in susceptible and resistant populations of *Aphis gossypii* from Fars province, Iran, using CDNB and GSH as substrates. Data are means ± SE. The means followed by different letters are significantly different at $p < 0.05$.

Although a significant specific difference in the activity of GST was noticed in resistant population, but GST's do not play a role in the imidacloprid resistance because DEM did not display synergism in resistant population, where the synergism ratio was 1.18 (Table 1). Li & Han (2007) also reported that in *A. gossypii* DEM had little synergistic effect on imidacloprid on both resistant and susceptible strains. Maybe the higher activity of GST in resistant population is because of history of exposure to other insecticides in addition to imidacloprid before collection as described in materials and methods. Another probability is genetic variation that naturally exists between populations.

**Cytochrome $P_{450}$ activity and Monooxygenases contents**

Cytochrome $P_{450}$ monooxygenases activity showed significant differences between susceptible and resistant populations (Fig. 3). Specific activity showed 2.1-fold higher activity in resistant population. Experiments with different synergists delivered the same conclusion, as cytochrome $P_{450}$ resulted in a higher synergism ratio (SR=5.82) in resistant populations. In addition to cytochrome $P_{450}$ activity assay, heme peroxidase assay also has been used in resistance experiments and increased heme contents has been known related to resistance in insects and mites (Memarizadeh et al., 2013; Tiwari et al., 2011). Therefore, for more confidence from the amount of cytochrome $P_{450}$, heme peroxidase assay was done. This assay does not measure mono oxygenase activity but titrates the amount of heme bound in the insect homogenate (Damayanthi & Karunaratne, 2005).

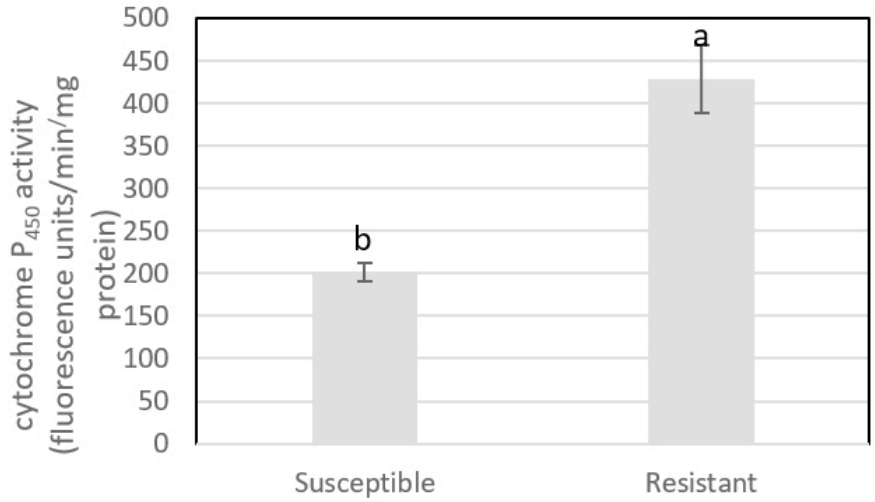


Fig. 3. Cytochrome $P_{450}$ activities in susceptible and resistant populations of *Aphis gossypii* using 7-ethoxycoumarin as substrates. Data are means ± SE. The means followed by different letters are significantly different at $p < 0.05$.

Involvement of mono oxygenases in the imidacloprid resistance of *A. gossypii* was indirectly tested by quantifying bound heme. The results indicated that heme contents in the resistant population was 2.66-fold higher than that in the susceptible population (Fig. 4).

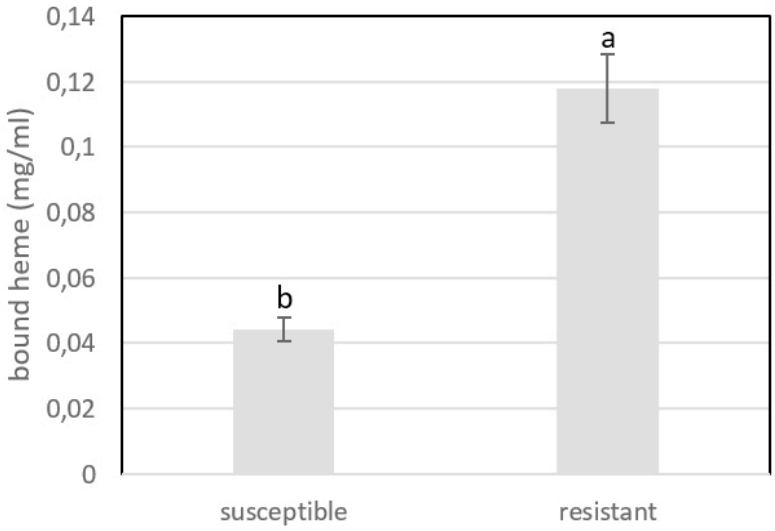


Fig. 4. Means ± SE of heme content in the resistant and susceptible populations of *Aphis gossypii.* The means followed by different letters are significantly different at $p < 0.05$.

Results of this assay confirmed the high amount of cytochrome $P_{450}$. Therefore, both enzymatic assays and synergism studies indicated that $P_{450}$- monooxygenase are key factors in imidacloprid detoxification and resistance development. In literature, resistance to imidacloprid has been attributed to increased rates of detoxification, especially enhanced oxidative detoxification of this pesticide by over-expressed $P_{450}$ monooxygenases. In *A. gossypii* (Shi, 2012), *M. persicae* (Philippou et al., 2010; Puinean et al., 2010b), *Drosophila* spp. (Diptera: Drosophilidae) (Daborn et al., 2001), brown planthoppers (*Nilaparvata lugens* Stal; Hemiptera: Delphacidae) (Puinean et al., 2010a; Wen et al., 2009; Zewen et al., 2003) and in *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Karunker et al., 2008; Karunker et al., 2009; Nauen & Denholm, 2005), $P_{450}$- monooxygenase is thought to be one of the most important mechanisms for imidacloprid resistance.

New insecticides are increasingly difficult and costly to develop; therefore, it is important that the value of the currently used insecticides is not lost to agriculture through the development of insecticide resistance. The ability to understand the status of insecticide resistance, along with the underlying genetics and the risk of resistance development, can significantly contribute to the development of sustainable pest management strategies. More exposure to pesticides may increase resistance, and it should be notable by the pesticide users. Changes in $LD_{50}$ values for imidacloprid are occurring rapidly, which likely is caused by a wide scale use of imidacloprid. Use of imidacloprid is a major factor for the occurrence and development of resistance to this insecticide in Iran. On the other hand, the resistance dynamics studies show that if we stop the spraying pressure, resistance to imidacloprid is not stable (Wang et al., 2009). If the use of these pesticides is interrupted there is great potential for the return of the sensitivity of aphids.

Since imidacloprid is so widely used to control cotton aphid in Iran and with growing concerns about imidacloprid tolerance in many insect species, it is important to develop baseline information for monitoring imidacloprid resistance to the *A. gossypii*. This research provides a basis for developing successful resistance-management plans and efficacy of neonicotinoids in use for aphid control. Resistance needs time to establish and the susceptibility of aphids should be monitored carefully over the next years to keep the effectiveness of this class of compounds as long as possible. This would enable local advisors and farmers to implement the appropriate control strategies to sustain high yields and crop quality.

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