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The effect of esterase activity in resistance of *Aphis gossypii* to selective insecticides

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Abstract

Esterase-mediated metabolic resistance more or less happens in all insects and all classes of insecticides in ester group. In the current study, the inhibitory effect of oxydemeton-methyl and pirimicarb on acetylcholinesterase (AChE) activity and their insecticides and imidaclopride extracted from two populations of *Aphis gossypii* (cotton aphid and melon aphid) on carboxylesterase activity were compared. Results showed that cotton aphid esterase activity against two substrates including α– and β-naphthyl acetate was 124.6 and 71.6 U/mg and for melon aphid 33.7 and 53 U/mg, respectively. Native electrophoresis showed that cotton aphid has more esterase bands than melon aphid (5 versus 3 bands). Cholinesterase inhibitory test showed that 50% of the enzyme activity of cotton aphid inhibited by 776.2 and 958.7 ppm of oxydemeton-methyl and pirimicarb, respectively, while 50% of the enzyme activity of melon aphid inhibited by 225.8 and 741.3 ppm of oxydemeton-methyl and pirimicarb, respectively. Thus, cotton aphid is more resistant to selected insecticides than melon aphid.

Key words: *Aphis gossypii*, electrophoresis, carboxylesterase, acetylcholine esterase, oxydemeton-methyl, imidaclopride, pirimicarb.

Introduction

Cotton aphid (*Aphis gossypii*) has a worldwide distribution and causes damage to numerous economically important crops [17]. In recent years, it has been considered as a serious pest in main cotton production areas of Iran [16]. Outbreaks of *Aphis gossypii* on cotton and melon fields in Torbatejam (Iran) happen in different times. For cotton the outbreaks are synchronized to *Bemezia tabaci* (the key pest of cotton), both controlled with one spraying; but for melon the outbreaks happen in two times; one happening in the first growth season that is usually controlled with insecticides, whereas the second happened in the end of growing season, when the persistence of insecticide spraying against key pests of melon is finished, therefore it is not controlled. Various insecticides from different groups have failed to control this pest [9, 12]. In addition, the development of resistance to chemical treatments by the *Aphis gossypii* has also been reported [9, 10, 32].

Esterases are frequently implicated in the resistance of insects to organophosphorus (OP) compounds, carbamates and pyrethroids through gene amplification, upregulation, coding sequence mutations or a combination of these mechanisms [15]. Increase of carboxylesterase activity [4], rarely decrease of carboxylesterase activity [3], excessive production of different forms of carboxylesterase [20], overproduction of esterase [27] and qualitative changes in enzyme structure [18] are responsible for esterase-mediated metabolic resistance. Acetylcholinesterase (AChE) is a serine hydrolase, which catalyzes the hydrolysis of acetylcholine. This enzyme is the target of organophosphate and carbamate insecticides which the serine of the active site of phosphorylates or carbamoylates blocking the hydrolysis of the neurotransmitter acetylcholine. The post-synaptic membrane then remains depolarized and synaptic transmission cannot take place, as a result, the insect dies [5].

In Iran, selected insecticides are commonly used to control various aphids on different crops, not only in the fields and gardens, but also in greenhouse. As in the case of other organophosphate and carbamate insecticides, pirimicarb and oxydemeton-methyl exert their effects by inhibiting esterases, especially AChE. Imidaclopride from neonicotinoid group acts on the nicotinic acetylcholine receptor, causing the insect to reduce or stop feeding, and reduces mobility [2]. Furthermore, the production of different forms of carboxylesterase is also reported to be the cause of resistance to insecticides in insects [20, 31]. Effective proactive insecticide resistance monitoring will contribute significantly to maintaining the capacity for effective insecticidal control [8].

Mechanisms of insecticide resistance in *Aphis gossypii* Glover in Iran have been poorly investigated. In this paper, collections of *Aphis gossypii* (cotton aphid and melon aphid) was collected from cotton and melon fields of Torbatejam (Iran) and after mass rearing in laboratory conditions, effect of selective insecticides on carboxylesterase and acetylcholinesterase activity extracted from two aphid populations was studied to determine existence probability of relative resistance to these insecticides**.**

Materials and Methods

Insect rearing: Stock colonies of *A. gossypii* used in these experiments were collected from melon and cotton farms of Torbatejam, Iran. The melon aphid colony reared on *Cucumis melo* var. Khatooni seedlings and cotton aphid colony reared on *Gossypium hirsutum* var. Varamin seedlings were handled in

greenhouse at 20±2°C, a 16 h light: 8 h dark cycle and relative humidity of 55±5% as described by Lashkari *et al*.[13]. These colonies were reared for several generations and apterous adults from these colonies were used for the insecticide tests.

Insecticides and chemicals: Three insecticides used in this experiment consist of pirimicarb 50% wettable powder (China's Jecom Company), imidacloprid (Confidor®) 35% suspension concentrate (German's Bayer Company) and oxydemeton-methyl (Metasystox-R®) 25% emulsifiable concentrate (German's Bayer Company). Acetylthiocholine iodide (ATChI), 5, 52-dithiobis-(2-nitrobenzoic acid) (DTNB), α– and β-naphthyl acetate and other biochemical reagents belonged to Sigma.

Enzyme preparation: For carboxylesterase, each ten female adults were ground in 400 µl of distilled water homogenized by a simple homogenizer on the ice dishes. The crude homogenates were centrifuged at 18,000 g for 30 min at 4°C. For AChE, 10 apterous adults (50 mg) were homogenized with 800 µl pre-cold homogenization buffer (0.01 mol/l sodium phosphate buffer, (pH 7.0), containing 1% Triton X-100) and centrifuged at 18,000 g for 15 min at 4°C. The supernatant was collected and used as enzyme solution.

Carboxylesterase assay: General esterase activity was assayed based on the method of Van Asperen [30]. The general buffer was 0.02 M, pH 7.0 phosphate buffer. α– and β-Naphthyl acetate were used as substrates. Twenty µl of supernatant mixed with 20 µl of substrate and 280 µl of phosphate buffer was incubated at 8°C for 30 min in a water-bath. Color development after incubation was obtained with adding 64 µl of an indicator solution mixture of sodium dodecylsulphate-fast blue B salt (SDS-FBS) and read at 700 nm on a spectrophotometer against a control that lacked enzyme. This experiment was treated for melon and cotton aphids with 2 substrates in 3 replications.

Electrophoresis*:* Esterases were separated by discontinuous, native polyacrylamide gel electrophoresis (PAGE) following the method described by Zhou *et al*.[33]. It was performed in a vertical mini protean II electrophoresis apparatus (Bio-Rad, Richmond, CA) using a 8% separating gel and 4.5% stacking gel with a continuous tris/glycine running buffer system (100 ml, pH 8.8). Samples were diluted 1:1 with 2 sample buffer [20% sucrose (w/v), 0.1% bromophenol blue and glycerol in 50 mM tris/glycine running buffer (pH 8.8)]. Gels were run at 150 V constant voltage for 3 hours at 4°C. Esterase bands were visualized by incubating the gels in 170 µl of 0.02 M sodium phosphate buffer (pH 7.0), with 2% (v/v) 30 mM α-naphthyl acetate and 30 mM β-naphthyl acetate dissolved in 50 ml of distilled water at 25°C for 60 min, before adding 60 µl of fast blue salt solution. Then gel was hold in acetic acid 7% and scanned by gel-documentation.

Acetylcholinesterase (AChE) inhibition assay*:* AChE inhibition by oxydemeton-methyl and pirimicarb were determined according to the method of Li and Han [14], using ATChI as substrate. Briefly, the reaction mixture consisting of 50 µl DTNB (0.18 mmol/l), 50 µl ATChI (0.675 mmol/l) and enzyme preparation (50 µl) was prepared. The inhibition of both insecticides was determined by adding 50 µl of various concentrations of them to the substrate. Absorbance was recorded by a spectrophotometer at 470 nm after 10 min in water-bath. These assays were treated for 3 replications. I_{50} values for the AChE of two populations were estimated by probit analysis using the POLO-PC computer program.

Results

Activities of carboxylesterase: Carboxylesterase from cotton aphid showed a significantly higher inhibition to both substrates than melon aphid ($P<0.05$). So, the esterase activity of cotton aphid was 1.6 and 1.3 fold higher than those of the melon aphid for α-NA and β-NA, respectively (Tables 1 and 2).

Table 1. Activity of carboxylesterase in cotton aphid (*Aphis gossypii*).

Variance	Standard error	Moderate absorbance of Wavelength (nm)	Substrate
20.33	4.51	124.66	α-naphthyl acetate
22.33	4.72	71.66	β-naphthyl acetate

Table 2. Activity of carboxylesterase in melon aphid (*Aphis gossypii*).

Variance	Standard error	Moderate absorbance of Wavelength (nm)	Substrate	Standard error
20.33	4.50	74.33	α-naphthyl acetate	4.50
13	3.36	53	β-naphthyl acetate	3.36

Electrophoresis*:* The presence of extra esterases was detected in native PAGE. A total of five esterases was observed in the cotton aphid, whereas three of these esterases appeared in melon aphid (Fig. 1). All esterases were able to use α– and β-naphthyl acetate.

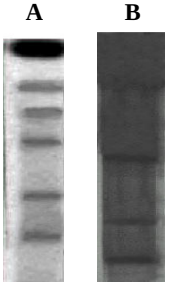

Figure 1. Polyacrylamide gels of esterase activities toward α– and β-naphthyl acetate in adult aphid from cotton (A) and melon (B) population of *Aphis gossypii*.

Inhibition of acetylcholinesterase by oxydemeton-methyl and pirimicarb: The inhibitory results showed that cotton aphid's AChE was significantly more insensitive to pirimicarb and much more insensitive to oxydemeton-methyl than melon aphid (Tables 3 and 4). So I_{50} ratio of cotton aphid to melon aphid for pirimicarb and oxydemeton-methyl was 1.3 and 3.4 fold higher, respectively. Percent inhibition of oxydemeton-methyl and pirimicarb on AChE in two populations of *Aphis gossypii* is shown in Figs 2 and 3.

Discussion

Xenobiotic resistance in insects has evolved predominantly by increasing the metabolic capability of detoxificative systems and/or reducing xenobiotic target site sensitivity [15]. Resistance of *A.gossypii* to organophosphorus, carbamate and neonicotinoid insecticides was almost detected to be associated to increase in

Table 3. Estimating I_{50}, confidence interval and lines responses' parameters of cotton aphid (*Aphis gossypii*) to pirimicarb and oxydemeton-methyl insecticides.

X^2 (d.f.)	Slope± SE	Confidence interval (95%)	I_{50}	Insecticide
3.3(3)	2.821±0.205	560-981.4	741.3	Pirimicarb
6.41(3)	2.241±0.175	180-286.3	226	Oxydemeton-methyl

Table 4. Estimating I_{50}, confidence interval and lines responses' parameters of melon aphid (*Aphis gossypii*) to pirimicarb and oxydemeton-methyl insecticides.

X^2(df)	Slope± SE	Confidence interval (95%)	I_{50}	Insecticide
1.4(3)	2.480±0.190	849-1087	958.7	Pirimicarb
9.72(3)	2.55±0.192	531-1144.6	776	Oxydemeton-methyl

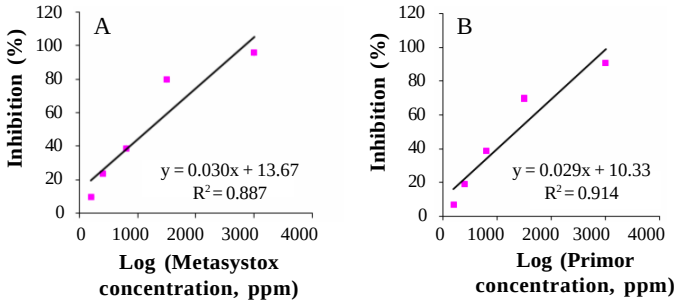

Figure 2. Percent inhibition of AChE in cotton aphid in relation to the applied oxydemeton-methyl (A) and pirimicarb (B) concentrations.

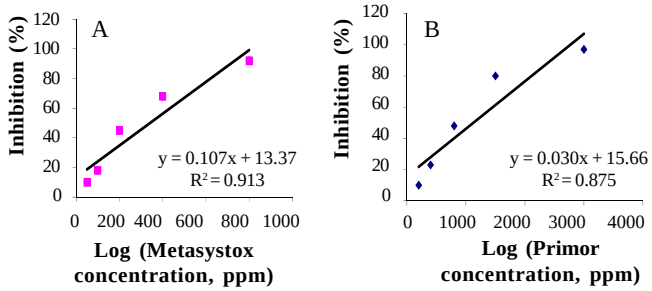

Figure 3. Percent inhibition of AChE in melon aphid in relation to the applied oxydemeton-methyl (A) and pirimicarb (B) concentrations.

activity of carboxylesterases (as sequestering proteins) [1, 4, 7, 23]. In many cases, resistance to insecticides, such as oxydemeton-methyl, imidaclopride and pirimicarb, in *A.gossypii* was correlated to esterase overexpression [10, 22, 32].

In this study, carboxylesterase activity of the *A. gossypii*, using α– and β-naphthyl acetate as substrate for insecticides, varied significantly among the cotton and melon aphid clones. Cotton aphid esterase activity against α– and β-naphthyl acetate was 1.6 and 1.3 fold higher than that of melon aphid, respectively. These results showed relative resistance of cotton aphid compared to melon aphid. Therefore, the use of insecticides was closely correlated with elevation of naphthyl esterase activity. In previous studies, Suzuki *et al.* [28] confirmed this correlation for *A. gossypii* fenitrothion resistance using α-naphthyl acetate and Oppenoorth [19], for organophosphate and pyrethroid insecticides. In some cases, excessive production of different isozymes of carboxylesterase in cotton aphid confers selective advantage to insecticide resistance [20, 31]. To come into view that presence of high activity of esterases is attributing to appearance of extra bands of esterases in native page [32].

In this study, the esterase pattern and activity were detected by electrophoresis. A qualitatively good correlation between the results obtained from the *in vitro* carboxylesterase assays and electrophoretic analysis (5 bands for cotton aphid and 3 bands for melon aphid) showed higher esterase sensitivity to inhibition in cotton aphid population than in melon aphid population. Similar correlation was also obtained for resistant clones of Japanese *Aphis gossypii* to pirimicarb and malathion insecticides by Takada and Murakami [29]. O'Brien *et al.* [18] by isoelectric focusing technique showed the affinity of different bands in organophosphate resistance of *A.gossypii* on cotton. Furk *et al.* [6] by polyacrylamide gel electrophoresis, starch gel electrophoresis and isoelectric focusing production showed that esterase patterns of pirimicarb-resistant *A. gossypii* (on chrysanthemums) consistently differed in both number of bands and migration rates from the susceptible type (on cucumber). According to assessments, generally, both qualitative and quantitative differences between the resistant and susceptible strains of cotton aphid cause resistance to insecticides [18, 20]. For example, presence of resistant-conferring co-migrating bands (E-3, E-7, E-9 and E-10) in resistant strains of *A.gossypii* gives a vivid qualitative difference between resistant and susceptible strains [21]. In contrast, in some insects, reduction in carboxylesterase activity is reported to be associated with resistance to organophosphorus insecticides. For example, in some malathion-resistant strains of *M. domestica*, that have high ali-esterase, low organophosphorus hydrolase, and intermediate malathion carboxylesterase (MCE) activities have been reported [3]. For *A.gossypii*, this phenomenon was detected in a laboratory-selected omethoate-resistant strain from China [25]. Acetylcholinesterase is our interest because insensitive AChE appears to play a role in the resistance and acts in conjunction with metabolic detoxification to confer overall resistance to pyrethroids, OPs and carbamates [24]. However, based on our esterase assays and esterase pattern, cotton aphid was more tolerant to selected insecticides than melon aphid. This also may be due to the insensitive AChE.

Based on I_{50}s values (the concentration required to inhibit 50% of AChE activity), cotton aphid population's AChE was more resistant to the inhibitory action of pirimicarb, especially oxydemeton-methyl insecticide, than melon aphid population (1.3 and 3.4 fold, respectively). Since I_{50}s values for pirimicarb in both populations were close, it suggested that melon aphid has high potential to develop cross-resistance to oxydemeton-methyl. Finally, difference in esterase activities was certain reason of relative resistance to pirimicarb between two populations. On the other hand, oxydemeton-methyl-insensitive acetylcholinesterase affects more than elevated carboxylesterase activity in creating higher resistance in cotton aphid than in melon aphid. Similar results was obtained by Sun *et al.* [26] for the organophosphate resistance in some Chinese strains of this aphid. Also Suzuki *et al.* [27] detected a reduced sensitivity of acetylcholinesterase instead of carboxylesterase activity. This was certain reason for the pirimicarb resistance in *A. gossypii.* Furthermore, Takada and Murakami [29] showed that the clones of this aphid with very high esterase activity were moderately resistant to malathion and very highly resistant to pirimicarb. These researchers concluded that malathion resistance is positively correlated with high esterase

activity, whereas pirimicarb resistance is not necessarily so and another mechanism is also responsible for resistance to pirimicarb. When different resistance mechanisms are synchronized, presence of insensitive acetylcholinesterase considerably increases resistance, but it is very difficult to separate values of resistance created by insensitive acetylcholinesterase from detoxifier esterase's. In pirimicarb-resistant and -sensitive *A. gossypii*, synergic effects of both mechanisms created high resistance that it was not possible to estimate LC_{50}. On the other hand, acetylcholinesterase is not a target site for imidaclopride, therefore, its I_{50} values were not detected for *A. gossypii*. Of course, other assays showed that cotton aphid has higher resistance than melon aphid due to a combination of elevated carboxylesterase activity and existence of extra forms of carboxylesterases. Wang *et al*. [32] showed that activities of α-naphthylacetate (α-NA) esterases and acetylcholinesterase (AChE) of imidacloprid-resistant *A. gossypii* was significantly higher than imidacloprid-susceptible strain and these cases for both strains were also significantly higher for cotton (*Gossypium hirsutum* L.) than cucumber (*Cucumis sativa* L.).

Present study has provided some basic information on the esterases of both melon and cotton aphid populations that are useful for understanding the mechanisms of insecticide resistance in *Aphis gossypii*. As both populations of *Aphis gossypii* that exhibit varying tolerance to insecticides become available, they are useful for further comparative toxicology, biochemical and molecular studies.

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