Research Article

Resistance mechanisms of a field population of diamond back moth, *Plutella xylostella* (Lepidoptera: Plutellidae) to current organophosphate pesticides

Maryam Zolfaghari¹, Mohammad Ghadamyari¹ and Reza Hassan Sajedi²

¹. Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.
². Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

Abstract: The toxicological and biochemical properties of four organophosphate (OP) insecticides, chlorpyrifos, diazinon, phosalone and dichlorvos, were examined in terms of the diamond back moth, *Plutella xylostella* (L.) susceptible (Gu-S) and resistant (Kar-R) to OPs. The Kar-R population had significantly high resistance to chlorpyriphos (69.3 fold), medium resistance to diazinon (14.49-fold) and phosalone (10.3-fold), and had less resistance to dichlorvos (5.17-fold) compared to Gu-S population. DEM and TPP reduced Chlopyrifos resistance of Kar-R population as an inhibitor of glutathione S-transferase (GST) and esterases (EST), respectively. Biochemical studies clarified that GST and EST kinetic parameters in the Kar-R population were significantly higher than parameters of Gu-S population. Moreover, this study examined the Kinetics of hydrolysis of acetylthiocholine iodide, butyrylthiocholine iodide as artificial substrates by AChE of resistant and susceptible population. IC₅₀ of monocrotophos, neostigmine bromide and eserine were also determined on AChE of resistant and susceptible populations. Kinetic analysis and inhibition tests indicated that an alteration in AChE of Kar-R population has an effect on both kinetic and inhibition results. The results distinctly showed that multiple mechanisms such as GST, esterases and altered AChE created chlorpyrifos resistance in the Kar-R and insensitivity of AChE is a significant factor for resistance to conventional OP compounds.

Keywords: *Plutella xylostella*, organophosphate pesticides, biochemical mechanisms, detoxification enzymes, acetylcholinesterase

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most devastating insect pests of cruciferous vegetables and has caused US $ 27 billion worth of global crop losses and control expenses (Zalucki et al., 2012). Short production time, high fecundity, seasonal migration patterns and a wide host plant range have turned DBM into a significant agricultural pest. DBM has increased a resistance to conventional insecticides that have been used intensively and extensively for many decades (Sayyed et al., 2008). Available insecticides have been rendered ineffective against the DBM, an urgent action is required to manage the resistance and expand the lifespan of current insecticides.

Organophosphates (OPs) are mostly used to control the agricultural pests in Iran. The OP insecticides cause toxicity by unalterable inhibition of acetylcholinesterase (AChE), which...
resolves the excitatory neurotransmitter, acetylcholine. AChE is the main target site of both organophosphate and carbamate insecticides, which blocks the enzyme activity by covalently phosphorylating and carbamylating the serine remains contain the active site gap (Corbett, 1974; Fournier, 1993). Chlorpyrifos is a wide spectrum, non-systemic and relatively toxic organophosphate insecticide that has been commonly used for more than 40 years to control many different insect pests. In California, on the average 1.45 million pounds of active ingredient of chlorpyrifos products was used annually between 2002 and 2012 (Lori and Peter, 2014). Many crop insect pests, such as Bemesia tabaci (Zhang et al., 2012), Laodelphax striatellus (Zhang et al., 2013) and Sogatella furcifera (He et al., 2015) had high level resistance to chlorpyrifos. Phosalone, diazinon and dichlorvos are the other OP pesticides which are used widely against P. xylostella in Iran. There are some reported cases of high horn fly resistance to diazinon (Beak et al., 2005) and phosalone in Agonosccna pistacies population (Alizadeh et al., 2014). According to the resistance mechanisms, improved activities of carboxyl esterase (Sun et al., 1978; Noppun et al., 1987) and glutathione S-transferase (Cheng et al., 1983) have been listed as degradation factors, even a decrease in sensitivity of the target enzyme, AChE, to OP has as well been presented (Sudderuddin and Kok, 1978; Noppun et al., 1983). Insensitive AChE has been reported in more than 30 insecticide resistant pest species. The two spotted spider mites were the first reports of OP resistant strains (Smissaert, 1964; Voss and Matsumura, 1964; Kozaki et al., 2001).

This study was undertaken to compare some toxicological and biochemical properties of detoxification enzymes (ESTs and GST) and cholinesterase between two resistant (Kar-R) and susceptible (Gu-S) field populations of P. xylostella.

**Materials and Methods**

**Insects**

The chlorpyrifos-susceptible (GU-S) population was collected from Rasht, Guilan province, Iran and the chlorpyrifos-resistant (Kar-R) population from Karaj, Alborz province, Iran. The larvae were reared on Brassica rapae and the adults were fed on 10% honey-water. Insect populations were maintained at 25 ± 2 °C and 70 ± 10% relative humidity under a photoperiod of 16:8 h (L: D).

**Insecticide and chemicals**

Chlorpyrifos (40% EC), diazinon (60% EC), phosalone (35 EC) and dichlorvos (50% EC) were obtained from Bayer and used in the bioassays. Fast blue RR salt was obtained from Fluka (Fluka, Buchs, Switzerland). Aldicarb 98% purity, eserine, neostigmine, carbofuran 95% purity, paraoxan and carbaryl, α-naphtyl acetate (αNA), β-naphtyl acetate (β-NA) and α-naphtylbutyrate (α-NB) were purchased from Sigma (www.sigmaaldrich.com). Monocrotophos and phosphamidon with 99.9% purity were obtained fromAccustandard (www.accustandard.com). Acetylthiocholine iodide (ATC), S-butylthiocholine iodide (BTC) and 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Wako (www.wako-chem.co.jp). Piperonyl butoxide (PBO), diethyl maleate (DEM), triphenyl phosphate (TPP) and all other chemicals were purchased from Merck (Darmstadt, Germany) and were reagent grade.

**Insecticide**

Toxicity of insecticides was measured using a standard leaf-dip bioassay with the leaves of cauliflower. Using commercial formulations, further serial dilutions were prepared in distilled water with Triton X-100 (0.1%) as a sticker to obtain five concentrations for each insecticide. The leaves were thoroughly washed in distilled water containing 0.1% Triton X-100 and dried at room temperature. Bioassays were conducted on third-instar larvae of P. xylostella. Leaf discs (5 cm) were dipped in separate concentrations (2000-3000-4000-6000-9000 (Kar-R) and 25 -35 -60-100, 150 (GU-S) of chlorpyrifos for 30s and after that treated leaf discs were air dried at room temperature for 1 h. The discs individually were transferred to plastic petri dishes (7 cm diameter). In control, the leaf discs were immersed in distilled

404
water containing 0.1% Triton X-100. Concentrations covering 10% to 90% mortality and each concentration had eight replicates and ten third instar larvae of the same age were used per replicate. If larvae were not able to move in a coordinated way when touched with a brush, the larvae were considered as dead. Mortality was documented after 48 h and the LC$_{50}$ and their confidence limits were estimated using POLO-PC software (LeOra Software, 2007).

Synergism assay

To determine synergistic effects, stock solutions (10 g litre$^{-1}$) of PBO, a MFO inhibitor, DEM, a GST inhibitor, and TPP, an esterase inhibitor, were prepared in 100% acetone (MERK). These solutions were then diluted in distilled water containing 200 µl litre$^{-1}$ spreading agent before synergism tests. Synergism was measured using the above described leaf-dip bioassay. Preliminary tests (maximum concentrations that caused no mortality) indicated that the concentrations of PBO, DEM and TPP for the susceptible population, respectively, were 50, 100 and 100 mg litre$^{-1}$ and 100, 180 and 200 mg litre$^{-1}$ for resistant population, respectively. To test the effect of PBO, DEM and TPP on the efficacy of chlorpyrifos, synergist was added to each of various concentrations of chlorpyrifos. Control for each strain were used. Mortality was scored after 48 h. LC$_{50}$ values were calculated by Probit regression using POLO-PC software (Kodwo and Tanaka, 2005). The synergism ratio (SR), which is a measure of synergistic effect, was calculated by dividing the LC$_{50}$ of the population treated with chlorpyrifos alone by the LC$_{50}$ of population treated with chlorpyrifos plus synergist.

Enzyme assays

EST

EST assays were performed according to the method of Van Asperen (1962). α-NA and β-NA were used as substrates. For esterase enzyme assay, ten third instar larvae were homogenized in 600 µl of 0.02 M phosphate buffer, containing 0.05% (v/v) Triton X-100 and pH 7.4 on ice, then were centrifuged at 11,000 × g for 10 minutes at 4°C. The kinetic parameters of the EST enzyme were analyzed using different concentrations (0.2-0.4-0.8-1.6-3.2-6.4 mM) of the substrates. 12.5 µl of supernatant was mixed with 50 µl of substrates, 112.5 µl of the phosphate buffer and after all 50 µl of 0.2% Fast Blue RR salt was added to mixture. The naphthol production was monitored using a microplate reader (Awareness Technology Inc, Stat Fax® 3200®) as a kinetic mode by assessing absorbance at 450 and 540 nm for α-NA and β-NA substrates, respectively. To enable calculation of the amount of naphthol produced in the process of esterase assay, a standard curve of absorbance for determining the amount of naphthol produced was created. The kinetic parameters were determined from Lineweaver–Burk plots. All measurements were done in triplicate.

GST

Ten third-instar larvae were homogenized in 400 µl of 0.02 M phosphate buffer, pH 7.4. Enzyme preparation was same as that pointed out above for esterase but without Triton X100. Subsequently, the homogenates were centrifuged for 10 minutes in 11,000 × g at 4°C. Activity was measured using 1-chloro-2,4-dinitrobenzene (with CDNB) and reduced glutathione (GSH) as substrates according to Habig et al. (1974) with slight modifications. For the test, 15 µl enzyme, 100 µl CDNB (1.2 mM) and 100 µl GSH (10 mM) were added to microplate. Enzyme activity was measured by monitoring the fluctuation in absorbance at 340 nm for 10 minutes with a microplate reader (Awareness Technology Inc, Stat Fax® 3200). Different concentrations of CDNB (0.01-5 mM) at fixed concentration of GSH (10 mM) were used to determine kinetic parameters, the $K_m$ and $V_{max}$ values were estimated using Lineweaver-Burk plots.

AChE assay

AChE activity and its kinetic parameters were measured with two artificial substrates, ATC and BTC, along with the modified method of Ellman et al. (1961). Increase in absorbance was recorded at 415 nm using a microplate, as described by Moores et al. (1988). One
thousand third instar larvae of DBM were collected and homogenized, on ice, in 30 ml (15 larvae in 500 μl) of 0.1 M phosphate buffer (pH 7) containing 0.5% Triton X-100 with a glass tissue grinder. The homogenate was centrifuged at 11,000 × g and 4 °C for 10 min and then supernatant used as enzyme solution. The reaction mixture contained 100 μl phosphate buffer (20 mM, pH 7.4), 20 μl DTNB (5, 5-dithio-bis 2-nitrobenzoic acid), 40 μl ATC or BTC and 40 μl of enzyme solution. The AChE activity was determined (every 5 min for 35 min) by measuring OD at 415 nm, using a microplate reader (Awareness technology Stat fax 3200®). Kinetic parameters (Km and Vmax) of AChE and activities were measured by various concentrations (0.5-50 mM) of ATC and BTC, and a double reciprocal plot was generated.

**Total protein assay**
Protein concentration was determined by the Bradford assay (Bradford, 1976) using BSA (Bovine serum albumin) as a standard protein. To determine the total protein, 10 μl of enzyme extract was added to 500 μl of Bradford reagent and the absorbance was read at 630 nm.

**Inhibition assay of AChE**
For inhibition assay, the enzyme solution was pre-incubated with various concentrations (0.5-50 mM) of monocrotophos, eserine, neostigmine bromide for 10 min at room temperature. Afterward buffer and DTNB were added to mixture. Residual activity was assessed by measuring absorbance at 412 nm kinetically using the standard assay method as mentioned above. Is0 value was assessed by probit analysis (Finney, 1971) and Sigma plot software. Experiments were done in triplicate. The Km and Vmax values were estimated with Lineweaver-Burk plots.

**Statistical analysis**
The mean values of the recorded data was subjected to analysis of variance (ANOVA), followed by Tukey’s test when significant differences were found at P = 0.05 using SAS program (SAS Institute, 2004).

**Results**

**Resistance level to OP insecticides**
When compared to the susceptible population, the Kar-R population exhibited 69.3-, 14.49-10 and 5-fold resistance ratio to chlorpyrifos, diazinon, phosalone and dichlorvos, respectively. Among the four organophosphorus pesticides tested, the Kar-R population had the highest resistance to chlorpyrifos (Table 1).

**Synergistic effect**
The effects of three inhibitors on chlorpyrifos toxicity in the Kar-R and susceptible populations are shown in Table 2. In Kar-R population, the synergistic ratios of DEM, PBO, TPP on chlorpyrifos toxicity were 1.26, 1- and 1.56-fold, respectively. In susceptible moth, synergistic ratios of DEM, PBO and TPP on chlorpyrifos toxicity were observed as 1.3, 1.12 and 1.06-fold, respectively. However, PBO have the similar synergism in the two populations (1.06 and 1-fold). Result shows TPP has higher synergism in the Kar-R population (1.56-fold), which implies that ESTs could be involved somewhat in the metabolic detoxification of chlorpyrifos. The resistance was not completely suppressed by synergists. Therefore, it is possible to consider another mechanism of resistance besides the metabolism by GST.

**Detoxification enzymes**
Results of kinetic parameters of EST, GST and MFO in resistant and susceptible populations of *P. xylostella* are summarized in Table 3. Both α-NA and β-NA were used to measure esterase activities. The specificity constant (Vmax/Km) and specific activity (SA) of esterase for both model substrates was more noticeable in the resistant population than that in Gu-S population. Vmax/Km values for male and female of the Kar-R population was higher than that of Gu-S population when α-NA and β-NA used as substrate. The GST activity was measured using CDNB as a substrate (Table 3). GST activity in Kar-R population was higher than that of Gu-S population and their specificity constants were for females1.9-fold and for males 1.26 fold that in the Gu-S population, respectively.
AChe activity and its kinetic parameters

Kinetic parameters of AChE and its activity are shown in Table 4. As shown, the Km values of Gu-S Male and female populations were higher than that of Kar-R population for the two (ATC, BTC) substrates. It appears that increase in Km in Gu-S population led to low affinity of the enzyme for the substrate without changing the reaction characteristics (Fig. 1).

Table 1 Response of susceptible (Gu-S) and Resistant (Kar-R) populations of Plutella xylostella to chlorpyrifos, diazinon, phosalone and dichlorvos.

<table>
<thead>
<tr>
<th>Population</th>
<th>Insecticide</th>
<th>LC50 (μg/ml)</th>
<th>95% CI</th>
<th>Slope (SE)</th>
<th>χ² (df)</th>
<th>RR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kar-R</td>
<td>Chlorpyrifos</td>
<td>4300.42</td>
<td>2722.6 - 7276.3</td>
<td>2.75 (0.42)</td>
<td>4.21 (3)</td>
<td>69.30 (53.2 - 90.4)</td>
</tr>
<tr>
<td>Kar-R</td>
<td>Diazinon</td>
<td>2899.80</td>
<td>2385.7 - 3617.9</td>
<td>2.63 (0.43)</td>
<td>1.95 (3)</td>
<td>14.49 (8.6 - 20.4)</td>
</tr>
<tr>
<td>Kar-R</td>
<td>Phosalone</td>
<td>987.79</td>
<td>673.6 - 1433.6</td>
<td>2.57 (0.51)</td>
<td>5.11 (3)</td>
<td>10.30 (7.5 - 14.1)</td>
</tr>
<tr>
<td>Kar-R</td>
<td>Dichlorvos</td>
<td>658.44</td>
<td>436.1 - 914.9</td>
<td>1.75 (0.29)</td>
<td>0.66 (3)</td>
<td>5.17 (3.5 - 7.7)</td>
</tr>
<tr>
<td>Gu-S</td>
<td>Chlorpyrifos</td>
<td>61.97</td>
<td>51.1 - 95.7</td>
<td>2.75 (0.42)</td>
<td>1.13 (3)</td>
<td>1</td>
</tr>
<tr>
<td>Gu-S</td>
<td>Diazinon</td>
<td>200.00</td>
<td>132.2 - 183.9</td>
<td>3.84 (0.59)</td>
<td>1.14 (3)</td>
<td>1</td>
</tr>
<tr>
<td>Gu-S</td>
<td>Phosalone</td>
<td>92.28</td>
<td>72.4 - 112.1</td>
<td>3.00 (0.57)</td>
<td>0.29 (3)</td>
<td>1</td>
</tr>
<tr>
<td>Gu-S</td>
<td>Dichlorvos</td>
<td>122.68</td>
<td>103.3 - 149.2</td>
<td>3.00 (0.48)</td>
<td>1.05 (3)</td>
<td>1</td>
</tr>
</tbody>
</table>

¹95% confidence interval.
²RR: Resistance ratio = LC50 of the resistant population/LC50 of the susceptible population.

Table 2 Effect of piperonyl butoxide (PBO), diethyl maleate (DEM) and triphenyl phosphate (TPP) on chlorpyrifos toxicity in Plutella xylostella populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Synergist</th>
<th>N</th>
<th>L50 as % of 95 CI (μg/ml)</th>
<th>Slope (SE)</th>
<th>χ² (df)</th>
<th>SR as % of 95 CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gu-S</td>
<td>without</td>
<td>400</td>
<td>61.97 (51.10 - 95.65)</td>
<td>2.75 (0.42)</td>
<td>1.13 (3)</td>
<td>---</td>
</tr>
<tr>
<td>PBO</td>
<td>400</td>
<td>58.21 (47.34 - 91.89)</td>
<td>2.80 (0.44)</td>
<td>1.10 (3)</td>
<td>1.06 (0.86 - 1.40)</td>
<td></td>
</tr>
<tr>
<td>DEM</td>
<td>400</td>
<td>55.00 (45.50 - 89.44)</td>
<td>2.80 (0.44)</td>
<td>0.61 (3)</td>
<td>1.12 (0.88 - 1.50)</td>
<td></td>
</tr>
<tr>
<td>TPP</td>
<td>400</td>
<td>47.18 (35.49 - 56.30)</td>
<td>3.80 (0.80)</td>
<td>2.20 (3)</td>
<td>1.30 (0.97 - 1.69)</td>
<td></td>
</tr>
<tr>
<td>Kar-R</td>
<td>without</td>
<td>400</td>
<td>4300.42 (3722.6 - 7276.3)</td>
<td>2.75 (0.42)</td>
<td>4.21 (3)</td>
<td>---</td>
</tr>
<tr>
<td>PBO</td>
<td>400</td>
<td>4258.46 (3279.0 - 5280.2)</td>
<td>3.10 (0.51)</td>
<td>2.40 (3)</td>
<td>1.00 (0.78 - 1.34)</td>
<td></td>
</tr>
<tr>
<td>DEM</td>
<td>400</td>
<td>3391.00 (2617.0 - 4094.0)</td>
<td>3.30 (0.62)</td>
<td>0.30 (3)</td>
<td>1.26 (1.04 - 1.66)</td>
<td></td>
</tr>
<tr>
<td>TPP</td>
<td>400</td>
<td>2742.80 (1869.2 - 3463.9)</td>
<td>3.20 (0.76)</td>
<td>1.71 (3)</td>
<td>1.56 (1.21 - 2.50)</td>
<td></td>
</tr>
</tbody>
</table>

Synergistic Ratio (SR) = LC50 of insecticide/LC50 of (synergist + insecticide). Gu-S and Kar-R indicate susceptible and resistant populations respectively.

Table 3 Kinetic parameters and specific activity of detoxification enzymes in susceptible (Gu-S) and resistant (Kar-R) populations of Plutella xylostella.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Population</th>
<th>Enzyme</th>
<th>Substrate</th>
<th>Km (μg/ml)</th>
<th>Vmax (nmol/min.mg protein)</th>
<th>Vmax/Km (nmol/min.mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Kar-R</td>
<td>EST</td>
<td>o-NA</td>
<td>0.44 ± 0.02a</td>
<td>0.78 ± 0.09a</td>
<td>0.13 ± 0.005a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>β-NA</td>
<td>0.17 ± 0.003a</td>
<td>0.65 ± 0.007a</td>
<td>0.09 ± 0.006a</td>
</tr>
<tr>
<td>Gu-S</td>
<td>EST</td>
<td>GST</td>
<td>CDNB</td>
<td>2.10 ± 0.009a</td>
<td>2.20 ± 0.008a</td>
<td>46.00 ± 6.000a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>o-NA</td>
<td>0.23 ± 0.009b</td>
<td>1.30 ± 0.01b</td>
<td>0.10 ± 0.007b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>β-NA</td>
<td>0.12 ± 0.005b</td>
<td>0.85 ± 0.01b</td>
<td>0.09 ± 0.005b</td>
</tr>
<tr>
<td>Male</td>
<td>Kar-R</td>
<td>EST</td>
<td>o-NA</td>
<td>0.42 ± 0.017a</td>
<td>0.60 ± 0.04a</td>
<td>0.10 ± 0.002a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>β-NA</td>
<td>0.16 ± 0.005a</td>
<td>0.50 ± 0.009a</td>
<td>0.07 ± 0.002a</td>
</tr>
<tr>
<td>Gu-S</td>
<td>EST</td>
<td>GST</td>
<td>CDNB</td>
<td>2.40 ± 0.02a</td>
<td>2.20 ± 0.006a</td>
<td>40.00 ± 3.000a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>o-NA</td>
<td>0.20 ± 0.005b</td>
<td>1.10 ± 0.008b</td>
<td>0.08 ± 0.006b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>β-NA</td>
<td>0.14 ± 0.008b</td>
<td>0.80 ± 0.009b</td>
<td>0.09 ± 0.005b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CDNB</td>
<td>1.50 ± 0.009b</td>
<td>1.66 ± 0.006b</td>
<td>25.00 ± 3.000b</td>
</tr>
</tbody>
</table>

Means of kinetic parameters followed by the same letters in each column are not significantly different (Tukey’s test, P < 0.05).

EST: Esterase, GST: glutathione-S-transferase, o-NA: o- napthyl acetate, β-NA: β-napthyl acetate, CDNB: 1-chloro-2, 4 dinitrobenzene special activity (nmol/min.mg protein) ± SE.
Table 4 Substrate specificities and kinetic parameters (mean ± SE) of AChE from susceptible (Gu-S) and resistant (Kar-R) populations of Plutella xylostella.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Population</th>
<th>Substrate</th>
<th>SA</th>
<th>Km</th>
<th>Vmax</th>
<th>Vmax/Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Gu-S</td>
<td>ATC</td>
<td>0.570 ± 0.080a</td>
<td>0.650 ± 0.040a</td>
<td>0.058 ± 0.010a</td>
<td>0.089 ± 0.018a</td>
</tr>
<tr>
<td></td>
<td>BTC</td>
<td>0.029 ± 0.006a</td>
<td>16.130 ± 0.050a</td>
<td>0.021 ± 0.006a</td>
<td>0.0013 ± 0.0008a</td>
<td></td>
</tr>
<tr>
<td>Kar-R</td>
<td>ATC</td>
<td>0.310 ± 0.050b</td>
<td>0.222 ± 0.010b</td>
<td>0.028 ± 0.009b</td>
<td>0.126 ± 0.009b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BTC</td>
<td>0.015 ± 0.008b</td>
<td>8.280 ± 0.080b</td>
<td>0.027 ± 0.004b</td>
<td>0.0032 ± 0.0001b</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Gu-S</td>
<td>ATC</td>
<td>0.520 ± 0.100a</td>
<td>0.570 ± 0.020a</td>
<td>0.041 ± 0.005a</td>
<td>0.071 ± 0.085a</td>
</tr>
<tr>
<td></td>
<td>BTC</td>
<td>0.022 ± 0.004a</td>
<td>16.100 ± 0.050a</td>
<td>0.018 ± 0.003a</td>
<td>0.0011 ± 0.0005a</td>
<td></td>
</tr>
<tr>
<td>Kar-R</td>
<td>ATC</td>
<td>0.280 ± 0.010b</td>
<td>0.190 ± 0.008b</td>
<td>0.020 ± 0.008b</td>
<td>0.100 ± 0.005b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BTC</td>
<td>0.013 ± 0.004b</td>
<td>9.140 ± 0.090b</td>
<td>0.027 ± 0.005b</td>
<td>0.0029 ± 0.0003b</td>
<td></td>
</tr>
</tbody>
</table>

Means of kinetic parameters followed by the same letters in each column are not significantly different (Tukey’s test, P < 0.05). ATC: Acetylthiocholine iodide, BTC: Butyrylthiocholine iodide specific activity.

Figure 1 Lineweaver-Burk plots of ATC and BTC hydrolysis by AChE in susceptible (Gu-S) and resistant (Kar-R) populations of Plutella xylostella. Hydrolysis of ATC by female of Gu-S (FS) and Kar-R populations (FR) (A), and male of Gu-S (MS) and Kar-R populations (MR) (B). Hydrolysis of BTC by female of Gu-S and Kar-R populations (C), and male of Gu-S and Kar-R populations (D).
Inhibition of AChE

The effects of OPs and carbamates on AChE activity from mass homogenates of resistant and susceptible populations were determined, and the results are shown in Table 5 and Fig. 2. Compared with IC₅₀ values for the Gu-S, the resistant population (Kar-R) demonstrated 11, 11, 5, 3 and 2-fold resistance in female and 10.2, 10.2, 4.2, 3 and 2-fold in male to monocrotophos, neostigmine and eserine, carbaryl and phosphamidon, separately. In contrast, there is no noteworthy difference in inhibition of AChE by paraoxan, carbofuran and aldicarb among Gu-S and Kar-R populations. These results imply that resistance to chlorpyrifos in Kar-R population causes simultaneous insensitivity to other inhibitors such as monocrotophos, neostigmine and eserine, carbaryl and phosphamidon.

Discussion

DBM has gained resistance against many insecticides because of its short lifespan, high reproduction, and the high selection pressure with insecticides (Zhao et al., 2002, Sayyed and Wright, 2006, Pu et al., 2010, Zhou et al., 2011), biotic potential, overlapping generations and the high migratory ability (Talekar and Shelton, 1993, Sarfraz et al., 2006). In this study, a field population (Kar-R) of the DBM collected from Karaj County, Alborz, Iran, was proved to have marginal to high resistance to selected OP insecticides compared to the susceptible population (Gu-S). The findings expressed a high degree of resistance to chlorpyrifos compared to diazinon, phosalone and dichlorvos (Table 1).

For chlorpyrifos, LC₅₀ value of Kar-R was about 69-fold higher than the susceptible population. Chlorpyrifos has been used to control DBM and other insect pests for many years in Iran and the world (Pastor and Sinègre, 1978; Milio et al., 1987; Rust and Reiterson, 1991; Archer, 1994; Guides et al., 1996; Liu et al., 2005; Curtis and Pasteur, 2009; Zhang et al., 2012).

Table 5 IC₅₀ values of in vitro inhibition of AChE activity in the chlorpyrifos-resistant and susceptible populations of Plutella xylostella.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Inhibitor</th>
<th>Guilan IC₅₀</th>
<th>Guilan 95% CL</th>
<th>Karaj IC₅₀</th>
<th>Karaj 95% CL</th>
<th>IR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Aldicarb</td>
<td>6 × 10⁻⁸</td>
<td>3 × 10⁻⁸ - 1.2 × 10⁻⁴</td>
<td>5.5 × 10⁻⁵</td>
<td>2.8 × 10⁻⁵ - 10⁻⁴</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Monocrotophos</td>
<td>6 × 10⁻⁵</td>
<td>3 × 10⁻⁵ - 1.2 × 10⁻⁴</td>
<td>7 × 10⁻⁴</td>
<td>2.5 × 10⁻⁴ - 1.2 × 10⁻⁴</td>
<td>11.0*</td>
</tr>
<tr>
<td></td>
<td>Eserine</td>
<td>7 × 10⁻⁵</td>
<td>10⁻⁵ - 10⁻⁷</td>
<td>4 × 10⁻⁷</td>
<td>10⁻⁷ - 8 × 10⁻⁷</td>
<td>5.0*</td>
</tr>
<tr>
<td></td>
<td>Neostigmine</td>
<td>7 × 10⁻⁷</td>
<td>3 × 10⁻⁷ - 1.4 × 10⁻⁶</td>
<td>8 × 10⁻⁶</td>
<td>2 × 10⁻⁶ - 3 × 10⁻⁵</td>
<td>11.0*</td>
</tr>
<tr>
<td></td>
<td>Phosphamidon</td>
<td>6.1 × 10⁻⁷</td>
<td>4 × 10⁻⁵ - 1.17 × 10⁻³</td>
<td>1.2 × 10⁻⁴</td>
<td>10⁻⁴ - 5.4 × 10⁻⁵</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Carbofuran</td>
<td>5 × 10⁻⁷</td>
<td>10⁻⁷ - 8 × 10⁻⁷</td>
<td>5 × 10⁻⁷</td>
<td>4 × 10⁻⁷ - 7.7 × 10⁻⁷</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Paraoxan</td>
<td>10⁻⁵</td>
<td>4 × 10⁻⁶ - 3.2 × 10⁻⁴</td>
<td>3 × 10⁻⁵</td>
<td>10⁻⁵ - 8 × 10⁻⁵</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Carbaryl</td>
<td>4 × 10⁻⁶</td>
<td>2.6 × 10⁻⁶ - 6.2 × 10⁻⁶</td>
<td>9 × 10⁻⁶</td>
<td>10⁻⁶ - 1.6 × 10⁻⁵</td>
<td>3.0</td>
</tr>
<tr>
<td>Female</td>
<td>Aldicarb</td>
<td>6 × 10⁻⁵</td>
<td>3 × 10⁻⁵ - 1.2 × 10⁻⁴</td>
<td>5.3 × 10⁻⁵</td>
<td>2.9 × 10⁻⁵ - 10⁻⁴</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Monocrotophos</td>
<td>6.1 × 10⁻⁵</td>
<td>3 × 10⁻⁵ - 1.2 × 10⁻⁴</td>
<td>4.6 × 10⁻⁴</td>
<td>1.8 × 10⁻⁴ - 3 × 10⁻⁵</td>
<td>10.2*</td>
</tr>
<tr>
<td></td>
<td>Eserine</td>
<td>7 × 10⁻⁸</td>
<td>10⁻⁸ - 10⁻⁷</td>
<td>3 × 10⁻⁷</td>
<td>6.8 × 10⁻⁸ - 5 × 10⁻⁷</td>
<td>4.2*</td>
</tr>
<tr>
<td></td>
<td>Neostigmine</td>
<td>6.8 × 10⁻⁷</td>
<td>3 × 10⁻⁷ - 1.4 × 10⁻⁶</td>
<td>7 × 10⁻⁶</td>
<td>2 × 10⁻⁶ - 3 × 10⁻⁵</td>
<td>10.2*</td>
</tr>
<tr>
<td></td>
<td>Phosphamidon</td>
<td>6 × 10⁻⁵</td>
<td>4 × 10⁻⁵ - 1.17 × 10⁻³</td>
<td>3 × 10⁻⁵</td>
<td>10⁻⁵ - 6 × 10⁻⁵</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Carbofuran</td>
<td>5 × 10⁻⁷</td>
<td>10⁻⁷ - 8 × 10⁻⁷</td>
<td>5 × 10⁻⁷</td>
<td>4 × 10⁻⁷ - 7.7 × 10⁻⁷</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Paraoxan</td>
<td>10⁻⁵</td>
<td>4 × 10⁻⁶ - 3.2 × 10⁻⁴</td>
<td>3 × 10⁻⁵</td>
<td>10⁻⁵ - 8 × 10⁻⁵</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Carbaryl</td>
<td>4 × 10⁻⁶</td>
<td>2.6 × 10⁻⁶ - 6.2 × 10⁻⁶</td>
<td>9 × 10⁻⁶</td>
<td>10⁻⁶ - 1.6 × 10⁻⁵</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Insensitivity Ratios (IR) = IC₅₀ of Kar-R population/IC₅₀ of Gu-S population.
* Statistically significant at 95% level (P < 0.05).
Some researchers have reported the resistance of this pest to some OP compounds. Sun et al. (1978) reported 14-fold resistance to diazinon in Taiwan and Yu and Nguyen (1992) published 73- and 21-fold resistance of DBM respectively to diazinon and chlorpyrifos in Florida. Enhanced activities of GST (Cheng et al., 1983) and carboxylesterase (Sun et al., 1978; Noppun et al., 1987) have been explained as degradation factors related to the OP-resistance and even modification of acetylcholinesterase (AChE) to an insensitive form of the target enzyme (Sudderuddin and Kok, 1978; Noppun et al., 1983). Synergists with blocking definite insecticide metabolic enzymes are used to improve the toxicities of insecticides against insect pests and the degree of synergism can suggest whether or not conceived detoxification enzymes metabolize the pesticides (Yang et al., 2001). In this research, we used three synergists (TPP, an EST inhibitor; PBO, a P450
inhibitor; and DEM, a GST inhibitor). As shown in Table 2, synergism effect triggered a reduction in LC50 values of OP on resistant population (SR: 1.26 and 1.56-fold for DEM and TPP, respectively). Thus, these results indicated that metabolic enzymes especially EST play a role in chlorpyrifos resistance. We compared the EST and GST kinetic parameters and specific activities between the Kar-R and Gu-S populations to confirm the possible contributions of these major detoxification enzymes in chlorpyrifos resistance in the Kar-R. Our results expressed that EST and GST activities in the Kar-R were significantly higher than activities in the Gu-S. In fact, results expressed that EST and GST cannot be the only cause that involves in chlorpyrifos resistance, therefore other mechanisms such as target site resistance should be involved in it. Kinetic parameters of this enzyme and inhibitory effect of monocrotophos, neostigmine and eserine as AChE inhibitor were studied to determine the role of AChE insensitivity in resistance mechanisms. There was an insensitive AChE to OP insecticides in several insects, including mosquitos, housefly (Kozaki et al., 2009; Walsh et al., 2001) aphids (Gao et al., 2002; Andrews et al., 2014), Drosophila (Mutero et al., 1994; Menozzi et al., 2004), and spider mites (Zamani et al., 2014) and even has been observed in P. xylostella on diazinon (sun et al., 1978), phenthoate (Noppun et al., 1987), chlorpyrifos (Cho et al., 2006), prothiofos (Beak et al., 2005; Lee et al., 2007). Our study indicated that the specific activity of AChE related to ATC was 1.83-fold higher in the Gu-S (female) and 1.85 (male) compared to the Kar-R. KM and Vmax values of AChE in Kar-R are about two fold less compared to those from the Gu-S population. That shows AChE had different dependency to substrates (ATC, BTC). Different studies show some different results of AChE KM and Vmax values (Raymond et al., 1986; Zhu and Clark, 1994; Stumpf et al., 2001; Gao and Zhu, 2002; Ren et al., 2002; Yu, 2006; Chai et al., 2007; Hsu et al., 2008; Alizadeh et al., 2014). Female and male in Kar-R population with almost same specific activity, KM and Vmax values probably both are involved in resistance (Table 4). These results indicated that male and female of Kar-R population have modified acetycholinesterase and that AChE in both sexes is assumed to be related to OP resistance. Decreased sensitivity of AChE has also been determined as one of the common mechanisms of resistance to OP compounds in many insects (Huang and Han, 2007). Our kinetic analyses clearly showed that AChE in the Kar-R population was significantly different from the Gu-S AChE. Such differences were indicated by not only the reduced sensitivity levels to different OP inhibitors (Table 5), but also the decreased dependency on ATC and BTC (Table 4). These results highly expressed that reduced sensitivity of AChE also played a significant role to increase OP resistance in the Kar-R. It is concluded that chlorpyrifos resistance in the Kar-R was partly due to the EST and GST mediated metabolic detoxification as shown by significant synergistic effect of DEM and TPP on the toxicity of chlorpyrifos, and significantly increased GST and EST activities. In addition, increased activity and reduced sensitivity of AChE also increased chlorpyrifos resistance in the Kar-R.

Acknowledgements

The authors gratefully acknowledge the use of the services and facilities of Department of Plant Protection, University of Guilan.

References


Ren, X. X., Han, Z. J. and Wang, Y. C. 2002. Mechanisms of monocrotophos resistance in cotton bollworm, Helicoverpa armigera...
Resistance mechanisms of P. xylostella to organophosphates

J. Crop Prot.


سنگ‌تیرگانه مقاومت جمعیت‌شک‌پایه پشت‌الماسی به حشره کش‌های ارگانوفسفر راک (Lepidoptera: Plutellidae)

مريم دولت‌القاضی، محمد قدرباًی و رضا حسن‌سادات

1- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه گيلان، رشت، ايران.
2- گروه بیوشیمی، دانشکده علوم‌پزشکی، دانشگاه تبریز، مرس، تهران، ایران.

یست الکترونیکی نویسنده مسئول مکاتبه: m.zolfaghari_89@yahoo.com

دریافت: 27 آذر 1397، پذیرش: 30 تیر 1398

چکیده: ویژگی‌های سیستمی‌سازی و بیوشیمیایی چهار حشره کش ارگانوفسفر، کلرپیریفس، دیازینون، فورالوس و دیکلروس در جمعیت‌های شبه‌پیرویتی‌شناخته شده کلیم حساس و متقاوم (Gu-S) مقاومت فاصله‌ای به کلرپیریفس Kar-R مقاومت ملی‌نوردی به کلرپیریفس Kar-R، مقاومت متوسط به دیازینون (4.94 کیلوگرم) و مواد مشابه به دیازینون (5.17 کیلوگرم) در مقایسه با جمعیت Gu-S داشت. دیاتیل‌مالات و تریفین‌فسفات به عنوان یکی از داروهای تعیین شده مربوط به جمعیت Kar-R گذشته گل‌ماکونی اس-ترانسفر (GST) و استراز جمعیت Kar-R به‌طور معنی‌داری بیشتر از باسکارهای جمعیت Gu-S جایگزین بوده است. علاوه بر این، در این تحقیق سیستمی‌های هیدرولیز استریکس تیوکولین بید و بوتیل Tioکولین بیدی به‌عنوان یک نشانه منحنی استیل کولین استر در جمعیت‌های مقاوم و حساس مورد بررسی قرار گرفت. 

IC50 مواد بررسی قرار گرفت: ۴۹.۲ کیلوگرم و ۴۹.۴ کیلوگرم برای دیازینون و فورالوس. نتایج دیگری پرداخته بوده است که تعداد نشان داد که سازگاری و تطبیقی میزان حساسیت کلرپیریفس به کلرپیریفس در جمعیت Kar-R و عدم حساسیت کلرپیریفس به کلرپیریفس در جمعیت Gu-S است. نتایج بیشتر از باج‌هایی مقاوم به کلرپیریفس در جمعیت Kar-R و عدم حساسیت کلرپیریفس به کلرپیریفس در جمعیت Gu-S است.

واژگان کلیدی: شیپرپدین‌شناخته، حشره کش‌های ارگانوفسفر، سازگاری‌های بیوشیمیایی، آنزیم‌های

سیستمی‌کننده کولین استر