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مقاومت به حشره‌کش دلتامترین در برخی از جمعیت‌های شب پره پشت الماسی *Plutella xylostella* در ایران

Deltamethrin resistance in several populations of diamondback moth, *Plutella xylostella* in Iran

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چکیده

شب پره پشت الماسی یا بید کلم *Plutella xylostella* L. در برابر بسیاری از آفتکش‌ها از جمله پایرتروئیدها مقاومت توسعه یافته‌ای نشان داده است. در تحقیق حاضر سمیت دلتامترین روی لارو سن سوم شش جمعیت از این حشره با استفاده از روش غوطه‌ور سازی برگ بررسی شد. نتایج نشان داد که جمعیت‌های مختلف این حشره حساسیت متفاوتی در برابر دلتامترین دارند. جمعیت‌های مقاوم شب پره پشت الماسی با استفاده از فشار گزینشی طی ۱۵ نسل در شرایط آزمایشگاه به وجود آمدند و حساسیت آنها به دلتامترین مورد ارزیابی قرار گرفت. در این جمعیت‌های مقاوم نسبت مقاومت به طور قابل توجهی افزایش پیدا کرد. اگرچه سینرژیست‌های DEM وTPP هیچ نوع اثر سینرژیستی روی دلتامترین نداشتند، سینرژیست PBO به طور قابل توجهی سمیت دلتامترین در جمعیت‌های مقاوم را کاهش داد. سینرژیست DEF نیز اثر سینرژیستی نسبتا خوبی داشت. مطالعات آنزیمی نشان دادند که فعالیت آنزیم‌های مونواکسیژناز و استراز در جمعیت‌های مقاوم بسیار بیشتر از فعالیت آنزیم گلوتاتیون اس-ترانسفراز می‌باشد. در بررسی مقاومت تقاطعی، نتایج نشان داد که جمعیت بسیار مقاوم شب پره پشت الماسی در برابر حشره‌کش‌های هگزافلومورون و ایندوکساکارب مقاومت تقاطعی بالایی را نشان می‌دهد همچنین در این جمعیت هیچ نوع مقاومت تقاطعی با حشره‌کش آبامکتین دیده نشد.

واژه‌های کلیدی

سم زدایی،
آفت کش،
سینرژی،
کلم،
آفت

Introduction

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a significant and serious pest of Brassica crops including broccoli, Brussels sprout, cabbage, cauliflower, Chinese cabbage, collard, mustard, rapeseed, radish and turnip (Furlong *et al*., 2013). In tropical and sub-tropical areas, *P. xlostella* causes damage over the year (Wang and Wu, 2012). The cost of managing *P. xylostella* varies from US$ 4 to 5 billion per year (Zalucki *et al*., 2012). Most costs are related to the use of insecticides (Bôas *et al*., 2004) because insecticides are still the major tools in controlling insect pests (Sun and Gao, 2012). This pest has shown different levels of resistance to many groups of insecticides and it is considered as one of the 20 critical resistant insect pests (Shelton *et al*., 2000). During recent years in Iran, this pest has caused many problems. High populations of the pest and their damages have led farmers to use a combination of different insecticides at doses higher than recommended and at multiple times.

Deltamethrin is the most widely used pesticide for controlling *P. xylostella* and has been frequently applied for controlling other cabbage pests such as *Pieris brassicae* L. (Lepidoptera: Pieridae) and *Pieris rapae* L. (Lepidoptera: Pieridae) in Iran (Hasanshahi *et al*., 2012). This pyrethroid insecticide pyrethroid insecticide paralyzes the insect nervous system rapidly causing a fast knockdown effect (Almasi *et al*., 2013). Hence, vast applications of this insecticide have led to the appearance of resistant populations of *P. xylostella* to pyrethroid insecticides (Liu *et al*., 1981).

Development of insecticide resistance is now an important area for chemical control of many insect pests (Liu and Yue, 2000; Zhao *et al*., 2017). It is necessary to improve management strategies to delay or event prevent the development of insecticide resistance in pest populations. Previous studies have proven that resistance to many insecticides from different groups of *P. xylostella* was caused by the elevated level of glutathione S-transferase (GST) enzymes, increase of monooxygenase (MFO) enzymes and esterase (EST) enzymes activity (Gong *et al*., 2013; Ishtiaq *et al*., 2012; Qian *et al*., 2008). Synergists like piperonyl butoxide (PBO), diethyl maleate (DEM), S,S,S-tributyl phosphorotrithioate (DEF), and triphenyl phosphate (TPP) can suggest theses biochemical mechanisms (Yu, 2008).

Insecticides with cross-resistance share some resistance mechanism and treatment with one of them may result in the resistance to all of them. Thus, these insecticides cannot be used rotationally (Qian *et al.*, 2008). There are a few studies on cross resistance between deltamethrin, abamectin and inoxacarb. Also, Cross resistance between deltamathrin and some other insecticides like thiodicarb, hexaflumuron or flubendiamide was not studied previously and this is the first evaluation in this regard. Although, correlations between pesticide resistance and enzyme activity have been widely studied by many researchers, there are a few studies on resistance patterns of deltamethrin in *P. xylostella* populations. Moreover, the actual status of deltamethrin resistance in Iranian populations of *P. xylostella* is not well-documented. The aims of this study were to determine the resistance status of some Iranian populations of diamondback moth to deltamethrin and to investigate some possible mechanisms of deltamethrin resistance in the resistant strains. Cross resistance to some other insecticides was also examined in high resistance strain of *P. xylostella*. Such investigations can provide help to reduce the risk of deltamethrin resistance development in diamondback moth.

Materials and Methods

Insects: In this study six different Iranian populations of *P. xylostella* were investigated. These strains were collected from some main *brassica* growing areas of Iran: Ardabil (Ardabil Province, Iran) (38°12′ N, 48°39′ E), Flaverjan (Isfahan Province, Iran) (32°29′ N, 51°33′ E), Karaj (Alborz Province, Iran) (35°48′ N, 51°00′ E) Naqadeh (West Azerbaijan Province, Iran) (36°57′ N, 45°25′ E), Tehran (Tehran Province, Iran) (36°15′ N, 50°03′ E) and Urmia (West Azerbaijan Province, Iran) (37°35′ N, 45°05′ E). For each location, information about used insecticides was achieved from appropriate growers or state extension personnel. Field-collected 3rd or 4th instar larvae or pupae of *P. xylostella* were transferred to greenhouse of The Department of Plant Protection in Urmia University. The populations were separately mass reared in cages (50 cm × 40 cm × 30 cm) and on leaves of cabbage *Brassica oleracea* L. (Brassicacea) plants. The insectaries were maintained under greenhouse conditions at 25±1 ^{o}C, 60-70% relative humidity and photoperiod of 16: 8 (light: darkness). Adult moths were fed with 10% honey-water solution. For adult ovipositions, potted radish seedlings *Raphanus sativus* L. (Brassicacea) were placed in adult cages and were replaced every one or two days. After being collected from the cages, the potted radish seedlings were put under greenhouse conditions where the eggs hatched and then second instar larvae transferred to larvae cages. Every day fresh leaves of cabbage were supplied to the larvae in the cages. This procedure was repeated for different strains of *P. xylostella* to study the toxicity of deltamethrin and resistance to it.

Insecticides and chemicals: Insecticides applied for bioassay were Deltamethrin (Decis®, EC 2.5%, Golsam, Iran), Flubendiamide (Takumi®, 20% WG, Nihon Nohgaku, Japan), Indoxacarb (Avanut®, 150 SC, DuPont, France), Hexaflumuron (Consult®, 10% EC, DowAgrosciences Company), Abamectin (Abamectin®, 1.8 EC, Partonar, Iran) and Thiodicarb (Larvin®, 80% DF, Moshkfam Fars, Iran). Tween 80, sodium dodecyl sulfate (SDS), and *a*-naphtol were purchased from Merck (Darmstadt, Germany). 1-Chloro-2, 4-dinitrobenzene (CDNB), hydrogen peroxide, reduced glutathione (GSH), *a*-Naphthyl acetate (*a*-NA),

S,S,S-Tributyl phosphorotrithioate (DEF), diethyl maleate (DEM), fast blue RR salt, piperonyl butoxide (PBO), triphenyl phosphate (TPP), 3,3',5,5'-Tetramethylbenzidine (TMBZ), cytochrome c, commassie brilliant blue G, and bovine serum albumin (BSA) were purchased from Sigma Aldrich (Taufkirchen, Germany). The other chemicals were obtained from commercial companies in IRAN.

Bioassays: The leaf dipping method reported by Shelton *et al*. (1993) was used for studying the toxicity of deltamethrin on third larvae of F1 generation. Insecticide solutions with different concentrations (at least five concentrations) were prepared with distilled water and Tween 80 (0.05%) as wetting agent. Leaf discs (5.5 cm in diameter) were collected from cabbage host plant and dipped in the prepared solutions of deltamethrin for 20 s. Then, the leaf discs were dried at room temperature for 1-2 h. Once air dried, each leaf-disc was placed in plastic petri dish (6 cm in diameter). 20 third instar larvae were placed into each petri dish. Leaves treated with only distilled water and Tween 80 were used as control. Mortality was recorded at 48 h after treatment. The larvae that did not move when stimulated with a fine-haired brush were counted as dead. Each test was replicated three times. Ardabil strain, which was found most susceptible to deltamethrin (and the other used insecticides) showed the lowest LC_{50}. Accordingly, it was reared in the laboratory for several generations without exposure to insecticides and used as susceptible strain. Naqadeh, Urmia and Flaverjan populations showed considerable resistance to deltamethrin; therefore, they were reared under deltamethrin selection pressure (with LC_{50} of the insecticide). Other populations were discarded. The surviving pests were reared to produce the next generation. After about 15 generations, new levels of resistance were evaluated and high resistant populations were considered for biochemical tests.

Synergism assays: For the analysis of the effects of synergists on the toxicity of deltamethrin, the synergists PBO, DEM, DEF, and TPP were dissolved in acetone. These synergists were normally considered as inhibitors of MFO, GST, EST and carboxyl esterase enzymes, respectively (Askari Saryazdi *et al*., 2014). Using leaf disc bioassay method as mentioned above, maximum sublethal doses (doses that have the highest synergistic effect as well as the lowest mortality in the treatment) were determined to be 300, 500, 150 and 90 mg l^{-1}, respectively. The test larvae were treated topically with 1 µl of synergist solution on the dorsal thoracic segments of the third instar larvae 1 h before they were fed with deltamethrin treated leaves. All above tests were performed in three replications. Mortality was assessed after 48 h.

Cross-resistance evaluation: Cross-resistance between deltamethrin and five other insecticides was evaluated on high deltamethrin resistant (Nagadeh) and susceptible (Ardabil) strains of *P. xylostella*. The insecticides used were abamectin, indoxacarb, hexaflumuron, thiodicarb and flubendiamide. The same leaf-dipping method as mentioned above was used to assess the toxicity of various insecticides.

Protein assay and preparation of enzyme extracts: The protein concentration of the enzyme solution was measured according to Bradford (1976) method, using bovine serum albumin (0.4-2 mg/ml) as standard. For the preparation of enzymes, sixty larvae of third instar were collected for three replications. These larvae were starved for 2h to remove digested food particles. In each replication about 20 larvae were homogenized with 500 µl ice-cold homogenization buffer. After centrifugation at 10000g for 20 min at 4 °C, the supernatants were collected and used as enzyme resources for the analysis of the activity of EST, MFO and GST. Homogenization buffers used for esterase, glutathione S-transferases and mixed function oxidase were 0.02 M sodium phosphate (pH=7 containing 0.3% Triton X-100), 0.1 M sodium phosphate (pH=6.5) and 0.625 M potassium phosphate buffer (pH=7.2), respectively.

Detoxification enzyme assay: Enzyme analyses were performed using procedure outlined by WHO (1998).

Glutathione S-transferases assay: Glutathione S-transferases activity towards the substrate CDNB and reduced GSH was measured using the method of Habig *et al*. (1974) The survey mixture consisted of 190 µl 10 mM reduced GSH solution (in 0.1 M sodium phosphate buffer pH=6.5) and 10 µl 63 mM CDNB solution (in methanol). To this reaction mixture, 10 µl enzyme extract was added. Optical density was read at 340 nm every 30 seconds for 5 minutes at 27 °C. The non-enzymatic reactions of CDNB and GSH without homogenate with buffer only were served as blanks. For estimation of the activity, changes in the absorbance per minute were converted into µmol CDNB conjugated/min/mg protein based on the extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Esterase assay: Evaluation of esterase activity was carried out based on the method proposed by Van Asperen (1962). The enzyme activity was measured using *a*-NA as substrate. The volume of reaction mixture per wells of microplate (Synergy HT, BioTek Instruments, Winooski, VT) was 220 µl containing 20 µl enzyme extract and 200 µl 30 mM substrate solution (100 µl *a*-NA dissolved in 10 ml sodium phosphate buffer (0.02 M, pH=7.2)). In this experiment phosphate buffer without enzyme was used as control. The reaction mixture was incubated at 25°C for 15 min. After incubation, 50 µl fast blue RR salt solution (150 mg fast blue RR salt dissolved in 15 ml distilled water and 35 ml 5% SDS) was added to stop the reaction. The reaction mixture was incubated at 25-27°C for 5 min and color development was accomplished. The absorbance was measured at 450 nm. The amount of the obtained product was estimated from alpha naphtol standard curve. Esterase activity was indicated as µmol of alpha naphthol/min/mg protein.

Monooxygenase assay: For evaluation of MFO activity, heme-peroxidase method, measurement of total amount of iron-containing protein, was used (Brogdon *et al*., 1997). This is a simple method for titration of heme bound in samples. The assay measures the heme content, which is mainly associated with cytochrome P450 in non-blood feeding insects. Total reaction volume was 325 µl. Briefly,

20 µl enzyme extract was transferred to a microtiter plate using a micropipette. 80 µl 0.625 M potassium phosphate buffer (pH=7.2) and 200 µl TMBZ working solution (5 ml methanol solution of TMBZ (0.01 g) with 15 ml sodium acetate buffer (0.25 M, pH=5)) were then added into each well and finally 25 µl 3% hydrogen peroxide (H_2O_2) solution was added. After 30 min incubation at room temperature in darkness, the absorbance of reaction product was recorded at 630 nm. In blank well, potassium phosphate buffer was added instead of the enzyme. Purified cytochrome c was used as standard.

Statistical analyses: The bioassay data were analyzed by probit analysis using SPSS software v.17.0. Synergism ratios were calculated as SR = LC_{50} value of insecticide alone/ LC_{50} value of insecticide with synergist. Resistance ratios (RR) were assessed as RR= LC_{50} value of resistant strain / LC_{50} value of susceptible strain. The amount of cross resistance (CR) was assessed by dividing LC_{50} value of resistant strain by LC_{50} value of susceptible strain. If this value was smaller than 1, it indicated no cross resistance and values of higher than 1 indicated positive cross resistance (Ramasubramanian and Regupathy, 2004). Data from enzymatic assays were analyzed by one-way ANOVA and Tukey's test ($P \leq 0.05$) using SPSS.

Results and Discussion

Resistance status

Concentration-mortality responses to deltamethrin in six *P. xylostella* field populations collected from different regions of Iran during 2016-2017 are presented in Table 1. The magnitude of variation was observed among LC_{50} values of different populations. Compared to susceptible population, very high levels of resistance were detected in Naqadeh population whose LC_{50} value was equivalent to 4333 mg l^{-1}. Resistance to deltamethrin was the lowest in Tehran and Karaj populations whose LC_{50} values of them were 508 and 331 mg l^{-1}, respectively. These populations showed only small difference in resistance to deltamethrin compared to susceptible population (Ardabil). The RR_{50}s derived from LC_{50} values were 20.75 and 21.84 for Urmia and Flaverjan populations, respectively.

Deltamethrin resistance selection: Third instar larvae of different *P. xylostella* populations were selected with deltamethrin for 15 generations (Table 2). The results revealed that Naqadeh population was highly resistant to deltamethrin showing high resistance ratio compared to susceptible strain (91.87-fold). The development of resistance in this strain was considerable. Likewise, resistance ratios of 82.84 and 70.42 were determined for Urmia and Flaverjan populations, respectively. Development of resistance in these populations was also considerable. Thus, the above results indicated that *P. xylostella* under selection pressure of deltamethrin showed higher degree of resistance.

Synergism of PBO, DEM, DEF and TPP: The synergism of PBO, DEM, DEF and TPP on deltamethrin was tested in susceptible and resistant strains of *P. xylostella*. The results are shown in Table 3. PBO showed an obvious synergism effect in three resistant strains.

Table 1. Toxicity of deltamethrin on third instar larvae of *P. xylostella* (F1)

strain	n	Slope ± SE	LC_{50} (mg l^{-1})	x^2 (df)	RR_{50}[a]
Ardabil	300	1.42 ± 0.24	165 (130-218)	1.75 (3)	1.00
Flaverjan	300	2.11 ± 0.36	3604 (3063-4339)	0.65 (3)	21.84
Karaj	300	1.42 ± 0.25	331 (260-437)	1.74 (3)	2
Naqadeh	300	2.18 ± 0.38	4333 (3703-5182)	1.08 (3)	26.26
Tehran	300	1.63 ± 0.24	508 (420-628)	1.45 (3)	3.08
Urmia	300	2.09 ± 0.36	3424 (2906-4127)	2.40 (3)	20.75

[a]RR_{50}, resistance ratio at LC_{50} level = LC_{50} of the resistant strain to LC_{50} of susceptible (Ardabil) strain.

Table 2. Toxicity of deltamethrin to third instar larvae of *P. xylostella* after 15 generations of selection with deltamethrin

strain	Slope ± SE	LC_{50} (mg l^{-1})	x^2(df)	RR_{50}[a]
Ardabil	1.42 ± 0.46	94 (82-109)	1.68 (3)	1.00
Flaverjan	2.42 ± 0.24	6620 (5196-8720)	1.73 (3)	70.42
Naqadeh	3.26 ± 0.38	8636 (7417-10255)	1.36 (3)	91.87
Urmia	3.66 ± 0.28	7787 (6336-9849)	0.65 (3)	82.84

[a]RR_{50}, resistance ratio at LC_{50} level = LC_{50} of the resistant strain to LC_{50} of susceptible (Ardabil) strain.

The PBO synergistic ratios were 1.20, 7.36, 5.76 and 5.26 for Ardabil, Flaverjan, Naqadeh and Urmia strains, respectively. No significant changes in mortality were found when TPP and DEM treatments were applied in conjunction with deltamethrin and no synergistic effect was observed neither in resistant strains nor in the susceptible strain. DEF showed a moderate synergism in Ardabil, Flaverjan, Naqadeh and Urmia strains (SRs were 1.27, 3.99, 3.74 and 3.12, respectively).

Cross resistance study: The high deltamethrin resistant strain (Naqadeh) was tested for cross resistance to different insecticides. The results are shown in Table 4. The results indicate that Naqadeh strain exhibited high cross resistance to hexaflumuron (CR= 32.18) and indoxacarb (CR= 16.78). Small cross resistance to thiodicarb (CR= 3) and flubendiamide (CR= 4.67) was also witnessed. There was no obvious cross resistance to abamectin (CR= 1).

Enzyme assay: Results of enzyme activity are shown in Figures 1, 2 and 3. There was no significant difference in GST activity between susceptible strain (Ardabil) and resistant strains (Flaverjan, Naqadeh and Urmia) ($P>0.05$, $F=8.29$, df= 3, 8). However, no correlation between resistance level and GST activity was found. EST enzyme activity was increased from 19.32 μmol min^{-1} mg $protein^{-1}$ in susceptible strain (Ardabil) to 57.63, 67.97 and 54.24 μmol min^{-1} mg $protein^{-1}$ in resistant Flaverjan, Naqadeh and Urmia strains, respectively ($P<0.05$, $F=330.72$, df= 3, 8). The MFO activity was significantly higher in resistant strains than in susceptible strain ($P<0.05$, $F=198.47$, df= 3, 8). These values were 0.39, 0.38, 0.47 and 0.09 μmol min^{-1} mg $protein^{-1}$ for Flaverjan, Naqadeh, Urmia and Ardabil strains, respectively.

Table 3. Toxicity of deltamethrin with and without synergists on third instar larva of *P. xylostella*

strain	synergist	n	Slope ± SE	LC_{50} (mg l^{-1})	x^2 (df)	SR[a]
Ardabil	None	300	2.66 ± 0.46	94 (82-109)	1.68 (3)	-
	DEF	300	1.27 ± 0.22	74 (56-100)	1.62 (3)	1.27
	DEM	300	1.27 ± 0.53	92 (70-126)	1.58 (3)	1.08
	PBO	300	1.76 ± 0.30	78 (64-97)	1.52 (3)	1.20
	TPP	300	1.15 ± 0.20	120 (107-136)	1.33 (3)	0.78
Flaverjan	None	300	1.42 ± 0.24	6620 (5196-8720)	1.73 (3)	-
	DEF	300	1.46 ± 0.22	1660 (1303-2187)	1.77 (3)	3.99
	DEM	300	1.27 ± 0.21	5785 (4412-7876)	2.08 (3)	1.14
	PBO	300	1.55 ± 0.27	899 (721-1157)	1.83 (3)	7.36
	TPP	300	1.12 ± 0.19	4172 (3071-5915)	0.84 (3)	1.59
Naqadeh	None	300	2.26 ± 0.38	8636 (7417-10255)	1.36 (3)	-
	DEF	300	1.27 ± 0.22	2307 (1760-3140)	2.03 (3)	3.74
	DEM	300	2.02 ± 0.34	6640 (5604-8059)	1.46 (3)	1.30
	PBO	300	1.06 ± 0.18	1498 (1084-2166)	2.02 (3)	5.76
	TPP	300	2.00 ± 0.34	5492 (4628-6675)	0.64 (3)	1.57
Urmia	None	300	1.66 ± 0.28	7787 (6336-9849)	0.65 (3)	-
	DEF	300	1.10 ± 0.19	2497 (1830-3557)	2.32 (3)	3.12
	DEM	300	1.25 ± 0.22	5828 (4435-7955)	1.90 (3)	1.34
	PBO	300	1.20 ± 0.20	1479 (1111-2048)	1.41 (3)	5.26
	TPP	300	1.08 ± 0.18	4917 (3577-7063)	2.14 (3)	1.58

[a] SR, synergistic ratio = LC_{50} of deltamethrin alone / LC_{50} of deltamethrin + synergist.

Table 4. Toxicity of different insecticides to the Naqadeh and Ardabil strains of *P. xylostella*

insecticide	strain	n	Slope ± SE	LC_{50} (mg l^{-1})	x^2 (df)	CR[a]
Abamectin	Ardabil	300	1.21 ± 0.20	2 (1.5-2.8)	0.95 (3)	1
	Naqadeh	300	0.89 ± 0.15	2 (1.5-3)	0.88 (3)	
Indoxacarb	Ardabil	300	1.09 ± 0.18	2.8 (2-4)	1.57 (3)	16.78
	Naqadeh	300	1.12 ± 0.19	47 (35-67)	1.12 (3)	
Hexaflumuron	Ardabil	300	1.37 ± 0.23	17 (13.5-23)	0.96 (3)	32.18
	Naqadeh	300	5.22 ± 0.89	547 (512-589)	1.47 (3)	
Thiodicarb	Ardabil	300	2.5 ± 0.42	58 (50-67)	0.66 (3)	3.03
	Naqadeh	300	0.79 ± 0.13	176 (114-287)	1.06 (3)	
Flubendimide	Ardabil	300	1.48 ± 0.26	3 (3-4)	3.47 (3)	4.67
	Naqadeh	300	1.67 ± 0.28	14 (12-18)	1.44 (3)	

[a] CR (cross resistance ratio) = LC_{50} of resistance strain (Naqadeh) / LC_{50} of susceptible strain (Ardabil)

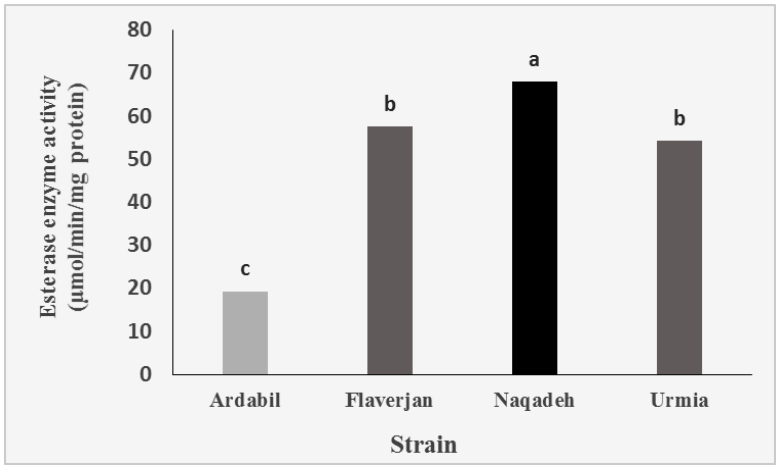

Figure 1. Esterase activity in resistant strains (Flaverjan, Naqadeh and Urmia) and susceptible strain (Ardabil) of *P. xylostella*. Data are presented as means ± SE. None similar letters on the top of columns indicate a significant difference in enzyme activity (P<0.05)

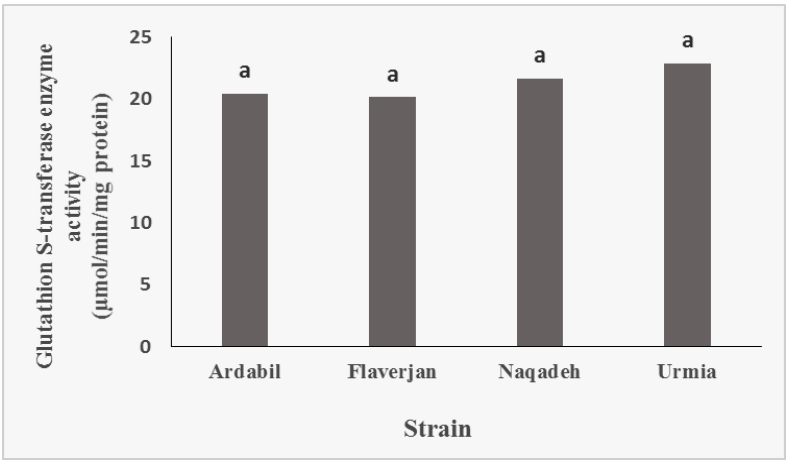

Figure 2. Glutathione S-transferase activity in resistant strains (Flaverjan, Naqadeh and Urmia) and susceptible strain (Ardabil) of *P. xylostella*. Data are presented as means ± SE. Similar letters on the top of columns indicate no significant difference in enzyme activity (P>0.05)

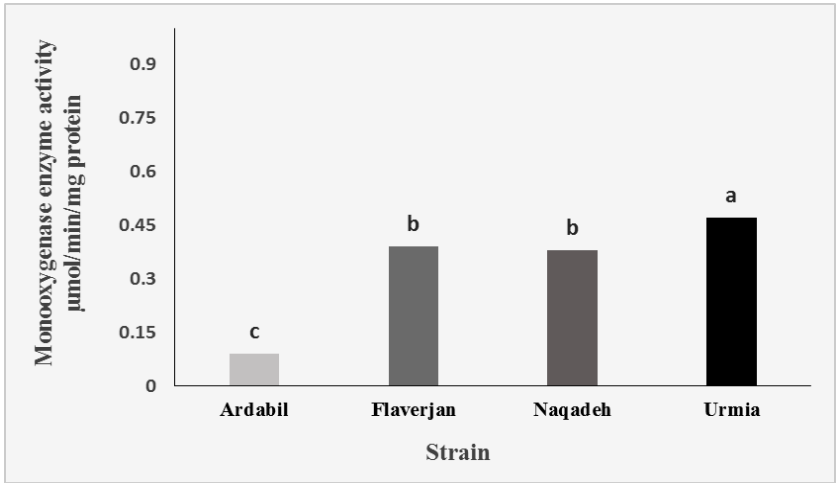

Figure 3. Monooxygenase activity in resistant strains (Flaverjan, Naqadeh and Urmia) and susceptible strain (Ardabil) of *P. xylostella*. Data are presented as means ± SE. None similar letters on the top of columns indicate a significant difference in enzyme activity (P<0.05)

The results of our study demonstrated that field collected and geographically separated populations of *P. xylostella* show reduced susceptibility to deltamethrin. This is the first documented case study of diamondback moth deltamethrin resistance in Iran. Among all populations, Ardabil strain was more susceptible to deltamethrin. Susceptibility to deltamethrin in Flaverjan, Naqadeh and Urmia strains was much lower compared with the susceptible (Ardabil) strain. These high levels of resistance may be due to over-reliance on deltamethrin or other pyrethroid insecticides for the control of *P. xylostella* in these areas. Indeed, in the areas where Flaverjan, Naqadeh and Urmia populations were collected, farmers also applied deltamethrin for controlling other cabbage pests such as *P. brassicae* and *P. rapae*. Reduction in susceptibility of Tehran and Karaj strains was less than Flaverjan, Naqadeh and Urmia strains. This can be because of the fact that in this area, according to the documented reports, farmers use insecticides of different groups such as indoxacarb, hexaflumuron, fenpropathrin, deltamethrin, chlorpyrifos, etc (Hasanshahi and Jahan 2012). According to our results, significant difference found in the slopes of dose-mortality curves of field populations and it showed high genetic variation in these populations. The slope for Karaj and Tehran strains were 1.42 and 1.63 compared with 2.11, 2.18 and 2.09 for Flaverjan, Naqadeh and Urmia strains, respectively. To investigate the resistance mechanisms toward deltamethrin, we selected resistant strains (Flaverjan, Naqadeh and Urmia). After 15 generations of selection, resistance ratios for Flaverjan (RR= 70.42), Naqadeh (RR= 91.87) and Urmia (RR= 82.84) strains were increased compared with susceptible (Ardabil) population (Table 2). Balasubramani *et al*. (2008) reported that a 15-fold increase was observed in LC_{50} value of the resistant *P. xylostella* strain after nine generations of selection with deltamethrin. Sayyed *et al*. (2005) also demonstrated that in *P. xylostella* selection with deltamethrin increased RR (>230-fold) compared with unselected population after nine generations.

Results of application of PBO, DEF, DEM and TPP on three deltamethrin resistant strains and susceptible strain, suggested that TPP and DEM had no synergistic effect on deltamethrin and did not significantly break the resistance. Results demonstrated that EST and GST are not involved in deltamethrin resistance in *P. xylostella*. PBO considerably increased deltamethrin toxicity in resistant strains. This result showed that MFO may be the main metabolic enzymes involved in the resistance of diamondback moth to deltamethrin. According to the obtained results, DEF slightly increased the toxicity of deltamethrin in resistant strains and it may be the case that EST enzymes also have enhanced resistance to deltamethrin in *P. xylostella*. Abro *et al*. (2013) suggested the involvement of MFO and GST in the resistance of this pest to lambdacyhalothrin based on their synergism studies. Miyata *et al*. (1986) also reported that PBO and DEF enhanced the toxicity of some pyrthroids including permethrin, cypermethrin, deltamethrin, and fenvalerate and synergistic ratio for these insecticides with PBO and DEF were (2.7, 2.1), (17.7, 1.6), (47.0, 1.1) and (30.0, 1.1), respectively. Ishtiaq *et al*. (2012) reported that MFO and EST were responsible for deltamethrin resistance in *Spodoptera exigua* L. (Lepidoptera: Noctuidae). Results obtained by these authors are in agreement with our findings.

Previous studies have shown that two insecticides may demonstrate cross resistance when they had the same effect on the activity of detoxification enzymes (Rodriguez *et al*. 2002). In this study, Naqadeh strain showed noticeable cross-resistance toward hexaflumuron (CR= 32.18), indoxacarb (16.78), flubendiamide (4.67) and thiodicarb (3). The MFO is responsible for resistance towards pyrethroids, organophosphates, DDT and insect growth regulators (Viswan *et al*. 2016). The concluded high level of MFO (in Naqadeh strain) in the present study proposed that the detoxification by this enzyme could be implicated in the cross resistance with mentioned insecticides. For resistance management, deltamethrin with hexaflumuron, indoxacarb, thiodicarb and flubendiamide is not recommend for rotational use. Our results showed that indoxacarb can achieve effective control of deltamethrin resistant *P. xylostella* population (Naqadeh) and Nehare *et al*. (2010) also demonstrated that organophosphate- and pyrethroid-resistant strains of this pest exhibited positive cross resistance to indoxacarb which agrees with our findings. Because there was very low or no cross resistance with abamectin, there shoud be no problem in using deltamethrin in alternation with this compound. Ishtiaq *et al*. (2012) found that abamectin had no effect on deltamethrin selected strain of *S. exigua*, and they found no cross resistance of abamectin with deltamethrin.

Since GST was not notably different in resistant strains (Flaverjan, Naqadeh and Urmia) compared with susceptible strain (Ardabil), it may be concluded that GST activity did not play a key role in conferring deltamethrin resistance to diamondback moth. Gong *et al*. (2013) reported that there was no relation between GST activity and resistance to beta cypermethrin (another pyrethroid insecticide). According to our results, determination of EST and MFO activity indicated that resistant strains had the highest activities of these enzymes. These results were in accordance with previous studies on different pyrethroid resistant species. Similarly, Gunning *et al*. (1996) reported increase in EST activity in pyrethroid-resistant strains of Australian *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) and some authors have reported the involvement of MFO as a potential factor in pyrethroid resistance in different pest species (Picollo *et al*., 2005; Lin *et al*., 2009; Gao *et al*., 2012). The results matched well with resistance levels and synergism data and leads us to the conclusion that MFO and EST to be involved in deltamethrin resistance in diamondback moth. Pesticide resistance may also be caused by many other mechanisms and factors such as regional, genetic, and environmental differences among different strains. Hence, further investigations are needed in these regards.

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Deltamethrin resistance in several populations of diamondback moth, *Plutella xylostella* in Iran

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Abstract

The diamondback moth, *Plutella xylostella* L. has been developed resistance to many groups of pesticides including of pyrethroids. The present study was conducted to evaluate the toxicity of deltamethrin on the third instar larvae of six populations of the pest using leaf dipping method. The results showed that different populations had different susceptibilities to deltamethrin. At the LC_{50} level, the resistance ratios of the Urmia, Flaverjan, Karaj, Tehran and Naqadeh populations to deltamethrin were 20.75, 21.84, 2.00, 3.08 and 26.26-fold. Resistant populations were selected for 15 generations and their susceptibility to deltamethrin was evaluated. Resistance levels were noticeably increased in these strains and equaled with 91.87, 82.84 and 70.42-fold in Naqadeh, Urmia and Flaverjan populations, respectively. Although, DEM and TPP had no synergistic effect on deltamethrin, treatment with PBO significantly decreased the toxicity of deltamethrin in the tested resistant strains. DEF also exhibited a moderate synergism with deltamethrin. Enzyme analysis proved that the activity of monooxygenase and esterase enzymes in the resistant strains were much stronger than that of glutathione S-transferase. The results showed that the high resistant strain (Naqadeh) of *P. xylostella* selected by deltamethrin exhibited high cross resistance to hexaflumuron and indoxacarb. This strain also had moderate positive cross resistance to flubendiamide and thiodicarb.

Key words: cabbage pest, synergism, chemical pesticide, detoxification