

## Research Article

# Characteristic of resistance to dichlorvos and biochemical mechanisms in the greenhouse strains of *Frankliniella occidentalis* (Thysanoptera: Thripidae)

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**Abstract:** The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is an invasive pest in greenhouse with high potential to cause damage to crops. There are a limited number of effective insecticides to manage this pest and several cases of chemical control failures have been reported in Iran which can be due to resistance to insecticides. To evaluate the status of insecticide resistance and possible resistance mechanisms, eight Iranian strains of *F. occidentalis*, collected from Tehran, Markazi, Alborz, Qazvin, Isfahan, Yazd (M and B) and Kerman provinces, were assayed against dichlorvos as a recommended insecticide for chemical control of thrips. Compared with the susceptible strain (Isfahan), two strains collected from Yazd had the lowest susceptibility to dichlorvos (Resistance Factor = 2.14 and 2.04 fold). Bioassay by synergists and enzyme assays demonstrated interfering of carboxyl esterase and glutathion S- transferase in Yazd M strain. The esterase inhibitor, triphenyl phosphite (TPP), and Glutathione S-transferase inhibitor, diethyl maleate (DEM), synergized the toxicity of dichlorvos in the Yazd M strain, (Synergistic Ratio = 5.28 and 1.79 fold, respectively). Also, carboxylesterase (for  $\alpha$ - naphthyl acetate and  $\beta$ - naphthyl acetate) and glutathion S-transferases activities in this population were 1.69, 7.31 and 0.97 fold higher than in the Isfahan strain. Furthermore, dichlorvos resistance did not significantly diminish after several months. Based on our results, we suggest that dichlorvos should be removed from the control program of this pest.

**Keywords:** carboxylesterase, glutathione s-transferase, bioassay, stability of resistance

## Introduction

The western flower thrips (WFT), *Frankliniella occidentalis* is one of the most destructive pests that attacks different family of agricultural, horticultural and ornamental crops throughout

the world (Lewis, 1997; Wang *et al.*, 2015). This highly polyphagous pest damages crop directly and indirectly. Direct damage occurs by feeding on plant tissues (Bielza *et al.*, 2008). Indirect damage is due to transmitting plant viruses of the genus *Tospovirus* (Riley *et al.*, 2011). Damage caused by this pest is estimated at millions of € worldwide (Mirnezhad *et al.*, 2010). Accordingly, it is considered the most important species in the thysanoptera order (Reitz, 2009).

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The main strategy to manage WFT populations is the use of insecticides whereas in any given situation and crop, the range of compounds and formulations that are effective for the control of thrips is quite limited (Contreras *et al.*, 2008). Also, control of this pest is difficult due to some behavioral and physiological features such as polyphagous nature, high reproductive potential, short generation time, haplodiploid reproductive system, cryptic habits (thigmokinetic behavior) and insecticide resistance (Jensen, 2000b; Contreras *et al.*, 2010; Wu *et al.*, 2018). *Frankliniella occidentalis* is the insect pest with a high potential to develop resistance to insecticides and it is capable to maintain resistance for a long time in the absence of insecticide pressure (Contreras *et al.*, 2008; Demirozer *et al.*, 2012). Insecticide resistance in WFT populations has been reported to various insecticide classes including organophosphorus (OPs), carbamates, pyrethroids, avermectins and spinosins (Immaraju *et al.*, 1992; Brødsgaard, 1994; Zhao *et al.*, 1995; Jensen, 2000a; Espinosa *et al.*, 2002; Bielza *et al.*, 2007; Contreras *et al.*, 2010; Chen *et al.*, 2011; Meng *et al.*, 2018).

There are several reports on the mechanisms of insecticide resistance in *F. occidentalis*. Major mechanism of resistance to insecticides is metabolic (increased detoxification) and in some cases (spinosad) in target site insensitivity (Immaraju *et al.*, 1992). WFT can develop resistance to insecticides using more than one mechanism, and multiple mechanisms can combine to contribute to each insecticide resistance. These factors have led to a large number of ways by which WFT has developed resistance to many insecticides (Demirozer *et al.*, 2012). OPs resistance related to esterase activity has been previously reported in western flower thrips (Jensen, 2000a; Maymo *et al.*, 2002). WFT was officially reported for the first time in 2004 in Iran and has spread throughout most provinces for almost fifteen years (Jalili Moghadam and Azmayeshfard, 2004). In Iran, control of WFT involves repeated application of insecticides. Dichlorvos is a recommended insecticide used extensively and intensively to control WFT (Gholami and Sadeghi, 2016). Recently, the

farmers have reported the insufficient control of the WFT by dichlorvos. Multiple applications and selective pressure of insecticides are potential causes of reports of control failures of this pest.

Therefore, the purpose of the current study is to define the status of dichlorvos resistance, stability of insecticide resistance and the mechanisms that confer resistance to dichlorvos in greenhouse strains of *F. occidentalis* collected from several provinces in Iran.

## Materials and Methods

### Insect strains

To assess resistance development to dichlorvos in *F. occidentalis*, eight greenhouse strains were collected from different provinces in Iran including Tehran (THN), Alborz (ABZ), Markazi (MRI), Esfahan (IHN), Kerman (KMN), Yazd (YZM and YZB) and Qazvin (QZN) during 2017 to 2018. Characteristics of the greenhouse strains of WFT used in the bioassays and enzyme activity assays are summarized in Table 1. These strains were reared under insecticide-free conditions in room chamber on fresh green bean pods (*Phaseolous vulgaris*) at  $25 \pm 1$  °C,  $65 \pm 5\%$  R.H. and a photoperiod of 16 L:8 D in the Department of Plant Protection, the Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (Gholami *et al.*, 2015).

### Insecticides and chemicals

Commercial formulation of dichlorvos EC50% (IRAC code 1B) (GolShimi Sepahan Co., Ltd., Iran) was used in insecticide bioassay. The synergists, piperonyl butoxide (PBO; 90%), triphenyl phosphate (TPP; 97.2%), diethyl maleate (DEM; 97%) and the chemicals, 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH; 98%) were obtained from Merck (Darmstadt, Germany).  $\alpha$ -naphthyl acetate ( $\alpha$ -NA; 98%),  $\beta$ -naphthyl acetate ( $\beta$ -NA), fast blue RR salt, bovine serum albumin (BSA), coomassie brilliant blue G-250, 5,5'-dithio-bis-2-nitrobenzoate (DTNB; 99%) and acetylthiocholine iodide (ATChI; 98%) were purchased from Sigma Aldrich (St. Louis, MO, USA).

**Table 1** Characteristics of the greenhouse strains of *Frankliniella occidentalis* (location, date and host plant source) used in the bioassays and biochemical assays

Code of population	Location of collecting site (Province)	Latitude (N) and longitude (E)	Date of collection	Insecticide regime used	Host plant	Pests on host plant
IHN	Isfahan (Isfahan)	32°39'16.66" N, 51°40'4.74" E	2017 December	Deltamethrin and OPs insecticides	Pepper	Whitefly-Thrips-Mite
THN	Varamin (Tehran)	35°19'30.87" N, 51°38'49.92" E	2017 June	samples were collected 10 days after Oberon treatment	Eggplant	Thrips-Mite
MRI	Aaveh (Markazi)	35°48'10.20" N, 50°25'32.33" E	2017 August	samples were collected 10 days after mixture of dichlorvos and abamectin treatment	Eggplant	Whitefly-Thrips-Mite
ABZ	Nazar Abad (Alborz)	35°45'52.1"N, 50°50'21.9"E	2017 September	thirty selection steps with dichlorvos and abamectin	Strawberry	Thrips-Mite
QZN	Alborz Industrial Park (Qazvin)	36°10'51.8" N, 50°05'06.5" E	2017 September	eight selection steps with dichlorvos and abamectin, samples were collected 20 days after abamectin, malathion and imidacloprid treatment	Cucumber	Whitefly-Thrips-Leafminer
YZM	Khavidak (Yazd M)	31°79'98.65" N, 54°50'81.09"E	2018 May	Deltamethrin and dichlorvos-	Eggplant	Thrips-Whitefly-Mite
YZB	Ahmad Abad (Yazd B)	31°47'08.9" N, 54°21'13.3" E	2018 June	Spinosad, malathion, dichlorvos and proteus	Eggplant	Thrips-Mite
KMN	Jiroft (Kerman)	30°51'51.8" N, 50°05'06.5" E	2018 May	eight selection steps with and OPs insecticides	Strawberry	Thrips-Mite

### Bioassay

The green bean pod sections-dipping method was used in bioassay based on the method described by IRAC (test method 014) ([www.irc-online.org](http://www.irc-online.org)). The LC<sub>50</sub> of dichlorvos were estimated on the collected strains. A hole (3 cm in diameter) was made on each plastic Petri dish cap and was covered with silk mesh to provide adequate ventilation. The sections of green bean pod, 2 cm in length, were completely immersed in insecticide solutions for 30 seconds and were air-dried. Then, they were put into a Petri dishes (one section per each Petri dish) and twenty 2<sup>nd</sup> instar larvae were placed into each dish. Petri dishes were kept at 25 ± 1 °C under a L16: D8. Deionized water was used as a control. At least five concentrations and three replicates were used per concentration. Mortality was recorded 24 hours after treatment. Thrips were considered dead when they were unable to move due to brush stimulation

### Synergistic effect

A concentration of 1000 mg/l of the synergists (mortality less than 10%) was applied 2 hours before the insecticide treatment. The synergists were dissolved in acetone and added to deionized

water (40: 60% v: v) at the desired concentration and Petri dishes were coated with 2 ml of synergist solution for 10 min before discarded. The 2<sup>nd</sup> instar larvae were incubated in the synergist coated plates for 2 hours before transferring to the new Petri dishes containing insecticide-treated green bean pods. Mortality was recorded after 22 hours (Nazemi *et al.*, 2016). Deionized water was used as a control and assays were replicated three times.

### Stability of resistance

A decline in resistance to dichlorvos in all strains were estimated by dividing the secondary LC<sub>50</sub> value (obtained from the second bioassay after several months of the rearing) by the primary LC<sub>50</sub> value (Contreras *et al.*, 2008).

### Detoxification enzyme assays

#### Protein measurement

Total protein content for all enzyme extract samples was determined according to Bradford (1976). The bovine serum albumin was used as a standard.

#### Carboxylesterase activity

According to Chen *et al.* (2011), about 80 2<sup>nd</sup> instar larvae of each strain were homogenized in 0.5 ml

phosphate buffer (0.1 M, pH 7.5) containing Triton X100 (0.1% v/v). The homogenates were centrifuged at 10,000 g for 5 min at 4 °C. The supernatants were separated as an enzyme source and were kept at -80 °C. Carboxylesterase activity using  $\alpha$ -NA and  $\beta$ -NA as substrates were measured according to the method of van Asperen (1962) with slight modification. Briefly, 200  $\mu$ l of 30 mM  $\alpha$ -NA or  $\beta$ -NA, 20  $\mu$ l of enzyme preparation and 50  $\mu$ l Fast Blue RR (0.01% w/v) were added to each well of a 96-well plate. Optical density of  $\alpha$ -naphthol and  $\beta$ -naphthol were recorded continuously for 20 min using a microplate reader at 450 and 540 nm, respectively (Elx808, Bio-Tek Instruments Inc., Winooski, VT, USA). Carboxyl esterase activity was presented as  $\mu$ mole min<sup>-1</sup>mg<sup>-1</sup> protein.

#### GST activity

About 80 2<sup>nd</sup> instar larvae of each strain were homogenized in 0.5 ml phosphate buffer (0.1 M, pH 7.5) and homogenates were centrifuged at 10,000g for 5 min at 4 °C. The supernatants were separated to measure the activity of GST and were kept at -80 °C. GST activity was determined using CDNB as substrate according to the method of Chen et al. (2011) with slight modification. Ten  $\mu$ l of enzyme extract was mixed with 200  $\mu$ l of 63 mM CDNB and 10 mM reduced glutathione (GSH) in 0.1 M sodium phosphate buffer (pH 6.5). Any change in absorbance was recorded continuously for 20 min at 340 nm using the microplate reader. The activity of GST was determined using the molar extinction coefficient of 9.6 mM<sup>-1</sup> cm<sup>-1</sup> for the CDNB (Habig et al., 1974).

#### Acetylcholinesterase (AChE) activity

AChE activity was determined according to the method of Ellman et al. (1961) with slight modification, using ATChI as substrate. Briefly, the reaction mixture consisting of ATChI (1.5 mM), DTNB (1 mM) and enzyme preparation (20  $\mu$ l) was prepared in a final volume of 270  $\mu$ l with phosphate buffer (0.1 M, pH 8.0). The absorbance was measured continuously every 1 min for 20 min at 405 nm. Enzyme activity was calculated using the extinction coefficient of 13.6 mM<sup>-1</sup> cm<sup>-1</sup> for 5-thio-2- nitrobenzoate (Ellman et al., 1961).

#### Data analysis

Bioassay data were analyzed by probit using the Polo-Plus software version 2.0 (LeOra Software, Berkeley, CA). Mortality was corrected using Abbott's formula for each probit analysis (Abbot, 1925). Resistance factors (RF) were calculated by dividing the LC<sub>50</sub> of the resistant strain by the LC<sub>50</sub> of the susceptible strain. Synergistic ratios (SR) were calculated by dividing the LC<sub>50</sub> of the insecticide alone by the LC<sub>50</sub> of the insecticide plus synergist (Brødsgaard, 1994; Espinosa et al., 2005). Statistical difference of the resistance to insecticides between strains was estimated by Lethal Dose Ratio (LDR) and the 95% confidence intervals of resistance factors and synergism factor were estimated by using the method described by Robertson et al. (2017). Enzyme activities were stated as mean  $\pm$  standard error of the mean (SE). Statistical analysis was performed by analysis of variance (ANOVA), and comparisons of the means were made using Tukey's test in SAS 9.1 (SAS, 2004) (P-value < 0.05).

#### Results

##### Resistance to dichlorvos

Variation in susceptibility among the eight greenhouse strains was moderate in the case of dichlorvos (Table 2). The most sensitive strain was IHN. The highest LC<sub>50</sub> value was observed in YZM strain (RF = 2.14 fold). By the LDR analysis, LC<sub>50</sub> value of IHN, THN and QZN were statistically significantly different in comparison to YZM strain, whereas there were no significant differences among the MRI, ABZ, YZM, YZB and KMN strains (P-value < 0.05). In the susceptible strain (IHN), synergistic effects were not observed, when DEM, PBO and TPP were applied with dichlorvos. Whereas toxicity of dichlorvos was synergized by DEM (SR = 1.79) and TPP (SR = 5.28) (Table 2) in the YZM strain. Also, PBO had no effect on the activity of detoxifying enzymes.

##### Stability of resistance to dichlorvos

Results of the stability of insecticide resistance in *F. occidentalis* are shown in Table 3. There were no changes in susceptibility of the IHN strain to insecticides after rearing in the

laboratory in the absence of selection pressure for 8 months. In the YZM strain, RF values showed stability, without significant difference during 5 months (from RF<sub>50</sub> = 2.14 to 2.12 fold).

### Metabolic enzyme activities

#### Carboxyl esterase

Among the populations, the YZM expressed higher levels of carboxyl esterase activity towards  $\alpha$ -NA and  $\beta$ -NA ( $3.75 \pm 0.42$  and  $5.01 \pm 0.06$  mmol product min<sup>-1</sup>mg<sup>-1</sup> protein, respectively), in which carboxyl esterase activity was 1.69 and 7.31 fold higher than that of susceptible strain (IHN). However, the lowest activity was obtained from the QZN strain activity ratio of which were 0.59 and 1.23 for  $\alpha$ -NA and  $\beta$ -NA, respectively. The analysis variance of carboxylesterase activities showed

significant difference between YZM and IHN strains (F-value = 16.73, P-value < 0001, df: 7). (Table 4).

#### Glutathione S-transferases and acetylcholinesterases

GST activity in the ABZ strain was 0.93- fold higher than that of IHN strain. However, GST activity was significantly higher in KMN than IHN (AR = 1.33 fold). All strains except the KMN and MRI were not significantly different in GST activity (F-value = 19.26, P-value < 0001, df: 7). (Table 5). Also, results of AChE activity from different strains are presented in Table 5. The YZM had the most AChE activity among the tested strains (AR = 14.3 fold) (F-value = 17.54, P-value < 0001, df: 7).

**Table 2** Lethal concentrations, slopes, resistance ratios and synergistic ratios of dichlorvos against different strains of *Frankliniella occidentalis*.

Strain <sup>1</sup>	Treatment/synergist	Number of Insects	Slope ( $\pm$ SE)	LC <sub>50</sub> (mg formulated litre <sup>-1</sup> ) (95% CL)	RR <sub>50</sub> <sup>2</sup> (95% FL)	SR <sub>50</sub> <sup>3</sup> (95% FL)
IHN <sup>4</sup>	Dichlorvos	265	2.81 $\pm$ 0.32	334.10 (283.49-389.33)	1.0	-
	+ DEM	226	4.05 $\pm$ 0.67	448.3 (370.48-519.69)	1.34 (1.07-1.68)	0.74 (0.59-0.93)
	+ PBO	218	3.92 $\pm$ 0.71	364.15 (284.86-427.96)	1.08 (0.85- 1.39)	0.91 (0.71-1.17)
	+ TPP	211	3.13 $\pm$ 0.44	322.53 (258.50-361.28)	0.96 (0.75-1.24)	1 (0.8-1.33)
THN	Dichlorvos	274	4.96 $\pm$ 0.66	369.97 (317.31-415.65)	1.10 (0.9-1.36)	-
	+ DEM	299	6.37 $\pm$ 0.83	416.67 (378.05-450.13)	1.24 (1.04-1.49)	0.88 (0.75-1.03)
	+ PBO	260	2.31 $\pm$ 0.37	174.34 (118.33-220.7)	0.52 (0.37-0.72)	2.12 (1.53-2.92)
	+ TPP	328	2.72 $\pm$ 0.36	209.82 (132.51-269.77)	0.62 (0.48-0.8)	1.76 (1.39-2.23)
MRI	Dichlorvos	295	5.04 $\pm$ 0.84	598.59 (514.78-667.28)	1.79 (1.44-2.18)	-
	+ DEM	280	2.86 $\pm$ 0.66	541.26 (396.54-646.2)	1.62 (1.24-2.11)	1.1 (0.86-1.41)
	+ PBO	298	2.33 $\pm$ 0.44	480.46 (356.58-589.51)	1.43 (1.34-2.18)	1.24 (0.84-1.19)
	+ TPP	274	2.10 $\pm$ 0.27	274.18 (207.46-339.63)	0.82 (0.61-1.09)	2.18 (1.66-2.86)
ABZ	Dichlorvos	290	8.41 $\pm$ 1.45	673.39 (605.57-721.57)	2.01 (1.68-2.4)	-
	+ DEM	226	5.17 $\pm$ 0.72	540.2 (478.25-594.34)	1.61 (1.33-1.95)	1.24 (1.09-1.42)
	+ PBO	218	4.87 $\pm$ 0.69	614.55 (552.2-675.49)	1.83 (1.52-2.21)	1.09 (0.96-1.24)
	+ TPP	230	4.78 $\pm$ 0.65	516.34 (454.81-569.57)	1.54 (1.27-1.87)	1.30 (1.13-1.49)

Table 2 continued.

Strain <sup>1</sup>	Treatment/synergist	Number of Insects	Slope (± SE)	LC <sub>50</sub> (mg formulated litre <sup>-1</sup> ) (95% CL)	RR <sub>50</sub> <sup>2</sup> (95% FL)	SR <sub>50</sub> <sup>3</sup> (95% FL)
QZN	Dichlorvos	284	5.49 ± 0.86	433.23 (359.42-486.68)	1.29 (1.04-1.6)	-
	+ DEM	226	14.18 ± 3.19	552.48 (475.59-581.78)	1.65 (1.39-1.96)	0.78 (0.66-0.92)
	+ PBO	198	3.42 ± 0.62	424.15 (338.02-494.97)	1.26 (1-1.61)	1.02 (0.81-1.28)
	+ TPP	216	3.69 ± 0.56	299.21 (202.36-376.6)	0.89 (0.7-1.14)	1.44 (1.14-1.82)
YZM	Dichlorvos	273	1.46 ± 0.19	715.64 (522.98-951.76)	2.14 (1.53-2.99)	-
	+ DEM	251	1.32 ± 0.20	397.79 (258.7-550.7)	1.19 (0.8-1.77)	1.79 (1.12-2.87)
	+ PBO	251	1.32 ± 0.20	432.78 (280.87-603.58)	1.29 (0.86-1.93)	1.65 (1.03-2.65)
	+ TPP	259	1.11 ± 0.21	135.34 (51.01-226.13)	0.4 (0.2-0.8)	5.28 (2.54-10.98)
YZB	Dichlorvos	263	1.32 ± 0.19	684.34 (477.38-932.92)	2.04 (1.42-2.94)	-
	+ DEM	269	1.32 ± 0.19	302.69 (195.15-446.76)	0.95 (0.62-1.45)	2.26 (1.28-3.6)
	+ PBO	275	1.28 ± 0.19	352.53 (220.51-493.14)	1.05 (0.69-1.6)	1.94 (1.17-3.22)
	+ TPP	255	0.95 ± 0.18	218.86 (89.07-358.74)	0.65 (0.34-1.25)	3.12 (1.54-6.34)
KMN	Dichlorvos	217	4.32 ± 0.76	691.79 (615.31-773.55)	2.07 (1.7-2.5)	-
	+ DEM	210	4.06 ± 0.68	584.8 (507.15-653.78)	1.75 (1.43-2.13)	1.18 (1-1.39)
	+ PBO	207	4.32 ± 0.69	540.13 (464.69-603.26)	1.61 (1.32-1.97)	1.28 (1.08-1.51)
	+ TPP	203	3.56 ± 0.68	375.85 (272.65-446.44)	1.12 (0.85-1.47)	1.84 (1.43-2.35)

<sup>1</sup> IHN (Isfahan), THN (Tehran), MRI (Markazi), ABZ (Alborz), QZN (Qazvin), YZM (Yazd M), YZB (Yazd B), KMN (Kerman).<sup>2</sup> Resistance ratio: ratio of LC<sub>50</sub> estimations between resistant and susceptible populations.<sup>3</sup> Synergist ratio: ratio of LC<sub>50</sub> estimations between a population treated with insecticide alone and the same population treated with a mixture of insecticide and synergist. <sup>4</sup> Susceptible population.Table 3 Over time development of resistance to dichlorvos against different strains of *Frankliniella occidentalis*.

Strain <sup>1</sup>	Insecticide	Month	Number of Insects	Slope (mean ± SE)	LC <sub>50</sub> (mg formulated litre <sup>-1</sup> ) (95% CL)	RR <sub>50</sub> <sup>2</sup> (95% FL)
IHN <sup>3</sup>	Dichlorvos	8	250	2.20 ± 0.28	280.28 (227.51-337.5)	0.83 (0.65-1.07)
THN	Dichlorvos	10	243	3.14 ± 0.49	487.68 (408.06-576.5)	1.45 (1.16-1.83)
MRI	Dichlorvos	12	312	1.12 ± 0.28	724.04 (474.27-1454.64)	2.16 (1.3-3.6)
ABZ	Dichlorvos	7	228	9.31 ± 1.55	672 (604.17-722.23)	2.01 (1.68-2.4)
QZN	Dichlorvos	11	227	2.32 ± 0.74	856.68 (672.74-1466.05)	2.56 (1.85-3.55)
YZM	Dichlorvos	5	273	1.31 ± 0.18	710.75 (501.25-972.76)	2.12 (1.48- 3.05)
YZB	Dichlorvos	4	263	1.18 ± 0.18	558.67 (360.61-788.17)	1.67 (1.11-2.51)
KMN	Dichlorvos	4	216	4.12 ± 0.81	751.01 (665.73-856.3)	2.24 (1.84- 2.73)

<sup>1</sup> IHN (Isfahan), THN (Tehran), MRI (Markazi), ABZ (Alborz), QZN (Qazvin), YZM (Yazd M), YZB (Yazd B), KMN (Kerman).<sup>2</sup> Resistance ratio: ratio of LC<sub>50</sub> estimations between resistant and susceptible populations. <sup>3</sup> susceptible population.

**Table 4** Mean esterase activities with  $\alpha$ -naphthyl acetate ( $\alpha$ -NA) and  $\beta$ -naphthyl acetate ( $\beta$ -NA) of the studied strains of *Frankliniella occidentalis*.

Strain <sup>1</sup>	Alpha (Mean $\pm$ SE) <sup>2,3</sup>	Activity ratio (R/S) <sup>4</sup>	Beta (Mean $\pm$ SE) <sup>2,3</sup>	Activity ratio (R/S) <sup>4</sup>
IHN <sup>5</sup>	2.20 $\pm$ 0.24 B-C	1	0.68 $\pm$ 0.03 D	1
THN	2.68 $\pm$ 0.13 B	1.21	1.65 $\pm$ 0.07 C	2.41
MRI	2.58 $\pm$ 0.12 B-C	1.17	2.75 $\pm$ 0.2 B	4.01
ABZ	1.38 $\pm$ 0.15 D	0.62	1.04 $\pm$ 0.05 C-D	1.52
QZN	1.32 $\pm$ 0.08 D	0.59	0.84 $\pm$ 0.07 C-D	1.23
YZM	3.75 $\pm$ 0.42 A	1.69	5.01 $\pm$ 0.06 A	7.31
YZB	3.67 $\pm$ 0.22 A	1.66	3.03 $\pm$ 0.24 B	4.42
KMN	1.88 $\pm$ 0.25 C-D	0.85	3.21 $\pm$ 0.67 B	4.68

<sup>1</sup> IHN (Isfahan), THN (Tehran), MRI (Markazi), ABZ (Alborz), QZN (Qazvin), YZM (Yazd M), YZB (Yazd B), KMN (Kerman).<sup>2</sup> carboxylesterase activity was expressed as  $\mu$ mol naphthol/min/mg protein.<sup>3</sup> Means followed by the same letter within a column are not significantly different (Duncan multiple comparison test, P-value < 0001).<sup>4</sup> Activity ratio = R activity/S activity.<sup>5</sup> susceptible population.**Table 5** Mean Glutathione S- transferase and acetylcholinesterase activities with acetylcholine iodide of the studied strains of *Frankliniella occidentalis*.

Strain <sup>1</sup>	Glutathione S- transferase (Mean $\pm$ SE) <sup>2,3</sup>	Activity ratio (R/S) <sup>5</sup>	Acetylcholinesterase (Mean $\pm$ SE) <sup>2,4</sup>	Activity ratio (R/S) <sup>5</sup>
IHN <sup>6</sup>	0.51 $\pm$ 0.01 B	1	0.006 $\pm$ 0.004 D	1
THN	0.42 $\pm$ 0.01 C	0.82	0.040 $\pm$ 0.01 B	6.66
MRI	0.29 $\pm$ 0.02 D	0.56	0.026 $\pm$ 0.005 B-C	4.30
ABZ	0.48 $\pm$ 0.02 B-C	0.93	0.027 $\pm$ 0.001 B-C	4.50
QZN	0.46 $\pm$ 0.01 B-C	0.90	0.011 $\pm$ 0.001 C-D	1.83
YZM	0.54 $\pm$ 0.01 B	1.05	0.086 $\pm$ 0.007 A	14.30
YZB	0.50 $\pm$ 0.04 B-C	0.97	0.024 $\pm$ 0.003 B-C-D	4
KMN	0.68 $\pm$ 0.01 A	1.33	0.035 $\pm$ 0.002 B	5.83

<sup>1</sup> IHN (Isfahan), THN (Tehran), MRI (Markazi), ABZ (Alborz), QZN (Qazvin), YZM (Yazd M), YZB (Yazd B), KMN (Kerman).<sup>2</sup> Means followed by the same letter within a column are not significantly different (Duncan multiple comparison test; P < 0001).<sup>3</sup> Glutathione S- transferase activity was expressed as  $\mu$ mol glutathione conjugated/min/mg protein.<sup>4</sup> Acetylcholinesterase activity was expressed as  $\mu$ mol 5-thio-2- nitrobenzoate/min/mg protein.<sup>5</sup> Activity ratio = R activity/S activity.<sup>6</sup> Susceptible population.

## Discussion

Based on the results of present study, the level of dichlorvos resistance was higher in the southern provinces (Yazd and Kerman), whereas, in the central areas such as Alborz, Tehran and Markazi, LC<sub>50</sub> values were lower (Table 2). This is the result of different patterns of the insecticide usage and management programs of greenhouse pests in different provinces of Iran. In the southern regions, the OPs have been used for a long time (maybe for 20 years, based on the year of registration of OPs in Iran), which can affect the OPs efficacy on strains of WFT distributed in these areas. These provinces are the main pillars of

producing greenhouse products such as cucumber, pepper, eggplant and ornamental (Baniameri, 2009). Therefore, conventional insecticides, such as OPs, have been used intensively. That is why the variation of resistance to dichlorvos among all tested strains is low (RF from 1.10 to 2.14 fold). In Alborz, Markazi and Qazvin as noted in Table 1, dichlorvos has been used with other insecticides of different chemical classes. But the application of OPs has been less than other chemical classes such as neonicotinoids, pyrethroids and avermectins. Resistance to dichlorvos in *F. occidentalis* (Brødsgaard, 1994; Macdonald, 1995; Jensen, 2000b), *Trialeurodes vaporariorum* (Sanderson &



Roush, 1992) and *Tetranychus cinnabarinus* (Guo *et al.*, 1998) have been reported by other researchers. Herron and James (2005) found that in the fipronil and spinosad resistant strain of western flower thrips, resistance ratio to dichlorvos increased from 0.9 to 3.5 fold after two years. MacDonald (1995) reported the 25-fold dichlorvos resistance ratio among seventeen different strains of WFT in the England and Ireland. There was a relationship between dichlorvos  $LC_{50}$  value in different strains of WFT and the number of applications of this insecticide in the nurseries sampled. The three strains with the lowest  $LC_{50}$  were not exposed to dichlorvos for one year prior to sampling, but six resistant strains were severely affected by dichlorvos selective pressure.

By removing a special insecticide from the chemical control program, susceptibility of the thrips maybe restored within several generations. In certain cases, resistance is persevered over many generations after the removal of selection pressure (Contreras *et al.*, 2008). Our results implicated that resistance in YZM and KMN strains was maintained after 5 and 4 months without dichlorvos exposure, respectively (Table 3). Raymond *et al.* (1993) suggested the slight decrease in resistance is due to the existence of alleles responsible for amplifications of detoxification enzyme. A slight reduction in resistance levels may be due to the negative effects of resistance genes on fitness components without insecticide selection pressure or lack of full consolidation of resistance genes in the gene reservoir (Saddiq *et al.*, 2016). The decline in the persistence of insecticide resistance in insecticide free-conditions varies with the resistant genotype, nature of selecting agent and intensity of resistance (Vastrad *et al.*, 2004). According to Uyenoyama (1986) when the insecticides are removed from chemical control of a pest, a reduction in the level of resistance would be produced by reapplying the original selective pressure after a while. Contreras *et al.* (2008) reported that in the very highly resistant strain of *F. occidentalis* to acrinathrin, resistance was maintained with little change after 5 and 8

months without acrinathrin exposure. In another strain, resistance to methiocarb was maintained after 5 months without selection pressure. Also, a slight decrease to formetanate, was observed in the absence of selection pressure for 8 months. Investigation of resistance stability is an important issue in insecticide resistance management (IRM). The stability of insecticide resistance had been already verified in WFT.

The resistance level of an insect depends on factors such as detoxification enzymes including P450 monooxygenase, esterase, and GST (Jensen, 2000a; Scott and Wen, 2001; Stevenson *et al.*, 2012). The experiments to evaluate the role of metabolic detoxification mechanisms by synergists confirmed that carboxyl esterase and GSTs are involved in resistance to dichlorvos therefore we assayed the activity of these two enzymes in all tested populations (Table 2). However, synergists are not entirely specific to each detoxification enzyme so results obtained from synergists must be considered with caution (Espinosa *et al.*, 2005; Lopez-Soler *et al.*, 2011). Resistance caused by carboxylesterase can be due to different non-specific isozymes that hydrolyze or sequester insecticides (Montella *et al.*, 2012; Teese *et al.*, 2013). Also, the variation in resistance levels is usually associated with variation in the number of copies of each gene as carboxylesterase isozyme patterns change with the strain (Devonshire, 1989). Also, it has been proposed that resistance may result from a mutation in a carboxyl esterase that simultaneously reduces activity and confers an OPs hydrolase activity (Newcomb *et al.*, 1997). The results of the carboxylesterase activity measurement in eight Iranian strains of WFT showed that the highest activity was related to two strains from Yazd province (M and B) (activity ratio for  $\alpha$ - NA was 1.69 and 1.66 fold and for  $\beta$ - NA was 7.31 and 4.42 fold). Also, glutathione S-transferase activity was significantly different only in KMN (1.33 times) compared to susceptible strain. Therefore, higher activity of carboxylesterase and glutathione S-transferase in these strains indicates the involvement of the enzyme-



dependent detoxification system in resistance to dichlorvos. The correlation between changes of carboxylesterase and dichlorvos resistance in *Culex pipiens* with three esters (Ester8, Ester9 and EsterB10) has been documented (Liu *et al.*, 2011). Maymo *et al.* (2002) found that in WFT strains selected with methiocarb and acrinatrin and some field strains, increased activity of carboxylesterase and glutathione S-transferase caused resistance compared to susceptible strain. Ferrari *et al.* (1993) demonstrated 10-fold carboxylesterase activity in a dimethoate and formetanate-resistant strain of *Scirtothrips citri* compared to the susceptible strain (resistance ratio = 35 and 3.9 fold, respectively). They reported that the reason for resistance to these insecticides is the increased activity of carboxylesterase. High levels of carboxylesterase and glutathione S-transferase activity in insecticide-resistant thrips have been reported in various studies (Immaraju *et al.*, 1990; Zhao *et al.*, 1995; Jensen 2000a; Saha *et al.*, 2012). On the contrary, in a strain selected with diazinon, carboxylesterase activity was somewhat lower than the reference strain. In addition, staining for carboxylesterase in electrophoresis showed more bands of carboxylesterase in the reference strain. In addition, no difference was found between GST activities. These results showed that carboxylesterase and GST were not involved in resistance (Zhao *et al.*, 1994).

Also, differences of 14.3 fold in activity ratio were observed for AChE between the YZM and IHN strains. High enzyme activity probably indicates less susceptibility of the AChE to inhibition by OPs that occurred in the resistant population. Also, over-production of AChE could cause the high AChE activity, which in turn leads to decreasing neurotransmitter level as a relevant effect of OPs (Bourguet *et al.*, 1997).

Nevertheless, insensitivity of AChE in studied strains should be specified to provide a better insight into the effect of this enzyme in the resistance to dichlorvos. Study on the selected strain with diazinon also revealed that although the level of insensitivity of this enzyme was similar in both susceptible and resistant strains, it

caused resistance to diazinon (Zhao *et al.*, 1994). Thus, the role of this mechanism in insecticide resistance of WFT is not well understood. In another experiment, an increase in the insensitivity of AChE to OPs and carbamates has been suggested as a reason for resistance in *S. citri* (Ferrari *et al.*, 1993). In three strains KMN, YZB and YZM, the activity of carboxylesterase, GST and AChE were increased. The reason for high resistance of these three strains to dichlorvos may be due to overlapping activity of these enzymes (Gong *et al.*, 2013). Results of our study suggest that enhanced detoxification by carboxylesterase play a significant role in resistance to dichlorvos. Due to the high potential of western flower thrips to develop insecticide resistance, the most important aspect of its chemical control would be precaution in the implementation of any factor used to control this pest. If farmers would have a high awareness of pest resistance to insecticides, they would be more careful about the use of certain chemical compounds. For insecticide resistance management towards dichlorvos; actions should be considered on the base of the regional history of chemical control and schedule rotational use of insecticides in each province.

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## بررسی مقاومت به دی کلرووس و سازوکارهای بیوشیمیایی آن در جمعیت‌های گلخانه‌ای تریپس *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) غربی گل

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**چکیده:** تریپس غربی گل با نام علمی *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) یکی از آفات مهم گلخانه‌ای با پتانسیل بالای ایجاد خسارت است. تعداد حشره‌کش‌های مؤثر برای مدیریت این آفت بسیار محدود بوده و موارد متعددی از ناکارآمدی کنترل شیمیایی این آفت در ایران گزارش شده است که می‌تواند ناشی از مقاومت به حشره‌کش‌ها باشد. به منظور بررسی وضعیت مقاومت به حشره‌کش‌ها و سازوکارهای مقاومت، هشت جمعیت مختلف از *F. occidentalis* جمع‌آوری شده از استان‌های تهران، مرکزی، البرز، قزوین، اصفهان، یزد (B و M) و کرمان، نسبت به دی کلرووس به عنوان حشره‌کش توصیه شده برای کنترل تریپس در ایران مورد سنجش قرار گرفتند. در مقایسه با جمعیت حساس جمع‌آوری شده از اصفهان، دو جمعیت از استان یزد (Yazd B و Yazd M) کم‌ترین حساسیت را نسبت به دی کلرووس داشتند (نسبت مقاومت: ۲/۱۴ و ۲/۰۴ برابر). همچنین زیست‌سنجی با استفاده از سینرژست‌ها نقش استرازاها و گلوکاتینون‌اس ترنسفراس را در مقاومت به دی کلرووس تأیید کرد. مهارکننده استراز (تری فنیل فسفات-TPP) و مهارکننده گلوکاتینون‌اس ترنسفراس (دی اتیل مالئات-DEM) باعث افزایش سمیت دی کلرووس در جمعیت Yazd M شدند (نسبت سینرژستی به-ترتیب برابر با ۵/۲۸ و ۱/۷۹ برابر). همچنین، فعالیت استرازاها در جمعیت Yazd M (برای سوبسترای آلفانفتیل استات و بتانفتیل استات) و گلوکاتینون‌اس ترنسفراس‌ها در مقایسه با جمعیت اصفهان به ترتیب ۱/۶۹، ۷/۳۱ و ۰/۹۷ برابر به دست آمد. همچنین، مقاومت به دی کلرووس بعد از چندین ماه کاهش معنی‌داری نداشت. براساس نتایج مطالعه حاضر، می‌توان پیشنهاد کرد که دی کلرووس از برنامه کنترلی این آفت حذف گردد.

**واژگان کلیدی:** استراز، گلوکاتینون‌اس ترنسفراس، زیست‌سنجی، یزد، پایداری مقاومت