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Research Article

Resistance levels and underlying mechanisms to imidacloprid and chlorpyrifos in greenhouse populations of *Trialeurodes vaporariorum* (Hem., Aleyrodidae) from Iran

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Abstract. To evaluate resistance levels and potential resistance mechanisms to chlorpyrifos and imidacloprid in *Trialeurodes vaporariorum*, six populations were collected from greenhouse-growing regions of Iran and assessed using leaf-dip bioassays followed by probit analysis of LC_{50} values and their 95% confidence limits. Compared with the susceptible FR population, the FL population exhibited the highest resistance to chlorpyrifos (15.04-fold) and imidacloprid (13.62-fold). Synergism assays with piperonyl butoxide (PBO), triphenyl phosphate (TPP), and diethyl maleate (DEM) showed that PBO and TPP produced strong synergistic effects against both insecticides in the FL population, whereas DEM showed only limited synergistic effects. Enzyme activity assays corroborated the synergism results, indicating that elevated esterase (EST) and mixed-function oxidase (MFO) activities, but not glutathione S-transferase (GST) activity, were associated with resistance in *T. vaporariorum*. Acetylcholinesterase (AChE) kinetic analysis showed that the Km and Vmax values in the FL population were approximately twofold lower and 2.5-fold higher, respectively, than those in the FR population, suggesting altered AChE kinetic properties. In addition, AChE inhibition assays revealed reduced sensitivity to most inhibitors in the FL population, whereas increased sensitivity to fosthiazate was observed. Overall, the findings suggest that reduced AChE sensitivity, together with elevated EST and MFO activities, may contribute to resistance to chlorpyrifos and imidacloprid in Iranian populations of *T. vaporariorum*. These findings provide a basis for resistance monitoring and support the development of effective insecticide resistance management strategies.

Keywords: *Trialeurodes vaporariorum*, Enzyme Inhibitors, Chlorpyrifos, Imidacloprid, Resistance

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Introduction

In recent years, the cultivation of greenhouse crops in Iran has expanded due to limited water resources and the need to optimize use of water, soil, and other resources. However, these crops are attacked by various pests, such as *Trialeurodes vaporariorum* Westwood, the greenhouse whitefly (Hemiptera: Aleyrodidae) (Byrne & Bellows Jr, 1991; Martin *et al.*, 2000). This species is a major pest of vegetable, horticultural, and ornamental crops worldwide. Both nymphs and adults feed on phloem sap, causing direct damage by removing plant nutrients, reducing plant vigor, inducing chlorosis, and suppressing plant growth. In addition, honeydew excretion promotes the development of sooty mold on plant surfaces, reducing photosynthetic efficiency and crop quality (Manzari & Fathipour, 2021). More importantly, *T. vaporariorum* acts as a vector of several economically important plant viruses, resulting in substantial economic losses in greenhouse production systems (Navas-Castillo *et al.*, 2011).

Because insect pests cause substantial economic losses in greenhouse crops, various control methods, including integrated pest management (IPM), have been proposed. One of these methods is biological control using natural enemies such as *Encarsia formosa* Gahan (Van Lenteren *et al.*, 1996) and *Chrysoperla carnea* (Stephens) (Senior & McEwen, 1998). Other control methods include physical controls with yellow sticky traps, cultivation of trap plants (Matu *et al.*, 2021; Moreau & Isman, 2011), and plant essential oils (Choi *et al.,* 2003). Historically, Iranian growers have relied primarily on repeated applications of insecticides such as imidacloprid, thiamethoxam, chlorpyrifos, pyriproxyfen, buprofezin, and pymetrozine to control *T. vaporariorum* (Norbakhsh, 2017). Among these insecticides, chlorpyrifos and imidacloprid are most widely used because of their low cost and availability.

Imidacloprid was the first neonicotinoid to be commercially introduced worldwide in 1991 (Bass *et al.*, 2015). Because of its systemic activity, imidacloprid has been widely adopted for the control of sucking insect pests. Imidacloprid disrupts the function of nicotinic acetylcholine receptors in sucking insects such as whiteflies (Casida & Durkin, 2013). Chlorpyrifos is one of the organophosphate insecticides widely used in Iran for its effectiveness, broad spectrum, and cost-effectiveness. At the molecular level, this compound binds to the active site of acetylcholinesterase (AChE) and inactivates the enzyme through serine phosphorylation, ultimately causing insect mortality (Fournier & Mutero, 1994). *T. vaporariorum* is a highly polyphagous species, feeding on plants belonging to at least 82 families (Mound & Halsey, 1978). Biological characteristics such as polyphagy, a short generation time, multiple generations per year, and high reproductive capacity predispose this species to the development of insecticide resistance (Kapantaidaki *et al.*, 2018; Pappas *et al.*, 2013). Insecticide resistance is a major challenge in pest management because it reduces insect susceptibility to insecticides and compromises control efficacy. Consequently, growers often increase insecticide application rates and spraying frequency, thereby promoting pest resurgence, outbreaks of secondary pests, environmental contamination, increased production costs, and further crop losses (Forgash, 1984; Pandian & Ramesh, 2020).

The expansion of greenhouse crop cultivation, the high cost of biological control, and the short generation time of *T. vaporariorum* have led growers to rely extensively on repeated insecticide applications. Consequently, chemical control remains a key component of *T. vaporariorum* management in Iran. As mentioned above, imidacloprid and chlorpyrifos are among the insecticides most commonly used to control this pest. Despite the widespread use of insecticides in Iranian greenhouse production systems, information on the resistance status and underlying resistance mechanisms of *T. vaporariorum* populations remains limited. Understanding resistance mechanisms and monitoring insecticide resistance are essential components of integrated pest management (IPM), providing the basis for resistance management strategies that help preserve the effectiveness of existing insecticides (Balaska *et al.*, 2020; Denholm & Rowland, 1992). Monitoring insecticide susceptibility in *T. vaporariorum* populations facilitates resistance management, helps delay the development and spread of resistance, and supports the selection of effective insecticides. Therefore, one of the objectives of the present study was to determine the resistance status of several Iranian populations of *T. vaporariorum* to imidacloprid and chlorpyrifos. In addition, elucidating the mechanisms underlying insecticide resistance is widely recognized as an important step toward sustainable resistance management (Van Leeuwen *et al.*, 2010). Two major mechanisms of insecticide resistance have been described in insect pests: target-site resistance and metabolic resistance (Van Leeuwen & Dermauw, 2016). In target-site resistance, reduced insecticide sensitivity results from mutations in target-site genes that decrease insecticide binding. In metabolic resistance, detoxification enzymes enhance the detoxification, sequestration, or metabolic degradation of insecticides, thereby reducing their effective concentration at target sites. The major enzyme families involved in metabolic resistance include esterases (ESTs), glutathione S-transferases (GSTs), and cytochrome P450 monooxygenases (P450s) (Van Leeuwen *et al.*, 2009). Therefore, the present study evaluated the activities of the major detoxification enzymes (P450s, GSTs, and ESTs) using synergist bioassays and biochemical assays to investigate their potential contribution to insecticide resistance.

Materials and methods

Whitefly strains

Five field populations of *T. vaporariorum* were collected from commercial greenhouses in Jiroft (JR), Filestan (FL), Shahriar (SA), Aveh (AE), and Zanjan (ZN), Iran, between 2019 and 2021 (Table 1).

Table 1. Information about the collection of *Trialeurodes vaporariorum* populations in Iranian greenhouses.

Collection site	Host	Strain's full name	Abbreviated name of the strains	Collection date
Fardis (Alborz province)	Sage	Fardis	FR	2019
Zanjan (Zanjan province)	Strawberry	Zanjan	ZN	2020
Filestan (Tehran province)	Chrysanthemums	Filestan	FL	2021
Shahriar (Tehran province)	Tomato	Shahriar	SA	2021
Aveh (Markazi Province)	Rosa	Aveh	AE	2020
Jiroft (Kerman Province)	Tomato	Jiroft	JR	2020

The susceptible FR population was collected from ornamental scarlet sage (*Salvia splendens*) in Fardis, Karaj, Iran, where insecticides had not been applied and, therefore, had not been exposed to insecticide selection pressure. Each field population was maintained separately on tomato plants (*Solanum lycopersicum* var. *cerasiforme*) in net cages (100 × 50 × 50 cm) at 25 ± 2°C, 60% relative humidity, and a 16:8 h (light:dark) photoperiod.

Insecticide and chemicals

Chlorpyrifos (40% EC) and dichlorvos (50% EC) were obtained from Arishimi Chemical Co., whereas imidacloprid (35% SC) was obtained from Maher Chemical Co. Fast Blue RR salt was purchased from Fluka (Buchs, Switzerland). Aldicarb (98% purity), carbofuran (99.5% purity), carbaryl, α-naphthyl acetate (α-NA), and β-naphthyl acetate (β-NA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphamidon (99.9% purity) was obtained from AccuStandard (New Haven, CT, USA). Fosthiazate, acetylthiocholine iodide (ATCI), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Wako (Osaka, Japan). Piperonyl butoxide (PBO), diethyl maleate (DEM), triphenyl phosphate (TPP), and all other chemicals were purchased from Merck (Darmstadt, Germany) and were of reagent grade.

Bioassays

The leaf-disc dip method was used to evaluate the toxicity of imidacloprid and chlorpyrifos against adult female *Trialeurodes vaporariorum*. Briefly, tomato leaf discs (5.5 cm in diameter) were immersed in serially diluted insecticide solutions or distilled water (control) for 15 s. The treated leaf discs were placed on a 1% agar layer in Petri dishes (6 cm diameter) and allowed to air-dry. Approximately 20 adult whiteflies were transferred from the rearing cages onto each treated leaf disc using a mouth aspirator. The Petri dishes were sealed with ventilated lids and maintained at 26 ± 2°C, 65 ± 5% relative humidity, and a 16:8 h (light:dark) photoperiod. Mortality was assessed after 24 h for chlorpyrifos and 48 h for imidacloprid. Individuals that showed no movement after gentle probing with a fine camel-hair brush were considered dead. Six concentrations of imidacloprid (25, 50, 100, 250, 500, and 1000 ppm) and six concentrations of chlorpyrifos (300, 600, 1250, 2500, 5000, and 10000 ppm), each with three replicates, were tested to obtain mortality ranging from 25% to 75%.

Synergism test

To investigate resistance mechanisms, the FR and FL populations were exposed to three synergists: piperonyl butoxide (PBO, MFO inhibitor), diethyl maleate (DEM, GST inhibitor), and triphenyl phosphate (TPP, esterase inhibitor). The synergists were dissolved in acetone and Triton X-100 and applied using the leaf-disc dip bioassay described above. Based on preliminary bioassays, the highest concentration causing less than 10% mortality was selected as the working concentration for each synergist. To evaluate the contribution of detoxification enzymes to insecticide resistance, PBO, TPP, and DEM were used at concentrations of 100, 50, and 150 ppm, respectively, for the FR population, and 250, 150, and 300 ppm, respectively, for the FL population. Each synergist concentration was mixed with serial concentrations of each insecticide before the bioassays. The synergistic ratio (SR) was calculated using the following equation:

$SR = LC_{50}$ (insecticide alone) / LC_{50} (insecticide + synergist)

Enzymatic assays

The P450 enzyme activity

Cytochrome P450 content (heme-associated oxidase level) was estimated using the TMBZ-based heme-peroxidase assay (William & Janet, 1997). In brief, 40 whiteflies of each strain were homogenized in 100 µl sodium phosphate buffer (0.1 M, pH 7) at 4 °C. The homogenates were centrifuged at 10,000 × g at 4 °C for 15 min, and the supernatants were used to measure the general oxidase level. Twenty microliters of the prepared enzyme, 80 µl sodium phosphate buffer (0.1 M, pH 7), 200 µl TMBZ (3,3,5,5-Tetramethyl Benzidine) solution, and 25 µl H2O2 (3%) were added into the wells of a 96-well microplate. The microplate was incubated for 2 hours at 25 °C before absorbance was read at 450 nm using a microplate reader (with slight modifications; Tiwari *et al.*, 2011). The value of P450 was estimated using the standard cytochrome C curve.

Total GST activity

The Activity of GST was measured by 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates (Habig *et al.*, 1974). Forty adult whiteflies were manually homogenized in 160 µl Tris/HCl buffer (0.05 M, pH 7.5) and centrifuged at 10,000 × g at 4 °C for 10 min. The total reaction volume contained 15 µl of the supernatant, 100 µl of CDNB (1.2 mM), and 100 µl of GSH (10 mM, dissolved in distilled water). The control sample included a non-enzymatic reaction of CDNB with GSH without homogenate. The change in absorbance was recorded continuously by the microplate reader at 20 sec intervals for 10 min at 340 nm and 27 °C (Tiwari *et al.*, 2011). At least three replicates were used for each strain. GST activity was represented based on the formation of the GSH conjugate with CDNB, using the extinction coefficient of $9600\ M^{-1}\ cm^{-1}$

Total esterase activity

Total esterase activity was measured (Van Asperen, 1962) with slight modification using α-NA and β-NA as substrates (Mahdavi Moghadam *et al.*, 2012). Forty adults from each strain were homogenized in 100 µL ice–cold sodium phosphate buffer (10 mM, pH 7, and containing 0.1% of Triton X-100). The homogenates were then centrifuged at 10,000 × g at 4°C for 15 min. The supernatants were used as the enzyme source. To each of the wells, 112.5 µL of the 0.1 M phosphate buffer (pH 7.0), 12.5 µL enzyme sample, 25 µL substrates (6.4 mM), and 50 µL of the fastblue RR (0.2% in distilled water) were added, respectively. The amount of naphthol produced was continuously measured using a microplate reader (State Fax 3200, Awareness Technology, USA) at 450 and 540 nm for α-NA and β-NA, respectively, over 30 min. Treatment without an enzyme source was used as a control for the non-enzymatic reaction. Using different doses of naphthol, a standard curve was constructed. The absorbance value of the samples was converted to the amount of product formed using the standard curve.

AChE kinetics

The kinetic parameters Km and Vmax of total AChE from six strains of *T. vaporariorum* were determined according to Moores *et al.*, 1996, with slight modifications. AChE activity was continuously monitored using a microplate reader at 405 nm for 30 minutes across eight ATC concentrations (0.125-25 mM). The Km and Vmax values were estimated from Lineweaver-Burk plots.

AChE inhibition

AChE inhibition was measured in *T. vaporariorum* strains according to Byrne and Devonshire (1997), with slight modifications. At least six concentrations with three replicates were tested for each inhibitor: phosphamidon (5×10^{-4}–7×10^{-2} mM), carbaryl (1×10^{-4}–2.5×10^{-2} mM), dichlorvos (1×10^{-6}–1×10^{-2} mM), aldicarb (1×10^{-4}–1×10^{-2} mM), carbofuran (5×10^{-6}–5×10^{-3} mM), and fosthiazate (1×10^{-3}–5×10^{-3} mM). Briefly, 40 µl of inhibitor and 40 µl of enzyme extract were added to each well and incubated for 15 min at 25 °C. Absorbance was recorded after the addition of 20 µl DTNB, 100 µl phosphate buffer, and 40 µl ATC using a microplate reader at 5-min intervals for 30 min at 405 nm and 25 °C. Protein content was determined according to Bradford (1976) using bovine serum albumin as the standard (Bradford, 1976).

Data analysis

Lethal concentration (LC_{50}) values and their 95% confidence limits (CLs), IC_{50} values, resistance ratios (RR), and synergistic ratios (SR) were estimated using PoloPlus software (Robertson *et al.*, 2017). Resistance ratios were calculated as:

RR = LC_{50} (field population) / LC_{50} (susceptible population)

The 95% confidence limits for LC_{50} and IC_{50} values were estimated using probit analysis in PoloPlus. Log-dose probit-mortality regression lines were generated for all whitefly populations. Enzyme activity data were analyzed using one-way analysis of variance (one-way ANOVA), followed by Tukey's HSD test for multiple comparisons, in Minitab (Version 16, 2015).

Results

Toxicity effects of chlorpyrifos and imidacloprid on *T. vaporariorum* strains

Bioassay results for the six populations are presented in Table 2. The highest and lowest LC_{50} values for chlorpyrifos were observed in the FL (7770.400 mg L^{-1}) and FR (516.447 mg L^{-1}) populations, respectively. Similarly, for imidacloprid, the highest and lowest LC_{50} values were recorded in the FL (491.578 mg L^{-1}) and FR (36.088 mg L^{-1}) populations, respectively. The highest and lowest resistance ratios (RRs) for chlorpyrifos were 15.04-fold and 1.63-fold in the FL and JR populations, respectively. The corresponding RRs for imidacloprid were 13.62-fold and 3.14-fold in the FL and JR populations, respectively (Table 2).

Efficacy of PBO, TPP, and DEM

In this study, the FR and FL populations were selected for synergistic testing. The effects of three synergists on the toxicity of chlorpyrifos and imidacloprid are presented in Table 3. In the FL population, the highest resistance ratio (RR) was observed for chlorpyrifos + DEM (20-fold), followed by chlorpyrifos (15.04-fold), imidacloprid (13.62-fold), and imidacloprid + DEM (11.16-fold) (Table 3). PBO and TPP produced the strongest synergistic effects against both imidacloprid (SR = 5.60 and 5.21, respectively) and chlorpyrifos (SR = 6.72 and 6.16, respectively) in the FL population (Table 3). These combinations increased insecticide toxicity and reduced the apparent resistance level, as reflected by lower LC_{50} values. In contrast, DEM showed only limited synergistic effects against imidacloprid and chlorpyrifos (SR = 1.8 and 1.9, respectively). In the FR population, none of the synergists produced an appreciable effect on the toxicity of either insecticide.

a: Confidence interval at 95%

b: LC_{50} value in FL strain with synergists/LC_{50} value in FR strain with synergists according to Robertson and Preisler (1992).

c: LC_{50} Value without Synergist/LC_{50} Value with Synergist According to Robertson and Preisler (1992).

* Statistically Significant at 95% Level (P < 0.05).

Table 2. Toxicity of Imidacloprid and Chlorpyrifos to different populations of *Trialeurodes vaporariorum.*

Insecticide	Population	LC_{50} (mg/l) (95%CL)a	Slope ± SE	X2(df)	RRb (mg/l) (95%CL)
Chlorpyrifos	FR	516.44 (432.66 - 601.55)	2.03±0.22	12.82 (18)	1
	ZN	7700.77 (7145.61 - 8348.95)	4.08±0.45	7.59 (18)	14.91 (12.44 – 17.86)*
	FL	7770.40 (7254.93 - 8330.88)	4.77±0.42	13.45 (18)	15.04 (12.59 – 17.97)*
	SA	2831.03 (2259.84 - 3616.9)	1.32±0.18	16.26 (18)	5.48 (4.13 – 7.27)*
	AE	870.41 (749.74 - 1025.05)	2.32±0.23	19.29 (18)	1.68 (1.35 – 2.09)*
	JR	845.19 (746.08 - 953.39)	2.62±0.25	13.87 (18)	1.63 (1.33 – 2)*
Imidacloprid	FR	36.08 (31.93 - 41.02)	2.62±0.24	11.66 (18)	1
	ZN	230.38 (198.54 - 269.07)	2.18±0.18	23.51 (22)	6.38 (5.28 – 7.69)*
	FL	491.57 (395.75 - 615.08)	1.42±0.15	9.92 (18)	13.62 (10.57 – 17.54)*
	SA	166.31 (138.39 - 202.29)	2.1±0.22	24.12 (18)	4.6 (3.78 – 5.6)*
	AE	113.47 (101.31 - 126.38)	3.03±0.27	16.32 (18)	3.14 (2.65 – 3.71)*
	JR	132.21 (115.29 - 150.77)	2.41±0.23	14.26 (18)	3.66 (3.04 – 4.4)*

Table 3. Synergistic activity of TPP, DEM and PBO on Imidacloprid and Chlorpyrifos efficacy in *Trialeurodes vaporariorum* strains.

Population	Insecticide+Synergist	LC_{50} (mg/l) (95%CL)a	Slope ± SE	X2(df)	RRb (mg/l) (95%CL)	SRc(mg/l) (95%CL)
FR	Chlorpyrifos	516.44 (432.66-601.55)	2.03±0.22	12.82 (18)		
	Chlorpyrifos+TPP	191.56 (158.51-231.25)	1.68±0.16	9.4 (18)		2.69 (2.1-3.46)*
	Chlorpyrifos+DEM	356.72 (265.81-438.23)	2.56±0.27	32.9 (18)		1.44 (1.14-1.82)*
	Chlorpyrifos+PBO	169.54 (145.31-199)	2±0.2	9.36 (18)		3 (2.42-3.82)*
	Imidacloprid	36.08 (31.93-41.02)	2.62±0.24	11.66 (18)		
	Imidacloprid+TPP	27.55 (24.57-30.82)	2.95±0.26	13 (18)		1.3 (1.1-1.55)*
	Imidacloprid+DEM	24.43 (21.99-27.03)	3.42±0.29	6.21 (18)		1.47 (1.25-1.73)*
	Imidacloprid+PBO	15.62 (13.72-17.59)	2.88±0.26	14.4 (18)		2.3 (1.93-2.75)*
FL	Chlorpyrifos	7770.40 (7254.93-8330.88)	4.77±0.42	13.45 (18)	15.04 (12.59-17.97) *	
	Chlorpyrifos+TPP	1261.38 (1078.55-1470.53)	2.15±0.18	16.73 (18)	6.58 (5.15-8.14) *	6.16 (5.19-7.3)*
	Chlorpyrifos+DEM	7098.85 (6591.46-7626.84)	4.48±0.41	14.88 (18)	20 (16.61-23.84) *	1.09 (0.99-1.21)
	Chlorpyrifos+PBO	1155.02 (659.26-2186.4)	1.13±0.12	61.51 (18)	6.81 (4.95-9.36) *	6.72 (5.06-8.94)*
	Imidacloprid	491.57 (395.75-615.08)	1.42±0.15	9.92 (18)	13.62 (10.57-17.54) *	
	Imidacloprid+TPP	94.26 (82.15-109.35)	2.19±0.26	7.27 (18)	3.42 (2.85-4.1) *	5.21 (4-6.77)*
	Imidacloprid+DEM	272.88 (241.88-307.68)	2.7±0.25	15.52 (18)	11.16 (9.52-13.09) *	1.8 (1.4-2.31)*
	Imidacloprid+PBO	87.71 (78.26-98.61)	2.74±0.27	12.27 (18)	5.61 (4.73-6.65) *	5.6 (4.37-7.18)*

a: Confidence Interval at 95%, b: Resistance Ratio (RR)= LC_{50} of any Population/LC_{50} of FR, * Statistically Significant at 95% Level ($P < 0.05$)

Enzyme activity of P450, EST, and GST

The activities of P450-dependent monooxygenases, GSTs, and esterases (ESTs) in *T. vaporariorum* populations were assessed using biochemical assays with artificial substrates. The results are presented in Figs. 1, 2, and 3.

Monooxygenase content

Significant differences in cytochrome P450 content were detected among populations (Fig. 1). Among the tested populations, the highest and lowest cytochrome P450 contents were observed in the FL population (0.0011 units of cytochrome P450 per whitefly) and the susceptible FR population (0.00026 units of cytochrome P450 per whitefly), respectively. The cytochrome P450 content of the FL population was 5.83-fold higher than that of the susceptible FR population.

EST activity

Total, esterase (EST) activity in the six populations was determined spectrophotometrically using α-NA and β-NA as substrates (Figure 2). The FL population showed significantly higher EST activity than the susceptible FR population, with 2.26-fold and 2.0-fold increases toward α-NA and β-NA, respectively. No significant difference in α-esterase activity was observed between the FL and ZN populations. Similarly, no significant difference in β-esterase activity was detected among populations except between the FL and FR populations.

GST activity

Significant differences in GST activity were observed among populations (Figure 3). The AE population exhibited the highest GST activity (0.323 µmol glutathione conjugated min^{-1} mg^{-1} protein), whereas the FL population showed the lowest GST activity (0.157 µmol glutathione conjugated min^{-1} mg^{-1} protein).

Response of *T. vaporariorum* strains AChE to inhibitors

AChE from the FL population exhibited reduced sensitivity to the tested inhibitors, requiring higher inhibitor concentrations to achieve comparable inhibition than the FR population, except for fosthiazate (Table 4). Carbofuran and carbaryl exhibited the lowest IC_{50} values, whereas phosphamidon and fosthiazate exhibited the highest IC_{50} values (Table 4). The highest AChE insensitivity ratio (IR) was observed in the FL population against

carbofuran (IR = 32). Nevertheless, no significant differences in sensitivity to fosthiazate were observed among the FR, AE, and JR populations (P > 0.05).

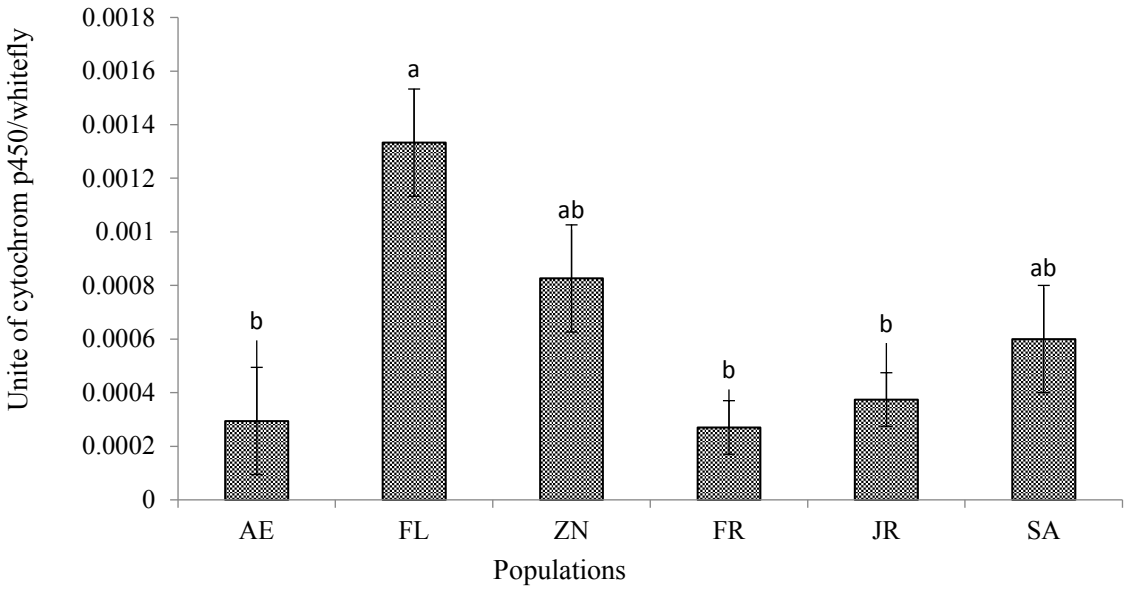

Fig. 1. Comparisons of the total P450 content between the *Trialeurodes vaporariorum* strains. The Tukey's test of the means compared P450 content among the six strains. Different letters over the bars indicate a significant difference at P<0.05. Vertical bars are the Mean (±SE) of three replicates.

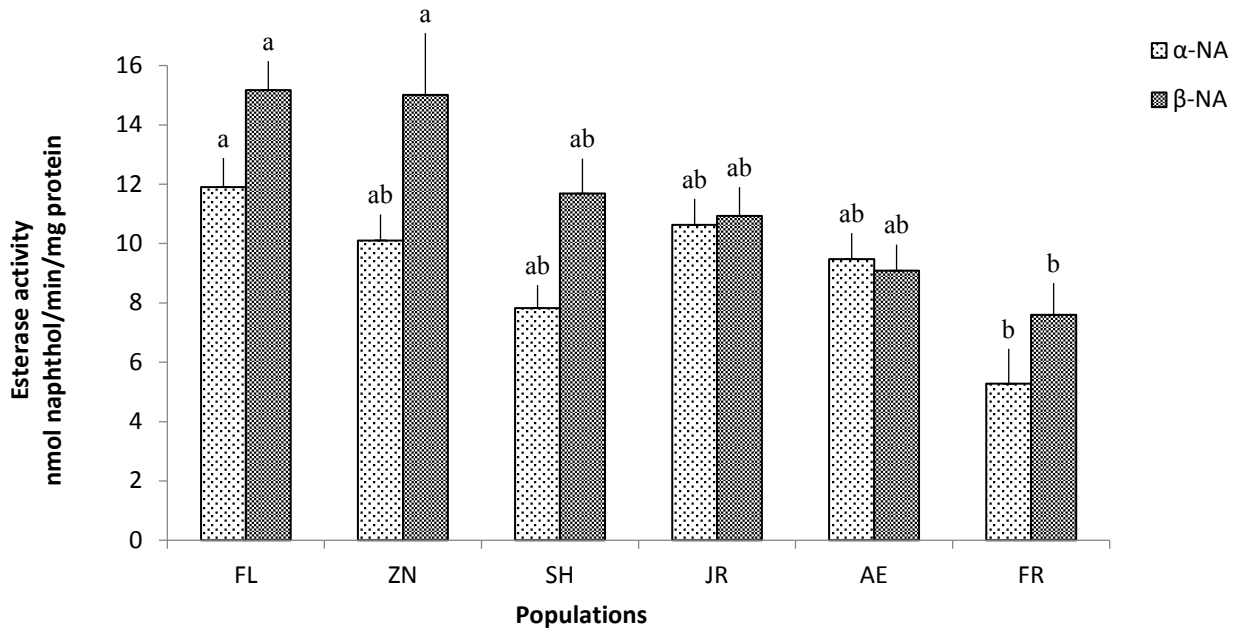

Fig. 2. General esterase activity in six strains of *Trialeurodes vaporariorum* using α-NA and β-NA substrates. Results are shown as the mean ± SE (n=3) of esterase activities. The letters on top of columns show a significant difference in esterase activities (nmol naphthol/min/mg Protein) between strains at P<0.05 (Tukey's Test).

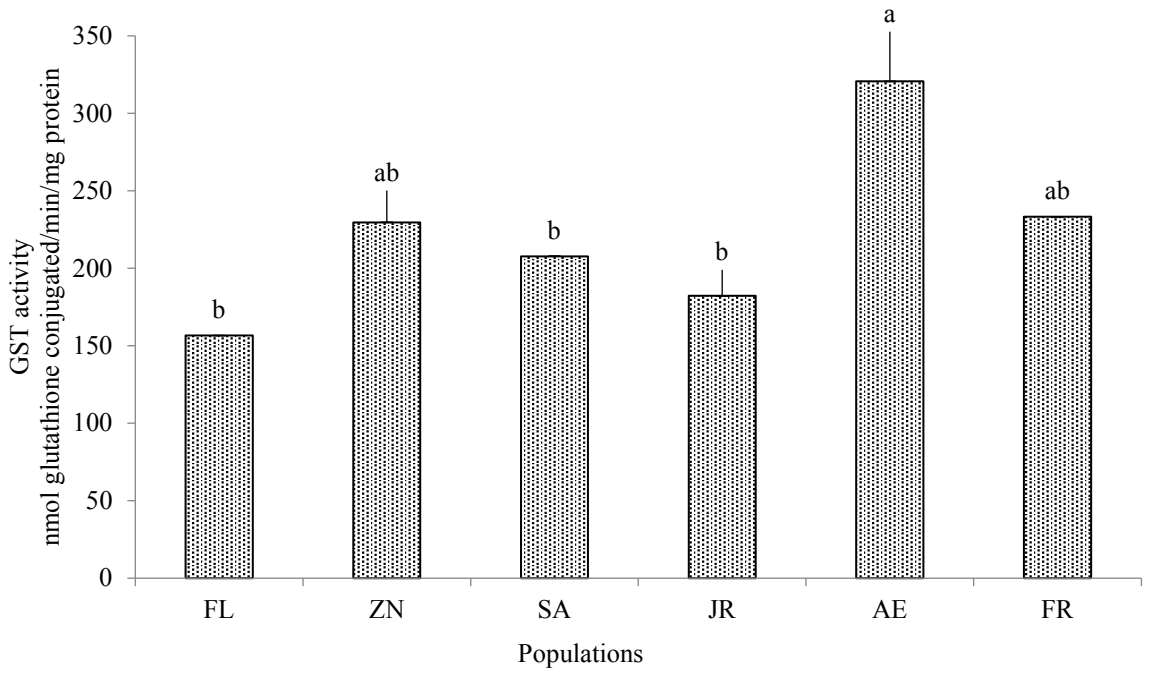

Fig. 3. GST activity in six strains of *Trialeurodes vaporariorum*. Results are presented as the mean ± SE (n=3) of GST activities (nmol glutathione conjugated/min/mg protein). Different letters over the bars shows significant differences between GST activities among populations at P<0.05 (Tukey's Test). Vertical bars are the mean (±SE) of three replicates.

Kinetics of AChE

To estimate the kinetic parameters, the effects of different ATCI concentrations were tested (Table 5). The enzyme's response to changes in substrate concentration indicates that the KM value of the resistant population is the lowest and differs by approximately a factor of 2 from that of the susceptible population. On the other hand, AChE from SA, a population with moderate resistance, had the lowest affinity for the substrate among the populations. The kinetic parameters of AChE were determined using different concentrations of ATCI (Table 5). Among the tested populations, the FL population had the lowest KM value (0.317 ± 0.08 mM), whereas the SA population had the highest (0.881 ± 0.03 mM). The highest Vmax was observed in the FL population (6.68 ± 0.07 mM/min/mg protein), while the lowest Vmax was recorded in the SA population (2.26 ± 0.03 mM/min/mg protein). Catalytic efficiency (Vmax/Km) also varied among populations, with the highest value in the FL population (21.07 ± 0.68) and the lowest in the SA population (2.50 ± 0.10).

Discussion

Published reports on *T. vaporariorum* resistance to chlorpyrifos and imidacloprid remain limited, and one of the earliest documented studies in this area was reported by Gorman *et al.* (2007). To our knowledge, no published study has investigated resistance to these insecticides in Iranian populations of *T. vaporariorum*. Therefore, the present study evaluated the resistance status of greenhouse whitefly populations to chlorpyrifos and imidacloprid in Iranian greenhouses. The results revealed varying levels of resistance among the tested populations. The toxicity of imidacloprid against greenhouse whitefly was significantly higher than that of chlorpyrifos (Table 2). The tested populations exhibited varying levels of susceptibility to imidacloprid and chlorpyrifos. The highest and lowest resistance levels to both insecticides were observed in the FL and FR populations, respectively. These findings demonstrate variation in resistance levels among *T. vaporariorum* populations and indicate the presence of insecticide resistance in greenhouse populations from Iran. The observed differences in resistance levels among

populations may be associated with differences in insecticide application histories in Iranian greenhouse production systems. Pappas *et al.* (2013) demonstrated differences in the LC_{50} values of thiacloprid and imidacloprid against various populations of *T. vaporariorum* in Greece. Research in Turkey found high to moderate resistance to neonicotinoids across various populations of *Bemisia tabaci* Gennadius (İlken & Şahin, 2017).

Table 4. IC_{50} values of organophosphate and carbamate compounds on *Trialeurodes vaporariorum* AChE activity.

Insecticide	Population	IC_{50}	Slope ± SE	95% confidence intervals (CI)	RFa
Aldicarb	FR	9×10^{-4}	0.850 ± 0.088	5×10^{-4} - 16×10^{-4}	
	ZN	20×10^{-4}	1.06 ± 0.096	15×10^{-4} - 26×10^{-4}	2.19 (1.43 – 3.36) *
	FL	15×10^{-4}	0.986 ± 0.091	7×10^{-4} - 37×10^{-4}	1.69 (1.09 - 2.61) *
	SA	13×10^{-4}	1.01 ± 0.093	10×10^{-4} - 18×10^{-4}	1.5 (0.98 – 2.31)
	AE	9×10^{-4}	1.039 ± 0.093	7×10^{-4} - 12×10^{-4}	1 (0.66 – 1.57)
	JR	9×10^{-4}	0.793 ± 0.087	6×10^{-4} - 13×10^{-4}	1 (0.63 – 1.65)
Carbaryl	FR	15×10^{-4}	0.947 ± 0.091	11×10^{-4} - 20×10^{-4}	
	ZN	12×10^{-3}	0.464 ± 0.086	5×10^{-3} - 5×10^{-2}	8.17 (2.92 – 22.9) *
	FL	16×10^{-3}	0.442 ± 0.086	6×10^{-3} - 8×10^{-2}	10.54 (3.32 – 33.44) *
	SA	10×10^{-3}	0.513 ± 0.087	3×10^{-3} - 14×10^{-3}	6.91 (2.8 – 17) *
	AE	3×10^{-3}	0.904 ± 0.095	1×10^{-3} - 8×10^{-3}	2.32 (1.46 – 3.67) *
	JR	2×10^{-3}	0.555 ± 0.084	1×10^{-3} - 4×10^{-3}	1.64 (0.9 – 2.99)
Carbofuran	FR	2×10^{-5}	1.285 ± 0.096	1×10^{-5} - 4×10^{-5}	
	ZN	3×10^{-5}	1.036 ± 0.076	1×10^{-5} - 5×10^{-5}	1.1 (0.77 - 1.56)
	FL	9×10^{-4}	1.190 ± 0.087	3×10^{-4} - 4×10^{-3}	32 (22.9 – 45.86) *
	SA	4×10^{-4}	1.297 ± 0.086	3×10^{-4} - 8×10^{-4}	17.4 (12.67 – 24.02) *
	AE	8×10^{-5}	0.781 ± 0.061	4×10^{-5} - 1×10^{-3}	2.98 (2.03 – 4.38) *
	JR	1×10^{-4}	0.969 ± 0.078	1×10^{-4} - 3×10^{-4}	0.676 (0.45 – 0.99) *
Dichlorvos	FR	2×10^{-4}	0.434 ± 0.054	1×10^{-4} - 3×10^{-4}	
	ZN	2×10^{-3}	0.482 ± 0.058	1×10^{-3}- 9×10^{-3}	11.57 (4.96 – 26.98) *
	FL	8×10^{-4}	0.377 ± 0.054	4×10^{-4} - 1×10^{-3}	3.98 (1.64 – 9.67) *
	SA	4×10^{-3}	0.333 ± 0.055	1×10^{-3} - 1×10^{-2}	19.04 (6.17 – 58.72) *
	AE	4×10^{-4}	0.404 ± 0.054	2×10^{-4} - 8×10^{-4}	2.08 (0.88 – 4.89)
	JR	5×10^{-4}	0.388 ± 0.054	3×10^{-4} - 1×10^{-3}	2.68 (1.12 – 6.4) *
Phosphamidon	FR	41×10^{-3}	1.065 ± 0.093	19×10^{-3}- 16×10^{-2}	
	ZN	28×10^{-3}	1.464 ± 0.108	12×10^{-3}- 10×10^{-2}	0.68 (0.46 – 1)
	FL	45×10^{-3}	1.055 ± 0.095	19×10^{-3}- 28×10^{-2}	1 (0.68 – 1.74)
	SA	21×10^{-3}	1.262 ± 0.096	13×10^{-3}- 4×10^{-2}	0.52 (0.35 – 0.78) *
	AE	16×10^{-3}	1.034 ± 0.084	6×10^{-3}- 8×10^{-2}	0.4 (0.26 – 0.61) *
	JR	11×10^{-3}	0.866 ± 0.079	6×10^{-3}- 22×10^{-3}	0.27 (0.17 – 0.42) *
Fosthiazate	FR	27×10^{-3}	4.27 ± 0.201	25×10^{-3}- 28×10^{-3}	
	ZN	22×10^{-3}	2.73 ± 0.155	18×10^{-3}- 26×10^{-3}	0.81 (0.7 – 0.94) *
	FL	$23\times10^{-}$	3.07 ± 0.162	20×10^{-3}- 26×10^{-3}	0.86 (0.75 – 0.99) *
	SA	$^{3}22\times10^{-}$	2.41 ± 0.153	27×10^{-3}- 36×10^{-3}	0.81 (0.7 – 0.94) *
	AE	$^{3}24\times10^{-}$	3.38 ± 0.168	23×10^{-3}- 25×10^{-3}	0.9 (0.79 – 1)
	JR	$^{3}24\times10^{-3}$	3.28 ± 0.165	22×10^{-3}- 25×10^{-3}	0.89 (0.78 – 1)

a Resistance Factor (RF) = IC_{50} of field populations/ IC_{50} of Fardis-susceptible population., * Significantly different from the susceptible population at 95% Level ($P < 0.05$)

Table 5. Kinetic parameters and catalytic efficiency of AChE in six populations of *Trialeurodes vaporariorum.*

Population	KM (mM) + SE	Vmax (mM/min/mg protein) + SE	Vmax/KM + SE
FR	0.654 + 0.04	2.64 + 0.07	4.03 + 0.3
ZN	0.429 + 0.01	5.29 + 0.03	12.33 + 0.5
FL	0.317 + 0.08	6.68 + 0.07	21.07 + 0.68
SA	0.881 + 0.03	2.26 + 0.03	2.5 + 0.1
AE	0.483 + 0.005	3.25 + 0.08	6.72 + 0.2
JR	0.583 + 0.07	3.88 + 0.004	6.65 + 0.57

Insecticide resistance in *B. tabaci* populations collected from commercial cruciferous vegetable fields in Shangjie, Minhou County, Fujian, China, was investigated. Compared with insecticide-susceptible *B. tabaci* populations, the RRs in the field population were 21.8 for chlorpyrifos and 8.0 for imidacloprid (Kang *et al.*, 2006). Earlier investigations into the dynamics of resistance have shown that insecticide resistance is unstable and decreases under reduced selection pressure (Kang *et al.*, 2006; Salehi-Sedeh *et al.,* 2020; Wen *et al.*, 2009). Given the highest slope of the dose-response curve in the FL population, this strain exhibits relatively high homogeneity. The resistance to chlorpyrifos and imidacloprid observed in this study may be associated with enhanced detoxification enzyme activity together with reduced sensitivity of the target enzyme in the nervous system. Synergist bioassays showed that PBO and TPP, but not DEM, produced the strongest synergistic effects against both chlorpyrifos and imidacloprid in the FL population (Table 3). PBO increased the toxicity of chlorpyrifos and imidacloprid by 6.72- and 5.6-fold, respectively, reducing the corresponding resistance ratios from 15.04- to 6.81-fold and from 13.62- to 5.21-fold. However, none of the synergists completely suppressed resistance to either insecticide. These findings suggest that, in addition to cytochrome P450- and esterase-mediated detoxification, other mechanisms may also contribute to resistance in the FL population.

The enzyme assay results were consistent with the synergism studies and suggest that cytochrome P450 and esterases are likely involved in resistance to chlorpyrifos and imidacloprid in *T. vaporariorum* (Figs. 1, 2, 3). Therefore, in both synergistic studies and biochemical assays, the involvement of oxidases and esterases in *T. vaporariorum* resistance to chlorpyrifos and imidacloprid was confirmed. Some researchers showed that hydrolase, monooxygenase, and GST were involved in imidacloprid resistance in *B. tabaci* (Salehi-Sedeh *et al.*, 2020). PBO (a monooxygenase inhibitor) and TPP (an esterase inhibitor) reduced LC_{50} RR of chlorpyrifos (5.3- and 8.5-fold) and imidacloprid (5.1- and 8-fold) in *B. tabaci*, respectively (Kang *et al.,* 2006). Esterase, P450, and GST were involved in chlorpyrifos resistance in *T. urticae* populations. However, resistance to chlorpyrifos was not entirely suppressed by PBO, DEM, and TPP, suggesting that additional mechanisms may be involved (Zamani *et al.*, 2014). The results of the synergism and biochemical assays suggest that cytochrome P450-mediated detoxification and esterase activity may contribute to resistance to chlorpyrifos and imidacloprid in the FL population. Similar findings have also been reported in other arthropod pests, including *Bemisia tabaci* and *Tetranychus urticae*, where detoxification enzymes have been associated with resistance to these insecticides. However, resistance to chlorpyrifos and imidacloprid was not completely suppressed by the synergists, suggesting that additional resistance mechanisms may also be involved. Further molecular studies are required to determine whether altered gene expression or changes in the target enzyme contribute to the observed resistance. These findings may help inform future resistance management strategies for *T. vaporariorum*, including insecticide rotation and the judicious use of synergists.

One approach to evaluating enzyme insensitivity to insecticides is the analysis of kinetic parameters. Since Km reflects substrate affinity, the lower Km observed in the FL population suggests that AChE has a higher affinity for its substrate than that in the other populations (Devonshire & Moores, 1984). This observation was consistent with the higher resistance level detected in the FL population; however, further mechanistic studies are required to determine the biological significance of this association. A significant difference in Km was observed between the FL population and the FR (2.06-fold) and SA (2.77-fold) populations, suggesting differences in the kinetic properties of AChE among populations. The higher Vmax observed in the FL population, together with its lower Km value, suggests altered kinetic properties of AChE compared with the other populations. Although these kinetic changes do not establish a causal mechanism of resistance, they are consistent with the variation in resistance levels observed among *T. vaporariorum* populations. Similar associations between altered AChE kinetic properties and insecticide resistance have been reported in other arthropod species (Fournier & Mutero, 1994).

The differences in AChE activity, KM, and Vmax among populations indicate distinct changes at the target site within the AChE active site (Berrada *et al.*, 1994). Mutated AChE has been reported in some insects and acarine species (Devonshire & Moores, 1984; Fournier & Mutero, 1994; Hall & Malcolm, 1991; Smissaert, 1964). Differences in AChE activity, Km, and Vmax among populations suggest possible alterations in the active site or catalytic properties of AChE (Berrada *et al.*, 1994). Previous studies have shown that mutations in AChE can contribute to insecticide insensitivity in insects and mites (Devonshire & Moores, 1984; Fournier & Mutero, 1994; Hall & Malcolm, 1991; Smissaert, 1964). Since most mutations associated with AChE insensitivity occur

at the catalytic triad of the enzyme, the possible difference in the catalytic Activity of AChE in *T. vaporariorum* populations may be due to one or more of the mutations identified by Mutero *et al.* (1994). They identified five point mutations related to insecticide resistance: Phe-115(78) to Ser, Ileu-199(Val- 129) to Val, Ileu-199(Val-129) to Thr, Gly-303(227) to Ala, Phe-368(288) to Tyr. Each mutation can confer a specific resistance, making an insect resistant to one insecticide and susceptible to another (Fournier, 2005). Also, insensitive AChE, higher specific Activity, and other available factors can cause changes in kinetic parameters (Alizadeh *et al.*, 2014). Another hypothesis is that the populations probably have allelic diversity of insensitive AChE (Moores *et al.*, 1996). However, it is not possible to say which mutations should be assessed with additional molecular assays. OP and carbamate insecticides target AChE, which plays a vital role in the insect nervous system. Therefore, researchers have focused on this enzyme (Fournier & Mutero, 1994).

The IC_{50} values showed that AChE from the FR population was more sensitive to all tested inhibitors than AChE from the FL population, except for fosthiazate. In contrast, the FL population showed increased sensitivity to fosthiazate, suggesting possible negative cross-resistance. This pattern may be associated with alterations in AChE properties that reduce sensitivity to some inhibitors while increasing susceptibility to others, possibly because different inhibitors interact differently with the altered active site of AChE. Similar observations have been reported in *Myzus persicae*, *Aphis gossypii*, *Leptinotarsa decemlineata*, and *Agonoscena pistaciae* (Ahangaran & Alizadeh, 2008; Ghadamyari *et al.*, 2008; Villatte *et al.*, 1999; Zhu *et al.*, 1996). Among the tested inhibitors, carbofuran and carbaryl produced the highest AChE insensitivity ratios (IRs) in the FL population (32.00 and 10.54, respectively). These findings may reflect differences in insecticide selection pressure among greenhouse populations (Dittrich *et al.*, 1985). For dichlorvos, the SA (19.04) and ZN (11.57) populations exhibited the highest IR values, whereas both populations showed the lowest IR values (0.81) for fosthiazate. In addition, no significant difference in AChE sensitivity to phosphamidon was detected between the susceptible FR population and the FL population ($P > 0.05$). These contrasting inhibition profiles suggest that the tested populations differ in their AChE sensitivity to individual inhibitors, possibly reflecting differences in insecticide exposure history or alterations in AChE properties. Furthermore, the IC_{50} values suggest that the SA and ZN populations exhibited lower AChE sensitivity to dichlorvos and aldicarb, respectively, than the FL population, despite exhibiting lower overall resistance levels to chlorpyrifos and imidacloprid. Our results suggest that resistance in the FL population is associated with reduced AChE sensitivity together with elevated esterase activity and cytochrome P450-mediated detoxification. These findings are consistent with previous studies in whiteflies and other insect pests, in which altered AChE sensitivity and increased detoxification enzyme activity were associated with resistance to organophosphates, carbamates, and neonicotinoids (Basij *et al.*, 2017; Dittrich *et al.*, 1990; Kang *et al.*, 2006; Pan *et al.*, 2019; Prabhaker *et al.*, 1988).

Author's Contributions

Seyed Ebrahim Shafiei: Conceived the study, conducted the research, collected and identified the insect populations, performed the experiments, analyzed the data, and contributed to manuscript preparation; **Mohammad Ghadamyari**: Supervised the research, contributed to the study design, interpreted the results, and critically revised the manuscript; **Hadi Mosallanejad**: Provided scientific consultation, contributed to the interpretation of the results, and critically revised the manuscript; **Elaheh Shafiei Alavijeh**: Contributed to manuscript writing, English editing, scientific revision, data interpretation, and preparation of the final version of the manuscript.

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Data Availability Statement

The data availability statement is not applicable

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Ethics Approval and Consent to Participate

This study involved only insect specimens (*T. vaporariorum*). All applicable institutional and national guidelines for the ethical use of experimental organisms were followed. This article does not involve human participants or vertebrate animals performed by the authors.

Conflict of Interest

The authors declare no conflicts of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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Research Article

سطوح مقاومت به ایمیداکلوپرید و کلرپیریفوس و مکانیسم‌های مرتبط در جمعیت‌های گلخانه‌ای *Trialeurodes vaporariorum* (Hem., Aleyrodidae) از ایران

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چکیده: برای ارزیابی سطوح مقاومت و مکانیسم‌های مقاومت بالقوه به کلرپیریفوس و ایمیداکلوپرید در *Trialeurodes vaporariorum*، شش جمعیت از مناطق کشت گلخانه‌ای ایران جمع‌آوری و با استفاده از زیست‌سنجی غوطه‌وری برگ و به دنبال آن تجزیه و تحلیل پروبیت مقادیر LC_{50} و حدود اطمینان ۹۵٪ آنها ارزیابی شدند. در مقایسه با جمعیت حساس FR، جمعیت FL بالاترین مقاومت را به کلرپیریفوس (۱۵/۰۴ برابر) و ایمیداکلوپرید (۱۳/۶۲ برابر) نشان داد. سنجش‌های هم‌افزایی با پیپرونیل بوتوکسید (PBO)، تری‌فنیل فسفات (TPP) و دی‌اتیل مالئات (DEM) نشان داد که PBO و TPP اثرات هم‌افزایی قوی علیه هر دو حشره‌کش در جمعیت FL ایجاد می‌کنند، در حالی که DEM فقط اثرات هم‌افزایی محدودی نشان داد. سنجش فعالیت آنزیم، نتایج هم‌افزایی را تأیید کرد و نشان داد که افزایش فعالیت‌های استراز (EST) و اکسیداز با عملکرد مختلط (MFO)، اما نه فعالیت گلوتاتیون S–ترانسفراز (GST)، با مقاومت در *T. vaporariorum* مرتبط بودند. تجزیه و تحلیل سینتیکی استیل کولین استراز (AChE) نشان داد که مقادیر Km و Vmax در جمعیت FL تقریباً دو برابر کمتر و ۲.۵ برابر بیشتر از جمعیت FR بود، که نشان‌دهنده تغییر خواص سینتیکی AChE است. علاوه بر این، سنجش‌های مهار AChE کاهش حساسیت به اکثر مهارکننده‌ها را در جمعیت FL نشان داد، در حالی که افزایش حساسیت به فوستیازات مشاهده شد. به طور کلی، یافته‌ها نشان می‌دهد که کاهش حساسیت AChE، همراه با افزایش فعالیت‌های EST و MFO، ممکن است در مقاومت به کلرپیریفوس و ایمیداکلوپرید در جمعیت‌های ایرانی *T. vaporariorum* نقش داشته باشد. این یافته‌ها مبنایی برای نظارت بر مقاومت و پشتیبانی از توسعه استراتژی‌های موثر مدیریت مقاومت به حشره‌کش‌ها فراهم می‌کند.

کلمات کلیدی: سفید بالک گلخانه، بازدارنده آنزیمی، کلرپایریفوس، ایمیداکلوپرید، مقاومت

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