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RESEARCH

Status and mechanism of insecticide resistance of *Ceratitis capitata* (Diptera: Tephritidae) populations in East Mediterranean Region in Türkiye

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Abstract C*eratitis capitata* Wied. (Diptera: Tephritidae), a major pest of citrus, stone fruit, and subtropical plants, represents significant economic threats. This study evaluated the resistance levels of eight *C. capitata* populations from the East Mediterranean region of Türkiye to esfenvalerate, spinosad, tau-fluvalinate, and malathion using bioassays, biochemical analyses, and molecular techniques. The activities of Esterase, Glutathione S-transferase (GST), and Monooxygenase were quantified, along with the expression of eleven CYP4, CYP6, CYP9, and CYP12 genes. A grower survey indicated that 70% of respondents predominantly rely on pyrethroid insecticides. Resistance factors (RF), based on LC_{50} values of field populations relative to a susceptible strain, ranged from 10.16 to 23.80 for esfenvalerate, 2.75 to 7.54 for malathion, 1.93 to 3.31 for spinosad, and 3.02 to 12.40 for tau-fluvalinate. Strong correlations were detected between monooxygenase activity and esfenvalerate resistance, as well as between GST activity and pyrethroid resistance. Biochemical assays revealed that the Çağlarca population exhibited the highest Esterase, GST, and Monooxygenase activity, while Dalakderesi also showed notably elevated levels of all three enzymes, indicating a strong association between enzymatic detoxification and resistance levels. Residue trials demonstrated that spinosad exhibited the highest initial adult mortality within 24 h, whereas tau-fluvalinate had the lowest efficacy. By the 10th day, all insecticides had lost effectiveness, except spinosad, which significantly reduced larval emergence. Gene expression analyses revealed upregulation of CYP6A51-2, implicating its role in pyrethroid resistance. These findings indicate low to moderate levels of insecticide resistance in *C. capitata* populations, emphasizing the necessity for integrated resistance management strategies, such as rotating insecticides with different modes of action, to sustain effective control.

Keywords Biochemical · Bioassay · Gene expression · Insecticide resistance · Mediterranean fruit fly · And monooxygenase

Introduction

The Tephritidae family represents a prevalent pest group with detrimental impacts on fruit production and export (Ricalde et al., 2012; Tiring & Satar, 2021). A prominent member of this family is the Mediterranean fruit fly (Medfly), *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), which is subject to zero-tolerance status under international quarantine

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regulations. The mere presence of this pest in a single fruit within an export shipment can result in the rejection of the entire consignment, potentially leading to the suspension of agricultural product exports (Başpınar et al., 2009; Halila, 2024; Szyniszewska & Tatem, 2014).

The Mediterranean fruit fly exhibits polyphagous behavior, targeting more than 300 hosts, including temperate and subtropical fruits, some vegetables, and ornamental plants (Christenson & Foote, 1960; Demirdere, 1961; Liquido et al., 1991; Satar & Tiring, 2016; Yayla & Satar, 2018). Originating from the African Sahara, this pest has spread globally, particularly in tropical and subtropical regions, becoming a pervasive issue in many countries bordering the Mediterranean, including the Mediterranean and Aegean regions of Türkiye (Israely et al., 2004; Ricalde et al., 2012; Tiring, 2015; Vera et al., 2002). In Türkiye, the Medfly poses a significant threat to fruits such as citrus, persimmon, pomegranate, peach, nectarine, and fig, with a particular preference for tangerines and easily peelable varieties, such as those in the Satsuma group (Demirdere, 1961; Thomas et al., 2007; Tiring & Satar, 2021). Medfly larvae feed on the fleshy part of the fruit, causing depressions in the fruit peel. Affected fruits are discarded before harvest and cannot be exported (Demirdere, 1961; Tiring & Satar, 2021).

The economic damage threshold is set at 0.5 flies per trap per day. To maintain population levels below this threshold, synthetic insecticides—primarily pyrethroids and organophosphates—have been widely used in commercial orchards (da Roza Harter et al., 2024). Various methods, including chemical control via cover sprays, bait sprays, mass trapping, and the sterile insect technique, are globally employed for Medfly control (Hallouti et al., 2024; Martinez-Ferrer et al., 2010; Tiring & Satar, 2022; Yayla & Satar, 2018). Malathion bait sprays have been in use since 1956; however, concerns over the side effects of organophosphorus insecticides led to the exploration of alternative control options, culminating in the discovery of Spinosad and its bait spray formulation (Burns et al., 2001). Bait sprays offer several advantages, including targeting the adult stage, prolonged effectiveness, and the absence of fruit coating requirements (Broughton et al., 2015). In Türkiye, both malathion and spinosad bait sprays have been employed for Medfly control (Yayla & Satar, 2018). However, cover sprays have become the predominant method for suppressing *C. capitata* populations. Despite the increased use of insecticides against Medfly, which remains subject to strict quarantine regulations, satisfactory control has not been achieved (Yayla & Satar, 2018). The indiscriminate application of chemical treatments has contributed to the development of resistance in pest populations over time. Resistance in *C. capitata* to various insecticides has been reported in bioassays conducted in different countries (Magana et al., 2007; Couso-Ferrer et al., 2011; Voudouris et al., 2018; Abdel-Razek & Abd-Elgawad, 2021; Al-Katib, 2024; Mansy et al., 2024).

The determination of resistance mechanisms using biochemical and molecular methods has become a crucial research area following the confirmation of insecticide resistance in *C. capitata.* Biochemical studies on Medfly have revealed a correlation between esterase enzyme activity and resistance to organophosphorus insecticides (Castells-Sierra, 2023; El-Agamy et al., 2021; Kakani & Mathiopoulos, 2008). Molecular studies have further elucidated resistance mechanisms, demonstrating that malathion resistance is associated with a target-site mutation in the acetylcholinesterase enzyme (ace1 gene, Gly328 Ala) (Magaña et al., 2008). Additionally, target-site insensitivity has been identified as a major factor contributing to malathion resistance (Couso-Ferrer et al., 2011; Vontas et al., 2011; Gazit & Akiva, 2017; Magaña et al., 2008; El-Agamy et al., 2021). Moreover, mutations that introduce premature stop codons in the gene encoding the target α6 subunit of the nicotinic acetylcholine receptor (Ccα6) have been linked to spinosad resistance in both laboratory strains and field populations (Guillem-Amat et al., 2023).

In *C. capitata*, 103 CYP genes and 9 pseudogenes have been identified, including a distinct cluster of 18 consecutive CYP genes, 13 of which belong to the CYP6 A subfamily. Genes associated with insecticide resistance predominantly belong to the CYP4, CYP6, CYP9, and CYP12 families (Guillem Amat, 2019). Monooxygenase enzyme activity has been linked to pyrethroid resistance, and gene expression studies focusing on CYP6 A51 genes have demonstrated significant upregulation in resistant populations (Arouri et al., 2015; Tsakireli et al., 2019). A deeper understanding of the molecular mechanisms underlying

resistance may provide comprehensive insights into the challenges posed by *C. capitata* in pest management strategies.

This study addresses a significant knowledge gap regarding the impact of chemical insecticide use for controlling *Ceratitis capitata* in Türkiye's East Mediterranean Region. We first conducted a producer and dealer survey to identify frequently used insecticides and areas with reported control failures. Building on this, we assessed the resistance levels of local *C. capitata* populations through bioassays, and further characterized the underlying mechanisms using biochemical and molecular techniques. The integration of these approaches enabled a comprehensive evaluation of resistance dynamics in the field. By linking field-level insecticide use with laboratory-based evidence of enzymatic detoxification and gene expression, this study provides critical insights into the current status of insecticide resistance. These findings form a foundation for the development of sustainable, region-specific resistance management strategies.

Materials and method

Field sampling

Populations of *Ceratitis capitata* were systematically collected from citrus orchards across eight distinct locations where chemical control had proven challenging. The selection of these locations was determined through extensive consultations with producers and dealers in the provinces of Adana (Karayusuflu, Kayışlı), Hatay (Erzin, Misis), and Mersin (Bekirde, Çağlarca, Dalakderesi, Tepeköy), in the East Mediterranean Region of Türkiye in 2018 (Fig. 1, Table 1). For comparative purposes, the Sarıçam population, maintained at a citrus research plantation under the Department of Plant Protection at Çukurova University, was chosen as the susceptible reference population. This population had not been exposed to insecticides for an extended period, making it a suitable benchmark for susceptibility in the subsequent experiments.



Fig. 1 Distribution of sampling locations of *Ceratitis capitata* populations in southern Türkiye. The yellow marks indicate the provinces sampled in the East Mediterranean Region (Adana, Hatay, Mersin). Although citrus is the main horticulture crop grown in the region, more than 80% of Turkey Production includes many types of fruits such as stones fruits, pome fruits, pomegranates, persimmon and figs (satellite image from Anonymous, 2019)

Table 1 The locations where samples were collected to determine the resistance levels of *Ceratitis capitata*

Location	Province	Altitude	Field Size (daa)	Crop	Coordinate
Dalakderesi	Mersin	390	40	Peach/Plum	36°55′37"N, 34°34′01"E
Çağlarca	Mersin	1080	50	Peach	37°0′58"N, 34°24′45"E
Tepeköy	Mersin	1330	25	Peach/Pear	36°55′03"N, 34°17′41"E
Bekirde	Mersin	40	55	Pear	36°52′11"N, 34°41′17"E
Kayışlı	Adana	10	60	Mandarin	36°53′51"N, 35°14′38"E
Karayusuflu	Adana	30	50	Persimmon	36°53′4"N, 35°14′32"E
Misis	Adana	100	30	Mandarin	36°52′11"N, 34°41′17"E
Erzin	Hatay	90	60	Mandarin	36°57′11"N, 35°36′38"E
Sarıçam (Sussceptible)	Adana	50	35	Mandarin	37°01′48"N, 35°21′44"E

Culturing Ceratitis capitata populations

Infested fruits were carefully collected from the field and transported to the laboratory for further study. Under controlled laboratory conditions, the fruits were placed in plastic cages containing a 5 cm layer of perlite at the bottom. The environmental conditions were maintained at 25 ± 1 °C, a 16:8-h (L:D) photoperiod, and 70–80% relative humidity. Pupae were obtained by sieving the perlite approximately 4–6 days after larval development within the fruits. The pupae were then transferred daily onto moistened perlite within cylindrical plastic cages, covered with fabric, and kept in a climate chamber at 25 ± 1 °C. Upon emergence, adult flies were carefully transferred into 3-L cylindrical plastic cages. To nourish them, a sponge soaked in an artificial diet was provided inside the cage. The diet consisted of 20 mL water, 10 mL molasses, 5 g sugar, and 10 g brewer's yeast, placed in a petri dish, as described by Satar et al. (2016). Adult flies, aged 3–5 days and fed on fresh daily diets, were then used in subsequent insecticide resistance experiments, ensuring a controlled and standardized approach to assessing the resistance levels of C. *capitata* populations under laboratory conditions.

Insecticides

In the provinces of Adana, Hatay, and Mersin, the selection of pesticides for this study was based on insights gathered from 100 producers and dealers via face-to-face and telephone interviews. This collaborative effort aimed to reflect the real-world agricultural practices in these regions. The following insecticides were selected for bioassay experiments: Esfenvalerate 200 g/L (Sumigold (EC), Sumitomo Chemical), Malathion 25 g/L (Malathion (WP), Koruma Tarım), Spinosad 0.24 g/L (Success (CB), Dow AgroScience), Tau-fluvalinate 240 g/L (Mavrik (EW), ADAMA Turkey Tarım San. and Tic. Ltd. Şti.). These active ingredients represented the key insecticides commonly used in the study regions, allowing for a comprehensive assessment of their efficacy and impact on *C. capitata* populations.

Bioassay studies

Esfenvalerate, Malathion, Spinosad, and Tau-fluvalinate were serially diluted 5–7 times in sterile water to obtain a range of concentrations suitable for experimentation. The specific concentrations used for each C. capitata population were determined based on preliminary trials assessing insecticide efficacy (Table 1). Specifically, Esfenvalerate (Bekirde_7200 - 37.50, Erzin_4800 - 37.50, Çağlarca_9600 - 37.50 ppm, Dalakderesi_4800 - 37.50 ppm, Karayusuflu_4800 - 18.750 ppm, Kayışlı_4800 - 18.750 ppm, Misis_9600 - 37.50 ppm, Sussceptible_300 - 4.69 ppm, Tepeköy_9600 - 37.50 ppm), Malathion (Bekirde_3200 - 25, Çağlarca_1600 - 12.50 ppm, Dalakderesi_3200 - 20 ppm, Erzin_2400 - 25, Karayusuflu_1000 - 12.50 ppm, Kayışlı_1200 - 20 ppm, Sussceptible_400 - 6.25 ppm, Tepeköy_3200 - 12.50 ppm), Spinosad (Bekirde_1000 - 13.89, Çağlarca_2000 - 15.62 ppm, Dalakderesi_2000 - 15.62 ppm, Karayusuflu_2000 - 15.62 ppm, Kayışlı_1000 - 15.62 ppm, Sussceptible_500 - 7.81 ppm, Tepeköy_2000 - 15.62 ppm), and Tau-fluvalinate (Bekirde_1800 - 9.37,

Çağlarca_4800 - 18.75 ppm, Dalakderesi_2400 - 25 ppm, Erzin_1800 - 15 ppm, Karayusuflu_3000 - 18.75 ppm, Kayışlı_2400 - 12.50 ppm, Misis_2400 - 9.370, Sussceptible_600 - 2.35 ppm, Tepeköy_2400 - 9.37 ppm) concentrations were adjusted based on preliminary trials considering *C. capitata* populations and efficacy of the respective insecticides. Sterile water served as the control treatment in all experiments. Bioassays were conducted using a Potter Spray Tower (Burkard), ensuring the uniform application of insecticides. The experimental unit consisted of circular cells constructed from 2.0 cm high plexiglass, enclosed between two circular glass plates (diameter: 12 cm, thickness: 0.4 mm). The Spray Tower was calibrated to apply 2.0 ± 0.2 mg/cm^2 of insecticide onto each glass plate. The diluted insecticide solutions were sprayed onto one side of the glass plate and allowed to air dry. The treated glass plates were then positioned face-to-face, as described by Satar et al. (2012). For each insecticide concentration, four replicates were established, with five female and five male adults of C. capitata released into each experimental arena. After a 24-h exposure period, mortality was assessed by recording the number of dead and surviving individuals.

Residual effects of insecticides on mortality and reproduction of *Ceratitis capitata* through surface of treated fruit

The residual effects of insecticides on the mortality and reproductive potential of *C. capitata* were investigated. Label doses of malathion (400 g/10 L water + 500 mL hydrolyzed protein), spinosad (1 L/10 L), esfenvalerate (40 mL/100 L), and tau-fluvalinate (40 mL/100 L) were applied using a backpack sprayer (3 bar pressure) in a 12-year-old Mandarin (Satsuma variety) plot located at the Çukurova University Research Field, known to harbor a susceptible *C. capitata* population. Water served as the control treatment.

Each insecticide treatment was applied to two 12-year-old Satsuma trees. Sampling was conducted on the 1 st, 4 th, 7 th, and 10 th days post-application. Weather conditions during the application and sampling period in September were characterized by sunny skies and a mean temperature of 27–28 °C. On each sampling date, three sets of four fruits were collected from trees treated with each insecticide and transferred to the laboratory for residual bioassay testing. Four fruits were placed together in a 3-L ventilated cylindrical cage lined with a plastic fabric cover on both sides and the top. The cage also had a 1 cm hole on one side for feeding *C. capitata* individuals. Ten mated adults (5♀:5♂), aged 8–10 days, were released into each cage. Three replicates were performed for each insecticide on each of the four sampling dates. The cages were kept at 25 ± 1 °C, 70 ± 10% RH, and 16:8-h (L:D) photoperiod conditions for 24 h. Adults were fed via a yellow sponge soaked in an artificial diet and water, as described by Satar et al. (2016).

Mortality of released adults was recorded after 24 h, and surviving individuals were removed from the cages. Subsequently, the fruits exposed to the adults were kept for 10 days in a climatic chamber under the same conditions to assess reproductive performance. The fruits were then dissected under a stereo microscope to count the larvae. A 10-day period was considered sufficient for the transition from egg to larval stage.

Biochemical studies

Esterase and Glutathione-S-transferase (GST) enzyme activities were quantified following the methodology outlined by Stumpf and Nauen (2002), with modifications to accommodate the 96-well plate format. Protein content was determined at 600 nm using the Bradford method (Bradford, 1976), with Bovine Serum Albumin as a standard for calibration.

For esterase enzyme activity, 1-naphthyl acetate was used as the substrate. Three adult specimens were homogenized in 600 µL of Tris–HCl buffer (0.05 M, pH 7.5) containing 0.1% Triton X- 100. After centrifugation at 10,000 g for 5 min at 4 °C, the supernatant, serving as the enzyme source, was collected and diluted tenfold before experimentation. For the assay, 25 µL of the supernatant was mixed with 25 µL of phosphate buffer (0.2 M, pH 6), followed by the addition of 200 µL of substrate solution. The substrate solution contained Fast Blue RR salt in sodium phosphate buffer (0.2 M, pH 6.0), with a final concentration of 1 mM 1-naphthyl acetate. These reaction mixtures were then dispensed into individual wells of a microplate. Total esterase activity was assessed kinetically in triplicate at 450 nm for 7 min at 30 °C using a Multiskan Go microplate reader (Thermo

Fisher Scientific, MA, USA). Wells containing buffer alone were used as controls to monitor non-enzymatic reactions.

For Glutathione-S-transferase (GST) enzyme activity, the same supernatant obtained from the esterase assay was used. In this assay, 100 µL of the supernatant was combined with 100 µL of a CDNB solution (final concentration: 0.4 mM 1-chloro-2,4-dinitrobenzene (CDNB) in 0.1% (v/v) ethanol) and 4 mM reduced glutathione (GSH). The reaction mixtures were dispensed into individual wells of the microplate, and absorbance was measured kinetically for 5 min at 340 nm and 25 °C using the microplate reader. Three replicates were performed for both esterase and GST enzyme activity to ensure accuracy and reliability.

Cytochrome P450 activity was measured via the O-deethylation of 7-ethoxycoumarin, following the methodologies of Rauch and Nauen (2003) and Satar et al. (2018). Frozen medflies (≥ 10 mg, –80 °C) were homogenized in Na/K-phosphate buffer (0.1 M, pH 7.6) containing EDTA, DTT, and sucrose. The homogenate underwent sequential centrifugation at 5,000 g, 15,000 g, and 100,000 g, after which the microsomal pellet was resuspended and used as the enzyme source.

Reactions were set up in a 96-well plate with microsomal fractions, buffer, and 7-ethoxycoumarin, and were initiated by adding NADPH (1 mM final concentration). After incubation at 30 °C for 30 min, self-fluorescent NADPH was removed using oxidized glutathione and glutathione reductase. The reaction was stopped with acetonitrile in TRIZMA buffer (pH 10). 7-Hydroxycoumarin levels were quantified using a fluorimeter (Victor 3 V, PerkinElmer) at 465 nm (excitation: 390 nm). Monooxygenase enzyme activity could not be determined for the Kayışlı and Erzin populations due to insufficient material.

Molecular studies

Total RNA isolation was performed using TRIzol reagent (Invitrogen, Germany) following the manufacturer's protocol. Five wingless adult medfly were weighed and used for RNA extraction. The quantity and quality of the isolated RNA were assessed using a spectrophotometer (Multiskan Go, Thermo Fisher Scientific, MA, USA) with a µDropPlate (Thermo Fisher Scientific, MA, USA). Additionally, RNA integrity was confirmed via electrophoresis on a 1.5% agarose gel.

For cDNA synthesis, the AB High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, MA, USA) was used according to the manufacturer's instructions. The reaction mixture contained 2 µL of 10 ×RT Buffer, 1 µL of 25 ×dNTP Mix (100 mM), 1 µL of MultiScribe Reverse Transcriptase, 2 µL of 10 ×RT random primer, 1 µL of RNase Inhibitor, and nuclease-free H_2O. The PCR conditions were set as follows: 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min. The synthesized cDNA was diluted 50-fold and stored at − 20 °C until further use in Real-time PCR reactions. Eleven primers, specifically targeting P450 monooxygenase genes, were selected for Real-time PCR based on their previously reported high expression levels (Arouri et al., 2015; Tsakireli et al., 2019) (Table 2).

The PCR reaction was carried out in a total volume of 40 µL, consisting of 2 µL of cDNA (equivalent to 5 µg), 2 µL of each primer, and 20 µL of SYBR Green mix. The PCR protocol included an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 20 s. At the end of the reaction, a melting curve analysis was performed to assess the specificity of the amplification products.

Data analysis

Dose–response data were analyzed using Probit analysis with Polo Plus software (LeOra Software, Berkeley, CA). The resistance factor (RF) was calculated by dividing the LC50 of field strains by that of the susceptible reference strain. Resistance levels were classified based on the criteria established by Hayashi (1983): low (< 10), medium (10–40), high (41–160), and very high (> 160). Mortality in insecticide treatments was adjusted using Abbott's formula (Abbott, 1925).

For residue effect trials, mortality data were arcsine transformed (SQRT[% mortality]) for variance stabilization. The normality of the data was tested using the Shapiro–Wilk test. Non-normal data (collected on the 4 th, 7 th, and 10 th days) were analyzed using the Kruskal–Wallis test, while normally distributed data were analyzed using one-way ANOVA, followed by Tukey's HSD test ($p < 0.05$). For non-normal data requiring further analysis, Tamhane's T2 test

Table 2 The primers targeted specifically P450 Monooxygenase

CYP family	n	GenBank Accession number	Size Bp	Forward	Reverse
CYP4	1	XM_004520608	106	GTATTGGCAACCGATTTGCT	GTGGCATTGAACGAGGTCTT
	2	XM_004521002	140	CGTTGATCGGTGTTGTTTTG	CGAACAGCCCAATACCAAAT
CYP6	1	XM_004520247	138	CCATTGGAACGCTGTTCTTT	AGGGATCACGTATGGTCTGC
	2	XM_004534802	113	CTTCCTCGGTTTGCTTATCG	CCGCCAGTATAATGGGCTTA
	3	XM_004534804	149	CCCGATCCAGAAAAGTTTGA	TATGTGAGACCGACCAACGA
	4	XM_004522816	148	CGCTATGATGCAGACGAAAA	ACGCACCACATTCAGGTGTA
	A51 - 1	XM_004534804**	130	CGGAATACTTCCCTGATCCA	TATGTGAGACCGACCAACGA
	A51 - 2		160	GCTCGTGCTCAGTGTTACGA	CTTTGTAGTGCGCCATTCCT
CYP 9	1	XM_004526487	101	GCGACAATGTGTCATGAGCTG	GACAAGGGCTTGCCTCCA
CYP 12	1	XM_004521275	147	ACTCAGCCTGGCAAAGAATC	CCAGTGGAAACGGGATACAT
	2	XM_004521276	88	ATCCATGCTGGGAGGTAAGA	GTTGTGGTATTCGCCTACGG
Rpl19*	1			AACAAACGTGTACTGATGG	CACGTACTTTATGTCGTCTG

*endogen controls (Sagri et al., 2017; Tsakireli et al., 2019)

**Two or three different pairs of primers were used for these genes (Arouri et al., 2015)

($\alpha = 0.05$) was applied. All statistical analyses were conducted using SPSS Version 26.

Enzyme activities (Esterase, GST, and Monooxygenase) were quantified as nmol/min/mg protein and analyzed using ANOVA, followed by Tukey's HSD test ($p = 0.05$) for mean comparisons in SPSS Version 26.

Pearson's correlation coefficient was used to assess relationships between enzyme activities (Esterase, GST, and Monooxygenase) and insecticide resistance (RF values), as well as to identify potential cross-resistance patterns by comparing resistance factors across different insecticides. The statistical significance threshold was set at $p < 0.05$, and non-significant correlations were excluded from graphical representation.

Relative gene expression was quantified using the 2^(-ΔΔCt) method (Livak & Schmittgen, 2001) in R software (version 4.2.0). Gene expression differences across populations were analyzed using the Kruskal–Wallis test, with Dunn's test and Bonferroni correction applied for post hoc analysis (Xu et al., 2017). Statistical significance was set at $p < 0.05$, with significant differences between populations indicated by asterisks (* or **). Gene expression data were visualized using ggplot2.

Linear regression analyses were performed in R software (version 4.2.0) to examine the relationship between glutathione S-transferase (GST) activity and cytochrome P450 monooxygenase (CYP) gene expression. Scatter plots were generated, and Pearson correlation coefficients (r) were calculated for each CYP gene to assess their relationship with GST activity. Statistical significance was determined at $p < 0.05$, and only significant correlations (R^2 values) were considered for further interpretation.

Results

Bioassay studies

A survey of 100 producers and dealers provided insights into the prevalent methods for *controlling C. capitata* in the provinces of Adana, Hatay, and Mersin, Türkiye. Results revealed that 45% of respondents used esfenvalerate, 25% used tau-fluvalinate, 15% used malathion, 10% used spinosad, and 5% employed other methods. Notably, 68% of producers reported failures in effectively controlling the Medfly, 22% achieved moderate success, and only 10% reported satisfactory control (Fig. 2).

In the East Mediterranean Region of Türkiye, the resistance levels of eight *C. capitata* populations to spinosad, esfenvalerate, malathion, and tau-fluvalinate were evaluated based on data obtained from the questionnaire (Table 3). LC50 values varied, with esfenvalerate ranging from 313.1 mg/cm^2 (Dalakderesi)

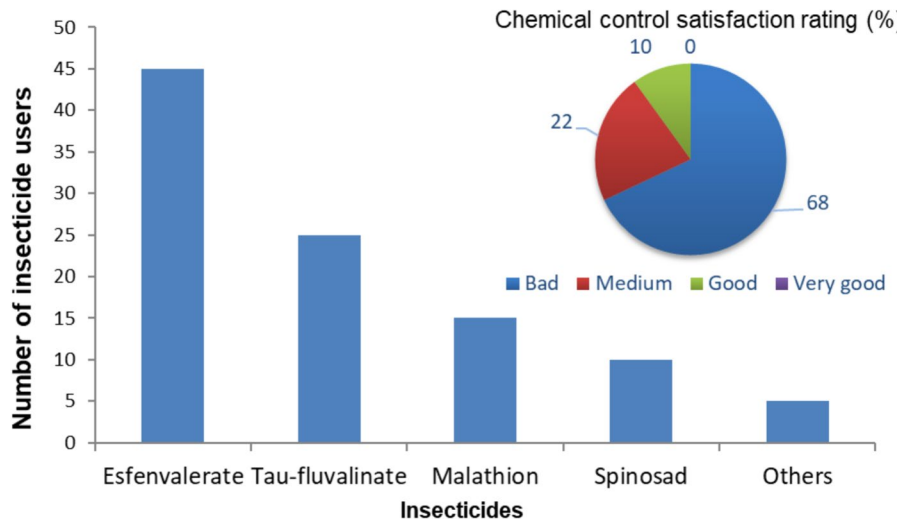


Fig. 2 Insecticide use based on the survey of 100 orchard owners and dealers regarding *Ceratitis capitata* control in the Adana, Hatay, and Mersin provinces of Türkiye

to 735.3 mg/cm^2 (Çağlarca), malathion from 127.3 mg/cm^2 (Kayışlı) to 349.1 mg/cm^2 (Tepeköy), spinosad from 97.2 mg/cm^2 (Bekirde) to 166.9 mg/cm^2 (Karayusuflu), and tau-fluvalinate from 107.7 mg/cm^2 (Kayışlı) to 445.5 mg/cm^2 (Çağlarca). Çağlarca exhibited the highest resistance level for esfenvalerate (RF: 23.80), followed by Misis and Bekirde. Similarly, Tepeköy demonstrated higher malathion resistance (RF: 7.54), followed by Çağlarca and Dalakderesi. The spinosad resistance factor ranged from 1.92 to 3.31, with Karayusuflu exhibiting the maximum resistance Among populations, Çağlarca exhibited the highest resistance level to esfenvalerate (RF: 23.80), followed by Misis and Bekirde. Tepeköy demonstrated the highest malathion resistance (RF: 7.54), followed by Çağlarca and Dalakderesi. For spinosad, resistance factors ranged from 1.92 to 3.31, with Karayusuflu exhibiting the maximum resistance. Çağlarca also displayed the highest tau-fluvalinate resistance (RF: 12.40), while Kayışlı had the lowest (RF: 3.02).

Importantly, very high LC90 values across populations suggested the presence of individuals with elevated resistance levels to all insecticides. Furthermore, the highest resistance was observed for esfenvalerate, while the lowest resistance was found for spinosad. The slope of most log-dose curves ranged between 1 and 1.5 (Table 3), indicating a heterogeneous resistance structure in these populations. (Table 3).

The correlation analysis between insecticide resistance factors revealed multiple significant relationships, suggesting potential cross-resistance among different insecticides. A strong positive correlation was observed between esfenvalerate and tau-fluvalinate resistance ($r = 0.72$, $p < 0.05$). Additionally, esfenvalerate resistance showed a moderate correlation with malathion resistance ($r = 0.67$, $p < 0.05$). A significant correlation was also detected between esfenvalerate and spinosad resistance ($r = 0.64$, $p < 0.05$). Similarly, spinosad and tau-fluvalinate resistance exhibited a strong correlation ($r = 0.71$, $p < 0.05$). Furthermore, a moderate correlation was identified between malathion and tau-fluvalinate resistance ($r = 0.60$, $p < 0.05$).

Corrected mortality and reproduction of ceratitis capitata from residual treatments

The highest mortality rates were observed in adults exposed to treated fruits on the first day after spraying, with spinosad inducing the most significant effect, resulting in 83% mortality, followed by malathion (55%), esfenvalerate (21%), and tau-fluvalinate (14%) ($F = 86.33$, $df = 3$, $P < 0.000$). On the fourth day after spraying, the ranking remained consistent, albeit with decreased mortality rates for all insecticides. By the fourth day, mortality rates had declined substantially compared to the first day, with an approximately 50% reduction in the spinosad treatment, 33% in malathion, 86% in esfenvalerate, and 100% in tau-fluvalinate. On the seventh and tenth days after exposure, there was no significant treatment effect, and mortality, if any, remained below

Table 3 Resistance levels of *Ceratitis capitata* populations to Esfenvalerate, Malathion, Spinosad, and Tau-fluvalinate

Active ingredient	Population	n*	LC_{50} (mg/cm$^{2)}$ (95% confidence limits)	LC_{90} (mg/cm^2) (95% confidence limits)	Slope ± SE	RF**
Esfenvalerate	Susceptible	340	30.8 (22.7–41.3)	203.5 (131.928–389.6)	1.56 ± 0.191	-
	Bekirde	315	472.6 (328.7–663.7)	5775.3 (3429.4–12,403.0)	1.18 ± 0.135	15.34
	Çağlarca	340	735.3 (511.1–1073.3)	11,395.0 (6114.4–28,875.0)	1.08 ± 0.124	23.80
	Dalakderesi	315	313.1 (214.2–442.5)	3552.7 (2080.0–8099.1)	1.22 ± 0.155	10.16
	Erzin	310	399.5 (284.0–560.7)	4225.2 (2471.3–9479.4)	1.25 ± 0.150	12.97
	Karayusuflu	320	352.4 (246.3–508.8)	5116.5 (2816.3–12,391.0)	1.10 ± 0.125	11.43
	Kayışlı	320	329.3 (232.5–469.8)	4335.0 (2455.6–9966.6)	1.15 ± 0.127	10.69
	Misis	340	488.9 (334.9–703.4)	7583.2 (4242.5–17,899.0)	1.08 ± 0.124	15.87
	Tepeköy	310	378.1 (248.8–554.1)	7123.3 (3869.4–17,883.0)	1.01 ± 0.121	12.27
	Susceptible	330	46.3 (32.9–63.7)	367.7 (223.5–811.9)	1.42 ± 0.191	-
Malathion	Bekirde	350	234.6 (166.1–327.5)	2443.6 (1449.4–5360.5)	1.26 ± 0.154	5.07
	Çağlarca	320	285.6 (210.5–401.7)	2201.7 (1304.3–4856.5)	1.45 ± 0.172	6.17
	Dalakderesi	340	266.1 (135.5–554.3)	6623.7 (2194.7–70,082.0)	0.92 ± 0.122	5.75
	Erzin	340	215.2 (147.7–306.9)	2795.7 (1572.6–6834.0)	1.15 ± 0.143	4.65
	Karayusuflu	320	155.7 (117.7–206.8)	1009.9 (663.4–1845.1)	1.58 ± 0.172	3.37
	Kayışlı	330	127.3 (93.8–172.0)	1026.1 (644.5–2040.2)	1.41 ± 0.162	2.75
	Tepeköy	390	349.1 (241.5–525.1)	5721.9 (2903.7–16,045.0)	1.06 ± 0.123	7.54
	Susceptible	340	50.4 (38.8–66.1)	331.5 (215.4–628.3)	1.57 ± 0.213	-

Table 3 (continued)

Active ingredient	Population	n*	LC_{50} ($mg/cm^{2)}$ (95% confidence limits)	LC_{90} (mg/cm^{2}) (95% confidence limits)	Slope ± SE	RF**
Spinosad	Bekirde	320	97.2 (72.9–129.6)	639.7 (417.9–1186.5)	1.57 ± 0.176	1.93
	Çağlarca	300	148.9 (106.2–206.2)	1443.9 (877.6–3012.3)	1.30 ± 0.151	2.95
	Dalakderesi	320	115.6 (81.7–159.1)	1076.9 (672.1–2150.5)	1.32 ± 0.154	2.29
	Erzin	270	145.4 (98.6–201.8)	1364.5 (872.9–2630.8)	1.32 ± 0.161	2.88
	Karayusuflu	320	166.9 (122.3–227.7)	1365.8 (854.7–2703.9)	1.40 ± 0.158	3.31
	Kayışlı	300	131.3 (95.6–181.2)	1038.1 (625.6–2285.6)	1.43 ± 0.183	2.61
	Tepeköy	330	140.3 (101.5–190.9)	1170.1 (741.5–2269.5)	1.39 ± 0.158	2.78
	Susceptible	330	35.9 (25.8–49.6)	358.8 (220.0–723.3)	1.28 ± 0.139	-
Tau-fluvalinate	Bekirde	320	116.8 (81.5–164.9)	1545.7 (901.564–3391.1)	1.14 ± 0.128	3.25
	Çağlarca	400	445.5 (308.4–658.6)	6954.3 (3626.6–18,804.0)	1.07 ± 0.129	12.40
	Dalakderesi	370	233.1 (173.6–312.9)	1611.1 (1037.5–3062.8)	1.53 ± 0.173	6.49
	Erzin	330	143.7 (100.6–203.1)	1858.5 (1066.4–4260.5)	1.15 ± 0.135	4.03
	Karayusuflu	340	320.8 (225.8–454.9)	4108.9 (2338.9–9529.8)	1.16 ± 0.135	8.94
	Kayışlı	350	107.7 (69.1–160.7)	2305.7 (1187.3–6553.2)	0.96 ± 0.125	3.02
	Misis	330	237.2 (169.8–338.9)	2786.8 (1581.4–6374.7)	1.20 ± 0.131	6.60
	Tepeköy	340	296.2 (206.4–445.9)	4489.7 (2293.9–12,440.0)	1.09 ± 0.127	8.25

*n = number of individuals tested, **RF = Resistance factor_ Field population LC_{50} value divided by Susceptible population LC_{50} value

10% ($P > 0.05$, Table 4). Although no statistically significant differences were detected ($P > 0.05$), biological relevance remains, as some treatments showed consistently lower larval counts over time, likely due to residual effects and sublethal impacts.

The number of *C. capitata* larvae emerging from treated fruit over a 10-day period is summarized in Table 5. Among the tested insecticides, spinosad exhibited the lowest number of larvae per fruit across all time points, with an initial mean of 1.3 ± 1.33 larvae per fruit on the first day, which slightly increased to 4.0 ± 2.00 larvae per fruit by the 10 th day. Malathion also resulted in relatively low larval counts, particularly on the first day (1.7 ± 1.67 larvae per fruit), but increased over time, reaching 4.7 ± 2.33 larvae per fruit on the 10 th day. Conversely, esfenvalerate and tau-fluvalinate treatments led to higher larval emergence, with initial values of 4.0 ± 2.00 and 4.3 ± 2.19 larvae per fruit, respectively, increasing to 6.3 ± 0.33 and 6.0 ± 0.57 larvae per fruit by the 10 th day. The control group

Table 4 Percentage corrected mortality (mean ± SE) of *Ceratitis capitata* adults exposed to fruits collected from Satsuma trees at 1, 4, 7, and 10 days after conducting insecticide sprays on the trees

Treatment active ingredient	n	1 st day	4 th day	7 th day	10 th day
Esfenvalerate	30	20.69 ± 3.450 a	3.33 ± 3.333 a	0.00 ± 0.000 a	0.00 ± 0.000 a
Malathion	30	55.17 ± 3.447 b	36.67 ± 3.333 b	3.33 ± 3.333 a	0.00 ± 0.000 a
Spinosad	30	82.76 ± 3.450 c	43.33 ± 3.333 b	6.67 ± 0.333 a	3.33 ± 3.333 a
Tau-fluvalinate	30	13.79 ± 3.446 a	0.00 ± 0.000 a	0.0 0 ± 0.000 a	0.00 ± 0.000 a

*Treatment mortality was adjusted using mortality in control and Abbott's formula (Abbott, 1925). Following confirmation of normality and homogeneity test, all parameters were analyzed using a parametric Tukey's test ($p < 0.05$) for data with a normal distribution (1 st day) or a nonparametric Kruskal–Wallis H test ($p < 0.05$) for data that did not follow a normal distribution (4 th, 7 th, and 10 th days) according to the Shapiro–Wilk normality test using SPSS software. Means within a column with different letters are statistically different (TUKEY-HSD multiple comparison test, $\alpha = 0.05$)

Table 5 Mean ± SE number of larvae from the fruit of treated trees exposed to adults of *Ceratitis capitata* (5 male: 5 female) for 24 h following a 10-day post-exposure period at 25 ± 1 ℃, 70 ± 10% RH and 16:8 L:D conditions

Active ingredient	Number of fruits*	1 st day*	4 th day*	7 th day*	10 th day*
		Number of larvae per fruit			
Esfenvalerate	12	4.0 ± 2.00	4.0 ± 2.08	5.3 ± 0.33	6.3 ± 0.33
Malathion	12	1.7 ± 1.67	4.7 ± 0.33	3.7 ± 1.86	4.7 ± 2.33
Spinosad	12	1.3 ± 1.33	1.7 ± 1.67	3.3 ± 1.67	4.0 ± 2.00
Tau-fluvalinate	12	4.3 ± 2.19	4.0 ± 2.00	6.0 ± 0.58	6.0 ± 0.57
Control	12	8.0 ± 0.58	8.7 ± 0.33	8.3 ± 0.33	8.7 ± 0.33

*There were no significant differences among means with a column (TUKEY multiple comparison test, $\alpha = 0.05$)

exhibited the highest larval counts at all time points, remaining consistently above 8.0 larvae per fruit.

No significant differences were observed among the treatments within each time point, as determined by the Tukey multiple comparison test ($\alpha = 0.05$). However, the trend in larval emergence indicates that spinosad provided the most effective suppression of larval development, while esfenvalerate and tau-fluvalinate were the least effective treatments over the 10-day post-exposure period (Table 5).

Biochemical analyses

The biochemical enzyme activities of Glutathione S-transferase (GST), Esterase, and Monooxygenase were assessed in different *C. capitata* populations (Fig. 3). GST activity varied among the populations, with Çağlarca exhibiting the highest activity (1.554 ± 0.2340 nmol/min/mg protein), while the Susceptible population displayed the lowest (0.191 ± 0.0086 nmol/min/mg protein). Other populations showed intermediate levels of GST activity. Statistical analysis revealed significant differences among populations ($F = 85.191$, $df = 8, 18$, $p < 0.001$), with Çağlarca and Dalakderesi populations exhibiting significantly higher GST activity than the Susceptible strain ($p < 0.001$), suggesting a potential role in detoxification mechanisms. Fold-change analysis indicated that GST activity increased by 8.15-fold in Çağlarca, 6.41-fold in Dalakderesi, and 3.21-fold in Bekirde compared to the Susceptible population (Fig. 3).

Esterase activity was highest in Çağlarca (1.82 nmol/min/mg protein), followed by Dalakderesi (1.39 nmol/min/mg protein) and Bekirde (0.68 nmol/min/mg protein). The Susceptible population displayed the lowest esterase activity (0.07 nmol/min/mg protein). Fold-change analysis indicated a substantial increase in resistant populations, with Çağlarca exhibiting a 26.71-fold rise in activity, followed by Dalakderesi with a 20.46-fold increase compared to the Susceptible strain. Other resistant populations displayed fold changes ranging from 3.67 to 9.94 (Fig. 3).

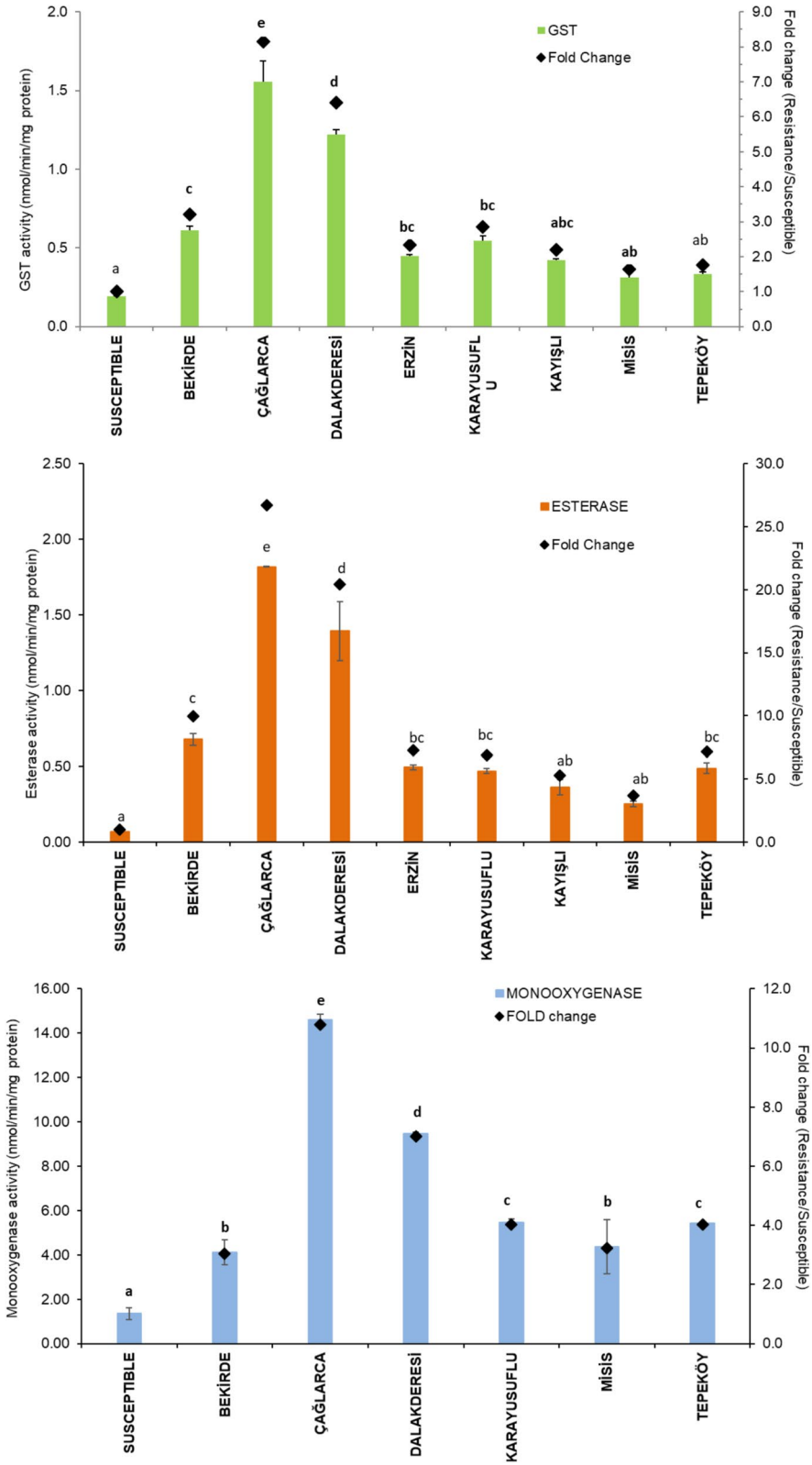


Fig. 3 Esterase, Glutathione S-transferase (GST), and Monooxygenase enzyme levels (Mean ± SE) and fold changes compared to control in *Ceratitis capitata* populations collected from Adana (Karayusuflu, Kayışlı*, Misis), Hatay (Erzin*), and Mersin (Bekirde, Çağlarca, Dalakderesi, Tepeköy). Each bioassay involved three replicates of three individuals, and enzyme activities were assessed kinetically. Esterase activity was measured at 450 nm for 7 min at 30 °C using a Multisikan Go microplate reader. GST activity was measured at 340 nm for 5 min at 25 °C using the microplate reader. Monooxygenase activity was assessed by quantifying 7-hydroxycoumarin levels using a fluorimeter (Victor 3 V, PerkinElmer) at 465 nm (excitation: 390 nm). Protein content was determined using the Bradford method (Bradford, 1976). Wells containing buffer alone were used as controls to monitor non-enzymatic reactions. Columns with means containing the same letter are not significantly different by Tukey's HSD multiple range test ($P < 0.05$). * Monooxygenase enzyme activity could not be determined

Monooxygenase enzyme activity varied significantly among the tested *C. capitata* populations. One-way ANOVA revealed a highly significant difference among populations ($F = 631.908$, $df = 6, 14$, $p < 0.001$), indicating substantial variability in metabolic detoxification potential. The Tukey HSD test ($\alpha = 0.05$) classified the populations into five statistically distinct subsets, further confirming these differences. The Susceptible population exhibited the lowest monooxygenase activity (1.35-fold), while the highest levels were recorded in Çağlarca (14.59-fold) and Dalakderesi (9.50-fold). Intermediate activity levels were observed in Tepeköy (5.44-fold) and Karayusuflu (5.46-fold), whereas Misis (4.37-fold) and Bekirde (4.11-fold) showed slightly lower activity. The highly significant differences among populations ($p < 0.001$) suggest that monooxygenase-mediated metabolic detoxification plays a crucial role in insecticide resistance, with Çağlarca and Dalakderesi populations exhibiting the most pronounced resistance potential.

Correlation analysis between bioassay results and enzyme activity revealed a moderate relationship between Esterase activity and Malathion resistance ($r = 0.39$, $p = 0.334$), suggesting that additional mechanisms may contribute to detoxification. A strong association was observed between Monooxygenase activity and resistance to Esfenvalerate ($r = 0.94$, $p = 0.002$), reinforcing its critical role in pyrethroid metabolism and suggesting its potential as a biomarker for Esfenvalerate resistance. Additionally, GST activity exhibited a significant correlation with pyrethroid resistance, particularly with Esfenvalerate ($r = 0.67$, $p = 0.048$) and Tau-fluvalinate ($r = 0.69$, $p = 0.042$). This further supports its involvement in detoxification processes, potentially enhancing metabolic resistance against these insecticides (Fig. 4).

The relative expression levels (REL) of several detoxification-related cytochrome P450 (CYP) genes varied significantly across populations (Fig. 5). Specifically, CYP4 -2, CYP6 -1, CYP6 -2, CYP6 -3, CYP9 -2, CYP12 -2, CYP6 A51 -1, and CYP6 A51 -2 exhibited statistically significant differences in expression among populations ($p < 0.05$), as confirmed by the Kruskal–Wallis test, followed by Dunn's multiple comparison test with Bonferroni correction. However, CYP4 -1, CYP6 -4, CYP9 -1, and

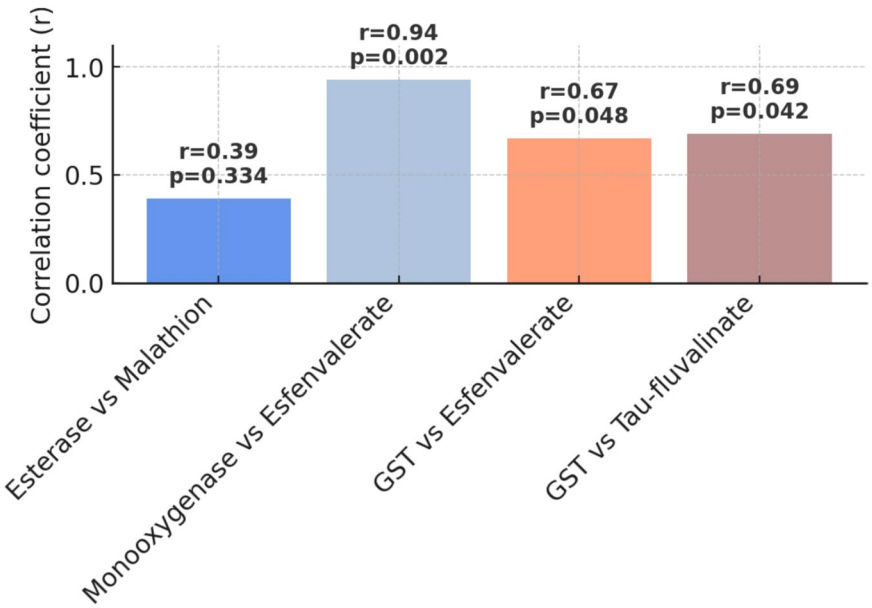


Fig.4 Correlation between enzyme activity and insecticide resistance in *Ceratitis capitata* populations. The bars represent Pearson correlation coefficients (r) for the relationships between enzyme activity (Esterase, GST, and Monooxygenase) and resistance to different insecticides. Significant correlations are highlighted, including strong associations between Monooxygenase activity and Esfenvalerate resistance, as well as GST activity and resistance to Pyrethroids. No significant correlation was observed between Esterase and Pyrethroids, or between Monooxygenase and Organophosphates. Error bars are not included as correlation coefficients are statistical measures rather than direct experimental values

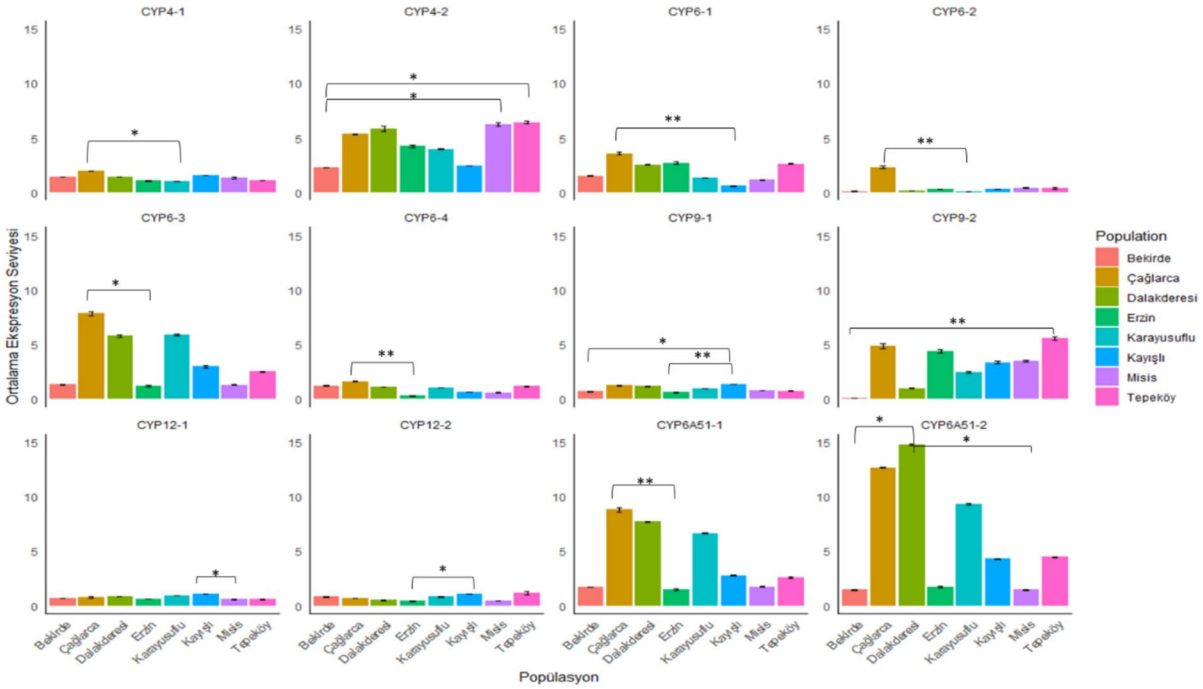


Fig. 5 Gene expression levels are shown for different *Ceratitis capitata* populations, measured and compared using the Kruskal–Wallis test and Dunn's test with Bonferroni correction. Each panel represents a different gene, with significant differences indicated by * ($p < 0.05$) and ** ($p < 0.01$). The populations are color-coded in the bar graphs. These graphs highlight that gene expression levels show significant differences between certain populations

CYP12 -1 showed no significant differences in their expression levels across populations ($p > 0.05$), indicating that their expression remained stable. Across all populations, the relative expression levels of CYP4 -1, CYP6 -2, CYP6 -4, CYP9 -1, CYP12 -1, and CYP12 -2 remained below a two-fold increase compared to the susceptible population, suggesting minimal upregulation of these genes. In contrast, CYP6 -1 exhibited a broader range of expression, with fold changes ranging from 1 to 4, indicating variable upregulation across populations. CYP4 -2 and CYP9 -2 were significantly upregulated, with fold changes between 1 and 6, suggesting a potential role in detoxification processes.

Notably, CYP6 -3 and CYP6 A51 -1 showed high expression in Dalakderesi, Karayusuflu, and Çağlarca populations, with fold increases ranging from 6 to 9 relative to the susceptible population. Among all the analyzed genes, CYP6 A51 -2 exhibited the highest expression levels, with a fold increase ranging from 9 to 15 relative to the susceptible population (Fig. 5). The significant upregulation of this gene suggests a crucial role in detoxification processes, potentially contributing to metabolic resistance mechanisms in *C. capitata.* Pairwise comparisons from the Dunn test further confirmed statistically significant differences in the expression of these genes among certain populations ($p < 0.05$), supporting their involvement in potential metabolic resistance mechanisms.

Correlation analysis revealed varying degrees of association between Glutathione S-transferase (GST) activity and the expression of different Monooxygenase (CYP) genes (Fig. 6). To better interpret these relationships, correlations were categorized as strong, moderate, and weak. Strong correlations were observed between CYP6 -1 and GST ($r = 0.52$, $p = 0.018$), indicating a potential link between GST activity and CYP6 -1-mediated detoxification processes. Moderate correlations were found between CYP6 A51 -2 ($r = 0.39$, $p = 0.046$), CYP4 -2 ($r = 0.38$, $p = 0.051$), and CYP6 A51 -1 ($r = 0.32$, $p = 0.072$) with GST activity. Weak or non-significant correlations were observed for several CYP genes, including CYP4 -1 ($r = 0$, $p = 0.998$), CYP12 -2 ($r = 0$, $p =$

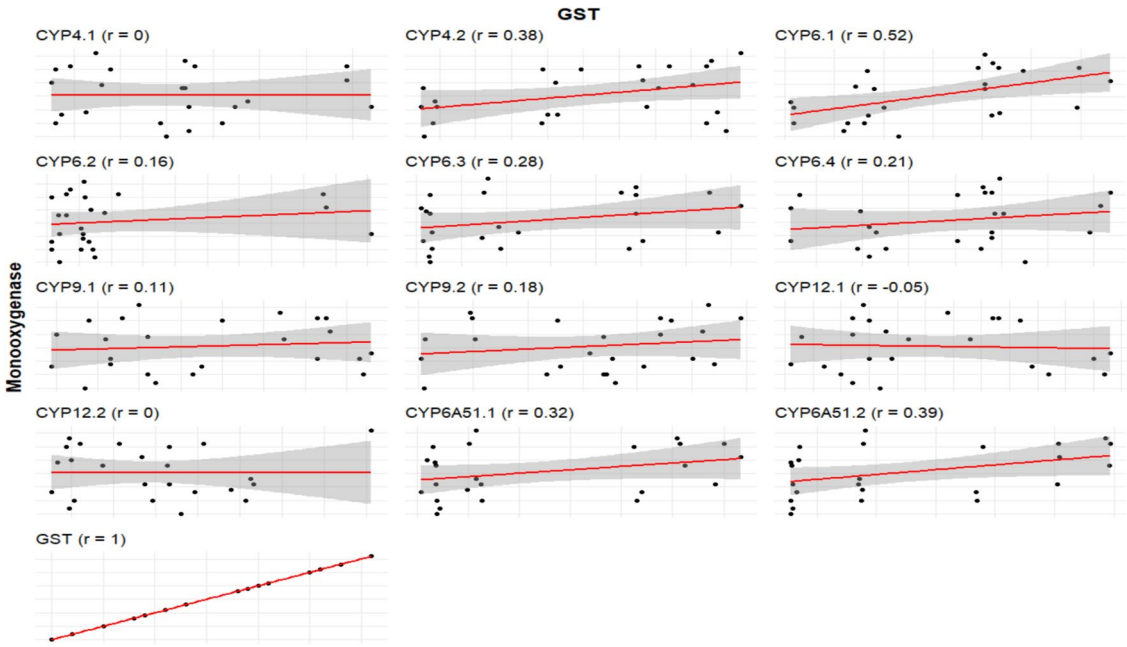


Fig. 6 Scatter plots showing the correlation between Glutathione S-transferase (GST) activity and the expression of different Monooxygenase (CYP) genes. Each plot represents the relationship between GST activity and a specific CYP gene, with black dots indicating individual data points and the red line representing the linear regression trend. The Pearson correlation coefficient (r) is displayed for each comparison. Genes such as CYP6.1 (r = 0.52), CYP6 A51.2 (r = 0.39), and CYP4.2 (r = 0.38) exhibited moderate positive correlations with GST activity, while CYP4.1 (r = 0) and CYP12.2 (r = 0) showed no significant correlation

0.995), and CYP12 -1 (r = − 0.05, p = 0.825), suggesting that these genes do not contribute directly to GST-mediated metabolic pathways. Other genes, such as CYP9 -1 (r = 0.11, p = 0.564), CYP9 -2 (r = 0.18, p = 0.341), CYP6 -3 (r = 0.28, p = 0.152), and CYP6 -4 (r = 0.21, p = 0.251), displayed weak positive correlations, but these were not statistically significant.

Discussion

Insecticide resistance management is a paramount concern in the chemical control of agricultural pests. As pests become less susceptible to insecticides, the efficacy of these chemicals diminishes accordingly. Consequently, a common error among producers is attempting to replicate past successes by escalating insecticide dosages. Unfortunately, this practice not only fosters the development of insecticide resistance but also increases costs and environmental impact. The indiscriminate and unmonitored application of insecticides accelerates the emergence of resistance (Tosun & Delen, 1996).

In recent years, *C. capitata*, which previously had 7–8 generations annually in the 1990 s (Özkan, 1993), has increased to 9–10 generations per year in the East Mediterranean due to rising temperatures (Satar & Tiring, 2016; Satar et al., 2016). Notably, from 2018 to 2019, it exhibited year-round activity, facilitated by warmer winters (Tiring & Satar, 2021). Moreover, grapefruit production in Türkiye faced setbacks after the 2000 s due to health concerns, leading farmers to leave unwanted fruits on trees. These fruits, similar to late Valencian varieties, became a source of sustenance and overwintering sites for Medfly, which then spread to surrounding stone fruits. This shift from vegetable and grain production to fruit crops has made *C. capitata* a year-round challenge (Satar et al., 2016). As a result, farmers increased insecticide use, worsening resistance issues. Our survey captured these changes and assessed *C. capitata* resistance mechanisms to esfenvalerate, spinosad,

tau-fluvalinate, and malathion in response to these ecological shifts.

Based on survey data obtained from producers and dealers, it was found that 70% of the region relies on pyrethroid-based insecticides for controlling *C. capitata.* Producers reported applying insecticides 6–7 times per production season to manage fruit flies, expressing dissatisfaction with the efficacy of these treatments. Bioassay studies confirmed a significant level of resistance in populations subjected to high rates of pyrethroid application. Our analysis revealed resistance rates ranging from low to moderate. Notably, among the insecticides tested, esfenvalerate exhibited the highest resistance factor, followed by tau-fluvalinate. The Çağlarca population exhibited the highest pyrethroid resistance, highlighting the importance of rotating with alternative active ingredients to mitigate resistance development.

While esfenvalerate is not licensed for use against *C. capitata* or citrus in Türkiye, it is authorized for controlling Lepidoptera, Psyllid, and select Cicadellidae species in apple, pistachio, tomato, cotton, and grape cultivation. Previous research has documented resistance phenomena in these pests within Türkiye (Uğurlu, 2001). Recent residue studies revealed that unlicensed esfenvalerate ranks highest among detected pesticide residues in Turkish citrus fruits (Dinçay et al., 2013). Similarly, tau-fluvalinate lacked licensing for citrus application during our research. Some pesticides face licensing hurdles due to their failure in side-effect tests on biological control agents, a criterion rigorously enforced by the Ministry of Agriculture and Forestry before granting licenses in citrus-growing regions. This regulatory challenge may drive farmers to resort to unlicensed products. The unregulated application of these pesticides leads to resistance issues and residue buildup on fruits, posing significant concerns (Vontas et al., 2011; Guillem-Amat et al., 2020; Castells-Sierra, 2023).

In our study, we detected malathion resistance within a lower range. Interestingly, malathion was not extensively used for controlling the Medfly in Türkiye until the 2010 s, despite its long-standing availability as a chemical agent. Although it has been registered in Türkiye since the 1960 s and employed in agricultural practices across various crops to combat insect species such as aphids, cotton weevils, leafhoppers, mosquitoes, certain beetles, and the Medfly, resistance development has been documented in some insect populations (Öngören et al., 1975, 1977). Persistent challenges with the Medfly compelled farmers to turn to this older, cost-effective product due to the necessity for repeated applications. Numerous global studies on Medfly resistance have revealed varying degrees of resistance to malathion across populations and countries (Magaña et al., 2007; Couso-Ferrer et al., 2011; Vontas et al., 2011; Gazit & Akiva, 2017; El-Agamy et al., 2021; Al-Katib, 2024; Salustino et al., 2024). For instance, Magaña et al. (2007) reported resistance levels ranging from 6- to 201-fold in *C. capitata* populations causing significant damage to citrus fruits in Spain. Similarly, Couso-Ferrer et al. (2011) observed 176-fold resistance to malathion in Spain. In contrast, our study indicates relatively lower resistance levels, likely due to malathion being one of the least utilized insecticides, as reported in our questionnaire survey. However, when evaluating malathion resistance among populations, notably higher resistance levels were found in Tepeköy, Çağlarca, Bekirde, and Dalakderesi districts, despite resistance factors being less than 10. Interviews with dealers and farmers corroborated higher malathion usage in these regions, aligning with the observed resistance factor results.

Ceratitis capitata exhibited the lowest resistance to spinosad among the tested active ingredients. Spinosad resistance was also detected at an insignificant level in Greece (Voudouris et al., 2018). In Egypt, an average of 125 ppm spinosad caused 70% and 62% mortality in laboratory and field strains, respectively (Abdel-Razek & Abd-Elgawad, 2021). The Kayışlı population showed the least resistance to most insecticides, except for spinosad. Despite minimal spinosad resistance, vigilance against potential resistance emergence is warranted.

When the slope coefficient exceeds 2, it indicates a homogeneous distribution of insecticide resistance within the population, whereas a slope coefficient below 2 suggests a heterogeneous resistance structure (Yu, 2008). In cases of homogeneity, the logarithmic dose-% mortality curve appears steep, while a flatter slope signifies a heterogeneous structure (Finney, 1952). Most log-dose curves in our study exhibited slopes between 1 and 1.5, indicating heterogeneity in resistance structures among populations. Notably, rural areas in the region contain hobby gardens with various citrus, stone, and pome fruits, serving as additional hosts for the Medfly. Furthermore, the city's

landscaping efforts have extensively incorporated sour orange trees along sidewalks in the city center. The potential spread of sensitive Medfly populations from untreated areas to commercial production regions could contribute to population heterogeneity.

In our study, significant positive correlations were observed between esfenvalerate and tau-fluvalinate, esfenvalerate and spinosad, and malathion and tau-fluvalinate. These correlations indicate potential cross-resistance mechanisms among different insecticides. The moderate correlation between malathion and tau-fluvalinate resistance suggests that while resistance mechanisms may overlap, metabolic detoxification pathways could play a key role in cross-resistance. Additionally, the strong correlation between esfenvalerate and spinosad resistance implies that oxidative metabolism, particularly through monooxygenase activity, may contribute to the detoxification of both compounds. Similarly, previous research in Spain detected cross-resistance between malathion and lambda-cyhalothrin (Couso-Ferrer et al., 2011). The lambda-cyhalothrin-resistant strain also exhibited high cross-resistance to deltamethrin (150-fold) and etofenprox (243-fold), whereas lower cross-resistance (3–sixfold) was observed for organophosphates (methyl-chlorpyrifos and malathion), spinosad, and lufenuron (Arouri et al., 2015). Castells-Sierra (2023) further confirmed that the mechanisms conferring lambda-cyhalothrin resistance also provide cross-resistance to deltamethrin. The high resistance levels observed in Spain were attributed to the widespread use of malathion for *C. capitata* control. Although malathion has not been used since 2009, researchers emphasized that resistant alleles may persist in the field and could be rapidly selected if cross-resistant insecticides are applied without proper management (Castells-Sierra, 2023). To mitigate this risk, integrated pest management (IPM) strategies should be adopted, including insecticide rotation, the use of biocontrol agents, and resistance monitoring to prevent the resurgence of resistant populations.

The experiment conducted to assess the effects of pesticide residues on *C. capitata* mortality rates corroborated findings from laboratory bioassays, revealing that the labeled dose of pyrethroid insecticides with high resistance levels proved ineffective. Among the tested insecticides, spinosad exhibited the highest efficacy, persisting until the fourth day after fruit spraying. However, the effectiveness of all insecticides declined after the fourth day, with no discernible impact on *C. capitata* survival by the tenth day. Notably, tau-fluvalinate exhibited lower control efficacy compared to other insecticides. Furthermore, the number of larvae hatching from eggs was significantly reduced, particularly with spinosad treatment, although it increased after the seventh day as spinosad's residual effect waned. Rahman and Broughton (2016) investigated the residual effects of insecticides on different life stages of Medfly and found that fenthion had the longest-lasting effect on fruit surfaces, killing 44% of adults after seven days, compared to 17% for thiacloprid and 30% for clothianidin. In larval control, none of the insecticides were 100% effective, but fenthion resulted in the lowest number of pupae. Similarly, another study found that the lambda-cyhalothrin-resistant *C. capitata* strain exhibited reduced fecundity and embryo-to-pupal viability, prolonged development, and increased adult weight and longevity under laboratory conditions. Notably, the egg count on the first day post-insecticide application was nearly half that of the second and third days. Additionally, fecundity was lower compared to both the control and the malathion-resistant strains (Guillem-Amat et al., 2020).

Residue effect experiments demonstrated spinosad's highest efficacy in terms of adult mortality and reduced larval emergence compared to other insecticides. However, this high efficacy comes at a cost, requiring skilled workers and frequent reapplications, thereby limiting its practicality in field applications. Nonetheless, the efficacy and rapid degradation of spinosad on fruit surfaces make it a valuable option for *C. capitata* control due to its lower environmental and human health impact. Additionally, it is crucial to monitor populations exhibiting low resistance levels, as these populations may serve as breeding grounds for highly resistant strains in the future.

Biochemical analyses revealed that the highest esterase enzyme activity was observed in the Çağlarca population, followed by Dalakderesi and Bekirde, while other populations exhibited moderate increases. The high esterase activity in Çağlarca, which showed resistance levels similar to those of other resistant populations, indicates a strong association with metabolic resistance, as demonstrated in previous studies (Castells-Sierra, 2023; El-Agamy et al., 2021; Kakani & Mathiopoulos, 2008; Koren et al., 1984; Magaña et al., 2008).

These findings support the hypothesis that increased esterase activity is a key determinant of organophosphate resistance in *C. capitata*. However, the moderate correlation between esterase activity and malathion resistance suggests that while esterase enzymes contribute to organophosphate detoxification, additional enzymatic pathways or target-site mutations may also play a role. Molecular studies have linked malathion resistance to target-site mutations in the acetylcholinesterase enzyme (ace1 gene, Gly328 Ala) (Magaña et al., 2008), reinforcing the role of target-site insensitivity in resistance mechanisms. Further genetic investigations into the ace1 region in these populations would provide valuable insights into resistance development.

Monooxygenase enzyme activity exhibited a 14.59-fold increase in the Çağlarca population and a 9.50-fold increase in the Dalakderesi population, suggesting that these populations may have developed an enhanced detoxification capacity contributing to resistance. The strong relationship between esfenvalerate resistance and monooxygenase activity further supports the hypothesis that increased metabolic capacity enhances resistance to this insecticide class. Monooxygenases are well known for their role in pyrethroid detoxification, and the observed correlation suggests their significant contribution to resistance development. While these enzymes are primarily associated with pyrethroid metabolism, their involvement in organophosphate resistance remains unclear. Further biochemical and molecular analyses are necessary to assess whether monooxygenases in *C. capitata* populations play a direct role in organophosphate metabolism or if other detoxification pathways are involved. However, previous research found no significant differences in P450 content in microsomal fractions between susceptible and resistant *C. capitata* strains (de Larriva, 2007). Similarly, NADPH-cytochrome c reductase activity did not show significant variation, suggesting that while monooxygenases contribute to insecticide detoxification, their specific role in resistance mechanisms requires further study (Couso-Ferrer et al., 2011). Additionally, a comparative analysis of detoxification enzyme activity in susceptible and resistant *C. capitata* strains showed that P450 cytochrome c reductase activity was slightly lower in resistant strains (5.4 vs. 4.2 nmol/min/mg protein), further highlighting the complexity of metabolic resistance mechanisms (Arouri et al., 2015).

Pyrethroid resistance in insects is primarily driven by three major mechanisms: target-site insensitivity, metabolic detoxification, and decreased cuticular penetration (Hemingway et al., 2004). Synthetic pyrethroid-based insecticides, such as esfenvalerate and tau-fluvalinate, primarily act on voltage-gated sodium channels (Scharf, 2003). However, metabolic detoxification, particularly through cytochrome P450 monooxygenases (CYPs), has been widely associated with insecticide resistance (Feyereisen, 2012).

Our findings suggest that metabolic detoxification plays a significant role in pyrethroid resistance in *C. capitata*, particularly through the upregulation of specific CYP genes. The highest expression levels were observed for CYP6 A51 - 2, CYP6 - 3, and CYP6 A51 - 1, particularly in the Dalakderesi, Karayusuflu, and Çağlarca populations, where expression levels were 7–15-fold higher compared to the susceptible population. These results align with previous studies that demonstrated the involvement of CYP6 genes in metabolic resistance (Arouri et al., 2015; Tsakireli et al., 2019). In contrast, genes such as CYP4 - 1, CYP6 - 4, CYP9 - 1, and CYP12 - 1 exhibited no significant variation across populations, indicating that not all CYP genes contribute equally to detoxification mechanisms. Dunn's test showed significant differences for some genes between populations, suggesting variations in gene expression. While Dunn's test identifies these differences as significant, it is important to note that these differences may not be substantial or consistent across all comparisons. Therefore, while some genes demonstrate significant expression differences, they do not universally indicate major variation in gene expression across all populations.

Correlation analysis suggests that certain cytochrome P450 enzymes contribute to detoxification processes associated with GST activity. CYP6 - 1 and CYP6 A51 - 2 showed significant positive correlations with GST, indicating a potential interaction in metabolic detoxification pathways. Moderate correlations for CYP4 - 2 and CYP6 A51 - 1 suggest a weaker association, while CYP12 - 1 and CYP12 - 2 exhibited no significant correlation, implying a minimal or indirect role in GST-mediated detoxification. Consistent with previous findings in insecticide-resistant populations (Hu et al., 2024), the upregulation of GSTs and P450 s in detoxification, particularly in the midgut, appears to be an adaptive mechanism enhancing insecticide resistance. These results

reinforce the importance of metabolic detoxification pathways when developing resistance management strategies.

Overall, our findings highlight that insecticide resistance in *C. capitata* populations is primarily driven by the activity of detoxification enzymes such as Esterase, GST, and Monooxygenase, with notable regional differences. The observed correlation between enzyme activity and CYP gene expression supports the role of metabolic detoxification in resistance, while the significant upregulation of specific CYP genes suggests local adaptation or selection pressure. To confirm their functional roles—particularly in pyrethroid metabolism—further studies using tools like RNAi or CRISPR-Cas9 are recommended. Broadening the molecular scope to include a wider range of P450 genes and evaluating GST and Esterase gene expression will offer a more complete picture of resistance mechanisms and inform better-targeted management strategies.

Ensuring sustainable *C. capitata* control requires the integration of biopesticides and IPM strategies. Although chemical control, mass trapping, and biological methods can offer temporary suppression, their long-term effectiveness is often compromised by resistance development, pest resurgence, and behavioral adaptations. Excessive reliance on insecticides, without complementary cultural or biotechnical practices, has further diminished treatment efficacy.

Our results also underscore the environmental and health risks associated with the unauthorized use of insecticides, such as esfenvalerate. Addressing these risks through stricter regulatory oversight should be a key component of resistance mitigation efforts.

While this study provides a timely snapshot of resistance patterns, future research should incorporate longitudinal monitoring to capture resistance trends. Economic analyses evaluating the costs of resistance and the benefits of IPM adoption would further support decision-making and field-level implementation. Ultimately, region-specific management plans that integrate molecular diagnostics, routine surveillance, and a balanced combination of chemical, biological, and cultural tactics will be critical to ensuring long-term, sustainable control of *C. capitata*.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Consent to publication All authors contributed critically to the drafts and gave final approval for publication.

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