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RESEARCH

Biochemical and molecular analyses of insecticide resistance in greenhouse populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Türkiye

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Abstract The sweet potato/cotton whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most destructive vegetable pests in greenhouses in Türkiye. While the preferred method of control primarily relies on insecticides such as neonicotinoids, organophosphates and pyrethroids, their intensive use has caused whiteflies to rapidly become resistant. Samples were collected from 13 Turkish *B. tabaci* greenhouse populations. Bioassays from the Marmara Region identified as high as a 53-fold increase in resistance to chlorpyrifos-ethyl, a 303-fold increase to imidacloprid and a 282-fold increase to acetamiprid. Bioassays in the Central Anatolia Region reported a 76-fold increase in resistance to deltamethrin and—15-fold increase to thiamethoxam. Most of the populations showed cross-resistance for neonicotinoids. All collected *B. tabaci* populations were determined to be MEAM 1 (also referred to as biotype B) for diagnostic band $E_{0.14}$. The $E_{0.14}$ esterase variant that helps to diagnose biotype B insects was found in all individuals from field populations. Almost all populations showed higher non-specific esterase, glutathione-S-transferase (GST) and monooxygenase activity when compared to susceptible SUD-S population. AChE insensitivity assays for individual *B. tabaci* suggest a target-site modification as a mechanism of resistance to chlorpyrifos-ethyl. This is the first report of AChE variants identified based on their sensitivities to chlorpyrifos ethyl-oxon and pirimicarb in Turkish *B. tabaci* populations. Fixed ace mutations in the target-site of organophosphates and pyrethroids were identified in six populations. Resistance to organophosphates and neonicotinoids were at least partially related to both ace mutations and insensitive AChE and monooxygenase activities, respectively. The results will help develop effective resistance management programs of *B. tabaci* in Türkiye.

Keywords Whitefly · Acetamiprid · Imidacloprid · Thiamethoxam · Chlorpyrifos-ethyl · Deltamethrin

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Introduction

Türkiye is among the top four countries in the world for the number of greenhouse facilities and ranks second in Europe after Spain. Greenhouse capacity has reached 79 thousand hectares, with an average yearly yield of approximately 7.9 million tons of vegetables with a value of 1.5 billion U.S. dollars (TUIK, 2020). The sweet potato or cotton whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most destructive pests of vegetables, horticultural and ornamental crops worldwide (Byrne & Bellows, 1991; Horowitz et al., 2020; Oliveira et al., 2001; Stansly & Naranjo, 2010). This pest causes extensive losses both directly, by feeding on plants, and indirectly, through honeydew excretions in which saprophytic fungi grow, leading to inhibition of photosynthesis. Adult whiteflies also play an important role in the transmission of more than 100 pathogenic plant viruses (Hogenhout et al., 2008; Jones, 2003). Whiteflies are difficult to control at all life stages due to their rapid reproductive rate, their preferred location on the under-surface of leaves, and their wide host range (Oliveira et al., 2001). The current management of *B. tabaci* is mostly dependent on chemical insecticides. However, the intensive use of chemical insecticides in closed areas such as greenhouses causes whitefly populations to rapidly become resistant and can hamper management programs.

Previous studies showed that *B. tabaci* could develop high levels of resistance against some classes of insecticides such as chlorinated hydrocarbons, organophosphates (OPs), carbamates, synthetic pyrethroids, insect growth regulators and neonicotinoids in many agricultural crops around the world (Bass et al., 2015; Erdogan et al., 2008; Horowitz et al., 2005, 2020; Houndéte et al., 2010; Kontsedalov et al., 2012; Roditakis et al., 2005; Wang et al., 2010). In this manner, frequent application of large amounts of these chemicals leads to economic losses and high human and environmental health risks. Therefore, it is important to monitor the development of resistance and understand which mechanisms are selected (Criniti et al., 2008). In 2022, over 55,000 tonnes of pesticides were used in Türkiye, at a cost of approximately 2 billion US dollars, according to data from the Turkish Ministry of Agriculture and Forestry, General Directorate of Food and Control in Türkiye (Erdoğan, 2024). It is critical to identify resistance as early as possible in order to implement insect resistance management strategies. Insecticide resistance studies in *B. tabaci* in Türkiye have been scarce, and primarily related to neonicotinoid insecticides, used especially in the Mediterranean region. Notably, there are different biotypes in this pest, and MEAM 1 (formerly B biotype) and MED (formerly Q biotype) *B. tabaci* have been reported in Türkiye (Dağlı et al., 2020; Erdogan et al., 2008, 2011; Satar & Ulusoy, 2016; Yükselbaba & Ali, 2022; Yükselbaba et al., 2012).

In the present study, we investigated the resistance status of Turkish whitefly populations against pyrethroids, neonicotinoids and organophosphates through bioassays, measurement of enzyme activities, and examination of mutations at the target sites of pyrethroids and organophosphates.

Materials and methods

Insect populations

Thirteen *B. tabaci* populations were collected from greenhouses located in Mugla (Aegean), Eskisehir (Central Anatolia), Zonguldak, Bartin (Black Sea) and Bilecik (Marmara) in Türkiye (Table 1).

In addition to these populations, several reference populations were obtained. MED and MEAM 1 populations of *B. tabaci* were kindly provided by the Plant Protection Institute of Heraklion, Greece, and Cukurova University, Türkiye, respectively, to assist in the classification of the collected populations of *B. tabaci*. A BCP-susceptible population of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) from the John Innes Centre in the UK was used to discriminate whitefly species. An insecticide-susceptible laboratory reference population (SUD-S) was obtained from the Rothamsted Research Institute, UK (Table 2).

A minimum of 100 individuals at all life stages were collected from each population of interest and transported to an insect-rearing room. Each population was reared on eggplants (*Solanum melongena* var. Kemer) until the F2 generation. The susceptible reference strain SUD-S, initially collected on cotton (*Gossypium hirsutum* L.) in Sudan in 1978 and obtained from Rothamsted Research, UK, had been raised since 1985 on *Gossypium hirsutum* 'Deltapine' with no insecticide application. All populations

Table 1 The origin, host, region, coordinate and date of sampling of *Bemisia tabaci* populations used in the present study

Population	Origin	Host plant	Region	Coordinate	Year
Bilecik 1	Bilecik	Cucumber	Marmara	40.13211N 30.17462E	11.07.2014
Bilecik 2	Bilecik	Bean/ Cucumber	Marmara	40.07940N 30.27983E	11.07.2014
Bilecik 3	Bilecik	Eggplant / Cucumber	Marmara	40.05703N 30.25778E	11.07.2014
Mihalgazi	Eskisehir	Tomatoes	Cent. Anatolia	40.02426N 30.57895E	13.09.2013
Saricakaya 1	Eskisehir	Cucumber	Cent. Anatolia	40.034381N 30.64116E	13.09.2013
Saricakaya 2	Eskisehir	Cucumber	Cent. Anatolia	40.03656N 30.62356E	13.09.2013
Derbent	Bartin	Cucumber	Black Sea	41.54150N 32.46762E	30.05.2013
Geriskatirci	Bartin	Eggplant	Black Sea	41.55553N 32.4383E	29.05.2013
Caycuma 1	Zonguldak	Cucumber	Black Sea	41.38811N 32.09058E	29.05.2013
Caycuma 2	Zonguldak	Cucumber	Black Sea	41.38367N 32.09472E	13.09.2013
Fethiye 1	Mugla	Tomatoes	Aegean	36.63739N 29.19317E	14.09.2013
Fethiye 2	Mugla	Tomatoes / Bean	Aegean	36.63078N 29.16220E	14.09.2013
Fethiye 3	Mugla	Cucumber	Aegean	36.64806N 29.21063E	14.09.2013

Table 2 Whiteflies used as a standard for identification, bioassay, and biochemical assay of *Bemisia tabaci* populations of the present study

Population	Species	Origin	Host	Year
SUD-S (Susceptible)	*Bemisia tabaci*	Sudan	Cotton	1978
MED	*Bemisia tabaci*	Crete/Greece	Nightshade	2012
MEAM 1	*Bemisia tabaci*	Adana/Türkiye	Eggplant	2010
BCP (Susceptible)	*Trialeurodes vaporariorum*	UK	Tobacco	1987

were maintained in different insect rearing rooms on eggplants (*S. melongena* var. Kemer) under 16 h:8 h L:D photoperiod and 27 ± 2 °C, 50–60% RH without exposure to insecticides in separate cabinets made of plexiglass (50 × 75 × 36 cm) to prevent contamination among populations.

Bioassays

All tested insecticides were specifically selected from different groups of pesticides that are registered to control *B. tabaci* in Türkiye. Formulated insecticides used in the bioassays were acetamiprid (Mospilan 20% by weight SP, Sumitomo), imidacloprid (Confidor 350 g L^{-1} SC, Bayer Crop Science), thiamethoxam (Actara 240 g L^{-1} SC, Syngenta), chlorpyrifos-ethyl (Dursban 4, 480 g /l EC, Corteva) and deltamethrin (Decis, 25 g L-1 EC, Bayer Crop Science). The doses were prepared before the experiments were initiated.

Two bioassay methods were conducted. Neonicotinoids (acetamiprid, acetamiprid and thiamethoxam) were tested by exposing insects to leaves treated systemically with these insecticides, and pyrethroid (deltamethrin) and organophosphate

(chlorpyrifos-ethyl) were tested by exposing insects to residues of these insecticides in a leaf-dip bioassay. Systemic bioassays with acetamiprid, imidacloprid and thiamethoxam were performed on five-day-old whitefly adult females according to Cahill et al. (1996). Fully expanded eggplant leaves were cut and the petiole immediately dipped into the insecticide solution or control in plastic cups for at least 40 h in an illuminated fume hood. Leaves dipped in distilled water were used as untreated controls. Leaf discs (37 mm in diameter) were cut from treated eggplant leaves using a cork borer and laid abaxial side up on a bed of agar (1%) previously poured into the base of plastic bioassay dishes (39 mm in diameter and 5 mm in depth). Each systemic bioassay consisted of three replicates of 20–30 five-day-old whitefly adult females per concentration, with at least five concentrations (plus an untreated control) per bioassay. Leaf dip bioassays with chlorpyrifos-ethyl and deltamethrin were carried out on five-day-old whitefly adult females following the procedure of Cahill et al. (1995). A stock solution was diluted to required concentrations with water containing 0.02% aqueous solution of the non-ionic wetter Triton X-100. Leaf discs (37 mm in diameter) were cut from eggplants and dipped into the insecticide solution for 20 s, and then air-dried for at least 2 h in a fume cabinet. The dried eggplant leaf disks were placed onto agar as described above (abaxial side up in the bioassay dishes). Each leaf dip bioassay consisted of three replicates of 20–30 five-day-old whitefly adult females per concentration, with at least five concentrations (plus an untreated control) per bioassay.

For both bioassays, adult females were collected with a mouth aspirator anaesthetized with CO_2 and immobilised on a black cloth-covered ice cassette. Twenty to thirty females were placed on each leaf disc. Bioassay dishes were then covered with a close-fitting, ventilated lid. Once insects became active, bioassay dishes were inverted so that leaf discs were abaxial side down and adult whiteflies oriented normally. Bioassay dishes were maintained in a climate cabinet at 25 ± 1 °C, 16 h:8 h L:D photoperiod, 50–60% RH and scored for mortality after 72 h for systemic bioassays and 48 h for leaf -dip bioassays.

Electrophoresis of non-specific esterases for *Bemisia tabaci* species determination

Polyacrylamide gel electrophoresis (PAGE) was used to discriminate between species and *B. tabaci* species complex as suggested by Gorman and Denholm (2000). For mass homogenates, 25 whitefly adult females from each population were homogenised in 20 µl of 1.6% Triton X-100 in distilled water, containing 10% sucrose and 0.01% bromocresol purple in an Eppendorf tube. The volume was adjusted to 90 µl with the same solution. A supernatant was obtained by applying 14,000 g centrifugation for 5 min at 4 °C and 7.5 µl aliquots of each homogenate were loaded onto a polyacrylamide gel as per Ornstein and Davis (1964). Gels were run at 250 V for 2 h at 4ºC and stained for 45–60 min in darkness at room temperature using 0.25 mL of 30 mM 1-naphthyl butyrate and 0.2% Fast Blue RR in 50 mL of phosphate buffer (pH 6.0). PAGE analysis of each population was repeated at least twice to confirm the consistency of banding patterns. After fixing in 7% acetic acid for 1 day, gels were photographed.

Glutathione-S-transferase activity

The glutathione-S-transferase activity was measured using the method of Habig et al. (1974). For mass homogenates, 10 whitefly adult females from each population were collected in Eppendorf tubes and homogenized in 50 µl Tris–HCl buffer (pH 7.5). The homogenate was centrifuged at 13,000 g at 4 °C for 5 min, and the supernatant was used as the enzyme source. These homogenates were then separated into 40 and 5 µl aliquots providing replicate plates for GST and total protein assays, respectively. For measurements of glutathione S transferase activity, 100 µl of GSH (final concentrations 0.4 mM) (Glutathione reduced) and 100 µl of CDNB (final concentrations 0.4 mM) (1-chloro-2,4-dinitrobenzene) was added to 100 µl aliquots of diluted insect homogenates. Reactions were then read kinetically at intervals of 10 s for 20 min at a wavelength of 340 nm at room temperature, using a Versamax kinetic microplate reader (Molecular Devices). Measurements of the total protein contents of whiteflies were determined according to Bradford (1976). For this, 5 µl of the homogenate prepared for the GST assay was transferred to a clean microplate well. After the addition of 250 µl of

Bradford reagent, the reaction was left for 15–20 min for incubation. The first row of wells served as a control without homogenate. The reactions were monitored at a wavelength of 620 nm using a Versamax microplate reader. GST activities of the samples were presented after correction for the amounts of protein present.

Total esterase activity

Esterase activities were conducted as described by Gomori (1953) with modifications by Grant et al. (1989). Mass homogenates of 10 adult females from each population were prepared using an Eppendorf compatible homogenizer in 50 µl 0.02 M phosphate buffer (0.02 M, pH 7.0), containing 0.1% Triton X-100. Following homogenization, the supernatant was collected after a 5 min centrifugation at 4 °C, at 14,000 rpm. From this homogenate, 10 µl was poured into a well of a microplate. The reaction was started by adding 200 µl of 1- naphthyl butyrate in 0.6% Fast Blue RR salt (diazotized 4-(benzoylamino)-2,5-dimethoxyaniline/ZnCl2) to give a final substrate concentration in the assay of 10 mM. The first row of wells was used as a control without homogenate. Activity was measured at 450 nm using a Versamax kinetic microplate reader (Molecular Devices, Menlo Park). Readings were taken for 5 min at 10 s intervals. The rate (mOD min^{-1}) was calculated by the integrated software Softmax Pro 5.2.

Monooxygenase activity

Cytochrome P450 activity was measured using the fluorometric assay method of Desousa et al. (1995). For mass homogenates, 100 whitefly adult females for each population were collected and homogenized in phosphate buffer (pH 7.6). The homogenate was centrifuged at 14,000 g at 4 °C for 5 min, and the supernatant was used as the enzyme source. 50 µl of the homogenate was transferred to a clean white microplate well, and 80 µl of a prepared substrate solution with 7-ethoxycoumarin (ECOD) was added. A microplate was prepared in this manner for each population and was incubated at 30 °C for 10 min. The reaction was initiated by adding 10 µl of 9.6 mM NADPH (β-Nicotamid adenine dinucleotide phosphate reduced tetrasodium salt). The amount of 7-hydroxycumarin released during the incubation was measured by SpectraMax M2 spectrofluorometer for 1 h in a SpectraMax M2 spectrofluometer and rates calculated by the integrated software, Softmax Pro 5.2. The excitation wavelength was 390, emission wavelength set to 460 nm taking readings at 30 s intervals. The non-microsomal pelleted cells were used as the control.

Insensitive acetylcholinesterase (AChE) by fluorometric method

Target-site insensitivity to AChE that would be associated with changes in susceptibility was evaluated for each whitefly population by measuring AChE sensitivity in the presence of chlorpyrifos-ethyl-oxon and pirimicarb. Inhibition of AChE in individuals was measured fluorometrically as described by Moores et al. (2000). Individual whiteflies were homogenized in separate wells of a microplate (Nunc) in 10 lµ of 0.1 M phosphate buffer, pH 7, containing 0.1% Triton X-100 (Roche Diagnostics GmbH, Manheim). A further 95 µl of the same buffer was added, remixed, and left for 10 min to solubilize. Replicate aliquots, equivalent to 0.25 of a whitefly individual, were then added to adjacent wells of fluorometric microtiter plates (Solid White). Diagnostic inhibitor concentrations were determined from preliminary experiments. In the absence and presence of diagnostic concentrations of the inhibitor, all homogenates were left for 10 min of incubation. A further 100 µl of ATChI was added to each aliquot. Reactions were monitored for 10 min in a SpectraMax M2 spectrofluometer and rates calculated by the integrated software, Softmax Pro 5.2. The excitation wavelength was 390 nm and the emission wavelength set to 473 nm. Final substrate and CPM concentrations were 0.5 mM and 1.0 mM respectively. To limit fluorescence caused by non-enzymatic substrate hydrolysis, ATChI solutions were always freshly prepared.

Resistance mutation diagnostic assays

DNA was extracted from a pool of 20 individuals using the "High Pure PCR Template Preparation (Roche)" kit, following the manufacturer's instructions. A total of 45 µl of genomic DNA was then obtained at concentrations ranging from 55 ng / µl to

146 ng / µl. Finally, whitefly DNA was put through PCR Amplification and purification of PCR products. The primers used to amplify L925I kdr gene include Bt-kdr-F1 5'-GCCAAATCCTGGCCAACT-3' and Bt-kdr-RIntr1 5'-GAGACAAAAGTCCTGTAGC-3' and to amplify F331W ace gene include Bt-ace-F 5'-TAGGGATCTGCGACTTCCC-3' and Bt-ace-R 5'-GTTCAGCCAGTCCGTGTACT-3'.

PCR-RFLP assays were conducted to identify the resistant mutations using the methods of Tsagkarakou et al. (2009). Female whiteflies obtained from the thirteen populations were analyzed for kdr (L925I) and ace (F331W) resistance mutations. Amplicons from 13 different populations were cut at 37 °C with *Dde*I restriction enzyme (Thermo) and ace amplicons at 65 °C with *Bsr*I (Thermo) restriction enzyme. For kdr, digestion of 184 bp PCR product was considered susceptible, as *Dde*I recognizes one site in the amplified fragment of the susceptible allele (L925), yielding two fragments of 124 and 60 bp, while the amplified fragment of the resistant allele (I925) does not contain a restriction site for *Dde*I, resulting in an intact fragment of 184 bp (Tsagkarakou et al., 2009). For ace, bands of 200 bp and 80 bp were considered susceptible (SS), while bands of 140 bp, 80 bp and 60 bp were considered as resistant (RR) populations upon digestion by *Bsr*I (Tsagkarakou et al., 2009). Finally, PCR-RFLP products were checked in 3.5% agarose gel electrophoresis.

Data analysis

Control mortality (consistently $< 10\%$ for field populations) was corrected by using Abbott's formula (Busvine, 1971). LC_{50} values were considered significantly different ($P < 0.05$) if their 95% confidence intervals did not overlap (Robertson et al., 2017). LC_{50} values were obtained by probit analysis (Finney, 1964), with the program POLO-PC (Leora Software, 1994). LC_{50} values (with confidence limits) and slopes of dose–response relationships were reported, and resistance factors were calculated by dividing LC_{50} values for field populations by the relevant LC_{50} value of the reference susceptible SUD-S population. The Pearson Correlation Coefficient statistic was conducted to explore correlation coefficients between monooxygenase activity, GST activity, esterase activity and all LC_{50} values (IBM, 2012).

Results

Bioassay studies

Increases in resistance factors for imidacloprid ranged between 4 and 303-fold. The most resistant population was Bilecik 2 (303-fold), followed by the Mihalgazi (88-fold) and Saricakaya 2 (73-fold) populations. The lowest resistance factor detected was in the Fethiye 1 population (fourfold) (Table 3).

Resistance to acetamiprid ranged from a 12-fold to a 282-fold increase in Fethiye 1 and Bilecik 2 populations, respectively (Table 4). The Saricakaya 2 population had the second-highest resistance factor (91-fold) with acetamiprid.

The highest resistance factors for thiamethoxam were found in the Mihalgazi and Geriskatirci populations with 15-fold increases (Table 5). The Derbent population in the Black Sea Region showed the lowest resistance to thiamethoxam.

Several resistance factors for chlorpyrifos-ethyl exceded a 30-fold increase, including up to Bilecik 3 (53-fold), followed by Derbent (44-fold) and Caycuma 1 (35-fold) populations. The lowest resistance factor was detected in the Fethiye 3 and Fethiye 1 populations with 1.4-fold and 1.9-fold increases, respectively (Table 6).

The highest resistance factor increases for deltamethrin were found in Mihalgazi (76-fold) and Bilecik 3 (65-fold) populations (Table 7). The Saricakaya 2 population in the Central Anatolia Region and Bilecik 2 in the Marmara Region showed the lowest resistance increase to deltamethrin at 20-fold.

Electrophoresis of non-specific esterases for *Bemisia tabaci* species determination

PAGE analysis revealed that esterase-banding patterns of all collected *B. tabaci* populations had a similar banding pattern, and the $E_{0.14}$ band was dominant in both greenhouse populations and the reference MEAM 1 *B. tabaci* (see supplementary information). All tested populations had a darker band sequence. This indicated that the esterase activities of tested populations were high.

Table 3 Log-dose probit mortality data for several populations of *Bemisia tabaci* tested with imidacloprid and resistance factors in systemic bioassays

Insecticide	Population	LC_{50} (mg/l^{-1})	CI (95%)[a]	Slope ± SE	*P* Value	RF[b]
IMIDACLOPRID	SUD-S	1.754	0.513–3.726	1.367(±0.191)	0,00	–
	Fethiye 1	7.237	3.102–14.504	0.830(±0.086)	0,00	4.13
	Fethiye 2	26.892	6.584–52.988	1.011(±0.235)	0,00	15.33
	Fethiye 3	78.713	37.911–215.843	0.929(±0.103)	0,00	44.88
	Saricakaya 1	38.394	18.183–97.010	0.700(±0.099)	0,00	21.89
	Saricakaya 2	128.232	37.154–2439.970	0.353(±0.093)	0,00	73.11
	Mihalgazi	154.257	39.562–6514.420	0.471(±0.110)	0,00	87.95
	Caycuma 1	22.607	0.248–83.011	0.679(±0.211)	0,00	12.89
	Caycuma 2	49.475	31.469–69.066	1.768(±0.259)	0,00	28.21
	Geriskatirci	36.827	19.169–79.834	0.901(±0.092)	0,00	21.00
	Derbent	22.152	9.869–40.328	1.041(±0.122)	0,00	12.63
	Bilecik 1	38.026	20.714–63.453	1.223(±0.157)	0,00	21.68
	Bilecik 2	531.474	220.318–10074.40	0.968(±0.211)	0,00	303.01
	Bilecik 3	26.868	11.727–48.092	1.131(±0.143)	0,00	15.32

[a]Confidence intervals 95%

[b]Resistance Factor = LC_{50} of greenhouse population/LC_{50} of SUD-S

Table 4 Log-dose probit mortality data for several populations of *Bemisia tabaci* tested with acetamiprid and resistance factors in systemic bioassays

Insecticide	Population	LC_{50} (mg/l^{-1})	CI (95%)[a]	Slope ± SE	*P* Value	RF[b]
ACETAMIPRID	SUD-S	0.394	0.048–1.011	0.792(±0.184)	0,88	–
	Fethiye 1	4.699	0.078–20.6130	0.804(±0.153)	0,00	11.92
	Fethiye 2	8.776	2.152–24.216	1.286 (±0.124)	0,00	22.27
	Fethiye 3	8.545	1.391–28.902	0.925(±0.100)	0,00	21.68
	Saricakaya 1	13.734	8.698–18.065	4.761(±1.078)	0,57	34.85
	Saricakaya 2	36.000	15.524–70.003	1.679(±0.267)	0,01	91.37
	Mihalgazi	14.128	4.058–2.973	2.342(±0.679)	0,93	35.86
	Caycuma 1	17.337	6.881–32.758	1.118(±0.142)	0,01	44.00
	Caycuma 2	13.578	3.601–31.286	1.165(±0.190)	0,05	34.461
	Geriskatirci	7.055	3.039–13.747	1.511(±0.140)	0,00	17.91
	Derbent	5.916	2.064–14.296	1.103(±0.142)	0,00	15.015
	Bilecik 1	9.725	3.376–21.797	1.667(±0.155)	0,00	24,68
	Bilecik 2	111.115	65.888–220.437	1.039(±0.156)	0,59	282.01
	Bilecik 3	18.012	5.447–27.305	2.912(±0.812)	0,36	45.72

[a]Confidence intervals 95%

[b]Resistance Factor = LC_{50} of greenhouse population/LC_{50} of SUD-S

Glutahione-S-transferase activity

In the present study, whitefly populations showed GST activities using CDNB as a substrate (Table 8). All field populations exhibited GST activities between 1.41 and 1.84 logmO.D./min./µg protein.

Gerişkatirci, Fethiye 1, and Derbent exhibited higher activity with values of 1.67 logmO.D./min./

Table 5 Log-dose probit mortality data for several populations of *Bemisia tabaci* tested with thiamethoxam and resistance factors in systemic bioassays

Insecticide	Population	LC_{50} (mg/l^{-1})	CI (95%)[a]	Slope ± SE	*P* Value	RF[b]
THIAMETHOXAM	SUD-S	2.300	0.595–5.072	1.106(±0.166)	0,25	–
	Fethiye 1	22.891	6.377–74.954	0.872(±0.144)	0,00	9.95
	Fethiye 2	14.648	11.056–18.880	2.560(±0.348)	0,73	6.37
	Fethiye 3	18.990	11.633–28.096	2.353 (±0.366)	0,28	8.26
	Saricakaya 1	21.911	13.065–34.655	1.389(±0.194)	0,28	9.53
	Saricakaya 2	13.054	3.158–27.608	1.555(±0.244)	0,00	5.68
	Mihalgazi	34.354	17.896–57.226	1.174(±0.261)	0,82	14.94
	Caycuma 1	11.500	8.139–14.684	3.108(±0.590)	0,91	5
	Caycuma 2	22.537	7.465–53.791	1.490(±0.202)	0,00	9.80
	Geriskatirci	34.040	21.181–47.306	2.083(±0.417)	0,79	14.80
	Derbent	8.863	2.594–20.446	1.558(±0.184)	0,00	3.85
	Bilecik 1	15.638	10.755–21.130	2.725(±0.396)	0,27	6.80
	Bilecik 2	14.949	2.456–29.388	2.134(±0.567)	0,23	6.50
	Bilecik 3	17.411	9.527–26.717	1.466(±0.259)	0,33	7.57

[a]Confidence intervals 95%

[b]Resistance Factor = LC_{50} of greenhouse population/LC_{50} of SUD-S

Table 6 Log-dose probit mortality data for several populations of *Bemisia tabaci* tested with chlorpyrifos-ethyl and resistance factors in leaf dip bioassays

Insecticide	Population	LC_{50} (mg/l^{-1})	CI (95%)[a]	Slope ± SE	*P* Value	RF[b]
CHLORPYRIFOS-ETHYL	SUD-S	6.594	2.862–10.982	1.886(±0.386)	0,97	–
	Fethiye 1	12.226	4.272–26.060	1.360(±0.148)	0,00	1.854
	Fethiye 2	24.382	14.428–41.851	1.922(±0.184)	0,00	3.697
	Fethiye 3	9.377	4.451–16.038	2.726(±0.369)	0,00	1.422
	Saricakaya 1	214.793	98.557–383.475	1.458(±0.286)	0,16	32.574
	Saricakaya 2	200.080	123.155–307.637	1.695(±0.248)	0,33	30.342
	Mihalgazi	170.796	69.835–232.406	5.015(±1.212)	0,01	25.90
	Caycuma 1	230.436	167.413–296.170	3.106(±0.535)	0,85	34.946
	Caycuma 2	137.489	71.105–290.030	1.273(±0.168)	0,01	20.850
	Geriskatirci	159.203	103.795–198.358	4.477(±0.823)	0,08	24.14
	Derbent	291.224	158.774–532.535	1.512(±0.256)	0,14	44.164
	Bilecik 1	173.289	47.597–48.777	1.463(±0.297)	0,03	26.28
	Bilecik 2	116.863	37.974–222.396	1.307(±0.238)	0,18	17.72
	Bilecik 3	349.927	170.857–758.336	1.173(±0.175)	0,11	53.07

[a] Confidence intervals 95%

[b] Resistance Factor = LC_{50} of greenhouse population/LC_{50} of SUD-S

µg protein, 1.69 logmO.D./min./µg protein, and 1.84 logmO.D./min./µg protein, respectively. In other words, field populations revealed higher GST activities than the susceptible reference population SUD-S. However, major differences in GST activities were not found between populations, suggesting that resistance developed against the insecticides tested in our study is not related to GST activity. This finding is

Table 7 Log-dose probit mortality data for several populations of *Bemisia tabaci* tested with deltamethrin and resistance factors in leaf dip bioassays

Insecticide	Population	LC_{50} (mg/l^{-1})	CI (95%)[a]	Slope ± SE	*P* Value	RF[b]
DELTAMETHRIN	SUD-S	1.764	0.103–4.231	0.936(±0.162)	0,00	–
	Fethiye 1	57.849	17.398–98.895	2.005(±0.246)	0,00	32.394
	Fethiye 2	61.322	39.812–82.468	3.273(±0.382)	0,00	34.763
	Fethiye 3	41.910	32.531–49.678	3.916(±0.640)	0,00	23.758
	Saricakaya 1	93.040	54.894–127.400	2.262(±0.425)	0,00	52.743
	Saricakaya 2	35.108	5.136–58.262	2.438(+0.765)	0,00	19.902
	Mihalgazi	134.722	83.190–185.357	2.169(+0.307)	0,00	76.373
	Caycuma 1	82.808	57.352–102.969	3.480(+0.662)	0,00	46,94
	Caycuma 2	42.394	5.436–84.869	1.389(±0.385)	0,00	24.032
	Geriskatirci	51.303	15.314–84.006	1.883(±0.462)	0,00	29.083
	Derbent	105.537	11.649–201.472	1.285(±0.308)	0,00	59.828
	Bilecik 1	64.699	25.914–88.643	3.511(±0.846)	0,00	36,68
	Bilecik 2	35.287	8.569–58.420	2.298(±0.600)	0,00	20.00
	Bilecik 3	114.289	78.363–148.856	2.376(±0.296)	0,00	64.79

[a] Confidence intervals 95%

[b] Resistance Factor = LC_{50} of greenhouse population/LC_{50} of SUD-S

Table 8 Glutathion-S-transferase, esterase, and monooxygenase activities of *Bemisia tabaci* populations

Population	GST Activity (logmO.D./min./µg protein)	Esterase Activity (logmO.D./min./whitefly)	Monooxygenase Activity (nmoles hydroxycoumarin/mg protein/hr)
SUD-S	1.42	2.36	4.90
Fethiye 1	1.69	2.57	5.26
Fethiye 2	1.56	2.67	5.30
Fethiye 3	1.55	2.68	5.40
Saricakaya 1	1.58	2.62	5.32
Saricakaya 2	1.48	2.69	5.44
Mihalgazi	1.61	2.34	5.43
Caycuma 1	1.45	2.52	5.09
Caycuma 2	1.57	2.08	4.87
Geriskatirci	1.67	2.04	5.06
Derbent	1.84	2.15	5.05
Bilecik 1	1.41	2.36	5.25
Bilecik 2	1.60	2.57	5.15
Bilecik 3	1.60	2.67	5.29

also supported by the lack of significant correlation coefficients between GST activity and all LC_{50} values (see supplementary information).

Total esterase activity

The highest esterase activity was found in the Saricakaya 2 population with a value of 2.69 logmO.D./min./whitefly, followed by Fethiye 3 with a value of 2.68 logmO.D./min./whitefly, then by Fethiye 2 and Bilecik 3 with values of 2.67 logmO.D./min./whitefly (Table 8). There was no significant difference in esterase activity among the tested field populations. This finding is also supported by the lack of significant correlation coefficients between esterase activity and all LC_{50} values (see supplementary information).

Monooxygenase activity

As was expected, SUD-S had one of the lowest activity levels of monooxygenase activity while the Mihalgazi and Saricakaya 2 populations from the Central Anatolia Region showed the highest activity with 5.44 and 5.43 nmoles hydroxycoumarin/mg protein/hr, respectively. The Central Anatolia Region populations generally revealed higher resistance factors for all tested neonicotinoid insecticides. Field populations of the Central Anatolia Region had higher P450 activities than the susceptible reference population (Table 8). There were no significant correlation coefficients between P450 activity and all LC_{50} values (see supplementary information).

Insensitive acetylcholinesterase (AChE) by fluorometric method

The field populations exhibited varying rates of modified AChE activity, with a sensitive and an insensitive group (Fig. 1). The AChE activity of the susceptible SUD-S population was inhibited by chlorpyrifos-ethyl oxon and pirimicarb compared to the tested field populations.

Resistance mutation diagnostic assays

PCR-RFLP diagnostic assays were used to analyze field-collected females for the presence of the L925I mutation in the *para*-type sodium channel (pyrethroid) as per Tsagkarakou et al. (2009). As the amplified fragment of the resistant allele (I925) does not contain a restriction site for *Dde*I, the kdr mutation seems to be fixed in field-collected populations based on the presence of an 184 bp band (Fig. 2).

The presence of four bands (200 bp, 140 bp, 80 bp, and 60 bp) following *Bsr*I digestion showed that the ace mutation (F331W) was not fixed in some of the populations. On the other hand, the mutation was fixed in Caycuma 1, Bilecik 1, 2, and 3, Saricakaya 2, and Fethiye 3 (Fig. 3).

Discussion

Levels of pyrethroid, organophosphate, and neonicotinoid resistance in *B. tabaci* were identified from greenhouses located in the Aegean, Central Anatolia, Black Sea, and Marmara regions of Türkiye in this study. Various levels of cross-resistance in *B. tabaci* populations to all tested neonicotinoid insecticides were also identified. In particular, the Bilecik 2 and Sarıcakaya 2 populations showed very high resistance levels (303- and 66-fold, respectively), while also showing cross-resistance to acetamiprid (282- and 91-fold, respectively). Biological, operational, and ecological factors can cause variation in cross-resistance in *B. tabaci* among neonicotinoids in different regions (Prabhaker et al.,

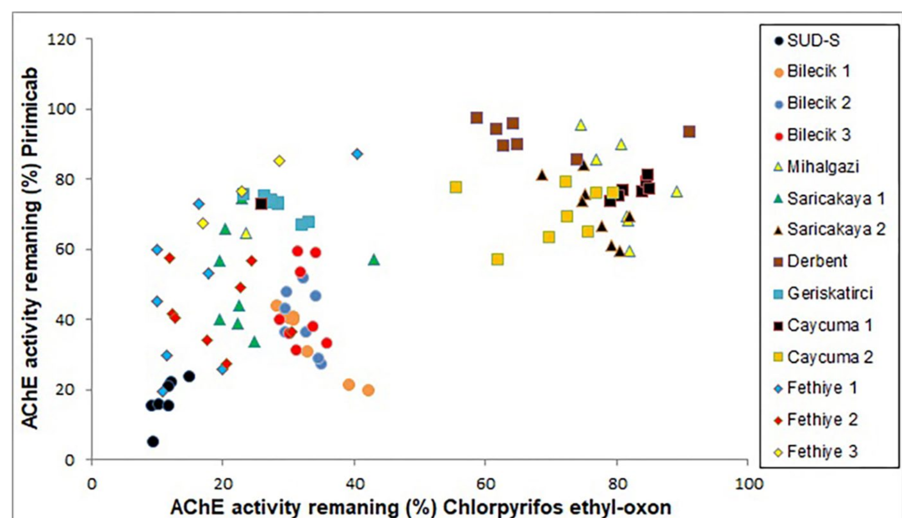

Fig. 1 Bivariate plot of mean % activity remaining during inhibition of AChE from populations of *Bemisia tabaci* by 10 µM chlorpyrifos-ethyl-oxon and 100 µM pirimicarb

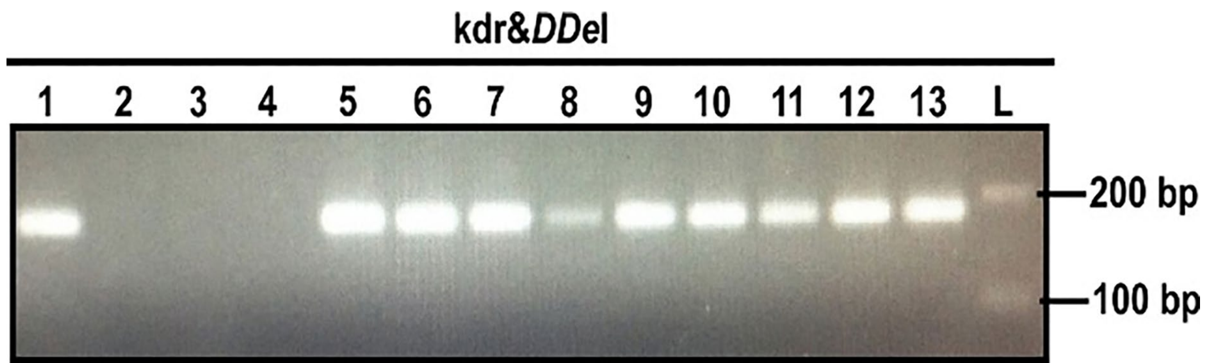

Fig. 2 PCR-RFLP detection of the kdr mutation in field populations of *Bemisia tabaci*. The PCR product was digested with the restriction enzyme *Dde*I (**1:** Caycuma 1, **2:** Caycuma 2, **3:** Derbent, **4:** Geriskatirci, **5:** Bilecik 3,**6:** Bilecik 2, **7:** Bilecik 1, **8:** Saricakaya 1, **9:** Saricakaya 2, **10:** Mihalgazi, **11:** Fethiye 1, **12:** Fethiye 2, **13:** Fethiye 3)

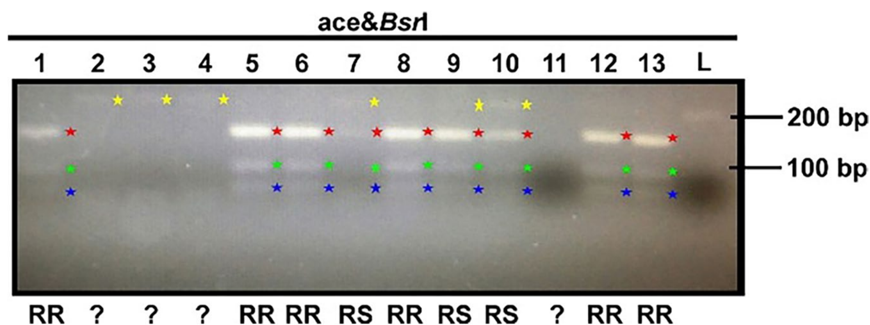

Fig. 3 PCR-RFLP detection of the F331W ace mutation in field populations of *Bemisia tabaci*. The PCR product was digested with the restriction enzyme *Bsr*I (**1:** Caycuma 1, **2:** Caycuma 2, **3:**Derbent, **4:**Geriskatirci, **5:** Bilecik 3, **6:** Bilecik 1, **7:** Saricakaya 1, **8:** Saricakaya 2, **9:** Mihalgazi, **10:** Fethiye 1, **11:** Fethiye 2, **12:** Fethiye 3, **13:** Bilecik 2)

2005). Although greenhouse agricultural production has started recently in the Aegean, Central Anatolia, Black Sea, and Marmara regions, neonicotinoid insecticides are no longer consistently effective. If the individuals in the population are homogeneous in terms of sensitivity, a steep logarithmic dose-probit line is formed, while with increasing heterogeneity in the population, the slope becomes flat. As the homogeneity of the resistance genes increases, the logarithmic dose-probit line becomes steeper again (Hoskins & Gordon, 1956). The slope of the logarithmic dose probit line against these insecticides was flat in field populations of *B. tabaci*. The reduced slope of the logarithmic dose-probit curves suggests heterogeneity in neonicotinoid resistance in these field populations of whiteflies.

In Türkiye, *B. tabaci* resistance to neonicotinoids is a serious problem, especially in the Mediterranean region. Satar et al. (2018) indicated that neonicotinoid usage expanded 900-fold from 2001 to 2011 in Türkiye, and a number of studies have found moderate to high levels of resistance to neonicotinoids in *B. tabaci* in Türkiye (Bahsi et al., 2012; Balkan & Kara, 2020; Dağlı et al., 2007; Sahin & Ikten, 2017; Satar et al., 2018). Similarly, high levels of resistance to imidacloprid were found in whitefly populations (up to 1958-fold) in Crete (Greece), which was strongly attributed to the frequency of neonicotinoid applications (Roditakis et al., 2009), while both MEAM1 and MED populations of *B. tabaci* had 28 to 1900-fold resistance to imidacloprid in China (Wang et al., 2010). On the other hand, low resistance levels to

acetamiprid were observed in *B. tabaci* (RF 7–12) in Cyprus (Vassiliou et al., 2011), while a 33.0-fold resistance to acetamiprid was found in China (Lou et al., 2010). Kontsedalov et al. (2012) found a 74-fold acetamiprid resistance in MED *B. tabaci* in Israel. Bahsi et al. (2012), Sahin and Ikten (2017), and Satar et al. (2018) found that acetamiprid resistance was 299-fold, 30.4-fold, and 68.2-fold in the Mediterranean region of Türkiye, respectively. These differences among levels of neonicotinoid resistance may be attributed to the application frequency, dosage of the applied insecticide and history of insecticide applications.

The highest resistance factors in the present study were found with chlorpyrifos-ethyl (44 to 53-fold) and deltamethrin (65 to 76-fold). Resistance to pyrethroids and organophosphates have already been documented in both *B. tabaci* and *T. vaporariorum* in different regions of Türkiye (Bahsi et al., 2012; Dittrich et al., 1990; Erdogan et al., 2008, 2012, 2021). Resistance to pyrethroids and organophosphates in *B. tabaci* have been reported in different countries such as Greece, USA etc. (Horowitz et al., 1988, 2020; Prabhaker et al., 1988; Dittrich et al., 1990, 1990; Byrne & Devonshire, 1991, 1993; Denholm et al., 1998; Moores et al., 2000; Roditakis et al., 2006, 2011). *Bemisia tabaci* individuals collected in the present study from greenhouses in Türkiye were assigned to the geographically widespread MEAM1 of *B. tabaci*. The MEAM1 of *B. tabaci* is known to be common in Türkiye and is the dominant species in vegetable growing areas (Bayhan et al., 2006; Erdogan et al., 2008, 2011; Ikten et al., 2007; Karut et al., 2014, 2015; Sahin & Ikten, 2017; Satar & Ulusoy, 2016; Yükselbaba & Ali, 2022).

Although some populations were collected from similar places and even from the same host plant, the resistance level of the populations was different. For example, Bilecik 1, 2, and 3 populations exhibited significantly different resistance ratios to imidacloprid; Saricakaya 1 and 2 populations exhibited significantly different resistance ratios to acetamiprid, imidacloprid and thiamethoxam. These differences may be due to the application frequency, dosage of the applied insecticide and history of insecticide applications. In addition, greenhouses are of varying sizes and each greenhouse owner uses different agricultural practices, control methods, irrigation and fertilization systems. Pests are therefore exposed to different pesticide regimes, which can trigger different physiological and genetic factors that can lead to the development of resistance (Satar et al., 2018). Some growers try to minimize input costs, including those related to insecticides. This can lead to the development of different resistance levels, even if in the similar area.

Total esterase activity can be linked to organophosphate and pyrethroid resistance through enhanced activity in *B. tabaci* (Byrne & Devonshire, 1991; Denholm et al., 1998; Dittrich et al., 1990; Erdogan et al., 2008; Horowitz et al., 1988; Prabhaker et al., 1988; Roditakis et al., 2006). Our results showed enhanced esterase activities among most field populations, suggesting that the resistance developed against tested pyrethroid insecticides was likely at least partially to account for the esterase activity. This conclusion is supported by other studies conducted in both *B. tabaci* and *T. vaporariorum* in Türkiye (Erdogan et al., 2008, 2021) and in other countries (Dittrich et al., 1990; Horowitz et al., 1988; Prabhaker et al., 1988; Roditakis et al., 2006). Although several studies reported that GST can be involved in the resistance mechanism in the sweet potato whitefly (Prabhaker et al., 1988), we did not find evidence of GST as a resistance mechanism in our study. Whitefly populations in our study exhibited increased levels of 7-ethoxycoumarin O-deethylase (ECOD) activity relative to the susceptible SUD-S (Table 8). There was also no positive relationship between cytochrome P450 activity and insecticide resistance in the present study. Although high factors of resistance for neonicotinoid resistance have been associated with enhanced cytochrome P450 enzyme activities, it is unlikely to be the only mechanism (Satar et al., 2018). The authors found that elevated cytochrome P450 monooxygenases were associated with neonicotinoid resistance in the Mediterranean Region of Türkiye and their results also support our conclusions. Enhanced detoxification is reported as the main mechanism for conferring resistance against imidacloprid in strains of both *B. tabaci* MEAM1 and MED (Horowitz et al., 2020; Karunker et al., 2008, 2009; Nauen et al., 2002; Rauch & Nauen, 2003; Roditakis et al., 2011; Wang et al., 2009).

The most effective mechanism of resistance to organophosphates in *B. tabaci* and *T. vaporariorum* is likely the modification of the AChE enzyme, which is the target site of organophosphate and carbamate insecticides (Byrne & Devonshire, 1993; Dittrich

et al., 1990; Erdogan et al., 2008, 2012; Moores et al., 2000). Our study showed that the field populations had varying rates of modified AChE activity. There were clearly two groups on the bivariate plot, with the insensitive group consisting of Caycuma 1, Caycuma 2, Derbent, Saricakaya 2, and Milhagazi (Fig. 1). This is the first report of different levels of insensitive AChE enzyme variant in Turkish *B. tabaci* MEAM 1 populations that may also confer insensitivity to chlorpyrifos-ethyl oxon and therefore resistance to chlorpyrifos-ethyl. AChE insensitivity assays for individual *B. tabaci* suggest a target-site modification as a mechanism of resistance to chlorpyrifos-ethyl. Results showed that the ace mutation was fixed in Çaycuma 1, Bilecik 3, Bilecik 1, Sarıcakaya 2, Fethiye 3, and Bilecik 2 populations. Tsagkarakou et al. (2009) identified a fixed ace mutation in whitefly populations; however, the populations showed a low resistance level to pirimiphos methyl. The authors suggested that the ace mutation may have been selected by repetitive treatments with carbamates or other organophosphates and not by prolonged exposure to pirimiphos methyl. Tsagkarakou et al. (2009) results support our results related to a fixed ace mutation and modified AChE activity in whitefly populations but showed low resistance levels to chlorpyrifos ethyl.

Conclusion

The present study revealed varying levels of neonicotinoid, pyrethroid, and organophosphate resistance in greenhouse *B. tabaci* populations in different regions of Türkiye. Our study can help to improve insect resistance management programs in greenhouses in Türkiye. Growers should use different control tactics such as cultural and biological control (preserving and releasing parasitoids and predators) for more sustainable agriculture. When considering new classes of insecticides such as Insect Growth Regulators and bioinsecticides with IPM approaches, pest scouting, the use of economic thresholds, the application rate, the number of applications, and intervals specified in the label should be strictly followed. It is recommended that the consecutive (repetitive) use of any insecticide in the same production season should be avoided, and rotation of different modes of action should be used. Regular monitoring of *B. tabaci* species and the status of insecticide resistance will help to make strategic decisions to control *B. tabaci* populations.

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Data availability The experimental data is available with the corresponding author in the study.

Declarations

Competing interest The authors declare no competing interests.

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