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Insecticide resistance status and vector potential of *Bemisia tabaci* populations on vegetable crops in Türkiye

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ABSTRACT

The sweet potato whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), is an economically important polyphagous pest species with a global distribution. This pest not only causes direct damage by sucking plant phloem sap but also transmits viruses and excretes honeydew, which can lead to the formation of black sooty mould, thereby intensifying its significance in agricultural regions. Although chemical insecticides have been extensively used for whitefly control, the development of resistance leading to control failures has been frequently documented. Here, we have initially assessed the efficacy of four commonly used insecticides (acetamiprid, sulfoxaflor, spirotetramat, cyantraniliprole) against whitefly populations through greenhouse trials across eight distinct locations. Additionally, we conducted a comprehensive molecular screening of 35 field populations to identify resistance mutations at the insecticide target sites and to detect plant pathogenic viruses. The results revealed that sulfoxaflor and cyantraniliprole exhibited the highest efficacy against nymphal stages of whiteflies, whereas acetamiprid was determined to be the most effective insecticide against adult stages. Several well-known target-site mutations in acetylcholinesterase (F331W), voltage-gated sodium channel (VGSC; M918L, L925I, T929V), and acetyl-CoA carboxylase (A2083V) were found to be widespread in *Turkish B. tabaci* populations. Additionally, two mutations, I936V and I936F, previously associated with pyrethroid resistance, were identified for the first time in the VGSC of *B. tabaci*. Conversely, no amino acid substitutions were detected in the amplified fragments of the ryanodine and nicotinic acetylcholine receptors. Furthermore, tomato chlorosis virus (ToCV) was detected in five field populations from Antalya. The widespread distribution of whitefly populations with multiple resistance mutations underscores the necessity of implementing integrated pest management programs in Turkish vegetable production areas.

1. Introduction

The southern part of Türkiye boasts the largest greenhouse area and is recognized as the country's vegetable factory, with Antalya hosting more than 40% of all greenhouses nationwide (TSI, 2023). However, the favorable climate conditions for vegetable production also foster the rapid development of several plant pests, leading to substantial economic losses (İnak et al., 2019). The intensive use of insecticides to control these notorious pests is, therefore, widespread. In 2022, the country utilized a total of 12,205 metric tons of insecticides, nearly doubling the amount used a decade earlier, which amounted to 7264 metric tons in 2012 (TSI, 2023). Among these pests, one of the most

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common and destructive is *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), also known as the sweet potato whitefly.

Bemisia tabaci is recognized as a species complex, with the Mediterranean (MED) and Middle East – Asia Minor (MEAM1) being the most well-known (De Barro et al., 2011; Firdaus et al., 2013). It is a significant pest capable of feeding on hundreds of host plants, resulting in direct economic losses of up to billions of dollars (Henneberry and Faust, 2008). Additionally, this pest can transmit over 200 virus diseases, such as begomoviruses, leading to potential losses amounting to billions of dollars (Hogenhout et al., 2008; Jones, 2003; Lapidot and Polston, 2010; Navas-Castillo et al., 2011). Furthermore, the excretion of honeydew by this pest promotes the growth of black sooty mould, impairing photosynthesis and exacerbating the overall level of damage and quality of produce (Oliveira et al., 2001).

Various control methods, such as host plant resistance and biological control, are available for managing whiteflies (Li et al., 2021; Nombela and Muñiz, 2010; Perring et al., 2018; Sani et al., 2020). Nevertheless, the predominant control method on a global scale remains the application of chemical insecticides (Horowitz et al., 2020; Palumbo et al., 2001). The vector characteristics of whiteflies also contribute to the extensive use of insecticides, particularly in situations where notorious virus diseases are identified, and economic injury levels are not well-established. However, instances of failures in pest management are frequently reported, with resistance development considered a primary contributing factor (Horowitz et al., 2020).

B. tabaci can rapidly develop resistance due to its short life cycle, high fecundity, and multivoltine nature (Basit, 2019; Horowitz et al., 2020). More than 750 cases have been reported for the development of resistance in *B. tabaci* populations to over 65 insecticides (Mota-Sanchez and Wise, 2023). Physiologically, insecticide resistance can occur through toxicokinetic and toxicodynamic processes in arthropods (Feyereisen et al., 2015). The former often involves the participation of detoxification enzymes, leading to a reduced amount of the toxic substance before reaching its target site via metabolism and/or sequestration. Furthermore, efflux pumps such as ATP-binding cassette (ABC) transporters can effectively decrease xenobiotic toxicity by reducing their concentration (Amezian et al., 2024; Dermauw and Van Leeuwen, 2014). Toxicodynamic resistance is frequently associated with target-site mutations, leading to decreased binding, as well as altered expression by duplication or deletion of the target site (Feyereisen et al., 2015). In many cases, resistance is complex and involves multiple mechanisms that additively or synergistically contribute to the phenotype. The effective management of insecticide resistance can only be achieved with a comprehensive understanding of both the resistance status and underlying mechanisms, coupled with a determination of their distribution (Van Leeuwen et al., 2020). Monitoring of insecticide resistance is typically conducted under laboratory conditions. However, it is important to note that stable laboratory conditions may not always accurately reflect the complex field conditions influenced by multiple factors (Ffrench-Constant, 2013).

Acetylcholinesterase (AChE) inhibitors such as organophosphates (OPs) and carbamates have long been used to control insect pests including whiteflies in Türkiye. Although most of the insecticides within these chemical groups have been banned, pirimiphos-methyl is still largely used for whitefly control (PPPD, 2023). Target-site insensitivity was suggested to be the major mechanism for resistance development across arthropods (Fournier, 2005; Lee et al., 2015). In the case of *B. tabaci*, an F331W (*Torpedo californica* numbering) mutation in AChE1 was associated with decreased susceptibility (Alon et al., 2008).

Pyrethroids act on voltage-gated sodium channel (VGSC), responsible for the rapid influx of sodium ions into the cell, leading to a change in membrane potential and the initiation of an action potential (Dong et al., 2014; Scott, 2019). Cypermethrin, etofenpyrox, lambda-cyhalothrin (in combination with buprofezin) are registered insecticides targeting whiteflies in Türkiye (PPPD, 2023). So far, three mutations — M918L, L925I and T929V (*Musca domestica* numbering) in the VGSC — have been reported to confer resistance in whiteflies (Morin et al., 2002; Roditakis et al., 2006).

Cyclic keto-enols inhibit acetyl-CoA carboxylase (ACC), resulting in a decrease in lipid content (Lümmen et al., 2014). Spiromesifen is known to be effective against both spider mites and whiteflies (Bretschneider et al., 2007), while spirotetramat can control both aphids and whiteflies with its unique phloem and xylem mobility (Nauen et al., 2008; Toprak et al., 2024). A target-site mutation, A2083V (*B. tabaci* numbering), in a highly conserved region of the carboxyltransferase domain (CT) in ACC of *B. tabaci* was uncovered and its role on keto-enol resistance was functionally validated (Lueke et al., 2020).

Neonicotinoids specifically bind to and activate nicotinic acetylcholine receptors (nAChRs), leading to the prolonged opening of the ion channels (Jeschke and Nauen, 2008). Along with aphids, acetamiprid has been used widely against whiteflies in Türkiye (PPPD, 2023). Although point mutations in the β1 subunit of nAChR of aphids were reported to confer resistance to neonicotinoids (Bass et al., 2011; Xu et al., 2022), no point mutations in nAChRs were found in whiteflies for a long time. Recently, Yin et al. (2024) reported two mutations (A58T and R79E; *B. tabaci* numbering) in the target-site and validated their role in resistance via transgenic flies and 3D modelling.

Diamide insecticides selectively act on ryanodine receptors (RyRs), pivotal proteins responsible for orchestrating calcium release, a process vital for the regulation of muscle contraction (Jeanguenat, 2013; Sattelle et al., 2008; Toprak et al., 2021). Although several target-site mutations were uncovered in lepidopteran species such as I4790 M/K and G4946 E/V (*Plutella xylostella* numbering) (İnak et al., 2021; Richardson et al., 2020), no target-site mutations have been reported in whiteflies so far, despite the widespread use of cyantraniliprole across the world, including in Türkiye (PPPD, 2023).

Resistance to traditional insecticide groups such as organophosphates, pyrethroids and insect growth regulators was documented as early as 15 years ago in Turkish populations of *B. tabaci* (Bahşi et al., 2012; Erdogan et al., 2008). On the other hand, resistance to neonicotinoids have also been reported in *B. tabaci* populations collected from the Mediterranean Region of Türkiye more recently (İkten and Şahin, 2017; Mohammed et al., 2020; Satar et al., 2018). In addition, high to extremely high levels of resistance ratios have been reported for spirotetramat and pyriproxyfen in whitefly populations from Antalya (Yükselbaba and Alı, 2022). These results clearly indicate the growing resistance issue, especially in southern Türkiye. A recent study revealed the presence of AChE (F331W) and VGSC (L925I) mutations via PCR-RFLP method in several *B. tabaci* populations collected over a decade ago across Türkiye (Erdogan et al., 2024). Beyond these few reports, no studies have yet investigated the presence and distribution of target-site resistance mutations, underscoring the imperative need for further data acquisition.

In the present study, we evaluated the efficacy of four commonly used insecticides (acetamiprid, sulfoxaflor, spirotetramat, cyantraniliprole) on nymphs and adult stages of *B. tabaci* populations in greenhouse conditions at eight different locations. Next, we performed an area-wide molecular screening for mutations at the target-site of widely used insecticides in 35 field-collected *B. tabaci* populations. All populations were molecularly identified, and their cryptic species status was determined based on mitochondrial cytochrome oxidase subunit 1 (COI) sequences. Last, the presence of plant pathogenic viruses in *B. tabaci* populations was determined, and the vector potential of whiteflies in vegetable production areas of Türkiye was discussed.

2. Materials and methods

2.1. Bemisia tabaci populations

A total of 35 *B. tabaci* populations were collected from vegetable crops from different geographical regions of Türkiye (Fig. 1, Table S1). The white flies were stored in 90% ethanol and RNAlater (Thermo Fisher

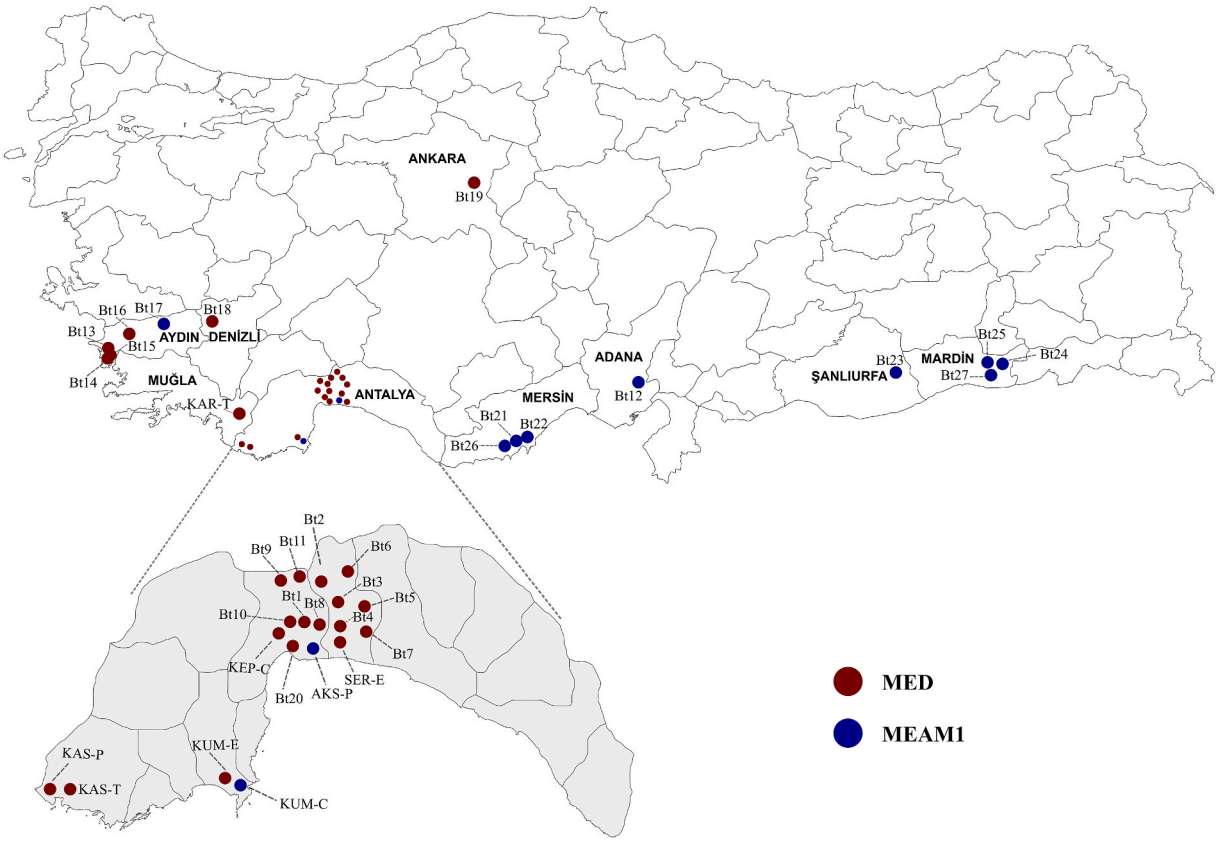

Fig. 1. Geographical map depicting the sampling locations of *Bemisia tabaci* populations across Türkiye. Red and blue dots indicate MED and MEAM1 species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Scientific, USA) at −20 °C for a maximum of 3 months until used in DNA and RNA extraction, respectively.

2.2. Greenhouse trials

Commercial formulations of four insecticides (acetamiprid, sulfoxaflor, spirotetramat, cyantraniliprole) were used in greenhouse trials. Detailed information for insecticides, such as trade names, field concentrations, IRAC mode of action group is presented in Table 1.

Greenhouse insecticide trials were performed in eight different locations in southern Türkiye (mainly in Antalya) in 2021 (Fig. 1), according to EPPO (European and Mediterranean Plant Protection Organization) field trial guidelines for whiteflies (PP1/036(3)), with slight modifications (EPPO, 2009). Trials were initiated when an average of more than 5 nymphs or pupae of *B. tabaci* were detected on a single leaf during a systematic edge-to-center examination of at least 40 plants within the trial area. A randomized complete block design was employed, consisting of three replications of 20 m^2 plots, each containing at least 40 plants per treatment. One row of plants was used as a no-spray buffer zone between each plot. Registered field concentrations of selected insecticides were used using an engine backpack sprayer (Oleo-Mac SP126, Italy) equipped with a full cone nozzle (1 mm diameter) (Table 1), while control plots were treated with water. Surviving adults were counted at 1 and 3 days after treatment (DAT) while surviving nymphs were counted at 6, 10, and 17 DAT. Random sampling of two leaves from the middle sections of the 10 plants in each plot was performed to assess mortality rates. Detailed information on greenhouse trials such as plant varieties, greenhouse temperature, and application time is given in Table S2.

The treatment results were evaluated using the Henderson–Tilton formula. Before conducting analyses, we assessed the equality of variances among groups using Levene's test and tested for normality of the distribution of data using the Kolmogorov–Smirnov test. The data were analyzed via one-way ANOVA using the MINITAB Release 16 package program. Mean differences were then grouped using Tukey's test at a significance level of $\alpha = 0.05$.

2.3. DNA/RNA extraction and cDNA synthesis

DNA and RNA were isolated from the pools of 10 adult flies using the DNeasy Blood & Tissue Kit (Qiagen, Germany) and PureLink RNA Mini Kit (Thermo Fisher Scientific, USA), respectively, following the

Table 1
Detailed information on insecticides used in greenhouse trials.

Active ingredient	Trade name	Field concentration	AI field rate (g ha^{-1})	IRAC MoA[a]	Registration date
Acetamiprid	Mospilan 20 SP	30 g/100 L	60	4A	1996
Sulfoxaflor	Breaker 240 SC	30 ml/100 L	72	4C	2016
Spirotetramat	Movento 240 SC	100 ml/100 L	240	23	2009
Cyantraniliprole	Benevia 100 OD[b]	100 ml/100 L	100	28	2015

[a] Insecticide Resistance Action Committee (www.irac-online.org) mode of action classification.
[b] Applied in combination with adjuvant (Codacide oil) at a rate of 250 ml per 100 L.

manufacturer's instructions. The quality and quantity of the extractions were assessed using a spectrophotometer (Thermo Scientific NanoDrop™ 2000, USA) and gel electrophoresis. Complementary DNA (cDNA) synthesis was conducted with the iScript cDNA synthesis kit (BioRad, USA). The resulting DNAs/cDNAs were subsequently utilized for gene amplification and detection of plant pathogenic viruses through PCR.

In VGSC, we identified a combination of several mutations in some populations. Therefore, we extracted individual DNA from 25 female whiteflies to further investigate which combinations occur within a single haplotype. DNA extractions from individual flies were performed following the method used by İnak et al. (2024). Briefly, flies were crushed in a mixture containing 20 µl of STE buffer (10 mM Tris-HCL, 100 mM NaCl, 1 mM EDTA, pH 8) and 2 µl (10 mg/mL) proteinase K (Sigma-Aldrich). Then, the solutions were incubated at 37 °C for 30 min followed by proteinase K inactivation at 95 °C for 5 min. After centrifugation, the supernatants were used as a template for PCR reactions.

2.4. Species identification and phylogenetic analysis

Species identification was performed using a partial fragment of *cytochrome oxidase subunit I* (*COI*) sequence via BLASTn and phylogenetic analysis of both obtained and reference sequences. PCR amplifications were performed in total volume of 30 µl volume, containing 2 µl of DNA (90–250 ng/µl), 1 µl of both forward and reverse primer, 11 µl of ultrapure nuclease-free water, and 15 µl of EmeraldAmp® MAX PCR master mix (TaKaRa, Japan). The primers and PCR conditions used are presented in Table S3.

A maximum likelihood (ML) phylogenetic tree with 1000 bootstrap replicates was constructed using MEGA (Molecular Evolutionary Genetics Analysis) v11 (Tamura et al., 2021) to differentiate between *B. tabaci* species, specifically MED and MEAM1. Additionally, COI sequences from two reference complete mitochondrial genomes representing the MED (accession number: MH205753) and MEAM1 (accession number: KR559508) species of *B. tabaci* were integrated into the analysis. The HKY + G model was determined as the best-fit model based on the Bayesian Information Criterion (BIC) score in MEGA. Genetic variation among Turkish whitefly populations was assessed in MEGA using K2 parameters with 1000 bootstrap iterations.

2.5. Amplification of insecticide target-sites

Target-sites of commonly used insecticides in *B. tabaci* control, voltage-gated sodium channel (VGSC), acetylcholinesterase (AChE), acetyl-CoA carboxlyase (ACC), ryanodine receptor (RyR) and nicotinic acetylcholine receptor subunit 6 (nAChRα6) were screened for the presence of resistance mutations. Primers, along with the corresponding target genes and PCR conditions used to amplify those genes, are presented in Table S3. Primer3web version 4.1.0 (Untergasser et al., 2012) was used to design new primers based on the available full genome of *B. tabaci* (http://www.whiteflygenomics.org; Chen et al., 2016). The PCR components were the same as described above. PCR amplicons were sequenced using the same primers, except for VGSC (see Table S3). The obtained sequences were analyzed using BioEdit 7.0.5 (Hall, 1999) and Unipro UGENE software (Okonechnikov et al., 2012). The mutations were checked based on visual inspection of sequencing chromatographs (İnak et al., 2019).

2.6. Screening of plant pathogenic viruses

The presence of plant pathogenic viruses in field-collected *B. tabaci* populations was determined via PCR assays.

Two pairs of universal primer sets were employed for DNA viruses to screen for the genus Begomovirus, which belongs to the family Geminiviridae. Additionally, coat protein (CP) gene region-specific primers were utilized for tomato yellow leaf curl virus (TYLCV).

For RNA viruses, degenerate primer sets were employed for the molecular detection of members of the Potyvirus, Polerovirus, and Torradovirus genera. Furthermore, CP gene region-specific primers were applied for tomato chlorosis virus (ToCV; Crinivirus), southern tomato virus (STV; Amalygavirus), and cucumber mosaic virus (CMV; Cucumovirus).

Sequences of primers together with PCR conditions are presented in Table S3. PCR reactions were performed via EmeraldAmp® MAX PCR master mix (Takara, Japan) as described above. Positive control samples were included for each reaction.

A neighbour-joining (NJ) phylogenetic tree with the T92 model was constructed using MEGA (Molecular Evolutionary Genetics Analysis) v11 (Tamura et al., 2021) incorporating ToCV sequences obtained in this study along with those retrieved from the public GenBank database. An isolate of sweet potato chlorotic stunt virus (SPCSV; accession number LT993430) was designated as the outgroup.

3. Results

3.1. Species confirmation and phylogenetic analysis

BLAST analysis of COI sequences from sampled *B. tabaci* populations showed high similarity (>99.45%) to *B. tabaci* sequences in the public GenBank database. Based on the constructed phylogenic tree, 24 out of 35 populations were determined to be MED species, whereas 11 populations were MEAM1 species (Fig. 2). Overall genetic variation among Turkish whitefly populations was 2.59% ± 0.41 (SE).

3.2. Greenhouse trials

The efficacy of four insecticides against both larval and adult stages of *B. tabaci* under greenhouse conditions is presented in Fig. 3 (see also Tables S4–S6).

In the adult stages, acetamiprid caused significantly higher mortality at 1 and 3 DAT compared to other tested insecticides in all trials (Fig. 3, Table S4). Notably, even for acetamiprid, the mortality rates were consistently below 80% in the adult stages. Conversely, spirotetramat exhibited the lowest efficacy (28–42% at 1 DAT and 17–41% at 3 DAT) in the adult stages.

The efficacy of all insecticides, except acetamiprid, was higher on larval stages. Sulfoxaflor and cyantraniliprole exhibited the highest efficacy across all time points with average mortalities of 81.5% ± 0.75 (SE) and 81.0% ± 1.15 (SE) at 6 DAT, respectively, followed by spirotetramat, which exhibited 72.4% ± 0.81 (SE) mortality. Acetamiprid was the least efficient insecticide on the larval stages of whiteflies, and mortality rates were always lower than 70% (even lower than 50% for KUM-E and SER-E). In most cases, there were no significant differences between the different counting time points.

3.3. Screening of target-site mutations

The presence of target-site mutations in 35 field-collected *B. tabaci* populations is detailed in Table 2. All obtained samples exhibited the F331W mutation fixed in AChE, with the exception of population Bt27 where the mutation was present but not fixed. Three known VGSC mutations (M918L, L925I, T929V) were detected in 7, 34 and 11 populations, respectively. In addition, two novel mutations, I936V (ATT > GTT) and I936F (ATT > TTT), were found in 8 whitefly populations, but not fixed. While four populations (Bt1, Bt3, Bt6, Bt7) harboured all four VGSC mutations, none were fixed across all populations, except population Bt7 where the L925I mutation was fixed. Determination of VGSC haplotypes through individual genotyping revealed that the L925I and L929V mutations were never concurrently present within the same haplotype (Table S7).

Among the 35 Turkish *B. tabaci* samples, 26 populations had the A2083V mutation in ACC. Notably, this mutation was fixed in 10

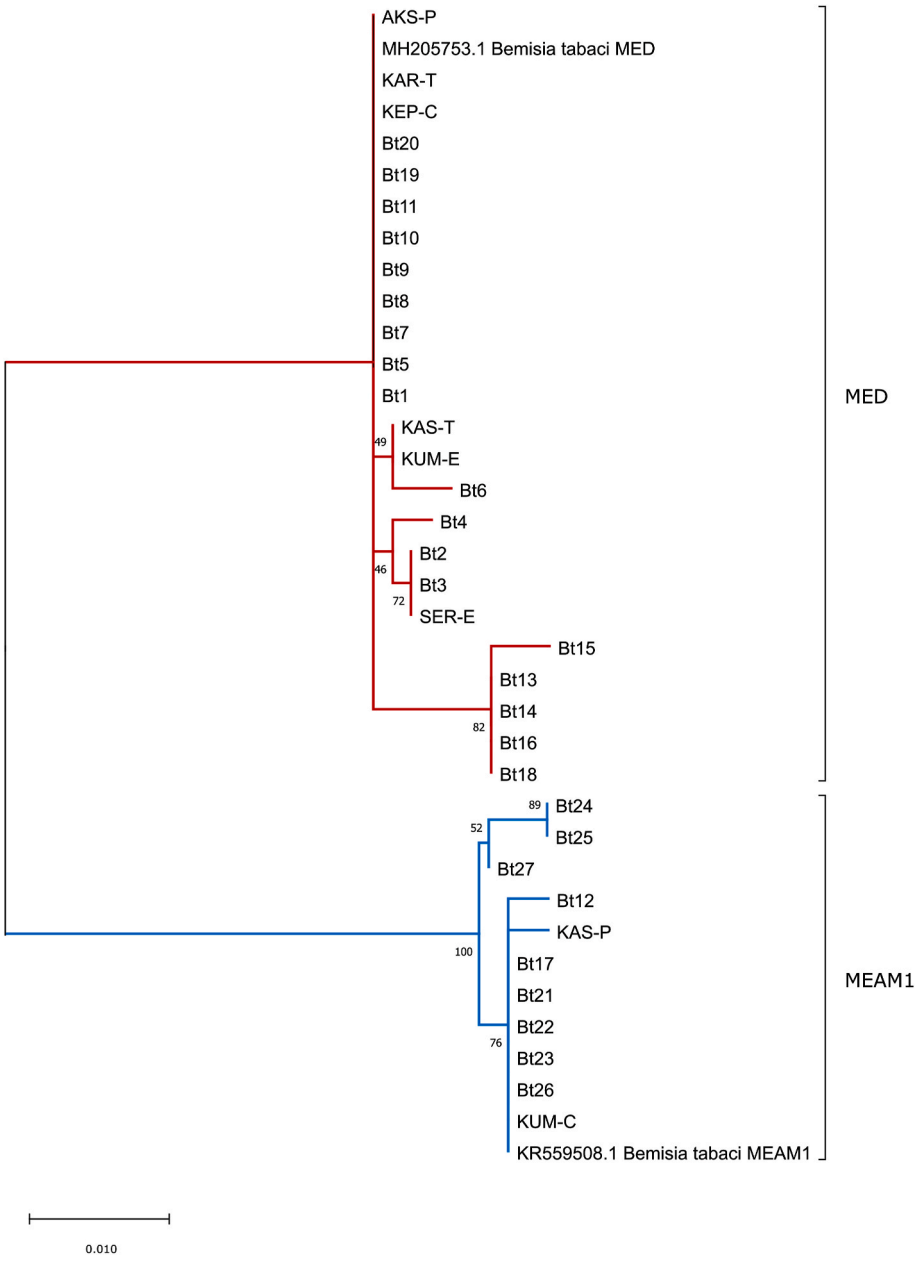

Fig. 2. A phylogenetic tree using the obtained and reference COI sequences to identify the species of *Bemisia tabaci* populations.

populations. On the other hand, no amino acid changes were detected in either RyR or nAChR.

3.4. Molecular screening of plant pathogenic viruses and phylogenetic analysis of ToCV

Molecular screening for the viruliferous status of whiteflies, utilizing both universal and specific primers, revealed the absence of almost all investigated plant pathogenic viruses. However, tomato chlorosis virus (ToCV) was detected in five whitefly populations (AKS-P, KAS-T, Bt2, Bt5, Bt9) based on coat protein (CP) sequences (accession numbers: PQ632950- PQ632954). BLASTn analysis of the obtained sequences demonstrated over 99% nucleotide sequence similarity with previously submitted ToCV sequences from Türkiye and Greece (Fig. 4). Phylogenetic analysis grouped the ToCV sequences into four distinct clusters. Isolates obtained in the present study mainly clustered with Turkish and Greek isolates, while isolates from South Korea, Albania, Brazil, Israel, and Pakistan were also present in a similar cluster. Conversely, isolates from China and Taiwan exhibited clear distinction from others.

4. Discussion

MEAM1 and MED whiteflies exhibit diverse host plant preferences, vector potentials and insecticide resistance due to distinct evolutionary trajectories among cryptic species (Watanabe et al., 2019; Gautam et al., 2022; Pym et al., 2023). In the present study, over 65% of all populations, including nearly 90% of those from Antalya, were identified as MED species. This finding is consistent with previous research that documented a higher frequency of the MED than MEAM1 species in Antalya (Karut et al., 2017). Whitefly populations in the provinces east of Antalya were predominantly of the MEAM1 species, while the MED species were widespread in the western provinces (Fig. 1), in line with Karut et al. (2017). A previous study suggested that Antalya may act as a transition zone between the MED and MEAM1 species, with a clearer pattern of species separation observed in the eastern and western areas of the region (Dağlı et al., 2020). Similarly, a comparable distribution

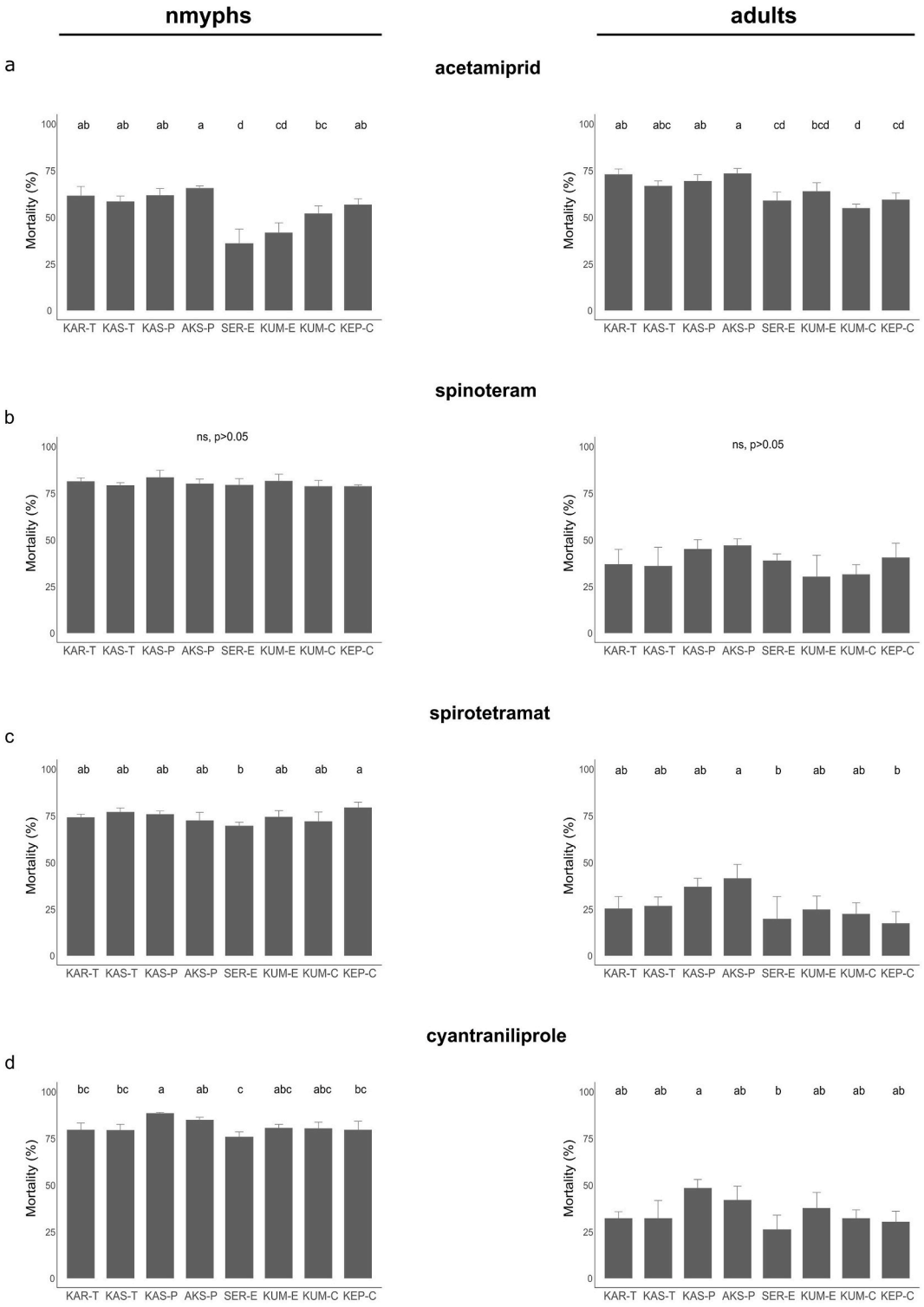

Fig. 3. Efficacy (% mortality ± SE) of a) acetamiprid, b) sulfoxaflor, c) spirotetramat and d) cyantraniliprole on the nymph and adult stages of *Bemisia tabaci* populations at 10 and 3 days after treatment (DAT), respectively, under greenhouse conditions. Different letters indicate statistically significant differences in insecticide efficacy for each trial ($p < 0.05$, Tukey test) and "ns" indicates no significant differences between the groups ($p > 0.05$)."

pattern was observed in the present study. However, additional sampling is needed to gain a better understanding of the spread of *B. tabaci* species across the country.

Although greenhouse trials have limitations compared to open field trials due to their inability to replicate the complexity and variability of field environments, they can still provide more reliable predictions of pest control under controlled conditions than laboratory tests. On the other hand, field and greenhouse trials offer less control over environmental factors, such as adult dispersal between plots, which can complicate the interpretation of results. Nevertheless, established field trial guidelines provide a standardized approach for assessing and comparing insecticide efficacies (EPPO, 2009).

In this study, we observed similar responses among whitefly populations in field trials, possibly due to shared chemical treatment

Table 2
The presence of known resistance mutations in acetylcholinesterase (AChE), voltage-gated sodium channel (VGSC) and acetyl-Coa carboxylase (ACC) in 35 field populations of *B. tabaci* across Türkiye.

Population	Biotype	AChE	VGSC				ACC
		F331W*	M918L	L925I	T929V	I936 V/F	A2083V
AKS-P	MED	W	M	I	T	I	V
KUM-C	MEAM1	W	M	I	T	I	V/A
KAR-T	MED	W	M/L	L	T/V	I/V	V/A
KAS-T	MED	W	M	I	T	I	V/A
KAS-P	MEAM1	W	M	I	T	I	A/V
KUM-E	MED	W	M	I	T	I	V
SER-E	MED	W	M	I	T	I	V/A
KEP-C	MED	W	M	I	T	I	V
Bt1	MED	W	M/L	L/I	T/V	I/V	V
Bt2	MED	W	M/L	I/L	T	I	V
Bt3	MED	W	M/L	I/L	T/V	I/V	V
Bt4	MED	W	M	I	T	I	V
Bt5	MED	W	M	I	T	I/V	V/A
Bt6	MED	W	M/L	I/L	T/V	I/V	V/A
Bt7	MED	W	M/L	I	T/V	I/V	V
Bt8	MED	W	M	I	T	I	V/A
Bt9	MED	W	M	I	T/V	I	V/A
Bt10	MED	W	M	I	T/V	I	V
Bt11	MED	W	M	I	T	I	V/A
Bt12	MEAM1	W	M	I	T	I	V/A
Bt13	MED	W	M	I	T	I	A
Bt14	MED	W	M	L/I	V/T	I/F	A
Bt15	MED	W	M	I/L	V/T	I	A
Bt16	MED	W	M	I	T/V	I	A
Bt17	MEAM1	W	M	I	T	I	V
Bt18	MED	W	M	I/L	T/V	I	A
Bt19	MED	W	M	I	T	I	V/A
Bt20	MED	W	M/L	I	T	I/F	A
Bt21	MEAM1	W	M	I	T	I	A/V
Bt22	MEAM1	W	M	I	T	I	A/V
Bt23	MEAM1	W	M	I	T	I	V/A
Bt24	MEAM1	W	M	I	T	I	A
Bt25	MEAM1	W	M	I	T	I	A
Bt26	MEAM1	W	M	I	T	I	V/A
Bt27	MEAM1	W/F	M	I	T	I	V/A

* When only a single letter appears in the list, the mutation is considered fixed. In cases where multiple alleles were present in the sample, amino acids are listed in decreasing order of estimated frequency.

practices among vegetable growers in trial sites located close to each other (see Table S2 for the insecticide treatment history at each location). Except for acetamiprid, the mortality rates of adult whiteflies remained below 50% in most cases for the other three insecticides. While this outcome aligns with expectations for spirotetramat, given its selective efficacy on immature stages of insects by inhibiting lipid biosynthesis, concerns arise from the evident loss of efficacy under field conditions for sulfoxaflor and cyantraniliprole. Although acetamiprid exhibited higher mortality rates in adults, its efficacy never surpassed 80% in any trial. Notably, the efficacy of insecticides on nymphal stages consistently exceeded that on adults, except for acetamiprid. The decreased efficacy of insecticides, particularly on adult stages, may complicate population-level control of whiteflies.

Resistance to neonicotinoids, including acetamiprid, has been reported in Turkish *B. tabaci* populations, partially attributed to increased protein levels of a P450 monooxygenase, CYP6CM1, as determined by a rapid lateral flow test (Nauen et al., 2015; Satar et al., 2018). Age-specific expression of *CYP6CM1*, resulting in higher susceptibility of nymphal stages compared to adults, has been documented in *B. tabaci* (Jones et al., 2011). Conversely, lower acetamiprid susceptibility in adult stages compared to nymph stages in Turkish whitefly populations was determined in the present study, similar to several Chinese populations reported by Chen et al. (2018). In light of the absence of target-site mutations in whitefly populations in our study, it is possible that the involvement of a P450 other than CYP6CM1 or a different detoxification enzyme may underlie these varying results. Therefore, further characterization of the molecular mechanisms underlying acetamiprid resistance in Turkish whitefly populations will contribute to designing robust resistance management programs.

The other three insecticides (sulfoxaflor, spirotetramat, cyantraniliprole) exhibited a consistent pattern in terms of mortality rates across both nymphal and adult stages. Their efficacy on nymphs ranged between 70 and 80%, with cyantraniliprole and sulfoxaflor demonstrating the highest effectiveness overall. Spirotetramat, known as a lipid synthesis inhibitor, is particularly effective in immature stages, hence its comparatively lower efficacy on adult flies in field trials. All tested populations harboured the A2083V mutation (being fixed in AKS-P, KUM-E, KEP-C) associated with spirotetramat resistance. This explains the relatively lower efficacy of spirotetramat on nymphs compared to the other two insecticides. Given the phenotypic strength and dominant nature of the A2083V mutation (Lueke et al., 2020), one might have expected an even greater reduction in efficacy. However, caution is warranted in directly correlating genotype with phenotype due to the limited number of individuals used in genotyping. Similar to spirotetramat, cyantraniliprole exhibited higher efficacy on nymphs than adults of *B. tabaci*, in line with previous findings (Chen et al., 2018; Grávalos et al., 2015). In contrast with our results, sulfoxaflor was reported to be more effective on adults than nymphs of *B. tabaci* by Chen et al. (2018), suggesting potential differences in resistance mechanisms between the Chinese and Turkish populations tested.

In addition to conducting field assays and genotyping on eight populations, a comprehensive molecular screening of target-site mutations was performed to enhance our understanding of the geographical distribution of resistance. Resistance to acetylcholinesterase (AChE) inhibitors in arthropods is frequently associated with target-site mutations (Lee et al., 2015; Alon et al., 2008) initially reported the F331W

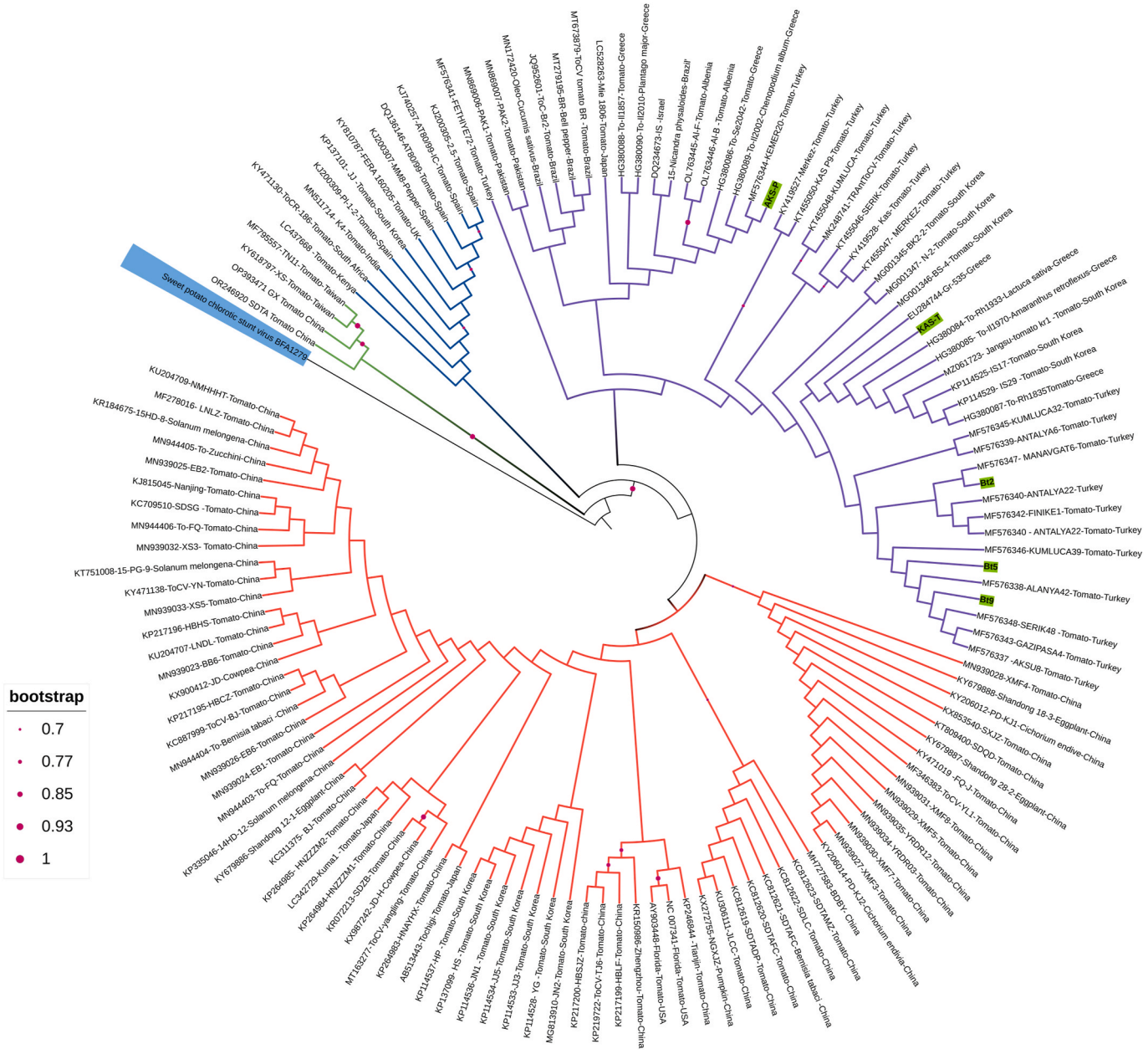

Fig. 4. Phylogenetic analysis of tomato chlorosis virus (ToMV) isolates obtained from Turkish *Bemisia tabaci* populations.

mutation within the acyl pocket of the active site gorge in the *ace1* gene of *B. tabaci*, which was subsequently corroborated by biochemical assays. This mutation has also been documented in spider mites as a major resistance mechanism to OPs (De Rouck et al., 2023). Unlike many other resistance mutations, the F331W mutation has been consistently found to be fixed in *B. tabaci* populations worldwide, including those in Australia, China, and Greece (Fang et al., 2022; Hopkinson et al., 2020; Tsagkarakou et al., 2009; Wang et al., 2020; Yuan et al., 2012). Similarly, this mutation was predominant in most of the Turkish populations previously studied (Erdogan et al., 2024). Consistent with previous findings, the F331W mutation was fixed in 34 out of 35 populations sampled in our study. Only one population (Bt27) exhibited the susceptible phenylalanine (F), albeit at a lower frequency (based on peaks in sequencing chromatography) compared to tryptophan (W). These results collectively indicate widespread and intensive usage of AChE inhibitors such as organophosphates (OPs) and carbamates across the globe, resulting in the fixation of resistance mutation. Surprisingly, Tsagkarakou et al. (2009) reported a *B. tabaci* population exhibiting phenotypic susceptibility to pirimiphos-methyl (an OP insecticide) despite the presence of the fixed F331W mutation, indicating that this mutation alone may not confer a high level of resistance to pirimiphos-methyl. This observation was further supported by biochemical assays demonstrating lower inhibition of pirimiphos-methyl when the mutation was tested alone in an AChE1-expressing baculovirus system compared to *B. tabaci* homogenate, which also contains other detoxification enzymes (Alon et al., 2008; Kim et al., 2022). Therefore, while many *B. tabaci* populations in our study exhibited the fixed mutation, it would be premature to interpret these findings solely in terms of pirimiphos-methyl resistance, as intensive use of other OP insecticides in the past might also have led to fixation. Nevertheless, the high prevalence of this mutation underscores the considerable risk of resistance development.

Mutations within the voltage-gated sodium channel (VGSC), the target site of pyrethroids, have been identified as the primary mechanism of resistance across arthropods (Dong et al., 2014). There are four domains (I-IV), including six transmembrane segments (S1-S6)

belonging to VGSC. Three mutations (M918L, L925I, T929V in the IIS4-S5 linker and IIS5) have been previously linked to pyrethroid resistance in *B. tabaci* (Morin et al., 2002; Roditakis et al., 2006). Electrophysiological experiments conducted using *Xenopus* oocytes have shown the critical role of these residues in conferring pyrethroid resistance (Rinkevich et al., 2013). Resistance-associated mutations were previously found in *B. tabaci* populations from China, Greece, and Australia (Fang et al., 2022; Tsagkarakou et al., 2009; Yuan et al., 2012). In the present study, while the occurrence of M918L and T929V mutations was less than 31% across populations, the L925I mutation was detected in almost all populations. The remarkably high frequency of the L925I mutation in Turkish *B. tabaci* populations (Erdogan et al., 2024; this study) may be linked to the continuous use of pyrethroids and the putative absence of a major fitness cost associated with this mutation (Tsagkarakou et al., 2009). Due to the use of pooled DNA in genotyping, we were unable to determine the combinations of mutations present via Sanger sequencing, which may result in the failure to detect genetic variations lower than 10–20% (Ishige et al., 2018; Njiru et al., 2023). Hence, we genotyped 20 individual female whiteflies from populations harbouring all four mutations to identify specific VGSC haplotypes. Our analysis revealed that the L925I and L929V mutations were never homozygously co-located on the same haplotype in the tested individuals. However, haploid males should be investigated in future studies to obtain more accurate results regarding haplotypes. Next to three well-known VGSC mutations, we discovered novel I936V/F mutations in eight *B. tabaci* populations. To the best of our knowledge, this is the first report of these mutations in *B. tabaci*. Although both the I936V and I936F mutations have been identified in various arthropods, such as *Helicoverpa zea* (Hopkins and Pietrantonio, 2010), *Dermanyssus gallinae* (Katsavou et al., 2020), *Cimex lectularius* (Dang et al., 2015) and *Myzus persicae* (MacKenzie et al., 2018), only the I936V mutation has been further shown to contribute to reduced sensitivity to pyrethroids through electrophysiological studies in *Xenopus* eggs (Usherwood et al., 2007). The prevalence of target-site mutations, coupled with the high pyrethroid resistance ratios observed in Turkish *B. tabaci* populations (Dağlı et al., 2020; Erdogan et al., 2008, 2024), underscores the urgent need for rotational strategies in the use of pyrethroids for whitefly control in the country.

Decreased susceptibility to cyclic ketoenols such as spiromesifen and spirotetramat has been previously documented in *B. tabaci* populations worldwide (Bielza et al., 2019; De Marchi and Smith, 2021; Peng et al., 2017; Yükselbaba and Alı, 2022). The use of synergistic chemicals in these strains failed to restore susceptibility in a ketoenol-resistant *B. tabaci* population from Spain, suggesting the potential presence of target-site resistance mechanisms (Bielza et al., 2019). In a follow-up study, the A2083V mutation within the carboxyltransferase (CT) domain of acetyl-CoA carboxylase (ACC) was identified and validated through CRISPR-Cas9 edited *Drosophila* flies (Lueke et al., 2020). Similar to its prevalence in Spain (Lueke et al., 2020), the A2083V mutation was found in 26 out of 35 Turkish *B. tabaci* samples, with 10 of these exhibiting fixation in all pooled individuals. Notably, all populations collected from Antalya harboured the A2083V mutation, indicating substantial selection pressure in that region. Conversely, the mutation was absent in more than half of the populations collected from locations outside the Mediterranean Region, highlighting the significance of local practice variations in resistance development.

Neonicotinoids such as acetamiprid have been extensively utilized to control *B. tabaci* populations (Nauen and Denholm, 2005). Despite the banning or restricting of some neonicotinoid members, acetamiprid remains widely employed for whitefly management in vegetable greenhouses and cotton fields. Only recently, a dual mutation at the target-site of a neonicotinoid-resistant Chinese whitefly was reported (Yin et al., 2024). However, none of the sampled *B. tabaci* populations in our study harboured any mutations at the target site.

Several mutations, including I4790M in helix S2 of RyR, have been identified in diamide-resistant lepidopteran species (Richardson et al., 2020). Since the presence of methionine (M) has been shown in many species belonging to insect orders, including Hemiptera, the isoleucine (I) at position 4790 was suggested as a selectivity switch for lepidopteran efficacy (Bailey et al., 2022; Richardson et al., 2020). Similarly, all sampled populations in the present study exhibited methionine at position 4790 in *B. tabaci*, for which cyantraniliprole is registered (Horowitz et al., 2020). Indeed, following CRISPR-Cas9 knock-in studies, this mutation was found to cause low resistance to anthranilic diamides such as chlorantraniliprole and cyantraniliprole (Douris et al., 2017; Wang et al., 2020). This partial selectivity might explain the higher registered dose of cyantraniliprole for *B. tabaci* (100 ml/100 L water) compared to lepidopteran pests such as *Tuta absoluta* and *Spodoptera littoralis* (60–75 ml/100 L water).

Whiteflies transmit several plant pathogenic viruses through non-persistent, semi-persistent, or persistent modes (Ghosh and Ghanim, 2021). Moreover, the development of insecticide resistance, high reproduction and dispersal rates, extensive polyphagy, and human activities have elevated whiteflies to the status of 'supervectors,' making them global pests and contributing to the emergence of numerous plant viruses (Gilbertson et al., 2015). In our study, although most of the screened plant virus diseases were not detected, five whitefly populations from Antalya were found to be viruliferous for Tomato chlorosis virus (ToCV). This finding is supported by reports of widespread ToCV presence in tomato plants in Antalya, which boasts a Mediterranean climate, unlike other regions characterized by colder winter months and higher-altitude growing areas (Yeşilyurt and Çevik, 2019). The high frequency of ToCV in Antalya is most likely associated with the intense activity of whiteflies in the region. Similarly, whiteflies have been associated with large vector populations in southern Spain, where ToCV outbreaks have impacted tomato production areas (Navas-Castillo et al., 2000). Except for AKS-P, all whitefly populations infected with ToCV observed in the present study belonged to the MED species, consistent with the findings of Shi et al. (2018), who hypothesized the superior vectoring capability of the MED over MEAM1 whiteflies for ToCV. Given the presence of ToCV and the dominance of the MED whiteflies in Antalya, increased attention should be directed toward monitoring and controlling the spread of ToCV. The use of insecticides such as flupyradifurone and acetamiprid, which disrupt the feeding behaviour of *B. tabaci*, may be prioritized in locations where ToCV infection is detected to quickly halt its spread (Maluta et al., 2021).

In conclusion, the loss of insecticide efficacies against *B. tabaci* populations has been observed in both the nymph and adult stages. In addition, the widespread distribution of known resistance mutations emphasizes the urgent need to implement integrated pest management strategies in whitefly control efforts, particularly in regions where they also serve as vectors for ToCV. Future studies should prioritize the precise determination of mutation frequency using ddPCR (Mavridis et al., 2022) and assess the potential of resistance mutations as molecular markers for the early and rapid detection of resistance development (Van Leeuwen et al., 2020).

CRediT authorship contribution statement

Arda İnak: Methodology, Investigation, Formal analysis. **Berke Demirci:** Investigation, Formal analysis, Data curation. **Esengül Erdem:** Formal analysis. **Filiz Randa-Zelyüt:** Visualization, Formal analysis. **Ali Karanfil:** Investigation. **Ahmednur Yusuf Idan:** Investigation. **Umut Toprak:** Formal analysis. **Emre İnak:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Thomas Van Leeuwen:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Declaration of competing interest

We have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2024.107097.

Data availability

Data will be made available on request.

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