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*Original article (Orijinal araştırma)*

# Susceptibility of Turkish Mediterranean populations of *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) to flupyradifurone, acetamiprid and sulfoxaflor

## Türkiye Akdeniz Bölgesi'ndeki bazı *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) popülasyonlarının flupyradifurone, acetamiprid ve sulfoxaflor'a duyarlılık seviyeleri

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## Abstract

*Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) is a major agricultural pest, and excessive insecticide use has reduced susceptibility in many populations, making resistance monitoring essential for effective resistance management. In this study, $LC_{50}$ values of *B. tabaci* populations collected in 2022 from the Mediterranean Region of Türkiye (Antalya, Mersin, Adana, and Hatay) were determined using the leaf-dip method for flupyradifurone, acetamiprid, and sulfoxaflor. Resistance ratios were calculated relative to the susceptible SUD-S strain. $LC_{50}$ values ranged from 1.16-6.92 mg (a.i.)/L for flupyradifurone, 7.06-43.39 mg (a.i.)/L for acetamiprid, and 0.96-72.68 mg (a.i.)/L for sulfoxaflor, with corresponding resistance ratios of 6.79-39.29, 38.2-234.5, and 3.83-290.7-fold. Resistance levels were low to high for flupyradifurone, high to very high for acetamiprid, and low to very high for sulfoxaflor. Given the high acetamiprid resistance, its use should be minimized, while rotation of sulfoxaflor and flupyradifurone with other modes of action is recommended. Although flupyradifurone is relatively new, the observed reduction in susceptibility highlights the need to investigate potential cross-resistance and underlying mechanisms.

**Keywords:** Insecticide resistance, leaf-dip, resistance monitoring, resistance ratio, whitefly

## Öz

*Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), önemli bir tarımsal zararlıdır ve aşırı insektisit kullanımı birçok popülasyonda duyarlılığın azalmasına yol açtığından, etkin bir direnç yönetimi için direnç izlemesi önemlidir. Bu çalışmada, Türkiye'nin Akdeniz Bölgesi'nden (Antalya, Mersin, Adana ve Hatay) 2022 yılında toplanan *B. tabaci* popülasyonlarının flupyradifurone, acetamiprid ve sulfoxaflor'a karşı yaprak-daldırma yöntemiyle $LC_{50}$ değerleri belirlenmiştir. Direnç oranları duyarlı SUD-S popülasyonuna göre belirlenmiştir. $LC_{50}$ değerleri flupyradifurone için 1.16-6.92 mg (e.m)./L, acetamiprid için 7.06-43.39 mg (e.m.)/L ve sulfoxaflor için 0.96-72.68 mg (e.m.)/L arasında gözlemlenirken; direnç oranları sırasıyla 6.79-39.29, 38.16-234.53 ve 38.2-234.5 ve 3.83-290.7 kat olarak bulunmuştur. Direnç düzeyleri flupyradifurone için düşük ile yüksek, acetamiprid için yüksek ile çok yüksek ve sulfoxaflor için düşük ile çok yüksek arasında değişmiştir. Yüksek acetamiprid direnci göz önünde bulundurularak, bu bileşiğin kullanımının minimize edilmesi; sulfoxaflor ve flupyradifurone'un ise farklı etki mekanizmalarına sahip insektisitlerle dönüşümlü kullanılması önerilmektedir. Flupyradifurone görece yeni olmasına rağmen gözlenen duyarlılık azalması, olası çapraz direnç ve altında yatan mekanizmaların araştırılmasının gerekliliğini vurgulamaktadır.

**Anahtar kelimeler:** İnsektisit direnci, yaprak daldırma, direnç takibi, direnç oranı, beyazsinek

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## Introduction

*Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) is reported as one of the insect species causing significant agricultural damage worldwide (Wang et al., 2020). *Bemisia tabaci* is a highly polyphagous pest, attacking more than 600 host plants and is responsible for transmitting over 200 viruses, such as Tomato Yellow Leaf Curl Virus (TYLCV) (Navas-Castillo et al., 2011). Cotton whitefly feeds on sap, and the honeydew they produce during feeding leads to sooty mold formation. This sooty mold covers the plant surface, obstructing photosynthesis and causing yield and quality losses (Hequet et al., 2007; De Barro et al., 2011).

With increasing global food demand, pesticide use has also increased steadily (Basit, 2019). Intensive pesticide use negatively affects human and environmental health, making it necessary to minimize pesticide use (Horowitz et al., 2020; Ali et al., 2021). Chemical control is widely preferred against *B. tabaci*. Consequently, reduced susceptibility to a wide range of insecticides has been reported in *B. tabaci* populations (Dittrich et al., 1990; Basit, 2019; Horowitz et al., 2020; Yükselbaba & Ali, 2022). Resistance has been defined as the survival of individuals after insecticide application and the transmission of this survival ability to subsequent generations (Ffrench-Constant & Roush, 1990). Insecticide resistance is known as a genetic condition involving various mechanisms such as target-site modifications or detoxification of the active ingredient (Horowitz et al., 2007). A total of 650 resistance studies have been reported for *B. tabaci*, and resistance to more than 60 active ingredients has been documented (APRD, 2018; Horowitz et al., 2020).

Broad-spectrum insecticides are commonly applied to manage *B. tabaci* (Basit, 2019; Horowitz et al., 2020). These include organophosphates, pyrethroids, spinosyns, neonicotinoids, sulfoximines, and butenolides (Sparks et al., 2013; Jeschke et al., 2015; Wang et al., 2020). Active ingredients that act as analogues of nicotinic acetylcholine receptors (nAChRs) are essential for effective pest management and were reported to account for 29% of the agricultural pesticide market in 2012 (Jeschke et al., 2015). However, loss of susceptibility to some of these compounds has been reported (Saleem et al., 2022). Therefore, new insecticidal compounds must be identified and developed. -

Flupyradifurone is an active ingredient belonging to the new butenolide class, characterized by distinct chemical and physical features, unique metabolic behavior in plants and insects, and suitability within an integrated pest management profile (Roffeni et al., 2014; Jeschke et al., 2015). Flupyradifurone can be translocated through the xylem. After foliar or soil application, it provides systemic protection and also exhibits translaminar activity (Jeschke & Nauen, 2007; Raupach et al., 2012; Roffeni et al., 2014). Consequently, flupyradifurone has shown efficacy against many pests resistant to neonicotinoids and other chemical classes and does not cause cross-resistance with other insecticides (Raupach et al., 2012; Jeschke et al., 2015).

Sulfoximines represent a new class of insecticides active against sap-feeding insects, with sulfoxaflor being the first commercially available compound of this class (Babcock et al., 2011; Sparks et al., 2013). Like several other insecticide classes sulfoxaflor acts on nAChRs in insects (Longhurst et al., 2013). However, sulfoxaflor shows efficacy against sap-feeding pests resistant to most insecticide classes (Sparks et al., 2013). The first insecticide containing sulfoxaflor was registered in Türkiye in 2016, and two formulations are currently available (Anonymous, 2023).

Among these classes, acetamiprid, belonging to the neonicotinoid group, is widely used to control a wide range of insect pests, such as Lepidoptera, Coleoptera, Hemiptera, and Thysanoptera (Wang et al., 2017). Acetamiprid binds to nAChRs in insects, blocking the nervous system and incapacitating the pest (Elbert et al., 2008). This active ingredient was registered in Türkiye in 1996 and continues to be used today for *B. tabaci* management (Anonymous, 2023).

In addition, *B. tabaci* is currently recognized as a complex of cryptic species, among which the Mediterranean (MED) and Middle East-Asia Minor 1 (MEAM1) genetic groups are the most widespread and economically important worldwide (De Barro et al., 2011). Previous studies have demonstrated that these genetic groups may differ considerably in their insecticide resistance profiles. MED populations have frequently been associated with higher resistance levels to several insecticide classes, particularly neonicotinoids, compared with MEAM1 populations (Horowitz et al., 2005). Sulfoxaflor and flupyradifurone have been reported to provide effective control of different *B. tabaci* genetic groups, including the MEAM1 and MED (Sparks et al., 2013; Nauen et al., 2015).

Numerous studies have examined insecticide susceptibility levels and resistance mechanisms in *B. tabaci* (Houndete et al., 2010; Bahşi et al., 2012; Dağlı et al., 2020; Mohammed et al., 2020; Wang et al., 2020; Yükselbaba & Ali, 2022). These studies aimed to determine the spatial and temporal variability of susceptibility loss, accurately identify resistant genotypes, provide early warning of potential resistance issues, and evaluate the effectiveness of resistance management strategies (Basit, 2019). Many studies have investigated susceptibility levels of *B. tabaci* to acetamiprid, reporting high levels of susceptibility loss in China, West Africa, Türkiye and Cyprus (Houndete et al., 2010; Vassiliou et al., 2011; Bahşi et al., 2012; Xie et al., 2014; Chen et al., 2018; Satar et al., 2018). Globally, studies examining sulfoxaflor have been conducted on populations collected from China, Mexico, Spain, Cyprus, the UK, and Italy, and susceptibility loss has generally been reported as low (Longhurst et al., 2013; Wang et al., 2017; Wang et al., 2019). In Türkiye, only one study has assessed sulfoxaflor resistance in *B. tabaci*, conducted in 2020 on Mersin populations (Mohammed et al., 2020). Studies on *B. tabaci* susceptibility to flupyradifurone are limited worldwide, with research conducted in both United States and China (Smith et al., 2016; Guo et al., 2020; Sparks et al., 2020; Wang et al., 2020). To the best of our knowledge, the susceptibility levels of *B. tabaci* to flupyradifurone have not yet been evaluated in Türkiye.

Considering the studies conducted to date, the number of insecticide resistance cases continues to increase steadily. The time, effort, and high cost required to discover and develop new insecticides also continue to rise (Sparks & Nauen, 2015). Likewise, minimizing the likelihood of resistance development to existing products is equally important, as many cases lack alternative compounds with similar properties or lower cost (Raupach et al., 2012; Jeschke et al., 2015; Sparks & Nauen, 2015). Therefore, regular monitoring of insecticide resistance levels has become increasingly important, since resistance monitoring plays a key role in the development of effective resistance management strategies (Sparks & Nauen, 2015). Studies aimed at understanding resistance status, resistance types, genetic basis, and potential for resistance development in *B. tabaci* are essential for designing effective resistance management programs. For this reason, this study assessed the current susceptibility levels of *B. tabaci* to flupyradifurone, acetamiprid, and sulfoxaflor, as an initial step toward developing effective resistance management strategies. Ten distinct *B. tabaci* field populations from Adana, Mersin, Antalya, and Hatay provinces in the Mediterranean Region were examined, and their lethal concentration values and resistance ratios were determined relative to a susceptible reference strain. This study aims to contribute to both the literature and resistance management strategies.

## Materials and Methods

### Insecticides

In the present work, three insecticides commonly used for the control of *B. tabaci* were evaluated: flupyradifurone from the butenolide class, acetamiprid from the neonicotinoid class, and sulfoxaflor from the sulfoximine class. Commercially available formulated products were used in all experiments. The recommended dose was selected according to the adult stage of the pest (Table 1).

Table 1. Information on the insecticides tested

| Trade Name | Active ingredient | Recommended dose | IRAC Chemical Group |
|---|---|---|---|
| SIVANTO SL 200 | 200 g/l Flupyradifurone | 60ml/100L | 4D |
| BREAKER 240 SC | 240 g/l Sulfoxaflor | 30ml/100L | 4C |
| MOSPILAN 20 SP | %20 Acetamiprid | 40g/100L | 4A |

### Field-origin *Bemisia* tabaci populations used in bioassay

The *Bemisia tabaci* field populations (Table 2) were collected from pepper and tomato greenhouses in selected provinces of the Mediterranean Region of Türkiye namely, Adana, Antalya, Hatay, and Mersin (Figure 1). Collected adults were transferred to climate-controlled chambers for population establishment and rearing. Whitefly populations were maintained in 10-L plastic cages. The opening of each cage was covered with a long fine mesh cloth to prevent insect escape or entry of external individuals. During watering or plant replacement, the mesh cover was carefully opened and immediately closed to minimize the risk of insect escape or the introduction of individuals from other populations, thereby preventing cross-contamination among populations.

Table 2. Geographic origins of *Bemisia tabaci* populations

| Population | District | Collection | Coordinates | Host | Group |
|---|---|---|---|---|---|
| SUD-S | Sudan | 1978 | - | *Gossypium hirsutum* | Q |
| DEM | Antalya-Demre | 01.07.2022 | 36º15'42.5"K29º59'23.2"G | *Capsicum annuum* | MED/Q |
| KUM | Antalya-Kumluca | 01.07.2022 | 36º18'56.9"K 30º20'41.7"G | *Solanum lycopersicum* | MED/Q |
| SER | Antalya-Serik | 22.07.2022 | 36º55'09.9"K 31º03'57.7"G | *Capsicum annuum* | MED/Q |
| GAZ | Antalya-Gaziler | 22.07.2022 | 36º59'31.3"K 30º47'06.1"G | *Capsicum annuum* | MEAM1/B |
| GAP | Antalya-Gazipaşa | 28.07.2022 | 36º15'53.6"K 32º18'32.0"G | *Solanum lycopersicum* | MEAM1/B |
| KAZ | Mersin-Kazanlı | 15.09.2022 | 36º49'02.2"K 34º46'14.8"G | *Solanum lycopersicum* | MED/Q |
| SIL | Mersin-Silifke | 15.09.2022 | 36º22'21.4"K 33º57'20.3"G | *Solanum lycopersicum* | MED/Q |
| DRY | Hatay-Dörtyol | 13.09.2022 | 36º52'17.5"K 36º12'12.3"G | *Capsicum annuum* | MEAM1/B |
| SAM | Hatay-Samandağ | 13.09.2022 | 36º04'19.8"K 35º59'10.8"G | *Capsicum annuum* | MED/Q |
| KRT | Adana-Karataş | 10.09.2022 | 36º34'46.1"K 35º21'45.7"G | *Solanum lycopersicum* | MED/Q |




Figure 1. *Bemisia tabaci* collection sites.

All populations were maintained on 25-30-day-old cotton plants, *Gossypium hirsutum* L. (Malvales: Malvaceae) cv. Carisma, grown under controlled conditions (26±1ºC, 60±10% relative humidity, and a 16:8 h light:dark photoperiod) without insecticide exposure. Cotton seeds (*cv. Carisma*) were obtained from Dr. Metin Durmuş Çetin, PhD, at the Western Mediterranean Agricultural Research Center (BATEM). Bioassays were conducted using individuals from the $F_1$ generation onward. To obtain the $F_1$ generation, at least 200 adults from field-collected populations ($F_0$) were transferred onto cotton leaves and allowed to oviposit.

The insecticide-susceptible reference population used in the study was the SUD-S population. This population was first obtained from cotton plants in Sudan in 1978 and subsequently reared under insecticide-free conditions for approximately 40 years. The SUD-S strain, provided by Raulf Nauen Bayer AG, Germany in 2021, has been kept in climate chambers without exposure to insecticides.

The genetic groups of the collected *B. tabaci* populations were determined based on DNA sequence analysis by amplifying a partial fragment of the mitochondrial cytochrome oxidase I (mtCOI) gene using the C1-J-2195 and TL2-N-3014 primers (Simon et al., 1994). All PCR amplifications, including reaction composition and thermal cycling conditions, were performed according to the protocol described by Yükselbaba & Ali (2022).

PCR amplification was performed with an initial 4 min denaturation at 94ºC, followed by 35 cycles of 94ºC for 40 s (denaturation), 48ºC for 40 s (annealing), and 72ºC for 50 s (extension), and a final extension at 72ºC for 5 min. The resulting PCR fragments, approximately 800 bp in length, were subsequently purified and sequenced. The obtained mtCOI sequences were aligned against reference sequences in GenBank using BLAST to assign each population to its corresponding genetic group. The sequences obtained in this study were deposited in GenBank under accession numbers PZ130074-PZ130083.

**Insecticide bioassays**

The lethal concentration values of *B. tabaci* populations for flupyradifurone, acetamiprid, and sulfoxaflor were determined using the leaf-dip bioassay method applied to adults (Roditakis et al., 2005; Wang et al., 2020).

For each insecticide, 5-7 concentrations inducing 0-100% mortality (Bielza et al., 2008) were suspended in distilled water supplemented with 0.2 g $L^{-1}$ Triton X-100. Cotton leaf discs (35 mm diameter) were prepared from the first true leaves of the same 25-30-day-old plants, using a steel cork borer. Leaf discs were individually dipped for 5 s into 100 mL plastic cups containing the appropriate insecticide concentration, while control discs were dipped into distilled water containing only 0.2 g $L^{-1}$ Triton X-100.

After dipping, leaf discs were placed on moist paper towels and left to dry for about 2 h. The dried discs were then transferred, with the abaxial surface facing upward, into 35-mm Petri dishes containing 1.5% (w/v) agar. Adult whiteflies, 1-5 days old, were dislodged by gently tapping the cotton plants on which the populations had been reared and allowed to fall directly onto the leaf discs. To ensure that the adults settled naturally on the leaf surface and to prevent escape, the Petri dishes were carefully inverted only once. During mortality assessments, the dishes were handled cautiously to avoid disturbing the insects, and no escape of adults was observed. At least 20 adults of mixed sex were placed on each disc.

Petri dishes were covered with a cotton mesh, with the leaf discs placed upside down, and incubated at 26±1ºC, 60±10% relative humidity, and a 16:8 h (light: dark) photoperiod for 72 h in a climate chamber. After the exposure period, mortality was assessed under a stereomicroscope. Individuals that showed no movement or response when gently touched with a soft brush were recorded as dead. Total numbers of live and dead whiteflies were recorded.

Each bioassay was conducted separately for each population and each insecticide, with at least five replicates per dose and at least 20 adult whiteflies per Petri dish. This resulted in a total of at least 100 insects tested per dose

**Data analysis**

$LC_{50}$ and $LC_{90}$ values, along with their 95% confidence intervals, were estimated using probit analysis performed with POLO-PC software (LeOra Software, 2008). Resistance ratios ($RR_{50}$) were determined by dividing the $LC_{50}$ value of each population by that of the SUD-S population. Populations were regarded as significantly different if their $LC_{50}$ 95% confidence intervals did not overlap (Chen et al., 2018; Yükselbaba & Ali, 2022). Resistance levels were classified as low resistance ($RR_{50}$=2-10), moderate resistance ($RR_{50}$=11-30), high resistance ($RR_{50}$=31-100), and very high resistance ($RR_{50}$ > 100) (Peng et al., 2017; Yükselbaba & Ali, 2022).

## Results

The *B. tabaci* populations were identified as MED and MEAM1 genetic groups, and their susceptibility to flupyradifurone, acetamiprid, and sulfoxaflor was assessed. Based on the bioassays, the LC values of *B. tabaci* populations for flupyradifurone are outlined in Table 3. The $LC_{50}$ values were estimated at 1.16-6.92 mg (a.i.)/L. Among the tested populations, DRY population had the lowest $LC_{50}$ value, whereas the GAZ population had the highest. The $LC_{50}$ of the SUD-S was 0.18 mg (a.i.)/L. In Figure 2, the minimum and maximum concentrations, the recommended field dose tested for flupyradifurone, and the corresponding average mortality rates were presented.

Table 3. Lethal concentration values of flupyradifurone for *Bemisia tabaci* populations

| Genetic group | Code | N | Slope±SE | $LC_{50}$ (mg a.i./L) CL | $LC_{90}$ (mg a.i./L) CL | H | $X^2$ | df | $RR_{50}$ |
|---|---|---|---|---|---|---|---|---|---|
| | SUD-S | 1191 | 0.99±0.06 | 0.18 (0.13-0.24) | 3.53 (2.35-5.81) | 0.81 | 27.5 | 34 | 1.0 |
| MED | SER | 1302 | 0.81±0.07 | 5.5 (3.20-8.55) | 209.8 (121.3-440.1) | 1.29 | 51.5 | 40 | 32.15 |
| | DEM | 1270 | 0.75±0.05 | 3.22 (2.23-4.52) | 162.7 (102.2-283.7) | 0.58 | 23.2 | 40 | 18.8 |
| | KUM | 1501 | 0.72±0.04 | 3.19 (2.27-4.4) | 191.9 (123.7-321.9) | 0.75 | 34.4 | 46 | 18.69 |
| | SAM | 1286 | 0.68±0.04 | 1.24 (0.85-1.78) | 95.58 (56.03-181.4) | 1.13 | 45.3 | 40 | 7.23 |
| | KRT | 1075 | 0.74±0.05 | 2.41 (1.67-3.44) | 132.9 (80.43-242.9) | 0.48 | 16.3 | 34 | 13.68 |
| | KAZ | 1264 | 0.79±0.05 | 2.57 (1.73-3.67) | 106.2 (69.12-177.9) | 0.53 | 20.7 | 39 | 14.62 |
| | SIL | 1069 | 0.743±0.05 | 1.98 (1.37-2.81) | 104.7 (63.54-190.3) | 0.46 | 15.7 | 34 | 11.22 |
| MEAM1 | DRY | 1278 | 0.71±0.04 | 1.16 (0.83-1.6) | 72.41 (45.52-125.1) | 0.54 | 21.4 | 40 | 6.79 |
| | GAZ | 1248 | 0.81±0.06 | 6.92 (4.69-9.78) | 268.8 (169.2-478.6) | 0.64 | 25.1 | 39 | 39.29 |
| | GAP | 1186 | 0.86±0.07 | 4.73 (3.11-6.79) | 145.4 (92.5-257.8) | 0.94 | 36.7 | 39 | 26.85 |

Number of individuals (N) used in bioassay, Chi-square ($X^2$), Confidents limits (CL), Resistance Ratio ($RR_{50}$:= $LC_{50}$ of the populations / $LC_{50}$ of the susceptible population), Degrees of freedom (df), Heterogenity (H).

The resistance ratios of the populations to flupyradifurone ranged between 6.79 and 39.29 fold, and based on these values, resistance levels were classified as low to high (Table 3). The confidence intervals of $LC_{50}$ values for *B. tabaci* populations were clearly distinct from those of the SUD-S population, indicating a significant loss of susceptibility. For flupyradifurone, slope values were also below 1 for all populations, and heterogeneity (H) values were mostly below 1, indicating heterogeneous population responses and reliable probit model fits.

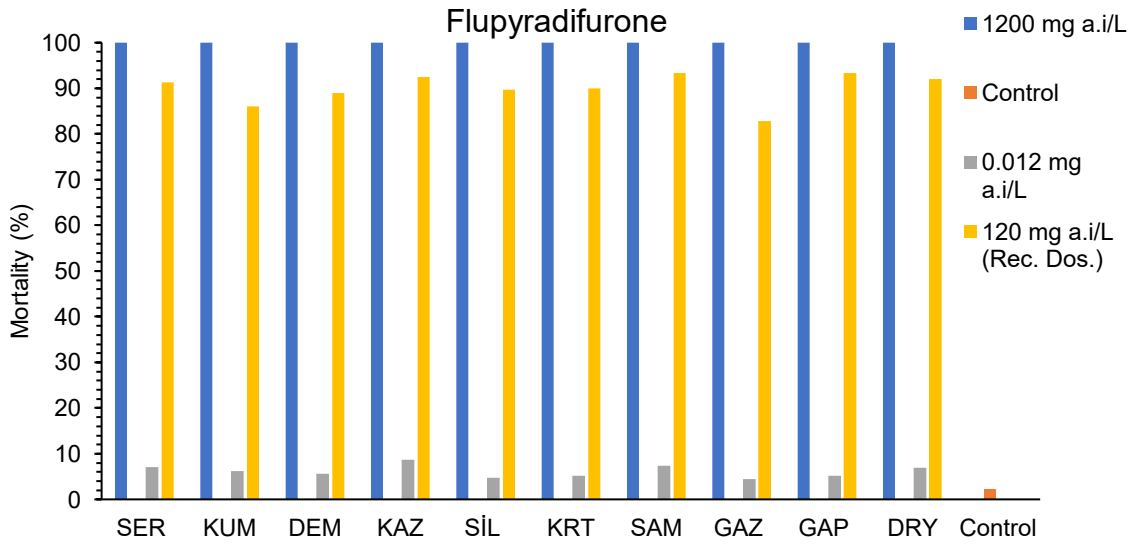



Figure 2. Observed mortality of *Bemisia tabaci* at the highest, lowest, and recommended doses of flupyradifurone.

The LC values of *B. tabaci* populations for acetamiprid are outlined in Table 4. $LC_{50}$ values were estimated at 7.06-43.39 mg (a.i.)/L. The lowest $LC_{50}$ was observed in the DRY, whereas the highest $LC_{50}$ was detected in the KAZ. The $LC_{50}$ value of the SUD-S for acetamiprid was 0.19 mg (a.i.)/L. In Figure 3, the minimum and maximum concentrations, the recommended field dose tested for acetamiprid, and the corresponding average mortality rates were presented. The $RR_{50}$ values for acetamiprid in the populations were estimated at 38.2-234.5 fold, indicating high to very high resistance (Table 4). Because the confidence intervals of $LC_{50}$ values among the populations were clearly distinct from those of the SUD-S population, susceptibility was significantly reduced. For acetamiprid, slope values were below 1 for all populations, indicating heterogeneous responses, and heterogeneity (H) values were generally close to or below 1, suggesting an acceptable fit of the probit models.

Table 4 Lethal concentration values for acetamiprid in *Bemisia tabaci* populations.

| Genetic group | Code | N | Slope±SE | $LC_{50}$ (mg a.i./L) CL (%95) | $LC_{90}$ (mg a.i./L) CF | H | $X^2$ | df | $RR_{50}$ |
|---|---|---|---|---|---|---|---|---|---|
| | SUD- | 108 | 0.93±0.05 | 0.19 (0.14-0.24) | 4.36 (2.88-7.14) | 0.85 | 29.0 | 34 | 1 |
| MED | SER | 126 | 0.73±0.06 | 33.37 (19.23-52.69) | 1936.8 (1022.2-4716.2) | 1.44 | 57.5 | 40 | 180.4 |
| | DEM | 138 | 0.67±0.04 | 32.58 (22.10-47.33) | 2757.4 (1527.7-5753.2) | 1.21 | 48.6 | 40 | 176.1 |
| | KUM | 132 | 0.78±0.07 | 37.52 (20.53-60.15) | 1653.1 (848.7-4492.7) | 1.83 | 73.2 | 40 | 202.8 |
| | SAM | 104 | 0.63±0.04 | 13.27 (8.91-19.63) | 1405.8 (761.5-2973.7) | 0.85 | 28.9 | 34 | 71.7 |
| | KRT | 994 | 0.76±0.05 | 21.49 (15.11-30.46) | 1035.9 (599.8-2039.1) | 0.37 | 19.7 | 40 | 116.2 |
| | KAZ | 160 | 0.91±0.07 | 43.39 (31.49-57.38) | 1130.6 (773.8-1819.8) | 0.84 | 41.9 | 50 | 234.5 |
| | SIL | 110 | 0.72±0.04 | 20.35 (14.52-28.17) | 1244.6 (751.9-2297.6) | 0.74 | 37.7 | 44 | 110 |
| MEAM1 | DRY | 113 | 0.75±0.04 | 7.06 (4.96-9.90) | 367.68 (228.9-646.2) | 0.28 | 9.5 | 34 | 38.2 |
| | GAZ | 110 | 0.77±0.05 | 8.45 (6.03-11.66) | 382.45 (242.7-657.9) | 0.84 | 27.8 | 33 | 45.67 |
| | GAP | 144 | 0.66±0.04 | 29.54 (17.95-46.89) | 2509.4 (1257.4-6201.1) | 1.93 | 79.2 | 41 | 159.7 |

Number of individuals (N) used in bioassay, Chi-square ($X^2$), Confidents limits (CL), Resistance Ratio ($RR_{50}$=$LC_{50}$ of the populations/$LC_{50}$ of the susceptible population), Degrees of freedom (df), Heterogenity (H).

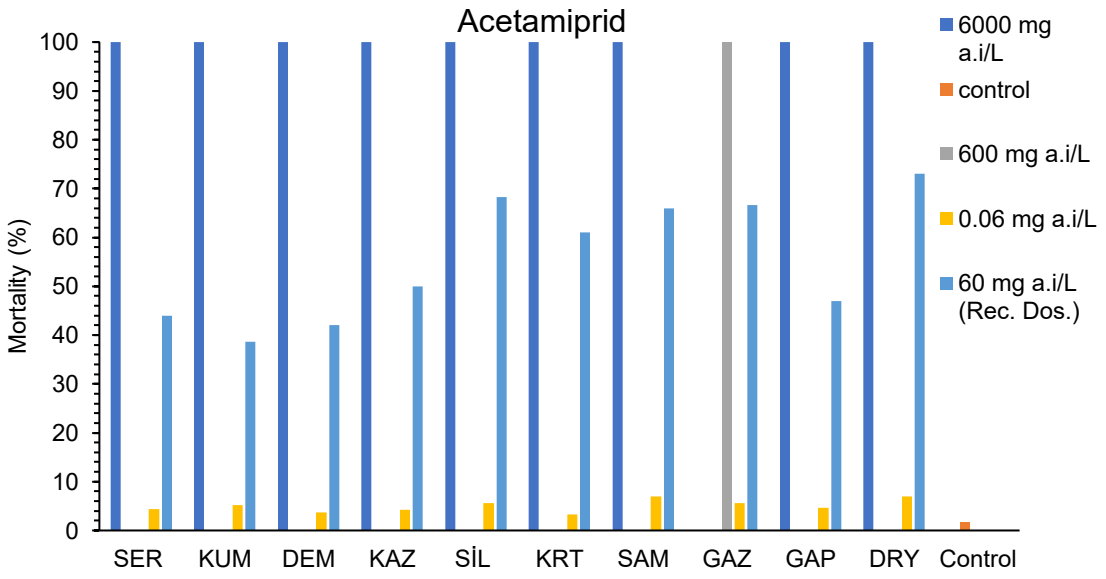



Figure 3. Observed mortality of *Bemisia tabaci* at the highest, lowest, and recommended doses of acetamiprid.

The LC values of populations for sulfoxaflor are presented in Table 5. $LC_{50}$ values were estimated at 0.96-72.68 mg (a.i.)/L. The lowest $LC_{50}$ was observed in the SAM, whereas the highest $LC_{50}$ was detected in the GAP. The $LC_{50}$ value of the SUD-S for sulfoxaflor was 0.25 mg (a.i.)/L. In Figure 4, the minimum and maximum concentrations, the recommended field dose tested for sulfoxaflor, and the corresponding average mortality rates were presented. The $RR_{50}$ values for sulfoxaflor in the populations were estimated at 3.83-290.7-fold, and resistance levels were classified as low, moderate, high, and very high (Table 5). The confidence intervals of $LC_{50}$ values for *B. tabaci* populations were clearly distinct from those of the SUD-S population, indicating a significantly reduced susceptibility. For sulfoxaflor, slope values remained below 1 for most populations, with heterogeneity (H) values close to or below 1, demonstrating heterogeneous susceptibility patterns and adequate fit of the probit models.

Table 5. Lethal concentration values of *Bemisia tabaci* (Genn.) populations for sulfoxaflor

| Genetic group | Code | N | Slope±SE | $LC_{50}$ (mg a.i./L) CL (%95) | $LC_{90}$ (mg a.i./L) CL (%95) | H | $X^2$ | df | $RR_{50}$ |
|---|---|---|---|---|---|---|---|---|---|
| | SUD-S | 12 | 0.91±0.05 | 0.25 (0.18-0.34) | 6.29 (3.97-10.94) | 1.16 | 42.8 | 37 | - |
| MED | SER | 16 | 0.74±0.05 | 6.68 (4.41-9.55) | 364.8 (236.1-622.9) | 0.56 | 27.5 | 49 | 26.8 |
| | DEM | 16 | 0.74±0.04 | 2.27 (1.64-3.09) | 126.2 (82.53-207.2) | 0.3 | 14.7 | 49 | 9.12 |
| | KUM | 16 | 0.98±0.12 | 8.63 (4.63-12.9) | 177.9 (110.6-385.1) | 1.28 | 60.3 | 47 | 34.65 |
| | SAM | 11 | 0.83±0.05 | 0.96 (0.69-1.32) | 33.39 (21.12-57.64) | 0.39 | 13.6 | 35 | 3.83 |
| | KRT | 13 | 0.79±0.05 | 2.12 (1.48-2.99) | 88.29 (55.20-155.28) | 0.79 | 28.4 | 36 | 8.51 |
| | KAZ | 14 | 0.68±0.04 | 1.88 (1.31-2.66) | 140.9 (87.17-246.4) | 0.29 | 12.0 | 41 | 7.53 |
| | SIL | 13 | 0.59±0.04 | 9.01 (5.93-13.55) | 1380.5 (734.5-2966.8) | 0.56 | 22.3 | 40 | 36.17 |
| MEAM1 | DRY | 11 | 0.75±0.04 | 1.3 (0.90-1.83) | 64.96 (40.06-115.45) | 0.43 | 14.7 | 34 | 5.2 |
| | GAZ | 11 | 0.80±0.05 | 2.81 (1.92-3.98) | 110.7 (69.77-194.1) | 0.81 | 27.6 | 34 | 11.28 |
| | GAP | 17 | 0.89±0.06 | 72.68 (54.83-95.02) | 2012.2 (1328.1-3357.1) | 0.69 | 32.6 | 47 | 290.7 |

Number of individuals (N) used in bioassay, Chi-square ($X^2$), Confidents limits (CL), Resistance Ratio ($RR_{50}$=$LC_{50}$ of the populations/$LC_{50}$ of the susceptible population), Degrees of freedom (df), Heterogenity (H).

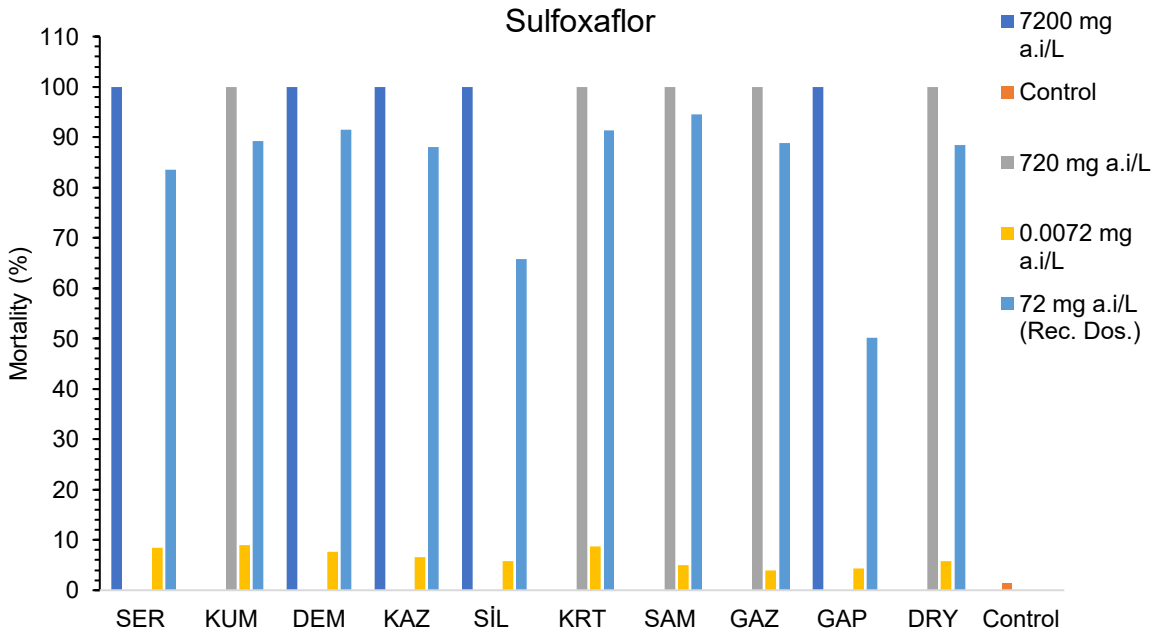



Figure 4. Observed mortality of *Bemisia tabaci* at the highest, lowest, and recommended doses of sulfoxaflor.

## Discussion

*Bemisia tabaci* is an important pest responsible for substantial yield and quality reductions in crops. Because chemical control is the primary method used against *B. tabaci*, a high level of loss of susceptibility to insecticides has been widely observed in this pest (Dittrich et al., 1990; Basit, 2019). Therefore, regular monitoring of insecticide resistance levels is essential for developing insecticide resistance management strategies and ensuring sustainable control of the target pest (Sparks & Nauen, 2015).

Susceptibility levels of *B. tabaci* populations from Antalya, Mersin, Adana, and Hatay in the Mediterranean Region were evaluated for flupyradifurone, acetamiprid, and sulfoxaflor. Based on insecticide bioassays, $LC_{50}$ values for flupyradifurone in *B. tabaci* populations were estimated at 1.16-6.92 mg (a.i.)/L, with resistance ratios ranging from 6.79 to 39.29-fold, indicating low, moderate, and high resistance levels. The $LC_{90}$ values for flupyradifurone ranged between 72.41 and 268.88 mg (a.i.)/L. Except for the Dörtyol, Samandağ, Silifke, and Kazanlı populations, all populations exhibited $LC_{90}$ values above the recommended field dose. Consequently, flupyradifurone is expected to be effective against the Dörtyol, Samandağ, Silifke, and Kazanlı populations, whereas its efficacy against the remaining populations is likely to be low.

The $LC_{50}$ values for acetamiprid in the populations ranged from 7.06 to 43.39 mg (a.i.)/L, with the $RR_{50}$ ranging from 38.16 to 234.53-fold, corresponding to high and very high resistance levels. The $LC_{90}$ values were estimated at 367.68-2757.36 mg (a.i.)/L, all exceeding the recommended field dose. These results indicate that acetamiprid is likely to be ineffective for controlling all *B. tabaci* populations evaluated in this study.

The $LC_{50}$ values for sulfoxaflor in *B. tabaci* populations were 0.96-72.68 mg (a.i.)/L, and $RR_{50}$ values were 3.83-290.7-fold, indicating low, moderate, high, and very high resistance levels. The $LC_{90}$ values were estimated at 33.39-2012.20 mg (a.i.)/L. Except for the Samandağ and Dörtyol populations, all populations exhibited $LC_{90}$ values above the recommended dose, suggesting that sulfoxaflor would have limited efficacy against most populations.

The observed resistance ratios provide important information on the potential efficacy of the tested insecticides under field conditions. Populations exhibiting higher RR values are expected to show reduced susceptibility, which may compromise the effectiveness of standard application rates or frequencies.

Conversely, populations with lower RR values are likely to respond more effectively to insecticide treatments. These results suggest that flupyradifurone could remain effective against several populations, whereas acetamiprid and sulfoxaflor may require adjusted management strategies in regions with highly resistant populations. The data can guide resistance management decisions, including rotation of insecticides with different modes of action and adjustments in application timing or frequency to mitigate resistance. The RR values, together with slope and heterogeneity data, provide a reliable basis for inferring relative resistance status and predicting potential biological impacts for pest control.

Although some general trends were observed between genetic groups, susceptibility differences among populations cannot be explained solely by MED versus MEAM1 classification. For acetamiprid, MED populations generally showed higher resistance, but one MEAM1 population also exhibited high resistance. For flupyradifurone, resistance levels were generally similar between MED and MEAM1 populations, indicating comparable susceptibility across genetic groups. For sulfoxaflor, the GAP population displayed the highest resistance, whereas other MEAM1 populations showed lower resistance than some MED populations. These patterns suggest that, in addition to genetic group, regional factors such as local insecticide use may contribute substantially to population-specific resistance levels. Overall, these findings highlight the importance of considering both genetic group and regional management practices when evaluating resistance and designing control strategies.

The slope values obtained from the probit analyses indicate variability in susceptibility among individuals within the tested populations. In general, slope values lower than 1 suggest a relatively heterogeneous response to the tested insecticides, whereas values higher than 2 indicate a more homogeneous susceptibility within the population (Robertson et al., 2017). In our study, slope values were below 1 for all populations, which is common for field-collected populations.

The heterogeneity values obtained from the probit analyses for the tested populations provide information on the goodness-of-fit of the probit model to the observed mortality data (Robertson et al., 2017). Values close to 1 indicate a good fit, values between 1 and 2 suggest an acceptable fit with minor variation, and values above 2 indicate poor model fit. Specifically, for flupyradifurone, heterogeneity values were below 1 except for two populations, which were 1.13 and 1.29; for sulfoxaflor, values were below 1 except for two populations, which were 1.16 and 1.28; and for acetamiprid, values ranged from 1.21 to 1.93 for four populations, while the others were below 1. All values remained below 2, indicating that the probit model provided an acceptable fit and that the $LC_{50}$ and $LC_{90}$ estimates can be considered reliable.

Flupyradifurone is a butenolide (4D) insecticide. To date, few studies have investigated the susceptibility levels of *B. tabaci* populations to flupyradifurone. Smith et al. (2016) evaluated the susceptibility of southern Florida populations of *B. tabaci* to flupyradifurone in the United States during 2013-2014. In their study, the insecticide was applied systemically to the leaves, followed by leaf disc sampling, and mixed-sex adults were used in the bioassays. They reported $LC_{50}$ values ranging from 0.011 to 1.471 mg (a.i.)/L, with resistance ratios ranging from 1.1 to 147.1-fold. Except for one population, lower $LC_{50}$ values were reported than those observed in the present study, while similar resistance ratios were detected in some populations. The observed differences in $LC_{50}$ values may be partly due to methodological differences. The extremely low $LC_{50}$ value (0.010 mg (a.i.)/L) of the susceptible population primarily accounts for the high resistance ratio observed. In addition, the low $LC_{50}$ values may reflect that sampling was conducted before flupyradifurone was registered in the United States, when its use was not yet widespread. Wang et al. (2020) reported $LC_{50}$ values ranging from 28.75 to 607.21 mg (a.i.)/L and resistance ratios between 1.37 and 29.03-fold for *B. tabaci* populations from Wuhan and Xiangyang, China. These $LC_{50}$ values were higher than those obtained in the present study. Comparable resistance ratios were observed in some populations, while higher resistance ratios detected in other populations may be explained by the high $LC_{50}$ value (20.91 mg (a.i.)/L) of the susceptible strain. Differences in $LC_{50}$ values may also be related to variation in the flupyradifurone formulation used. Guo et al. (2020) evaluated the susceptibility of 18 *B. tabaci* populations

in China to flupyradifurone and reported $LC_{50}$ values ranging from 6.4 to 93.3 mg (a.i.)/L, with resistance ratios between 0.5 and 7.0-fold. Compared with the present study, higher $LC_{50}$ values and lower resistance ratios were reported. These differences may be explained by the relatively high $LC_{50}$ value (13.3 mg (a.i.)/L) of the susceptible population. Differences in $LC_{50}$ values may also be related to the use of flupyradifurone in China since 2016 and as also suggested by Wang et al. (2020), to variation in the formulation used.

Acetamiprid is a neonicotinoid insecticide**.** Satar et al. (2018) determined the susceptibility of *B. tabaci* populations sampled in 2009 across the Mediterranean Region of Türkiye using female adults, and reported $LC_{50}$ values ranging from 39.9 to 213.1 mg (a.i.)/L, with resistance ratios ranging from 12.8 to 68.2 -fold. Vassiliou et al. (2011) assessed *B. tabaci* populations collected in 2006-2007 from Southern Cyprus, a region geographically close to Türkiye, and reported $LC_{50}$ values between 67.1 and 122.67 mg (a.i.)/L, with resistance ratios ranging from 7 to 12-fold. Wang et al. (2017) determined the susceptibility of Q-biotype *B. tabaci* populations collected in China between 2011 and 2013 and reported $LC_{50}$ values for acetamiprid ranging from 45.5 to 187.4 mg (a.i.)/L, with resistance ratios of 14.16-58.22-fold. The populations reported in the above-mentioned studies differ from those evaluated in the present study, with higher $LC_{50}$ values. Bahşi et al. (2012) reported $LC_{50}$ values ranging from 4.5 to 209.6 mg (a.i.)/L and resistance ratios between 6- and 299-fold for *B. tabaci* populations collected from districts of Antalya Province during 2007-2009. Xie et al. (2014) reported $LC_{50}$ values of 30.7-417 mg (a.i.)/L and resistance ratios of 1.40-19.0-fold for field and laboratory populations from China. Similarly, Basij et al. (2017) determined $LC_{50}$ values for acetamiprid against *B. tabaci* populations collected in Iran using adult females in the bioassays, reporting $LC_{50}$ values ranging from 4.96 to 865 mg (a.i.)/L with resistance ratios of 6.38-174.57-fold. While some $LC_{50}$ values overlap with those obtained in the present study, higher $LC_{50}$ values were also reported compared with our results. The lower $LC_{50}$ values observed in the present study may be explained by the reduced use of neonicotinoid insecticides in Türkiye following the ban on clothianidin, imidacloprid, thiacloprid, and thiamethoxam since 2019, resulting in decreased selection pressure on acetamiprid. In addition, the introduction of insecticides with different modes of action and their use in rotation may have further reduced acetamiprid application. Conversely, the higher $LC_{50}$ values reported in other studies may be related to earlier sampling periods, when acetamiprid use was more widespread and *B. tabaci* populations were exposed to greater selection pressure. Differences in resistance ratios may also be associated with higher $LC_{50}$ values of susceptible reference populations used for comparison, as well as regional differences in agricultural practices and insecticide use.

Sulfoxaflor is a sulfoximine (4C) insecticide and was registered for use in Türkiye in 2016 (Longhurst et al., 2013). Mohammed et al. (2020) investigated the susceptibility of *B. tabaci* populations from Mersin to sulfoxaflor and reported $LC_{50}$ values ranging from 31.13 to 51.13 mg (a.i.)/L, with resistance ratios between 1.0- and 1.86-fold. These results differ from the present study, as lower $LC_{50}$ values and lower resistance ratios were reported, whereas only one population in the present study exhibited a higher $LC_{50}$ value. This difference may be primarily explained by the relatively high $LC_{50}$ value (26.57 mg (a.i.)/L) of the susceptible strain used in that study, relative to the values observed for the reference SUD-s population in the present study, as well as by differences in the sulfoxaflor formulation (WG) applied. Wang et al. (2017) assessed the susceptibility of *B. tabaci* populations from China to sulfoxaflor and reported $LC_{50}$ values ranging from 3.86 to 29.04 mg (a.i.)/L with resistance ratios between 0.40 and 3.07-fold. While some $LC_{50}$ values partially overlap with those obtained in the present study, the generally lower resistance ratios and the higher $LC_{50}$ values observed in our study indicate differences between the studies. These differences may be related to the relatively high $LC_{50}$ value (9.44 mg (a.i.)/L) of the susceptible population used for comparison, as well as to the earlier sampling years (2013-2014), which likely resulted in lower selection pressure from sulfoxaflor compared with the populations examined in the present study. Chen et al. (2018) reported $LC_{50}$ values ranging from 10.55 to 146.19 mg (a.i.)/L for sulfoxaflor in *B. tabaci* populations from China. These results do not fully overlap with those of the present study, except for the Gazipaşa

population, which exhibited comparable $LC_{50}$ values. The higher $LC_{50}$ values reported may be associated with the earlier registration of sulfoxaflor in China (2014), leading to greater selection pressure compared with populations from Türkiye. As no susceptible reference population was included in that study, resistance ratios could not be directly compared with those obtained here. Longhurst et al. (2013) evaluated the susceptibility of a *B. tabaci* population known to possess multiple resistance using adult females in the bioassays and reported an $LC_{50}$ value of 4.99 mg (a.i.)/L and a resistance ratio of 2.77-fold for sulfoxaflor, indicating no loss of susceptibility. While these $LC_{50}$ values are comparable to those obtained for some populations in the present study, the higher resistance ratios observed in our study suggest differences that may be related to the earlier sampling period (1997-2008), during which populations were exposed to lower selection pressure from sulfoxaflor. Guo et al. (2020) reported $LC_{50}$ values ranging from 5.4 to 53.1 mg (a.i.)/L and resistance ratios between 0.3 and 3.2-fold for *B. tabaci* populations from China. Although some $LC_{50}$ values overlap with those obtained in the present study, the lower resistance ratios differ from our results. This difference may be primarily attributed to the relatively high $LC_{50}$ value of the susceptible population used for comparison, as well as to longer-term sulfoxaflor use leading to differences in selection pressure.

## Conclusion

The results of this study demonstrate that *B. tabaci* populations exhibit particularly high resistance levels to acetamiprid. Resistance to sulfoxaflor and flupyradifurone was generally low to moderate, although high and very high resistance levels were also detected for sulfoxaflor in some populations. Despite the relatively recent registration of flupyradifurone in Türkiye (2020), the observed resistance development may be associated with the shared mode of action on nicotinic acetylcholine receptors and could potentially be linked to other compounds targeting the same site. Continuous monitoring of all tested insecticides is recommended to follow changes in resistance levels and support the development of resistance management strategies.

The results of the laboratory resistance levels suggest that continuous and intensive use of sulfoxaflor and flupyradifurone could contribute to further resistance development. Therefore, rotation strategies incorporating insecticides with different modes of action are recommended as part of integrated resistance management programs. Given the consistently high resistance levels observed for acetamiprid, its use in *B. tabaci* control should be minimized and restricted to rotation programs with insecticides possessing different modes of action. Overall, the findings of this study provide valuable baseline data for future resistance monitoring efforts and contribute to the development of more sustainable management strategies for *B. tabaci* in Türkiye.

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