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Original article (Orijinal araştırma)

Insecticide resistance status of *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) populations to cyantraniliprole, pyriproxyfen and spirotetramat in Antalya (Türkiye)[1]

Antalya (Türkiye)'dan *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) popülasyonlarının cyantraniliprole, pyriproxyfen ve spirotetramata direnç düzeyleri

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Abstract

In the study, the susceptibility of twelve *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) MEAM1 and MED populations collected from tomato and pepper greenhouses in Antalya Province (Türkiye) in 2019 and 2020 to spirotetramat, pyriproxyfen and cyantraniliprole were determined. To determine the lethal concentrations (LC_{50}) for the populations, spirotetramat and pyriproxyfen were applied using leaf dipping method to second instar and eggs, respectively, while a systemic uptake method was used for testing the susceptibility of whitefly instars to cyantraniliprole. The resistance ratios were calculated by dividing the LC_{50} of the populations by the LC_{50} of a susceptible population. The LC_{50} of the populations ranged from 0.28 to $1.70x10^3$ mg a.i./l for pyriproxyfen, from 1.76 to 228 mg a.i./l for spirotetramat, and from 0.103 to 0.382 mg a.i./l for cyantraniliprole. Resistance ratios for pyriproxyfen were particularly high. For spirotetramat and cyantraniliprole resistance varied between 2.38 and 309, and 4.68 to 17.4 times, respectively. All populations were susceptible to cyantraniliprole, but some populations highly resistance to pyriproxyfen and spirotetramat. The results will be a valuable reference for future monitoring and management of insecticide resistance.

Keywords: Biotype, insecticide, resistance, susceptibility, whitefly

Öz

Çalışmada, Antalya İli (Türkiye)'nden 2019 ve 2020 yıllarında toplanan *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae)'nin oniki farklı MEAM1 ve MED popülasyonlarının spirotetramat, pyriproxyfen ve cyantraniliprole karşı duyarlılık düzeyleri belirlenmiştir. Popülasyonların lethal konsantrasyon (LC_{50}) değerleri spirotetramat ve pyriproxyfen için yaprak daldırma yöntemi ile sırasıyla 2. larva ve yumurta dönemine uygulanarak ve cyantraniliprole için sistemik alım yöntemi ile larva dönemine uygulanarak belirlenmiştir. Çalışmada popülasyonların LC_{50} değerlerinin duyarlı popülasyonunun LC_{50} değerine bölünmesiyle popülasyonların direnç katları belirlenmiştir. Popülasyonların LC_{50} değerleri, pyriproxyfen için 0.28 ila $1.70x10^3$ mg a.i./l, spirotetramat için 1.76 ila 228 mg a.i./l ve cyantraniliprole için 0.103 ila 0.382 mg a.i./l aralıklarında belirlenmiştir. Popülasyonların pyriproxyfen için direnç katları çok yüksek seviyede tespit edilmiştir. Direnç oranlarının spirotetramat için 2.38 ile 309 kat arasında ve cyantraniliprole için 4.68 ile 17.4 kat arasında değiştiği tespit edilmiştir. Sonuçlara göre, tüm popülasyonların cyantraniliprole karşı duyarlı olduğu tespit edilirken, pyriproxyfen ve spiroteramata karşı bazı popülasyonlarda yüksek düzeyde direnç tespit edilmiştir. Sonuçlar, insektisit direncinin gelecekte izlenmesi ve yönetimi için referans verileri içermektedir.

Anahtar sözcükler: Biyotip, insektisit, direnç, hassasiyet, beyazsinek

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Introduction

The cotton whitefly, *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae), is a polyphagous pest that causes significant damage to many vegetables and ornamental plants in tropical and subtropical regions (Frohlich et al., 1999; De Barro et al., 2000). *Bemisia tabaci* is a species complex that has been reported in all regions of the world, except Antarctica, because of its ready adaptability to new hosts, geographic regions and has been found in association with 600 plant species. (Martin et al., 2000). By feeding on the plant, it can cause about 50% loss of yield and promote sooty mold by secreting honeydew (Horowitz et al., 2003). It is also a vector of plant viruses such as tomato yellow leaf curl virus (TYLCV) (Horowitz et al., 2003).

Several biotypes of *B. tabaci* have been identified in different parts of the world (Perring, 2001) and this indicates that *B. tabaci* is a complex of species, genetic groups or biotypes. Differences in biological properties of the biotypes cause differences in sensitivity to insecticides (Perring, 2001; Abdullahi et al., 2003). Middle East-Asia Minor1 (MEAM1) (formerly B biotype) and Mediterranean (MED) (formerly Q biotype) are the two most widespread and devastating genetic groups in the Mediterranean Basin (Horowitz et al., 2005). The MEAM1 and MED were reported from Türkiye (Erdoğan et al., 2008; Yükselbaba et al., 2012; Karut et al., 2017; Dağlı et al., 2020a).

Chemicals are widely used to control this pest because they are easy to apply and have a quick effect. *Bemisia tabaci* populations have acquired resistance to insecticides and insect growth regulators such as organophosphates, pyrethroids, neonicotinoids, carbamates and juvenile hormone analogs as a result of widespread chemical use (Denholm et al., 1996; Horowitz et al., 1998, 2005; Elbert & Nauen, 2000; Gravalos et al., 2015). *Bemisia tabaci* ranks fifth of the 12 insect species with the highest insecticide resistance reported worldwide (APRD, 2018). Due to resistance problems, there is a need for a new chemical compound that are especially effective against the target pest and have low toxic effects on the environment. Spirotetramat is a novel insecticide belonging to the new chemical class of tetramic acid derivatives (Bielza et al., 2019). Tetramic acid derivatives affect the second and third instars of whiteflies, and its mode of action appears by inhibiting the lipid metabolism enzyme, acetyl-CoA-carboxylase, causing a decrease in total lipids (Bretschneider et al., 2003; Nauen et al., 2005). Diamides are the most exciting new class of insecticides developed recently. Diamide insecticides have a novel mode of action that acts on the ryanodine receptor in insects, no other synthetic insecticide has ever been used at this site of the insect, and have very low mammalian toxicity due to their specificity for insect ryanodine receptors (Gravalos et al., 2015). Pyriproxyfen is a juvenile hormone analog that inhibits hatching of eggs and suppresses adult emergence in whiteflies and other insects (Horowitz et al., 1999; Li et al., 2012).

Overall, resistance monitoring studies are important tools for early detection of a decrease in susceptibility to insecticides in pests known to be prone to development of resistance. There were studies conducted on determination of the resistance of *B. tabaci* to cyantraniliprole, spirotetramat and pyriproxyfen in a number of countries. Hopkinson & Pumpa (2019) studied the toxicity of spirotetramat, cyantraniliprole and dinotefuran to Australian *B. tabaci* MEAM1 populations. Gravalos et al. (2015) investigated the resistance and cross-resistance of *B. tabaci* Mediterranean strains collected from Greece, Italy and Spain to cyantraniliprole. Bielza et al., (2019) determined the resistance and cross-resistance status of Spanish *B. tabaci* populations against spiromesifen and spirotetramat compounds. Resistance of *B. tabaci* populations against pyriproxyfen have been documented from several countries including China, Egypt, Israel, Spain and the USA (Horowitz et al., 1999, 2002, 2005; El Kady & Devine, 2003; Fernandez et al., 2009).

There are limited studies on the resistance of *B. tabaci* to these insecticides in Türkiye. Erdoğan et al. (2008) determined the resistance level of *B. tabaci* populations to organophosphates, synthetic pyrethroids, and insect growth regulator in 2000 and 2001. Dağli et al. (2020b) determined the susceptibility of *B. tabaci* Mediterranean and Aegean populations collected between 2005 and 2006 to endosulfan, lambda-cyhalothrin

and imidacloprid. Bahşi et al. (2012) determined the resistance level of *B. tabaci* populations collected from Antalya and its districts to the chlorpyrifos-ethyl, cypermethrin and acetamiprid in between 2007 and 2009. Satar et al. (2018) studied the resistance of five *B. tabaci* populations collected from vegetable and cotton fields in provinces of Mediterranean Region of Türkiye to neonicotinoids in 2009. Mohammed et al. (2020) determined the resistance of *B. tabaci* populations collected from greenhouses in Mersin Province to the acetamiprid, imidacloprid, thiamethoxam, spinetoram, spinosad and sulfoxaflor in 2018.

Studies on the susceptibility of *B. tabaci* populations have shown that the risk of the development of resistance is high in regions where repeated insecticide applications are common during the growing season to control the pest (Wang et al., 2020). In these cases, the necessity of appropriate and regular resistance screening studies becomes clear. To the best of our knowledge, is that no research has been conducted in Türkiye on the susceptibility of *B. tabaci* populations to spirotetramat, and cyantraniliprole. This study is aimed to determine the susceptibility and current resistance of whitefly populations to spirotetramat, pyriproxyfen, and cyantraniliprole in the Western Mediterranean Region of Türkiye to design strategies for the control of *B. tabaci* and discuss the sustainability of these strategies. Based on the data obtained as a result of the research, the study is aimed to contribute to the design of resistance management methods to delay and prevent the development of resistance. The data obtained in this study will be informative in terms of comparison of the resistance level to the specified active substances. Additionally, it can be a valuable resource for researchers on this subject and will provide data containing important information from Türkiye

Materials and Methods

Insecticides

For all insecticide bioassays, commercial formulations of the diamide group- cyantraniliprole (Circaden 200SC 200 g/l, USA), tetramic acid spirotetramat (Movento SC100 100 g/l, Germany) and insect growth regulator pyriproxyfen (Admiral 10 EC 100 g/l, France) were used in the study.

Insects

Bemisia tabaci populations were collected from tomato and pepper greenhouses in Alanya, Demre, Gazipaşa, Gaziler, Kumluca, and Serik districts in Antalya Province, with at least 200 whiteflies in 2019 and 2020 (Table 1). Antalya Province is located in the Western Mediterranean Region of Türkiye. Antalya is greenhouse vegetable cultivation center of Türkiye with has 37% of the country's total greenhouse area with 27.8 kha of greenhouse cultivation and 48% of the greenhouse vegetable production (TUIK, 2019). In Türkiye, the amount of pesticide uses in 2018 was 60 kt and regionally, pesticides were mostly used in the Mediterranean Region with 29%. Antalya Province ranked first place with 8.6% of total pesticide use in Türkiye (Anonymous, 2021). The collected *B. tabaci* populations were maintained on cotton plants in climate chambers with 26 ± 1°C temperature, 60 ± 10% relative humidity (RH) and 16:8 h L:D photoperiod without any insecticide application. An insecticide susceptible reference population (SUD-S) was also used in the study. SUD-S, initially collected on cotton in Sudan in 1978, was obtained from Raulf Nauen (Crop Science Division R&D Bayer AG, Germany) where it has been maintained in the absence of insecticides for the past 40 years. SUD-S was maintained on cotton as above conditions.

Determination of the genetic groups of *Bemisia tabaci* populations

Genetic groups of *B. tabaci* populations used in the study were identified using sequence information of the mitochondrial cytochrome oxidase I (mtCOI) region. The mtCOI were amplified with specific primers "C1-J-2195 5'-TTGATTTTTTGGTCATCCAGAAGT-3', "TL2-N-3014 5'-TCCAATGCACTAATCTGCCATATTA-3' (Frohlich et al., 1999), as stated by Yükselbaba & Göçmen (2016). According to the protocol specified in Omega EZNA SQ Tissue DNA isolation kit, DNA isolation was made from 10 individual female *B. tabaci*

adults subjected from each population. Following DNA isolation, amplification of the mtCOI region was determined according to Yükselbaba & Göçmen (2016). The PCR products obtained were sequenced in both directions by BM Labosis (Ankara, Türkiye). Using the BLAST tool, the nucleotide sequences were compared to those in the GenBank database (www.ncbi.nlm.nih.gov). Sequences were added to the GenBank database under accession numbers ON738324- ON738335 (Table 1).

Insecticide bioassay

The LC_{50} and LC_{90} of the populations were determined separately for each active substance. Lethal concentrations were determined by using at 5 or more concentrations of each insecticide, giving mortality between 10 and 90%. For populations collected in 2019 and 2020 each dose was applied to 5 or more replicates.

Determination of susceptibility of *Bemisia tabaci* populations to pyriproxyfen

The baseline susceptibility of *B. tabaci* populations to pyriproxyfen were determined by applying leaf dipping method to *B. tabaci* eggs as described by Horowitz et al. (1999), Ma et al. (2010) and IRAC (2019) method 16. At least twenty whitefly adults from the population were aspirated with a mouth aspirator and placed in small cages. The cages were attached to a young cotton plant leaves with a clip and kept in climate chambers at 26 ± 1°C, 60 ± 10% RH and 16:8 h L:D photoperiod for 24 h to allow whiteflies to deposit eggs on the cotton leaves. Then the adults were removed and the total number of eggs on the leaves were determined and noted. There were at least 30 eggs per leaf. The leaves with whitefly eggs were dipped for 10 s in insecticide solutions containing Triton-X in a volume of 100 ml and dipped a pure Triton-X solution as a negative control. The treated leaves were checked about 8 days after the insecticide application. Mortality of the eggs was calculated by subtracting the live instars from the total number of eggs.

Determination of the susceptibility of *Bemisia tabaci* populations to spirotetramat

For spirotetramat, the leaf dipping method described by Bielza et al. (2019) was used with slight modification. At least 20 whitefly adults taken from the populations were placed in cages and young cotton plant leaves were attached to the cages to allow whiteflies to deposit eggs on the cotton leaves for 24 h. After 24 h the whiteflies were removed, and the young cotton plants were kept at 26°C, 60% RH and a16:8 h L:D photoperiod for 10 days to allow second instars to develop. Then, using a microscope, different stages of the whitefly on the leaves were removed and the second instar numbers were noted. There were at least 25 second instars per leaf. Then the leaves with second instars were dipped in 100 ml serial dilutions of insecticide solutions containing Triton-X and a negative control for 10 s. The treated leaves were checked 6 days after the application. Instars that developed to pupal stage were considered to be living, dried nymphs as well as those that did not develop and remained as 2nd instars were considered as affected and dead ones.

Determination of the susceptibility of *Bemisia tabaci* populations to cyantraniliprole

The lethal concentrations of *B. tabaci* populations against cyantraniliprole were determined by using the systemic uptake method described by Li et al. (2012) with some modifications. At least twenty adult whiteflies were taken from the populations and placed in cages. Young cotton plant leaves were attached to the cages to allow them to deposit eggs on the leaves for 24 h. After that whiteflies were removed and the number of eggs on the leaves were counted. There were at least 30 eggs per leaf. The shoots of the cotton plants were cut into ~13 cm lengths with the help of clean scissors and placed in tubes containing 20 ml of serial doses of cyantraniliprole containing Triton-X and a negative control. These tubes with cotton plant shoots were kept in a climate chamber with 26°C, 60% RH and a16:8 L:D photoperiod for 12 days after the application. The nymphs that developed to second instars were considered as alive. Mortality was determined by subtracting the number of living second instars from the total number of eggs (Li et al., 2012).

Data analysis

Lethal concentrations, 95% confidence interval and related parameters of the populations were determined by probit analysis POLO-PC (Leora Software 2008, Petulama, CA, USA). The resistance ratio (RR_{50}) of the populations was calculated by dividing their LC_{50} by the LC_{50} of SUD-S. *Bemisia tabaci* populations were considered significantly different when the 95% confidence interval of the LC_{50} of the *B. tabaci* populations did not overlap (Nauen et al., 2005; Gravalos et al., 2015). The insecticide resistance level was classified according to the criteria: low resistance (RR_{50} = 2-10); moderate resistance (RR_{50} = 11-30); high resistance (RR_{50} = 31-100); very high resistance (RR_{50} > 100) (Torres-Vila et al., 2002; Peng et al., 2017).

Results

In the study, the genetic groups of *B. tabaci* populations used in insecticide bioassays were determined as MED and MEAM1 groups (Table 1).

Table 1. Details of *Bemisia tabaci* populations used in the study

Population code	Location	Sampling date	Coordinates	Host	Group	Accession
ALN19	Alanya	27.06.2019	36°35'37" N, 31°51'33" E	*Solanum lycopersicum*	MEAM1	ON738334
ALN20	Alanya	20.06.2020	36°35'38" N, 31°51'45" E	*S. lycopersicum*	MEAM1	ON738335
DMR19	Demre	28.06.2019	36°24'24" N, 30°00'20" E	*S. lycopersicum*	MED	ON738326
DMR20	Demre	21.06.2020	36°25'51" N, 30°02'15" E	*Capsicum annuum*	MED	ON738327
GZP19	Gazipaşa	27.06.2019	36°16'46" N, 32°20'28" E	*S. lycopersicum*	MEAM1	ON738332
GZP20	Gazipaşa	20.06.2020	36°15'46" N, 32°19'28" E	*S. lycopersicum*	MEAM1	ON738333
GLR19	Gaziler	24.06.2019	36°99'58" N, 30°77'80" E	*S. lycopersicum*	MEAM1	ON738330
GLR20	Gaziler	19.06.2020	36°98'32" N, 30°76'05" E	*S. lycopersicum*	MEAM1	ON738331
KML19	Kumluca	28.06.2019	36°22'23" N, 30°17'50" E	*S. lycopersicum*	MED	ON738324
KML20	Kumluca	21.06.2020	36°30'23" N, 30°35'50" E	*C. annuum*	MED	ON738325
SRK19	Serik	27.06.2019	36°56'36" N, 31°2'28" E	*S. lycopersicum*	MED	ON738328
SRK20	Serik	19.06.2020	37°00'44" N, 31°03'53" E	*S. lycopersicum*	MED	ON738329
SUD-S	Sudan	1978	-	*Gossypium hirsutum*	-	

In the study, pyriproxyfen was applied to *B. tabaci* eggs. The LC_{50} of *B. tabaci* populations ranged between 0.28 and $1.70x10^3$ mg a.i./l (Table 2). The highest LC_{50} was found in GLR19 population, while GZP20 population had the lowest LC_{50}. Table 2 shows that the resistance ratios of the populations ranged from 350 to $2.12x10^6$. There were four orders of magnitude differences between the populations. The confidence interval of all populations did not overlap with the confidence interval for SUD-S.

The LC_{50} of the populations to spirotetramat were between 1.76 and 228 mg a.i./l (Table 3). The highest LC_{50} was determined in the GZP19 population, while the lowest LC_{50} was determined in the KML19 population. The resistance ratios of the populations ranged from 2.38 to 309. The confidence interval of SRK20 overlapped with the confidence interval of SUD-S. All other populations were significantly different from SUD-S.

Table 2. Susceptibility status of *Bemisia tabaci* populations to pyriproxyfen

Population	n	Slope ± SE	LC_{50} (mg a.i./l) (95% CL)	LC_{90} (mg a.i./l) (95% CL)	H	X^2	df	RR_{50}
ALN19	1856	1.03 ± 0.08	0.33 (0.18-0.51)	5.85 (3.86-10.4)	2.29	68.8	30	412
ALN20	1497	1.05 ± 0.05	0.34 (0.19-0.55)	5.70 (3.31-11.7)	3.89	97.0	25	425
DMR19	2182	1.34 ± 0.07	0.71 (0.34-1.15)	6.36 (3.75-5.23)	9.42	273	29	887
DMR20	1347	0.63 ± 0.04	3.65 (1.96-6.86)	967 (362-3.48x10^3)	2.14	44.9	21	4.56x10^3
GZP19	3080	0.90 ± 0.05	0.85 (0.16-2.24)	22.6 (8.73-117)	15.0	511	34	1.06x10^3
GZP20	1710	1.03 ± 0.05	0.28 (0.15-0.45)	4.96 (2.98-9.38)	3.97	103	26	350
GLR19	3805	0.38 ± 0.03	1.70x10^6 (711-5.06x10^3)	3.94x10^6 (499x10^3-12.2x10^6)	3.61	148	41	2.12x10^6
GLR20	1160	0.46 ± 0.04	291 (94.1-984)	186x10^3 (24.6x10^3-10.3x10^6)	3.20	70.5	22	363x10^3
KML19	3766	0.42 ± 0.03	243 (105-528)	121x10^3 (34.5x10^3-773x10^3)	5.73	223	39	304x10^3
KML20	2518	2.81 ± 0.32	564 (467-674)	1.61x10^3 (1.20x10^3-2.81x10^3)	1.99	37.8	19	705x10^3
SRK19	3415	0.48 ± 0.03	1.01x10^3 (308-4.19x10^3)	1.19x10^6 (104x10^3-300x10^6)	7.09	269	38	1.26x10^6
SRK20	2451	0.49 ± 0.03	134 (62.5-261)	57.9x10^3 (19.2x10^3-291x10^3)	3.43	85.7	25	167x10^3
SUD-S	2400	1.92 ± 0.18	0.0008 (0.0001-0.011)	0.0036 (0.0021-0.37)	11.7	351	30	1.00

n, number of individuals used in bioassay; $RR_{50,}$ ratio of LC_{50} of the test population and the susceptible population; H, heterogenity; X^2, Chi-square; and df: degrees of freedom

Table 3. Susceptibility status of *Bemisia tabaci* populations to spirotetramat

Population	n	Slope ± SE	LC_{50} (mg a.i./l) (95%CL)	LC_{90} (mg e.m./l) (95% CL)	H	X^2	df	RR_{50}
ALN19	1479	0.45 ± 0.03	10.8 (4.34-22.9)	7.56x10^3 (2.56x10^3-35.5x10^3)	2.04	69.4	34	14.6
ALN20	1357	0.59 ± 0.04	2.83 (1.12-5.84)	413 (201-1.03x10^3)	1.96	58.7	30	3.82
DMR19	2079	1.05 ± 0.09	156 (63.3-260)	2.59x10^3 (1.53x10^3-6.73x10^3)	3.36	141	42	211
DMR20	1107	0.52 ± 0.04	43.9 (24.2-79.8)	12.9x10^3 (4.65x10^3.-52.2x10^3)	1.68	40.4	24	59.3
GZP19	2108	1.21 ± 0.12	228 (124-346)	2.61x10^3 (1.36x10^3-10.3x10^3)	3.99	172	43	309
GZP20	1165	0.81 ± 0.11	18.2 (2.16-53.1)	699 (295-2.50x10^3)	2.23	62.6	28	24.6
GLR19	1782	0.99 ± 0.05	4.15 (2.51-6.27)	81.1 (52.4-138)	2.64	89.8	34	5.60
GLR20	986	0.51 ± 0.05	6.24 (1.04- 22.4)	2.05x10^3(517-16.7x10^3)	2.95	79.7	27	8.43
KML19	3102	1.18 ± 0.05	1.76 (1.25-2.38)	21.7 (15.5-32.7)	2.96	145	49	2.38
KML20	836	0.46 ± 0.06	92.7 (41.7-184)	57.7x10^3 (17.1x10^3-372 x10^3)	0.89	17.8	20	125
SRK19	2197	0.68 ± 0.03	2.32 (1.06-4.50)	182 (83.9-505)	5.80	203	35	3.14
SRK20	1462	0.59 ± 0.04	2.01 (0.93-3.89)	308 (132-970)	2.63	78.8	30	2.72
SUD-S	1792	1.29 ± 0.09	0.74 (0.46-1.02)	7.26 (4.93-13.2)	3.04	94.1	31	1.0

n, number of individuals used in bioassay; $RR_{50,}$ ratio of LC_{50} of the test population and the susceptible population; H, heterogenity; X^2, Chi-square; and df: degrees of freedom.

The LC_{50} of cyantraniliprole for the populations ranged from 0.103 to 0.382 mg a.i./l, and the LC_{50} of SUD-S was determined as 0.022 mg a.i./l (Table 4). KML20 population had the highest LC_{50}, while ALN19 population had the lowest LC_{50}. Based on the LC_{50} of the SUD-S population Table 4 shows that the resistance ratios of the populations ranged from 4.68-17.4 times. The confidence interval of the populations did not overlap with the confidence interval of SUD-S.

Table 4. Susceptibility status of *Bemisia tabaci* populations to cyantraniliprole

Population	n	Slope ± SE	LC_{50} (mg a.i./l) (95%CL)	LC_{90} (mg e.m./l) (95%CL)	H	X^2	df	RR_{50}
ALN19	1845	2.04 ± 0.19	0.103 (0.08-0.127)	0.44 (0.37-0.57)	1.44	36.1	25	4.68
ALN20	582	2.34 ± 0.31	0.155 (0.109-0.202)	0.55 (0.37-1.46)	2.81	64.6	23	7.04
DMR19	1288	2.12 ± 0.16	0.289 (0.241-0.343)	1.16 (0.89-1.70)	1.94	68	35	13.1
DMR20	929	1.64 ± 0.14	0.304 (0.257-0.360)	1.83 (1.35-2.76)	0.71	14.2	20	13.8
GZP19	2130	2.70 ±0.17	0.262 (0.167-0.364)	0.78 (0.54-1.47)	9.73	350	36	11.9
GZP20	1218	2.26 ± 0.15	0.301 (0.227-0.410)	1.11 (0.73-2.32)	5.26	121	23	13.7
GLR19	2364	2.65 ± 0.12	0.298 (0.264-0.333)	0.91 (0.78-1.10)	2.06	78.3	38	13.5
GLR20	1015	2.55 ± 0.19	0.272 (0.241-0.309)	0.87 (0.71-1.12	0.55	11.0	20	12.4
KML19	3300	2.84 ± 0.13	0.283 (0.193-0.423)	0.80 (0.52-1.76)	15.39	570	37	12.9
KML20	801	1.54 ± 0.13	0.382 (0.218-0.651)	2.59 (1.28-12.67)	6.34	146	23	17.4
SRK19	2749	2.04 ± 0.09	0.353 (0.313-0.398)	1.50 (1.21-2.00)	2.09	73	35	16.0
SRK20	1637	2.14 ± 0.13	0.346 (0.285-0.429)	1.37 (1.00-2.16)	2.41	57.9	24	15.8
SUD-S	1888	2.21 ± 0.11	0.022 (0.013-0.041)	0.083 (0.043-0.505)	26.97	566	21	1.0

n, number of individuals used in bioassay; $RR_{50,}$ ratio of LC_{50} of the test population and the susceptible population; H, heterogenity; X^2, Chi-square; and df: degrees of freedom.

Discussion

The idea of biotypes in *B. tabaci* was introduced in 1950s after *B. tabaci* populations could not be separated morphologically due to different biological characteristics (Perring, 2001). It has been suggested that host associations, virus-carrying capacity, as well as different susceptibility and resistance to insecticides, resulted in biological differences between biotypes (Horowitz et al., 2005; De la Rua et al., 2006). Khasdan et al. (2005) suggested that different resistance to insecticides in *B. tabaci* B and Q biotypes have an impact the spread and dynamics of the biotypes. Kontsedalov et al. (2012) associated the biotype changes with different susceptibility of biotypes to insecticides. In this study the populations were determined as MED and MEAM1 genetic groups. Confidence interval of MEAM1 and MED populations with the highest LC_{50} for pyriproxyfen were overlapped, similarly, no difference was observed in the confidence intervals of MEAM and MED populations with the lowest LC_{50} for pyriproxyfen. There was no difference in the confidence interval of MEAM and MED populations with highest LC_{50} and the lowest LC_{50} for spirotetramat and cyantraniliprole. No differences were observed in the susceptibility to these insecticides in the MEAM1 and MED populations.

Pyriproxyfen resistance was observed in this study for the first time in Türkiye, with a very high level of resistance in all populations compared to the LC_{50} of the SUD-S population. Despite the high resistance ratios, the LC_{90} of the populations collected from Alanya, Demre and Gazipaşa remained below the recommended dose. Thus, pyriproxyfen has a high chance of controlling these populations. Additionally, the LC_{90} of Gaziler, Kumluca and Serik populations were above the recommended dose. Therefore, it has been conducted that pyriproxyfen might not be effective for controlling *B. tabaci* in these three regions.

Similarly, Horowitz et al. (2002) observed high resistance (>500 times) to pyriproxyfen after three consecutive applications in rose greenhouses one year after pyriproxyfen was introduced in Israel. Fernandez et al. (2009) determined the susceptibility of six *B. tabaci* populations to pyriproxyfen in Spain in 2006. it was determined that the LC_{50} of the populations ranged from 15.4 to 402 mg a.i./l with resistance ratios between 0.7 and 19.3 times. Despite high LC_{50}, low resistance ratios were reported due to the reference population used in their studies. Hopkinson et al. (2020) determined the pyriproxyfen susceptibility of *B. tabaci* populations from cotton fields in Australia in 2017 with LC_{50} between 0.001 and 2.1 mg a.i./l and resistance ratios ranging from 0.10 to 96.9 times compared to the susceptible population. Wang et al. (2020) reported the LC_{50} of six *B. tabaci* populations collected from Shangdong Province, China for pyriproxyfen ranged from 15.3 to 59.0 mg a.i./l with resistance ratios between 1.44 and 5.55 times. In comparison to other studies, the LC_{50} they determined were higher, although they described the resistance of populations as low. Ma et al. (2007) determined the resistance level of six *B. tabaci* populations collected from the Xinjiang Province, China in 2004 and 2005 for pyriproxyfen. They found, the LC_{50} of the populations ranged from 0.021-0.037 a.i./l with resistance ratios between 22 and 37. Toscano et al. (2001) determined the LC_{50} of *B. tabaci* populations collected in Arizona and California, USA between 1997 and 1999 were in between 0.003 and 9.7 mg a.i./ml for pyriproxyfen. In their study, they found over three orders of magnitude variant in susceptibility to pyriproxyfen. Although LC_{50} and resistance ratios reported in some of these studies partially overlap to the LC_{50} of some populations in our study, our study differed in that it includes populations with higher LC_{50} and resistance ratios. In our study, we obtained high LC_{50} such as $1.70x10^3$ and $1.01x10^3$ mg a.i./l to pyriproxyfen. The primary reason for this high value is the licensed and extensive use of pyriproxyfen in the management of *B. tabaci* since 1995 in Türkiye. Very high resistance ratios were observed in our study. SUD-S is highly susceptible because it has been maintained for many years without being exposed to insecticide under laboratory conditions which resulted in high resistance ratios we recorded. Similarly, Bielza et al. (2007) reported about $3x10^6$ times resistance to spinosad in *F. occidentalis* populations. They noted that the highly sensitive laboratory strain results in very high rates of resistance in field populations.

Tetronic and tetramic acid derivatives, often known as ketoenols, have been approved for use against *B. tabaci* in Türkiye since 2009. In Türkiye, no research on the susceptibility of *B. tabaci* to spirotetramat has been conducted. We conduct the first study on spirotetramat susceptibility in *B. tabaci* populations in Türkiye. In the study, low to very high resistance in *B. tabaci* to spirotetramat were observed. Based on the LC_{90} of the populations, it was found that all populations except KML19 had LC_{90} above the recommended dose. Based on our findings, it was determined that spirotetramat could have a low success rate in controlling *B. tabaci* in the sampling regions. In parallel with our study, high resistance was reported in China and Spain. Peng et al. (2017) studied the resistance changes in *B. tabaci* Q biotype to spirotetramat from 2012 to 2016 in China. They determined that all populations showed an increase in resistance from a low level in 2012 to a moderate or high level in 2016. They found that the resistance of two populations had increased to 184 (1.40 mg a.i./l) and 544 (4.13 mg a.i./l) times in 2016. Bielza et al. (2019) determined the susceptibility of 19 *B. tabaci* field populations for spirotetramat in Spain. They reported that the LC_{50} of the most susceptible and resistant field populations were 14.2 and 306 mg a.i./l with resistance ratio of 6-130, respectively. Other research (mentioned below) partially overlap with our study's low LC_{50} and resistance ratios. Hopkinson & Pumpa (2019) reported the susceptibility status of *B. tabaci* populations to spirotetramat ranged from 2.80 to 5.98 mg a.i./l with a 2.1 times difference. Prabhaker et al. (2014) determined the susceptibility status of *B. tabaci* in Arizona and California to spirotetramat. They reported that Arizona and California *B. tabaci* populations had LC_{50} ranged from 1.02 to 7.02 µg a.i/ml, and from 0.91 to 13.5 µg a.i./ml, with a 7-14 times difference in resistance between populations, respectively.

Based on the LC_{50} of the susceptible population used in this study, all *B. tabaci* populations showed low to moderate resistance to cyantraniliprole. The LC_{90} of the populations were much lower than the 100 mg/l of cyantraniliprole recommended dose (Table 4). The lethal concentrations obtained indicate that there is still a high susceptibility in the populations to cyantraniliprole. One possible reason for the moderate resistance could be that populations were exposed to insecticides from different groups during the growing season and the high resistance found in the other insecticides used in the study support this theory. Cyantraniliprole has been approved in Türkiye since 2015 and it is still too early to observe any resistance to this insecticide in *B. tabaci*. When the reasons described above are considered, the resistance ratios found in the study against cyantraniliprole can be explained as natural variation. Similar to our study, Gravalos et al. (2015) reported that the LC_{50} of cyantraniliprole in 14 *B. tabaci* populations collected from resistance-prone regions of Greece, Italy, and Spain ranged from 0.011 to 0.116 mg a.i./l, with a difference of 10.5 times between the most and least susceptible populations. They determined that the 10.5 times difference was a natural variation and this could be related to the previous use of chlorantraniliprole and flubendiamide in these regions. Li et al. (2012) found the LC_{50} of *B. tabaci* populations collected from Arizona in 2008 and 2009 for cyantraniliprole were in between 0.015 and 0.191 µg a.i./ml, with resistance ratios ranging from 0.94 to 2.63 times. They reported that the difference in susceptibility against cyantraniliprole between populations was low, and this was due to natural variation. Susceptibility in *B. tabaci* to cyantraniliprole has also been reported in studies from Australia, China and the USA (Caballero et al., 2013; Xie et al., 2014; Hopkinson & Pumpa, 2019). According to the studies afore mentioned above, *B. tabaci* populations were found to be susceptible to cyantraniliprole, however, cyantraniliprole resistance was reported in *B. tabaci* populations in China. Wang et al. (2018) determined the resistance of adult *B. tabaci* populations to cyantraniliprole by leaf dipping method between 2015 and 2016 in China. They found the LC_{50} of the populations were between 5.53 and 27.4 mg a.i./l and between 14.1 and 40.4 mg a.i./l in 2015 and 2016, respectively. They determined that the resistance ratios were between 7.01 and 25.8 in the 2016 populations. They noted a significant increase in resistance in *B. tabaci* against cyantraniliprole within 2 years. In their study, cyantraniliprole was applied to *B. tabaci* adults by a different method than ours. Cyantraniliprole is more toxic to *B. tabaci* nymphal stage than to the adult stage (Caballero et al., 2013; Gravalos et al., 2015). The difference between the findings could be due to these factors.

In the present study, all populations were found to be highly resistant to pyriproxyfen, but susceptible to cyantraniliprole. Low, moderate and high resistance were observed in *B. tabaci* populations to spirotetramat. Based on these findings, it is strongly recommended to be careful when using formulations containing spirotetramat and pyriproxyfen. Also, rotation of insecticides from different classes should be considered when it comes to managing *B. tabaci* resistance. Cyantraniliprole can be used in rotation with pyriproxyfen and spirotetramat in *B. tabaci* management. To avoid the development of cyantraniliprole resistance, it is also recommended to avoid repeated use of insecticides containing cyantraniliprole in the control of *B. tabaci*. Insecticide usage is the primary strategy in the control of *B. tabaci*, which has resulted in development of resistance to many classes of insecticides. Insecticide resistance evaluation should be conducted regularly in intensive insecticide-using areas to detect early signs of the development of resistance. Considering the findings of the study, it is recommended to give priority to biological control and biotechnical control methods in effective control of *B. tabaci*.

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