

Original article (Orijinal araştırma)

Neonicotinoid resistance in adults and nymphs of *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) populations in tomato fields from Tokat, Turkey¹

Tokat (Türkiye) domates alanlarındaki *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) ergin ve nimf popülasyonlarında neonikotinoid direnci

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Abstract

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) is one of the most important agricultural pests in Turkey and in the world. This polyphagous pest is a highly efficient vectors of plant viruses and has the ability to rapidly develop resistance to diverse range of insecticides, hence controlling this pest is problematic. In this study, bioassays and biochemical tests were conducted to determine resistance to neonicotinoid in *B. tabaci* populations collected in 2017-2018 from Tokat (Turkey). According to the adult test results, resistance ratios for acetamiprid, imidacloprid and thiamethoxam were 5.64-16.8, 10.0-30.9 and 4.01-14.9, respectively. The highest resistance ratio for acetamiprid and thiamethoxam in the Pazar population were 16.8 and 14.9, respectively. The highest resistance ratio to imidacloprid was 30.9 in the TOGU campus population. According to the nymph test results, resistance ratios for acetamiprid, imidacloprid and thiamethoxam were 2.96-8.60; 4.29-8.74 and 2.48-4.88, respectively. Enzyme analysis revealed statistically higher metabolic resistance. Maximum enzyme activities were 4.37 and 3.79 pmol/min/mg protein for cytochrome P450 monooxygenase in TOGU campus and Pazar populations, respectively.

Keywords: Acetamiprid, *Bemisia tabaci*, imidacloprid, insecticide resistance, thiamethoxam

Öz

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) Türkiye ve dünyadaki en önemli tarım zararlılarından biridir. Bu polifag zararlı oldukça etkili bir bitki virüs vektörüdür ve çeşitli insektisitlere karşı hızla direnç geliştirme kabiliyetine sahiptir, bu yüzden zararlıyı kontrol etmek zordur. Bu çalışma 2017-2018 yıllarında Tokat (Türkiye)'tan toplanan farklı *B. tabaci* popülasyonlarının neonikotinoid grubu insektisitlere karşı direnç durumunu biyoassay ve biyokimyasal yöntemlerle belirlemek amacıyla yapılmıştır. Ergin testlerinde ortaya çıkan sonuçlara göre acetamiprid, imidacloprid ve thiamethoxam için direnç oranları, sırasıyla 5.64-16.8; 10.0-30.9 ve 4.01-14.9 arasındadır. Acetamiprid ve thiamethoxam için en yüksek direnç oranı, Pazar popülasyonunda sırasıyla 16.8 ve 14.9 'dur. Imidacloprid'e en yüksek direnç ise TOGU kampüs popülasyonunda 30.9'dur. Nimf testlerinde ortaya çıkan sonuçlara göre acetamiprid, imidacloprid ve thiamethoxam için direnç oranları, sırasıyla 2.96-8.60; 4.29-8.74 ve 2.48-4.88 arasındadır. Enzim analizi istatistiki anlamda yüksek metabolik direnci ortaya çıkarmıştır. Her üç insektisit içinde, TOGU kampüs ve Pazar popülasyonlarında en yüksek monooksijenaz P450 enzim aktivitesi sırasıyla 4.37 ve 3.79 pmol/dk/mg protein bulunmuştur.

Anahtar sözcükler: Acetamiprid, *Bemisia tabaci*, imidacloprid, insektisit direnci, thiamethoxam

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Introduction

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) is one of the most important pests worldwide (Anonymous, 2019a). This species was initially described by Gennadius in 1889 as *Aleyrodes tabaci* (Thomas, 2001). *Bemisia tabaci* was first recorded in Turkey in 1928 (Ulusoy et al., 1996; Ulusoy, 2001). It damages more than 600 host plants belonging to 63 families worldwide (Taylor, 2011).

Both adults and nymphs of *B. tabaci* suck the plant sap and severely reduce plant growth and health. In addition, during feeding, the honeydew that forms a sticky film on the leaves after a time supports sooty mold growth. This reduces the quality of the product and its market value. More importantly, *B. tabaci* is an important virus vector of more than 300 plant viruses that cause serious economic damage and major crop losses (Bedford et al., 1993, 1994; Markham et al., 1994; Paul et al., 2011; Gilbertson et al., 2015).

Bemisia tabaci has been recognized as highly cryptic species complex and recorded 24 biotypes which differ in host range, host plant adaptability, induction of phytotoxic reactions, insecticide resistance and virus-transmission capabilities among biotypes. However, biotype B and Q, two common biotypes, are particularly important plant pests (Boykin, 2014). B and Q biotypes have been identified in studies in Turkey (Bayhan et al., 2006; Ulusoy et al., 2007; Topakcı & Göçmen, 2011; Karut et al., 2012, 2014; Satar & Ulusoy, 2016).

This pest has an extraordinary potential to develop resistance to different insecticides (Denholm et al., 1998). Six hundred and thirty-one records of resistance in *B. tabaci* have been reported in the world, 250 of which are related to neonicotinoid group chemicals. There are 59 active ingredients in these records (APRD, 2019).

Neonicotinoids are the most widely used insecticides in the world. This group includes acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam. They have reached a share of around 25% in the global pesticide market with a monetary value of around 2.63 billion USD (Jeschke et al., 2011).

Neonicotinoids are highly effective insecticides that control many important pests (Nauen et al., 2008; Jeschke et al., 2011). These have been used effectively against various kinds of insect pests by different treatments in more than 120 countries for 25 years (Nauen et al., 2008; Bass et al., 2015). These chemicals target (nAChRs) in the insect central nervous system and are effective against a wide range of target species (Anonymous, 2019b). Neonicotinoids are selective agonists of the nicotinic acetyl choline receptors in the central nervous system of insects (Jeschke et al., 2011). The mode of action classification scheme of the Insecticide Resistance Action Committee (IRAC) lists seven commercial neonicotinoids in Group 4A (nAChR agonists) (Bass et al., 2015).

There are two major resistance mechanisms to insecticides in insect pests such as whiteflies. These are target site resistance and metabolic resistance. It has been determined that especially monooxygenase activity (P450) is caused by neonicotinoid resistance (Karunker et al., 2008; Roditakis et al., 2011; Nauen et al., 2015; Bass et al., 2015; Satar et al., 2018).

Continuous use of neonicotinoids has led to resistance in white flies. The resistance in *B. tabaci* has become a serious problem in various regions of the USA, European countries, China, Israel, Pakistan, including in Iran and Turkey over the last 25 years (Cahill et al., 1996; Elbert & Nauen, 2000; Nauen et al., 2002, 2008; Byrne et al., 2003; Horowitz et al., 2004; Roditakis et al., 2005; Feng et al., 2010; Luo et al., 2010; Schuster et al., 2010; Wang et al., 2010; Bahşi et al., 2012; Basit et al., 2013; Smith & Nagle, 2014; Basij et al., 2017; Naveen et al., 2017; Şahin & İkten, 2017; Satar et al., 2018). In agriculture, repeated insecticide applications lead to the development of resistance. It also increases the dependence on chemicals, increases the cost of production significantly and causes concerns in scientific communities (Naranjo & Ellsworth, 2009).

An increasing number of studies on neonicotinoid resistance in *B. tabaci* have been published. However, there is no study that determined the sensitivity of *B. tabaci* populations in tomato grown areas in Tokat Province, Turkey. Tomato is the most commonly produced product in this region and it is grown in 37.8% (~6000 ha) of vegetable production areas (Anonymous, 2019c). Although acetamiprid, imidacloprid and thiamethoxam are licensed against *B. tabaci* nymphs and adults, most of the studies to date have been performed on adult *B. tabaci* individuals (Nauen et al., 2008). In this study, nymph resistance was examined in addition to adults. For this reason, the aim was to determine the level of resistance to acetamiprid, imidacloprid and thiamethoxam in *B. tabaci* nymph and adult populations which are both harmful in tomato cultivation in Tokat Province.

Materials and Methods

Bemisia tabaci populations

Bemisia tabaci populations were collected from tomato production areas in Tokat. Populations were collected in July 2017 and August 2018 (Table 1). *Bemisia tabaci* were collected from at least 10 points in each tomato production area and brought to the laboratory in a cooler box within a few hours. The samples were identified using the keys of Martin et al. (2000).

Table 1. The collection places and dates of *Bemisia tabaci*

Location	Date	Coordinates
Yayladali (Susceptible)	24 July 2017	40.374527, 36.592487
TOGU campus (greenhouse)	25 July 2017	40.332352, 36.474065
Erbaa	26 July 2017	40.733764, 36.465677
Turhal	27 July 2017	40.311277, 36.282048
Zile	28 July 2017	40.215354, 35.651539
Pazar	4 August 2018	40.269830, 36.232960
Central	8 August 2018	40.340024, 36.414255
Niksar	17 August 2018	40.529501, 36.908518
Guryildiz	27 August 2018	40.341306, 36.363476

Insecticides and chemicals

Insecticides and chemicals

In this study, three neonicotinoid insecticides were selected. Active ingredients, commercial names and modes of action of insecticides used in this investigation are detailed in Table 2. 1,4-Dithioerythritol (DTT) (>98%), 1-chloro-2,4-dinitrochlorobenzene (CDNB) (99%), 7-ethoxycoumarin (99%), bovine serum albumin, ethylene diamine tetraacetic acid (EDTA) (>99%), fast blue RR salt, glutathione reductase, NADPH (97%) (tetrasodium salt), oxidized glutathione (≥98%), reduced glutathione (GSH) (≥98%), sucrose (≥99.5%), Tris-HCL buffer, Triton X-100, Trizma base (≥99.9%), and α-Naphthyl acetate (α-NA) (98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 2. Active ingredients and commercial names for neonicotinoids and their mode of action

Active ingredient (a.i)	Commercial name	IRAC mode of action*
Acetamiprid	Mospilan 20 SL, Nippon Soda Co.	Nerve action, Nicotinic acetylcholine receptor (nAChR) competitive modulators (4A)
Imidacloprid	Confidor SC 350, Bayer CropScience	Nerve action, Nicotinic acetylcholine receptor (nAChR) competitive modulators (4A)
Thiamethoxam	Actara 240SC, Sygenta	Nerve action, Nicotinic acetylcholine receptor (nAChR) competitive modulators (4A)

*(IRAC, 2020).

Rearing of *Bemisia tabaci*

Bemisia tabaci populations was reared in a laboratory. The adults were reared on tomato in net-covered cages (50 x 50 x 60 cm) at 25±1°C, 65±5% RH and 16:8 h L:D photoperiod. The tomato plants were produced at 25±1°C and 16:8 h L:D photoperiod in a controlled climate room. In 2017, a *B. tabaci* population was collected from Yayladali (Tokat, central) that has not been exposed to insecticide applications. This populations were maintained in a controlled climate room and used as susceptible reference in bioassay.

Bioassays Methods

Adult bioassay

To determine the resistance status of *B. tabaci*, LC₅₀ values were determined by modifying the IRAC 008 method (IRAC, 2016a). In this method, the three-leaf tomato plants were dipped in concentrations in six doses (5, 10, 25, 50, 100, 200 mg a.i./L) prepared for each active ingredient and in distilled water (as a control) for 5 s. The material was dried and then placed in glass containers with a bottom drilled diameter of 2-3 mm. In this way, with the help of aspirator, 20 adults were transferred into polystyrene container for bioassay and then the top of the containers were closed with a tulle cloth. In order to prevent the death of the plants in the cups, water was added in a second glass container with holes and these containers were placed in an insectarium at 25±1°C, 60-70%RH and 16:8 h L:D photoperiod. Bioassays were performed in three replicates. Mortality was recorded after 72 h.

Nymph bioassay

In order to determine the resistance status of *B. tabaci* nymphs, LC₅₀ values were determined by modifying the IRAC 016 method (IRAC, 2016b). Each of the leaves of the tomato plant in the same stage was cut into a rectangular shape about 4 x 6 cm in order to form a certain area and placed in empty cabins. Adult whiteflies were collected using the aspirator from the cages, and about 50 insects per leaf were left on plants whose leaves were cut into a rectangular shape. Adult whiteflies were left in cages until they laid eggs (24 h) and then all adults were removed from the cages. The leaves of the plants which were kept for 9 d were taken together with the nymphs and leaf dipping method was applied for 5 s. The rectangular leaves were dipped in six concentrations (5, 10, 25, 50, 100, 200 mg a.i./L) prepared for each active ingredient and in distilled water (as a control) for 5 s, then dried and placed into polystyrene containers drilled to the bottom with a diameter of 2-3 mm. The cups were covered with a thin tulle curtain and left to the insectarium at 25±1°C and 65±5% RH and 16:8 h L:D photoperiod. Bioassays were performed with three replicates. Nymphal mortality rates (adults were considered alive) were determined seven days after pesticide applications.

Biochemical assays

Bemisia tabaci populations collected from tomato production areas were placed in ice boxes and brought to the laboratory and stored at -80°C for enzyme analysis. The total protein amounts of *B. tabaci* individuals were determined according to the Bradford (1976) method, in which bovine serum albumin was used as a standard.

Determination of esterase activity

Twenty *B. tabaci* individuals were homogenized by pressing with plastic pestle in Eppendorf tubes containing 100 µl sodium phosphate buffer (0.1 M, pH 7.5) and 0.1% Triton X-100. This homogenate was used as an enzyme source after centrifugation at 10000 g at 4°C for 5 min. The supernatant taken from the upper portion of the Eppendorf tube as the enzyme source was diluted tenfold with distilled water. Twenty-five µl of supernatant and 25 µl phosphate buffer (0.2 M, pH 6) were added to the microplate cells. In the

study, 30 mg of fast blue RR salt was dissolved in 50 ml of 0.2 M sodium phosphate buffer and 500 µl of 100 mM α -naphthyl acetate was added to this mixture. The substrate solution obtained was added 200 µl to the microplate cells. Enzyme activity was determined with Infinite P200 Pro (Tecan) microplate reader at 23°C for 10 min at 450 nm (Stumpf & Nauen, 2002). Enzyme readings were made at three-times.

Determination of glutathione S-transferase activity

Glutathione S-transferase (GST) activity was determined using CDNB and GSH as substrate. Thirty *B. tabaci* adults were homogenized in 300 µl of Tris-HCL buffer (0.05 M, pH 7.5). The total reaction volume of each cell of the 96-cell plate with flat bottom was adjusted as 300 µl. As a result, the reaction consisted of 100 µl of supernatant, CDNB in buffer (containing 0.1% v/v ethanol) and reduced GSH (final concentration of 0.4 mM CDNB and 4 mM GSH). The change in absorbance was measured kinetically at 20°C and 340 nm for 5 min. The non-enzymatic reaction of CDNB and GSH was measured without homogenate as control (Rauch & Nauen, 2003). Enzyme assays were performed in three replicates.

Determination of cytochrome P450 monooxygenase activity

Monooxygenase enzyme activity which is dependent on Cytochrome-P450 was determined by O-deethylation of 7-ethoxycoumarin. Ten mg of *B. tabaci* frozen at -80°C were homogenized in Na/K phosphate buffer (0.1 M, pH 7.6, 1 mM EDTA, 1 mM DTT, 200 mM sucrose). The homogenate was centrifuged at 5000 g at 4°C for 5 min, and the obtained liquid fraction was centrifuged at 15,000 g for 15 min, then at 100,000 g for 60 min. The microsomal pellet remaining at the bottom of the Eppendorf tube was remixed in 300 µl buffer and used as an enzyme source. 50 µl of the microsomal fraction and 40 µl of Na/K phosphate buffer (0.1 M, pH 7.6, containing 1 µl of 40 mM 7-ethoxycoumarin in acetone) were placed in cells of 96-cell black plates. The reaction was initiated by adding 10 µl of watery NADPH to each cell. The final concentration was consisted of 1 mM NADPH and 0.4 mM 7-ethoxycoumarin. The plate was shaken and incubated at 30°C for 30 min. The NADPH which has fluorescence feature was removed by addition of 10 µl of oxidized glutathione (30 mM in water) and 10 of glutathione reductase (0.5 U). The reaction was stopped with 120 µl of 50% acetonitrile in Trizma base buffer (0.05 M, pH 10) after 10 min. The amount of 7-hydroxycoumarin released during the incubation was measured spectrofluorometer (Tecan) (390 extension and final 465 nm). The standard curve of 7-hydroxycoumarin was used to convert the optical density to pmol of product form. For each population, applications were repeated two times and non-microsomal pelleted cells were used as control (Rauch & Nauen, 2003).

Statistical analysis

Probit analyses of the concentration-dependent mortality data were calculated using PoloPlus (LeOra software, Berkley, CA, USA). Resistance ratios (RRs) were obtained by dividing LC₅₀ values by the corresponding value for the susceptible population. Data of enzyme activities were subjected to one-way ANOVA, and the means were compared using Tukey's HSD test ($P < 0.05$) (SPSS version 22.0, IBM Corp., Armonk, NY, USA).

Results

LC₅₀ and resistance ratios are given in Tables 3 and 4. The reference population was always the most susceptible population.

Resistance of adults

Acetamiprid resistance ratios were determined from 5.64 to 16.8. The most susceptible population was Guryildiz and the most resistant was the Pazar population. Slope values are between 1.42 and 2.51. Erbaa population is considered the most heterogeneous population since it shows the least slope of the regression line.

Imidacloprid resistance ratios ranged from 10.0 to 30.9, while the most susceptible Zile population was found to be the most resistant TOGU campus population. Slope values are between 1.41 and 2.56. The TOGU campus population, which showed the highest RR_{50} (30.9) among all test populations, displayed the slope of the lowest regression line (1.41).

Thiamethoxam resistance ratios were between 4.01 and 14.9, while it was the most susceptible Guryildiz population and the most resistant Pazar population. Slope values are between 1.41 and 2.47. Turhal population is considered the most heterogeneous population since it gave the least slope of the regression line.

Table 3. Log-dose probit mortality results for *Bemisia tabaci* adult populations tested with acetamiprid, imidacloprid, thiamethoxam

Insecticide	Population	n	Slope $\pm$ SE	LC ₅₀ mg(a.i.)/L (95% CL)	LC ₉₀ mg(a.i.)/L (95% CL)	RR ₅₀
Acetamiprid	Susceptible	420	1.79 $\pm$ 0.18	12.1 (9.4-4.9)	63.0 (47.8-91.5)	1.00
	Guryildiz	420	2.51 $\pm$ 0.24	68.1 (58.1-80.4)	220.7 (172.0-311.4)	5.64
	Erbaa	420	1.42 $\pm$ 0.16	90.4 (70.0-124.0)	722.3 (423.2-1630.2)	7.49
	Central	420	1.75 $\pm$ 0.19	96.8 (77.9-125.9)	525.2 (342.1-995.7)	8.02
	Zile	420	1.66 $\pm$ 0.19	122.1 (95.6-167.7)	728.3 (444.8-581.9)	10.12
	Turhal	420	1.72 $\pm$ 0.20	123.7 (97.4-168.5)	689.2 (425.6-447.0)	10.25
	Niksar	420	1.76 $\pm$ 0.21	135.5 (106.4-186.9)	728.3 (444.8-1581.9)	11.23
	TOGU campus	420	1.99 $\pm$ 0.28	187.1 (145.2-272.8)	826.9 (491.8-2015.0)	15.51
	Pazar	420	2.11 $\pm$ 0.31	202.9 (157.3-299.9)	819.5 (489.5-2025.3)	16.82
Imidacloprid	Susceptible	420	1.58 $\pm$ 0.18	8.6 (6.1-11.1)	55.7 (41.2-84.9)	1.00
	Zile	420	2.20 $\pm$ 0.24	85.8 (71.2-107.1)	327.3 (232.2-544.0)	10.02
	Turhal	420	1.57 $\pm$ 0.20	118.4 (90.1-172.2)	772.2 (437.6-1913.8)	13.83
	Guryildiz	420	2.56 $\pm$ 0.31	123.7 (104.3-152.3)	391.4 (284.5-640.7)	14.44
	Niksar	420	1.89 $\pm$ 0.24	136.8 (106.4-194.2)	652.2 (394.5-1473.9)	15.98
	Erbaa	420	1.97 $\pm$ 0.25	146.9 (117.1-199.4)	655.9 (415.9-1355.3)	17.15
	Central	420	1.73 $\pm$ 0.24	158.2 (118.8-241.8)	869.4 (483.0-2345.0)	18.48
	Pazar	420	2.15 $\pm$ 0.35	206.1 (154.9-331.7)	814.0 (460.2-2377.6)	24.07
	TOGU campus	420	1.41 $\pm$ 0.22	264.8 (178.5-507.7)	2139.4 (941.6-9452.4)	30.93
Thiamethoxam	Susceptible	420	1.63 $\pm$ 0.16	15.6 (12.2-19.4)	95.3 (69.9-145.6)	1.00
	Guryildiz	420	2.47 $\pm$ 0.23	62.5 (53.2-73.7)	206.4 (161.3-289.0)	4.01
	Niksar	420	1.57 $\pm$ 0.18	105.7 (82.8-144.0)	695.24 (421.3-1489.0)	6.79
	Central	420	1.60 $\pm$ 0.19	113.7 (89.0-155.5)	713.7 (431.8-1545.8)	7.30
	Zile	420	1.70 $\pm$ 0.20	120.5 (95.0-163.7)	681.5 (421.1-1426.8)	7.74
	Erbaa	420	1.71 $\pm$ 0.20	122.1 (96.2-166.1)	685.4 (423.4-1437.2)	7.84
	TOGU campus	420	1.40 $\pm$ 0.17	122.7 (92.4-179.7)	1014.4 (553.0-2639.2)	7.88
	Turhal	420	1.41 $\pm$ 0.17	124.9 (94.3-182.3)	1004.1 (550.0-2605.6)	8.02
	Pazar	420	1.77 $\pm$ 0.27	232.5 (169.8-384.0)	1227.9 (647.4-3842.1)	14.94

n: Number of whiteflies tested; SE: Standard Error; LC: Lethal Concentration; CL: Confidence Limits; RR: Resistance Ratio calculated as (LC₅₀ of field population) / (LC₅₀ of Susceptible population)

Resistance of nymphs

Acetamiprid resistance ratios were determined from 2.96 to 8.60. While the most susceptible population was Guryildiz, the most resistant was found TOGU campus. Slope values are between 1.12 and 1.97. Pazar population is considered the most heterogeneous population since it gave the least slope of the regression line.

Imidacloprid resistance ratios ranged from 4.29 to 8.74. Erbaa population was the most susceptible and Central population was the most resistant. Slope values are between 1.10 and 1.97. The TOGU campus population is considered the most heterogeneous population since it gave the least slope of the regression line.

Thiamethoxam resistance ratios were determined between 2.48 and 4.88. The most susceptible population was determined in Niksar and the most resistant population was found in Pazar. Slope values are between 1.19 and 2.07. Niksar population is considered the most heterogeneous population since it gave the least slope of the regression line.

Table 4. Log-dose probit mortality results for *B. tabaci* nymph populations tested with acetamiprid, imidacloprid, thiamethoxam

Insecticide	Population	n	Slope±SE	LC ₅₀ mg(a.i.)/L (95% CL)	RF ₅₀
Acetamiprid	Susceptible	1056	1.44±0.16	6.2 (4.0-8.4)	1.00
	Guryildiz	1077	1.31±0.14	18.3 (13.6-23.6)	2.96
	Erbaa	1073	1.70±0.16	21.1 (16.8-26.1)	3.42
	Turhal	1122	1.69±0.14	29.2 (23.9-35.4)	4.73
	Zile	1128	1.63±0.14	31.7 (25.8-38.8)	5.13
	Pazar	1108	1.12±0.13	33.8 (25.2-45.0)	5.47
	Central	1120	1.97±0.16	35.8 (29.9-42.8)	5.79
	Niksar	1131	1.80±0.14	36.7 (30.7-43.9)	5.94
	TOGU campus	1137	1.83±0.15	53.1 (44.4-64.3)	8.60
Imidacloprid	Susceptible	1004	1.17±0.16	4.1 (2.0-6.4)	1.00
	Erbaa	1086	1.41±0.14	17.6 (13.3-22.3)	4.29
	Turhal	1073	1.57±0.14	19.6 (15.5-24.1)	4.78
	Zile	1115	1.77±0.14	21.3 (17.5-25.7)	5.21
	TOGU campus	1119	1.10±0.12	27.1 (20.1-35.8)	6.63
	Pazar	1148	1.33±0.14	28.6 (22.2-36.7)	6.99
	Guryildiz	1099	1.42±0.14	28.7 (22.5-36.2)	7.01
	Niksar	1159	1.25±0.13	35.5 (27.3-46.2)	8.67
	Central	1100	1.97±0.16	35.8 (29.9-42.8)	8.74
Thiamethoxam	Susceptible	1086	1.76±0.16	8.1 (6.0-10.2)	1.00
	Niksar	1123	1.19±0.13	20.0 (14.8-26.1)	2.48
	Guryildiz	1081	1.87±0.15	20.2 (16.5-24.2)	2.50
	Erbaa	1114	1.60±0.14	20.6 (16.4-25.3)	2.55
	Zile	1117	1.80±0.14	23.0 (18.9-27.6)	2.85
	TOGU campus	1097	1.37±0.13	26.2 (20.4-32.9)	3.23
	Turhal	1036	1.96±0.15	31.3 (26.3-37.1)	3.88
	Central	1105	1.77±0.15	33.8 (27.7-41.0)	4.18
	Pazar	1141	2.07±0.16	39.4 (33.2-46.7)	4.88

n: Number of whiteflies tested; SE: Standard Error; LC: Lethal Concentration; CL: Confidence Limits; RR: Resistance Ratio calculated as (LC₅₀ of field population) / (LC₅₀ of Susceptible population).

Enzyme activity levels in populations

The results from the biochemical assays enzyme activities for the *B. tabaci* adult populations are given in Table 5. There was no statistical difference between populations in terms of GST and EST enzyme activities. For P450, the lowest enzyme activities ratios (1.68 and 1.65) were detected in Niksar and Central. The highest activity ratio was 4.20 in the TOGU campus population.

Table 5. Esterase (EST), glutathione S-transferase (GTS), cytochrome P450 monooxygenase (P450) activities for *B. tabaci* populations from Tokat

Population	EST (mOD/min/mgprotein)	GST (mOD/min/mgprotein)	P450 (pmol/min/mgprotein)*	P450 Ratio
Susceptible	0.8563	0.0070	1.0413 f	1.00
Erbaa	0.9652	0.0074	2.2498 cd	2.16
Guryildiz	1.1638	0.0090	2.3163 cd	2.22
Central	1.1845	0.0125	1.7553 e	1.68
Niksar	1.0570	0.0091	1.7185 e	1.65
Pazar	1.1275	0.0093	3.7933 b	3.64
TOGU Campus	1.3329	0.0087	4.3715 a	4.20
Turhal	1.2621	0.0079	2.4399 c	2.34
Zile	1.0520	0.0087	2.0549 de	1.97

*Values followed by the different letters are significantly different ($P < 0.05$) after Tukey's HSD test.

Discussion

In terms of population sampling region, adult resistance bioassay revealed different levels of resistance. In general, a high proportion of imidacloprid and acetamiprid resistance was found in almost all populations. In addition, it was concluded that there was moderate resistance for thiamethoxam in all populations.

It was determined that the LC_{50} values obtained for the three insecticides for the susceptible population were lower than the LC_{50} values of all other field populations. According to this result, the population was accepted as sensitive.

The Pazar population had the highest RR_{50} for three neonicotinoid group insecticides and showed a high resistance (Table 3). It was concluded that there is a high level of resistance due to the intensive cultivation, the presence of other pests in this region in addition to *B. tabaci* and the common use of neonicotinoid group preparations. Therefore, it is obvious that it will be useful to use different insecticide groups in the control of whiteflies in Pazar.

Different resistance levels have been determined in the studies of *B. tabaci* adults and neonicotinoid insecticides around the world. Schuster et al. (2006), Rao et al. (2012), Castle et al. (2013), Gnankine et al. (2013), Wang et al. (2016), Basij et al. (2017), Naveen et al. (2017), Hajjar et al. (2020) and Taquet et al. (2020) have worked on neonicotinoid resistance against *B. tabaci* in different countries and on different host plants. They have determined that *B. tabaci* has developed resistance at different rates.

In Turkey, Bahşı et al. (2012), investigated resistance levels and the potential of resistance development of acetamiprid, chlorpyrifos-ethyl and cypermethrin in *B. tabaci* populations collected from Antalya district. Resistance levels for acetamiprid, chlorpyrifos and cypermethrin were determined as 6-299, 2-16 and 1-22, respectively. In addition, 18 and 4 times increases in resistance levels of the populations selected with acetamiprid and chlorpyrifos-ethyl were determined. According to these results, Antalya populations of *B. tabaci* showed significant resistance to acetamiprid, chlorpyrifos and cypermethrin. Şahin & İkten (2017) studied the resistance of different *B. tabaci* populations collected from Antalya against to neonicotinoid group insecticides. They observed that LC_{50} resistance ratios were between 4.4-30.4 relative to a susceptible population for acetamiprid. Similarly, they found that durability for thiamethoxam ranged from 8.6 to 31.8 times compared to the susceptible population. Satar et al. (2018), showed that whiteflies were resistant to all neonicotinoids tested when their susceptible SUD-S strain and *B. tabaci* populations were compared. They reported that the highest resistance factor was 2060 for imidacloprid in Kumluca and 5.36 times for thiamethoxam in Samandağ.

Different levels of resistance have been determined. It can be said that there is moderate resistance to imidacloprid and low resistance to thiamethoxam in all populations when nymph resistance bioassay results are evaluated on the basis of population sampling regions. In addition, low resistance to acetamiprid was found in three populations and moderate resistance was found in five populations (Table 4). Compared to adult bioassay results with nymph resistance bioassay results, all populations were found to be more susceptible to three effective agents. This is thought to be due to incomplete body development in nymphs.

There are only a few reported studies on *B. tabaci* nymphs and neonicotinoid insecticides. Jones et al. (2011) applied imidacloprid against adults and nymphs in three *B. tabaci* populations and found that nymphs were more susceptible in all three populations. Nauen et al. (2008) evaluated age-specific resistance of *B. tabaci* to neonicotinoid insecticides. The highest resistance rate was 13 times in prepupa period and 580 times in adult stage. The findings of the current study (Table 4) were similar with the studies performed by the above authors, and it was confirmed that the nymphs are more susceptible than the adults of whiteflies.

In the current study esterase activity was determined, but no statistical difference was found between populations. Jeschke & Nauen (2005), reported that the difference in esterase activity is not related to neonicotinoid resistance but to organic phosphates. In the current study, similarly, low EST activity was detected in comparison with susceptible and resistant populations.

There was no statistical difference between the populations in GST activity. Neonicotinoid resistance is the result of monooxygenase enzyme activity rather than GST activity. Vontas et al. (2000) and Rauch & Nauen (2003) reported that the activity of this enzyme is generally associated with insecticide resistance of organic chlorinated and chlorinated hydrocarbon groups. Rauch & Nauen (2003) found that the highest GST activity was in the susceptible race USA-B and found no higher GST activity in any resistant population. Feng et al. (2010) did not observe any difference in terms of GST between two *B. tabaci* races. Basij et al. (2017) reported that susceptible *B. tabaci* race had higher GST activity than resistant ones. In the present study, low GST activity was found to be similar when the susceptible population was compared to resistant populations.

The most susceptible one of the nine populations used in the studies, was found to have the lowest P450 activity. The TOGU campus population was found to have 4.19 times more enzyme activity than the susceptible population. It had 3.64 times more P450 enzyme activity in the Pazar population. Cytochrome P450 is an enzyme that is effective in gaining resistance to neonicotinoid group preparations in insects. In the current study, cytochrome P450 enzyme activity paralleled the bioassay findings in terms of resistance to neonicotinoid group insecticide. As a result of this research, TOGU campus and Pazar cytochrome P450 activities, which are the highest resistant populations, were found to be higher than the susceptible populations. In this respect, it can be said that whiteflies develop resistance to these pesticides because neonicotinoid pesticides are commonly used in the areas where populations are collected. Nauen et al. (2002) and Rauch & Nauen (2003) found that neonicotinoid group resistance in B and Q biotypes collected from Spain, Germany and Israel was due to increased cytochrome P450-dependent monooxygenase activity. An important relationship between cytochrome P450-dependent monooxygenase activity and imidacloprid resistance level was also observed in Q biotypes of *B. tabaci* populations collected from Crete (Roditakis et al., 2009). Karunker et al. (2008) *B. tabaci* B and Q biotypes related to the high imidacloprid resistance to the cytochrome P450 gene CYP6CM1 in their study carried out, the most important resistance mechanism in all populations found that increased cytochrome P450 monooxygenase enzyme detoxification. Wang et al. (2009) applied imidacloprid to *B. tabaci* s NJ (B biotype) population. They applied this process for 30 generations and obtained the NJ-lmi population. It was 490 times more resistance to imidacloprid. They found that the cause of resistance in the NJ-lmi population was related to the overproduction of cytochrome P450 monooxygenase enzyme. Feng et al. (2010) reported that cytochrome P450 monooxygenase activities increased by 1.21 and 1.68 times, respectively, as a result of biochemical analyzes of two populations. Rao et al. (2012) reported that resistance in biotype strains collected from China was caused by overexpression of cytochrome P450 monooxygenase gene CYP6CM1. Basij et al. (2017) studied the sensitivity of imidacloprid and acetamiprid of nine *B. tabaci* populations collected from different regions of Iran. They reported that the resistance ratio of the populations was between 9.72 and 205 for imidacloprid and 6.38 and 175 for acetamiprid. They found that cytochrome P450 monooxygenase enzyme activity was associated with imidacloprid and acetamiprid resistance. Therefore, they reported that cytochrome P450 monooxygenase is the only enzyme system responsible for neonicotinoid resistance in nine populations of *B. tabaci*.

Conclusions

In the current study, it was determined that *B. tabaci* had developed resistance to acetamiprid, imidacloprid and thiamethoxam. The LC₉₀ values of susceptible population for imidacloprid and thiamethoxam (55.7 and 95.3 mg a.i./L) were much lower than the recommended rates of those insecticides (350 and 240 mg a.i./L). The application of imidacloprid and thiamethoxam are prohibited by Ministry of Agriculture and Forestry, General Directorate of Food and Control in open agricultural open areas because of toxicity to bees. As a result of the current study, acetamiprid, which is not included in the ban, has been found to have moderate resistance.

The LC₉₀ values of the susceptible population for acetamiprid (62.99 mg a.i./L) are almost equal to the recommended rate of this insecticide (60 mg a.i./L). This indicates that sensitive *B. tabaci* can still be controlled under field conditions. However, in order to prevent the medium level *B. tabaci* resistance to rising to higher levels, insecticides, which have different mode of action, should be used in rotation. In order to fully understand the acetamiprid resistance, it is useful to perform multiple resistance studies and synergistic studies related to cytochrome P450 monooxygenase with other insecticides commonly used in the region.

According to these results, it is concluded that nymphs are more sensitive than adults. Therefore, it is thought that targeting nymphal stages will increase the success and prevent the development of resistance. In addition, insecticides should be used at an appropriate dose, the frequency of application should be reduced, and the control studies should be managed in a more sustainable manner by not using insecticides which have the same mode of action in a row. Continuous use of pesticides with the same mode of action in *B. tabaci* pest management leads to the elimination of susceptible populations and can also contribute to the development of cross-resistance. In this regard, resistance mechanisms should be studied in more detail. Defining resistance mechanisms helps overcome resistance management problems. Besides such studies, cultural, biological, biotechnical and other control measures should be used intensively.

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