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Abamectin, Hexythiazox and Spiromesifen Resistance in Populations of *Tetranychus urticae* Koch (Acari:Tetranychidae) Collected from Cucumber Greenhouses in Tokat Province[#]

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ABSTRACT

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most important pests in greenhouses. This harmful pest has rapidly developed high resistance to many classes of acaricides. In this study, *T. urticae* populations were collected from cucumber greenhouses in Gümenek village, Tokat, Turkey. Growers in this location use acaricides such as abamectin, hexythiazox and spiromesifen to suppress *T. urticae* populations. The study aimed to determine the resistance levels of *T. urticae* populations against to mentioned acaricides. The LC_{50} values of the collected populations were determined by the spray tower-leaf disc method. The resistance rates were found by dividing the LC_{50} values of the collected greenhouse populations by the LC_{50} value of the susceptible population. The resistance levels of *T. urticae* were determined to be between 1.88-2.14-folds against abamectin, 1.67-1.84 folds against hexythiazox and 1.77-2.09 folds against spiromesifen. According to these results, a low rate of resistance development was observed against abamectin, hexythiazox and spiromesifen.

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Introduction

Growers are faced with many plant protection problems in greenhouse cultivation around world. Pests are one of the problems and causes economic losses. Producers have to deal with many pests during the production season. Among them, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important one. It damages more than 1000 economic plant species (Migeon and Dorkeld, 2010). It causes by feeding on the parenchyma cells of more than 250 host plants (Den Boom et al., 2003). Acaricides are used intensively in the control of *T. urticae* to keep economic injury level low in the greenhouse.

Acaricide use is quite common in the world, and it was reported that the world acaricide market value was 900 million € in 2013. In Turkey, the use of acaricides, which was 902 tons in 2006, reached 2452 tons in 2017, and it is among the 10 countries that use the most acaricides in the world (Leeuwen et al., 2015). *T. urticae* quickly develops resistance to synthetic insecticides (Pimentel et al., 1992; Mansour et al., 2004) due to the high reproduction rate in many generations per year and its unique detoxification abilities (Khajehali et al., 2011; Çağatay et al., 2018). In addition, using chemicals more than the recommended dose, not rotating acaricides with different action mechanisms, and not spraying in accordance with the suitable techniques increase also the development of resistance. *T. urticae* is resistant to 96 compounds that belong to different chemical groups. Five hundred and forty-nine records of resistance status of *T. urticae* have been reported in the world and 73 of them are related to abamectin, 11 to hexythiazox, and 6 to spromesifen (Leeuwen et al., 2010; APRD, 2021).

Abamectin is classified in Group 6 of the mode of action (MoA) classification by the Insecticide Resistance Action Committee (IRAC) and allosterically activate glutamate-gated chloride channels (GluCls), causing paralysis (IRAC, 2022). Hexythiazox is a non-systemic acaricide with contact and stomach action from the thiazolidine group and has ovicidal, larvicidal and nymphicidal activities (Yorulmaz and Ay, 2013). Hexythiazox is classified in Group 10A of the MoA classification by the IRAC and inhibit the enzyme that catalyzes the polymerization of Chitin. Spiromesifen is classified in Group 23 of the MoA classification by the IRAC and inhibit acetyl coenzyme A carboxylase, part of the first step in lipid biosynthesis, leading to insect death (IRAC, 2022).

It is useful to monitor the resistance levels of pests against pesticides in order to make an effective chemical control. There is only one study on insecticide/acaricide resistance in Tokat province. The study was conducted in 2017-2018 to determine neonicotinoid resistance in *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) populations (Balkan and Kara, 2020). In this study, the resistance levels of *T. urticae* populations against abamectin, hexythiazox and spiromesifen were determined in cucumber greenhouses from Tokat, Turkey.

Materials and Methods

Collection of Tetranychus urticae, rearing conditions, and acaricides

The cucumber leaves infected by *T. urticae* from the greenhouse production areas where cucumbers are grown in the central district of Tokat province were put in plastic bags, labeled and brought to the Toxicology Laboratory (TOGU Faculty of Agriculture, Department of Plant Protection). *T. urticae* populations were reared on bean plants (*Phaseolus vulgaris* L.) at 26 ± 1°C, 55 ± 5% relative humidity and a photoperiod of 16:8 (L: D) in a climate room (Çağatay et al., 2018). The susceptible *T. urticae* population was brought to insect-rearing rooms. They were taken from a pesticide-free production area in 2021 and reared without exposure to any pesticide.

Abamectin (Torpedo 5 EC, Hektaş), hexythiazox (Twister 5 EC, Hektaş) and spiromesifen (Fibon SC, Hektaş) were used for toxicity studies.

Toxicity Assays

A leaf disc bioassay with a spray tower was used to determine the LC_{50} values of abamectin, hexythiazox, and spiromesifen for susceptible and greenhouse populations of *T. urticae* (Yaman et al., 2016; Solmaz et al., 2020). Bioassay studies were started after the populations had reached sufficient density in the rearing room. Three cm leaf discs were placed on 9 cm diameter polystyrene Petri dishes covered with cotton, and 25±3 mite larvae were transferred on these discs with a soft-tipped brush under the binocular. For the toxicity assay, three replicates of seven acaricide concentrations and a control were used. The concentrations were prepared by ½ serial dilution series in distilled water and were sprayed at a rate of 2 ml per leaf disc by a Potter spray tower (Burckard Manufacturing Co Ltd, Rickmansworth, Herts, UK) at 1 bar (Çağatay et al., 2018). Distilled water was applied in control groups. The leaf discs were kept at 25 ± 2°C and 60 ± 10% humidity (RH) in a polystyrene petri dish under a 16:8-h light: dark photoperiod (Solmaz et al., 2020). The mortality of *T. urticae* was determined by touching them with a fine brush. *T. urticae* that did not move any appendage were considered dead (Koh et al., 2009). Mortality was evaluated 24 hours after abamectin and 7 days after hexythiazox and spiromesifen applications, by counting the dead and alive specimens.

Statistical analysis

LC_{50} values with 95% confidence limits and the slope associated were calculated using the POLO-PC software (Leora software 1994). Resistance ratios (RR) were calculated by dividing the LC_{50} value of a treated greenhouse population by the LC_{50} value of the susceptible population. The RR_{50} were categorized according to Hayashi (1983) as follows: low, $RR_{50} \leq 10$; moderate, $10 < RR_{50} \leq 40$; high, $40 < RR_{50} \leq 160$; very high, $160 < RR_{50}$ (Koh et al., 2009).

Results and Discussion

The resistance rates of *T. urticae* populations collected from the greenhouse cucumber production areas in the central district of Tokat against abamectin, hexythiazox, and spiromesifen were determined according to the LC_{50} and LC_{90}. These values are given in Tables 2.1, 2.2 and 2.3 for abamectin, hexythiazox and spiromesifen, respectively.

The resistance rates of *T. urticae* populations against abamectin according to the LC_{50} values were determined as 1.88, 2.14, and 1.90-fold, respectively. The highest resistance rate against abamectin was determined in the Gümenek2 population, and the lowest resistance rate was determined in the Gümenek1 population (Table 1). Tirello et al. (2012) found 4.3 and 169.3-fold resistance levels against abamectin in *T. urticae* populations collected from greenhouses in Italy. Yorulmaz and Kaplan (2014) stated low resistance to abamectin in three and moderate resistance in 3 of the *T. urticae* populations collected from tomato greenhouses in Isparta province. Turan et al. (2016) determined a low (8.44-fold) resistance in one population and moderate (12-26.5 folds) resistance in 19 populations against abamectin in *T. urticae* populations collected from melon greenhouses in Antalya, Turkey. Yorulmaz and Kocaman (2017) determined 43.5-246.2 folds resistance against abamectin in *T. urticae* populations collected from cut flower greenhouses in Isparta, Turkey. Çağatay et al. (2018) found high resistance levels varying between 223 and 404 times in *T. urticae* populations collected from vegetable greenhouses in Antalya and Muğla, Turkey. Solmaz et al. (2020) found that abamectin resistance was low (1.61-fold) in one of the *T. urticae* populations collected from cut flower greenhouses in Antalya, Turkey, and very high (197-851 folds) in seven. Kirisik and Dagli (2021) found low resistance levels (1.3 to 2 folds) in all *T. urticae* populations obtained from different greenhouses in the coastal areas of Antalya, Turkey. In our study, a low level of resistance against abamectin was found in *T. urticae* populations.

The resistance rates of *T. urticae* populations against hexythiazox according to the LC_{50} value were determined as 1.73, 1.67, and 1.84-fold, respectively. The highest resistance rate against hexythiazox was determined in the Gümenek3 population, and the lowest resistance rate was determined in the Gümenek2 population (Table 2.2). Tirello et al. (2012) found 39.7- and 46.9-fold resistance levels against hexythiazox in *T. urticae* populations collected from commercial roses grown in greenhouses located near Treviso and Sanremo in Italy. Yorulmaz and Kaplan (2014) recorded low resistance (8.54–10.0 fold) against hexythiazox in all populations except ID4 (11.75-fold) in T. urticae populations collected from greenhouses in Isparta, Turkey.

Table 1. Abamectin resistance ratios on different *T. urticae* populations

Population	n*	Slope±SE	LC_{50} (mg a.i l^{-1}) (95% CL)	LC_{90} (mg a.i l^{-1})(95% CL)	RR_{50}**	RR_{90}***
Gümenek1	536	2.307±0.166	4.02(3.41-4.71)	14.4 (11.6-19.3)	1.88	2.49
Gümenek2	551	2.165±0.152	4.58 (3.77-5.53)	17.9(13.8-25.3)	2.14	3.09
Gümenek3	550	1.896±0.130	4.074 (3.47-4.77)	19.3 (15.3-25.9)	1.90	3.33
Susceptible	541	2.907±0.226	2.143 (1.77-2.55)	5.80(4.79-7.44)	-	-

*Number of individuals used in the experiment. **The resistance rate according to LC_{50}. ***The resistance rate according to LC_{90}.

Table 2. Hexythiazox resistance ratios on different *T. urticae* populations

Population	n*	Slope±SE	LC_{50} (mg a.i l^{-1}) (95% CL)	LC_{90} (mg a.i l^{-1}) (95% CL)	RR_{50}**	RR_{90}***
Gümenek1	548	1.749±0.126	4.54 (3.52- 5.79)	24.5 (17.3-40.0)	1.73	2.76
Gümenek2	554	1.318±0.108	4.38 (3.27-5.79)	41.1 (6.13-80.0)	1.67	4.63
Gümenek3	561	1.579±0.118	4.81 (3.71- 6.18)	31.2 (21.4-53.3)	1.84	3.51
Susceptible	557	2.416±0.172	2.61 (2.13- 3.18)	8.88 (6.89-12.5)	-	-

*Number of individuals used in the experiment. **The resistance rate according to LC_{50}. ***The resistance rate according to LC_{90}.

Table 3. Spiromesifen resistance ratios on different *T. urticae* populations

Population	n*	Slope±SE	LC_{50} (mg a.i l^{-1}) (95% CL)	LC_{90} (mg a.i l^{-1}) (95% CL)	RR_{50}**	RR_{90}***
Gümenek1	508	1.491±0.125	6.53 (5.34-8.01)	47.3 (33.1-76.2)	1.97	2.56
Gümenek2	514	1.439±0.121	6.94 (5.70-8.57)	53.9 (37.2-89.0)	2.09	2.91
Gümenek3	528	1.521±0.121	5.88 (4.79-7.27)	40.9 (28.7-66.2)	1.77	2.21
Susceptible	511	1.717±0.132	3.32 (2.78-3.94)	18.5 (14.2-26.0)	-	-

*Number of individuals used in the experiment. **The resistance rate according to LC_{50}. ***The resistance rate according to LC_{90}.

Alpkent et al. (2020) detected high resistance levels ranging from 169 to 465 times in *T. urticae* populations collected from greenhouses in Antalya, Turkey. In our study, low resistance against hexythiazox was determined in all *T. urticae* populations collected from cucumber-grown greenhouses.

The resistance rates of *T. urticae* populations against spiromesifen according to the LC_{50} value were determined as 2.09, 1.97, and 1.77-fold, respectively. The highest resistance rate against spiromesifen was determined in the Gümenek2 population, and the lowest resistance rate was determined in the Gümenek3 population (Table 2.3).

Yorulmaz and Kaplan (2014) stated that *T. urticae* populations collected from tomato greenhouses in Isparta province developed moderate resistance (8.16-22.82 folds). Sharma and Bhullar (2018) found moderate resistance to spiromesifen in *T. urticae* populations collected from vegetable-growing areas in different regions of India (11.14-21.40 folds). Kaur and Bhullar (2019) reported that *T. urticae* populations collected from cucumber (*Cucumis sativus* L.) fields in different regions of India developed low and moderate resistance to spiromesifen (1.88-16.05 folds). This study showed low resistance to spiromesifen, similar to the resistance rates of *T. urticae* populations collected from cucumber fields in Hoshiarpur (1.88-fold) and Patiala (2.02-fold) locations, India (Kaur and Bhullar, 2019). However, moderate resistance has been observed in other studies.

Conclusions

Determining the resistance level of local populations to acaricides is the first step in resistance management for *T. urticae* in the cucumber greenhouse. As a result of this study, it was determined that abamectin, hexythiazox and spiromesifen acaricides, which are widely used in cucumber greenhouses in Gümenek village of Tokat, cause low resistance in the *T. urticae* populations. However, due to the heterogeneity of some populations and the appropriate biology of *T. urticae*, it is thought that chemical control should be paid attention to and resistance levels should be checked at regular intervals. It is thought that the use of acaricides with a different mode of action by rotation, instead of using these acaricides at frequent intervals and one after the other, will adversely affect the development of resistance. It is important to determine the resistance levels for effective chemical control against pests.

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