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Determination of the Resistance Level of Two-Spotted Spider Mite (*Tetranychus urticae* Koch) Populations in Apple Orchards in Isparta Province Against Some Pesticides[*]

Elvan SÖKELİ[1] Recep AY[1] İsmail KARACA[1]

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Abstract: This study was conducted to determine the resistance of two-spotted spider mites to some pesticides (propargite, chlorpyrifos and abamectin) used in apple orchards around Isparta province where apple production is intense. The LC_{50} and LC_{90} levels of all populations to these pesticides were determined using Petri plate - spray tower method. The LC_{50} value of *Tetranychus urticae* Koch populations to propargite, chlorpyrifos and abamectin was found to be 137.69 – 295.07 µ l l^{-1} distilled water, 70.99 – 1219.46 µ l l^{-1} distilled water and 6.41 – 15.13 µ l l^{-1} distilled water, respectively. Resistant ratios of populations were calculated by dividing the LC_{50} values of field populations by that of a susceptible population of *T. urticae* strain GSS. The resistance ratios for all *T. urticae* populations ranged from <1.0 – 1.1 fold for propargite, 2.3 – 40.2 fold for chlorpyrifos and <1.0 – 1.4 fold for abamectin (based on LC_{50}). As a result of this study, it was found that *T. urticae* populations collected from apple orchards were resistant to chlorpyrifos but they were susceptible to propargite and abamectin.

Key Words: Abamectin, Chlorpyrifos, propargite, *p*esticides resistance, *Tetranychus urticae*, Tetranychidae

Isparta İlindeki Elma Bahçelerinde Zararlı Olan İki Noktalı Kırmızıörümcek (*Tetranychus urticae* Koch) Populasyonlarının Bazı Pestisitlere Karşı Direnç Düzeylerinin İncelenmesi

Öz: Bu çalışmada elmanın yoğun olarak üretildiği Isparta ilindeki elma bahçelerinde zararlı olan iki noktalı kırmızıörümcek populasyonlarının bazı kimyasallara (propargite, chlorpyrifos ve abamectin) karşı direnç düzeyleri incelenmiştir. Bütün populasyonların bu ilaçlara karşı LC_{50} ve LC_{90} düzeyleri petri kabı – ilaçlama kulesi metodu kullanılarak belirlenmiştir. *T. urticae* Koch populasyonlarının propargite, chlorpyrifos ve abamectin'e karşı LC_{50} değerleri sırasıyla 137.69 – 295.07 µ l l^{-1} saf su, 70.99 – 1219.46 µ l l^{-1} saf su ve 6.41 – 15.13 µ l l^{-1} saf su olarak bulunmuştur. Elma bahçelerinden toplanan *T. urticae* populasyonlarının direnç oranı standart hassas populasyon GSS ile oranlanarak bulunmuştur. Populasyonların direnç oranları propargite için <1.0 – 1.1 kat, chlorpyrifos için 2.3 – 40.2 kat ve abamectin için <1.0 – 1.4 kat bulunmuştur (LC_{50}'ye göre). Bu sonuçlara göre elma bahçelerinden toplanan T. *urticae* populasyonları chlorpyrifos'a karşı dirençli fakat propargite ve abamectin'e karşı duyarlı bulunmuştur.

Anahtar Kelimeler: Abamectin, chlorpyrifos, *p*ropargite, pestisid direnci, *Tetranychus urticae*, Tetranychidae

Introduction

Isparta province is the most important apple production centre of Turkey. It provides 21.1 % (about 500 thousand tones) of the total apple production of Turkey (Yıkar 2003). This area has suitable climate and soil structure for apple production. With the increase in apple production areas, the number of economically harmful insects also increases (Uygun et al. 2002). Spider mites are among the harmful pests in apple orchards. In this study, two-spotted spider mite (*Tetranychus urticae* Koch) was determined as the common species. *T. urticae* has a worldwide distribution and a large number of host plants (Tsagkarakou et al. 2002). Chemical control is preferred for spider mites in most apple orchards in Turkey. Therefore, spider mites are under hard selection pressure because of the consistent use of

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[1] Süleyman Demirel University, Faculty of Agriculture Plant Protection Department-Isparta

pesticides against it. Since spider mites longevity was short and they have many generations per year, they can develop resistance to pesticides.

In this study, the susceptibility level of *T. urticae* that was collected from different apple orchards was tested against three pesticides (propargite, chlorpyrifos, abamectin). One of the pesticides is propargite which is a selective acaricide registered for controlling spider mites in many crops. Propargite has been registered since 1996 to be used against *T. viennensis* Zacher in apple orchards. Chlorpyrifos is a broad-spectrum insecticide-acaricide and has contact effect. This insecticide is registered to be used against many insects on fruits such as aphids, *Hyphantria cunea* (Drury), *Synanthedon myopaeformis* Bkh., *Cydia pomonella* (L.), *Quadraspidiotus perniciosus* Comst, *Zeuzera pyrina* L. and leaf rollers. It has been used in apple orchards since 1985. Abamectin is an insecticide-acaricide and was registered in 1991 against *T. urticae* on vegetables (Anonymous 2002).

The objective of this study was to test susceptibility of *T. urticae* collected from apple orchards in Isparta using propargite, chlorpyrifos and abamectin.

Materials and methods

Spider mite populations: Nine different populations of *T. urticae* Koch were collected from apple orchards in Isparta (Table 1). These populations were cultured on bean plants in a climate chamber with 26 ± 2 °C temperature, 60 - 65 % r.h. and a photoperiod of 16 : 8 h (Light : Dark). A susceptible strain (GSS) was obtained from Rothamsted Experimental Station, Harpenden (England) and reared under same climate chamber conditions since 2001. GSS population has been maintained in England as a laboratory culture, since 1965 (Dennehy et al. 1993). Synchronized cultures of *T. urticae* were produced from each of nine stock populations and the GSS population for using in bioassays. Adult females were transferred from stock populations to bean leaves in small plastic containers.

Pesticides: Pesticides used in the experiments were propargite (Omite® Süper 570 EW) 570 gl^{-1}, chlorpyrifos (Dursban 4 EC) 480 gl^{-1} and abamectin (Agrimec EC) 18 gl^{-1}. Six different concentrations of these pesticides were prepared by mixing with 100 ml distilled water to obtain $X^{1/2}$ intervals (Anonymous 1969). The maximum dose of used pesticides is; 1000 - 4000 $\mu l\ l^{-1}$ in distilled water for propargite, 4000 $\mu l\ l^{-1}$ in distilled water for chlorpyrifos and 100 $\mu l\ l^{-1}$ in distilled water for abamectin.

Bioassays: Pesticide applications were done using spray tower-petri dish method as previously reported by Kabir and Chapman (1997), Campos et al. (1997) and Ay (2005). Pesticides prepared in different doses were applied to the internal surfaces of lids and bases of 50-mm-diameter plastic petri dishes and allowed to dry for 30 min at 26 ± 2 °C. For each application, 1 ml suspension was sprayed on each base and lid pair by a Potter spray tower (Auto-Load; Burcard Manufacturing Co. Ltd., Rickmansworth, Herts., UK) at 1 bar and 3 s settling time. Preliminary tests were conducted before each experiment to determine the range of concentrations that would produce 5 - 95 % mortality. All experiments were conducted in three replicates of six-concentration design (plus distilled water only, as control). Adult female mites (25 - 30) were transferred to each dish using a fine hairbrush. The dishes were then closed, sealed with parafilm to prevent the escape of mites, and placed in a growth chamber (16 : 8 h (L : D) at 26±2 °C and 60 - 65 % r.h.). Mortality, defined as the inability to move when prodded, was assessed after 24 h with the aid of a stereomicroscope in all experiments.

Statistical analyses: All data from each concentration-mortality experiment were pooled and subjected to probity analysis. LC_{50} and LC_{90} values with their 95 % confidence level (CL) and slopes ± S.E. of regression were estimated using the computer program POLO (LeOra Software 1994).

Results and discussion

Field populations, collected from apple orchards of Isparta in 2004 and one susceptible population (GSS) were examined for their resistance to propargite, chlorpyrifos and abamectin. Resistance rate of the populations were given in Table 2 - 4.

Susceptibility of these populations to propargite at LC_{50} value varies <1.0 to 1.1 fold**.** According to LC_{50} values, all populations were found to be more susceptible than GSS population. However, susceptibility degrees of the populations were decreased based on LC_{90} values in all populations (Table 2).

According to LC_{50} and LC_{90} values, resistance rates of all populations against chlorpyrifos ranges from 2.3 to 40.2 and 4.3 to 356.5 fold. Population of Gelendost 1 was more resistant when compared to other populations (Table 3).

Table 1. Origins of *Tetranychus urticae* populations

Species	Location of collection	Date of collection	Population Name
Tetranychus urticae	Kuleönü - Isparta	30.06.2004	Kuleönü
"	Gelendost - Isparta	15.07.2004	Gelendost 1
"	Gelendost - Isparta	15.07.2004	Gelendost 2
"	Eğirdir - Isparta	28.07.2004	Eğirdir
"	Aksu - Isparta	28.07.2004	Aksu 1
"	Aksu - Isparta	28.07.2004	Aksu 2
"	Gönen - Isparta	26.08.2004	Gönen
"	Uluborlu Isparta	26.08.2004	Uluborlu
"	Çünür - Isparta	18.08.2004	Çünür

Table 2**.** Probit statistics for susceptible population (GSS) and field populations of *T. urticae* tested against propargite (Omite) 570 g l^{-1}

Population Name	n*	Slope±SE	LC_{50} ($\mu l l^{-1}$) (0.95 CL)	LC_{90} ($\mu l l^{-1}$) (0.95 CL)	Resistance factor LC_{50}**	Resistance factor LC_{90}**
GSS (Susceptible)	**671**	**3.5±0.4**	**282.22** 193.85-364.49	**662.46** 503.54-1063.23	**-**	**-**
Kuleönü	**623**	**1.8±0.2**	**156.91** 10.9-20.5	**819.94** 605.84-1270.72	**< 1**	**1.2**
Gelendost 1	**646**	**1.4±0.2**	**295.07** 140.79-470.33	**2623.56** 1316.82-15154.97	**1.1**	**3.9**
Gelendost 2	**809**	**1.7±0.1**	**186.68** 105.40-280.22	**1038.00** 673.94-2004.20	**< 1**	**1.6**
Eğirdir	**698**	**1.4±0.2**	**158.90** 111.14-212.62	**1366.77** 930.32-2372.07	**< 1**	**2.1**
Aksu 1	**611**	**1.4±0.2**	**170.52** 102.29-255.93	**1414.89** 831.88-3422.83	**< 1**	**2.1**
Aksu 2	**599**	**1.1±0.1**	**144.93** 67.05-270.45	**2256.21** 955.84-12999.85	**< 1**	**3.4**
Gönen	**628**	**1.7±0.2**	**137.69** 98.42-180.45	**820.98** 616.02-1188.17	**< 1**	**1.2**
Uluborlu	**615**	**2.0±0.3**	**194.88** 130.81-256.01	**826.60** 615.43-1305.23	**< 1**	**1.2**
Çünür	**729**	**2.7±0.3**	**224.24** 117.21-335.74	**672.80** 437.30-1694.47	**< 1**	**1.0**

**Sample size refers to number of adult females
**Resistance factor = LC_{50} or LC_{90} of the field-collected / LC_{50} or LC_{90} of the susceptible population (GSS)

Susceptibility losses of *T. urticae* populations to abamectin were <1.0 - 1.4 fold and <1.0 - 1.6 fold according to LC_{50} and LC_{90} levels, respectively. Most *T. urticae* populations were more susceptible than the GSS population (Table 4).

In the present study, no important susceptibility loss was determined against two selective acaricides (propargite and abamectine) but, a high level of resistance was found against organophosphate chlorpyrifos with a broad-spectrum insecticide-acaricide effect. Producers prefer using broad-spectrum pesticides like chlorpyrifos against harmful insects in Turkey rather than selective pesticides. Results obtained in this study and those in Ay (2005) and Ay et al. (2005) support this phenomenon. Resistance against agricultural chemicals is dependent directly on the frequency of chemical use (Hoyt et al. 1985, Compos et al. 1996). The usages of broad - spectrum chemicals increases selection pressure, and have a negative effect on natural enemies. Therefore, natural enemy pressure becomes less over spider mites and aphids so their intensity increases for a short time. This situation makes chemical use an absolute necessity. Herron et al. (1998) had stated that the resistance selection is directly associated with the frequency of pesticide use, that most of the organophosphate insecticide – acaricides used for cotton are also applied to other pests as well as a result of which the selection pressures of these pesticides increase in *T. urticae* populations.

Table 3. Probit statistics for susceptible population (GSS) and field populations of *T. urticae* tested against chlorpyrifos (Dursban 4) 480 g l^{-1}

Population	n*	Slope±SE	LC_{50} ($\mu l l^{-1}$) (0.95 CL)	LC_{90} ($\mu l l^{-1}$) (0.95 CL)	Resistance factor LC_{50}**	Resistance factor LC_{90}**
GSS (Susceptible)	**774**	**2.3±0.2**	**30.33** 19.08-44.79	**108.77** 68.91-258.46	**-**	**-**
Kuleönü	**620**	**1.0±0.1**	**707.64** 305.34-1413.54	**13469.94** 4980.26-135278.16	**23.3**	**123.8**
Gelendost 1	**631**	**0.9±0.1**	**1219.46** 430.96-4170.16	**38776.06** 8463.47-5428631.49	**40.2**	**356.5**
Gelendost 2	**613**	**1.2±0.1**	**410.86** 234.88-637.65	**4575.63** 2570.66-11758.57	**13.5**	**42.1**
Eğirdir	**542**	**1.6±0.2**	**181.44** 52.87-361.24	**1094,18** 541.18-4443.50	**6.0**	**10.1**
Aksu 1	**607**	**2.9±0.3**	**166.93** 118.50-212.44	**464.90** 363.24-666.95	**5.5**	**4.3**
Aksu 2	**783**	**1.5±0.2**	**274.32** 99.17-477.04	**1985.32** 1185.65-4679.14	**9.0**	**18.3**
Gönen	**757**	**1.4±0.1**	**70.99** 20.89-141.41	**590.62** 292.62-2182.47	**2.3**	**5.4**
Uluborlu	**595**	**2.0±0.2**	**341.78** 194.79-493.80	**1455.29** 1017.98-2460.46	**11.3**	**13.4**
Çünür	**709**	**1.0±0.1**	**265.75** 136.40-441.50	**4700.96** 2549.02-11956.23	**8.8**	**43.2**

*Sample size refers to number of adult females
** Resistance factor = LC_{50} or LC_{90} of the field-collected / LC_{50} or LC_{90} of the susceptible population (GSS)

Table 4. Probit statistics for susceptible population (GSS) and field populations of *T. urticae* tested against abamectine (Agrimec)18 g l^{-1}

Population	n*	Slope±SE	LC_{50} ($\mu l l^{-1}$) (0.95 CL)	LC_{90} ($\mu l l^{-1}$) (0.95 CL)	Resistance factor LC_{50}**	Resistance factor LC_{90}**
GSS (Susceptible)	**720**	**2.1±0.2**	**10.91** 6.21-15.40	**45.67** 29.72-117.09	**-**	**-**
Kuleönü	**615**	**1.8±0.2**	**7.32** 4.27-11.11	**36.22** 22.40-80.96	**< 1**	**< 1**
Gelendost 1	**691**	**2.2±0.2**	**13.88** 10.50-17.27	**52.60** 41.80-71.15	**1.3**	**1.2**
Gelendost 2	**671**	**1.9±0.3**	**15.13** 3.69-24.35	**73.00** 46.96-239.67	**1.4**	**1.6**
Eğirdir	**618**	**2.1±0.2**	**6.99** 4.06-10.11	**28.13** 19.12-51.84	**< 1**	**< 1**
Aksu 1	**605**	**1.9±0.2**	**8.74** 5.62-12.42	**39.95** 26.64-74.65	**< 1**	**< 1**
Aksu 2	**611**	**1.7±0.2**	**7.06** 4.30-10.33	**40.63** 26.63 -75.64	**< 1**	**< 1**
Gönen	**613**	**1.5±0.1**	**7.40** 3.28-13.13	**56.55** 29.23-199.21	**< 1**	**1.3**
Uluborlu	**616**	**2.1±0.2**	**6.41** 5.07-7.83	**26.82** 21.31-35.86	**< 1**	**< 1**
Çünür	**607**	**1.7±0.2**	**8.48** 6.20-10.98	**45.82** 34.52-65.91	**< 1**	**1.0**

*Sample size refers to number of adult females
** Resistance factor = LC_{50} or LC_{90} of the field-collected / LC_{50} or LC_{90} of the susceptible population (GSS)

Sawicki and Denholm (1987) explained *T. cinnebarinus* Boisduval and *T. lombardinii* Pritchard & Baker's struggle only uses dimethoate in cotton orchards in Zimbabwe between 1960 – 1970 years. So, this species developed high resistance (about 1000 fold) against dimethoate and they were also resistant to organophosphate insecticide – acaricides.

The resistance rates of different *T. urticae* populations gathered from vegetable greenhouses of

Isparta for propargite, amitraz and abamectin was found to vary between <1.0 - 2.5, 1.2 - 2.1 and <1.0 - 2.9 fold (based on LC_{50}) (Ay et al. 2005). In a study conducted in Isparta, it was found that chlorpyrifos was used widely in apple orchards (Demircan and Yılmaz 2005). The rate of resistance against chlorpyrifos changed 8.00 – 1774.00 fold in *T. urticae* populations, collected by Ay (2005) from Isparta and Antalya vegetable greenhouses. Ay and Gürkan (2005) determined that resistance rates of *T. urticae* populations collected from cotton fields changed <1.0 - 669 fold against bifenthrin.

As it is seen, while there is no significant susceptibility loss against selective acaricides, there is a high level of resistance against chlorpyrifos in two-spotted spider mites.

Finally, the usage of selective acaricides like propargite and abamectin are advantageous against spider mites in apple orchards. Broad - spectrum pesticides such as chlorpyrifos should not be used for controlling pests on fruit crops like apple for fresh consumption.

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Correspondce adress:
Recep AY
Süleyman Demirel University, Faculty of Agriculture
Plant Protection Department -Isparta
Tel: 0 246 211 46 41
E-mail: recepay@ziraat.sdu.edu.tr