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The stability of abamectin resistance and the efficacy of spinosad in *Tetranychus urticae* Antalya population

Tetranychus urticae Antalya populasyonunda spinosad'ın etkisi ve abamectin direncinin stabilitesi

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ABSTRACT

The level and stability of abamectin resistance in a *Tetranychus urticae* Koch population collected from a greenhouse in Antalya (Turkey) exposed to long term intensive pesticide applications were determined. Multiple-resistance spectrum for dicofol and tetradifon and the efficacy of spinosad to larvae of abamectin-resistant *T. urticae* were also investigated. Laboratory bioassays were performed for abamectin and dicofol on adults, for tetradifon on eggs, and for spinosad on larvae. LC values and resistance ratios of populations were determined by the leaf-dip method. The resistance ratio to abamectin in *T. urticae* population collected from Altınova, Antalya (Turkey) was 643 fold. The population expressed a 10 fold multiple-resistance to dicofol but multiple-resistance to tetradifon was not significant. The resistance ratio to abamectin decreased from 643 to 11 fold in the population maintained pesticide-free for 20 months (~60 generations). Spinosad had significantly affected the abamectin-resistant *T. urticae* larvae with mortalities of 100 and 72 % at recommended dose and $1/10^{th}$ of it, respectively. Monitoring of resistance level in field populations should be continued. As a resistance management strategy, abamectin applications may be ceased in certain period in locations where abamectin resistance appears as a significant problem. The high efficacy of spinosad on abamectin-resistant *T. urticae* may be considered as an alternative for resistance management strategies with abamectin.

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ÖZ

Bu çalışmada Antalya'da uzun süre yoğun ilaç kullanılan bir seradan alınan *Tetranychus urticae* Koch populasyonunda abamectin'e direnç düzeyi ve abamectin direncinin stabilitesi belirlenmiştir. Abamectin'e dirençli populasyonda dicofol ve tetradifon'a çoklu direnç spektrumu ve ayrıca spinosad'ın abamectin'e dirençli *T. urticae* larvaları üzerindeki etkisi araştırılmıştır. Laboratuvar biyoesseylerinde, abamectin ve dicofol için ergin testi, tetradifon için yumurta testi, spinosad için larva testi düzenlenmiştir. Yaprak daldırma yöntemiyle populasyonların LC değerleri ve duyarlı populasyona göre direnç katları belirlenmiştir. Altınova, Antalya (Türkiye)'dan alınan *T. urticae* populasyonu abamectin'e 643 kat dirençli bulunmuştur. Abamectin'e dirençli populasyonda dicofol'e 10 kat çoklu direnç görülmüştür. Tetradifon'a ise çoklu direnç görülmemiştir. Yirmi ay (~60 generasyon) kadar ilaç baskısı olmaksızın devam ettirilen Altınova populasyonunda abamectin direnci 643 kattan 11 kata düşmüştür. Spinosad'ın abamectin'e dirençli *T. urticae* larvalarına karşı önemli düzeyde etkili olduğu tespit edilmiştir. Spinosad, serada önerilen tavsiye dozunda ve bunun 1/10 katında larvalar üzerinde sırasıyla % 100 ve % 72 ölüme yol açmıştır. Arazi populasyonlarında abamectin'e karşı direnç izlenmeye devam edilmelidir. Bir direnç yönetim taktiği olarak direnç sorunu olan lokasyonlarda abamectin kullanımına belirli süre ara verilmelidir. Spinosad'ın abamectin'e dirençli populasyona karşı yüksek düzeyde etkiye sahip olması, abamectin için yürütülen direnç yönetim programlarında bir alternatif olarak göz önüne alınabilir.

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, is a serious agricultural world-wide pest. The main host of this phytophagous spider mites are vegetables, fruits, cotton, strawberry, and ornamentals. Heavy infestations cause considerably yield losses and reduce fruit quality (He et al. 2009; Migeon and Dorkeld 2015; UC-IPM 2015). The two-spotted spider mites are common problem in greenhouse-growing vegetables in Mediterrenean Costal region of Turkey according to several survey studies (Ulubilir and Yabaş 1996; Bulut and Göçmen 2000).

Currently pesticides are widely and frequently used against the major pests including spider mites in Turkey. This strategy caused resistance development in pest populations (Yorulmaz and Ay 2009; Yorulmaz Salman and Kaplan 2014). The rise of insecticide resistance has caused two major problems in chemical management programmes. First, many insecticides or acaricides became ineffective to resistant pest populations at the recommended field doses. And secondly, the use of these ineffective pesticides resulted increase in application dose. This in turn adds to cost, creates environmental, ecological and health concerns in the production areas (Roush and Tabashnik 1990). Abamectin, an insecticide-acaricide registered in 1991 in Turkey, currently recommended to many insect and mite pests (Tosun and Onan 2014). However, low to high level resistance to abamectin in spider mite populations were reported (Campos et al. 1995; Campos et al. 1996; Sato et al. 2005; Yorulmaz and Ay 2009; He et al. 2009; Yorulmaz Salman and Kaplan 2014). Producers in Antalya have been complaining about ineffectiveness of abamectin. Thus, resistance status of two-spotted spider mite against abamectin should be screened routinely and several insecticide management tactics should be investigated for solution of resistance problem related with abamectin.

The aim of the study was to determine the level of resistance to abamectin in a *T. urticae* population collected from Altınova (Antalya) where population have been exposed to long term intensive field pesticide application. Then, stability of abamectin resistance was monitored in this population maintained pesticide-free in the lab for 60 generations. Multiple-resistance potential of the population to dicofol and tetradifon were also determined. Finally, the acaricidal effects of spinosad, commonly known as insecticide, on larvae of the population (abamectin-resistant) were examined with laboratory assays.

2. Materials and Methods

2.1. Insecticides and acaricides

Detailed information of acaricides and insecticides used in the study were given in Table 1. Abamectin, an insecticide-acaricide registered in 1991 in Turkey, currently recommended to many insect and mite pests (Tosun and Onan 2014). Dicofol and tetradifon, used for a long time against spider mites, were abandoned in Turkey in 2011. Spinosad was registered in 1998 and it is currently used as insecticides in Turkey (Tosun and Onan 2014).

2.2. Populations and rearing

A laboratory susceptible colony of *T. urticae* was maintained in a walk-in growth chamber for approx. four years without exposure to chemical application. The field population of *T. urticae* was collected from a cucumber greenhouse in the district of Altınova (Antalya) in 2006 where pesticides had intensively been used. Abamectin selected and unselected strains were established from the Altınova population. Altınova (original) strain was maintained pesticide-free for 20 months (~60 generations) and then this colony was used for reversion bioassays in the study. All colonies were reared on potted cowpea (*Vigna sinensis* L) at 25 ± 1 °C and photoperiod of 16:8 h L:D. Cowpea leaves were used to obtain leaf disks for bioassays. All adult females of the populations showed red color and are considered to be red form of *T. urticae* (Auger et al. 2013).

Table 1. Active ingredient (a.i.), commercial name and (IRAC) mode of action classification of pesticides.

Active ingredient	Commercial name and formulation	IRAC mode of action classification*
Abamectin	18 g l^{-1} EC Agrimec, Syngenta	6- Glutamate-gated chloride channel (GluCl) allosteric modulators, Nerve and muscle action
Dicofol	195 g l^{-1} EC Hekthane, Hektaş	Compounds of unknown or uncertain Mode of Action
Tetradifon	75.2 g l^{-1} EC Akardion V-18, Safa	12- Inhibitors of mitochondrial ATP synthase, Energy metabolism
Spinosad	Laser, SC 480 g l^{-1} Dow Agro Sciences	5- Nicotinic acetylcholine receptor (nAChR) allosteric modulators, Nerve action

*(IRAC 2016)

2.3. Laboratory bioassays

The leaf dip method was used in laboratory bioassays (Roditakis et al. 2005), which are similar to current IRAC methods recommended for spider mites (IRAC 2015a; 2015b). Laboratory bioassays were performed for abamectin and dicofol on adults, for tetradifon on eggs, and for spinosad on larvae.

2.3.1. Adult bioassays with abamectin and dicofol

The cowpea leaf disks (20 mm diameter) were dipped for 5 seconds into four to six concentrations of insecticides diluted in 100 ml distilled water containing 1 % Triton X-100. These serial insecticides concentration were adjusted to cause 0-100 % mortality in populations. Control disks were dipped only in distilled water with Triton X-100. Treated disks were placed on the wet cotton pads in petri dishes. After 2 h drying, adult *T. urticae* were transferred onto treated leaf disks using a fine brush. Mortalities were recorded after 3 days of exposure. Adult spider mites were considered as dead if they were unable to move when touched with a fine brush.

2.3.2. Egg bioassay with tetradifon

For tetradifon bioassay, leaf disks bearing 0-24 h old *T. urticae* eggs were dipped as described above and mortalities were scored after 6 days. The unhatched eggs were counted as dead. Three leaf disks were used for each insecticide concentration or water (control). Each disk included 10-15 adult or eggs. Three replicates were used in each bioassay concentrations.

2.3.3. Larvae bioassays with spinosad

In spinosad bioassay, leaf dip method similar to above mentioned was used. The full dose of spinosad [144 mg active ingredient (a. i.) l^{-1}] recommended for *Spodoptera littoralis* and 1/10th of it [14.4 mg (a. i.) l^{-1}] were used in this bioassay. Cowpea leaf disks (3 cm diameter) were dipped into spinosad diluted in 100 ml distilled water containing 1 % Triton X-100 for 5 seconds. Control disks were dipped into distilled water with Triton X-100. Treated leaf disks were dried for 2 h and were placed on an agar layer in the bottom of plastic vials. One day old larvae were placed on the treated leaf disks using fine brush under the stereomicroscope and covered with stretch film that were perforated with 20-30 holes using insect pin. In order to determine the effect of 6- days old residues of spinosad, cowpea leaf disks were taken from the leaves of plants treated 6 days before. Twenty replicates were used for the full dose assay, ten replicates were used for both 1/10th of full dose assay and 6-days old residues assay. The number of control replicates is equal to that of the insecticide treatments. Each replicate included 3-4 larvae. The mortalities were determined after 7-day of exposure and the data were adjusted for mortalities in control (Abbott 1925).

2.4. Selection with abamectin

A *T. urticae* subpopulation from the Altınova (original) population was subjected to selection with abamectin for 7 times in a 5 months at a dose of 0.9 mg (a.i) l^{-1}. The selected strain was used to determine both resistance potential to abamectin and multiple-resistance to dicofol in laboratory bioassays.

2.5. Data analysis

To calculate the LC (lethal concentration) values and their confidence limits, data were subjected to probit analysis (Polo-Plus, Probit and Logit Analysis, LeOra Software 2002-2015). Resistance ratios were calculated by dividing the LC_{50} of the field and selected strain by that of the laboratory susceptible strain. Failure of 95 % CL (confidence limits) to overlap at a given LC_{50} indicated significant difference.

3. Results

The LC_{50} values and resistance ratios for abamectin in Altınova populations are given in Table 2. Multiple-resistance ratios of Altınova population to dicofol and tetradifon are summurized Table 3 and Table 4, respectively. LC_{90} values and field recommended doses of acaricides are also presented in Table 2 - 4. The mortality ratios on *T. urticae* larvae in Altınova population in bioassay with spinosad are given in Table 5.

Resistance level of Altınova population for abamectin was 643 fold at LC_{50}. Also, LC_{90} value of this population was higher than recommended field dose of abamectin (Table 2).

Altınova population showed a 10 fold multiple-resistance to dicofol (Table 3). The multiple-resistance to tetradifon was 2 fold but this ratio was not significant according to confidence limits (Table 4).

Resistance ratio to abamectin has increased in Altınova population after selected with abamectin for 7 times (Table 2). Abamectin selection has also caused an increase of the multiple-resistance ratio to dicofol in Altınova population (173 fold) (Table 3).

The resistance ratio decreased to 19 and 11 fold in Altınova (original) population maintained without exposure to pesticides for 5 and 20 months, respectively (Table 2). Moreover, the LC_{90} value of Altınova population after 20 month was lower than that of the recommended field dose of abamectin (Table 3). However, multiple-resistance ratio to dicofol did not decrease in Altınova population 5 months later (Table 3).

Spinosad had significantly effected the *T. urticae* larvae. After seven days of exposure, mortalities at recommended dose and at 1/10th of that were found to be 100 and 72 %, respectively (Table 5). The mortality ratio in 6 - days old residues of the spinosad was 89 % (Table 5).

Table 2. Potential and stability of abamectin resistance in Altınova *Tetranychus urticae* population.

Populations	n[a]	slope±se	**LC_{50}** mg (a. i.) l^{-1} *confidence limits 95 %*	**Resistance level**[b]	**LC_{90}** mg (a. i.) l^{-1} *confidence limits 95 %*	Field dose[c] mg (a. i.) l^{-1}
Susceptible	330	1.9±0.5	**0.005** *0.001-0.008*	-	**0.02** *0.02-0.08*	4.5
Altınova	232	0.9±0.3	**3.215** *1.283-10.966*	**643**	**79.31** *17.9-48045.0*	4.5
Altınova - Selected	281	1.0±0.2	**5.932** *2.736-11.665*	**1186**	**111.95** *45.4-595.8*	4.5
Altınova 5-month later	324	0.7 ±0.1	**0.097** *0.014-0.356*	**19**	**8.7** *2.0-147.5*	4.5
Altınova 20-month later	494	1.2±0.1	**0.054** *0.035-0.085*	**11**	**0.70** *0.34-2.23*	4.5

[a] : number of individuals used for bioassay, [b]: field or selected-unselected population LC_{50} / susceptible population LC_{50}, [c] : Recommended field dose (Tosun and Onan 2014).

Table 3. Multiple-resistance spectrum for dicofol in abamectin-resistant *Tetranychus urticae* (Altınova) population.

Populations	n[a]	slope±se	**LC_{50}** mg (a. i.) l^{-1} *confidence limits 95 %*	**Resistance level**[b]	**LC_{90}** mg (a. i.) l^{-1} *confidence limits 95 %*	Field dose[c] mg (a. i.) l^{-1}
Susceptible	225	1.6±0.4	**1.3** *0.13-2.94*	-	**8.4** *3.8-112.1*	29.25
Altınova	242	1.2±0.3	**13.2** *4.79-45.08*	**10**	**148.4** *43.9-33958.4*	29.25
Altınova - Selected	276	1.7±0.3	**224.7** *153.4-355.9*	**173**	**1297.5** *678.8-5345.8*	29.25
Altınova 5-months later	313	1.3±0.4	**13.5** *7.4-23.1*	**10**	**131.9** *68.4-374.2*	29.25

[a]: number of individuals used for bioassay, [b]: field or selected-unselected population LC_{50} / susceptible population LC_{50}, [c]: Recommended field dose (Yücer 2007).

Table 4. Multiple-resistance spectrum for tetradifon in abamectin-resistant *Tetranychus urticae* (Altınova) population.

Populations	n[a]	slope±se	**LC_{50}** mg (a. i.) l^{-1} *confidence limits 95 %*	**Resistance level**[b]	**LC_{90}** mg (a. i.) l^{-1} *confidence limits 95 %*	Field dose[c] mg (a. i.)l^{-1}
Susceptible	206	1.0±0.2	**3.2** *1.4-7.1*	-	**64.5** *23.4-392.5*	112.8
Altınova	223	1.5±0.3	**7.5** *2.0-14.5*	**2**	**51.3** *24.4-437.5*	112.8

[a]: number of individuals used for bioassay, [b]: field population LC_{50} / susceptible population LC_{50}, [c]: Recommended field dose (Yücer 2007).

Table 5. Efficacy of spinosad against *Tetranychus urticae* larvae of abamectin-resistant (Altınova) population.

Dose mg (a. i.) l^{-1}	Mortality ratio (%)
144 (recommended dose)*	100
144 (6 days-old residue)	89
14.4 (1/10th of recommended dose)	72

*Recommended dose of *Spodoptera littoralis* (Tosun and Onan 2014).

4. Discussion

For this study, a *T. urticae* population was collected from Altınova (Antalya) where pesticides had been frequently used. As expected, a high level abamectin resistance up to 643 fold was detected in *T. urticae* Altınova population. Additionally, LC_{90} value of this population was higher than the recommended field dose of abamectin (Table 2). The results suggest that chemical management of *T. urtice* populations using abamectin may result in control failures. Resistance to abamectin in *T. urticae* were reported from Turkey and several other countries. *T. urticae* populations from vegetable greenhouses in Isparta (Turkey) were found to be 8 - 25 fold resistance to abamectin. In addition, the same populations expressed resistance against spiromesifen (8-23 fold) and hexythiazox (9-12 fold), (Yorulmaz Salman and Kaplan 2014). Campos et al. (1995) reported high variations in susceptibilities of California *T. urticae* populations to abamectin where resistance ratios were 1 - 685 fold at LC_{95}. *T. urticae* populations from Florida, Holland and Canary island were found to be 0.5 - 175 fold (Campos et al. 1996). Beer et al. (1998) reported that resistance to abamectin in *T. urticae* populations from pear orchards in Washington was at levels considered moderate to low (5-27 fold).

Additonal selections with abamectin on Altınova *T. urticae* population have led to increase in resistance ratio to abamectin when compared to that of the susceptible population with an increase from 643 to 1186 fold. However, the increase of resistance in the same population to abamectin was insignificant with only 2 fold when compared to that of the Altınova (original colony) (Table 2). The reasons may be that Altınova population had already a high level of resistance and additional (7 times) selections may not have been sufficient to cause a significant shift in resistance. Genetic inheritance of abamectin resistance in Altınova population has not been determined in present study, however, heritability of inheritance may have affected low level increase in resistance. The inheritance of abamectin resistance in the same species was reported to be incompletely recessive and response to laboratory selection for resistance against abamectin was slow (He et al. 2009). The resistance ratio was increased from 13 to 1597 fold in *T. urticae* California populations after selection with abamectin for 38 times in 9 months (Campos et al. 1995). Sato et al. (2005) reported abamectin selections on *T. urticae* Brazil populations caused an increase of the resistance ratio up to 342 fold. *T. urticae* population collected from greenhouse in Gazipaşa (Antalya-Turkey) were selected for 15 times and resistance increased to 35 fold in the selected population. Furthermore, abamectin-resistant population showed multiple-resistance to chlorpyrifos, propargite, clofentezine and fenpyroximate in the same study (Yorulmaz and Ay 2009).

Resistance to abamectin significantly decreased from 643 to 11 fold in Altınova population maintained without exposure to pesticides for 20 months (~60 generation). Additionally the LC_{90} value of Altınova population after 20 month was lower than recommended field dose of abamectin (Table 3).These results suggest abamectin resistance may revert in *T. urticae* Altınova (Antalya) population. Similarly, Sato et al. (2005) reported that abamectin resistance was unstable in *T. urticae* without selection pressures in Brazil. According to laboratory results from present study, resistance level of Altınova *T. urticae* population to abamectin decreased to a point that management using abamectin could be effective. The inheritance of abamectin resistance in *T. cinnabarinus* was detected as incompletely recessive (He et al. 2009). This genetic inheritance trait was compatible with unstability of abamectin resistance whithout pesticide pressure in present study.

Abamectin-resistant Altınova (original) population has showed 10 fold multiple-resistance to dicofol. Selection with abamectin for 7 times in Altınova population has resulted in significant increase in multiple-resistance ratio against dicofol from 10 to 173 fold.

Although, resistance to abamectin has significantly decreased, resistance to dicofol has not declined in Altınova population. This result suggested dicofol resistance was stable at least 5 month in *T. urticae* Altınova population. Dicofol is not suitable alternative for resistance management programme with abamectin. The dicofol has been abandoned in Turkey in 2011. The results support revoking the dicofol for resistance management in spider mites. However, dicofol is still recommended against spider mites in USA (UC-IPM 2015).

Abamectin-resistant Altınova (original) population showed insignificant multiple-resistance to tetradifon. Tetradifon may be an alternative acaricide for resistance management promramme against spider mites, however, this active ingredient was also abondaned in Turkey in 2011.

The laboratory test results indicated that spinosad has significantly affected the *T. urticae* larvae. Mortalities at recommended dose (144 mg a. i. l^{-1}, ~144 ppm) and at 1/10th (14.4 mg a. i. l^{-1}, ~14.4 ppm) of that were found to be 100 and 72 %, respectively, in 7-days of exposure (Table 5). A mortality of 89 % was also found after the 6 days exposure to residues of the spinosad (Table 5). An acaricidal effect of spinosad was reported where spinosad showed high efficacy to *T. urticae* population but low efficacy to *Panonychus ulmi* (Koch) (Villanueva and Walgenbach 2006). In this study, mortality ratios of larve in *T. urticae* laboratory population were 86, 92, 95 and 98 % at doses of 25, 55, 121 and 266 ppm, respectively, in bioassays using spinosad-treated leaf disks for 8-days of exposure. In addition, mortality of adult females of *T. urticae* were 63, 72, 76 and 81 % at doses of 25, 55, 121 and 266 ppm, respectively, after 4-days of exposure (Villanueva and Walgenbach, 2006). These studies suggested that spinosad had significant potential acaricidal effect on *T. urticae* populations. In the present study, the high level effect of spinosad on abamectin-resistant *T. urticae* population suggested that spinosad may be an alternative chemical in resistance management programme for *T. urticae* after field trials.

A high level of abamectin resistance was documented in the *T. urticae* population from Antalya, Turkey. A risk for further increase in resistance to abamectin and dicofol in abamectin-selected population was found. Significant decrease in level of resistance to abamectin in the field population after 20 months (~60 generations) without pesticides suggest that useful life of this acaricide may be increased if it is ceased in certain period in locations where abamectin resistance appears as a significant problem.

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