

Multiple resistance, detoxifying enzyme activity, and inheritance of abamectin resistance in *Tetranychus urticae* Koch (Acarina: Tetranychidae)

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Abstract: The present study investigated the biochemical mechanism of abamectin resistance in a *Tetranychus urticae* Koch population collected from a bean greenhouse (BEYO 2) in Beyobası village in Gazipaşa district, Antalya province, and maintained in the laboratory. $LC_{50, 60, 90}$ levels of BEYO 2 population of *T. urticae* were determined using a dry film method. The LC_{50} level of the BEYO 2 strain with abamectin was selected 15 times and was increased from 2.42 $\mu\text{L } 100 \text{ mL}^{-1}$ water to 38.67 $\mu\text{L } 100 \text{ mL}^{-1}$ water. A selected strain showing 35.05-fold resistance was named ABA 15 strain. It was investigated whether the resistant strain developed multiple resistance to different pesticide groups. The ABA 15 strain with abamectin resistance developed multiple resistance to chlorpyrifos, propargite, clofentezine, and fenpyroximate. The synergistic activity between abamectin and piperonyl butoxide (PBO), triphenyl phosphate (TPP), and S-benzyl-O,O-diisopropyl phosphorothioate (IBP) was studied in the resistant strain. Application of abamectin with synergists PBO, IBP, and TPP resulted in 1.76-, 2.43-, and 1.73-fold synergistic ratios, respectively, in the ABA 15 strain. The inheritance of resistance to abamectin of F1 females after reciprocal crosses between resistant and susceptible strains was maternal and paternal incompletely dominant. GSS (susceptible strain), BEYO 2, and ABA 15 strains were investigated in terms of the enzyme activities of esterase, glutathione S-transferase (GST), and monooxygenase (P450). No significant change was determined in esterase enzyme activities of the ABA 15 resistance strain to abamectin. The band density of esterase enzyme increased in the electrophoretic method. GST enzyme activity increased from 10.23 $\text{mOD min}^{-1} \text{mg}^{-1}$ protein to 12.36 $\text{mOD min}^{-1} \text{mg}^{-1}$ protein. The P450 enzyme activity was raised from 0.0017 $\text{mOD min}^{-1} \text{mg}^{-1}$ protein to 0.0039 $\text{mOD min}^{-1} \text{mg}^{-1}$ protein.

Key words: *Tetranychus urticae*, abamectin, inheritance, synergist, multiple resistance, detoxification enzymes

Abamectin'e dirençli *Tetranychus urticae* Koch (Acarina: Tetranychidae)'de çoklu direnç, detoksifikasyon enzimlerinin aktivitesi ve kalıtım

Özet: Bu çalışmada Antalya ili Gazipaşa ilçesi Beyobası'nda bulunan fasulye serasından toplanılarak laboratuvar koşullarında yetiştirilen *Tetranychus urticae* Koch popülasyonundan (BEYO 2) geliştirilen abamectin dirençli popülasyonda abamectin direncinin biokimyasal mekanizması belirlenmiştir. *Tetranychus urticae*'nin BEYO 2 ırkının $LC_{50,60,90}$ değerleri kuru film yöntemi ile belirlenmiştir. Abamectin ile 15 kez selekte edilen BEYO 2 popülasyonunun LC_{50} değeri 2.42 $\mu\text{L } 100 \text{ mL}^{-1}$ su'dan 38.67 $\mu\text{L } 100 \text{ mL}^{-1}$ su'ya yükselmiştir. Seleksiyon sonucu abamectin'e 35.05 kat abamectin direnç geliştiren popülasyonda ABA 15 ırkı olarak adlandırılmıştır. Dirençli ırkın farklı gruptan ilaçlara karşı çoklu direnç geliştirip geliştirmedeği incelenmiştir. Abamectin dirençli ABA 15 ırkı chlorpyrifos, propargite, clofentezine ve

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fenpyroximate karşı çoklu direnç geliştirmiştir. Ayrıca dirençli ırkta piperonyl butoxide (PBO), triphenyl phosphate (TPP) ve S-Benzyl-O,O-diisopropyl phosphorothioate (IBP) sinerjistlerinin abamectin ile birlikte etkinlikleri incelenmiştir. ABA 15 ırkında PBO, IBP ve TPP sinerjistleri ve abamectinin birlikte uygulanması sonucu 1.76, 2.43 ve 1.73 kat sinerjistik oranı elde edilmiştir. Dirençli ve hassas ırkı arasında yapılan resiprokal çaprazlamalar sonucu elde edilen F1 dişilerinde abamectin direncinin anne ve babadan eşik dominant olarak taşındığı belirlenmiştir. GSS (hassas populasyon), BEYO 2 ve ABA 15 ırkında esterase, glutathione S-transferase (GST) ve monooxygenase (P450) enzim aktiviteleri de incelenmiştir. ABA 15 ırkında esterez enzim aktivitesinde önemli bir değişiklik belirlenmemiştir. Ancak elektrofoterik yöntemde esterez enziminin bant yoğunluğunda artış gözlenmiştir. GST enzim aktivitesi 10.23 mOD min⁻¹ mg⁻¹ protein'den 12.36 mOD min⁻¹ mg⁻¹ protein'e yükselmiştir. P450 enzim aktivitesi 0.0017 mOD min⁻¹ mg⁻¹ protein'den 0.0039 mOD min⁻¹ mg⁻¹ protein'e artmıştır.

Anahtar sözcükler: *Tetranychus urticae*, abamectin, kalıtım, sinerjist, çoklu-direnç, detoksifikasyon enzimleri

Introduction

Tetranychus urticae Koch is one of the most polyphagous spider mite species and is a major pest in many cropping systems worldwide (Nauen et al., 2001). *Tetranychus urticae* can increase population density very rapidly in suitable host plant and climate conditions. *Tetranychus urticae* outbreaks in agricultural ecosystems result in large economic loss, particularly in greenhouses where mite populations can reach very high densities, due to the favorable temperature and availability of good quality food throughout the year (Tsagkarakou et al., 1999).

Acaricides have been widely used for mite control in glasshouses, orchards, and many other cropping systems (Van Leeuwen et al., 2006). Their high reproductive potential and extremely short life cycle, combined with the frequent applications of acaricides usually required to maintain mite populations below economic thresholds, facilitate the development of resistance in this species (Stumpf et al., 2001).

Abamectin (avermectin B₁) is the major fermentation component of avermectins derived from a soil actinomycete (*Streptomyces avermitilis*) and has strong insecticidal, nematocidal, and acaricidal activity (Putter et al., 1981; Wang and Wu, 2007). Abamectin was registered in 1981 and has been used to control *T. urticae* on many crops in Turkey (Ay et al., 2005). Abamectin is used to control insect and mite pests of a range of agronomic, fruit, vegetable, and ornamental crops and for the control of fire ants (Andrei, 2005; Ismail et al., 2007). It was reported in various previous studies that *T. urticae* developed resistance to abamectin (Campos et al., 1995; Campos et al., 1996; Stumpf and Nauen, 2002). Wang and Wu (2007)

found cross-resistance to imidacloprid and emamectin in an abamectin resistant *Bemisia tabaci* (Gennadius) population.

This study was conducted to confirm the resistance strain, ABA 15, in the laboratory in comparison with a susceptible strain, GSS, and to calculate resistance ratios. To understand the risk of resistance and inheritance to abamectin in *T. urticae*, multiple resistance, reciprocal crosses, and resistance mechanisms were investigated. Additionally, synergistic effects and detoxifying esterase, glutathione S-transferase (GST) and cytochrome P450 monooxygenase (P450) enzyme activities in the susceptible and resistant strain of *T. urticae* were also examined, in order to understand the possible mechanisms of abamectin resistance.

Materials and methods

Mite populations

The original population of *Tetranychus urticae* was collected in June 2003, from a commercial bean (*Phaseolus vulgaris* L.) greenhouse in Antalya province, Turkey. This population was named the BEYO 2. After collection, *T. urticae* was reared continuously on pinto bean plants under laboratory conditions at 26 ± 2 °C, 60 ± 5% RH, and a 16 h photo-period. A susceptible strain (GSS) was obtained from the Rothamsted Experimental Station, Harpenden (England) in 2001 and has since been reared in laboratory conditions. The abamectin resistant (ABA 15) strain had been produced by choosing a field colony (the BEYO 2) with abamectin for 15 selections.

Toxicity tests

These tests were conducted based on the method described by Ay (2005). The acaricide used was a commercial formulation of abamectin (Agrimec 18 g L⁻¹). The prepared suspension of abamectin was sprayed on the internal surfaces of lids and bases of 60 mm diameter plastic petri dishes and allowed to dry for 30 min. For each application, 1 mL of suspension was sprayed on each base and lid pair by a Potter spray tower (Burckard Auto-Load, Rickmansworth, Herts., UK) at 1 kPa. Adult female mites (@ 30) were transferred to each dish using a hairbrush, and the dishes were closed and sealed with Parafilm to prevent the spider mites from escaping. Thereafter, the mites in the dish were kept at 26 ± 2 °C, 60 ± 5% RH, and a 16 h photoperiod for 24 h after treatment. Survival of individual mites was determined by touching each mite with a fine brush. Mites unable to walk at least a distance equivalent to their body length were considered dead. Mortality tests were done before each experiment to determine a range of concentration that caused approximately 10%-95% mortality. Each experiment was conducted using 3 replicates of 7 concentrations (plus a control of distilled water only). Pooled data were subjected to probit analysis (POLO PC) (LeOra software, 1994), and LC_{50,60 and 90} with 95% CL were estimated. The LC₅₀ values of the selection strain were compared to those of the susceptible strain (GSS). A resistance ratio (RR) was calculated according to the following formula: RR = LC₅₀ value of the resistant strain / LC₅₀ value of the GSS strain.

Selection for resistance

Females of the original population (BEYO 2) were selected for resistance to abamectin under laboratory conditions from September 2006 to June 2007. Selection experiments were conducted by modification of the method proposed by Yang et al. (2002). At least 400 adult female mites were transferred from the BEYO 2 population into petri dishes (40 mites/petri dish) treated with abamectin concentrations equal to the LC₆₀ for that cycle. After 24 h, surviving mites were transferred back to the plants, and the populations were allowed to regenerate. The next selection cycle was conducted 2

or 3 generations (approximately 15-20 days) later, when the populations had increased. A bioassay using the selection acaricide was periodically conducted on mite strains when the number of surviving mites changed in the selection petri dishes. The new LC₆₀ was determined and applied to each generation for selection pressure.

Synergism test

The effects of abamectin and synergists were tested using the methods of Kim et al. (2004). Mixed-function oxidase (MFO) and esterase inhibitor piperonyl butoxide (PBO) and other esterase inhibitors S-benzyl-O, O-diisopropyl phosphorothioate (IBP), and triphenyl phosphate (TPP) were used to inhibit detoxification enzymes (Stumpf and Nauen, 2002; Kim et al., 2004; Young et al., 2005; Kang et al., 2006; Kim et al., 2006; Van Leeuwen and Tirry, 2007; Wang and Wu, 2007). Synergists were dissolved in acetone: distilled water (1:1) and 1 mL of suspension sprayed on the base and lid of petri dishes in the same manner as in the toxicity test, 30 min prior to the acaricide application. Only acetone + distilled water were applied to the control. Synergist solutions were prepared at the following concentrations: (mg L⁻¹): PBO (500), IBP (400), and TPP (125). A synergistic ratio (SR) was calculated using the following formula: SR = LC₅₀ of abamectin without synergist / LC₅₀ of abamectin with synergist.

Multiple resistance

Multiple resistance relationships between abamectin and 6 other acaricides were evaluated on both the selected and the susceptible strain of *T. urticae*. The pesticides were fenpyroximate (Meteor[®] (SC) 50 g L⁻¹), amitraz (Kortraz 20 EC 200 g L⁻¹), propargite (Komite EC 588 g L⁻¹), clofentezine (Apollo SC 500 g L⁻¹), chlorpyrifos (Dursban 4 EC 480 g L⁻¹), and bifenthrin (Talstar EC 100 g L⁻¹). All acaricides were commercially available in Turkey. The bioassay method used for all acaricides was the same as described previously for the toxicity test. The mortality data of each acaricide for the susceptible strain GSS and the selected resistant strain of *T. urticae* were subjected to probit analysis (POLO PC) (LeOra software, 1994). The multiple resistance

relationships between each acaricide and abamectin were analyzed based on whether or not there was overlap of 95% confidential intervals of LC_{50} values, estimated for each acaricide for the susceptible and resistant strains. The resistance ratio was calculated by division of LC_{50} of the resistant strain by the LC_{50} of the susceptible strain.

Crossing experiment

To estimate the dominance of resistance, the GSS and the abamectin resistant strain ABA 15 were reciprocally crossed to produce hybrid F1 females by placing 15 female teleiochrysalis of one strain and 30 adult males of the other strain on the upper side of a primary bean leaf, which was then placed on wet cotton in a petri dish. Directly after molting, the diploid females were fertilized by the haploid males, and 1 day later they began to lay eggs. Both males and females were removed after 5 days. If mating was successful, the haploid-diploid mating system resulted in F1 females. Resulting F1 females were then transferred to bean plants that were free of mites. The bioassay experiment was started when F1 females reached the adult stage. The bioassay method was the same as described previously in the toxicity test. The experiment was conducted using 3 replicates of 7 serially diluted concentrations (plus a control of distilled water only) covering the range of 10%-90% mortality.

The degree of dominance (D) of the resistance trait in the F1 females from both reciprocal crosses was estimated using the formula

$$D = (2X_2 - X_1 - X_3) / (X_1 - X_3)$$

where X_1 is the log of the LC_{50} of the resistant strain, X_2 is the log of the LC_{50} of the F₁ females, and X_3 is the log of the LC_{50} of the susceptible strain (Stone, 1968). This formula gives a value of -1 if resistance is fully recessive, a value of 0 if there is no dominance, and a value of 1 if resistance is fully dominant.

Biochemical assays

Esterase enzyme activity was detected by both gel electrophoresis and microplate reader methods, while glutathione S-transferase (GST) and monooxygenase (P450) activity was detected only by the microplate reader method. Sometimes one of the esterase bands and other times total esterase played a role in resistance. Thus, the activity of esterase enzyme was determined by using 2 methods.

Electrophoresis

Vertical slab polyacrylamide gel electrophoresis was performed following previously reported procedures by Walker (1994) and Goka and Takafuji (1992). The gels were 1 mm thick and 80 mm × 80 mm in area. Acrylamide concentrations were 7.5% in separating gels and 3.5% in stacking gels. Adult female mites were homogenized individually in 10 μL of 32% (w v⁻¹) sucrose with 0.1% Triton X-100 in well microplates by a multiple homogenizer. Electrophoresis was carried out at a constant current of 150 V at 5-8 °C for about 1.5 h. For esterase activity, gel was incubated for 30 min in a 0.02% (w v⁻¹) solution of a-naphthyl acetate in 0.2 M phosphate buffer (pH 6.5), which contained 1% acetone. Gel was then stained by placing in 0.4 (w v⁻¹) fast blue BB salt for 1 h. Placing the gel in 7.5% acetic acid stopped all staining reactions.

Photometric esterase assay

Esterase assays were performed according to Stumpf and Nauen (2002). The 10,000 g supernatant of mass homogenates of 100 adult females prepared in 500 μL of ice-cold 0.1 M sodium phosphate buffer, pH 7.5, containing 0.1% (w v⁻¹) Triton X-100, was diluted 10-fold and used as the enzyme source. Twenty-five microliter aliquots (0.5 mite equivalent) were added to the wells of a 96-well microplate, containing 25 μL of 0.2 M sodium phosphate buffer, pH 6.0. Wells with buffer-only served as a control for the non-enzymatic reaction. The assay was started by adding 200 μL of substrate solution to each well, giving a final volume of 250 μL. Substrate solution consisted of 15 mg of fast Blue RR salt dissolved in 25 μL of sodium phosphate buffer, pH 6.0, and 250 μL of 100 mM 1-naphthyl acetate in acetone. The esterase activity was measured continuously at 450 nm and 25 °C in a Versamax kinetic microplate reader (Molecular Devices) for 10 min, and analyzed utilizing Softmax PRO software to fit kinetics plots by linear regression.

Photometric glutathione S-transferase assay using 1-chloro-2,4-dinitrobenzene

Glutathione S-transferase activities were performed according to Stumpf and Nauen (2002). GST activity was determined using 1-chloro-2,4-dinitrobenzene and reduced glutathione (GSH) as substrate. One hundred adult females were

homogenized in 1000 μL of Tris-HCl (0.05 M, pH 7.5). The total reaction volume per well microplate was 300 μL , consisting of 100 μL of each supernatant (10,000 g, 5 min), CDNB (containing 0.1% (v v⁻¹) ethanol), and GSH in Tris-HCl (0.05 M, pH 7.5), giving final concentrations of 0.4 mM CDNB and 4 mM GSH. The change in absorbance was measured continuously for 5 min at 340 nm and 25 °C using the Versamax kinetic microplate reader (Molecular Devices). The nonenzymatic reaction of CDNB with GSH measured without homogenate served as a control.

Photometric monooxygenase assay using the *O*-demethoxylation of *p*-nitroanisole

Assays examining the *O*-demethoxylation of *p*-nitroanisole (PNOD) by cytochrome P450 monooxygenase were conducted using the procedures developed by Rose et al. (1995). For the PNOD assay, 100 adult females were homogenized on ice in 200 μL , in homogenization buffer (0.05 M Tris - HCl + 1.15% KCl + 1 mM EDTA, pH (7.7)). The homogenate was centrifuged at 4 °C, 20,000 $\times g$ for 20 min. Then 100 μL of 2 mM *p*-nitroanisole solution, 45 μL of enzyme, and 45 μL of homogenization buffer were added to each well. The microplate was incubated for 5 min at 30 °C and the reaction initiated by the addition of 10 μL of 9.6 mM NADPH. The absorbance was read in the Versamax kinetic microplate reader (Molecular Devices) at 405 nm and 30 °C for 15 min.

The activity of all enzymes was analyzed by Softmax PRO software and presented as $\text{mOD min}^{-1} \text{mg}^{-1}$ proteins. The data were analyzed using the general linear model (GLM) procedure of SAS (1999) by using strains in the model and PDIFF statement was used to compare the strains' enzyme activity means for dependent variables. An alpha level of 0.05 was regarded as significant.

Results

Selection for resistance

The ABA 15 strain of *Tetranychus urticae* was analyzed for abamectin in terms of the resistance. After 15 selections for abamectin resistance, the LC_{50} of the BEYO 2 strain increased from 2.42 $\mu\text{L 100 mL}^{-1}$ water to 38.67 $\mu\text{L 100 mL}^{-1}$ water (Table 1). LC_{60} and

LC_{90} values increased depending on the number of selections. After 15 selections with abamectin, 35.05-fold resistance was observed and the strain was named the "ABA 15" strain.

Multiple resistance

The toxicity of 6 pesticides to the susceptible strain GSS and the resistant strains ABA 15 of *T. urticae* was examined using the petri dish-spray tower. The activities of the 6 different pesticides against both strains of *T. urticae* are shown in Table 2. The ABA 15 strain showed multiple resistance to clofentezine, chlorpyrifos, fenpyroximate, and propargite. No resistance was detected for the selective acaricide amitraz and insecticide/acaricide bifenthrin.

Synergistic effects

In order to gain information on the abamectin resistance mechanisms, synergists such as PBO (MFO and esterase inhibitor), IBP, and TPP (esterase inhibitors) were used for the bioassay (Table 3). Treatment with IBP exhibited a markedly higher level of synergism to abamectin in the ABA 15 strain (SR = 2.43). Treatment with PBO and TPP resulted in 1.76- and 1.73-fold increased synergisms respectively in the ABA 15 strain. In comparison, 2.02-, 1.74-, and 2.09-fold synergism by PBO, IBP, and TPP was determined in the susceptible strain, GSS (Table 3).

Mode of inheritance of abamectin resistance

In an attempt to estimate the dominance of the resistance, individuals of the susceptible (GSS) and resistant (ABA 15) strains were reciprocally crossed to produce hybrid F1 females. The response of these F1 females showed that the resistance trait was inherited from both the maternal and paternal line and was incompletely dominant (Table 4). The dominance value was found to be 0.84 in the ABA 15♀ \times GSS♂ crossed F1 offspring and 0.82 in the GSS♀ \times ABA 15♂ crossed F1 offspring.

Detoxifying enzyme activity

The activity of esterase, GST and P450 enzymes was analyzed in susceptible strain GSS, parental strain BEYO 2, and the resistant strain ABA 15 selected with abamectin. While esterase enzyme activity was detected by gel electrophoresis and microplate reader methods, GST and P450 enzyme activity was detected only by the microplate reader

Table 1. Resistance ratio and LC₅₀ levels determined after selection with abamectin from the BEYO 2 and GSS strains (µL formulation 100 mL⁻¹ of distilled water).

Strains	n ^a	Slope ± SE (0.95 CI ^b)	LC ₅₀ (µL 100 mL ⁻¹) (0.95 CI ^b)	LC ₆₀ (µL 100 mL ⁻¹) (0.95 CI ^b)	LC ₉₀ (µL 100 mL ⁻¹) LC ₅₀ ^c	RR
BEYO 2	718	0.952 ± 0.098	2.42 (1.80-3.21)	4.47 (3.37-6.16)	53.86 (30.95-120.00)	2.19
select 1	727	1.059 ± 0.103	3.51 (2.67-4.60)	6.09 (4.65-8.28)	57.05 (34.59-115.47)	3.18
select 2	715	1.073 ± 0.101	3.67 (2.83-4.75)	6.40 (4.89-8.48)	57.55 (35.51-112.84)	3.33
select 3	721	1.074 ± 0.102	5.62 (4.34-7.34)	9.68 (7.42-13.25)	87.86 (52.88-179.41)	5.10
select 4	721	1.036 ± 0.102	6.25 (4.77-8.23)	10.98 (8.34-15.19)	108.04 (63.50-230.20)	5.67
select 5	727	1.074 ± 0.101	7.04 (5.44-9.11)	12.12 (9.36-16.30)	109.89 (67.57-216.70)	6.38
select 6	720	1.231 ± 0.106	9.93 (7.88-12.40)	15.95 (13.16-19.66)	109.20 (73.33-186.65)	9.00
select 7	726	1.100 ± 0.101	9.74 (7.55-12.46)	16.56 (12.93-21.78)	142.54 (90.76-265.56)	9.00
select 8	726	1.086 ± 0.098	11.79 (9.26-14.98)	20.18 (15.86-26.49)	178.41 (113.01-334.46)	10.69
select 9	730	1.086 ± 0.101	14.45 (11.15-18.57)	24.74 (19.24-32.70)	219.04 (138.11-415.13)	13.10
select 10	728	1.091 ± 0.102	19.33 (14.94-24.92)	33.00 (25.58-44.02)	289.06 (180.29-557.54)	17.53
select 11	723	1.004 ± 0.096	21.40 (16.51-27.69)	38.25 (29.49-51.67)	404.07 (241.04-837.83)	19.40
select 12	722	1.030 ± 0.097	24.56 (19.03-31.59)	43.28 (33.59-57.81)	431.00 (262.84-861.49)	22.27
select 13	721	1.081 ± 0.098	27.53 (21.53-35.00)	47.21 (37.08-61.95)	421.64 (266.90-792.25)	24.96
select 14	725	1.133 ± 0.104	34.98 (27.23-44.66)	58.54 (45.83-76.87)	472.97 (303.86-869.44)	31.71
ABA 15	723	1.339 ± 0.102	38.67 (29.90-49.67)	65.75 (51.15-87.11)	567.20 (357.58-1075.31)	35.05
GSS	721	1.098 ± 0.098	1.10 (0.86-1.40)	1.87 (1.47-2.467)	16.19 (10.25-30.36)	-

^a Total number of mites used, ^b Confidence Limit, ^c Resistance ratio = LC₅₀ value of resistance strain / LC₅₀ value of the GSS strain

Table 2. Toxicity of 6 acaricides to the susceptible (GSS) and abamectin resistant (ABA 15) strains of *Tetranychus urticae*.

Chemical	Strain	n ^a	Slope ± se	LC ₅₀ (µL 100 mL ⁻¹) (0.95 CI ^b)	LC ₉₀ (µL 100 mL ⁻¹) (0.95 CI ^b)	RR ^c
Chlorpyrifos	GSS	720	1.772 ± 0.135	2.59 (2.13-3.08)	13.70 (10.92-18.18)	-
	ABA 15	721	1.341 ± 0.111	8.41 (5.98-11.33)	75.89 (49.07-143.83)	3.24
Amitraz	GSS	724	1.508 ± 0.114	30.03 (24.63-36.20)	212.67 (160.27-304.96)	-
	ABA 15	722	1.258 ± 0.108	31.13 (24.50-38.89)	324.93 (226.44-525.27)	1.03
Propargite	GSS	724	1.214 ± 0.101	12.75 (10.17-15.82)	144.89 (99.71-238.14)	-
	ABA 15	723	1.329 ± 0.107	27.16 (22.00-33.24)	250.20 (178.76-388.49)	2.12
Clofentezine	GSS	716	1.387 ± 0.108	2.86 (2.32-3.47)	24.02 (17.75-35.479)	-
	ABA 15	724	1.482 ± 0.121	9.88 (6.34-14.37)	72.44 (43.74-165.57)	3.45
Fenoxypriate	GSS	720	1.349 ± 0.104	6.61 (5.42-7.98)	59.01 (43.201-88.37)	-
	ABA 15	725	1.282 ± 0.113	17.23 (13.40-21.71)	172.35 (119.90-280.11)	2.60
Bifenthrin	GSS	527	1.493 ± 0.154	54.53 (34.03-85.54)	393.62 (206.87-1426.58)	-
	ABA 15	725	1.216 ± 0.107	45.73 (35.99-57.55)	518.34 (348.96-884.22)	< 1

^a Total number of mites used, ^b Confidence interval, ^c Resistance ratio, LC₅₀ for the ABA 15 strain / LC₅₀ for the GSS strain

Table 3. Toxicity of abamectin with and without synergist to susceptible, GSS and abamectin-resistant ABA 15 strains of *Tetranychus urticae* (μL of formulation 100 mL^{-1} of distilled water).

Treatment	n ^a	Slope \pm SE	LC ₅₀ ($\mu\text{L } 100 \text{ mL}^{-1}$) (0.95 CI ^b)	LC ₉₀ ($\mu\text{L } 100 \text{ mL}^{-1}$) (0.95 CI ^b)	SR ^c
ABA 15 strain					
Abamectin only	723	1.099 \pm 0.102	38.67 (29.90-49.67)	567.20 (357.58-1075.31)	-
Abamectin + PBO	723	1.382 \pm 0.113	21.93 (17.47-27.00)	185.68 (135.97-278.31)	1.76
Abamectin + IBP	720	1.333 \pm 0.109	15.90 (11.60-20.89)	145.57 (97.85-256.43)	2.43
Abamectin + TPP	723	1.519 \pm 0.110	22.30 (17.83-27.41)	194.22 (141.62-292.77)	1.73
GSS strain					
Abamectin only	721	1.098 \pm 0.098	1.10 (0.86-1.40)	16.19 (10.259-30.363)	-
Abamectin + PBO	721	1.522 \pm 0.121	0.54 (0.40-0.70)	3.78 (2.62-6.33)	2.02
Abamectin + IBP	720	1.183 \pm 0.104	0.63 (0.49-0.80)	7.66 (5.17-13.01)	1.74
Abamectin + TPP	724	1.487 \pm 0.120	0.52 (0.34-0.75)	3.83 (2.37-8.20)	2.09

^aTotal number of mites used, ^bConfidence interval, ^cSynergistic ratio: LC₅₀ for abamectin alone / LC₅₀ for abamectin with synergist

Table 4. LC₅₀ values for abamectin against adult females of the susceptible strain GSS and abamectin resistant strain ABA 15 of *Tetranychus urticae* and their reciprocal crosses (μL of formulation 100 mL^{-1} of distilled water).

Strain	N ^a	Slope \pm se	LC ₅₀ ($\mu\text{L } 100 \text{ mL}^{-1}$) (0.95 CI)	RR ^b	D ^c
ABA 15	723	1.339 \pm 0.102	38.67 (29.90-49.67)	35.05	-
GSS	721	1.098 \pm 0.098	1.10 (0.86-1.40)	-	-
F1 (ABA 15♀ \times GSS ♂)	724	1.223 \pm 0.107	29.372 (23.05-36.91)	26.62	0.84
F1 (GSS ♀ \times ABA 15♂)	724	1.288 \pm 0.108	28.56 (22.74-35.44)	25.90	0.82

^aTotal number of mites used, ^bRR Resistance ratio, ^cD Degree of dominance

method. There was no significant difference in esterase activity between the parental BEYO 2 and the resistant ABA 15 strains; however, the increase in the esterase enzyme activity of the resistant ABA 15 strain was significantly higher than the susceptible GSS strain ($P < 0.05$) (Table 5). In addition, the band intensity of esterase enzyme was increased in ABA 15 in the electrophoresis method, compared to the parental strain BEYO 2 (Figure 1). In particular, the density of the Est 3 band was markedly increased in the ABA 15 resistant individuals. GST enzyme activity increased from $10.23 \text{ mOD min}^{-1} \text{ mg}^{-1}$ protein to $12.36 \text{ mOD min}^{-1} \text{ mg}^{-1}$ protein after selection (Table 5). However, the GST activity of the susceptible GSS strain was found to be significantly

higher than the abamectin resistant ABA 15 resistant strain ($P < 0.05$). P450 enzyme activity rose from $0.0017 \text{ mOD min}^{-1} \text{ mg}^{-1}$ protein to $0.0039 \text{ mOD min}^{-1} \text{ mg}^{-1}$ protein (Table 5). The P450 activity was significantly higher in the resistant ABA 15 strain than in both the parental BEYO 2 strain and the susceptible GSS strain ($P < 0.05$).

Discussion

It seems that resistance developed rapidly in spider mites exposed to the insecticides used in the chemical control. Van Leeuwen and Tirry (2007) reported that *T. urticae* developed resistance against most of the chemicals used.

Table 5. Esterase, GST and P450 activities in susceptible strain GSS, parental strain BEYO 2 and abamectin resistant strain ABA 15 of *Tetranychus urticae* ($P < 0.05$)^a.

Enzyme	Strain	n ^b	Specific activity (\pm SE) mOD min ⁻¹ mg ⁻¹ proteins	R/S ^c
Esterase	GSS	4	10.35 (\pm 0.35) b	-
	BEYO 2	4	13.72 (\pm 0.30) a	1.32
	ABA 15	3	14.33 (\pm 0.35) a	1.38
GST	GSS	5	13.73 (\pm 0.32) a	-
	BEYO 2	5	10.23 (\pm 0.25) c	0.74
	ABA 15	5	12.36 (\pm 0.25) b	0.90
P450	GSS	3	0.0016 (\pm 0.0006) b	-
	BEYO 2	3	0.0017 (\pm 0.0006) b	1.06
	ABA 15	4	0.0039 (\pm 0.0005) a	2.43

^a: Means with different letters in column for each enzyme are significantly different ($P < 0.05$), ^b: number of replicates, ^c: enzyme activity BEYO 2 or ABA 15 / enzyme activity GSS strain.

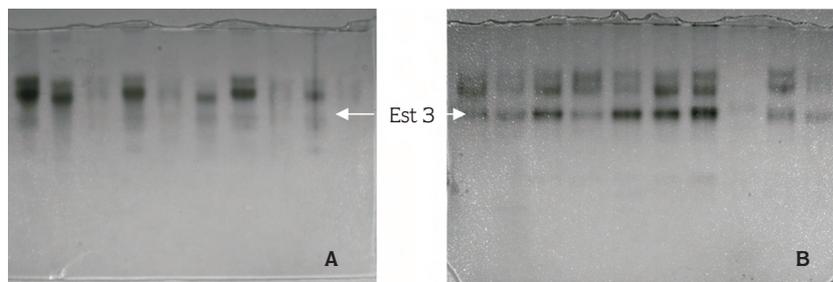


Figure 1. Esterase zones in the different strains of the *Tetranychus urticae* individuals (A, esterase zones of the BEYO 2 strain and B, esterase zones of the ABA 15 strain).

The resistance development in the BEYO 2 increased according to the number of selections. The strain BEYO 2, selected 15 times with abamectin, has a resistance ratio varying between 2.19- and 35.05-fold. The abamectin resistant ABA 15 strain developed multiple resistance against chlorpyrifos, propargite, clofentezine and fenpyroximate, 3.24-, 2.12-, 3.45-, and 2.60-fold, respectively. However, multiple resistance to amitraz and bifenthrin was not observed. Sato et al. (2005) reported that *T. urticae* populations developed 342-fold resistance to abamectin after 5 selections and that, in the resistant population, the cross resistance was determined against milbectin 16.3-, fenpropathrin 3.20-, and

chlorfenapyr 2.23-fold; no cross resistance was determined against fenpyroximate, cyhexatin, propargite, or dimethoate. Scot et al. (1991), working with house flies (*Musca domesticate* L.), reported that there was no increase in (cross) resistance to crotoxyphos, dichlorvos, dimethoate, tetrachlorvinphos, permethrin, dieldrin, or lindane following abamectin selection. No cross resistance of METI resistant strain AKITA could be found between abamectin and the ovo-larvicide hexythiazox and clofentezine (Nauen et al., 2001). Kim et al. (2004) found the fenpyroximate resistant strain showed moderate levels of cross resistance to abamectin, fenbutatin oxide, fenpropathrin, pyridaben,

pyridaben + bifenthrin, and tebufenpyrad. The results of the present study led us to consider that the insecticides chlorpyrifos, propargite, clofentezine, and fenpyroximate should not be used in abamectin-resistant spider mite populations when its usage is not absolutely necessary. The reciprocal crossing was made to determine the inheritance manner of the resistance. The resistance in the F1 females was determined to be carried from mother and father with the incompletely dominant genes. Devine et al. (2001) reported that reciprocal crossing experiments between mitochondrial electron transport inhibitor acaricide resistance strain TUKA 4 and susceptible strain GSS showed that the resistance phenotype is incompletely dominant and capable of being inherited both paternally and maternally. Sato et al. (2005) found that resistance is unstable in the absence of abamectin selection pressure, under laboratory conditions. Abamectin resistance in 2 isogenic strains of the Colorado potato beetle was determined to be autosomal, incompletely recessive, and polygenic (Argentine et al., 1992).

The PBO, IBP, and TPP synergists have increased the effect of abamectin on ABA 15 and susceptible strains. No statistically significant differences were found between the esterase enzyme values of the resistant and the parental strains ($P > 0.05$). However, the Est 3 bands in the resistant strain showed greater density when compared to the parental strain; in addition, when the effect of esterase inhibitor synergists was taken into consideration, the Est 3 band might have an effect on abamectin resistance. The GST enzyme activity obtained with kinetic readings showed a statistically significant increase in the ABA 15 strain compared with the BEYO 2 strain, but the greatest GST activity was determined in the susceptible strain. The P450 enzyme showed a statistically significant increase in the resistant strain, compared with the susceptible and parental strain. The effect of P450 inhibitor PBO synergists led to the consideration that the P450 enzyme might have an

effect on the abamectin resistance. Stumpf and Nauen (2002) determined that the NL - 00 and COL - 00 strain of *T. urticae* showed abamectin resistance 1.6- and 1.6-fold esterase, 5.7- and 12.7-fold MFO, and 2- and 1.6-fold GST activity compared to the susceptible strain GSS. Although all 3 of the enzymes activities increased when compared to their parental strains, the GST enzyme activity of abamectin resistant ABA 15 was found to be lower than that of the susceptible GSS strain. According to the results obtained in the present study, the GST enzyme does not have a role in abamectin resistance in *T. urticae*. The increase in the esterase enzyme according to the microplate results was found to be insignificant. However, there are some important differences in the parental strain when compared to the resistant ABA 15 strain in terms of esterase enzyme bands obtained from polyacrylamide gel results. The density of the Est 3 band was increased. Wang and Wu (2007) showed that PBO synergists increased the effectiveness of abamectin, and that P450 and GST enzyme activities affect abamectin resistance in *B. tabaci*. The increase in the P450 enzyme in abamectin resistant strain ABA 15 was significant compared to the susceptible strain and the parental strain.

The results of our study indicate that the abamectin resistance has an incompletely dominant heritage characteristic. The enzymes P450 and the Est 3 band may play a role in the abamectin resistance mechanism in *Tetranychus urticae* Koch.

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