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Analysing resistance of different *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) strains to abamectin insecticide

[*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'nın farklı türlerinde abamektin insektisitine karşı dayanıklılığın analizi]

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ABSTRACT

Objective: *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), tomato leafminer, is an oligophagous insect. Larvae of *T. absoluta* can destroy especially tomato plants which lead to important yield loss in this economically valuable crop. Chemical control through insecticides has been a main method of controlling it in farming areas all over the world. However, continues application of certain registered insecticide such as abamectin might lead to resistance development in *T. absoluta*. The aim of this study was to monitor resistance status of abamectin insecticide and analyse resistance mechanisms of this insecticide in *T. absoluta* field populations from three districts of Turkey by using bioassay and biochemical methods.

Methods: Bioassays and Biochemical assays.

Results: Bioassay results showed that while Adana and Antalya strain of *T. absoluta* showed low resistance (3.03- and 2.3-fold) to abamectin insecticide, Ankara strain of *T. absoluta* was not resistant to abamectin (1.31-fold). Biochemical analysis displayed that CYP450-PNOD activities showed 2.55 and 1.95-fold increase compared to susceptible population in Adana and Antalya field populations, respectively. Furthermore, GST-CDNB activities showed statistically significant ($p<0.05$) 1.3-fold increase only in Adana population. Although EST-α-NA activities showed 3.41-fold increase only in Ankara field population, this field population did not display a significant resistancy to abamectin.

Conclusion: Consequently, cytochrome P450 monooxygenase enzymes seemed to have a major role in abamectin resistance development in field populations of *T. absoluta* from Turkey. In addition, GSTs possibly have supportive role such as reducing oxidative stress that developed during metabolism of abamectin in resistant field populations of *T. absoluta*.

Key Words: Abamectin, bioassay, CYP450 monooxygenases and glutathione S-transferases, *Tuta absoluta*.

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), domates güvesi, olifag bir böcektir. *T. absoluta*'nın larvaları özellikle domates bitkisine zarar verebilirler, böylece ekonomik olarak değerli bu üründe ciddi ürün kaybına neden olurlar. Tüm dünyadaki tarım alanlarında bu zararlının kontrolünde kullanılan ana yöntem insektisitler yoluyla yapılan kimyasal mücadeledir. Ancak, abamektin gibi ruhsatlı bazı ilaçların sürekli olarak kullanılması *T. absoluta*'da dayanıklılık gelişimine neden olabilmektedir. Bu çalışmanın amacı biyoanaliz ve biyokimyasal yöntemleri kullanarak Türkiye'nin üç farklı bölgesindeki *T. absoluta* tarla populasyonlarında abamektin dayanıklılığının tespiti ve bu dayanıklılığın mekanizmasını analiz etmektir.

Metod: Biyoanalizler ve Biyokimyasal analizler.

Bulgular: Biyoanaliz sonuçları *T. absoluta*'nın Adana ve Antalya türlerinde abamektin'e karşı düşük seviyede (3.03- ve 2.3-kat) dayanıklılık olduğunu gösterirken, Ankara türünde ise abamektin'e (1.31-kat) karşı dayanıklılık olmadığını gösterdi. Biyokimyasal analiz sonuçları ise CYP450-PNOD aktivitesinin hassas populasyona göre Adana ve Antalya tarla populasyonlarında sırasıyla 2.55 ve 1.95-kat arttığını göstermektedir. Ayrıca, GST-CDNB aktivitesinin sadece Adana tarla populasyonunda istatistiksel olarak anlamlı 1.3-kat artış gösterdi. EST-α-NA aktivitesi ise abamektine karşı dayanıklılık göstermeyen Ankara tarla populasyonunda 3.41-kat artış gösterdi.

Sonuç: Sonuç olarak, Türkiyedeki *T. absoluta* tarla populasyonlarında sitokrom P450 monooksijenaz enzimlerinin abamektine karşı dayanıklılık oluşumunda başlıca rolü oynadıkları görülmektedir. Ayrıca, abamektine dayanıklılık gösteren tarla populasyonlarında glutatyon S-transferazlar'ın abamektin'in metabolizması sırasında oluşan oksidatif stresin azaltılmasını sağlayarak dayanıklılık oluşumuna katkı sağlayan bir rolü olabilir.

Anahtar Kelimeler: Abamektin, biyoanaliz, CYP450 monooksijenazlar ve glutatyon S-transferazlar, *Tuta absoluta*.

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Tomato, *Lycopersicon esculentum* Mill., is one of the most important economical crops in Turkey. According to Turkish Statistical Institute, Turkey produced 11.350.000 tons tomato in 2012 [1]. Food and Agricultural Organisation (FAO) reported that Turkey is fourth largest producer of tomato in the world [2]. Tomato farming is generally carried out under greenhouses as well as in outdoor areas with the highest gross financial return to farmers in Turkey.

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), tomato leafminer, is a neotropical oligophagous insect that attacks solanaceous crops. Larvae of *T. absoluta* can destroy tomato plants during all growth stages by producing large galleries in tomato leaves, burrowing stalks, apical buds, green and ripe fruits [3,4]. The fruit borer activity of larva results in major economic impact. It can also cause important yield losses in different production regions and under diverse production systems [3,5]. Although it is originally described in Peru, it is widespread throughout South America [6-9]. It has become one of the important pests of tomato crops in many South American countries since 1960s [4,10,11]. Although it was one of the important pests of tomato, it was restricted to the Latin America. When it introduced in Europe in 2006 at the first time [12], it started to threat global tomato production in the world [13].

T. absoluta (Meyrick) (Lepidoptera: Gelechiidae) is one of the main pests of tomato in Turkey. In 2009, *Tuta absoluta* (Meyrick) was initially reported at Izmir (Aegean Region) and Çanakkale (Marmara Region) provinces in Turkey [14]. Then, it spread throughout the country. Furthermore, in 2010, there were damage reports from 39 provinces in Turkey [15]. In addition, Karut et al [16] reported that *T. absoluta* was recorded for the first time on 29th April 2010 in Mersin (Mediterranean region).

In order to control *T. absoluta* in agricultural lands, chemical insecticides have been used to control it in all farming areas. However, resistance development against applied insecticides has been reported by several studies [17-20]. Although there were not any registered insecticides for *T. absoluta* in Turkey until 2009. Now there are some registered insecticides such as abamectin used against it in Turkey. In 2010, chemical insecticides were applied for 160.680 da areas to control *T. absoluta* in Turkey. After that, integrated pest management program was planned for 11.569.728 da in 2011 [15]. However, it is difficult to achieve effective control of *T. absoluta* with insecticides because of the mine-feeding behaviour of its larvae, deficient spraying technology and resistance development.

One of the main important insecticide resistance development mechanisms is increasing insecticide metabolism in insect body with certain enzymatic systems like esterases (EST), glutathione S-transferases (GST) and cytochrome P-450 monooxygenases (CYP450). Biochemical activity assays are useful techniques for analysing these metabolic resistance development mechanisms at molecular level. Therefore, these assays were useful to analyse changes of EST, GST and CYP450 enzyme systems in *T. absoluta* under abamectin stress.

Bioassay is a method that use organism such as *T. absoluta* to estimate toxicity of selected insecticides on that organism. Determination base line toxicity of insecticides is important for insecticide resistance management and early detection of resistance development. Thus, resistance status of abamectin, registered and commonly used insecticide for *T. absoluta*, was analysed with this method.

In present study, we describe bioassay of abamectin insecticide together biochemical assays in order to determine toxicity of tested insecticide and characterize molecular mechanisms in three field populations of *T. absoluta* from Turkey.

Materials and Methods

Insects

T. absoluta field samples were obtained from *T. absoluta* infested tomato fields in Adana, Ankara and Antalya provinces during 2011-2012 years (Fig. 1). A susceptible strain

Figure 1. Exact location of the collection sites of *Tuta absoluta* Populations in Turkey Circles (O) indicate sampling locations.

of *T. absoluta* was obtained from Bayer CropScience (Monheim, Germany). Larvae were fed on tomato plants in the laboratory. Bioassays were done with L2 larva of *T. absoluta* (usually 150-180 individuals). In addition, whole adult larvae were used for cytosol preparation to obtain enzyme source for biochemical activity assays.

Bioassay method

Abamectin insecticide (Vertis 1.8 CS) was obtained from an agrochemical company of Turkey, Baydar Tarım. Bioassay experiments were done by using leaf-dip bioassay method with L2 larvae of *T. absoluta* according to IRAC susceptibility test method series, (version 3) method no: 022. The dilutions of abamectin insecticide were prepared with distilled water containing 0.2% Triton X-100 at different concentrations. These insecticide solutions were prepared in different doses from 0.14 to 4.5 ppm for abamectin insecticide. 0.2% Triton X-100 containing water solution was used as a control, as well. Firstly, tomato leaflets were taken from the young plants. Then, five to seven doses (0.14, 0.28, 0.56, 1.125, 2.25 and 4.5 ppm) of this insecticide and 20-30 larvae for each dose were used to estimate the lethal concentration 50% (LC_{50}) value. The tomato leaves after dipping into the prepared solutions for 3 seconds; they were dried on towel paper and placed into the petri dishes. Prior to placing the leaflets in the petri dishes, a slightly moistened filter paper was placed on the bottom of each cell. Finally, the larva was placed onto this leaves. After this application, bioassay trays were stored in an area where a temperature of 25±2°C, 60-65% room humidity and 16:8 light/dark photoperiod regime.

Data analysis

The dead or alive larva was scored after 72 hours. Larvae, unable to make coordinated movement from gentle stimulus with a seeking pin or fine pointed forceps to the posterior body segment, are considered to be "dead". The LC_{50} values were calculated by probit analysis using POLO PLUS software.

Resistance ratios (RRs) were calculated by dividing the LC50 value of each field strain by the LC_{50} value of the susceptible strain of *T. absoluta*. According to the calculated RRs that level of insecticide resistance was classified as reported by Torres-Vila et al [21]. Susceptible (RR=0-1), low resistance (RR=2-10), moderate resistance (RR=11-30), high resistance (RR=31-100).

Preparation of cytosols from Tuta absoluta larvae for CYP450, GST and esterase assays

Batches (25mg) of adult *T. absoluta* (≈10 larvae) were homogenized in 1 ml of 100 mM potassium phosphate buffer, pH 7.4, containing 1 mM DTT, 1 mM EDTA and 1 mM PMSF with an ultraturrax homogenizer on ice. The homogenate was centrifuged at 10,000 x g for 30 min at 4°C. The supernatant was used as the enzyme source for CYP450, GST and esterase assays. The protein concentrations in the prepared crude extracts were determined by the method of Bradford (1976) [22].

Determination of Cytochrome P450 monooxygenases activity towards p-nitro anisole (p-NA)

Assay of O-demethylation of p-nitroanisole (PNOD) activity by CYP450s was determined according to the method of Rose et al [23]. Reactions were carried out in 96 well micro plates by monitoring p-nitrophenol formation in a final volume of 200 µl at 405 nm using p-nitroanisole (p-NA) as a substrate at 30 s intervals for 15 min at 30 °C. Each reaction mixture contained 100 mM potassium phosphate buffer, pH: 7.4, containing 0.5 mM NADPH, 1 mM p-NA and 90 µg proteins in a final volume of 200 µl. The molar extinction coefficient for p-nitrophenol at 405 nm was determined by preparing standard curves and was used to calculate CYP450-PNOD activities as pmole/min/mg protein.

Determination of GST enzyme activity towards 1-chloro-2,4-dinitrobenzene (CDNB)

Glutathione S-transferase activity measurements were done by a modified Habig et al [24] method. 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate. Each reaction mixture contained 100 mM potassium phosphate buffer, pH: 7.4, 1 mM GSH, 1 mM CDNB and ≈ 2 µg cytosolic proteins in a final volume of 250 µl in 96 well plate at 340 nm wavelength. The reactions were started by the addition of enzyme-containing protein extracts into each well. GST-CDNB activity measurements were done automatically every 20 s for 10 min at 25 °C. Finally, the rate of reaction (dA/dt) in each well was determined separately. The extinction coefficient for CDNB at 340 nm is 9.6 $mM^{-1}cm^{-1}$. This value was adjusted for the path length of the solution in the well (0.65 cm) and used for calculation of GST-CDNB activities. The GST-CDNB activities were expressed as nmol/min/mg protein.

Determination of esterase activity towards α-naphthylacetate (α-NA)

Esterase-α-NA enzyme activity was determined in 96 well micro plates by monitoring α-naphthol formation in a final volume of 250 µl at 450 nm using α-naphthyl acetate (α-NA) as a substrate at 15 s intervals for 10 min according to the method of van Asperen [25]. Each reaction mixture contained 200 mM potassium phosphate buffer, pH: 6.0, containing 6 mg fast blue RR salt, 1 mM α-NA and 5 µg proteins in a final volume of 250 µl. The molar extinction coefficient for α-naphthol at 450 nm is 9.25 $mM^{-1}cm^{-1}$ [26] was used to calculate activities as nmol/min/mg protein.

Statistical analysis of enzyme activity assays

Similarity or difference between in measured CYP450, GST and esterase activities were calculated with student-t test by MINITAB 15.0 statistics software.

Results

Bioassay results

Results of abamectin insecticide bioassays with the Adana, Ankara and Antalya strains of *T. absoluta* are summarized in Table 1. LC_{50} values obtained from Adana, Ankara and Antalya strains were compared to that of susceptible strains to calculate resistance ratios.

Resistance ratio of tested abamectin insecticide in Adana field strain was 3.03-fold compared to susceptible population. Similarly, *T. absoluta* field population from Antalya showed 2.3-fold resistance ratio. However, a resistance ratio of tested abamectin insecticide was 1.31-fold in Ankara strain of *T. absoluta*. While Adana and Antalya strain of *T. absoluta* showed low resistance (3.03- and 2.3-fold) to abamectin insecticide, Ankara strain of *T. absoluta* was not resistant to abamectin insecticide (1.31-fold).

All the measurements for enzyme activities were performed with individually prepared *T. absoluta* samples. Enzyme activity determination experiments were done in triplicate. The mean of three measurements was accepted as a sample value for that assay. Accordingly, the average of sample values of that population for each assay was accepted as a population value.

CYP450-PNOD enzyme activities of Tuta absoluta strains

CYP450-PNOD activities of *T. absoluta* showed similar pattern in Adana and Antalya populations. CYP450-PNOD activities showed statistically significant ($p<0.05$) 2.55 and 1.95-fold increase compared to susceptible population in Adana and Antalya field populations, respectively (Table 2). However, Ankara field population did not show statistically significant ($p<0.05$) increase in CYP450-PNOD activities. The highest difference in measured CYP450-PNOD activities (2.55 fold) was observed in Adana population.

GST-CDNB enzyme activities of Tuta absoluta strains

In order to analyse GST enzyme systems in *T. absoluta* samples, CDNB, general substrate for GSTs, was used

Table 1. Toxicity of Abamectin Insecticide in Adana, Ankara and Antalya Strains of *Tuta absoluta*

Insecticide	Strains	Number of insect[a]	LC_{50}[b]	Slope±SE (95% CL)	Hetero-genity	Resistance Ratio[c]
Abamectin	TUTAB	150	0.342 (0.276-0.420)	3.102±0.47	0.55	–
	ANKARA	180	0.448 (0.294-0.684)	1.60±0.34	0.143	1.31
	ADANA	180	1.034 (0.781-1.359)	1.97±0.3	0.55	3.03
	ANTALYA	180	0.798 (0.260-1.556)	2.009±0.32	1.64	2.3

[a]Number of larvae used in the experiment; [b]ppm; [c]LC50 value of field population / LC50 value of susceptible population.
TUTAB: Susceptible Tuta absoluta strain obtained from Germany; ADANA: Field strain of Tuta absoluta collected from cotton fields in Adana province; ANKARA: Field strain of Tuta absoluta collected from cotton fields in Ankara province; ANTALYA: Field strain of Tuta absoluta collected from cotton fields in Antalya province.

Biochemical Activity Assays

Table 2. CYP450-PNOD Enzyme Activities of *Tuta absoluta* Strains

Strain	Sample size (n)	CYP450-PNOD Activity[a]	Fold increase IN CYP450-PNOD Activity[b]
TUTAB	26	78.2±20	1
Adana	22	199.4*±11.3	2.55-fold
Ankara	15	79.8±29.3	No significant change
Antalya	20	152.6*±5.4	1.95-fold

[a]pmole min^{-1} mg $protein^{-1}$ ±Standard Error of Mean; [b]CYP450-PNOD activity value of field strain / CYP450-PNOD activity value of susceptible strain; *Value significantly different from the susceptible strain ($p<0.05$) with Student-t test.
TUTAB: Susceptible Tuta absoluta strain obtained from Germany; ADANA: Field strain of Tuta absoluta collected from cotton fields in Adana province; ANKARA: Field strain of Tuta absoluta collected from cotton fields in Ankara province; ANTALYA: Field strain of Tuta absoluta collected from cotton fields in Antalya province.

Table 3. GST-CDNB Enzyme Activities of *Tuta absoluta* Strains

Strain	Sample size (n)	GST-CDNB Activity[a]	Fold increase in GST-CDNB Activity[b]
TUTAB	26	304.6± 12.3	1
Adana	22	389.5*±23.9	1.3-fold
Ankara	18	276.4±10.9	No significant change
Antalya	20	289.6±10.2	No significant change

[a]nmole min^{-1} mg $protein^{-1}$ ±Standard Error of Mean; [b]GST-CDNB activity value of field strain / GST-CDNB activity value of susceptible strain; *Value significantly different from the susceptible strain ($p<0.05$) with Student-t test.
TUTAB: Susceptible Tuta absoluta strain obtained from Germany; ADANA: Field strain of Tuta absoluta collected from cotton fields in Adana province; ANKARA: Field strain of Tuta absoluta collected from cotton fields in Ankara province; ANTALYA: Field strain of Tuta absoluta collected from cotton fields in Antalya province.

Table 4. EST-α-NA Enzyme Activities of *Tuta absoluta* Strains

Strain	Sample Size (n)	EST-α-NA Activity[a]	Fold increase IN EST-α-NA Activity[b]
TUTAB	26	25.61± 1.83	1
Adana	22	30.9±2.44	No significant change
Ankara	18	87.32*±7.46	3.41 fold
Antalya	20	22.53±0.68	No significant change

[a]nmole min^{-1} mg $protein^{-1}$ ±Standard Error of Mean; [b]EST-α-NA activity value of field Strain / EST-α-NA activity value of susceptible strain; *Value significantly different from the susceptible strain ($p<0.05$) with Student-t test.
TUTAB: Susceptible Tuta absoluta strain obtained from Germany; ADANA: Field strain of Tuta absoluta collected from cotton fields in Adana province; ANKARA: Field strain of Tuta absoluta collected from cotton fields in Ankara province; ANTALYA: Field strain of Tuta absoluta collected from cotton fields in Antalya province.

in biochemical activity determinations. GST-CDNB activities results displayed different activity patterns in field populations of *T. absoluta*. While GST-CDNB activities showed statistically significant ($p<0.05$) 1.3-fold increase only in Adana population, Ankara and Antalya field populations did not show statistically significant ($p<0.05$) increase compared to susceptible population (Table 3).

EST-α-NA enzyme activities of Tuta absoluta strains

Enzymatic activity analysis of esterases from *T. absoluta* was done with EST-α-NA assay. It was found that increases in EST-α-NA activities were statistically significant only in the Ankara population (Table 4). EST-α-NA activities showed statistically significant ($p<0.05$) 3.41-fold increase compared to susceptible population only in Ankara field population. However, Adana and Antalya field populations did not show statistically significant ($p<0.05$) increase in EST-α-NA activities.

Discussion

In this study, the existence of abamectin resistance in Turkish populations of *T. absoluta* has been experimentally demonstrated for the first time. Low level abamectin resistance was observed in Adana and Antalya field populations of *T. absoluta*. However, a meaningful abamectin resistance was not detected in Ankara population. The difference at resistance levels indicates presence of differential selection pressures such as use of different amount of abamectin insecticide in different areas where *T. absoluta* samples collected. Also, genetic diversity in the resistance mechanisms among *T. absoluta* populations may affect degree of resistance [27].

The populations of *T. absoluta* from Adana and Antalya fields showed different pattern for cytochrome P450-monooxygenases (CYP450-PNOD) and esterases (EST-α-NA) enzyme activities. While CYP450-PNOD enzyme activities increased significantly ($p<0.05$), EST-α-NA activities were not changed significantly ($p<0.05$) in Adana and Antalya fields compared to susceptible population. Slight increase in CYP450-PNOD activities (2.55-fold in Adana strain, 1.95-fold in Antalya strain) might be explained with low level abamectin resistance (3.3-fold in Adana strain, 2.3-fold in Antalya strain). As cytochrome P450 monooxygenases and esterases generally take a role in direct metabolism of several insecticides [28-30], abamectin stress seems to induce cytochrome P450-monooxygenases activation in Adana and Antalya field populations of *T. absoluta*. Consequently, significant increases in CYP450-PNOD enzymatic activities display that cyto-

chrome P450-monooxygenases might have a major role in abamectin related resistance development in Adana and Antalya field populations of *T. absoluta*.

Siqueira et al [18] suggested that esterases have a major role in abamectin resistance of *T. absoluta* populations from Brazil. However, our results did not support their findings since esterase activity in resistant strains of *T. absoluta* did not show an increase in our study. On the other hand, cytochrome P450-monooxygenase activity in resistant strains increased with increasing resistancy to abamectin in *T. absoluta* from Turkey. Our results suggested that cytochromeP450 plays a major role in detoxification of abamectin in *T. absoluta* pulations from Turkey. Similarly, Reyes et al [31] suggested that increased cytochrome P450- monooxygenase activity would play major role in spinosad resistance of *T. absoluta* populations in Chile.

According to activity results of Ankara population, it was seen that *T. absoluta* insects from this population showed no statistically significant ($p<0.05$) change in CYP450-PNOD activity compared to susceptible population. Interestingly, EST-α-NA activity (3.41-fold) showed statistically significant ($p<0.05$) change in this population. Although Siqueira et al [18] reported that esterases of *T. absoluta* have a major role in abamectin resistance from Brazil, it was not detected any resistance to abamectin insecticide in Ankara population with bioassay experiments. This increased esterase activity might be related with resistance development against other insecticide(s) in this population. For example, Siqueira et al [32] suggested that esterases have a role in cartap resistance of *T. absoluta* populations from Brazil.

In general, GSTs have a role in direct metabolism of some insecticides from organophosphate and organochlorine groups [33]. But, they do not participate in direct metabolism of other insecticides such as pyrethroid insecticides [34,35]. If they don't play a role in direct metabolism of insecticide, it is suggested that they may play a role in reducing oxidative stress by conjugating reactive species and detoxifying lipid peroxidation products [36]. Furthermore, they may have a role in the prevention or repair of oxidative damage, induced by insecticide exposure [37]. Bioassay analysis displayed that there was low level resistance to abamectin in Adana and Antalya field populations. In the literature there are contradictory results between GST-CDNB activity levels and abamectin resistance. For example, while increased GST-CDNB activities were determined in abamectin resistant Tetranychus urticae and Bemisia tabaci [38,39], GST-CDNB activity was not changed in abamectin resistant *T. absoluta* in Brazil [18]. Furthermore, it should be also considered that GST activity levels depend on metabolic rate of animals, increased respiration of mitochondria leads to accumulation of reactive oxygen species [40]. According to these findings, 1.3 fold increase in GST-CDNB activities in Adana population suggested that GSTs seem to play a minor role in metabolic resistance of abamectin by reducing oxidative stress resulted from metabolism of this insecticide. In addition, similar results from researchers in South America were reported, too [18,31,32]. They reported that GSTs have a minor role in resistance development in *T. absoluta* against cartap and abamectin in Brazil and spinosad in Chile. On the contrary, low level abamectin resistance in Antalya strain was not resulted in enhanced GST-CDNB activity. This unchanged GST-CDNB activity level in Antalya strain seems to be caused by decreased metabolic rate of abamectin, which produces considerably less reactive oxygen species.

In conclusion, abamectin resistance was determined in Turkish populations of *T. absoluta*. Cytochrome P450 enzymes was found to have a major role in abamectin resistance development in field populations of *T. absoluta* from Turkey, and GSTs might have a minor role in this resistance development. Whereas, esterase activity did not show an increase in resistant larvae populations suggesting that it may not have an important function in abamectin resistance mechanisms in field populations of *T. absoluta* from Turkey.

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Conflict of Interest

There are no conflicts of interest among the authors.

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