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Orijinal araştırma (Original article)

Insecticide resistance in two populations of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) from Turkey

Türkiye'deki iki *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) populasyonunda insektisit direnci

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Summary

In this study, the resistance of two (Aydın and Urla) populations of *T. absoluta* against five commonly used insecticides (indoxacarb, spinosad, azadirachtin, chlorantraniliprole and metaflumizone) were determined. Further, the activity of insecticide detoxifying enzymes [gluthation-S-transferase (GST) and esterase (EST)] was also evaluated to confirm the resistance. Aydın population of *T. absoluta* had higher resistant values 8-fold, 3.79-fold, 6.4-fold and 1.84-fold for indoxacarb, metaflumizone, spinosad and chlorantraniliprole, respectively against all insecticides except azadirachtin compared to the Urla population. It was determined that in comparison with *T.absoluta* population from Aydın, the Urla population can be more susceptible to other insecticides except azadirachtin. GST enzyme activity was 1.5-fold higher in Aydın than the Urla populations, however, EST enzyme had similar activity in both the populations. The results of study imply that *T. absoluta* populations from Aydın (Turkey) can be resistant against indoxacarb, metaflumizone, spinosad and chlorantraniliprole. Increased GST enzyme activity in resistant populations confirms this resistance development. Insecticides of plant origin like azadirachtin, for which least insecticide resistance was recorded, may be applied in combination with other methods to effectively control *T.absoluta*.

Keywords: *Tuta absoluta*, resistance, enzymes, leaf dip

Özet

Bu çalışmada *T. absoluta*'nın iki populasyonunun (Aydın ve Urla) en çok kullanılan 5 insektisite karşı (indoxacarb, spinosad, azadirachtin, chlorantraniliprol ve metaflumizon) direnç durumları tespit edilmiştir. Ayrıca detoksifikasyon enzimlerinin [glutation-S-transferaz (GST) ve esteraz (EST)] aktiviteleri de direnci doğrulamak için saptanmıştır. *T.absoluta* Aydın populasyonunun azadirachtin hariç diğer insektisitlere karşı (Urla populasyonuna göre) sırasıyla indoxacarb, metaflumizon, spinosad ve chlorantaraniliprol'e karşı 8, 3.79, 6.4 ve 1.84 kat direnç geliştirdiği gözlenmiştir. Aydın populasyonu ile karşılaştırıldığında Urla populasyonunun azadirachtin hariç diğer insektisitlere karşı daha duyarlı olabileceği saptanmıştır. GST enzim aktivitesi Urla populasyonuna göre Aydın populasyonunda 1.5-kat fazla iken, EST enzimi için her iki populasyonda benzer sonuçlar bulunmuştur. Çalışmanın sonuçları, Aydın (Türkiye)'dan alınan bir *T. absoluta* populasyonunun indoxacarb, metaflumizon, spinosad ve chlorantraniliprol'e karşı dirençli olabileceğine işaret etmektedir. Dirençli populasyondaki artan GST enzim aktivitesi, bu direnç gelişimini doğrulamaktadır. En az insektisit direnci rapor edilmiş olan bitkisel kaynaklı insektisit azadirachtin diğer metodlarla birlikte bu zararlının etkili bir şekilde kontrol edilmesinde uygulanabilir.

Anahtar sözcükler: *Tuta absoluta,* direnç, enzimler, yaprak daldırma

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Introduction

Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was first detected in South America (Silva et al., 2011) then it was recognized in eastern Spain in 2006. It rapidly invaded European and the North African Mediterranean Basin Countries (Desneux et al., 2010, 2011). By August 2009, it had been detected in the Urla region of Turkey (Kılıç, 2010). From there, it dispersed to Antalya Kumluca Turkey in January 2010 (Erler et al., 2010) and has now spread to Aydın, Turkey.

Tuta absoluta is the main pest of tomato which damages the leaves, stalks, flowers and fruits. If no preventative measures are adopted, this pest can destroy 80-100% tomato crops in both greenhouse and open-field tomato production (Desneux et al., 2010). Each insect can lay 260 eggs on the leaves of tomato plant; often resulting in up to 12 generations of insects (Silva et al., 2011). Larvae produce large galleries in tomato plant leaves, burrows stalks, buds and fruits (Cáceres, 1992; Lietti et al., 2005). Because of the leaf mines, the yield of the tomato might be greatly reduced. For instance, significant crop losses were reported in Spain, Italy and Greece (Roditakis et al., 2013b). Crop reduction due to leaf damage was identified as key reason for reduced export of tomatoes from USA, Canada, China and India (Desneux et al., 2011).

Due to its impact, different techniques were implemented for the management of this pest, such as monitoring with pheromone traps or using natural enemies. The most substantial control method has been the application of insecticides for sufficient and rapid control. However, repeated chemical control often results in the resistance development in insects to the registered active ingredients. Resistance is accelerated by multiple applications of the insecticide. Reports have shown that some producers apply insecticide 36 times during vegetation period (Picanço et al., 1995). Other researchers have documented the resistance mechanisms and levels of this pest (Lietti et al., 2005; Durmuşoğlu et al., 2011; Dağlı et al., 2012)

Recent studies have reported insecticide resistance development of *T. absoluta* in diverse tomato production regions. For example, field populations of Adana and Antalya strain of *T. absoluta* showed low resistance to abamectin insecticide while Ankara strain of *T. absoluta* was not resistant to abamectin (Konuş, 2014). Low or no resistance levels were found toward pyrethroids, abamectin, spinosad, *Bacillus thuringiensis* and the mixture deltamethrin+ triazophos, however indoxacarb and chitin synthesis inhibitors showed high resistance (Silva et al., 2011). *T. absoluta* has also shown resistance to indoxacarb and chlorantraniliprole in three laboratories from different countries (Greece, Spain and Italy) (Roditakis et al., 2013b). *T. absoluta* insecticide resistance has been attributed to the combination of the insecticide's mode of action and its detoxification by special enzymes. A variety of enzymes and their roles against insecticides have been studied. For instance, the registered insecticide spinosad, has a unique and powerful action mechanism on lepidopteran larvae (Reyes et al., 2012), is a nicotinic acetylcholine receptor (n AchR). Allosteric activators have been found to directly impact the activity of esterases (IRAC, 2013). Metaflumizone, which is specifically developed against Lepidoptera species, is a voltage-gated sodium channel blocker. Indoxacarb is also a voltage-gated sodium channel blocker and is bioactivated by esterases in the target insects (Ahmad & Hollingworth, 2004). Although azadirachtin has unknown modes of action, the insects may become more resistant owing to the role of neem in reducing enzyme levels through blockage of protein synthesis (Lowery & Smirle, 2000). Chlorantraniliprole has a novel mode of action, ryanodine receptor modulator, it controls external Ca^{2+} entry and ryanodine receptor channels (RyRs).

The aim of this study was to evaluate the toxicities of five insecticides on two populations of *T. absoluta* in order to establish if there are differences in susceptibility in those populations.

Materials and Methods

Biological material (*Tuta absoluta* / Insects + Rearing + Plant material)

Tuta absoluta populations were collected from tomato infested fields in Urla (Kuşçular, Yağcılar) and Aydın (Sultanhisar, İncirliova, Germencik) Turkey during summer in 2011 and 2012. Larvae infested leaves were collected, transferred to cages (40 x 40 x 40 cm), and kept in a climate chamber which had a constant temperature of 25 ± 1°C, relative humidity 65% and 16:8 h light: dark photoperiod. Larvae were then fed with tomato leaves from plants cultivated under greenhouse conditions without any insecticide treatment. In the cages water, sugar, and pollen mixture was used to endorse adult population.

The Newton tomato variety was used in the experiment. The plants were irrigated and inspected every second day. Infested leaves were removed and destroyed to prevent cross breeding from unknown strain. Tomatoes leaves without insecticides were used in this study to prevent any bias due to the presence of mites. Urla and Aydın populations were reared in different climate chambers to prevent cross contamination. The insects from these climatic chambers were collected when the adults reached an age of 13-15 days. If the number of eggs were not adequate, plants were then removed and new plants were placed for the insects' oviposition progress. After 8 days, second instar larvae were collected and used for the experiments.

Insecticides

The insecticides chlorantraniliprole (Altacor^{R} 35 WG), metaflumizone (Alverde^{R} SC) and indoxacarb (Avaunt150SC) were provided by BASF. Spinosad (Laser SC 480 g/l) was provided by Dow Agrosciences. Azadirachtin 10 g/l (Neemazal^{R}-T/S) was provided by Trifolion-M GmbH Germany.

Bioassay

Toxicity of insecticides on *T. absoluta* was evaluated by the IRAC method (IRAC, 2013). Second instar larvae (4-5 mm in size) were collected from infested leaves of tomato plants in the cages. It was ensured that larvae had never been in starvation stress. F2 generation larvae were obtained and these were used to homogenize the larvae which was recommended in IRAC 022 method. Tomato leaves were collected from top third of the plant ensuring similar size and placed in a moist paper towel to avoid wilting. Commercial insecticide formulations were used in a leaf dip bioassay which is the most efficient method for detection of the toxicity of insecticide formulations for *T. absoluta* (Galdino et al., 2011). The control leaves dipped in the solvent without the insecticide and other leaves were dipped individually in the different solutions for 3 seconds with agitation, making sure that the surfaces of the leaves were covered by respective insecticides; then the treated leaves were dried. Two petri cups per concentration, and six concentrations plus one control per insecticide were used. It is summed up a total of 14 petri cups per insecticide. This method was applied for 5 insecticides individually. The bottom of each cup was covered with moistened filter paper and these were then labeled according to the solution used. The insecticide treated two leaves were then placed individually in the respective petri cups. Two insecticide treated leaves were placed in 9 cm diameter petri dishes.

By using a fine soft brush, 10 larvae were put on each leaf, with two leaves used for each insecticide concentration. Six different concentrations were used for each insecticide. Leaves not treated with insecticides and the same numbers of larvae were used as a control. Mortality was calculated after 72 h using a fine soft brush. If larvae did not move when brushed, they were assumed as dead, while if they moved they were assumed as alive. Also, if they were moribund larvae, they were assumed alive.

Activities of the two enzymes gluthathion S transferase (GST) and esterase (EST) of second instar larvae were measured. Larvae extracts were prepared using Reyes method (Reyes et al., 2012). Protein contents of larvae extracts were determined by Bradford method (Bradford, 1976). The same number of

larvae was used for evaluating EST and GST enzyme activity. Thirty larvae for each replication were collected from the leaves of tomatoes, with analysis repeated six times for each treatment. Thus for each sampling site, 180 larvae were collected. They were homogenized on ice in 50 µl Hepes buffer (50 mM, pH 7.0) by using pestils. Homogenates were centrifuged at 15,000 g for 15 min at 4 °C. The supernatants of each sample were used to measure enzyme activity.

Substrate β-naphtyl acetate (β-NA) was used to determine esterase activity according to Walker (1998). Absorbance was determined by a micro plate ELISA spectrophotometer reader. Each well contained 10 µl extract and185 µl β-NA (0.03 mM, final concentration in well). After 20 min incubation at 30 °C, 55 µl Fast Garnet (0.4%) and SDS (2.5%) were added to the solution. Absorbance at 590 nm of each well was measured after 20 min incubation in the dark room. EST activity was determined as n mol β-NA mg protein^{-1} min^{-1}.

GST activity was determined by using monochlorobimane (MCB) as a substrate. Thirty µl of extract, 168 µl and 100 mM GSH in Hepes Buffer (50 mM pH 7.0) and 2 µl 30 mM MCB were added to each well. Fluorescence was measured after 20 min incubation at 22 °C with 450 nm emission and 380 nm excitation filter. Activities were determined as unit activity mg protein^{-1} min^{-1} because of the absence of bimane- glutathione adduct.

Data analysis

Insecticide bioassay data were subjected to probit analysis, using Polo Plus Program (LeOra Software). The LC_{50} and LC_{90} values, linearity of dose-mortality response and slope were computed (Robertson et al., 2003). The mortality values of the control treatment of each insecticide were used to perform the probit analysis, for calculating the regression line, the slope and the LCs' for each insecticide. The LCs' of the susceptible population was used for the calculation of the Resistance Factor (RF) for each insecticide The population which has a minor value of LC assumed as susceptible population since there was no susceptible strain available for this insect to evaluate resistance factor.

The mean activity values of the two enzymes, EST and GST were calculated by Student Newman Keuls analyses with SPSS program (Abdi & Williams, 2010). This test offered comparison of the enzyme values of Urla and Aydın populations.

Results

Resistance factors of insecticides

Aydın and Urla populations of *T. absoluta* responded differently to applied insecticides in the experiment (Table 1). In contrast to Urla population of *T. absoluta*, the Aydın population was found to have developed a resistance against all insecticides (except azadirachtin). On the other hand, Urla populations were noted to have a weak resistance only against azadirachtin. The highest insecticide resistance in *T. absoluta* Aydın populations was found against indoxacarb followed by spinosad. LC_{50} resistance ratio of indoxacarb was eight fold higher in Aydın population compared to Urla population. LC_{50} and LC_{90} values for indoxacarb were 215.26 mg L^{-1} and 695.64 mg L^{-1} in Aydın population, respectively. On the other hand, LC_{50} value was 26.81 mg L^{-1} and LC_{90} value was 144.61 mg L^{-1} in Urla population. The resistance factor of azadirachtin was the lowest among the insecticides studied (Table 1). LC_{50} values of Aydın and Urla populations were 19.18 mg L^{-1} and 23.60 mg L^{-1}, respectively. *T. absoluta* exhibited three-fold increase in LC_{50} by metaflumizone in Aydın population as compared with Urla. For metaflumizone, LC_{50} values of 2091.4 mg L^{-1} and 550.47 mg L^{-1} were found for Aydın and Urla populations, respectively. RF values indicated that the Aydın population of *T. absoluta* was six fold higher resistant than the Urla population for spinosad and the resistance factor of Aydın was also higher. LC_{50} values for Aydın and Urla were found to be 0.7 mg L^{-1} and 0.11 mg L^{-1}, respectively. The LC_{50} ratio of

the Aydın population to the Urla population for chlorantranilprole was 1.84, and its LC_{50} values were 15.35 mg L^{-1} and 8.36 mg L^{-1} for Aydın and Urla, respectively.

Table 1. Toxicity data of indoxacarb, azadirachtin, metaflumizone, chlorantraniliprole and spinosad against Aydin and Urla population of *Tuta absoluta*

	Number Tested	Slope	X^2	LC_{50} (95% CL)	LC_{90}(95% CL)	RF(95% CL)
Indoxacarb						
Aydın	140	1.940	1.300	215.26	695.64	
				(162-360)	(399-2704)	
Urla	140	1.700	2.800	26.81	144.61	
				(13-39)	(91-394)	
Aydın/Urla						8.02
						(4.4-14.4)
Azadirachtin						
Aydın	140	1.060	1.508	19.18	303.00	
				(10-42)	(93-19343)	
Urla	140	1.800	6.600	23.60	115.23	
				(10-60)	(49-2888)	
Urla/Aydın						1.23
						(0.2-1.1)
Metaflumizone						
Aydın	140	1.270	0.140	2091.40	21356.00	
				(1160-12092)	(5721-531398)	
Urla	140	1.807	0.356	550.47	2818.20	
				(374-795)	(1653-8360)	
Aydın/Urla						3.79
						(1.6-5.6)
Spinosad						
Aydın	140	1.751	0.463	0.70	275.00	
				(0.140-2.770)	(37-25985)	
Urla	140	0.305	0.495	0.11	66.00	
				(0.013-0.440)	(10-4812)	
Aydın/Urla						6.40
						(0.6-49.5)
Chlorantraniliprole						
Aydın	140	0.210	0.848	15.35	63.25	
				(10.950-21.360)	(40-144)	
Urla	140	0.200	1.220	8.36	34.34	
				(5.390-11.570)	(22-73)	
Aydın/Urla						1.84
						(1.1-2.8)

LC_{50} in mg L^{-1}; LC_{90} in mg L^{-1}; RF= Resistance Factor (LC_{50}value of insecticide in Aydın/ LC_{50}value of insecticide in Urla or LC_{50}value of insecticide in Urla / LC_{50}value of insecticide in Aydın)

EST and GST activities

EST activities of the Aydın and the Urla populations were 0.110±0.02 n mol β-NA/mg protein/min and 0.097±0.03 n mol β-NA/mg protein/min, respectively (Figure1.A). There was no significant difference ($p>0.05$) between the EST activities of the Aydın and Urla populations of *T. absoluta*. GST activities of the Aydın and Urla populations were 1.905±0.55 unit activity/mg protein/min and 1.237±0.718 unit activity/mg

protein /min, respectively (Figure 1.B). Aydın population showed a significant lower value of GST activity (P< 0.05) than the Urla population.

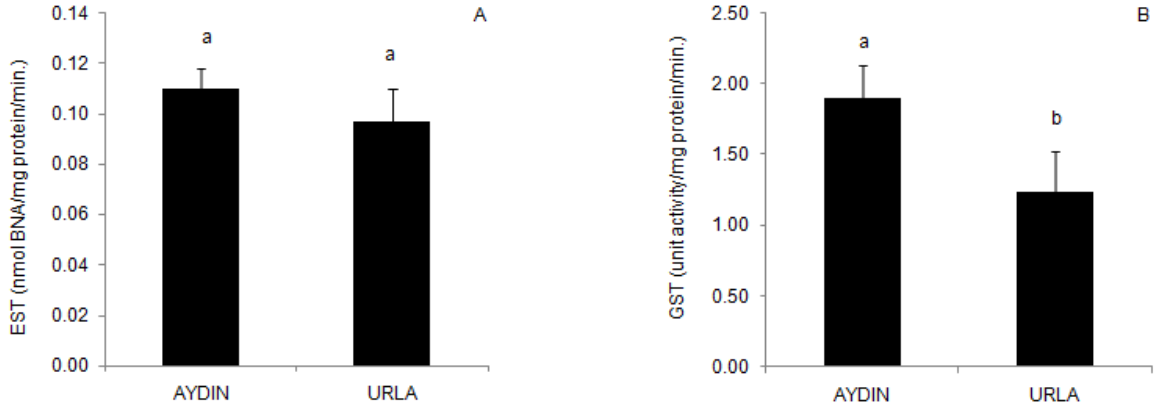

Figure 1. A) Esterase activity ranges of *T. absoluta* populations in Aydın and Urla, B) Gluthathion-S-transferase activity ranges of *Tuta absoluta* populations in Aydın and Urla.
Different letters on the bars indicate statistical differences (P < 0.05), Student Newman Keuls Analysis

Discussion

In this study, the toxicities of five insecticides registered to control *T. absoluta* in two population from Turkey were determined, and the possible responsible enzymes for induction of insecticide resistance were investigated. Previously, several resistance studies have been done due to the spatial dependence of *T. absoluta*. For example, in Brazil, the use of pyrethroid and organophosphorus compounds was suggested to be avoided due to the high resistance ratio of *T. absoluta* to those compounds (Branco et al., 2001). Deltamethrin was found the least toxic to *T. absoluta* while mevinphos was found as the most toxic compound in Africa and Chile (Salazar & Araya, 2001). Previously, resistance to indoxacarb was estimated since this insecticide was registered worldwide for the control of moths. In this study, it was observed that indoxacarb resistance was 8.02-fold higher in the Aydın population in relation to Urla. Recent studies have also reported cases of insecticide resistances in *T. absoluta* from many parts of world. For example, in a study from Greece, Roditakis et al. (2013a) found LC_{50} values of indoxacarb for resistant *T. absoluta* populations 4, 10 and 12-fold higher compared with the susceptible populations. Similarly, Silva et al. (2011) reported 27.5-fold resistance development against indoxacarb in resistant populations than the susceptible populations. The resistance ratio of indoxacarb was found 15-fold higher compared with a laboratory susceptible population for *Spodoptera litura* (Lepidoptera: Noctuidae) (Silva et al., 2011). The increased resistance was attributed to the activity of GST enzyme (Ahmad et al., 2008).

According to our studies, metaflumizone was comparable between the study groups in the experiment. *T. absoluta* showed 3.79-fold resistance in Aydın compared to the Urla population. Roditakis et al. (2013b) reported that *T. absoluta* showed 5-fold resistance to metaflumizone in Crete province of Greece when compared to the susceptible strain. Contrarily, metaflumizone was reported effective against *T. absoluta* in Italy (Nannini et al., 2011). Although metaflumizone is a new semicarbazone insecticide, frequent usage of this insecticide could have led to the development of resistance in *T. absoluta*.

Currently, historically used insecticides are being replaced by novel ones because of the cross and multiple resistances among insect pests (Metcalf, 1967; Singh et al., 2005; Cordova et al., 2006; Rattan, 2010). Spinosad is one among the alternative class of insecticides such as organophosphates,

carbamates and pyrethroids (Cleveland et al., 2002). Spinosad which is most commonly used against a range of insects including Lepidoptera (El-Mageed & Elgohary, 2006) was found an effective insecticide for *T. absoluta* when the sample sites were compared in our studies. The Urla populations were susceptible to this insecticide while the Aydın population had 6-fold resistance with respect to the Urla population. In another study, it was established that resistance to spinosad, which is a neurotoxic insecticide, ranged between 1.2 to 4.8-fold in Brazil (Silva et al., 2011). Reyes et al. (2012) reported that four of the five field populations showed significantly lower susceptibility to spinosad, when compared with the susceptible strain. In a study from Egypt, it was determined that spinosad resistance could contribute to a decrease in AchE activity (El-Mageed & Elgohary, 2006). Further, EST and mixed function oxidase (MFO) can also reduce the efficacy of spinosad in *T. absoluta* (Reyes et al., 2012). The lower variability of LC_{50} was observed between Urla and Aydın for Azadirachtin i.e. 1.23. Previously, researchers have reported that effectiveness of azadirachtin against *T. absoluta*. For example, in as study from Brazil, the azadirachtin was found to cause almost complete mortality of *T. absoluta* populations (Tomé et al., 2013). The other studies also report a higher mortality of this insect pest through application of azadirachtin (Gonçalves-Gervásio & Vendramim, 2007; Durmuşoğlu et al., 2011). Many studies have reported the resistance development in insect pest against chlorantraniliprole (Astor & Scals, 2009; Roditakis et al., 2013a; Roditakis et al., 2013b). In our study, *T. absoluta* showed 1.83-fold resistance to chlorantraniliprole, whereas European populations of *T. absoluta* showed 1 to 6-fold resistance to chlorantraniliprole (Roditakis et al., 2013b).

In this study, the results regarding insecticide detoxifying enzymes support the observed resistance ratios of insecticides. Aydın population was found to possess significantly higher GST activity compared to the Urla population. In accordance with our results, some recent studies confirm an increased enzyme activity in the insecticide resistant insect populations. For example, 1.3-fold higher GST activity was reported for the *T. absoluta* populations which had developed a resistance against abamectin in two provinces of Turkey (Konuş, 2014). Another study from South America indicated that enzymes (oxidase, GST, EST) activities differed significantly in susceptible and resistant populations of *T. absoluta* (Reyes et al., 2012). Radwan & Taha (2012) had reported that dinotefuran, imidacloprid, fenoxycarb, phenthoate and thiocyclam insecticides exposure causes reduction or increase in the activity of AChE and GST enzymes.

In conclusion, the susceptibility of *T. absoluta* to registered insecticides has decreased in certain agricultural fields of Turkey. This insect may become a major pest of several crops if proper alternative control measures are not adopted. The resistance development in *T. absoluta* was confirmed by increased activity of GST enzyme. According to the results from the two population, azadirachtin was found to have developed no or very weak resistance against *T. absoluta* populations which may be due to a different mode of action of this insecticide. Future research work may be carried out to evaluate the synergistic relationship between enzymes to develop alternative management strategies.

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