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Article

Target-site insensitivity to some acaricides in a field population of *Tetranychus urticae* Koch (Acari: Tetranychidae) from Egypt

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ABSTRACT

The control of the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, a worldwide agricultural pest, is largely dependent on pesticides. However, their efficacy is often encountered by the development of resistance. The present study was conducted to evaluate resistance levels to five pesticides with different modes of action in field population of *T. urticae* collected from tomato plants grown in open fields at Bernasht village, El-Ayat, Giza Governorate, Egypt, using leaf-dip technique. These pesticides were dimethoate, chlorpyrifos, and ethion (organophosphates), etofenprox (pyrethroids), and abamectin (avermectins). Moreover, the common resistance mutations associated with different groups of pesticides in *T. urticae* was identified by quantitative sequencing. Very high resistance levels of TSSM to organophosphate pesticides were observed in Eg-Bernasht population. Also, the nucleotide sequences were aligned, megablasted, and compared for similarity with the corresponding genes in different databases such as NCBI nucleotide collection (nr/nt). Of the five common mutations associated with organophosphate and carbamate pesticides, three mutations were identified in Eg-Bernasht population: a threonine residue was replaced by alanine (T280A), glycine was replaced by alanine (G328A), and phenylalanine was replaced by tryptophan (F331W). One of the three common mutations associated with pyrethroid pesticides, alanine was replaced by aspartic acid (A1215D). Further two substitutions of amino acids, leucine to valine (L988V) and aspartic acid to glutamic acid (D1569E) were also identified. The common mutations associated with the target site of avermectins (glutamate-gated chloride channels, *GluCl1*, *GluCl3*) and etoxazole pesticide (chitin synthase, *CHS1*) were absent in Eg-Bernasht population. Also, with glutamate-gated chloride channel genes in one clone of Eg-Bernasht population, two substitutions of alanine to leucine (A308L) and valine to leucine (V309L) were identified. The results obtained from the current investigation indicated that four common mutations associated with pesticide resistance were present in the field population of *T. urticae*. These mutations were T280A, G328A, and F331W in the acetylcholinesterase (*AChE*) gene and A1215D in the voltage-gated sodium channel (*VGSC*) gene. An improved understanding of acaricides resistance in TSSM is important to maintain the efficacy of these chemicals for the control of this pest in tomato and other crops. We recommend focusing on the use of integrated pest management programs and pesticide alternatively to manage resistance.

KEY WORDS: Acaricides; quantitative sequencing; resistance; target-site point mutations; two-spotted spider mite.

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INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* (Acari: Tetranychidae), is a worldwide polyphagous pest. It attacks more than 1000 host plants, including crops of major economic importance (Migeon *et al.* 2010). In Egypt, it became one of the most important pests of many greenhouses and field vegetable crops, particularly Solanaceae and Cucurbitaceae, which can establish high population densities and cause serious damage to tomato plants.

Several groups of acaricides have been used to control *T. urticae*, such as organophosphates (OPs), carbamates, pyrethroids, and avermectins. However, this pest has rapidly developed resistance to almost every type of acaricide, which has been linked to control failure. The accelerated development of resistance in *T. urticae* is not only due to the extensive exposure to acaricides but is also exacerbated by its biology, namely an extremely short life span with relatively high fecundity and arrhenotokous reproduction (Grbić *et al.* 2011; Van Leeuwen *et al.* 2013). As its name implies, *T. urticae* is able to produce webs from silk glands located at each palp (Alberti and Crooker 1985). The webbings made by *T. urticae* may work as a shelter to avoid pesticide exposure and protect it from other life-threatening conditions, including wind, rain, and natural enemies (Davis 1952). Moreover, *T. urticae* undergoes diapause in soil, tree bark, ground cover, and dried leaves when decreasing temperature, photoperiod, and decline in the quality of food supply occur due to plant senescence (Veerman 1985). Diapause facilitates *T. urticae* adaptation to the agroecosystems and offers a refuge to escape pesticide exposure.

The foregoing biological characteristics, in addition to other factors, have made *T. urticae* a very useful model for studying resistance mechanisms and their evolution in the field. These factors are: (i) *T. urticae* is a globally distributed and highly polyphagous species (Migeon *et al.* 2010); (ii) this species has been found to be resistant to 94 unique insecticide/acaricide active ingredients in 468 documented cases worldwide (Van Leeuwen *et al.* 2015); and (iii) it has structured populations (Tsagkarakou *et al.* 1998, 1999; Navajas *et al.* 2002), very often reproductively isolated (De Boer 1985), suggesting strong barriers in gene flow among populations.

The mechanisms of pesticide resistance exhibited by arthropods typically evolve along several trajectories, including behavioral avoidance (Wada-Katsumata *et al.* 2013), decreased cuticular penetration (Koganemaru *et al.* 2013), enhanced sequestration, metabolic detoxification (Feyereisen 2012), and target site insensitivity (Liu *et al.* 2006; Zhu *et al.* 2014). Among these, target site insensitivity to acaricides in *T. urticae* has been investigated extensively. For example, several mutations in the acetylcholinesterase (*AChE*) gene have been documented in OP-resistant *T. urticae* strains from Europe and Korea (Khajehali *et al.* 2010; Kwon *et al.* 2010b).

Two mutations were identified in the glutamate-gated chloride channel (*GluCl*) genes that are correlated with abamectin resistance in *T. urticae* populations (Kwon *et al.* 2010c). Studies have reported that resistance to bifenazate commonly used for *T. urticae* control was tightly linked to multiple mutations at the Quinol oxidation (Qo) site of mitochondrial cytochrome *b* (*cytb*) (Van Leeuwen *et al.* 2008; Van Nieuwenhuyse *et al.* 2009). Pyrethroid resistance in *T. urticae* has been associated with several amino acid substitutions in the voltage-gated sodium channel (*VGSC*) gene (Tsagkarakou *et al.* 2009; Kwon *et al.* 2010a). Additionally, recent studies revealed that a mutation on the chitin synthase (*CHS1*) gene may contribute to resistance to etoxazole (Van Leeuwen *et al.* 2012; Demaeght *et al.* 2014), hexythiazox, and clofentezine (Demaeght *et al.* 2014) in *T. urticae*.

The current study aimed to identify the resistance mutations associated with the target sites of several acaricide classes in *T. urticae*, collected from open tomato fields at Bernasht village, El-Ayat, Giza Governorate, Egypt, using quantitative sequencing. This study may help in the implementation of appropriate resistance management tactics and addressing local control programs.

MATERIALS AND METHODS

Tetranychus urticae *populations*

A field resistant population of *T. urticae* was collected from tomato plants (*Solanum lycopersicum* L.) grown in open fields at Bernasht village, El-Ayat, Giza Governorate, Egypt. Also, the susceptible population of *T. urticae* was collected from castor bean plants (*Ricinus communis* L.), growing in the farm of Faculty of Agriculture, Cairo University, without exposure to pesticides during the last 10 years. Both populations were reared on leaves of copperleaf shrubs (*Acalypha wilkesiana* Müll. Arg.) in an incubator at 28 ± 2 °C, 65 ± 5% relative humidity (RH%), and (L14:D10 h photoperiod) to maintain a sustained colony during the experiment.

Pesticides tested

Three OP pesticides, dimethoate (Tepro-One 40% EC), chlorpyrifos (Tak 48% EC), and ethion (Endo 50% EC), one pyrethroid pesticide, etofenprox (Primo 10% SC), and one avermectin pesticide, abamectin (Agromec 1.8 EC) were bioassayed. The recommended application rates at field on insect or mite pests were 200, 250, 200, 93.75, and 40 mL/100 L, respectively. These pesticides were chosen because they are commercial pesticides frequently used on tomato plants against many pests in the above-mentioned study area and belong to different classes of chemicals with different modes of action.

Bioassays

Seven aqueous concentrations of dimethoate (200, 400, 600, 800, 1200, 2400, and 4800 ppm), chlorpyrifos (240, 480, 960, 1440, 2400, 4800, and 9600 ppm), ethion (250, 500, 750, 1000, 1500, 3000, and 6000 ppm), etofenprox (5, 10, 30, 50, 90, 180, and 360 ppm) and abamectin (0.9, 1.8, 3.6, 5.4, 7.2, 14.4, and 28.8 ppm) were prepared based on formulation dilution. These concentrations were chosen based on the field recommended dose. Leaf discs (2-cm diameter) of fresh leaves of copperleaf shrubs (*A. wilkesiana*) were separately dipped in each concentration for 1 min. and air-dried for one h at laboratory conditions. Five leaf discs were placed on moist cotton in Petri dish (12-cm diameter). Each concentration was replicated five times, with 20 adult females of *T. urticae* each. Parallel distilled water-treated control groups were also conducted five times, with 20 adult females each. The bioassay was conducted on 4th generation of adult females from the sustained colony (once a deutonymph turns into an adult). This technique was separately conducted for each population. Mortality count was recorded 24 h post-treatment. The criterion for mortality was an inability on the part of mites to walk at least one body length when lightly prodded. Petri dishes were incubated at 28 ± 2 °C and 60 ± 5% R.H. Mortality (%) was corrected by Abbott's formula (Abbott 1925). Corrected mortality was then subjected to Probit analysis (Finney 1971) to estimate the LC_{50}, LC_{90}, 95% confidence limits (CL), slopes ± SE, and Chi-square for each pesticide, using the LdP Line® computer program (Bakr 2000). The LC_{50} and LC_{90} values of the field resistant population were compared to those of the susceptible population.

DNA extraction and PCR

The spider mite was identified by light microscope based on morphological characteristics (Zhang *et al*. 2002). Also, the DNA of three samples of *T. urticae* Eg-Bernasht field population was extracted to identify the common resistance mutations associated with different groups of pesticides by quantitative sequencing.

DNA extraction of mass mite genomic DNA was prepared by crushing approximately 120 adult females with a plastic pestle in a 1.5-ml micro-centrifuge tube, using gene JET genomic DNA purification kit (Thermo Scientific # K0721).

PCR amplification was performed by standard PCR protocol, using 50 ng genomic DNA , 0.5 mM concentration primers (specific primers for each gene, Table 1), 0.2 mM concentration (dNTP Mix, 2 mM each), 2.5 µl of 10X Dream Taq buffer (Thermo Scientific), Dream-Taq DNA polymerase (1 unit), and nuclease-free water up to 25 µl. The thermal conditions were 95 °C for 5 min followed by 35 cycles of 95 °C for 30s, 72 °C for 60 s, and final extension at 72 °C for 10 min (annealing 52–58 °C, Table 1). After completion of the PCR, a 20-µl aliquot of the reaction was loaded onto 1.0% agarose gel stained with ethidium bromide and visualized under UV light by a gel documentation system. The following DNA fragments were cut and purified, using a TI-ANgel Midi purification kit (TIANGEN DP209 Company).

Table 1. Characterization of primers which were used in this study.

No.	Primer name	Gene	Sequence (5′ - 3′)	Annealing Temp. (°C)	Amplified (bp)	Reference
1	UAF_119	*AChE*[*]	GCCTATCAGTAAACATTTGG	52 °C	*639*	Ilias *et al.* (2014)
	UAR_280		AAACCTCGGGATCTTCATCG			
2	UAF_1552		ACCTGCCTCTGAATTGGTTG		571	
	UAR_331		TGCAGGCGTATAACCATGAAC			
3	UAF_INTRON		CTGGATGGGAGTTATTCATG		560	
	UAR_INTRON		TACATCAACTCAGTATGCTAG			
4	KdrF4	*VGSC*[**]	CAACATTCAAAGGTTGGACAAT	54 °C	*226*	Khajehali *et al.* (2010)
	KdrR1		TCTTCCGTCATCAACATCTCC			
5	KdrF2		TGCATCTCAATTGTCCAAGG		225	
	KdrR2		GTTTCTTCCAGGCAACATGG			
6	KdrF5		TGATTGTTTTCCGTGTCCTG		292	
	KdrR5		CTGCGAAGCTGCTTAAGTCC			
7	GluCl1_F1	*GluCl1*[†]	TTGGATTGACCCTAACTCAGCA	58 °C	*263*	Dermauw *et al.* (2012)
	GluCl1_R1		TTGCACCAACAATTCCTTGA			
8	GluCl3_F1	*GluCl3*	CCGGGTCAGTCTTGGTGTTA		*251*	
	GluCl3_R1		CACCACCAAGAACCTGTTGA			
9	TuCHS1m_diaF	*CHS1*[‡]	GTCCGCTTGTTATGCACTACT	57 °C	*541*	Van Leeuwen *et al.* (2012)
	TuCHS1_diaR		GCCACCAAGTGGGTCAAGAT			

[*] Acetylcholinesterase (*AChE*) gene.
[**] Voltage-gated sodium channel (*VGSC*) gene.
[†] Glutamate-gated chloride channel (*GluCl1*, *GluCl3*) genes.
[‡] Chitin synthase 1 (*CHS1*) gene.

Analyses of the PCR products

PCR products were purified using QIA quick PCR purification kit (Qiagen, Cat. # 28104) and sequenced directly on PCR product with the original PCR primers (Table 1). Sequencing reactions were performed by a 3500 Genetic Analyzer Applied Biosystems according to Sanger and Coulson (1975). Detection of resistance associated point mutations DNA, massively extracted from *T. urticae* samples collections, was used as a template for PCR amplification of target gene fragments encompassing the insecticide resistance mutations, using the specific primers listed in Table 1. DNA sequences were aligned using bioinformatics tools such as nucleotide BLAST in the National Center for Biotechnology Information (NCBI).

RESULTS

Susceptibility and resistance level of Tetranychus urticae *to some pesticides*

At LC$_{50}$, the calculated dimethoate and chlorpyrifos resistance ratio for Eg-Bernasht field population was high (27.76- and 31.98-fold value, respectively), the resistance ratio for abamectin was moderate (18.90-fold value) and low for ethion and etofenprox (11.90- and 8.45-fold value, respectively). Resistance ratios relative to the response of the susceptible population ranged from 8.45- to 31.98-fold values for all tested pesticides (Table 2). The decrease in resistance ratio value may be due to conducting a bioassay on 4th generation of adult females from the sustained colony of both Eg-Bernasht field resistant (R) and susceptible (S) populations of *T. urticae*.

The Eg-Bernasht field population of *T. urticae* showed high LC_{50} values to chlorpyrifos and dimethoate (8794 and 7106 ppm, respectively). Abamectin was the most toxic pesticide against *T. urticae* population, with LC_{50} values of 1.27 and 24 ppm, and with LC_{90} values of 5.68 and 93 ppm for susceptible and resistant populations, respectively.

Table 2. Mortality response and resistance ratio of Eg-Bernasht field resistant (R) and susceptible (S) populations of *Tetranychus urticae* to some pesticides.

Pesticides	Population	LC_{50} (ppm) (95% CL)	LC_{90} (ppm) (95% CL)	Slope ± SE	Chi-square	Resistance factor LC_{50}*
Dimethoate (Tepro-One 40% EC)	S	**256** (205−303)	**1083** (915−1348)	**2.04 ± 0.19**	**7.21**	**-**
	R	**7106** (5298−10908)	**37682** (21402−89916)	**1.77 ± 0.20**	**3.03**	**27.76**
Chlorpyrifos (Tak 48% EC)	S	**275** (210−337)	**1322** (1099−1670)	**1.88 ± 0.18**	**4.96**	**-**
	R	**8794** (6958−11996)	**53905** (33367−107095)	**1.63 ± 0.15**	**4.13**	**31.98**
Ethion (Endo 50% EC)	S	**398** (278−493)	**1221** (994−1843)	**2.63 ± 0.22**	**15.02**	**-**
	R	**4736** (3975−5914)	**19124** (13573−30677)	**2.11 ± 0.18**	**8.21**	**11.90**
Etofenprox (Primo 10%SC)	S	**7.26** (5.59−8.94)	**41.83** (33.8−56.97)	**1.69 ± 0.16**	**2.17**	**-**
	R	**61** (52−75)	**272** (192− 448)	**1.98 ± 0.20**	**7.59**	**8.45**
Abamectin (Agromec 1.8 EC)	S	**1.27** (1.02−1.51)	**5.68** (4.77−7.06)	**1.97 ± 0.17**	**3.30**	**-**
	R	**24** (19−49)	**93** (79−369)	**2.20 ± 0.20**	**19.78**	**18.90**

[*] Resistance ratio = LC_{50} of the field-collected resistant population (R)/LC_{50} of the susceptible population (S).
- The bioassay was conducted on 4th generation of adult females from the sustained colony.

Comparison of Tetranychus urticae *AChE gene amino acid sequence polymorphism between Eg-Bernasht field population and GenBank databases*

On comparing amino acid sequence of *AChE* gene in Eg-Bernasht field population with the reference GenBank populations, three mutations of the five common ones associated with OPs and carbamates pesticides were identified in Eg-Bernasht population (Table 3, Figs. 1, 2). These mutations were T280A (391) at position 280, threonine was replaced by alanine, G328A (436) at position 328, glycine was replaced by alanine, and F331W (439) at position 331, phenylalanine was replaced by tryptophan. Moreover, one of the five common mutations was absent in Eg-Bernasht population; A201S (309) at position 201, with one substitution; alanine to serine.

Comparison of Tetranychus urticae *VGSC, GluCl1, GluCl3 and CHS1 genes amino acid sequence polymorphism between Eg-Bernasht field population and some populations in GenBank databases*

On comparing amino acid sequence of *VGSC* gene in Eg-Bernasht field population with some populations in GenBank databases, one of the three common mutations associated with pyrethroid pesticides was identified in Eg-Bernasht population; alanine was replaced by aspartic acid at position 1215 (A1215D). Moreover, two of the three common mutations were absent in Eg-Bernasht population: phenylalanine to isoleucine at position 1538 (F1538I) and leucine to valine at position 1024 (L1024V) (Table 4). In addition, two substitutions of amino acids, leucine to valine at the position 988 (L988V) and aspartic acid to glutamic acid at the position1569 (D1569E) were identified in Eg-Bernasht population.

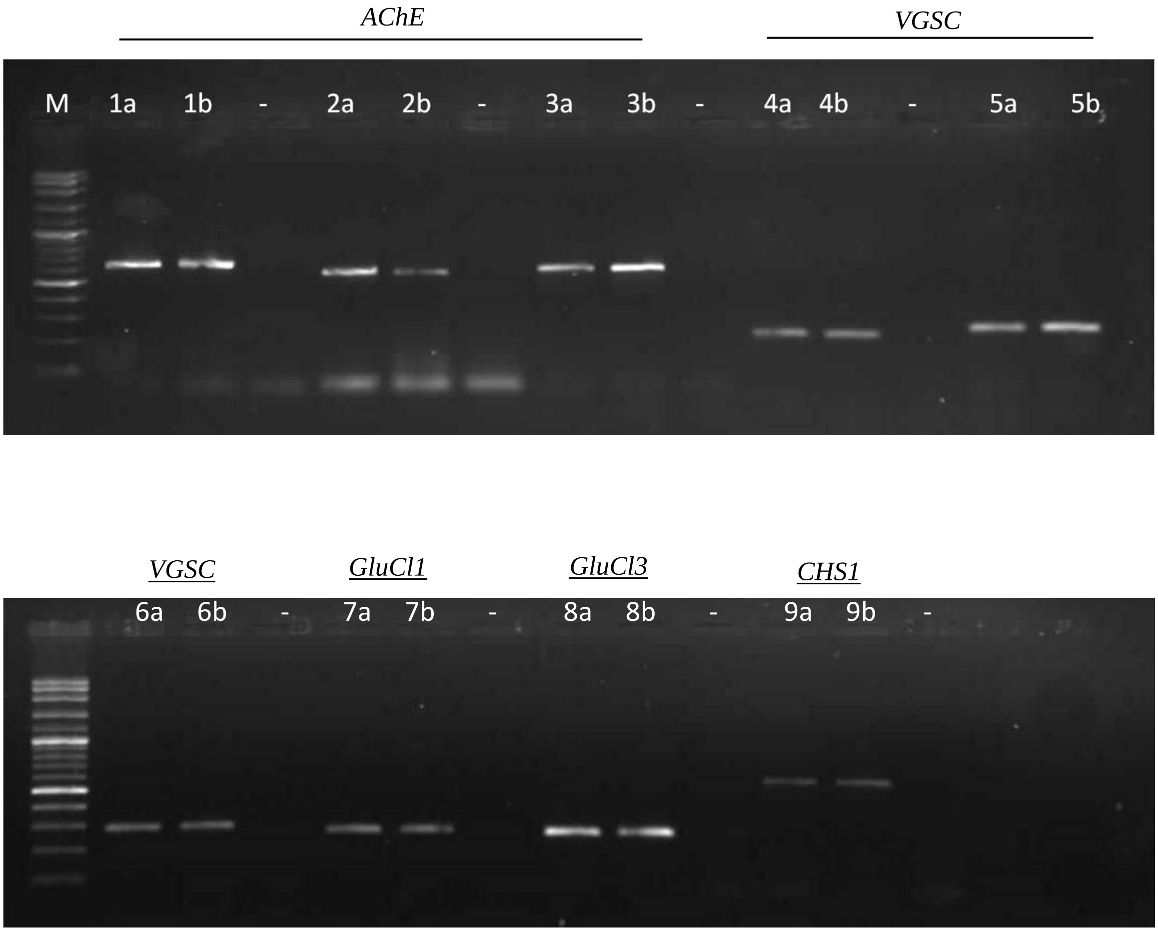

Figure 1. Products were imaged on a gel post electrophoresis. *Tetranychus urticae* acetylcholinesterase (*AChE*) gene fragment amplified expected sizes (639 bp, 571 bp, and 560 bp), using specific primers sets (1, 2, and 3, respectively). *Tetranychus urticae* voltage-gated sodium channel *(VGSC)* gene fragments were amplified products (226 bp, 225 bp, and 292 bp), using specific primers primer sets (4, 5, and 6, respectively). *Tetranychus urticae* glutamate-gated chloride channel (*GluCl1*, *GluCl3*) genes and chitin synthase 1 (*CHS1*) gene amplified products (263 bp, 251 bp, 541 bp), using primer sets (7, 8, and 9 respectively). EtBr was added to the gel before electrophoresis to a final concentration of 0.5 µg/ml, followed by separation at 100 V for 1 h. The gel was exposed to UV light and the picture was taken with a gel documentation system. Letter a: sample 1, letter b: sample 2, (-): no DNA templates. M:100 bp DNA ladder.

Table 5 presents the comparison of amino acids in Eg-Bernasht field population and GenBank databases populations. The two common mutations associated with avermectin pesticides were absent in Eg-Bernasht population (G314D and G326E), with two substitutions: glycine to aspartic acid at position 314 and glycine to glutamic acid at position 326.

Also, in one clone of Eg-Bernasht population, two substitutions of amino acids: alanine to leucine at position 308 (A308L) and valine to leucine at position 309 (V309L) were identified. While, in case of Eg-Bernasht population, *CHS1* gene did not detect any common mutations associated with etoxazole pesticides.

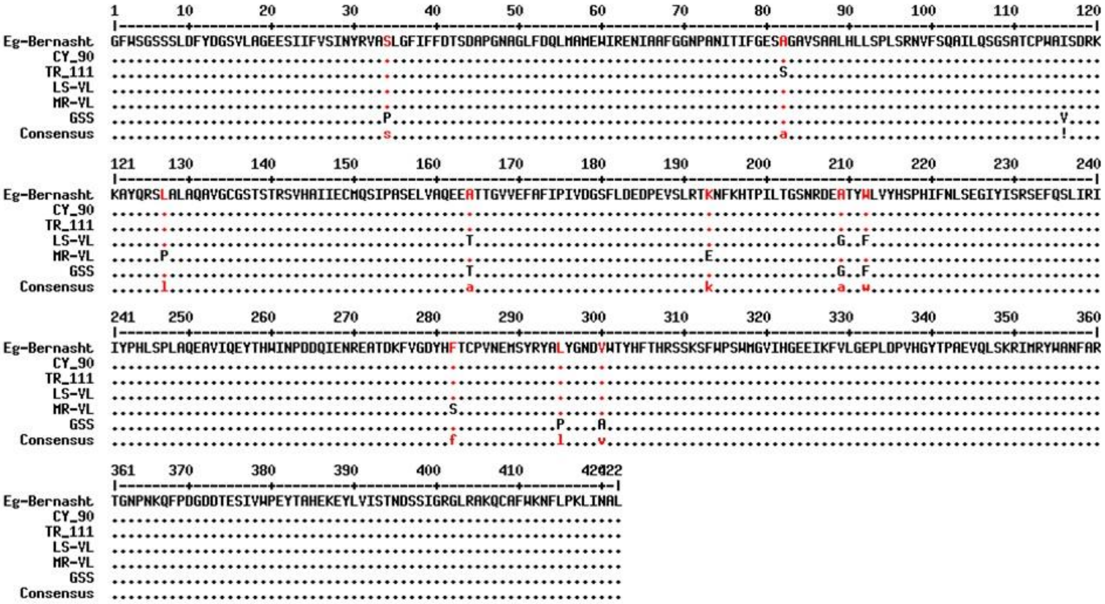

Figure 2. Multiple alignment of the amino acid sequences of *T. urticae* acetylcholinesterase (*AChE*) between Eg-Bernasht population and GenBank published populations. *Tetranychus urticae* AChE sequences were performed local as well as global alignments, using BLASTX search protein databases, using a translated nucleotide (NCBI). Three different specific primer sets (1, 2, and 3; Table 1) contained 422 amino acids of *AChE* gene. Dots: indicate amino acid similarity. Digital number: amino acid position on the AChE protein.

Table 3. Comparison of amino acid sequence polymorphism of *T. urticae* acetylcholinesterase (*AChE*) gene between Eg-Bernasht field population and populations in GenBank databases.

Populations-clones (GenBank accession)	**Sources and Host plant**	**Country**	**Year**	**Mutation position in acetylcholinesterase (*AChE*) gene**			**Reference**
				(T280A)* **(391)****	**(G328A)*** **(436)****	**(F331W/y)*** **(439)****	
Eg-Bernasht-1©	Field (Tomato)	Egypt	2014	A	A	W	This study
Eg-Bernasht-2©				A	A	W	
Eg-Bernasht-3©				A	A	W	
CN_128 (AHX97744)	Field (Eggplant)	China	2011	T	G	F	Ilias *et al.* (2014)
CY_90 (AHX97740)	Field (Eggplant)	Cyprus	2010	A	A	W	
TR_111 (AHX97742)	Field (Tomato)	Turkey	2011	A	A	W	
IL_120 (AHX97741)	Field (Squash)	Israel	2011	A	G	W	
ES_131 (AHX97745)	Field (Clementine)	Spain	2011	A	G	W	
CY_91 (AHX97751)	Field (Tomato)	Cyprus	2010	T	G	W	
ZA_147 (AHX97747)	Field (Apple)	South Africa	2012	T	G	W	
GR_81 (AHX97752)	Field (Citrus)	Greece	2010	A	G	Y	
CY_92 (AHX97743)	Field (Bean)	Cyprus	2010	T	G	Y	
KE_155 (AHX97754)	Field (Bean)	Kenya	2012	T	G	Y	

* Numbers refer to the position in mature *AChE1* of *Torpedo californica* (Massoulié *et al.* 1992).
© Not recorded in GenBank.
** Numbers in brackets refer to the position in *T. urticae AChE1* precursor (Khajehali *et al.* 2010).

Table 3. Continued.

Populations-clones (GenBank accession)	**Sources and Host plant**	**Country**	**Year**	**Mutation position in acetylcholinesterase (*AChE*) gene**			**Reference**
				(T280A)* **(391)****	**(G328A)*** **(436)****	**(F331W/y)*** **(439)****	
MR-VL (GQ461339)	Greenhouse (Beans & Ornamentals)	Belgium	2003	A	A	W	Khajehali *et al.* (2010)
ATHRos-Pm (GQ461345)	Greenhouse (Roses)	Greece	2007	A	A	W	
GSS (GQ461348**)**	Lab (BayerCropScience)	Germany	1965	T	G	F	
LS-VL (GQ461336)	Lab (Roses)	Belgium	2000	T	G	F	
SAMB (GQ461344)	Lab (Sambucus)	Netherlands	1965	T	G	F	

* Numbers refer to the position in mature *AChE1* of *Torpedo californica* (Massoulié *et al.* 1992).
© Not recorded in GenBank.
** Numbers in brackets refer to the position in *T. urticae AChE1* precursor (Khajehali *et al.* 2010).

Table 4. Comparison of amino acid sequence polymorphism of *T. urticae* voltage-gated sodium channel *(VGSC)* gene between Eg-Bernasht field populations and the reference GenBank and Ilias *et al.* (2014) populations.

Populations-clones (Genbank accession)	**Mutation position in voltage-gated sodium channel *(VGSC)* gene**					**Reference**
	(L1024V)*	**(A1215D)***	**(F1538I)***	**(D1569E)****	**(L988V)****	
Eg-Bernasht-1©	L	D	F	E	V	This study
Eg-Bernasht-2©	L	D	F	E	V	
Eg-Bernasht-3©	L	D	F	E	V	
S (AFU35097)	L	-	F	D	L	Gao *et al.* (2011)
R-Fe (AFU35098)	-	A	I	D	L	
GS Fen-R (ADB92110)	L	A	F	D	L	Shen *et al.* (2009)
GSS (ACR14823)	-	A	F	D	-	Tsagkarakou *et al.* (2009)
CREVeg-Bf (ACR14822)	-	-	I	D	L	
ATHRos-Bf (ACR14821)	-	D	-	-	-	
ATHRos-Bf (ACR14825)	-	D	-	-	-	
CREVeg-Bf (ACR14826)	L	D	-	-	L	
JP_143©	L	D	I	-	-	Ilias *et al.* (2014)
GR_82©	L	D	F	-	-	
CY_114©	V	D	F	-	-	
FR_80 ©	L	A	I	-	-	
GE_GSS©	L	A	F	-	-	
IR_159©	L	A	I	-	-	
KE-154©	L	D	I	-	-	

\- Not examined.
© Not recorded in GenBank.
* Common mutation and numbers refer to the position in *Musca domestica* according to Ilias *et al.* (2014).
** New mutation.

Table 5. Comparison of amino acid sequence polymorphism of *T. urticae* glutamate-gated chloride channel (*GluCl1*, *GluCl3*) genes and chitin synthase 1 (*CHS1*) gene between Eg-Bernasht field population and the reference GenBank and Ilias *et al.* (2014) populations.

	Mutation position					
Populations-clones (Genbank accession)	**glutamate-gated chloride channel (*GluCl1*, *GluCl3*) genes**				**Chitin synthase 1 (*CHS1*) gene**	**Reference**
	(A308L)**	**(V309L)****	**(G314D)***	**(G326E)***	**(I1017F)***	
Eg-Bernasht-1©	L	L	G	G	I	This study
Eg-Bernasht-2©	A	V	G	G	I	
Eg-Bernasht-3©	A	V	G	G	I	
ISR (AGA16509)	A	V	G	-	-	Memarizadeh *et al.* (2012)
Marathonas (AFG29908)	-	-	-	E	-	Dermauw *et al.* (2012)
Marathonas (AFG29906)	A	V	D	-	-	
005 (AFG28414)	-	-	-	-	I	Van Leeuwen *et al.* (2012)
GSS (AFG28413)	-	-	-	-	I	
EtoxR (AFG28419	-	-	-	-	F	
005 (AFG28416)	-	-	-	-	F	
London (AFG28412)	-	-	-	-	I	
TuSB9 (AFG28418)	-	-	-	-	F	
JP_143©	-	-	G	G	F	Ilias *et al.* (2014)
GR_82©	-	-	D	E	F	
CY_114©	-	-	G	G	I	
FR_80©	-	-	G	G	F	
GE_GSS©	-	-	G	G	I	
IR_159©	-	-	G	E	I	
KE-154©	-	-	G	G/E	F	

- Not examined.

© Not recorded in GenBank.

* Common mutation and numbers refer to the position in *Musca domestica* according to Ilias *et al.* (2014).

** New mutation.

DISCUSSION

Susceptibility of TSSM to the tested pesticides varied significantly between Eg-Bernasht population and the susceptible population. These findings confirm resistance of Eg-Bernasht population to pesticides, with exhibiting the higher LC_{50} values especially Ops, such as chlorpyrifos and dimethoate compared with the susceptible population. These outcomes are consistent with those obtained by Brown *et al.* (2017), who reported that two populations of *T. urticae* in Louisiana were highly resistant to abamectin, with corresponding LC_{50} values of 0.082 and 0.184 ppm, and resistance ratios of 630 and 1415-fold. In contrast, one population from Mississippi was slightly resistant, with an LC_{50} value of 0.0021 ppm, and a resistance ratio of 11.1 compared with a susceptible control population. Also, Sato *et al.* (2005) reported a resistance ratio of 25 against abamectin for a population of TSSM collected from a strawberry farm in Brazil compared with a susceptible population. Similarly, Yang *et al.* (2001) observed that TSSM exposed to dimethoate developed 15.9-fold resistance level to bifenthrin compared to non-selected mites.

Known resistance mechanisms in TSSM are like other arthropods, which include enhanced glutathione S-transferase (GST), cytochrome P450-dependent monooxygenases (MFT), reduced penetration of acaricides and insecticides, and target site resistance (Knowles 1997; Stumpf and Nauen 2001).

Finally, very high resistance levels of TSSM to OPs were observed in Eg-Bernasht population. The resistance of *T. urticae* to pesticides may be due to the frequent use of pesticide, especially those based on the use of pesticides of the same group.

Also, we assessed the target mutations conferring resistance to five different classes of acaricides in a relatively small geographic scale (Bernasht village, El-Ayat, Giza Governorate, Egypt). The application history of the acaricides tested was unknown.

Many insecticide resistance mechanisms have been elucidated at the molecular level, particularly ones associated with alterations in the target sites of insecticides. Target site resistance mutations include L1024V and A1215D in the voltage-gated sodium channel (*VGSC*) gene, G314D and G326 in glutamate-gated chloride channel (*GluCls*) gene, G126S, 1136T, S141F, D161G, and P262T in the cytochrome b (*cytb*) and 11017F in the chitin synthase 1 (*CHS1*) gene (Van Leeuwen *et al.* 2008, 2012; Tsagkarakou *et al.* 2009; Van Nieuwenhuyse *et al.* 2009; Khajehali *et al.* 2010; Kwon *et al.* 2010a, b, c; Dermauw *et al*. 2012; Ilias *et al.* 2014).

In *T. urticae* only, one *AChE* gene occurs, which shares higher sequence similarity with *AChE1* gene of insects (Grbić *et al*. 2011). Five OP resistance mutations (G119S, A201S, T280A, G328A, and F331W) have been detected in the *AChE* gene in *T. urticae* (Ilias *et al*. 2014). To date, no explicit data exist associating the presence and frequency of each of the five mutations or their combinations with different OP resistant phenotypes neither with the OP selection pressure. Kwon *et al*. (2010c, 2012) reported that extensive *ace* duplication is another molecular mechanism of OP resistance in *T. urticae*.

Khajehali *et al.* (2010) examined the role of F331W and T280A in the AChE activity and insensitivity towards four OPs and one carbamate. Further, Kwon *et al.* (2012) characterized the kinetic properties of G119S, T280A, and F331W, and their combination based on the functional expression of various *T. urticae* AChE mutants. They determined that both mutations in the positions 119 and 331 were responsible for the *AChE* gene insensitivity towards monocrotophos, whereas T280A did not affect kinetics, but restored the catalytic reduction, thus, seemed to compensate for the fitness cost introduced from the other two mutations.

The study of Kwon *et al.* (2014) revealed that resistance to monocrotophos, fenpropathrin, and abamectin in *T. urticae* was primarily conferred by reduced sensitivity of respective target sites [i.e., acetylcholinesterase (TuAChE), voltage-sensitive sodium channel (TuVSSC) and glutamate-gated chloride channel (TuGluCl)], which are due to point mutations (G228S and F439W in TuAChE; L1022V in TuVSSC; G323D in TuGluCl). The two TuAChE mutations responsible for monocrotophos resistance were almost saturated in most field populations. The TuVSSC L1022V mutation tentatively associated with fenpropathrin resistance was also found in 9 field populations.

Comparison of resistant and susceptible *T. urticae* populations revealed that F1538I and L1024V were associated with pyrethroid resistance (Tsagkarakou *et al.* 2009). In this context, L1022V mutation has been proposed to play a major role in knockdown resistance to fenpropathrin, with a frequency of 24 % (Kwon *et al.* 2010a). In a recent monitoring study, Kwon *et al.* (2014) detected the L1024V mutation in a similar frequency in samples from the Republic of Korea. Surprisingly the A1215D substitution was the most frequent. The same pattern (high frequency of the A1215D mutation) was also observed in field collected samples from the Netherlands (Khajehali *et al.* 2011).

The TuGluCl G323D mutation conferring abamectin resistance was found only in a few field populations, suggesting that abamectin resistance is not yet widespread (Kwon *et al*. 2014). The low frequency of abamectin target site resistance mutations is in line with the limited number of resistance reports (Van Leeuwen *et al*. 2010).

In *CHS1* gene, the putative target of chitin synthase inhibitors such as etoxazole, the I1017F resistance mutation was found relatively widespread. This mutation has been recently associated with extremely high levels of etoxazole resistance scaling up to approximately 375,000 folds and found in a number of resistant populations derived from different locations (Van Leeuwen *et al.* 2012).

CONCLUSION

Previous experiences have underscored the benefits of alternating pesticide regimes for regaining control over outbreak-level of a given pest (Shaurub *et al*. 2021). To accomplish this, knowledge of the extent of susceptibility of *T. urticae* populations in different geographical areas is critical for measuring the trends in temporal and spatial resistance development of this pest. Resistance is also dependent on availability of refugia for susceptible populations, strength of selection, fitness cost of resistance alleles, and gene flow between populations (Hemingway *et al*. 2002). Studying the genetic structure of *T. urticae* population together with the identification of resistance genes will allow a better understanding of the evolution of resistance. In Egypt, overcoming resistance in *T. urticae* requires holistic management and the judicious use of new chemistries, such as juvenile hormone analogues and neonicotinoids, in parallel with routine monitoring of resistance levels to the diverse chemistries available. Together, such practices will help in the implementation of integrated management measures on an area-wide basis.

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عدم حســاسیت محل هدف به برخی کنه‌کش‌ها در جمعیت مزرعه‌ای *Tetranychus urticae* Koch (Acari: Tetranychidae)، از مصر

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چکیده

مهار کنهٔ تارتن دو لکه‌ای (TSSM)، *Tetranychus urticae* Koch، آفت کشاورزی در سراسر جهان، تا حد زیادی به آفت‌کش‌ها وابسته است. با این حال، اثربخشی آنها اغلب با ایجاد مقاومت مواجه می‌شود. مطالعه حاضر به منظور ارزیابی سطوح مقاومت به پنج آفت‌کش با حالت‌های مختلف عمل در جمعیت مزرعه‌ای *T. urticae* جمع‌آوری‌شده از بوته‌های گوجه‌فرنگی کشت‌شده در مزارع روباز روستای ایگ–برنشت، الآیات، استان غزه، مصر با استفاده از روش غوطه‌وری برگ انجام شد. این آفت‌کش‌ها عبارت بودند از: دیمتوات، کلرپیریفوس و اتیون (فسفره‌های آلی)، اتوفنپروکس (پیرتروئید)، و آبامکتین (اورمکتین). افزون بر این، جهش‌های مقاومت رایج مرتبط با گروه‌های مختلف آفت‌کش‌ها در *T. urticae* با توالی‌یابی کمی شناسایی شد. نتایج نشان داد که مقاومت بسیار زیادی در کنهٔ تارتن دو لکه‌ای به آفت‌کش‌های فسفره‌های آلی در جمعیت ایگ–برنشت مشاهده شد. همچنین، توالی‌های نوکلئوتیدی هم‌تراز، مگابلاست شدند و برای شباهت با ژن‌های مربوط در پایگاه‌های داده مختلف مانند مجموعه نوکلئوتیدی NCBI (nr/nt) مقایسه شدند. از پنج جهش رایج مرتبط با آفت‌کش‌های فسفرهٔ آلی و کاربامات‌ها، سه جهش در جمعیت ایگ–برنشت شناسایی شد. باقی‌مانده ترئونین با آلانین (T280A)، گلیسین با آلانین (G328A) و فنیل آلانین با تریپتوفان (F331W) جایگزین شد. یکی از سه جهش رایج مرتبط با آفت‌کش‌های پیرتروئید، آلانین با اسید آسپارتیک (A1215D) جایگزین شد. دو جایگزین دیگر از اسیدهای آمینه، لوسین به والین (L988V) و اسید آسپارتیک به اسید گلوتامیک (D1569E) شناسایی شد. جهش‌های رایج مرتبط با محل هدف اورمکتین‌ها (کانال‌های کلرید دردار با گلوتامات، *GluCl1*، *GluCl3*) و آفت‌کش اتوکسازول (کیتین سنتاز، *CHS1*) در جمعیت ایگ–برنشت وجود نداشت. همچنین با استفاده از ژن‌های کانال کلرید دردار گلوتامات در یک کلون از جمعیت ایگ–برنشت، دو جایگزینی آلانین به لوسین (A308L) و والین به لوسین (V309L) شناسایی شد. نتایج به‌دست‌آمده از این پژوهش نشان داد که چهار جهش رایج مرتبط با مقاومت به آفت‌کش‌ها در جمعیت مزرعه‌ای *T. urticae* شناسایی شد. این جهش ها T280A، G328A و F331W در ژن استیل کولین استراز (*AchE*) و A1215D در ژن کانال سدیم دارای ولتاژ (*VGSC*) بودند. درک بهتر از مقاومت به کنه‌کش‌ها در کنهٔ تارتن دو لکه‌ای برای حفظ عمر مفید این مواد شیمیایی برای کنترل این آفت در گوجه‌فرنگی و سایر محصولات مهم است. توصیه می‌شود برای مدیریت مقاومت بر استفاده از برنامه‌های مدیریت تلفیقی آفات و تناوب آفت‌کش‌ها تمرکز شود.

واژگان کلیدی: کنه‌کش‌ها؛ توالی‌یابی کمی؛ مقاومت؛ جهش نقطه محل هدف؛ کنهٔ تارتن دو لکه‌ای.

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