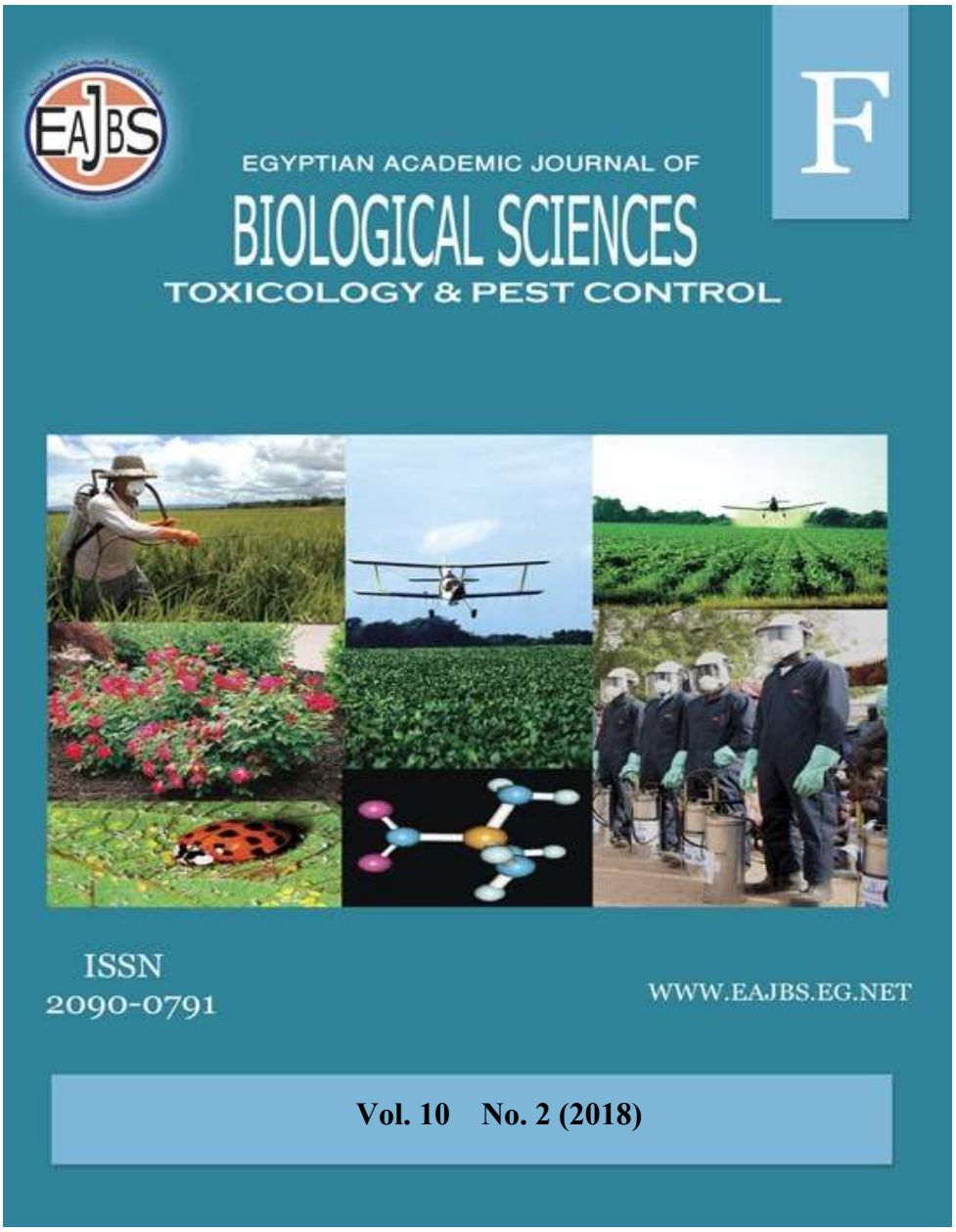

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www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 10(2): 89– 97(2018)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest control
ISSN: 2090 - 0791
www.eajbs.eg.net

Resistance of *Tetranychus uraticae* Koch to Some Compounds in Cotton Fields And Biochemical Resistance Mechanisms

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ARTICLE INFO

Article History
Received:15/11/2018
Accepted:22/12/2018

Keywords:
Tetranychus uraticae Koch, insecticide /acaricide résistance, resistance mechanisms.

ABSTRACT

The field populations of the two-spotted spider mite, *Tetranychus uraticae* Koch were collected from cotton fields at Sakha Agric. Res. Station Farm Kafr El-Sheikh, Egypt in 2017 season. Five compounds belonged to different groups were assayed by the leaf disc dip technique to determine levels of resistance to these compounds in addition to the activity of some detoxification enzymes. The field populations of *T. uraticae* showed variation in susceptibility to tested compounds, abamectin was the most toxic one, while chlorpyrifos and deltamethrin were the least toxic against laboratory and field populations. Moreover, the resistance ratios of fenpyroximate exhibited the high resistance level, while abamectin exhibited moderate resistance. Lower resistance levels were observed for Chlorfenapyr, deltamethrin and chlorpyrifos. Based on the susceptibility factor values, the tested compounds showed variation in susceptibility factor values, it's were exhibited low effective which susceptibility factor values were more than 0.5. On the other hand, the specific activity of mixed function oxidase and carboxylesterase were higher in field populations than that in laboratory strain. In contrast, there is an insignificant difference in glutathione-s-transferase activity between field and laboratory strain. The obtained results could be used as a basis for future resistance T. uraticae monitoring program in cotton fields

INTRODUCTION

The two-spotted spider mite, *Tetranychus uraticae* Koch (Acari: Tetranychidae) is one of the economically most important pests that causes significant damage to cotton, maize, ornamentals, vegetables, flowers, legumes, deciduous tree, citrus, etc. (Jappson *et al*., 1975; Helle and Sabelis, 1985 and Kasap,2005). This species is often difficult to manage because of its high reproductive potential, very short life-cycle and arrhenolokous reproduction, which support frequent acaricides and insecticides applications that often Leeds to development of resistance very rapidly (Luczynzki *et al.*, 1990 and Van Leeuwen *et al*., 2010). *T. uraticae* control has been used large a number of compounds with different chemical structure and mode of action such as organophosphates, pyrethroids and specific acaricides including Mitochondrial Electron Transport inhibitors (METIs), organotins and ketoenols. Due to widespread intensive applications of acaricides and insecticides, *T. uraticae* have developed a different

level of resistance to these acaricides/insecticides (Knowles, 1997; Dekeyser, 2005 and Van Leeuwen *et al*., 2009). The development of insecticide resistance is influenced by many factors, as genetics, biology /ecology and control operations (Georghiou and Taylor,1977).

There are many possible adaptations that statement an insect or mite to survive lethal doses of an insecticide/acaricide. These are classified based on their biochemical or physiological properties, such as mechanisms of decreased response to pesticides or mechanisms of decreased exposure (penetration, distribution, enhanced metabolism and excretion)(Roush and Tabashnik, 1990; Feyereisen,1995 and Taylor and Feyererisen,1996), and enhance metabolic detoxification by esterases ,glutathione-s- transferases,P450 monooxygenases are of major enzymes in resistance to insecticides in *T. uraticae* (Enayati *et a*l.,2005 and Li *et al*.,2007). Therefore, the continuous monitoring of *T. uraticae* resistance to different insecticides might be conducted reasonable scale to identify the efficiency of these insecticides, also it should be helpful in developing an integrated pest management program for *T. uraticae* control. The present study aimed to provide information to monitor the resistance of *T. uraticae* collected from cotton fields at Kafr El-Sheikh Governorate during 2017 cotton season to five compounds belonging to different groups, also the study included determination of some detoxification enzymes activity.

MATERIALS AND METHODS

Rearing Mite:

Two strains of the two-spotted spider mite, *Tetranychus uraticae* Koch, were used in this study. The laboratory strain was originated from unsprayed cotton fields at Sakha Agric. Station Farm and reared on sweet potatoes cuttings holding about 5 leaves placed in glass jars 50 ml containing tap water and renewed every 72 h when they get yellow color under laboratory conditions (25±2 C^0; 65±5% RH and 16 L:8 D photoperiod) as described by Dittrich (1962). Mites were transferred from old to young plants by cutting heavily infested leaves into small sections which were then placed on new plants. These colonies were kept on the rearing seedlings for one year without any exposure to insecticides, this laboratory strain was used as a reference strain, while the field strain was collected from cotton fields at Sakha Agric. Res. Station Farm, Kafr El-Shiekh, Egypt in August 2017 season and transferred to the laboratory to evaluate the toxicity tests.

Chemical Used:

In this study, the commercial formulations of the tested compounds were used
Fenpyroximate (Ortus 5% EC, NichinoAmericoAncor-Purishn), at the rate of 25 mg AL /l (ppm)
Chlorfenapyr (Challenger 36% SC, BASF Corpor), at the rate of 16.2 mg AL /l
Abamectin (Vertimec 1.8% EC, Syngenta Agro), at the rate of 7.2 mg AL /l
Chlorpyrifos (Dursban 48% EC, Dow Agro Sciences), at the rate of 16oo mg AL /l
Deltamethrin (Decies 2.5 % EC, Bayer Co.), at the rate of 29.2 mg AL /l

Toxicity Tests:

The leaf disc dip technique described by Pree *et al*., (1989) was used to compare the susceptibility of the laboratory and field colonies of *T. uraticae* to the tested compounds. Serial concentrations for each compound were prepared in distilled water. The sweet potato leaf discs (2 cm in diameter) were dipped in concentrations (mg AL /l) of various insecticides/acaricides for 10 seconds and left to dry, the discs

were put on wet cotton wool in Petri-dishes. Each concentrate was replicated four times. For each strain of *T. uraticae* ten adults' female stage (one day old) were transferred on each disc with a fine brush and kept under constant conditions (25±2 C^0; 65±5% RH and 16 L:8 D photoperiod). Mortality was recorded after 24 h from treatment, as mites were considered dead if appendages did not move when prodded with camels' hairbrush. Data were corrected for control mortality using Abbotts formula (Abbott,1925). The LC50, LC90 values and slopes for the tested compounds were estimated by probit analysis according to Finney (1971). A resistance ratio (RR) was determined by dividing LC50 of the field strain over that of the laboratory strain according to Georghiou (1972). Populations with an RR values of <20,20-50 and > 50 were classified as, low, moderate and high resistance, respectively according to Kerns and Gaylor(1992).

Enzymes Preparation:

To determine the activity of mixed function oxidase (MFO), carboxylesterase, glutathione-s-transferase (GST) and total protein in the field strain and laboratory strain. The adult mite females pre-starved for 6 hours, mite females (one day old), 500 adult females were homogenized in 2.5ml of 0.1 M phosphate buffer, pH 7.0 containing 0.05%(v/v) Triton-x-1oo on ice using a glass homogenizer. The homogenate was centrifuged at 8000 rpm for 15 minutes at 2 C^0in a refrigerated centrifuge. The supernatant was kept in the deep freezer at -20 C^0 until determination. The mixed function oxidase (MFO) activity was determined according to a slight modification of Hansen and Hodgson (1971), carboxylesterase activity was measured according to Simpson *et al*. (1964), glutathione-s-transferase (GST) by (Habig *et al*.,1974) and total protein (Bradford, 1976).

Statistical Analysis:

Data were analyzed statistically using t-test at p-value < 0.05 through SPSS computer program (2006).

RESULTS AND DISCUSSION

The field populations of the two-spotted spider mite, *Tetranychus uraticae* were collected from cotton fields at Kafr El-Sheikh Governorate during 2017 season and assayed to determine the levels of resistance to five compounds belonging to different groups, also the study involved the determination of some detoxification enzymes activity.

Susceptibility of *T. uraticae* to Tested Compounds:

Data in Table (1) revealed that abamectin was proved to be the most toxic compound against the laboratory strain of *T. uraticae* giving LC_{50} of 0.93 mg AI/l followed by fenpyroximate and Chlorfenapyr with LC_{50} values were 3.74 and 18.14 mg AI/l, respectively, while chlorpyrifos and deltamethrin were the least toxic compounds with LC_{50} values of 116.49 and 154.09 mg AI/l, respectively. The field strain of T. *uraticae was* collected from the cotton field during 2017 season was less susceptible to all tested compounds, this may be due to the indirect effect of these compounds used to control the different cotton pests in all cotton season. Abamectin was the most toxic one with LC_{50} value of 18.7 mg AI/l, while chlorpyrifos and deltamethrin was the least toxic one giving LC_{50} values of 455.05 and 696.67 mg AI/l, respectively. Chlorfenapyr and fenpyroximate exhibited moderate effect with LC_{50} values of 216.47and 242.71 mg AI/l, respectively without significant differences because of overlapping between their confidence limits. These results agreed with the finding of Farage (2011); Abd El-Mageed *et al*. (2013) and Kumari

et al. (2017) they found that abamectin was the most toxic to adults of *T. uraticae* followed by fenpyroximate and Chlorfenapyr.

However, the slope value is considered a good parameter for a reaction indicator between the tested compounds and target organism, which the high slope value means more homogeneity in the organism response towards the compound, while the low slope value indicates heterogeneity. The present data in Table (1) indicated that the highest degree of homogeneity for laboratory populations of *T. uraticae* was obtained towards fenpyroximate and deltamethrin with the slope values of 2.35 and 2.29, respectively, while abamectin, chlorfenapyr and chlorpyrifos exhibited the low slope values, this indicates heterogeneity in the mite response to these compounds. As for the field strain of *T. uraticae*, the populations reflected different degree of homogeneity in response to the tested compounds, in other words, the field strain was higher homogeneity in the response to tested compounds more than those of the laboratory strain, which means that the pesticide might have acted as a selection factor producing an organism strain produce an organism strain as genetically pure as possible. Regarding of resistance ratio, the laboratory strain was used as susceptible strain, the results in Table (1) revealed that the field strain of *T. uraticae* showed low resistance to chlorpyrifos, deltamethrin and Chlorfenapyr with resistance ratio of 3.91,4.52 and 11.93-fold, respectively, while abamectin exhibited moderate rate of resistance, as resistance ratio was 20.04-fold. Also, it was indicated that mite population recorded resistance ratio by 64.95-fold with fenpyroximate. This result might be due to the annually wide use of fenpyroximate and repeated application of this compound against cotton pests and other arthropods at Kafr El-Sheikh Governorate. The obtained results agreed with findings by Cho *et al*. (1995) and Goka (1998) they found that the high-level resistance of *Tetranycus. spp* with fenpyroximate, on the other hand, Kim *et al. (*2007) showed that high level of *T. uraticae* resistance to fenpyroximate, while abamectin resistance was moderate resistance rate. However, abamectin resistance in *T. uraticae was* reported worldwide (Campos *et al*.,1995; Stumpf and Nauen ,2002 and Kwon *et al.*,2010). In some of the studies, it was shown that resistance rate was related to the number and frequency of chemicals applied in a productive season and to the continuous use of compounds having the same effect mechanism (Campos *et al*.,1995).

From, the mentioned results, it can be concluded that the low slope value in mite population resistance to abamectin and Chlorfenapyr are associated by those of Hoskins and Gorden (1956) who reported that the first song in developing resistance is the decrease in the slope value. On the other hand, the slope values of the other tested compounds did not coincide with the level of resistance ratio.

Table (1): Toxicity of certain insecticides against laboratory strain and field strain of *Tetranychus uraticae* Koch collected from cotton fields during 2017 season under laboratory conditions

Insecticide	Laboratory strain			Field strain of 2017			R. R.**
	LC50 (mg AI/l)	C.L. of LC50*	Slope ± SE	LC50 (mg AI/l))	C.L. of LC50*	Slope ± SE	
Abamectin	0.93	0.16-1.67	1.26±0.35	18.7	14.85-23.24	1.99±.29	20.04
Chlorfenapyr	18.14	12.06-25.67	1.3±0.26	216.47	145.54-286.1	1.74±0.29	11.93
Fenpyroximate	3.74	2.98-4.59	2.35±0.32	242.71	205.15-290.3	2.55±.48	64.95
Chlorpyrifos	116.49	81.36-239.15	1.65±0.20	455.05	334.65-846.83	1.55±0.45	3.91
Deltamethrin	154.09	119.69-191.89	2.29±.27	696.67	546.82-852.04	2.48±.47	4.52

C.L. of LC50*= confidence limits R.R.**= resistance ratio ppm (mg AI/l)

The prophesy the ability of the tested insecticides at their recommended field rates (diluted in 300 water/ Feddan) to control *T. uraticae* population in cotton fields, susceptibility factor was calculated as described by Nazer *et al*. (1983), where the insecticides expected to be effective under field conditions are those having values less or equal to 0.5 only. The results in Table (2) showed variation in susceptibility factor related to the tested compound. From the above results, the all tested compounds exhibited low effect, where susceptibility factor values were more than 0.5. However, the susceptibility factor can be perfected by reducing the total spray volumes. Therefore, it can be concluded that using newer compounds with acaricidal activity should be applied to avoid control failures.

Table (2): Susceptibility factor of some compounds against colonies of *Tetranychus uraticae* Koch.collected from cotton fields during 2017 season at Kafr El-Sheikh Governorate

Insecticide	Recommended field conc. (mg AI/l)	LC90 of field strain	Susceptibility factor
Abamectin	7.2	82.45	11.45
Chlorfenapyr	162	1175.15	7.25
Fenpyroximate	25	773.99	30.96
Chlorpyrifos	1600	3057.6	1.9
Deltamethrin	**29.2**	**2289.02**	**78.39**

Susceptibility factor = LC_{90} of tested insecticide from LCP line/ Recommended field concentration (mg AI/l)

The specific activity of some enzymes in field and laboratory strains of *T. uraticae*:

Enzymes play a major role as mechanisms in insecticides/ acaricides resistance of mites, thus the specific activity of mixed function oxidases (MFO), glutathione-s-transferase (GST) and carboxylesterase was determined in laboratory and field strains, the data were presented in Table (3).

Mixed function oxidase (MFO):

The obtained data in Table (3) revealed that the specific activity of mixed function oxidase was significantly higher in field strain than that of laboratory strain by 1.16- fold, these results are in accordance with the resistance factors in Table (1). The increase oxidative detoxification via MFO might be involved METI (mitochondria electron transport inhibitor) acaricides resistance likely contribute to fenpyroximate and chlorfenapyr resistance in field strain, so MFO plays a major role in fenpyroximate and chlorfenapyr resistance in field populations of mites. The obtained results are agreed with those by Kim *et al* (2004) who Suggested that enhanced activities of both mixed function oxidase and esterase as confer with fenpyroximate resistance of the FR-20 strains of *T. uraticae,* furthermore Stumpf and Nauen (2002) founding that MFO and GST may be involved in abamectin resistance.

Table (3) Detoxifying enzyme activities in laboratory and field populations of *Tetranychus uraticae* Koch collected from cotton fields during 2o17 season at Kafr El-Sheikh Governorate

Enzyme	**Laboratory strain ± SD**	**Field strain± SD**	**enzyme activity[4]**	**R/S[5]**	**T-value**
MFO[1]	46.57 ± 3.5	54.13 ± 7.97	+16.23	1.16	2.86*
GST[2]	337.13 ± 9.82	345.93 ± 14.64	+2.61	1.03	0.86^{ns}
Carboxylesterase[3]	73.0 ± 6.25	138.67 ± 8.08	+89.96	1.9	10.47**

ns: the result is not significant at p <0.05 *: significant at p <0.05 **: high significant at p < 0.05

[1]Expressed as (u mol sub oxidized /min/ mg protein) [2]Expressed as (n mol sub conjugated /min/ mg protein)

[3]Expressed as (ug Meb/min /mg protein) [4]Value in brackets mean an increase (+%) or decrease (-%) in enzyme activity

[5]R/S, enzyme activity of field strain / enzyme activity of laboratory strain

Glutathione-s-transferase (GST):

GST in insects plays an important role in detoxification of insecticides (Clark, 1989). Data in Table (3), cleared insignificant differences in glutathione-s-transferase activity between the laboratory and field strains. Leading to the conclusion that this enzyme was no a major defense mechanism in the resistant strain of mite. The same results were found by Kim *et al*.,2004) who reported that was insignificant differences in GST activity between the S and FR-20 strains. In contrast, Farag (2011) and Memarizadeh *et al*. (2013) showed that GST activity was significantly higher in resistant strains of *T. uraticae* than those of the susceptible strain.

Carboxylesterase:

Esterase's play an important role in conferring or contributing to insecticides detoxification in insect and arthropod species (Motoyama and Dauterman (1974) and Saleh et al. (198٦). Data in Table (3) cleared that the specific activity of carboxylesterase was significantly increased in field strain recording 1.9-fold higher than that of laboratory strain, this means that carboxylesterase might be due to a major role in mechanisms detoxification in resistance strain of mite. These results agree with the finding of Gerson et al. (1991); Kim *et al*. (2004) and Memarizadeh *et al*. (2013) they found that esterases are associated with resistance to insecticides/ acaricides in mites.

Generally, mechanisms of resistance to insecticides/ acaricides in mite species are found to be reduced penetration, target site insensitivity (altered acetylcholinesterase and nerve insensitively) and enhanced metabolic detoxification by MFO, GST and/ or esterases has been considered as the main factor in acaricides resistance of mites.

From these results, it can be concluded that the field population of *T. uraticae* showed variation in susceptibility to the tested compounds, abamectin was the most toxic one, while chlorpyrifos and deltamethrin exhibited the least toxic effect against both laboratory and field populations. On the other words, fenpyroximate showed high resistance rate, while abamectin recorded moderate level resistance. The activity of MFO and carboxylesterase were higher in field populations than that in laboratory strain of mite. The obtained data can be used as a basis for future resistance *T. uraticae* monitoring program in cotton fields.

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ARABIC SUMMARY

مقاومة العنكبوت الأحمر لبعض المركبات المستخدمة فى حقول القطن وفاعلية الأنزيمات فى ازالة السمية

مديحة الصباحي حامد الديوى
معهد بحوث وقاية النباتات ـ مركز البحوث الزراعية ـ الجيزة ـ مصر

يهدف البحث لدراسة مستوى مقاومة العنكبوت الأحمر لخمس مبيدات تنتمى لمجموعات كيماوية مختلفة وذلك فى السلالة الحقلية التى تم جمعها من حقول القطن فى موسم القطن ٢٠١٧ م بمزرعة البحوث الزراعية بسخا وذلك بطريقة غمر الدسكات فى محاليل المبيدات المختبرة وتم دراسة النشاط النوعى لبعض الأنزيمات التى تلعب دورا هاما فى ازالة سمية هذه المبيدات ، حيث اوضحت النتائج بناءا على التركيز القاتل ل ٥٠% من الأفراد المعاملة تفاوت فى حساسية السلالة الحقلية والمعملية للعنكبوت الأحمر تجاه المبيدات المختبرة فكان مبيد ابامكتين اكثر المبيدات فاعلية بينما كلوربيروفوس ودلتاميثرن اقلها سمية. أظهرت السلالة الحقلية درجةعالية من المقاومة لمبيد فنبيروكسيمات ومقاومة متوسطة لمبيد ابامكتين ، وسجلت مبيدات كلورفنبير ودلتاميثرن وكلوربيروفوس أقل مستوى مقاومة خلال موسم الدراسة .أشارت النتائج بناءا على معامل الحساسية (التنبؤ بفاعلية المركبات عند استخدامها فى الحقل) انخفاض فى فاعلية المركبات المختبرة حيث كان معامل الحساسية اكبر من٠,٥، وكان النشاط النوعى لأنزيمى mixed function oxidase and carboxylesterase مرتفع فى السلالة الحقلية عنه فى السلالة المعملية ،بينما لم نجد فروق معنوية بين السلالتين فى نشاط انزيم glutathione-s-transferase .

بناءا على النتائج الموضحة يمكن استخدام هذه المعلومات فى وضع برنامج لمواجة مقاومة العنكبوت الأحمر فى حقول القطن .