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Resistance mechanisms to some pesticides in a field strain of the two-spotted spider mite *Tetranychus urticae*

Ayat K. El Gammal[1*], Akram M. Abouzied[2], Mohamed S. Salman[1], Ahmed H. Abo-Ghalia[2]
[1]Plant protection Department, Agriculture research center, Ismailia, Egypt
[2]Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

ABSTRACT

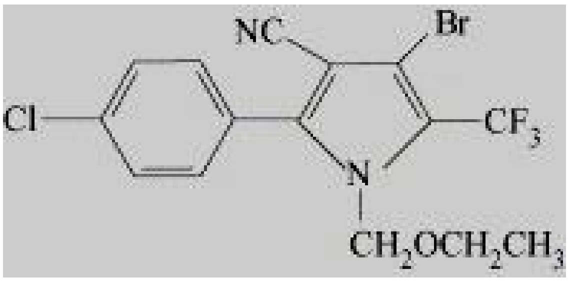
The two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) is a major pest affecting crops worldwide, for which biological control has not yet been achieved, therefore, acaricides are commonly used to control it. Spider mites are sap-sucking and can cause leaves and flowers to wilt and die. The entire mite life cycle takes about 2-3 weeks. All life stages of spider mites are usually found on the undersides of leaves, which make early detection difficult. The overall aims of the study described in this work were to study the molecular biology of resistance mechanisms against certain acaricides which currently available in some Ismailia regions (Egypt). *T. urticae* were collected from five different sprayed districts, together with a strain collected from unsprayed districts. The laboratory tests were carried out to establish the probit analysis of the sprayed strains comparing with the laboratory susceptible strains using two acaricides (abamectin and chlorfenapyr). Results of LC_{50} for abamectin in resistant strains obtained from different regions (El-Qassasin, Ismailia, Abu-Sueir, Faied and El-Qantara Gharb) and in susceptible strains were 1.32, 1.54, 6.18, 11.03, 26.02 and 0.233 respectively, while for chlorfenapyr were 1.174, 1.47, 15.58, 26.25, 46.16 and 0.22 respectively. Results revealed that all treatments in significant reduction of the mean numbers of moving stages of the mite populations compared with that of unsprayed control. In all counts, abamectin was affected more than chlorfenapyr against the target pest. All field strains showed high resistant comparing with susceptible laboratory strain.

Key words: Abamectin, chlorfenapyr, *Tetranychus urticae*, toxicity.

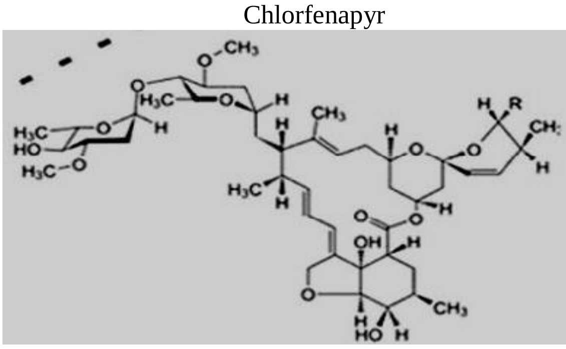
INTRODUCTION

The spider mite, *Tetranychus urticae* is an economically significant pest of vegetables and fruits crops (Benjamin *et al*., 2011). *Tetranychus urticae* was first reported in Brazil, where it is thought to have originated (Leeuwen, *et al.*, 2007). It is currently considered the most important dry-season arthropod pest of tomato in several eastern and southern African countries, including Egypt, where it also has extremely damaging effects on other crops grown as part of smallholder farming (beans, eggplants, etc.) (Ismail *et al.*, 2007). Currently, the most effective and widely used method for controlling *T. urticae* is through the use of chemical pesticides (Montasser *et al.,* 2011). Ismailia governorate has encouraged this through an initiative to supply subsidised pesticides to subsistence farmers. A primary threat imposed by intensive use of these compounds in both agriculture and as part of vector control strategies is the potential selection for resistance (Katsura and Tatsuya, 2009).

In Ismailia there have been several recent unpublished reports that abamectin and chlorfenapyr have good result to control mite populations. In the present study, reports of abamectin and chlorfenapyr resistance in *T. urticae* populations in Ismailia have been confirmed using acaricide bioassays (Katsura and Tatsuya, 2009; Ghadamyari and Sendi, 2008; Ochiai *et al*., 2007). The cloning and sequencing of regions (Abdollah *et al*., 2006; Deok *et al*., 2010), encoding domains II to IV of the *T. urticaei* para-type sodium channel (Katsura and Tatsuya, 2009; Dermaw *et al*., 2012) in two acaricides resistant Ismailia populations and in two acaricide-susceptible strains from laboratory strain (Leeuwen *et al*., 2004) in Ismailia Agricultural Research Station are described. Finally, how the knowledge and tools generated in this study can be used to develop more effective control manner aimed at reducing the damage to crops caused by *T. urticae* in Ismailia is discussed (Leeuwen *et al*., 2004).



Chlorfenapyr



Abamectin

MATERIALS AND METHODS

Maintenance of mite strains

T. urticae five strains were collected from Ismailia regions 2011 (Fig. 1). They were collected from eggplant (*Solanum melongena* L.). Collection areas are

* *Corresponding Author: ahmed_aboughalia@science.suez.edu.eg*

major horticultural centres in Ismailia governorate (Table 1). The mite sensitive colony was kept in a special cage 60x60x90 cm, with a net of stainless steel around the four sides and the top of the cage. The colony was reared under the laboratory conditions 25±2°C temperature, Lab. Relative humidity 65±5% and illumination for 24 hours. The sweet potato patches were changed after going to wilt about twice weekly in summer (Leeuwen *et al.,* 2005; Ismail *et al.,* 2007).

Insecticide bioassays

Mite cultures were age synchronized prior to bioassay by rearing adults only at each generation. Individuals used in bioassays were selected as mobile healthy adults by using binocular microscope and fine brush to transfer the selected as mobile healthy adults mites. Insecticide solutions were prepared using aqueous diluents control solutions consisted of the diluents only (Montasser *et al.,* 2011). A disc (2 cm diameter) cut from a clean potato leaf was placed ad axial side down put on wet cotton in Petri-dish (Bowie *et. al.,* 2001). Each Petri-dish was sprayed with a constant amount of the toxicant solution as an aqueous dilution using a glass manual atomizer (Sigma glass spray unit No. S 3135) for five seconds. At least five concentrations of each tested compound were used to draw the toxicity line. The treated Petri-dishes were left to dry and incubated under constant temperature of 25c° and lab. Relative humidity (65±5% RH) after 24 hours.

Dishes were incubated so that mites were oriented normally. Bioassays were held under standard rearing conditions (26±1◦C, 16 h day length) until endpoint (24h). Mortality was assessed using a binocular microsc-

Table (1): Collection records of *T. urticae* sampled in selected sites at Ismailia

Sample cod	Locality	Host plant	Density
A	Ismailia	Eggplant	+
B	Abu Sueir	Eggplant	+
C	ElQassasin	Eggplant	++
D	El Qantara Gharb	Eggplant	+++
E	Fayid	Eggplant	++

Density of infestation distributed over 1 inch² (100): High (+++) = over 100 individuals , moderate infestation (++) = (20:100)individual and low density of infestation in patches (+) = (1:20individual).

-ope, and individuals showing no sign of movement were scored as dead. Probit analyses of mortality data using LDP line programe (to calculate probity analysis according to finney (1971). generate estimated LC_{50} values and slopes with corresponding confidence limits. Resistance ratios for each strain and compound were calculated by dividing LC_{50} values by those of the most susceptible strain (Leeuwen *et. al.,* 2004).

RESULTS

All five *T. urticae* strains tested in this study showed low levels of mortality to chlorfenapyr. El-Qassasin, Ismailia and Abu Sueir regions in Ismailia had significant levels of resistance to the two acaricides compared with the other districts strains from Ismailia. In contrast, toxicity of LC_{50} values (Table 2, 3) for the more recently introduced acaracide abamectin (Avermectin) and chlorfenapyr (Challenger acaracide) were significantly different between strains.

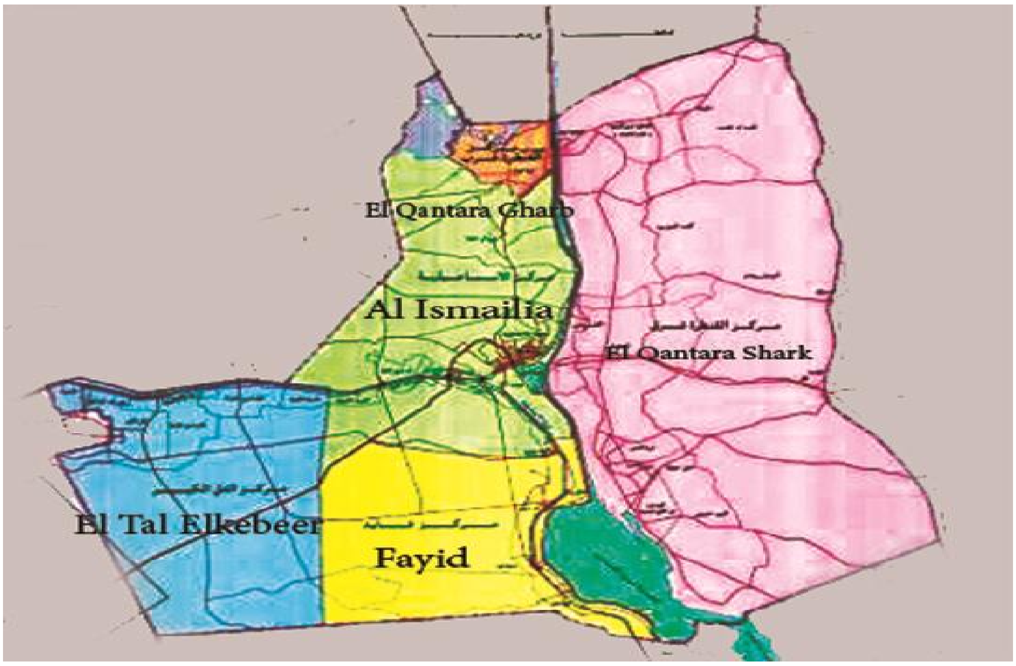


Figure (1): Map of Ismailia governorate showing the study area

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Table (2): Abamectin results for different regions at Ismailia governorate

Sample Districts	Slope	Toxicity index of LC_{90}	Toxicity index of LC_{50}	LC_{90}	LC_{50}
Sensitive	1.105	100	100	0.039	0.233
El-Qassasin	0.916	12.14	17.896	0.321	1.32
Ismailia	0.731	3.47	15.11	1.123	1.542
Abu-Sueir	1.51	0.47	3.768	8.286	6.184
Faied	1.246	0,28	2.11	13.917	11.035
El-Qantara Gharb	2.305	0.075	0.89	51.329	26.01

Table (3): Chlorfenapyr results for different regions at Ismailia governorate

Sample Districts	Slope	Toxicity index of LC_{90}	Toxicity index of LC_{50}	LC_{90}	LC_{50}
Sensitive	0.896	100	100	5.872	0.22
El-Qassasin	0.753	9.95	18.569	59.014	1.174
Ismailia	0.622	3.48	14.83	168.53	1.47
Abu-Sueir	1.326	4.0	1.376	146.75	15.85
Faied	0.886	0.799	0.83	734.92	26.25
El-Qantara Gharb	1.54	1.871	0.472	313.75	46.16

Susceptibility of abamectin and chlorfenapyr in strains of *T. urticae* resistant to other acaricides

Tables 2 and 3 clearly show that abamectin and chlorfenapyr were both effective in controlling *T. urticae* strains, abamectin also showed excellent efficacy against *T. urticae*. In contrast, an increase in numbers of *T. urticae* was observed in the chlorfenapyr treatments comparing in controlling strain untreated with the other resistant districts strains.

On the other hand table 4 clearly shows that toxicity of abamectin and chlorfenapyr were both effective in controlling *T. urticae* strains. abamectin also show excellent efficacy against *T. urticae* than chlorfenapyr by comparing values of five different districts to control sample and show that from upper go down to lower, El-Qassasin strain has higher toxicity index than the other strains to end with Fayid.

DISCUSSION

The acaricidal properties of abamectin and chlorfenapyr against *T. urticae* were determined that abamectin provides excellent control against adult stages of *T. urticae* at low concentrations but chlorfenapyr was less effective on the adult stages. Our data show that abamectin is more effective on *T. urticae* than chlorfenapyr. These unique features benefit both farmers and horticulturists. The results of selectivity experiments led to the conclusion that the number of surviving adults treated with abamectin less survive than that treated with chlorfenapyr.

Individuals treated with abamectin at a concentration of 2.5 mg/l of chlorfenapyr are not affected in the population density of *T. urticae*, but those treated with abamectin, decreased significantly the number of allowed population resurgence of *T. Urticae*. So, it may be safe to conclude that abamectin exerts its toxicity by a mechanism distinct from other acaricides. Therefore, abamectin has been categorized as a neurotoxic acaricide compound and classified by the Insecticide Resistance Action Committee (IRAC).

The present results show that abamectin and chlorfenapyr compounds represent acaricides with a very high specificity for phytophagous mites. Their efficacy and selectivity give support to their use as deal tools for the integrated pest management of tetranycid mites and as an environmentally friendly acaricide.

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ميكانيكية المقاومة لبعض مبيدات الآفات لسلالة حقلية من العنكبوت ذى النقطتين (تترنيكس يورتيكا)

آيات الجمال[1] – أكرم أبو زيد[2] – محمد سالمان[1] – أحمد أبوغالية[2]

[1] محطة بحوث الإسماعيلية - معهد بحوث وقاية النباتات - مركزالبحوث الزراعية – الدقى - الجيزة- مصر
[2] قسم علم الحيوان- كلية علوم - الاسماعيلية - جامعة قناة السويس

الملخص العربـــــى

يعتبر أكاروس العنكبوت ذى البقعتين من الآفات الهامة التي تصيب الكثير من النباتات فى بلاد متعددة ومنها مصر ولذا وجد من الواجب دراسة كيفية مكافحته. تمتص أفراد الآفة عصارة أوراق النباتات وأزهارها مما يسبب ذبولها. ومن المعلوم أن دورة حياة الحيوان تستغرق ٢-٣ أسابيع لكى تكتمل أطوارها. يقضى الطور البالغ الكثير من حياته فى الأرض على السطح السفلى للورقة مما يجعل ملاحظته صعبة. ولقد تمت دراسة تأثير إستخدام بعض المبيدات الأكاورسية ضد الحيوان موضع الدراسة وهى متاحة فى منطقة الاسماعيلية (مصر). تم تجميع الأكاروس من خمس مناطق مقارنتاً بسلالة حساسة معملية، وتمت الدراسة على مبيدين (الأبامكتين – الكلورفين بير).

أوضحت النتائج أنه هنك قلة ملحوظة فى عدد الأفراد الحية مقارنتاً بالميتة ومنها وضح أنه السلالات الحقليه لديها مقاومة أكثر منها فى السلالة الحساسة. ويأتى هذا فى ضوء المشاكل التى تصاحب إستخدام المبيدات فى المكافحة مثل ظاهرتى المقاومة لإستخدام المبيدات وكذا الأثر الباقى لها. وقد تم مناقشة ذلك بشىء من التفصيل.