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STUDIES ON SELECTION AND RESISTANCE MECHANISM BY ABAMECTIN AND ETOXAZOLE IN *Tetranychus urticae* (Acari.: Tetranychidae)

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ABSTRACT

This investigation was conductedto study the mode of development of resistance *Tetranychus urticae* against acaricides. A *T. urticae* strain was collected from cotton plants grown in the experimental farm of Sakha. After ten generations of selection with abamectin and etoxazole under laboratory conditions, the resistance strain to abamectin and etoxazole started slowly in the first generation until the fourth generation, then began to increase gradually increase until reached the highest peak at the end of the tenth generation (developed 140 and 37.45 fold resistance, respectively), suggesting that these glutathione-S-transferase (GST), acid phosphate, alkaline phosphate, ATPase and AChE enzymes are important in conferring abamectin and etoxazole resistance in *Tetranychus urticae*. The major resistance mechanism to abamectin was the increasing activities of glutathione-S-transferase (GST), acid phosphate, alkaline phosphate, ATPase and AChE. The activity in resistance strain developed 602.35, 203.52, 143.87, 8.93, and 1.31 fold comparable to that in the susceptible strain, respectively, with a percentage of increase equal to 419.38 % for alkaline phosphate. The activity of GST in the resistance strain for etoxazole developed 381.56 fold when compared with the susceptible strain. While the percentage of increase was 163.82 **%** for AChE.

INTRODUCTION

The two spotted spider mite, *T. urticae* is one of the most important and serious pests on cotton plants, and it is difficult to contro. Because of its high fecundity, short generation time, high rate of inbreeding and high possibility of acaricides exposure, its resistance to pesticides is a big problem compared to other crop pests. The development of resistance in susceptible strain of *T. urticae* for ten successive generations after exposure to compounds treated surface was developed slowly and the resistance increased gradually. Several investigators evaluated the resistance problem around the world; (Kobayashi *et al.* (2001), Lee *et al.* (2003) and Mario *et al*.(2005). Sato *et al*. (2005) found that the resistance to abamectin in *T. urticae* increased 13 fold. Mohamed (2006) and Kim *et al*. (2007). The resistance ratio was high resistant to abamectin He *et al*. (2009).

The enzymes plays an important role as biochemical mechanism for resistance to acaricides. Kwon *et al*. (2010**)** found that the percent increase in activity enzymes, it could be fairly concluded that the most inductive effect was obtained in case of the abamectin and etoxazole in glutathione-S-

transferase (GST), alkaline phosphate, acid phosphate, ATPase and AChE. Smissanert (1964), Voss and Matsumura (1964) and Lin *et al.* (2009).

Therefore, the present work was conducted to investigate the selection of *T. urticae* resistance strains at the laboratory. The difference of the activity of five detoxification glutathione-S-transferase (GST), acid phosphate, alkaline phosphate, ATPase and AChE enzymes of susceptible strain and resistance strains were compared, so as to investigate the biochemical mechanisms of the formation of resistance in *T. urticae* to abamectin and etoxazole.

MATERIALS AND METHODS

Selection pressure studies:

Resistance evaluation were made, using some compounds of acaricides (abamectin, etoxazole, comite and ortus), and tested using the leaf disc dipping technique against adults of the two-spotted spider mite, *T. urticae*. The field strain of the two-spotted spider mite, *T. urticae* was collected from cotton plants grown in the experimental farm of Sakha Agricultural Research Station, at Kafr EL- Sheikh. Each sample transferred to the laboratory, and reared under optimum laboratory conditions away from any acaricide contamination for three successive generations before starting in the selection pressure experiments. Selection pressure was carried out using concentrations that give about 50 % mortality of the adults for all tested compounds. Selection pressure was carried out by applying the toxicant solution on the leaf discs having the cuttings of sweet potato plants by dipping it for about 5 seconds, left to dry in each concentration both compounds and then number individuals tested were 50 individ. of *T. urticae* were transferred on each leaf discs in dishes. The mite numbers were recorded after every 24 and 48 hours from the application, the surviving mites were transferred on new sweet potatoes leaves away from any acaricides contamination and left to propagate. The colony to a level where it could again be selection pressured. At the end of the selection pressure when the mites began to tolerate, the toxicant increased the selection pressure generation after generation by increase in the concentrations ppm.

$$RR = \frac{LC_{50} \text{ of resistance strain}}{LC_{50} \text{ of susceptibility strain}}$$

Preparation of enzyme samples from *T. urticae*:

The homogenate of the two spotted spider mite adults were collected from the susceptible strain (300 individ.) and resistant strains tested in laboratory (300 individ. for each compound) after being starved for 6 hours. Each samples was ground in mortar to obtain fine powder, adult were homogenized in an ice solution of 0.25 M sucrose using a glass homogenizer. The homogenate was centrifuged and the supernatant was used as the enzymes sources (crude enzyme). Equal weights of each of the

selected mite samples were frozen at -2°C until needed. The suspensions were transferred to beakers with few drops of sodium azid (.2ml), according to El-doksh (2001).

Enzymes assay:

Protein determination:

In a clean glass tube, 50 µl from mite homogenate was added to 2ml Potassium iodide. The solution was mixed well and the resultant colour was measured after 10 minutes at 545 nm spectrophotometer. The same procedures was repeated using 50 µl of bovine albumin (standard) instead of sample homogenate. A control was included in which 50 µl distilled water was used instead of mite homogenate (Weichselbaum 1946).

Determination of acetylcholinesterase and Glutathion - S - transferas (GST) activity:

20µ ATchI, 100 µl. DTNB, 2.78 ml phosphate buffer and 100 µl of enzyme solution were mixed in the tube. A control was included for corporation in which 100 µl. distilled water was used instead of the enzyme solution. The reaction mixture was incubated for 30 min. in a water bath at 37°C. The absorbance was measured at 421 nm on a spectrophotometer (Rose and Wallbank 1986). While Glutathion - S - transferas (GST) 0.1 ml of sample (enzyme source), 0.1 ml of buffer were pipette into the tube, well mixed and placed into a water bath kept at 35°C for 4 min. to equilibrate, 0.01ml of DCNB was added and the mixture replaced into the water bath and absorbance was measured using the reagent blank as reference, two replicates were made for each assay.

Determination of acid and alkaline phosphates activity:

0.5 ml sodium tartrate solution was added, mixed well and incubated at 37°C for exact 30 min and after tha, 5.0 ml of NaOH, 0.02 N was added to each tube, then 0.1 ml of sample was added to the control tube and mixed well again. After that, the developed color was measured at wavelength of 405 nm spectrophotometer (Moss 1984). While alkaline phosphates four tubes of adult mite homogenate sample, standard tube, adult mite homogenate blank were used. 2 ml. of phenyl phosphate were transferred to each tube and incubated for 5 minutes at 37°C, 50 µ/L of adult mite homogenate were added to adult homogenate tube, while 50 µ/L of phenyl were added to standard tube, then all the four tubes were incubated for 15 minutes at 37°C, after that 0.5 ml of amino -4- antipyrine was added to each tube and mixed well, 0.5 ml of potassium ferricyanide was added to each tube and 50 µL of homogenate were added to homogenate control tube, while 50 µL distilled water were added to the reagent control tube, then mixed well and let stand for 10 minutes in the dark and measured at 510 nm spectrophotometer (Kind and King 1954).

Determination of ATPase activity:

In a test tube: 100 µl of sample (enzyme source) 775 µl of Tris buffer and 25 µl ATP solution were pipetted, the mixture was incubated for 15 min. at 37ºC in a shaking water bath, then 0.1 ml 50% trichloroacetic acid (TCAA) was added and the absorbance spectrophotometer at 840 nm was recorded (Taussky and Shorr 1953).

$$\% \text{ increase in the activity of tested enzymes} = \frac{\text{S.A. of enzymes in treatmint - S.A. in control}}{\text{S.A. of the enzymes in control}} \times 100$$

RESULTS AND DISCUSSION

Development of resistance to abamectin in *T. urticae*:

Results of the abamectin selection showed that the resistance in *T. urticae* increased in the generation that followed selection. Data obtained in Table (1) show the selection for 10 generations with abamectin. The resistance level in *T. urticae* was low until the second generation. Resistance started to increase in the third generation with the LC_{50} 0.12 ppm. (12.2 fold). Therefore, the resistance increased gradually, with LC_{50} values rising from approximately 1.4 ppm. (140 fold) in the tenth generation when compared with the susceptible strain.

It could be concluded that the highest degree of population homogeneity was obtained towards the third generation which had the highest slope value (3.2). While the fourth, ninth and tenth generations have the lowest slope values of 2.0, 1.7 and 1.6, respectively. first, second and Fifth, generations have slope values, 2.6, 2.5 and 3.1, respectively. Sato *et al*. (2005) reported that after 5 generations of selections with abamectin, resistance to abamectin in *T. urticae* increased 13 fold. Mario *et al*.(2005) indicated that the resistance ratio (R/S) at the LC_{50} reached 342 fold values of *T. urticae*, He *et al*. (2009), after 42 generations of selection, showed that the strain was 8.7-fold resistant to abamectin. Koh *et al*. (2009) found that the resistance ratios to abamectin were low (RR≤10) in all populations of *T. urticae*

Table (1): The selection of resistance to abamectin in *T. urticae.*

Generation tested	LC_{50} (ppm)	C.L. for LC_{50}		Slope value	Rr
		Lower	Upper		
Susceptible	0.01	0.022	0.03	2.3	-
1	0.04	0.034	0.04	2.6	4.00
2	0.06	0.036	0.15	2.5	6.00
3	0.12	0.116	0.12	3.2	12.0
4	0.13	0.120	0.14	2.0	13.0
5	0.14	0.140	0.15	3.1	14.0
6	0.36	0.250	0.46	0.9	36.0
7	0.36	0.250	0.46	0.9	36.5
8	0.43	0.260	2.46	1.3	43.0
9	0.67	0.28	1.70	1.7	67.0
10	1.40	0.56	3.60	1.6	140.0

Rr : Resistance ratio

Development of resistance to etoxazole in *T. urticae*:

The selection process of etoxazole susceptible and resistant strains is shown in Table (2). The resistance of *T. urticae* to etoxazole developed slowly in the first generation until the fourth generation (5.69 fold) and began to increase in the fifth generation (11.83 fold). It gradually increased until reached the highest peak at the end of the ninth and tenth generations, represented by 34.46 and 37.45 fold, respectively. Therefore, the resistance increased after ten generations of selection when compared with the susceptible strain, and the resistance level of etoxazole developed very fast.

Table (2): The selection of resistance to etoxazole in *T. urticae*.

Generation tested	LC_{50} (ppm)	C.L. for LC_{50}		Slope value	Rr
		Lower	Upper		
Susceptible	667.38	516.31	880.81	0.88	-
1	1000.0	1302.38	769.25	1.15	1.49
2	1050.0	1307.18	846.77	1.62	1.57
3	2623.56	1316.82	1515.47	1.40	3.93
4	3800.24	3500.2	4300.56	0.94	5.69
5	8000.00	9820.27	6517.81	1.58	11.98
6	13000.0	16741.81	10094.79	1.37	19.47
7	14500.0	18819.55	11171.89	1.33	21.72
8	16000.0	19682.21	13008.13	1.50	23.97
9	23000.0	31172.21	16974.17	1.70	34.46
10	25000.0	32389.33	19298.53	1.56	37.45

Rr : Resistance ratio

Referring to Table (2) it appears that the second, fifth and ninth, generations have the highest slope values (1.62, 1.58 and 1.70 respectively) followed by the eighth and tenth generations with slope values of 1.50 and 1.56, respectively. While the first and fourth generations were of the lowest slope values 1.15 and 0.94, respectively.

These results can be supported with those obtained by several investigators. Kobayashi *et al.* (2001) who reported a resistance ratio of >20,000 to etoxazole of *T. urticae*. Lee *et al.* (2003) found that *T. urticae* was highly resistant to etoxazole (RR 90.0).

Mechanisms of resistance in *T. urticae*:

All tested compounds were bioassayed for their effects on the specific activities of some enzymes. Results of specific activity of glutathione-S-transferase (GST), Alkaline phosphate, Acid phosphate, ATPase and Acetylcholinesterase (AChE) in *T. urticae* are shown in Table (3), and play an important role as biochemical mechanisms for resistance to acaricides. Specific activity of AChE and ATPase enzymes in *T. urticae* abamectin (R) strains 1.31 and 8.93 fold with percent of increase of 39.36 and 276.79 %, respectively and if compared with the susceptible strain 0.94 fold. Etoxazole resistance strain of *T. urticae* had the least specific activity of AChE and ATPase enzymes of 2.48 fold and 5.87 fold with increase of 163.82 and 147.67 %, respectively. Specific activity of GST and Alkaline phosphate of both resistant strains increased when compared with susceptible strain. However, only the difference between abamectin strain and susceptible strain

reached to 602.35 and 143.87 fold with percent of increase of 102.74 and 419.38 % which indicated into the formation of *T. urticae* abamectin resistance. Results, also indicated that these significant different were found between abamectin, etoxazole (R) strain and SS strain for GST and acid phosphate had reached the significant level to 381.56 and 108.32 fold with percent of increase of 28.42 % and 57.14 %, respectively.

Smissanert (1964) and Voss and Matsumura (1964) found that AChE activity of the O. P. resistance of *T. urticae*, was much lower than that of susceptible mite and the AChE in resistance mite was much less sensitive than that in susceptible mite. Kwon *et al*. (2010) indicated that the biochemical mechanisms of abamectin resistance in two *T. urticae* strains (PTF, 239 fold resistance; AbaR, 4753 fold resistance).

Table (3): Specific activity and percent of increase of enzymes in susceptible and resistant strains of *Tetranychus urticae*.

Compound	Specific activity enzymes				
	AChE	GST	Acid phosphate	Alkaline phosphate	ATPase
Susceptible(S)	0.94 b	297.10 c	68.93 c	27.70 bc	2.37 b
Abamectin(R)	1.31 ab	602.35 a	203.52 a	143.87 a	8.93 a
Etoxazol (R)	2.48 a	381.56 b	108.32 b	30.54 b	5.87 ab
% increase 0f enzymes activity					
Susceptible(S)	-	-	-	-	-
Abamectin(R)	39.36	102.74	195.25	419.38	276.79
Etoxazol (R)	163.82	28.42	57.14	10.25	147.67

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دراسات على انتخاب وميكانيكية المقاومة للابامكتين و الايتوكسازول فى العنكبوت الأحمر

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يعتبر العنكبوت الأحمر واحدا من أهم الآفات الاقتصادية التى تسبب خسائر فادحة لمحصول القطن المصري ولقد أظهرت النتائج المتحصل عليها أن الانتخاب لمدة عشرة أجيال متتالية باستخدام مركبات الابامكتن و الايتوكسازول أدى الى تطور مقاومة الاكاروس للمبيدات تدريجيا وتبدأ منخفضة فى الجيل الأول حتى الجيل الرابع و تبدأ تزداد بشكل تدريجي حتى تصل إلى نسبة عالية فى نهاية الجيل العاشر حيث وصلت إلى 140 مرة مع الابامكتن ،وعند إجراء الضغط الانتخابي لمركب الايتوكسازول على أفراد العنكبوت الأحمر أظهرت النتائج المتحصل عليها أن تطور المقاومة يزداد تدريجيا من الجيل الأول إلى الجيل العاشر بمقدار 37.45 ضعفا.

أظهرت النتائج أيضا أن إنزيمات الجلوتاثيون و الفوسفاتيز القلوي و الحامضى و ادينوزين - ترای – فوسفات و الكولين استريز لهما دور فى ميكانيكية المقاومة مع الابامكتن وزاد نشاط هذه الإنزيمات فى السلالة المقاومة كالاتى: 602.35 ,203.52 ,143.87 , 8.93, 1.31 ٪ على التوالى مقارنتا بالسلالة الحساسة ،و فى حالة السلالة المقاومة لمركب الايتوكسازول كان نشاط إنزيم الجلوتاثيون مرتفعا حيث وصل الى 381.56 ضعفا مع النسبة المئوية لزيادة نشاط الإنزيم 28 ٪ وعلى ذلك أظهرت السلالة المقاومة من أفراد العنكبوت الأحمر لمركب الايتوكسازول نشاطا عاليا لإنزيم الكولين استريز2,48 مع النسبة المئوية لزيادة نشاط الإنزيم 163.82 %.

قام بتحكيم البحث

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