Influence of Pesticides Selection Pressure on Protein Banding Patterns of the Two Spotted Spider Mite *Tetranychus urticae* Koch by SDS-PAGE

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ABSTRACT

The aim of the present study was to investigate the effect of three pesticides selection pressure on the protein banding of the two-spotted spider mite *Tetranychus urticae* Koch by SDS-PAG electrophoresis comparing. In this experiment, four batches of mite colonies reared for nine months away from any pesticide contamination were exposed for selection pressure of the tested pesticides. The first batch was subjected to successive selection with chlorfenapyr, the second patch was subjected to repeated selection of fenpyroximate, the third one for repeated selection of hexythiazox, whereas the 4th patch was left without any pesticide treatment as a check. Selection pressure was carried out at LC50 level of each of the tested toxicant. The selection was studied for 12 generations to follow up the development of resistance and the resistance ratio (RR) values were calculated. The highest RR value of F12 selected generation was for Fenpyroximate (14.32) followed by 12.06 for chlorfenapyr, while the lowest value was obtained by hexythiazox (4.22). Comparing the protein banding pattern of chlorfenapyr fenpyroximate, and hexythiazox resistant strains with the susceptible one, it was found that the protein bands with MW (54 and 36 KD) appeared only in susceptible strain, while disappeared in the other tested resistant strains of the two-spotted spider mite *T. urticae.* The protein band with MW 44 KD was appeared only in Hexythiazox resistant strain. The protein bands with MW 53 and 34 KD were only found in fenpyroximate resistant strain of *T. urticae*, whereas such bands were not found in the other strains. The protein bands pattern with MW 146, 35 and 31 KD were found only in hexythiazox and chlorfenapyr resistant strains of *T. urticae*. Such result may indicate that the mechanism of resistance strains is similar for both strains.

Key Words: *Tetranychus urticae*, SDS-PAGE, Pesticides pressure, Hexythiazox, Fenpyroximate, Chlorfenapyr.

INTRODUCTION

The two spotted spider mite *Tetranychus urticae* Koch is an important economically pests infesting many crops specially vegetables. The wide use of chemical compounds caused many problems such as pulation and chemical resistance endangering human health and wealth.

Molecular studies and protein electrophoresis have been used for the detection of genetic polymorphism to mites (Lewontin, 1991; Osakabe & Komazaki 1996; Navajas & Fenton 2000 and Smrz, 2000). Electrophoretic techniques used to study a wide range of problems, such as species identification (Avanzati *et al.*, 1994; Enohara & Amano, 1996 and Goka and Takafuji, 1997), genetic divergence between population (Navajas *et al.* 2000), feeding preferences of predatory mites (Solomon *et al.*, 1985) and paternity analysis (Yasui 1997).

The aim of this study was to investigate the effect of selection pressure with some pesticides on the protein banding patterns from chlorfenapyr fenpyroximate, and hexythiazox resistant strains of the two-spotted spider mite *T. urticae* by SDS-PAGE electrophoresis comparing with protein banding pattern of the susceptible strain.

MATERIALS AND METHODS

Chemicals Used

1. Growth regulator: Hexythiazox (Maccomite 10% WP)
trans-5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl -2-oxothiazolidine-3-carboxamide

2. Pyrrole compound: Chlorfenapyr (Challenger 36% SC)
4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile

3. Acaricide: Fenpyroximate (Ortus 5% SC)
1,1-dimethylethyl4-[(*E*)-[(1,3-dimethyl-5-phen-oxy -1*H*-pyrazol-4-yl) methylene] amino] oxy] methyl] benzoate

Rearing Mites under Pesticide Selection Pressure

This work aims to study the rate of development of resistance phenomenon to three selected pesticides, chlorfenapyr, fenpyroximate and hexythiazox. Therefore, four batches of mite colonies which had been reared for nine months away from any pesticide contamination were reared for selection pressure of the tested pesticides. The first batch was subjected to successive selection with chlorfenapyr, the second batch with fenpyroximate, the third one with hexythiazox, whereas the 4th was left free from any pesticide treatment as a check.

The selection pressure was carried out by dipping sweet potato cuttings each holding six leaves in water-dilution of the tested toxicant for 5 seconds. The treated cuttings were left for solvent evaporation. The old treated leaves were placed over the new treated ones to allow immigration of the mites to the later leaves. Selection pressure was carried out at LC_{50} level of each of the tested toxicant. The selection was studied for 12 generations to follow up the development of resistance.

For studying the level of resistance in the three selected strains, toxicity lines of the inducer against the adult females were established every generation using leaf disc residue film technique. All the results of the selected strains were compared with those of the laboratory susceptible strain.

SDS-Polyacrylamide Gel Electrophoresis of Protein

Sodium dodecyl sulfate polyacrylamide gel electrophoresis SDS-PAGE was used to separate the protein of selection pressure on the protein banding patterns from fenpyroximate, chlorfenapyr and hexythiazox resistant strains of the two-spotted spider mite *T. urticae* comparing with protein baning pattern of susceptible strain.

The protein contents were determined using the Lowery method (Lowery *et al*, 1951). An aliquot contain 100 µg protein was placed in a 2x volume of sample buffer containing 10% glycerol, 5% 2-mercaptoethanol, 2% SDS, 0.0725 Tris-HCl buffer (pH 6.8) and 0.01% bromophenol blue. Sample and the mixture tissue were heated in boiling water for 10 minutes.

An aliquot containing approximately 30 µg of protein of such preparation was loaded onto acrylamide gel and electrophoresis was carried out until the bromophenol blue track reached the bottom of the gel at 5 to 10 mA on a vertical plate gel containing 12.5% acrylamide separating gel and 1.0 cm of 5% acrylamide stacking gel. Gel and electrode buffer (pH 8.3) were prepared according to Laemmli (1970) and King & Laemmli (1971). The molecular weights of the protein bands on the SDS gels were determined by comparing the nobilities of the protein with proteins of known molecular weight according to Weber and Osborn (1969).

Following electrophoresis, the gels were fixed in 25% isopropanol and 10% acetic acid for 30 minutes. Protein bands were stained with a solution of 25 % methanol, 10 % acetic acid and 0.025 % coomassie blue R. 250 for several hours, and later distained by shacking in 10 % acetic acid the gels were placed on several layers of filter papers and slowly dried under vacuum. Data were analyzed by image analysis system to show the differentiations between species using protein marker as a stander.

RESULTS AND DISCUSSION

Developmental Pressure of Pesticides Selection on the Susceptibility of the Two-Spotted Spider Mite *T. urticae* Adult Females

Effect of Fenpyroximate

Data in table (1) showed the building up of the resistance to fenpyroximate in the laboratory strain of the two spotted spider mite during 12 generations. The results revealed that LC_{50} value generally increased from 2.19 ppm in parent generation to 11.96 ppm in the 6^{th} generation, while in the 9^{th} generation increased to 21.52 ppm and reached 31.5 ppm in the 12^{th} generation. Such result indicated that the resistance ratio (RR) increased to 14.32 folds in F12 selected generation at LC_{50}.

Concerning slope values F1 line had the flattest regression line (slope = 1.66), while F12 was the steepest regression line (slope = 1.38). This might be related to the increasing in homogenecity in F12 which was more than in the other selected generation.

The present data coincide with the results of Ioriatti *et al*. (2000) who used resistant ratios to evaluate the level of resistance; resistance was detected with discriminating doses. Resistance to

Table (1): Effect of three pesticides selection on the susceptibility of adult females of two-spotted spider mite *T. urticae.*

Pesticides	Chlorfenapyr		Fenpyroximate		Hexythiazox	
Generations	LC_{50}	R.r.*	LC_{50}	R.r.*	LC_{50}	R.r.*
Susceptible	1.59	-	2.19	-	5.06	-
1	1.69	1.06	2.41	1.20	5.08	1.01
2	1.71	1.08	2.64	1.20	5.50	1.09
3	3.07	1.93	2.74	1.25	5.83	1.15
4	3.61	2.27	10.26	4.76	5.33	1.05
5	4.85	3.05	11.89	5.41	5.69	1.13
6	4.82	3.03	11.69	5.44	6.25	1.24
7	9.23	5.81	16.90	7.69	6.62	1.31
8	9.05	5.98	17.17	7.81	10.74	2.12
9	11.79	7.42	21.52	9.79	10.98	2.17
10	14.96	9.41	23.16	10.53	19.79	3.91
11	18.23	11.47	31.02	14.11	20.73	4.1
12	19.18	12.06	31.50	14.33	21.34	4.22

*R.r. = Resistant ratio

fenpyroximate and propargite results widespread in all apple orchards investigated. No evidence of cross resistance between fenpyroximate and the other two METI (Mitochondrial Electron Transport Inhibitor) has been found in this study.

Effect of Chlorfenapyr

Data in table (1) showed the building up of the resistance to chlorfenapyr in the laboratory strain of two spotted spider mite during 12 generations. The result indicated that LC_{50} value generally increased from 1.59 ppm in parent generation to 4.82 ppm in the 6^{th} generation, while in the 9^{th} generation it increased to 11.79 ppm and reached 19.18 ppm in the 12^{th} generation. Such results indicated that the resistance ratio (RR) increased to 12.06 folds at F12 selected generation at LC_{50}.

Concerning slope values, F1 line had the flattest regression line (slope = 1.73), while F12 was the steepest regression line (slope = 1.12). This might be due to increase homogenecity in F12 which was more than in the other selected generation.

Effect of Hexythiazox

Data in Table (1) showed building up of the resistance to hexythiazox in the laboratory strain of two spotted spider mite during 12 generations. The results indicated that LC_{50} value generally increased from 5.06 ppm in parent generation to 6.62 ppm in the 7^{th} generation, while in the 8^{th} generation it increased to 10.74 ppm and reached 21.34 ppm in the 12^{th} generation. Such results indicated that the resistance ratio (RR) increased to 4.22 folds in F12 selected generation at LC_{50}.

Concerning slope values, F1 line had the flattest regression line (Slope = 1.34), while F12 was the steepest regression line (slope = 1.27). This might be correlated with the increasing in homogenecity in F12 which was more than in the other selected generation. The present data are coincided with the results of Flexner *et al.* (1995) who found that LC_{50}s in the consecutive hexythiazox program increased after the 5^{th} generation.

Influence of Pesticides Selection Pressure on Protein Banding Patterns of the Two-Spotted Spider Mite *T. urticae* by SDS-PAG Electrophoresis

The protein banding patterns in this technique were arranged according to their variable molecular weight. The protein banding patterns differences of those resistant strains and susceptible strain were examined by using SDS-PAGE electrophoresis technique (Figs 1 & 2). The results presented in

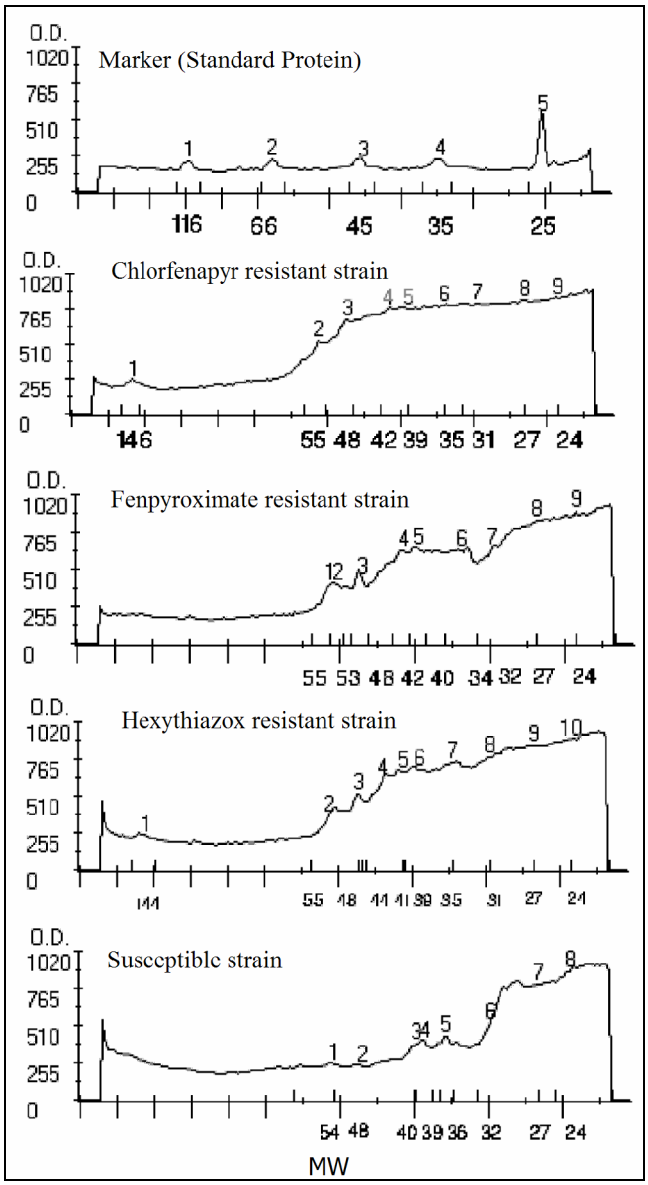

Fig. (1): Densitometric analysis of Silver stained protein bands.
MW = Molecular weight O.D.= Optical density

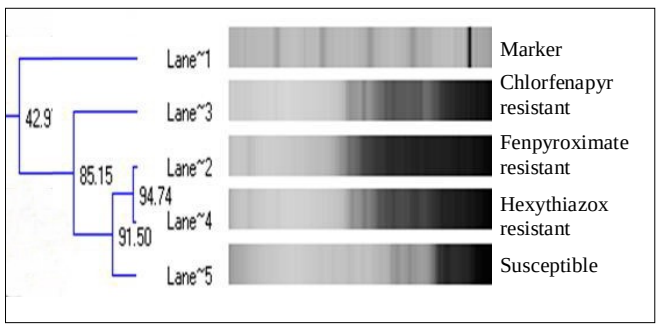

Fig. (2): Cluster analysis showing the similarity polymorphism of protein banding patterns obtained by SDS-PAGE.

Table (2): Protein banding patterns obtained by SDS-PAGE from Marker (Standard protein), Chlorfenapyr, Fenpyroximate, Hexythiazox-resistant strains and susceptible strain of *Tetranychus urticae* as total amount %.

MW KDa	Protein banding patterns as % Amt			
	Chlorfenapyr resistant strain	Fenpyroximate resistant strain	Hexythiazox resistant strain	Susceptible strain
146	0.72	0.00	0.89	0.00
55	1.47	2.31	32.71	0.00
54	0.00	0.00	0.00	5.63
53	0.00	4.51	0.00	0.00
48	2.82	3.03	0.30	17.95
44	0.00	0.00	3.67	0.00
42	29.37	4.23	0.00	0.00
41	0.00	0.00	0.20	0.00
40	0.00	4.46	0.00	0.40
39	25.82	0.00	25.67	4.20
36	0.00	0.00	0.00	3.12
35	20.99	0.00	20.41	0.00
34	0.00	8.23	0.00	0.00
32	0.00	45.40	0.00	31.98
31	8.48	0.00	5.75	0.00
27	4.64	13.84	5.04	7.76
24	5.42	13.99	5.37	28.96

M.W. = Molecular weight

Table (4): Protein banding patterns obtained by SDS-PAGE from marker (standard protein), Chlorfenapyr, Fenpyroximate, Hexythiazox resistant strains and susceptible strain of *Tetranychus urticae*.

MW KDa	Protein banding patterns of obtained SDS-PAGE from			
	Chlorfenapyr resistant strain	Fenpyroximate resistant strain	Hexythiazox resistant strain	Susceptible strain
146	+	-	+	-
55	+	+	+	-
54	-	-	-	+
53	-	+	-	-
48	+	+	+	+
44	-	-	+	-
42	+	+	-	-
41	-	-	+	+
40	-	+	-	+
39	+	-	+	+
36	-	-	-	+
35	+	-	+	-
34	-	+	-	-
32	-	+	-	+
31	+	-	+	-
27	+	+	+	+
24	+	+	+	+

Table (3): Protein banding patterns obtained by SDS-PAGE from Marker (Standard protein), Chlorfenapyr, Fenpyroximate, Hexythiazox-resistant strains and susceptible strain of *Tetranychus urticae* as peak area.

MW KDa	Peak area				± % change of peak area with control		
	Susceptible strain	Fenpyroximate resistant strain	Hexythiazox resistant strain	Chlorfenapyr resistant strain	Fenpyroximate resistant strain	Hexythiazox resistant strain	Chlorfenapyr resistant strain
146	2328	-	-	-	new band	-	-
144	-	-	3089	-	-	-	new band
55	5664	3027	113870	-	new band	new band	new band
54	-	-	-	5274	disappeared band	disappeared band	disappeared band
53	-	5922	-	-	-	new band	-
48	9166	3975	1053	16821	45.51	76.37	-1278.83
44	-	-	12761	-	-	-	new band
42	95380	5555	-	-	new band	new band	-
41	-	-	685	-	-	-	new band
40	-	-	-	378	disappeared band	disappeared band	disappeared band
39	83863	-	89345	3933	-2032.29	disappeared band	-2171.68
40	-	5858	-	-	-	new band	-
36	-	-	-	2926	disappeared band	disappeared band	disappeared band
34	-	10805	-	-	-	new band	-
35	68188	-	71027	-	new band	-	new band
31	27549	-	20017	-	new band	-	new band
32	-	59597	-	29969	disappeared band	-98.86	disappeared band
27	15056	18163	17528	7269	-107.13	-149.87	-141.13
24	17599	18368	18695	27135	35.14	32.31	31.10

$$\% \text{ change of peak area} = \frac{\text{Peak area of susceptible strain} - \text{peak area of resistant strain}}{\text{Peak area of susceptible strain}} \times 100$$

Table (2) showed that the protein band of 144 KD MW. was not present in all tested strains as well as in marker protein. The protein bands of MW 116, 66, 45 and 25 KD were found only in marker protein.

Comparing the protein banding pattern from fenpyroximate, chlorfenapyr and hexythiazox resistant strains with susceptible strain, it was found that the protein bands with MW (54 and 36 KD) appeared only in susceptible strain, while disappeared in the other tested resistant strains of *T. urticae* (Table 3).

The protein band with MW 44 KD appeared only in Hexythiazox resistant strain. The protein bands with MW 53 and 34 KD were only found in fenpyroximate resistant strain of *T. urticae*, whereas such bands were not found in the other strains. The protein bands pattern with MW 146, 35 and 31 KD were found only in hexythiazox and chlorfenapyr resistant strains of *T. urticae*; such result may indicate that the mechanisms of resistance strains are similar for both strains (Table 4).

From the above-mentioned results, it is suggested that such bands may include probably the protein enzymes of esterase and P450 mono-oxygenase (MO) as mentioned by Tomas Van Leeuwen *et al*. (2006). They showed that they both enzymes are responsible for increasing the resistance to chlorfenapyr in the two spotted spider mite *T. urticae* resistant strain. It was also clear from the above-mentioned results that the use of SDS-PAGE technique may be a good tool as a biochemical marker on identification of resistance of *T. urticae* to acaricides and sheds light on the protein patterns fractions in resistant strains of the two spotted spider mite.

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