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Studies on Selection and Resistance Mechanism by Some Acaricides in (Two Spotted Spider Mite, *Tetranychus urticae)*

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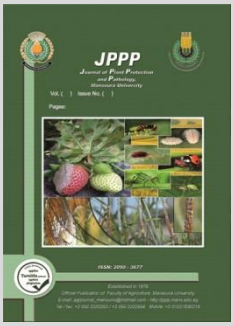

ABSTRACT

The progress of resistance was evaluated in susceptible strains of *Tetranychus urticae* to several chemicals, including Fenpyroximate, Cyhalothrin, Abamectin, and Ethion, and to measure the specific activity of Acetylcholine esterase (AChE) and Glutathione-S-transferase (GST) enzymes under laboratory conditions to understand the resistance mechanisms. The resistance levels to Fenpyroximate showed significant variability over two generations, with the ninth and tenth generations exhibiting high resistance levels of 65.63 fold and 76.73 fold, respectively. Resistance to Cyhalothrin increased over ten generations, achieving a 36.63-fold resistance. Abamectin resistance gradually rose, reaching 138.44-fold by the tenth generation. For Ethion, the ninth and tenth generations demonstrated resistance levels of 70.27-fold and 91.99-fold, respectively. The results indicated that the AChE enzyme's specific activities were significantly lower in the susceptible strain, with notable differences among the compounds. The Cyhalothrin-resistant strain of *T. urticae* exhibited the highest specific AChE activity at 2.23, compared to 0.83 in the susceptible strain. Conversely, the Abamectin-resistant strain showed the lowest specific AChE activity at 1.18. Ethion and Fenpyroximate significantly reduced AChE activity to 1.82 and 1.83, respectively. Additionally, higher GST activity in all resistant strains of *T. urticae* compared to the susceptible strain.

Keywords: Tetranychidae, Acaricides, Resistance, Fenpyroximate, Cyhalothrin.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae*, is a significant pest affecting a wide range of plants and is notoriously difficult to manage. This challenge is due to its high reproductive rate, brief time of generation, high levels of inbreeding, and frequent exposure to acaricides, which collectively contribute to its substantial resistance to pesticides compared to other crop pests. Resistance development in susceptible strains of *T. urticae* over ten successive generations exposed to treated surfaces occurred gradually, with resistance levels increasing steadily. Numerous researchers worldwide have investigated this resistance issue (Herron et al., 2018; Papapostolou et al., 2021). For instance, Sato et al. (2005) observed a 13-fold increase in abamectin resistance in *T. urticae*, a finding corroborated by Mohamed .(2006). Similarly, He et al. (2009) reported a high resistance ratio to abamectin.

Monteriro et al. (2015) and Ferreira et al. (2015) monitored abamectin resistance and highlighted the crucial role of enzymes as a biochemical mechanism in resistance to acaricides. The most significant increases in enzyme activity were induced by abamectin and etoxazole, specifically in glutathione-S-transferase (GST), alkaline phosphatase, acid phosphatase, ATPase, and acetylcholine esterase (AChE) (Smissanert 1964; Lin et al., 2009; Kwon et al., 2010). Nasr (2013) also noted that specific AChE activities were lower in resistant strains, while GST activity was higher. Consequently, the present study was undertaken to evaluate the development of resistance in *T. urticae* strains to various chemicals and to analyze the specific activities of AChE and GST enzymes in a laboratory setting to elucidate the mechanisms of resistance in *T. urticae*.

MATERIALS AND METHODS

Prey Cultures

We acquired colonies of the two-spotted spider mite, *Tetranychus urticae* (Koch) (Acarina: *Tetranychidae*), originally sourced from castor bean plants in Kafr El-Sheikh Governate. These colonies were then maintained under controlled laboratory conditions. The prey organisms were cultured at a temperature of 25 ± 2°C, with a 16-hour photoperiod to promote plant growth, while maintaining a relative humidity of 70 ± 5%.

Chemicals studied

Various compounds were employed for testing, with dosages calculated based on parts per million (ppm) of active ingredient's. The chemical names and formulations of the used materials are:

1- **Fenpyroximate (5% S.C):** tret-butyl (E)-a(1,3-dimethyl-5-phenoxy pyrazole-4-yl methylene-amino-oxy) –p-toluate, provided by Shoura Company.
2- **Cyhalothrin (5% E.C):** A mixture consisting of equal amounts of (S) -2cyano-3phenoxybenzy1 (z)-(1R3R)3(2chloro-3,3,3trifluoro propeny1) -2,2 dim ethyl cyclopropane carboxyl ate and (R) -acyno-phenoxybenzyl (Z)(1S, 3S) -3(2-chloro-3,3,3-trifluropropenyl)2,2-dimethyl cyclopropane carboxyl ate, manufactured by El-Help Company.
3- **Abamectin (1.8% E.C):** A blend comprising a minimum of 80% avermectin B1a (5-0-deinethyl avermectin A1a) and a maximum of 20% avermectin B1b [5-0-demethyl-25-de-(1-methylpropyl)-25-(1-methylethyl) avermectin A1a], supplied by Merck Company.
4- **Ethion (50% E.C):** 0, 0, 0, 0-tetraethyl s, s-methylene-bis

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(phosphoeodi-thioate), provided by El-Help Company.

Resistance investigations

To assess resistance levels, studies were conducted using the leaf disc dipping method with selected compounds (ethion, fenpyroximate, cyhalothrin, and abamectin) for adult specimens of the two-spotted spider mite, *T. urticae*. We determined the LC_{50} value and slope value, and then calculated the resistance ratio by comparing it to the susceptibility strain.

Selection pressure investigation

The field strain of the two-spotted spider mite, *T. urticae*, was gathered from cotton plants cultivated in the experimental farm of Sakha Agricultural Research Station in the Kafr Elsheikh region. Upon collection, each specimen was individually transferred to laboratory conditions and reared for four successive generations, ensuring an environment free from any acaricide contamination, before commencing the experiments by apply selective pressure.

Selection pressure was applied using concentrations that resulted in approximately 50% mortality (LC_{50}) of adult mites for all tested compounds. This pressure was administered using the toxicant solution to leaf discs containing sweet potato cuttings, with each disc dipped for approximately 5 seconds and then left to dry at each concentration. A total of 200 *T. urticae* individuals were tested on each leaf disc within dishes. Mite counts were recorded at 24 and 48-hour intervals post-treatment, with surviving mites subsequently transferred to fresh sweet potato leaves to avoid any contamination from acaricides. The colony was maintained at a level suitable for further selection pressure application.

As mites began to develop tolerance, the selection pressure was intensified across successive generations by incrementally increasing the concentrations of the toxicants, resulting in elevated LC_{50} values. Each concentration was replicated four times for all compounds. LC_{50} values, slope values, and resistance ratios were calculated at the conclusion of each generation and compared with those of the susceptible strain.

The resistance ratio (Rr) was computed according to the method described by Ibrahim (2000) as LC_{50} of the susceptibility strain divided by the LC_{50} of the resistant strain.

Preparation of enzymes samples from *T.* urticae

Adult specimens of the two-spotted spider mite were collected from both the susceptible strain (5 mg) and resistance strains tested in the laboratory (30 mg of fresh mite homogenate for each compound) after a 6-hour starvation period. All samples were finely ground in a mortar to take a powder, which was then suspended and homogenized in an ice solution containing sucrose (0.25 M), by a glass homogenizer. Following homogenization, after centrifugation of the mixture, the supernatant was collected as the enzyme source. Equal amounts of every chosen mite sample were frozen (-2°C) awaiting further use.

The suspensions were then divided into seven 70 ml beakers, with a few drops of sodium salt added to each. The samples were labeled accordingly (0.2 ml), as per El-Doksh's (2010) guidelines. The obtained supernatants were utilized for analysis in mite studies conducted at the analysis laboratory, Agricultural Research Center, Dokki, Giza, Egypt.

Enzyme activity assessment

1- Acetyl cholinesterase

Determination of acetylcholinesterase (AChE) activity was performed by Ellman et al. (1961) method, utilizing acetylthiocholine iodide (ATchI) as a substrate.

Reagents

1- Acetylthiocholine iodide (ATchI), 0.075 M, prepared by dissolving 21.67 mg /ml redistilled water.
2- Dithio-bisnitrobenzoic acid (DTNB) 5.5-dithiobis (2-nitrobenzoic acid (0. 01 M) prepared by dissolving weight of 39.6 mg in 10 ml of 0.1M-pH 7.4 phosphate buffer with 15 mg sodium bicarbonate.
3- Acetylthiocholine AchE Acetic acid + choline

Procedure

A mixture containing 20µ ATchI, 100 µl DTNB, 2.78 ml phosphate buffer, in addition to 100 µl of enzyme solution was prepared in a tube. The treatment of control included 100 µl distilled water in place of the enzyme solution. The reaction mixture was placed in a water bath at 37°C and incubated for 30 minutes. Absorbance was then measured at 421 nm using a spectrophotometer. Each assay was conducted with two replicates.

2- Glutathione-S-transferase (GST)

GST activity was determined using the calorimetric method outlined by Rose and Wallbank (1986), with 1,2-dichloro-4-nitrobenzene (DCNB) as the substrate. The absorbance of the yellow product was determined spectrophotometrically at 344 nm. The enzyme activity is quantified as the rate of change in absorbance over 4 min., This value can be converted to nanomoles per milligram of tissue using an extinction coefficient of 10 $Mm^{-1}cm^{-1}$ (as described by Yu (1982). Additionally, the specific activity is expressed as millimoles of dichloronitrobenzene hydrolyzed per minute per milligram of protein.

Reagents

1- 150 Mm, 1.2 dichloro -4nitrobenzene (DCNB).
2- Reduced Glutathione, 20 mM in 0.1M phosphate buffer, pH 7.5.

Procedure

0.1 ml of sample (enzyme source) and 0.1 ml of buffer were pipetted into a tube, mixed well, and incubated in a water bath at 35°C for 4 minutes. Then, 0.01 ml of DCNB was added, and the mixture was returned to the water bath. The measurement of absorbance was done by the reagent blank as a reference. Each assay was performed in two replicates.

Enzyme activity was determined according to Taussky,H.H and Shorr,E. (1953) and calculated using the following equations:

Specific activity = Absolute activity in a desired homogenate volume/mg protein in the same homogenate volume (Eq. 1).

Specific activity index = Specific activity of resistance strain/Specific activity of susceptible strain (Eq. 2).

% increase in the activity of tested enzymes = (Specific activity of enzymes in treatment - S.A. in control)/(Specific activity of the enzymes in control) × 100 (Eq. 3).

RESULTS AND DISCUSSION

Progress of T. urticae resistance to fenpyroximate

The results of selection pressure experiments revealed a gradual increase in resistance levels in *T. urticae* to Fenpyroximate over successive generations. Table (1) presents the data indicating the emergence of tolerance, starting from the first generation with a 1.86-fold increase in

resistance (LC_{50} values of 2.94 ppm). Subsequent generations demonstrated escalating resistance levels, with the fourth, fifth, and sixth generations exhibiting moderate resistance levels of 11.47, 22.54, and 38.21 fold, respectively.

High variability in resistance levels was observed, with the seventh and eighth generations showing moderate resistance (average RR of 39.48 and 42.17 fold) and LC_{50} values of 62.38 and 66.63 ppm, respectively. Very high resistance levels were evident in the ninth and tenth generations (average RR of 65.63 and 76.73 fold), and LC_{50} were 103.70 and 121.24 ppm, respectively.

As depicted in Table (1), the tenth generation displayed the highest slope value (2.51), after that the first and fourth generations and slope estimates were 1.93. Conversely, the sixth, eighth, and ninth generations exhibited the lowest slope estimates and were 0.98, 0.96, and 0.94, in that order.

Previous studies by Kim et al. (2007) reported high resistance levels to Fenpyroximate (RR, 68 fold), while Goka (1998) found a resistance ratio of 1265 fold for Fenpyroximate in their resistance strain of *T. urticae*. Additionally, Cho et al. (1995) indicated high resistance levels of T. urticae to Fenpyroximate.

Contrasting results were reported by Sharaf (1977), who observed only tolerance levels up to 2.33 fold in a strain of *T. cinnabarinus* after 18 generations of selection with LC50 level. Fahnbulleh (2007) found resistance factor values at LC50 for NOR4 and NOR5 (T. urticae) to be 0.62 and 0.75, respectively, indicating higher tolerance in GSS (T. urticae).

Moreover, Hammad (2005) reported a slope value of 2.87 for Fenpyroximate, and Van Leeuwen et al. (2020) emphasized the importance of molecular diagnostics in managing insecticide resistance in agricultural pests.

The percentage increase in activity of enzyme was estimated by El-Doksh (2001) procedure.

Progress of *T. urticae* resistance to Cyhalothrin

Table (2) presents the progression of resistance in susceptible strains of *T. urticae* over ten successive generations following exposure to cyhalothrin-treated surfaces. Resistance development to cyhalothrin was gradual, starting from the first generation with a modest increase in LC_{50} (1.48 fold), gradually escalating till the fourth generation (5.57 fold). Notably, resistance began to accelerate in the fifth generation, with an LC_{50} value of 7120.22 ppm (11.85 fold).

Subsequent generations demonstrated a steep rise in resistance levels, with the sixth, seventh, and eighth generations reaching high resistance levels of 19.05, 21.49, and 23.44, respectively. The resistance continued to escalate rapidly in the ninth and tenth generations compared to the susceptible strain, reaching peak levels of 34.08 and 36.63 fold, respectively.

Analysis of Table (2) reveals that the second, fifth, and ninth generations exhibited the greatest estimates of slope (1.46, 1.42, and 1.53, respectively), then the eighth and tenth generations (1.35 and 1.40 slope values, respectively). In contrast, the first and fourth generations displayed the least slope estimates of 1.04 and 0.85, respectively.

Table 1. The selection of resistance to Fenpyroximate in *Tetranychus urticae*

Generation tested	C.L. for LC_{50}			Slope Value	RR
	LC_{50} ppm	Lower	upper		
susceptible	1.58	1.40	1.78	2.48	-
1	2.94	2.57	3.41	1.93	1.86
2	5.15	4.05	6.66	1.55	3.26
3	12.99	11.30	15.55	1.60	8.22*
4	18.12	11.58	31.26	1.91	11.47**
5	35.61	30.58	43.26	1.25	22.54
6	60.37	48.79	77.59	0.98	38.21
7	62.38	51.10	79.60	1.08	39.48
8	66.63	54.53	82.50	0.96	42.17
9	103.70	71.51	154.51	0.94	65.63
10	121.24	110.14	139.58	2.51	76.73

RR. Resistance ratio * RR < 10 Tolerance **RR > 10 Resistance

Table 2. The selection of resistance to cyhalothrin in *Tetranychus urticae.*

Generation tested	C.L. for LC_{50}				
	LC_{50} ppm	Lower	upper	Slope Value	RR
susceptible	600.64	544.67	792.72	0.79	-
1	890.42	872.07	1592.28	1.04	1.48
2	924.81	876.39	1662.03	1.46	1.54
3	2334.92	1985.12	3163.86	1.26	3.89
4	3344.18	3310.18	3870.45	0.85	5.57*
5	7120.22	6838.18	9466.02	1.42	11.85**
6	11440.33	15067.62	18085.23	1.23	19.05
7	12905.32	11937.55	19054.62	1.20	21.49
8	14080.63	13713.98	20707.29	1.35	23.44
9	20470.52	18054.98	33276.69	1.53	34.08
10	22000.35	19150.37	35368.65	1.40	36.63

RR. Resistance ratio * RR < 10 Tolerance **RR > 10 Resistance

The observation of low slope values in certain generations suggests a degree of heterogeneity within the population to ward the tested compound. These results align with earlier studies. Youssef et al. (2011) observed resistance rates ranging from 37.45 fold for cyhalothrin in *T. urticae* populations. Similarly, Kobayashi et al. (2001) reported insensitivity to cyhalothrin in some *T. urticae* populations gathered from a specific geographical area, with resistance ratios exceeding 20,000. Lee et al. (2003) identified high resistance levels to cyhalothrin (R.R 90.0) in *T. urticae*

populations. Mohamed (2006) reported a slope value of 0.9891 for cyhalothrin, indicating resistance. Furthermore, Inak et al. (2019) documented resistance incidence and the existence of resistance mutations in *Tetranychus urticae* populations from vegetable crops.

Progress of *T. urticae* resistance to Abamectin

The advancement of resistance in susceptible strains of *T. urticae* was studied over ten successive generations following exposure to abamectin-treated surfaces. The results indicated a gradual increase in resistance with each generation under selection pressure. Table (3) shows the resistance levels across the ten generations.

Initially, the resistance intensity in *T. urticae* was low during the selection process up to the second generation. However, resistance began to manifest in the third generation, with an LC_{50} value of 0.107 ppm (11.89 fold). The sixth, seventh, and eighth generations exhibited high resistance levels of 35.56, 36.00, and 42.55 fold, respectively. Resistance continued to increase gradually, reaching LC_{50} values of 0.596 ppm (66.22 fold) in the ninth generation and 1.246 ppm (138.44 fold) for the tenth generation compared to the susceptible strain.

These findings contrast with those of Koh et al. (2009), who reported low resistance ratios (RR 10) to abamectin in all populations studied.

Regarding slope values, it is well known that they indicate the degree of homogeneity or heterogeneity in the population's response to the tested acaricides. A high slope value reflects a high degree of homogeneity within the population, suggesting similar responses to the tested compounds.

Table 3. The selection of resistance to Abamectin in *Tetranychus urticae*.

Generation tested	C.L. for LC_{50}				
	LC_{50} ppm	Lower	upper	Slope Value	RR
susceptible	0.009	0.007	0.027	2.07	-
1	0.036	0.034	0.044	2.34	3.96
2	0.053	0.050	0.135	2.25	5.89*
3	0.107	0.102	0.135	2.88	11.89**
4	0.116	0.106	0.140	1.80	12.89
5	0.125	0.114	0.153	2.79	13.88
6	0.320	0.305	0.414	0.81	35.56
7	0.324	0.313	0.422	0.81	36.00
8	0.383	0.314	0.437	1.17	42.55
9	0.596	0.521	0.693	1.53	66.22
10	1.246	1.204	1.440	1.44	138.44

RR. Resistance ratio * RR < 10 Tolerance **RR > 10 Resistance

Conversely, a low slope value indicates a high degree of heterogeneity within the population, suggesting varied responses to the tested acaricides. This heterogeneity may necessitate pooling modifier genes to develop resistance effectively. Based on the data in Table (3), the third generation exhibited the highest degree of population homogeneity with a slope value of 2.88. The first, second, and fifth generations also showed relatively high slope values of 2.34, 2.25, and 2.79, respectively. In contrast, the fourth, ninth, and tenth generations displayed the lowest slope values of 1.80, 1.53, and 1.44, respectively.

These findings align with Youssef et al. (2011), who reported that after 10 selection generations, the strain was 140-fold resistant to abamectin compared to the susceptible strain. Meng et al. (2000) found that Panonychus citri developed a 7.3-fold resistance to abamectin after five generations of selection, with a 13-fold increase compared to the initial level, indicating a potential for rapid resistance development in this species. He et al. (2009) observed an 8.7-fold resistance to abamectin after 42 generations of selection. Kim et al. (2007) noted moderate resistance levels to abamectin. Mario et al. (2005) reported that the resistance ratio (R/S) at the LC50 reached 342-fold in *T. urticae.* Kwon et al. (2010) investigated the biochemical mechanisms of abamectin resistance in two *T. urticae* strains, PTF239 and AbaR, which showed 239-fold and 4753-fold resistance, respectively. Ferreria et al. (2015) had resistant field populations of *Tetranychus urticae* to abamectin.

Progress of *T. urticae* resistance to Ethion

The LC_{50} estimates and ratios of resistance for ten generations of *T. urticae* populations are given Table (4). The study shows that resistance ratios were low initially, with the first generation exhibiting an LC_{50} estimate of 20.86 ppm and a resistance ratio of 1.92 fold. However, resistance gradually increased, reaching its peak by the tenth generation. Resistance levels began to rise significantly in the fourth generation, which had an LC_{50} value of 160.80 ppm (15.70 fold). The fifth, sixth, and seventh generations exhibited moderate resistance levels of 15.77, 25.95, and 34.33 fold, respectively. The eighth generation also showed moderate resistance with an LC_{50} value of 406.56 ppm and a resistance ratio of 39.70 fold.

Overall, most generations displayed high resistance levels (RR>10). Notably, the ninth and tenth generations showed very high resistance levels, with LC_{50} values of 719.54 ppm and 941.99 ppm, and resistance ratios of 70.27 and 91.99 fold, respectively. According to Table (4), the slope values indicate increased homogeneity in these generations, with values of 4.55 in the sixth generation and 4.50 in the ninth generation. The seventh and eighth generations had moderate slope values of 2.30 and 3.42, respectively, in addition decreased slope value of 0.90 was obtained in the tenth generation.

These findings are consistent with previous research. Sokel et al. (2007) reported that resistance ratios for *T. urticae* populations ranged from <1.1 fold for ethion. Goka,k.(1998) identified only low to moderate resistance to ethion in *T. urticae.* Kim et al. (2007) observed moderate resistance levels to ethion in T. urticae. Ay et al. (2005) suggested that resistance ratios for chemicals ranged from <2.5 for Propargite (Ethion) and <2.9 for abamectin, based on LC_{50} values. Papapostolou et al. (2021) found significant multiple insecticide resistance in *Tetranychus urticae* field populations.

Table 4. The selection of resistance to Ethion in Tetranychus urticae.

Generation tested	C.L. for LC_{50}				
	LC_{50} ppm	Lower	Upper	Slope Value	RR
susceptible	10.24	9.12	15.16	1.36	-
1	20.86	19.56	36.10	1.30	1.92
2	57.92	48.08	109.76	1.39	5.66
3	84.76	76.21	119.76	1.22	8.28*
4	160.80	151.24	218.43	0.95	15.70**
5	161.53	155.96	236.43	1.18	15.77
6	265.76	251.80	302.40	4.55	25.95
7	351.59	300.50	430.78	2.30	34.33
8	406.56	386.7	469.80	3.42	39.70
9	719.54	624.19	900.00	4.50	70.27
10	941.99	812.79	1529.10	0.90	91.99

RR. Resistance ratio * RR < 10 Tolerance **RR > 10 Resistance

Resistance process in *T. urticae*.

To elucidate the mechanism of resistance in *T. urticae*, the specific activity of certain enzymes was determined under laboratory conditions. Enzyme assays were performed on mite homogenates prepared from individuals exposed to each of the tested acaricides. The specific activities of these enzymes were measured to assess the impact of the acaricides. Enzymes play a major role in the resistance mechanism to acaricides in resistant mites (Ibrahim 2000).

activity of Acetyl cholinesterase in susceptible and resistant strains.

Acetylcholinesterase enzyme (AChE) plays a crucial role in the biochemical mechanisms underlying *acariacide* resistance. The specific activities of AChE were measured in both susceptible and resistant strains of *T. urticae*. Results presented in Table 5 and Figure 1 show a significant decrease in AChE activity in the susceptible strain. Additionally, differences were significant among the tested compounds.

The resistant strain of *T. urticae* to cyhalothrin exhibited the highest AChE specific activity value of 2.23 and a specific activity index of 2.37 compared to the susceptible strain's value of 0.83. Conversely, the abamectin-resistant strain had the lowest AChE-specific activity of 1.18 and a specific activity index of 1.25. These results are largely consistent with the resistance factors displayed in Tables 1, 2, 3, and 4. Specifically, ethion and fenpyroximate significantly increased AChE activity, with values of 1.82 and 1.83, and specific activity indices of 1.93 and 1.94, respectively.

Based on the percentage increase in enzyme activity, it can be concluded that the highest percentage increase in enzyme activity was observed in the cyhalothrin-resistant strain, with an increase of 147.44% compared to the susceptible strain. The abamectin-resistant strain showed the lowest percentage increase in enzyme activity, at 35.42%. Notably, both ethion and fenpyroximate caused significant increases in AChE activity, with percentage increases of 103.40% and 104.36%, respectively.

The involvement of AChE in organophosphate (O.P.) resistance has been reviewed by several authors. Smissanert (1964) and Voss and Matsumura (1964) showed that AChE activity in the homogenate of O.P.-resistant *T. urticae* was much less sensitive than in susceptible mites. Similarly, El-Nawawy et al. (1981) showed that AChE activity was higher in the susceptible strain than in the O.P.-resistant strain of S. littoralis.

In conclusion, the results in Table 5 suggest that AChE activity is not the primary resistance factor. El-Doksh (2001) reported that all tested inducers (ethion and fenpyroximate) increased AChE activity and reduced its sensitivity to inhibition by methomyl.

Table 5. Specific activity, specific activity index, and percentage of increase of AChE in susceptible and resistant strains of T. urticae

Compounds	Specific activity µmol/min./mg protein	Specific activity index	% increase of enzyme activity
Susceptible	0.83 e	-	-
Fenpyroximate	1.83 b	1.94	104.36
Cyhalothrin	2.23 a	2.37	147.44
Abamectin	1.18 d	1.25	35.42
Ethion	1.82 c	1.93	103.40

***Average followed by the same letter within a column have not significant difference at 0.05 level.**

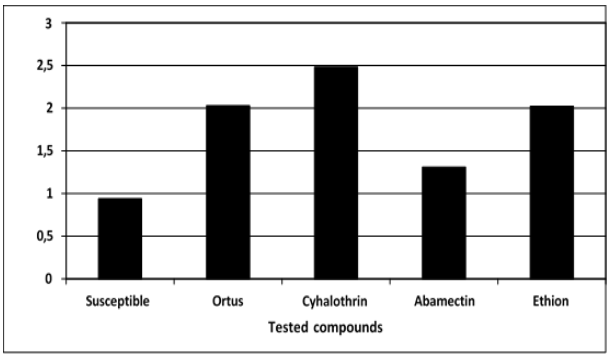

Figure 1. Specific activity of ache enzyme in susceptible and resistance strains.

The Role of Glutathione-S-transferase in *T. urticae* Resistance to Acaricides

Activity of Glutathione-S-transferase (GST) was examined in both susceptible and resistant strains. Numerous studies have underscored the significance of GST, alongside other enzymes like AChE, in insecticide metabolism, particularly in insects like houseflies. In the case of *Tetranychus urticae*, GST enzyme plays a pivotal role in the biochemical mechanism conferring resistance to acaricides.

The data presented in Table 6 and Figure 2 indicate a significant elevation in GST- transferas activity across all resistance strains compared to the susceptible strain for all tested acaricides. Specifically, there was a notable increase in specific activity in the resistant strains of *T. urticae*, with values reaching 556.01and 622.13 for ethion and Fenpyroximate, respectively, corresponding to specific activity indices of 2.09 and 1.86. Abamectin exhibited a moderately inductive effect, with a specific activity index of

1.83 and a value of 542.12. Conversely, cyhalothrin showed the least inductive effect, with a specific activity index of 1.15 and a value of 343.40.

A comparison of the percentage increase observed for the tested compounds revealed Fenpyroximate as the most potent inducer, with a percentage increase of 119.39 %, while abamectin and ethion displayed mean inductive effects, with increases of 92.47% and 97.15%, respectively. Cyhalothrin exhibited the least increase at 25.58%.

Further supporting evidence from Lin et al. (2009) suggests a significant elevation in GST activity in resistant strains in comparison with susceptible ones, particularly in the context of resistance to abamectin in *Tetranychus cinnabarinus*. The resistant strains displayed a 3.4-fold increase in GST activity in comparison with susceptible strains, indicating a consistent role of GSTs in the resistance mechanism across spider mite species.

Table 6. Specific activity, Specific activity index, and percentage of increase of GST in susceptible and resistance strains of *T. urticae*.

Compounds	Specific activity µmol/min./mg protein	Specific activity index	% increase of enzyme activity
Susceptible	267.39 e	-	-
Fenpyroximate	622.13a	1.86	119.39
Cyhalothrin	343.40d	1.15	25.58
Abamectin	542.12c	1.83	92.47
Ethion	556.01a	2.09	97.15

*** Average followed by the same letter within a column have not significant difference at 0.05 level.**

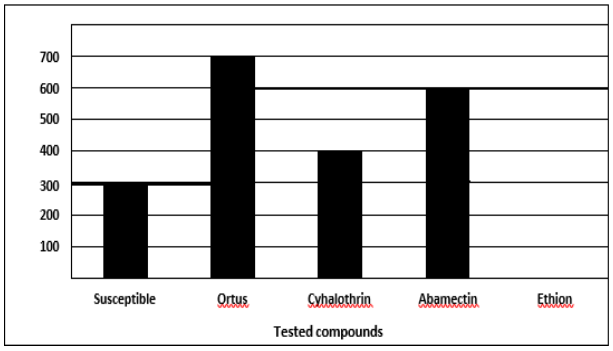

Figure 2. Specific activity of GST enzyme in susceptible and resistance strains.

RECOMMENDATION

It is recommended to implement integrated pest management (IPM) strategies that incorporate rotating acaricides that show altered approaches of action to minimize resistance advancement. Additionally, the significant increase in enzyme activities (AChE and GST) observed in resistant strains suggests that targeting these enzymes could be a potential approach for managing resistance. Continuous test of resistance levels in field populations is essential to inform timely adjustments in pesticide application programs, thereby enhancing the sustainability and effectiveness of pest control measures.

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دراسات على مقاومة الأكاروس العنكبوت الأحمر لبعض المبيدات

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الملخص

هدفت هذه الدراسة إلى تقييم تطور المقاومة في سلالات من العنكبوت الأحمر ذي البقعتين (Tetranychus urticae) الحساسة للعديد من المبيدات، بما في ذلك Fenpyroximate، Cyhalothrin، Abamectin، وEthion، وقياس النشاط النوعي لإنزيمي أسيتيل كولين إستريز (AChE) والجلوتاثيون إس-ترانسفيراز (GST) تحت ظروف المعمل لفهم آليات المقاومة. أظهرت مستويات المقاومة لـ Fenpyroximate تفاوتًا كبيرًا على مدى جيلين، حيث أظهرت الأجيال التاسعة والعاشرة مستويات مقاومة عالية بلغت 66.58 ضعفًا و78.72 ضعفًا على التوالي. زادت المقاومة لـ Cyhalothrin على مدى عشرة أجيال، محققةً مقاومة بمقدار 37.45 ضعفًا. ارتفعت المقاومة لـ Abamectin تدريجيًا، حيث وصلت إلى 140 ضعفًا بحلول الجيل العاشر. بالنسبة لـ Ethion، أظهرت الأجيال التاسعة والعاشرة مستويات مقاومة بلغت 71.8 ضعفًا و94.1 ضعفًا على التوالي. أشارت النتائج إلى أن الأنشطة النوعية لإنزيم AChE كانت أقل بشكل ملحوظ في السلالة الحساسة، مع وجود اختلافات ملحوظة بين المركبات. أظهرت السلالة المقاومة لـ Cyhalothrin من *T. urticae* أعلى نشاط نوعي لـ AChE بلغ 2.48، مقارنة بـ 0.94 في السلالة الحساسة. في المقابل، أظهرت السلالة المقاومة لـ Abamectin أدنى نشاط نوعي لـ AChE بلغ 1.31. قلل كل من Ethion وFenpyroximate نشاط AChE بشكل ملحوظ إلى 2.20 و2.30 على التوالي. بالإضافة إلى ذلك، كان نشاط GST أعلى بشكل ملحوظ في جميع السلالات المقاومة لـ *T. urticae* مقارنة بالسلالة الحساسة.