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Metabolic Resistance Mechanisms of Different Pesticides in The Two-Spotted Spider Mite, *Tetranychus urticae* Koch (Tetranychidae:Acari)

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ABSTRACT

An investigation was performed to determine the possible role of detoxification metabolism in resistant strains of *Tetranychus urticea* Koch.Adult females were subjected to selection pressure through 40 generations by LC50's and LC90's of Vertimec (Avermectines), Cypermethrin (Pyrethroids), Methomyl (Carbamates) and Malathion (O.P), were (41.308, 42.021, 44.881, and 49.265 folds) and (49.815 ,55.411 ,63.972 ,and 90.00 folds), respectively, whereas RRS slopes ranged from 0.884 to 1.395 folds. Comparative assay with esterases (EST) and mixed function oxidases(MFO) showed that LC50's of Mal-Resistant strains had higher EST. activiy (4.71) with lower MFO activity (3.80),while ,LC50's of Vert-Rresistant strains had lower EST. activiy (2.70) with higher MFO activity (4.26). Synergist experiments showed low synergism by S,S,S tributylphosphorotrithioate (DEF) of EST activiy ranged from 4.81 to 2.99 folds,while synergism by Piperonyl Butoxide (PBO) of MFO activity ranged from 4.27 to 3.82folds to $LC_{50's}$ pesticides resistant strains.

Key Words: *Tetranychus urticae*, Resistance, Selection Pressure, Avermectines, Pyrthroids, Carbamates OP's, Mixed Function Oxidases, Esterases, PBO, DEF

INTRODUCTION

Resistance to pesticides is conferred by genes controlling penetration, detoxification and sensitivity of the target protein (Brown, 1990). However, linkage relationships among these genes are not defined in most agricultural pests, especially mites. The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), a worldwide pest of many plant species, after having been exposed to many pesticides for many years has developed resistance to a wide range compounds. This resistance may develop quickly because of the mite has numerous annual generations and high frequency of spray applications. The evolution of resistance depends on gene flow among populations that colonize different habitats in many places (Tsagkarakou *et al*., 1996).

Metabolic resistance is the potential of pests to expel poisonous pesticides from their body through chemically driven deterioration. Three enzyme systems have been found to be involved in this resistance mechanism: Nonspecific esterases, mixed function oxidases and glutathione- S –transferases (GSTs) (Brogdon & McAllister, 1998). A considerable amount of evidence shows that esterases are connected with acaricide resistance in many spider mite strains. Kim *et al*. (2004) obtained resistant strains of *T. urticae* by sequential assortment with the acaricides: carbophenothion, ethion, dicofol, cyxehation and bifenthrin, and separated easterase isoenzyme by polyacrylamide gel electrophoresis. Therefore, it was able to detect different isoenzymes in the resistant and susceptible strains. The difference indicated that esterases are associated with the resistance mechanisms of the tested acaricides.Comparing with Vertimec resistance which resulted mainly from oxidative metabolism in many pests such as Colorado potato beetles (Argentine *et al.*, 1992), that a high resistance to abamectin resulted from increased cytochrome P-450 monooxygenase–mediated detoxification in the resistant strains. However, general esterases and carboxylesterase activities in the abamectin-resistant strains were significantly enhanced compared with the SS strain (Clark *et al*. ,1992).This result was most apparent in the carboxylesterase assay, which gave greater than two fold increase in hydrolyic activity to α-naphthyl butyrate in both abamectin –resistant strains compared with the SS strain. In Vertimec resistant strains of *T.urticae*,the effects of the synergists were evaluated in several resistant populations.PBO produced only low levels of synergism even in (S) and (R),as shown by their synergistic ratios 0.1 and 1.7respectively,which indicated that oxidative metabolism played its role with other metabolic systems in detoxification (Clark *et al*.,1992).

This study aimed to investigate the metabolic resistance in *T. urticae* adult females after selection with $LC_{50's}$ of Vertimec , Cypermethrin, Methomyl and Malathion based on changeable activity of

esterases and mixed function oxidases (MFO) with and without synergists.

MATERIALS AND METHODS

Pesticides and Chemicals:

The formulated pesticides used for leaf-dip bioassays were vertimec (Averrmectines) 1.8% EC from Syngenta Co.,cypermethrin(Pyrethroids) 50% EC from KZ Co., methomyl (Carbamates) 90%EC from KZ Co. and malathion (Organophosphates) 95%EC from KZ Co.

Maintenance of *Tetranychus urticae:*

Colonies of the spider mite, *T. urticae* were reared under laboratory conditions (25±2˚C, and 60±5%RH) at Plant Protection Research Institute branch, Dakahlia Governorate. They were reared on clean castor oil plants leaves placed on moist cotton wool pad in Petri-dishes .The colonies began with mites which were taken from infested castor oil plant leaves, then left for one year under lab. Conditions to get a homogenous and sensitive colonies.

Assessment of acaricidal activity:

In this respect, laboratory experiments were conducted to evaluate the activity of tested pesticides against *T. urticae* adult females. The leaf-dip technique described by Dittrich (1962) was used. Mortality percentages were determined and corrected by using Abott's formula (1925) and stastically analyzed according to Finney (1971) to estimate LC_{50}, LC_{90} and slope values.

Rearing of *T.urticae* colonies under pesticides selection pressure:

Treated mite colonies were reared under selection pressure of tested pesticides, Vertimec; Cypermethrin; Methomyl and Malathion. It was carried out by leaf–dip technique at LC_{50} level of each of the tested toxicants, from parent's generation till 40^{th} generation. For studying the levels of resistance in these selected strains ,toxicity lines of each acaricide against the adult females were established every two generations .All the results of the selected strains were compared with those of the laboratory susceptible strain to calculate the level of resistance for each compound.

Estimation of esterases activity:

EST activity was measured using α–Naphthyl Acetate (α-NA) by the method of Van Asperen (1962) with slight modifications .The reaction mixture contained 450 µl of potassium phosphate buffer (4mM,PH 6.8) and 50 µl of enzyme solution (from 0.01 gm of each stored sample) was incubated at 37 ˚C for 15 min after addition of 0.5ml of α-NA in ethanol.The reaction was stopped and color developed by adding 0.5 ml of dye solution (10glitre^{-1} diazoblue B salt + 50g litre^{-1} sodium lauryl sulfate) 2:5 by volume for 20 min. The absorbance was read at 600nm for α-NA by a Gilford 260PS spectro-photometer.

Estimation of oxidases assay:

MFO activity was measured using p-nitroanisole-O-demethylation by the method of Kim *et al*., (2004). The reaction mixture contained 50 µl of miocrosomal preparation (5-50 protein equivalents), 50 of NADPH–generating system (Magnesium chloride 12 mM, NADPH 2.7mM, NADP 8.1 mM, glucose-6-phosphate 240 Mm, glucose-6-phosphate dehydrogenase 25 units ml^{-1}), 390 µl of potassium buffer (0.1 M, PH = 7.4) and 10 µl of PNA in ethanol (0.05 mM). The reaction was run at 37˚C for 3 min. Absorbance was measured at 400nm by a Gilford 260PS spectrophotometer. The concentration of P-nitrophenol generated was determined from a standard curve.

Synergism Test:

Piperonyl Butoxide (PBO-Technical product from Sigma, Milwaukee, WI) and S,S,S tributylphosphorotrithioate (DEF-Technical product from ChemServices West Chester, PA) were used to inhibit detoxification mechanisms by esterases (EST) and mixed-function oxidses (MFO), respectively, by adding 10 µl of synergist to the mite homogenates in a final concentration of 10^{-7} M and was incubated for 5 min at 27˚C.

RESULTS AND DISCUSSION

The $LC_{50's}$ and $LC_{90's}$ for all resistant *T. urticae* adult females till 40^{th} generation, were considerably higher, and revealed large differences comparable with those of the reference strains.

Vertimec Resistance:

For the selection pressure by vertimec through 40 generations of *T. urticae* adult females, table (1) showed that LC_{50} and LC_{90} with upper and lower limits were 2099.38 (5941.245-741.830 ppm) and 7192.297 (20354.201-2541.448 ppm), respectively, which were significantly higher than susceptible strain.

The slope of concentration–mortality lines for susceptible and resistant populations varied from 4.20±0.53 to 5.86±0.14, respectively, with RR =1.395 folds, suggesting heterozygosity in adult females in each population till gain resistant 40^{th} generation.

Table (1): Development of resistance in *Tetranychus urticae* to Vertimec.

G.	LC_{50}(ppm)			LC_{90}(ppm)			Slope	Toxicity index	
	Main	U.L.	L.L	Main	U.L.	L.L		LC_{50}	LC_{90}
S.	50.822	111.30	23.206	144.38	316.192	65.613	4.20	100	100
F2	70.416	147.874	33.531	523.894	1100.178	249.473	3.14	70.17	27.56
F4	218.763	437.526	109.382	998.145	1996.29	499.073	2.79	23.23	14.46
F6	599.431	1192.868	301.222	2014.333	4008.523	1012.228	3.01	8.48	7.17
F8	714.379	1500.196	324.718	3520.141	7392.296	1676.258	3.24	7.11	4.10
F10	825.794	2064.485	330.318	4199.375	10498.438	1679.75	3.57	6.15	3.44
F20	1032.119	2745.437	384.631	5210.413	13859.699	1958.802	4.23	4.92	2.77
F30	1571.245	4415.199	559.162	6031.157	16947.551	2146.319	5.09	3.23	2.39
F40	2099.38	5941.245	741.830	7192.297	20354.201	2541.448	5.86	2.42	2.00

Table (2): Resistance ratios of Vertimec resistant adult females of *Tetranychus urticae* till 40th generation.

G.	LC_{50}			LC_{90}			RR
	RR50	RR50 U.L.	RR50 L.L	RR90	RR90 U.L	RR90 L.L	Slope
S.	--------	-------	------	-------	-------	-------	------
F2	1.386	1.329	1.44	3.629	3.479	3.802	0.748
F4	4.304	3.931	4.714	6.913	6.314	7.606	0.664
F6	11.795	10.718	12.98	13.952	12.677	15.427	0.717
F8	14.056	13.479	13.993	24.381	23.379	25.548	0.771
F10	16.249	18.549	14.234	29.086	33.203	25.601	0.85
F20	20.309	24.67	16.575	36.088	43.833	29.854	1.007
F30	30.917	39.67	24.956	41.773	53.599	32.712	1.212
F40	41.308	53.38	31.967	49.815	64.373	38.734	1.395

Table (3): Development of resistance in *Tetranychus urticae* to Cypermethrin.

	LC_{50}(ppm)			LC_{90}(ppm)			Slope	Toxicity index	
	Main	U.L.	L.L	Main	U.L.	L.L		LC_{50}	LC_{90}
S.	70.356	148.451	33.344	175.242	369.761	83.053	3.71	100	100
F2	90.197	200.237	40.629	591.146	1312.344	200.282	3.52	78.00	29.64
F4	219.185	515.085	93.27	992.535	2332.457	422.355	3.03	32.1	17.66
F6	435.753	1050.165	180.81	1299.247	3131.185	539.107	3.10	16.15	13.49
F8	612.133	1530.333	244.853	3216.366	8040.915	1286.456	2.27	11.49	5.45
F10	841.385	2238.084	316.310	5317.977	14145.819	1999.24	2.98	8.36	3.3
F20	1197.196	3352.149	427.57	6430.032	17752.09	2264.297	4.07	5.88	2.76
F30	2030.175	5745.395	717.376	7163.215	20271.898	2531.171	4.13	3.47	2.45
F40	2956.44	889.32	985.48	9710.337	29131.011	3236.779	4.45	2.38	1.80

Table (4): Resistance ratios of Cypermethrin resistant adult females of *Tetranychus urticae* till 40th generation.

G.	LC_{50}			LC_{90}			RR
	RR50	RR50 U.L.	RR50 L.L	RR90	RR90 U.L	RR90 L.L	Slope
S.	--------	-------	------	-------	-------	-------	------
F2	1.282	1.349	1.218	3.373	3.549	2.411	0.949
F4	3.115	3.47	2.797	5.664	6.308	5.411	0.817
F6	6.194	7.074	5.423	7.414	8.468	6.491	0.836
F8	8.701	10.309	7.343	18.354	21.746	15.491	0.612
F10	11.959	15.076	9.486	30.346	38.257	24.072	0.803
F20	17.016	22.581	12.823	36.179	48.01	27.263	1.097
F30	28.856	38.702	21.514	40.876	54.824	30.477	1.113
F40	42.021	59.7	29.555	55.411	78.783	38.972	1.199

Table (5): Development of resistance in *Tetranychus urticae* to Methomyl.

G.	LC_{50}(ppm)			LC_{90}(ppm)			Slope	Toxicity index	
	Main	U.L.	L.L	Main	U.L.	L.L		LC_{50}	LC_{90}
S.	140.617	295.296	66.960	401.599	803.198	191.238	3.90	100	100
F2	326.437	754.069	1441.315	985.114	2275.613	426.456	3.04	43.08	40.767
F4	539.332	1321.363	220.136	1423.773	3488.244	581.132	2.99	26.07	28.21
F6	746.543	1963.408	283.857	3335.146	8771.434	1268.116	2.76	18.84	12.04
F8	939.104	2554.363	345.259	5939.417	16155.214	2183.609	2.54	14.97	6.762
F10	1521.657	4428.022	522.906	8215.887	23908.231	2823.329	2.83	9.24	4.889
F20	3489.112	10467.336	1163.037	10625.143	31875.429	3541.714	3.09	4.03	3.779
F30	5114.725	15906.795	1644.606	11725.462	36466.187	3770.245	3.43	2.75	3.425
F40	6310.97	20195.104	1941.837	25691.159	82211.781	8028.487	3.52	2.23	1.563

Table (6): Resistance ratios of Methomyl resistant adult females of *Tetranychus urticae* till 40th generation.

G.	LC_{50}			LC_{90}			RR
	RR50	RR50 U.L.	RR50 L.L	RR90	RR90 U.L	RR90 L.L	Slope
S.	--------	-------	------	-------	-------	-------	------
F2	2.322	2.554	2.112	2.453	2.833	2.23	0.779
F4	3.835	4.475	3.290	3.545	4.343	3.039	0.767
F6	5.309	6.649	4.243	8.305	10.921	6.631	0.708
F8	6.678	8.650	5.161	14.789	20.114	11.418	0.651
F10	10.821	14.995	7.816	20.458	29.766	14.763	0.726
F20	24.813	35.447	17.385	26.457	39.686	18.52	0.792
F30	36.373	53.867	24.583	29.197	45.401	19.715	0.879
F40	44.881	68.389	29.479	63.972	102.356	41.982	0.903

Table (7): Development of resistance in *Tetranychus urticae* to Malathion.

G.	LC_{50}(ppm)			LC_{90}(ppm)			Slope	Toxicity index	
	Main	U.L.	L.L	Main	U.L.	L.L		LC_{50}	LC_{90}
S.	172.254	361.733	82.026	878.378	1844.594	418.275	3.52	100	100
F2	225.741	521.462	97.723	1315.112	3037.909	569.313	3.01	76.31	66.79
F4	546.132	1310.717	227.555	3741.557	8979.737	1558.982	2.53	31.54	23.48
F6	896.177	2240.443	358.471	9525.114	23812.785	3810.046	2.00	19.22	9.22
F8	1541.332	4161.596	570.864	11410.983	30809.654	4226.29	2.14	11.18	7.7
F10	2937.905	8226.134	1049.252	14998.983	41995.338	5356.548	2.98	5.86	5.86
F20	5001.419	15504.399	1613.361	39171.662	121432.15	12636.02	3.31	3.44	2.24
F30	6329.881	19749.299	2028.808	52110.339	162584.26	16702.032	3.43	2.72	1.69
F40	8486.113	25458.339	2828.704	79054.235	237162.71	26351.412	3.98	2.03	1.11

Table (8): Resistance ratios of Malathion resistant adult females of *Tetranychus urticae* till 40th generation

G.	LC_{50}			LC_{90}			RR
	RR50	RR50 U.L.	RR50 L.L	RR90	RR90 U.L	RR90 L.L	Slope
S.	--------	-------	------	-------	-------	-------	------
F2	1.311	1.442	1.191	1.497	1.647	1.361	1.169
F4	3.171	3.623	2.774	4.26	4.868	3.727	1.391
F6	5.203	6.194	4.37	10.844	12.909	9.109	1.76
F8	8.948	11.505	6.96	12.991	16.703	10.104	1.645
F10	17.056	22.741	12.7917	17.075	22.767	12.806	1.181
F20	29.035	42.861	19.669	44.595	65.831	30.21	1.063
F30	36.747	54.596	24.734	59.326	88.141	39.931	1.026
F40	49.265	70.379	34.485	90.00	128.572	63.00	0.884

Relative to the strain (S) ,the resistance ratios (RR's),showed in table (2), to vertimec were 41.308 and 49.815 folds as determined at LC50 and LC90 levels, respectively. Consequently they exhibited moderate levels of resistance (RR)≥40 ,according to Hayashi scale(1983).

Cypermethrin Resistance:

For the selection pressure by cypermethrin through 40 generations of *T. urticae* adult females, table (3) showed that LC_{50} and LC_{90} with upper and lower limits were 2956.44 (8869.32-985.48 ppm) and 9710.337 (29131.011-3236.779 ppm), respectively, which were significantly higher than susceptible strains.

The slope of concentration–mortality lines for susceptible and resistant populations varied from 3.71±0.23 to 4.45±0.14, respectively, with RR =1.199 folds, suggesting heterozygosity in adult females in each population till gain resistant 40^{th} generation.

Relative to the strain (S) ,the resistance ratios (RR's),showed in table (4), to cypermethrin were 42.021 and 55.411 folds as determined at LC50 and LC90,respectively.Thus, they exhibited moderate levels of resistance (RR)≥40 ,according to Hayashi scale(1983).

Methomyl Resistance:

For the selection pressure by methomyl through 40 generations of *T. urticae* adult females, table (5) showed that LC50 and LC90 with upper and lower limits were 6310.97(20195.104-1941.837) ppm and 25691.159 (82211.781-8028.487 ppm), respectively, which were significantly higher than susceptible strains.

The slope of concentration–mortality lines for susceptible and resistant populations varied from 3.90±0.08 to 3.52±0.15, respectively, with RR =0.903 folds, suggesting nearly heterozygosity in adult females in each population till gain resistant 40^{th} generation.

Relative to the strain (S) ,the resistance ratios (RR's),showed in table (6), to methomyl were 44.881 folds at LC_{50} level ,so it exhibited moderate levels of resistance (RR)≥40.

On the other hand, RR was 85.178 folds upon LC_{90} level, so it exhibited high level of resistance RR≤160.

Malathion Resistance:

For the selection pressure by malathion through 40 generations of *T. urticae* adult females, table (7) showed that LC_{50} and LC_{90} with upper and lower limits were 8486.113 (25458.339-2828.704 ppm) and 79054.235 (237162.71-26351.412 ppm), respectively, which were significantly higher than susceptible strains.

The slope of concentration–mortality lines for susceptible and resistant populations varied from 3.52±0.46 to 3.98±0.72, respectively, with RR =0.884 folds, suggesting nearly heterozygosity in adult females in each population till gain resistant 40^{th} generation.

Relative to the strain (S), the resistance ratios (RR's), showed in table (8), to malathion were 49.265 folds at LC_{50} level, so it exhibited moderate levels of resistance (RR)≥40 According to Hayashi scale (1983).

On the other hand, RR was 90.00 folds at LC_{90} level, so it exhibited high level of resistance RR≤160, according to Hayashi scale (1983).

Esterases Activity:

Data in table (9) referred to the changes of the rate of α-NA hydrolysis by vertimec, cypermetrin, methomyl and malathion resistant strains and laboratory strain of *T. urticae* adult females homogenates. The data generally revealed that all tested compounds caused increasing in α-NA hydrolysis in the tested strains in comparable with the laboratory strain. Malathion caused the highest level of increasing in α-NA hydrolysis (6.27) µg/mite/minute, followed by methomyl (4.05) µg/mite/minute,then cypermethrin (3.17) µg/mite/minute and finally vertimec (2.08) µg/mite/minute, while the laboratory strain recorded 1.33,1.09,0.92 and 0.77 µg/mite/minute as the rate of α-NA hydrolysis by malathion, methomyl, cypermethrin and vertimec, respectively.

Esterases activity of (R) populations of $LC_{50's}$ of vertimec, cypermetrin, methomyl and malathion towards α-NA is much higher than that of (S) in nearly 2.70, 3.45, 3.72 and 4.71 folds, in the same order.

Data in table (9) referred to the changes of the rate of α-NA hydrolysis by vertimec, cypermetrin, methomyl and malathion resistant strains and laboratory strain of *T. urticae* adult females homogenates in the presence of DEF as synergist. The data generally revealed that all tested compounds caused synergism of increasing in α-NA hydrolysis in the tested strains in comparable with

Table (9): Rate of α -NA hydrolysis (2.5 x 10^{-4} M) by (S) and (R) Strains homogenates of *Tetranychus urticae*, with and without DEF as an Synergist

Tested pesticides	Substrate hydrolysis and enzyme activity determination	Strains	
		S	R
Vertimec	α-NA	0.77	2.08
	*EST. A	------	2.70
	**α -NA+ DEF	0.91	2.72
	*EST. A	------	2.99
Cypermethrin	α -NA	0.92	3.17
	*EST. A	------	3.45
	**α -NA+ DEF	1.08	3.98
	*EST. A	------	3.69
Methomyl	α -NA	1.09	4.05
	*EST. A	------	3.72
	**α -NA+ DEF	1.53	6.11
	*EST. A	------	4
Malathion	α-NA	1.33	6.27
	*EST. A	------	4.71
	**α-NA+ DEF	1.36	6.54
	*EST. A	------	4.81

*EST.A = Esterases Activity = $\frac{\text{hydrolysis of α–NA in R Strain}}{\text{hydrolysis of α–NA in S Strain}}$

**α -NA+ DEF = Potentiation of synergist (DEF) to pesticide

Table (10): Rate of PNA hydrolysis (2.5 x 10-4 M) by (S) and (R) Strains of *Tetranychus urticae* homogenates , with and without PBO as an Synergist.

Tested pesticides	Substrate hydrolysis and enzyme activity determination	Strains	
		S	R
Vertimec	PNA	11.26	47.95
	* Oxidases A.	-----	4.26
	**PNA+PBO	12.26	52.29
	* Oxidases A.	-----	4.27
Cypermethrin	PNA	10.05	40.21
	* Oxidases A.	------	4
	**PNA+PBO	10.27	41.59
	* Oxidases A.	-----	4.05
Methomyl	PNA	9.05	35.12
	* Oxidases A.	-----	3.88
	**PNA+PBO	9.58	37.25
	* Oxidases A.	-----	3.89
Malathion	PNA	8.95	34.05
	* Oxidases A.	------	3.80
	**PNA+PBO	9.45	36.11
	* Oxidases A.	-----	3.82

*Oxidases A = Oxidases Activity = $\frac{\text{hydrolysis of PNA in R Strain}}{\text{hydrolysis of PNA in S Strain}}$

**PNA+PBO = Potentiation of synergist(PBO) to pesticide.

the laboratory strain.Malathion caused the highest level of increasing in α-NA hydrolysis (6.54) µg/mite/minute, followed by methomyl (6.11) µg/mite/minute, then cypermethrin (3.98) µg/mite/minute and finally vertimec (2.72) µg/mite/minute, while the laboratory strain recorded 1.36,1.53,1.08 and 0.91 µg/mite/minute as the rate of the synergism of α-NA hydrolysis by malathion, methomyl, cypermethrin and vertimec, respectively.

Rate of α-NA hydrolysis by (S) and (R) strains homogenates in the presence of DEF as synergist was also measured in case of treatments with vertimec, cypermethrin, methomyl and malathion as following, 2.99, 3.69, 4.00 and 4.81 folds, in the same order.

Mixed function Oxidases Activity:

Data in table (10) referred to the changes of the rate of PNA hydrolysis by vertimec, cypermetrin, methomyl and malathion resistant strains and laboratory strain of *T. urticae* adult females homogenates. The data generally revealed that all tested compounds caused increasing in PNA hydrolysis in the tested strains in comparable with the laboratory strain.Vertimec caused the highest level of increasing in PNA hydrolysis (47.95) µg/mite/minute ,followed by cypermethrin (40.21) µg/mite/minute, then methomyl (35.12) µg/mite/minute and finally malathion (34.05) µg/mite/minute, while the laboratory strain recorded 11.26,10.05,9.05 and 8.95 µg/mite/minute as the rate of PNA hydrolysis by vertimec, cypermethrin, methomyl and malathion, respectively.

Mixed function oxidases activity of (R) populations of $LC_{50's}$ of vertimec ,cypermetrin,methomyl and malathion towards PNA is much higher than that of (S) in nearly 4.26, 4.00, 3.88 and 3.80 folds in the same order.

Data in table (10) referred to the changes of the rate of PNA hydrolysis by vertimec, cypermetrin, methomyl and malathion resistant strains and laboratory strain of *T. urticae* adult females homogenates in the presence of DEF as synergist. The data generally revealed that all tested compounds caused synergism of increasing in PNA hydrolysis in the tested strains in comparable with the laboratory strain. Vertimec caused the highest level of increasing in PNA hydrolysis (52.29)µg/mite/minute, followed by cypermethrin (41.59) µg/mite/minute, then methomyl (37.25) µg/mite/minute and finally malathion (36.11) µg/mite/minute, while the laboratory strain recorded 12.26, 10.27, 9.58 and 9.45 µg/mite/minute as the

rate of the synergism of α-NA hydrolysis by malathion,methomyl,cypermethrin and vertimec, respectively.

Rate of PNA hydrolysis by (S) and (R) strains homogenates in the presence of PBO as synergist was also measured in case of treatments with $LC_{50's}$ of vertimec, cypermetrin, methomyl and malathion as following, 4.27, 4.05, 3.89 and 3.82 folds, in the same order.

The slope of concentration–mortality line is able to indicate phenotypic variations, including environmental as well as genetic variations (Hoskins, 1960; Tabashnik and Cushing, 1989). If environmental variations in a bioassay can be constantly maintained, with errors in the estimation kept small, the slope is probably sufficient to express any relationship between slope and genetic variations in the tolerance towards a pesticide. Resistance is usually evaluated by conducting laboratory bioassays, which are typically presented as the median lethal concentration (LC_{50}) and slope of the concentration–mortality line (Finney, 1971).

Side by side, the resistance ratio (RR) of each acaricide was calculated by dividing the LC50 value of a treated mite population by that of strain S. The RR's from resistant populations ranked into four categories, as suggested by Hayashi (1983); ≤10, >10,and≤40, >40 and ≤160,and >160, considered as low ,moderate, high and extremely high resistance, respectively. In the present study, using Hayashi scale it was found that most gained resistances ranged between moderate and high levels. Moreover, in the same trend, Shu *et al*. (2006) mentioned that decrease and increase in the slope suggested that the degree of heterozygosity increased and decreased over time through generations exposed to pesticides.

These results are not surprising, especially with the small number of chromosomes (n = 3) in *T. urticae*, which increases the possibilities of multiple-resistance development (Helle & Bolland 1967). The selection with an acaricide e.g. abamectin may select populations resistant to another group of acaricides e.g. pyrethroids, if the genes responsible for resistance to these two groups are located in the same chromosome (Omoto, 1995). Abamectin resistance in *T. urticae* was also reported by several authors (Campos *et al*., 1996, Beers *et al*., 1998). Stumpf & Nauen (2002), investigating enzymes involved in abamectin resistance in the two-spotted spider mite, observed that resistant strains (NL-00 and COL-00) presented several folds higher MFO (cytochrome P450-dependent monooxygenase) activity than the susceptible strain GSS. Abamectin resistance in strain NL-00 was strongly synergized by PBO (piperonyl butoxide) and DEM (diethyl maleate), suggesting that MFO and GST (glutathione S-transferases) may be involved in abamectin resistance (Stumpf & Nauen 2002). These results are in agreement with the present results, in which vertimec resistant strains recorded higher oxidases activity than all resistant strains of other tested pesticides. It is revealed that metabolic resistance of vertimec is depending on oxidases activity levels in the treated mites. On the contrary, Chuan-hua *et al*. (2009) mentioned that the major resistant mechanism to abamectin in *Tetranychus cinnabarinus* (Boisd.) was the increasing activities of carboxylesterases (CarE) with 2.7, glutathione-S-transferase (GST) with 3.4 and mixed function oxidase (MFO) with 1.4 folds contrasted to that in susceptible strain, respectively.

Stability of acaricide resistance has been studied for several compounds in *T. urticae* and other species of mites (Inoue 1980, Omoto *et al*., 1995; Stumpf & Nauen 2002 and Sato *et al*., 2004). Dicofol resistance was shown to be unstable in *Panonychus citri* (McGregor), in the absence of selection pressure (Inoue 1980). Lower variations in resistance frequency observed for populations with low percentage of resistant mites (Inoue 1980), corroborated the results obtained in this study with abamectin resistance in *T. urticae*. Although the abamectin resistance frequency decreased from 75% to less than 15% in six months in the population of *T. urticae*, abamectin resistance was shown to be stable in the laboratory at least over six months in a Dutch strain (NL-00) of two-spotted spider mite, collected from roses (Stumpf & Nauen 2002).

Similar trends were also observed for O.P's and Carbamates but with carboxylesterases levels which were at least two times higher in R than S females, as demonstrated by several previouus studies . These results can be explained based on two major resistance mechanisms concerning this type of pesticides. Firstly, the overproduction of one of two closely related carboxylesterases (E4-FE4) that sequester or degrade organophosphates and carbamates esters before they reach their target sites in the nervous system (Foster and Devonshire, 1999). Secondly mechanism, termed MACE (Modified Acetyl Choline Esterase), due to a modification to the O.P's and Carbamates target, AChE, confers strong resistance specially to dimethylcarbamates,primicarb and triazamate.) referred to the increased esterase level in (R) strains

because of the production of esterase isozyme that metabolises OP's.

Pyrethroids resistance associated with insensitivity of the sodium ion channel target and genetically linked to cytochrome oxidases as detoxification mechanism (Park and Brown, 2001). This explanation was proved by synergism i.e. propnylaryl ethers (Lee et al., 1999). Martinez-Torres *et al*. (1999) explained pyrethroids resistance upon a point mutation leading to a single amino acid substitution (leucine to phenylalanine) in a voltage–gated sodium channel protein that appears to be associated with resistance to several pyrethroids .It can be termed Knock-down resistance (Kdr). Concerning pyrethriods resistance, previous linkage proved that resistant cypermethrin strain showed high level of ixodases followed by resistant vertimec strain compared with other resistant strains.

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