IV- RESULTS

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1. Susceptibility levels of *Culex pipiens* larvae to certain insecticides .

Data of the insecticidal activities of the synthetic pyrethroid (Sumicidin) ; The organophosphorus (Fenitrothion and Malathion) and the carbamate (Propoxur) against the early fourth instar larvae of ***Culex pipiens*** resistant strain under laboratory conditions are presented is Tables (1-4) and shown graphically in Figs (1-4) . The obtained data indicated that :-

- High concentration was used to produce the larval mortalities in comparison with the levels below the discriminating doses which kill the susceptibil larvae
- The low doses used was found to be over than the recorded discriminating doses .
- The larvae are not exhibiting any apparent heterogenity regarding their response to different doses of the insecticides .

Table (1) : Susceptibility of the early fourth instar larvae of ***Culex pipiens*** to Sumicidin after 24 hours of exposure.

Concentrations in (ppm)	Averages of corrected mortality percent	LC_{50} (ppm)
	$\bar{X}$ ± S.E.	
0.040	*15.1 ± 0.15	0.06
0.045	32.0 ± 0.58	
0.070	62.1 ± 0.91	
0.098	86.1 ± 0.73	
0.150	99.1 ± 0.81	

Table (2) : Susceptibility of the early fourth instar larvae of ***Culex pipiens*** to Fenitrothion after 24 hours of exposure.

Concentrations in (ppm)	Averages of corrected mortality percent	LC_{50} (ppm)
	$\bar{X}$ ± S.E.	
0.09	*10.0 ± 0.14	0.2
0.14	24.0 ± 0.60	
0.22	62.0 ± 0.81	
0.55	87.0 ± 0.63	
0.70	98.4 ± 0.91	

* Each value represents the mean of five replicates ± standard error

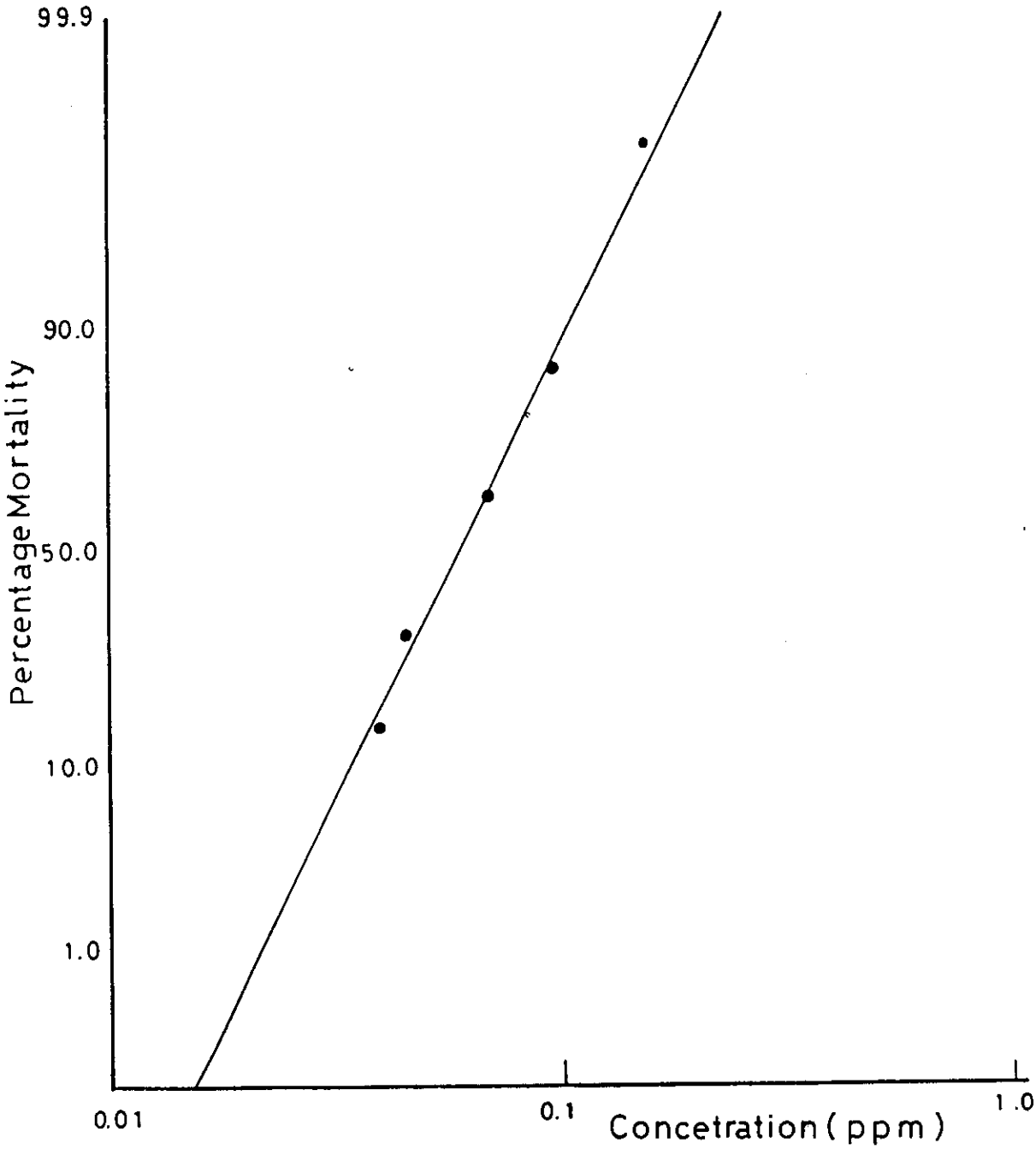

Figure (1) : Probit regression line of mortality among early fourth instar larvae of *Culex pipiens* after treatment with Sumicidin.

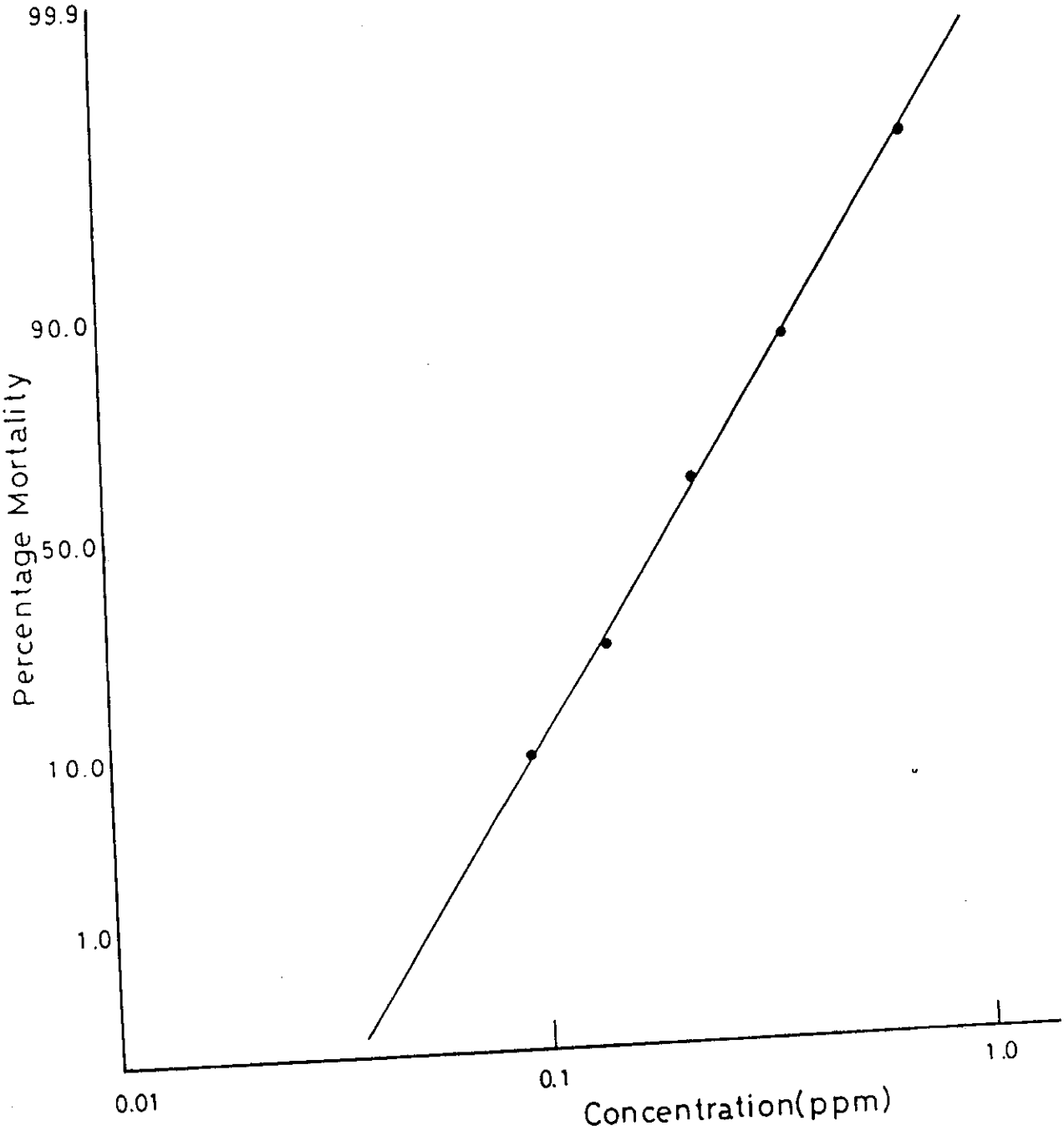

Figure (2) : Regression line of mortality among the *Culex pipiens* larvae after treatment with different concentrations of Fenitrothion.

Table (3) : Susceptibility of the early fourth instar larvae of ***Culex pipiens*** to Malathion after 24 hours of exposure .

Concentrations in (ppm)	Averages of corrected mortality percent	LC_{50} (ppm)
	$\bar{X}$ ± S.E.	
1.1	*30.1 ± 0.90	1.49
1.5	40.8 ± 0.81	
2.0	76.1 ± 0.93	
3.5	90.1 ± 0.21	
6.0	98.2 ± 0.13	

Table (4) : Susceptibility of the early fourth instar larvae of ***Culex pipiens*** to Propoxur after 24 hours of exposure.

Concentrations in (ppm)	Averages of corrected mortality percent	LC_{50} (ppm)
	$\bar{X}$ ± S.E.	
6.0	*38.1 ± 0.59	7.2 ppm
8.0	54.2 ± 0.84	
10.0	74.2 ± 0.92	
15.0	85.1 ± 0.99	
17.0	94.3 ± 0.95	

* Each value represents the mean of five replicates ± standard error

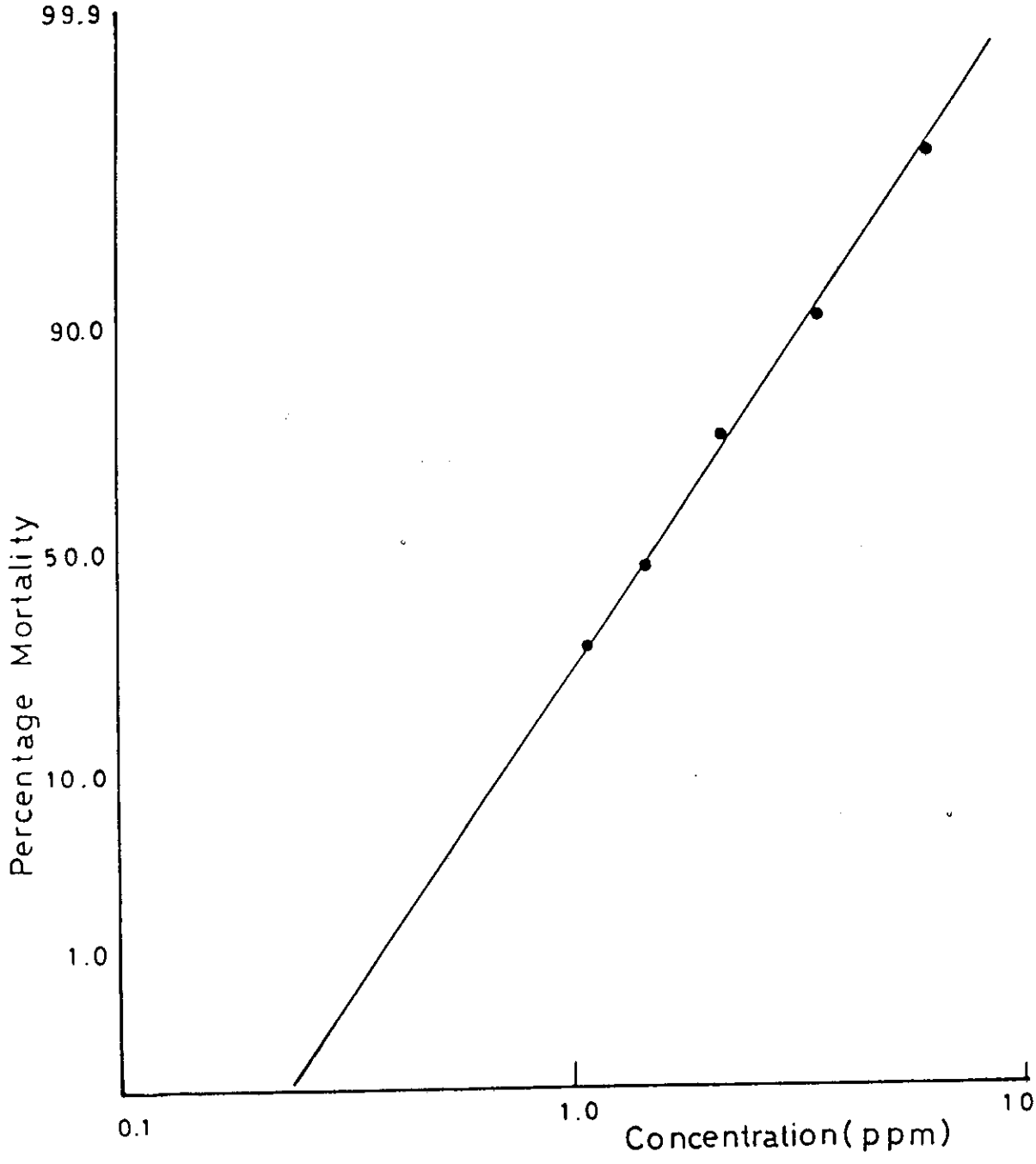

Figure (3) : Regression line of mortality among the *Culex pipiens* larvae after treatment with different concentrations of Malathion .

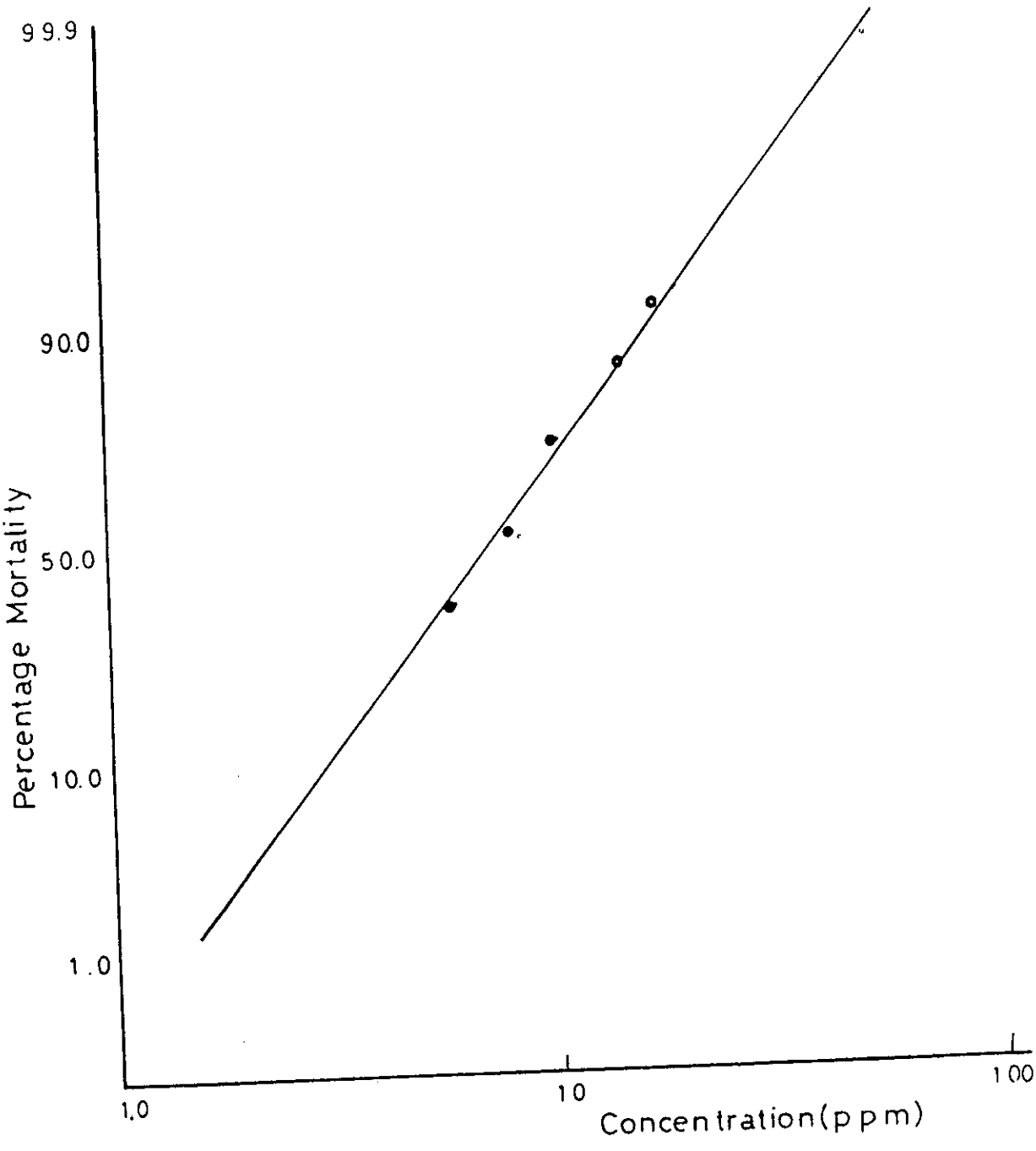

Figure (4) : Regression line of mortality among the *Culex pipiens* larvae after treatment with different concentrations of Propoxur .

2. Comparative susceptibility of *Culex pipiens* larvae to the tested insecticides

Based on the obtainded results of the previous series of experiments, the susceptibility of the early fourth ***Culex pipiens*** larval instar to the four used insecticides was compared in order to evaluate their larvicidal activity .

The tabulation of the estimated LC_{50}'s and the LC_{90}'s of each of the four tested insecticides in table (5) indicated that the larvicidal activity was relatively very high due to larval exposure to Sumicidin followed by Fenitrothion then Malathion and Propoxur, respectively .

Table (5) : Comparative susceptibility of the four used insecticides (Sumicidin, Fenitrothion , Malathion and Propoxur) against ***Culex pipiens*** larvae during 24 hour of exposure .

Insecticides used	Lethal concentrations in (ppm)		Relative effeciency
	LC_{50}	LC_{90}	LC_{50} / LC_{90}
Sumicidin	0.06	0.10	0.60
Fenitrothion	0.20	0.40	0.50
Malathion	1.49	3.10	0.47
Propoxur	7.20	16.50	0.44

3. Combined effect of the surfactants with the insecticides.

The purpose of the experiment is to investigate the synergistic effect of some surfactant additives i.e. Triton 20 , Triton X-100 and Tween 80 to certain insecticides i.e., Sumicidin, Fenitrothion, Malathion and Propoxur against ***Culex pipiens*** larvae .

A series of mixtures of each insecticides with different surfactants were tested against the ***Culex pipiens*** larvae. The results are presented in the (Tables 6-8) as the following .

3.1. Effect of Surfactants with the pyrethroid .

A mixture of Sumicidin and some surfactants Triton 20 , Triton X-100 were bioassayed against early fourth instar larvae of ***Culex pipiens***. The resulted larval mortality after 24 hours of exposure to the mixed suspension are graphically represented in Fig. (5) and tabulated in table (6) . Data indicated the following :

- ***Culex pipiens*** larvae are quite susceptible to the synergistic effect of the surfactants i.e. the larval mortality increased due to it's addition.
- The addition of the Triton 20, Triton X-100 and Tween 80 increased the toxicity of Sumicidin.
- Highly significant mortality resulted from the combination of Triton 20 followed by Tween 80 , but Triton X-100 showed a slight synergistic effect ($P < 0.05$) .

Table (6) : Percentage mortality of the early 4th instar larvae of *Culex pipiens* treated with Sumicidin in combination with the surfactants (Triton 20, Triton X-100 & Tween 80) at concentration (10 ppm).

Sumicidin Concentration / Surfactants used	Average of mortality (%)			
	LC_{50} (0.06 ppm)		LC_{30} (0.047 ppm)	
	Treated	Control	Treated	Control
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.
Triton 20	*100.0 ± 0.00	54.2 ± 1.0	98.6 ± 1.14	33.91 ± 2.11
Triton X-100	69.8 ± 3.56	51.3 ± 1.4	46.6 ± 1.94	32.11 ± 1.32
Tween 80	90.6 ± 2.10	52.3 ± 2.2	82.6 ± 2.07	34.24 ± 3.21

* Each value represents the mean of 5 replicate ± standard error .

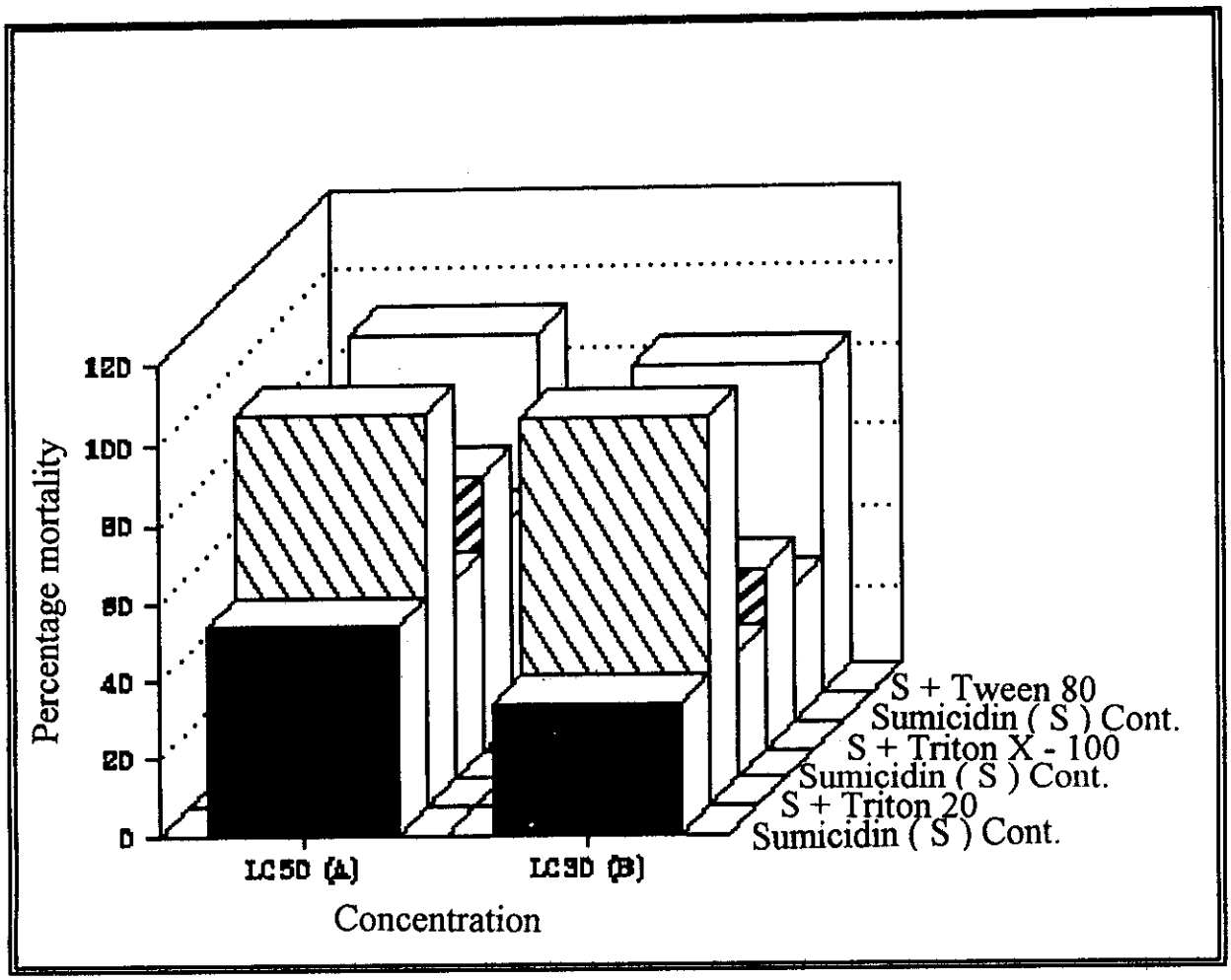

Figure (5) : The synergistic effect of surfactants (Triton 20, Triton X-100 & Tween 80) on the toxicity of Sumicidin with LC_{50} (a) and LC_{30} (b)

3.2- Effect of the surfactants with the organophosphorus .

The Fenitrothion and Malathion were tested against the ***Culex pipeins*** larvae when used in combination with the tested surfactants (Triton 20, Triton X-100 and Tween 80). The results of the tests are presented in table (7) and graphically illustrated in Figs. (6-7) According to the obtainded data, the following points are concerned :-

- A clear effect of Tween 80 on increasing the toxicity of Fenitrothion followed by Triton 20 then Triton X-100 . ($P<0.05$)
- Triton 20 resulted in a significant higher larval mortality when used incombination with Malathion followed by Tween 80 then Triton X-100 . ($P<0.05$)
- The resulted larval mortality percent from the use of Triton X- 100 with either of Fenitrothion or Malathion indicated its slight effect on increasing the toxicity of both of them .

Table (7) : Percentage mortality of the early 4th instar larvae of *Culex pipiens* treated with Fenitrothion or Malathion and in combination with each of the surfactants (Triton 20, Triton X-100 & Tween 80) at concentration (10 ppm).

Insecticides used / Surfactants used	Average of mortality (%)							
	Fenitrothion				Malathion			
	LC_{50} (0.2 ppm)		LC_{30} (0.15 ppm)		LC_{50} (1.49 ppm)		LC_{30} (1.1 ppm)	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.
Triton 20	*81.2±3.19	52.4±2.1	72.1±3.80	32.24±1.11	*91.6±2.40	55.11±2.20	81.0±2.91	33.91±1.1
Triton X-100	66.0±3.93	53.22±1.3	45.4±1.67	31.44±2.10	64.2±4.49	52.34±1.01	37.8±3.63	31.11±2.1
Tween 80	96.2±3.70	51.24±2.1	88.8±4.08	33.24±3.10	70.2±4.20	54.21±3.11	54.0±4.50	32.21±3.2

* The mean is the average of 5 treatment each of 5 replicates ± Standared error.

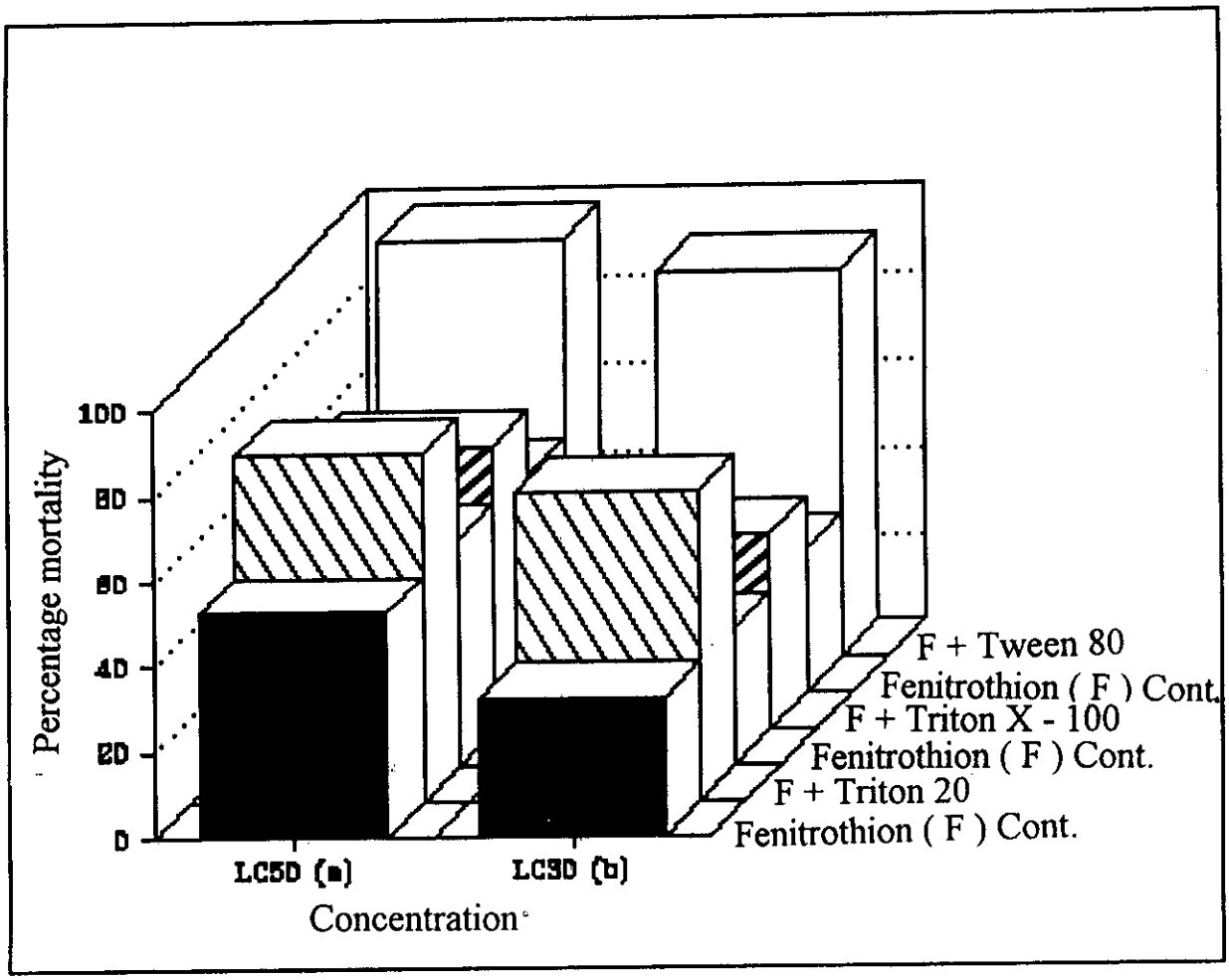

Figure (6) : The synergistic effect of surfactants (Triton 20, Triton X-100 & Tween 80) on the toxicity of Fenitrothion with LC_{50} (a) and LC_{30} (b)

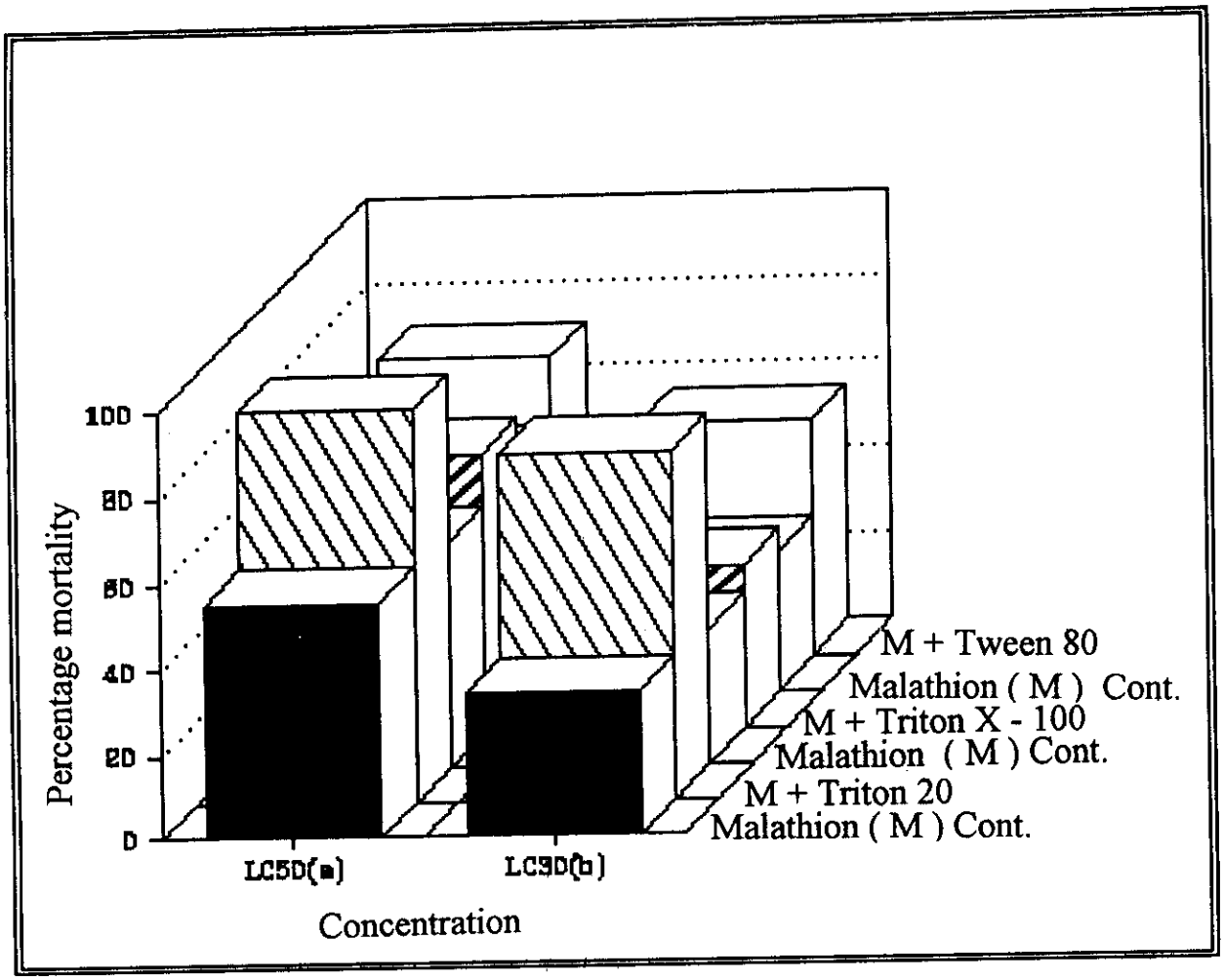

Figure (7) : The synergistic effect of surfactants (Triton 20, Triton X-100 & Tween 80) on the toxicity of Malathion with LC_{50} (a) and LC_{30} (b)

3.3- Effect of the surfactants with the carbamate .

The Propoxur was tested against the ***Culex pipiens*** larvae when used in combination with the surface active agent including Triton 20 , Triton X-100 and Tween 80 in order to detect their synergistic role . The obtainded resultes are tabulated in Table (8) and shown graphically in Fig. (8) . Data indicated the following :

- A slight synergistic effect of the Triton 20 followed by Tween 80 on increasing the toxicity of Propoxur. However the Triton X-100 almost have no synergistic effect on Propoxur .

Table (8) : Percentage mortality of the early 4^{th} instar larvae of ***Culex pipiens*** treated with Propoxur in combination with the surfactants (Triton 20, Triton X-100 & Tween 80) at concentration (10 ppm).

Propoxur Concentration / Surfactants used	Average of mortality (%)			
	LC_{50} (52.5 ppm)		LC_{30} (30.4 ppm)	
	Treated	Control	Treated	Control
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.
Triton 20	*65.6 ± 4.66	51.24 ± 3.10	40.8 ± 3.49	29.80 ± 2.3
Triton X-100	52.4 ± 3.50	50.11 ± 1.20	33.2 ± 2.58	30.00 ± 1.2
Tween 80	55.2 ± 3.34	49.22 ± 2.11	39.6 ± 3.97	31.89 ± 2.1

* Each value represents the mean of 5 replicats ± standard error .

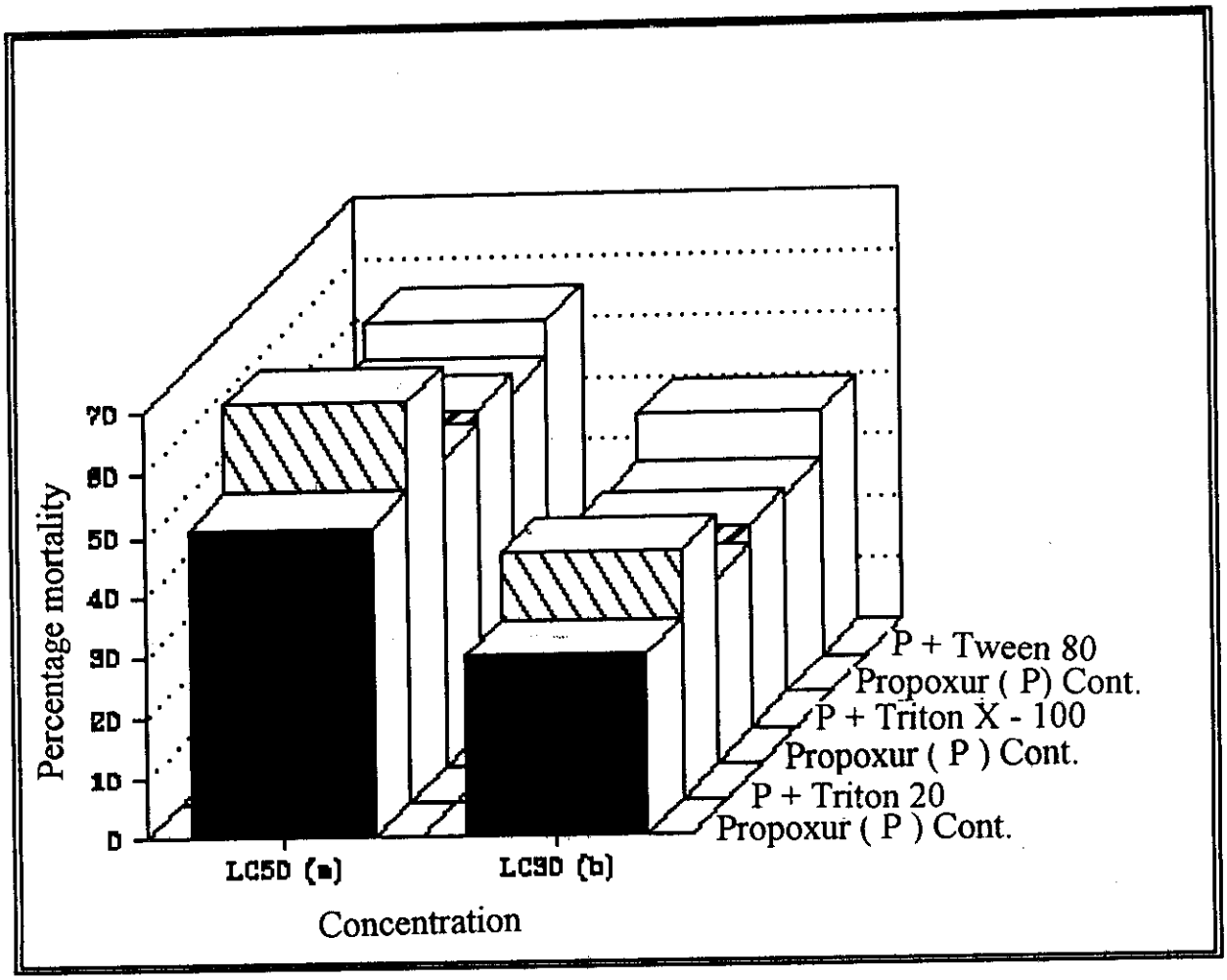

Figure (8) : The synergistic effect of surfactants (Triton 20, Triton X-100 & Tween 80) on the toxicity of Propoxur with LC_{50} (a) and LC_{30} (b)

4. Biochemical assays of some enzyme activity of *Culex pipiens* larvae in relation to the synergestic effect of surfactants to the insecticides

In this investigation the ATPase and acid phosphatase enzyme activity were studied .

4.1- ATPase .

Studies for the evaluation of the ATPase enzyme activity in the early fourth instar larvae of ***Culex pipiens*** were performed on untreated larvae and with that treated with either the LC_{50} of the tested insecticide alone or with its mixture with the surfactants at 10 ppm concentration, also control with the surfactants alone were done . Results are presented as the following :

4.1.1. Effect of Sumicidin and surfactants or both on ATPase activity :

Results concerning the effect of Sumicidin when tested separatly or in combination with the surfactants (Triton 20, Triton X- 100 & Tween 80) on ATPase of the ***Culex pipiens*** larvae are given in Table (9) and graphically illustrated in Fig. (9) . Data indicated that :

- Sumicidin shows a significant inhibition on ATPase enzyme after 3 hours of exposure compared to untreated larvae (54.96 %) .
- A relative increase in ATPase inhibition was due to synergistic effect of Triton 20 (78.14 %) followed by Tween 80 (64.75 %) .
- A negligable inhibition of ATPase activity occurred from the addition of Triton X-100 (55.05 %) in comparison with that treated

with the LC_{50} of Sumicidin alone (54.96 %) .

- Comparing the ATPase inhibition of the untreated larvae (control) with that treated with the surfactants alone indicate that Triton X- 100 showed moderate inhibition of ATPase than that treated with Triton 20 or Tween 80, however it has the lowest synergistic effect when combined with Sumicidin .

Table (9): Effect of Sumicidin (LC_{50}) on ATPase activity of *Culex pipiens* larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) at concentration level 10 ppm. Specific activity expressed as µ moles pi released / µ g protein / 45 minutes at $37^{o}C$.

Sumicidin LC_{50} (0.06 ppm) / Surfactants used	ATPase activity µ moles pi / µ g protein/45 min.		
	Treated	Control	Inhibition %
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	
	0.6630 ± 0.411	1.472 ± 0.041	54.96
Triton 20	0.3108 ± 0.581	1.422 ± 0.031	78.14
Triton X-100	0.6343 ± 0.012	1.411 ± 0.022	55.05
Tween 80	0.5129 ± 0.058	1.455 ± 0.042	64.75

N.B. The mean is the average of three samples ± standard error .

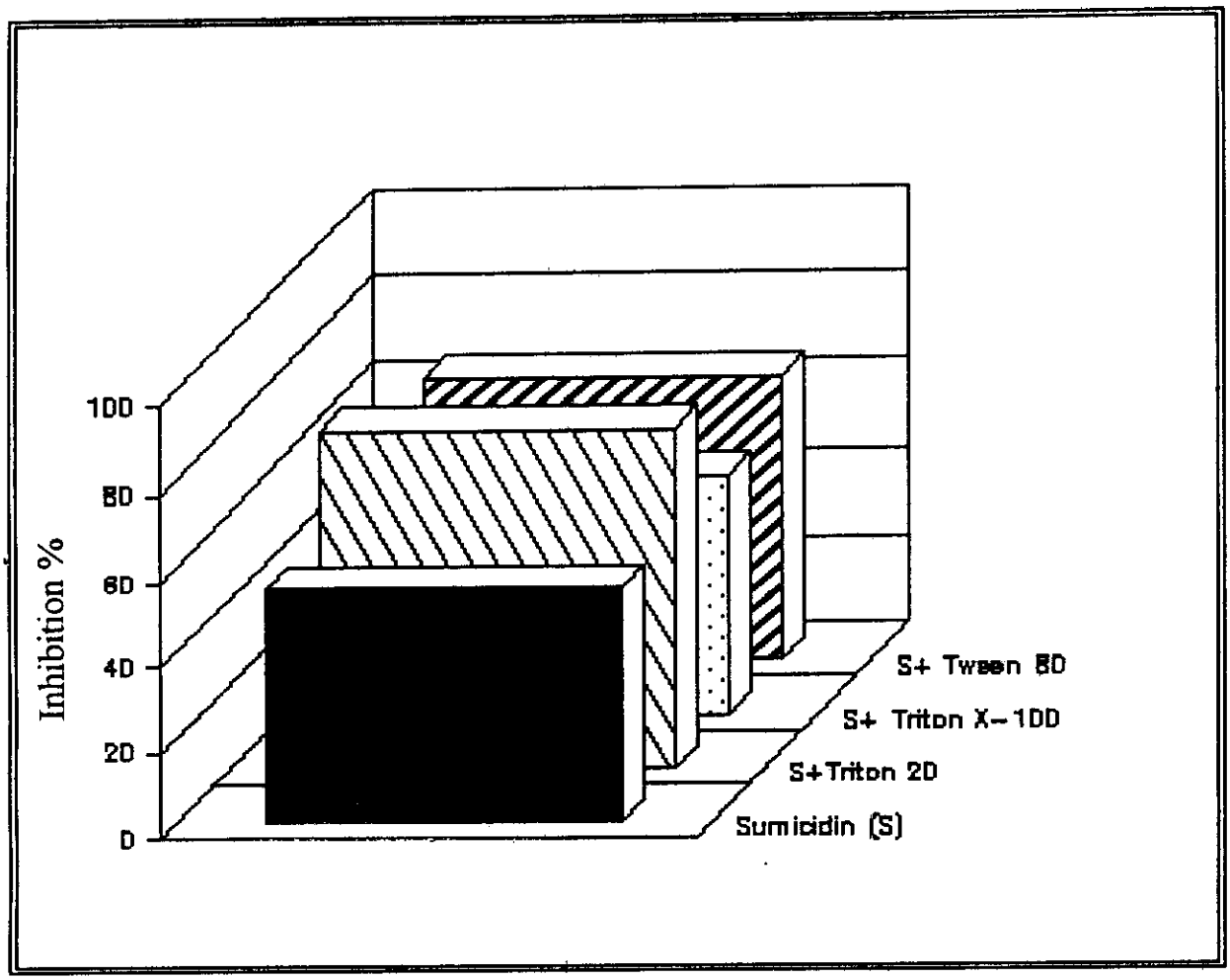

Figure (9) : Inhibitory action of Sumicidin and its mixtures with surfactants (Triton 20, Triton X-100 or Tween 80) on ATPase activity of *Culex pipiens* larvae

4.1.2. Effect of the organophosphorus and surfactants or both on ATPase activity .

The effect of Fenitrothion and Malathion and their mixtures with the surfactants on ATPase enzyme activity are presented in Table (10 & 11) and graphically illustrated in Figs . (10 & 11) respectively . Data obtained revealed the following :-

Moderate inhibitory action on ATPase enzyme occurred as a result of Fenitrothion treatment followed by Malathion treatment.

- Addition of Triton 20 , Triton X-100 & Tween 80 to Fenitrothion and Malathion induced a significant increase in the inhibition of ATPase enzyme .

Table (10): Effect of Fenitrothion (LC_{50}) on ATPase activity of *Culex pipiens* larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) at concentration level 10 ppm. Specific activity expressed as µ moles pi released / µ g protein / 45 minutes at $37^{o}C$.

Fenitrothion LC_{50} (0.2ppm) / Surfactants used	ATPase activity µ moles pi / µ g protein/45 min.		
	Treated	Control	Inhibition %
	$\overline{X}$ ± S.E.	$\overline{X}$ ± S.E.	
	0.794 ± 0.235	1.374 ± 0.031	42.21
Triton 20	0.6040 ± 0.412	1.365 ± 0.041	55.74
Triton X-100	0.7710 ± 0.013	1.364 ± 0.012	43.48
Tween 80	0.5099 ± 0.510	1.218 ± 0.041	58.14

N.B. The mean is the average of three samples ± standard error .

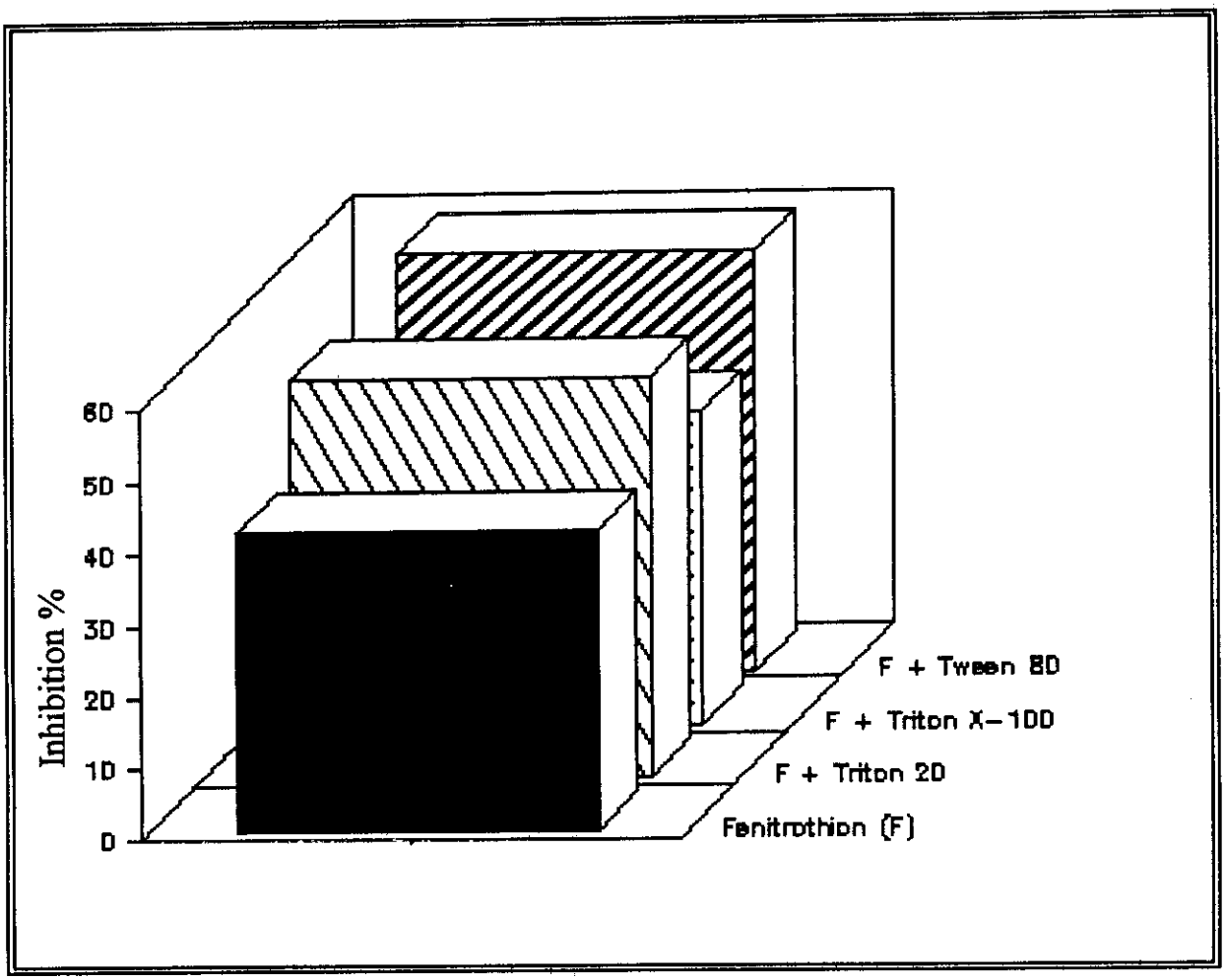

Figure (10) : Inhibitory action of Fenitrothion and its mixtures with surfactants (Triton 20, Triton X-100 or Tween 80) on ATPase activity of *Culex pipiens* larvae

Table (11): Effect of Malathion (LC_{50}) on ATPase activity of ***Culex pipiens*** larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) at concentration level 5 ppm. Specific activity expressed as µ moles pi released / µ g protein / 45 minutes at $37^{o}C$.

Malathion LC_{50} (1.49 ppm) / Surfactants used	ATPase activity µ moles pi / µ g protein/45 min.		
	Treated	Control	Inhibition %
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	
0.8193 ± 0.452	1.399 ± 0.041	41.44	
Triton 20	0.5943 ± 0.180	1.360 ± 0.051	56.30
Triton X-100	0.7631 ± 0.231	1.354 ± 0.032	43.64
Tween 80	0.6630 ± 0.481	1.395 ± 0.021	52.47

N.B. The mean is the average of three samples ± standard error .

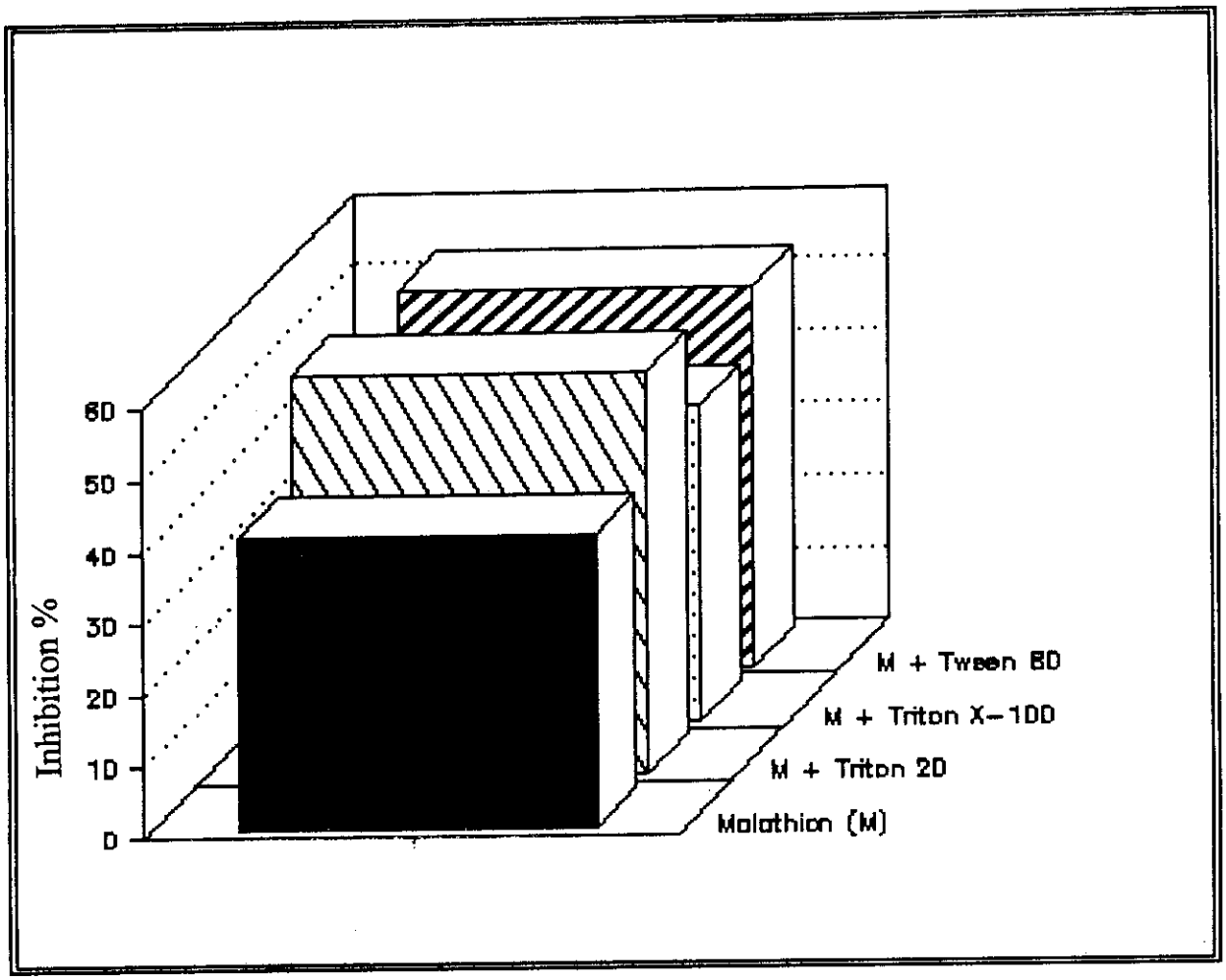

Figure (11): Inhibitory action of Malathion and its mixtures with surfactants (Triton 20, Triton X- 100 or Tween 80) on ATPase activity of *Culex pipiens* larvae

4.1.3. Effect of the Propoxur and the surfactants or both on ATPase activity

The effect of the addition of the surfactants (Triton 20 , Triton X-100 & Tween 80) to propoxur were presented in Table (12) and shown graphically in Fig. (12) . Data indicated that :

- Propoxur have a relative inhibitory effect of the ATPase enzyme.
- Addition of the surfactants Triton 20 , Triton X-100 & Tween 80 to Propoxur showed slight increase on the inhibition to the ATPase enzyme .

Table (12): Effect of Propoxur (LC_{50}) on ATPase activity of ***Culex pipiens*** larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) at concentration level 10 ppm. Specific activity expressed as µ moles pi released / µ g protein / 45 minutes at $37^{o}C$.

Propoxur LC_{50} (52.5ppm) / Surfactants used	ATPase activity µ moles pi / µ g protein/45 min.		
	Treated	Control	Inhibition %
	$\overline{X}$ ± S.E.	$\overline{X}$ ± S.E.	
	1.1413 ± 0.481	1.470 ± 0.021	22.36
Triton 20	0.8942 ± 0.521	1.321 ± 0.031	32.31
Triton X-100	0.9934 ± 0.201	1.300 ± 0.022	23.58
Tween 80	0.9521 ± 0.141	1.318 ± 0.031	27.76

N.B. The mean is the average of three samples ± standard error .

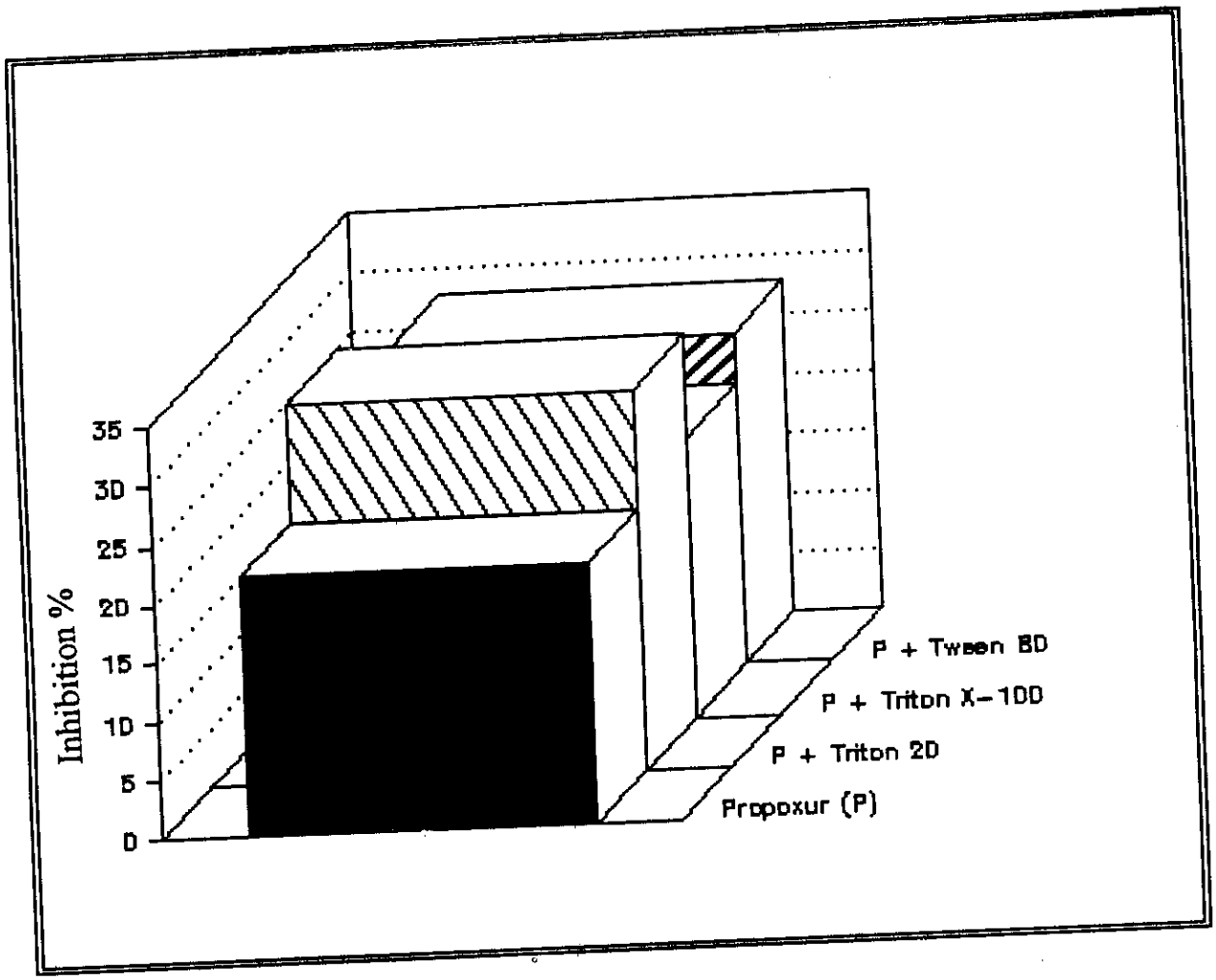

Figure (12) : Inhibitory action of Propoxur and its mixtures with surfactants (Triton 20, Triton X-100 or Tween 80) on ATPase activity of *Culex pipiens* larvae .

4.1.4. Comparative inhibition of ATPase enzyme regarding with the treatment by the insecticides or its mixture with the surfactants .

Percentages of ATPase inhibition of the ***Culex pipiens*** larvae after treatment with Sumicidin, Fenithrothion, Malathion and Propoxur inseciticides, or with their mixtures with Triton 20, Triton X-100 & Tween 80 surfactants, are represented in Table (13) and graphically illustrated in Fig. (13). Obtainded data indicated the following :

- The pyrethroid plays a very important role in the ATPase inhibition on concerning its toxicity to the ***Culex pipiens*** larvae followed by the organophosphorus and then the carbamate Propoxur with the remark that this action resulted after 3 hours of Sumicidin treatment while in case of Fenitrothion, Malathion & Propoxur after 6 hours of treatment
- Addition of Triton 20 to Sumicidin exhibited the highest inhibitory effect of the ATPase followed by its addition to Fenitrothion, Malathion & Propoxur .
- Addition of Tween 80 to Sumicidin revealed a significant inhibition of ATPase followed by its addition to Fenitrothion, Malathion & Propoxur respectively .
- From the forementioned results Triton X-100 was the least effective among the tested surfactant in increasing the inhibition of the ATPase when combined with the insecticides .
- Malathion and Fenitrothion showed the same response to the effect of the surfactants addition in increasing the inhibitory activity of the ATPase.

Table (13) : Percentage inhibition of ATPase of ***Culex pipiens*** larvae after Sumicidin, Fenitrothion , Malathion and Propoxur treatment or with their mixture with the surfactants (Triton 20 , Triton X-100 & Tween 80) .

Insecticides / Surfactants	Percentage of ATPase inhbition			
	Sumicidin	Fenitrotion	Malathion	Propoxur
	54.96	42.21	41.44	22.36
Triton 20	78.14	55.74	56.30	32.31
Triton X-100	55.05	43.48	43.64	23.58
Tween 80	64.75	58.14	52.47	27.76

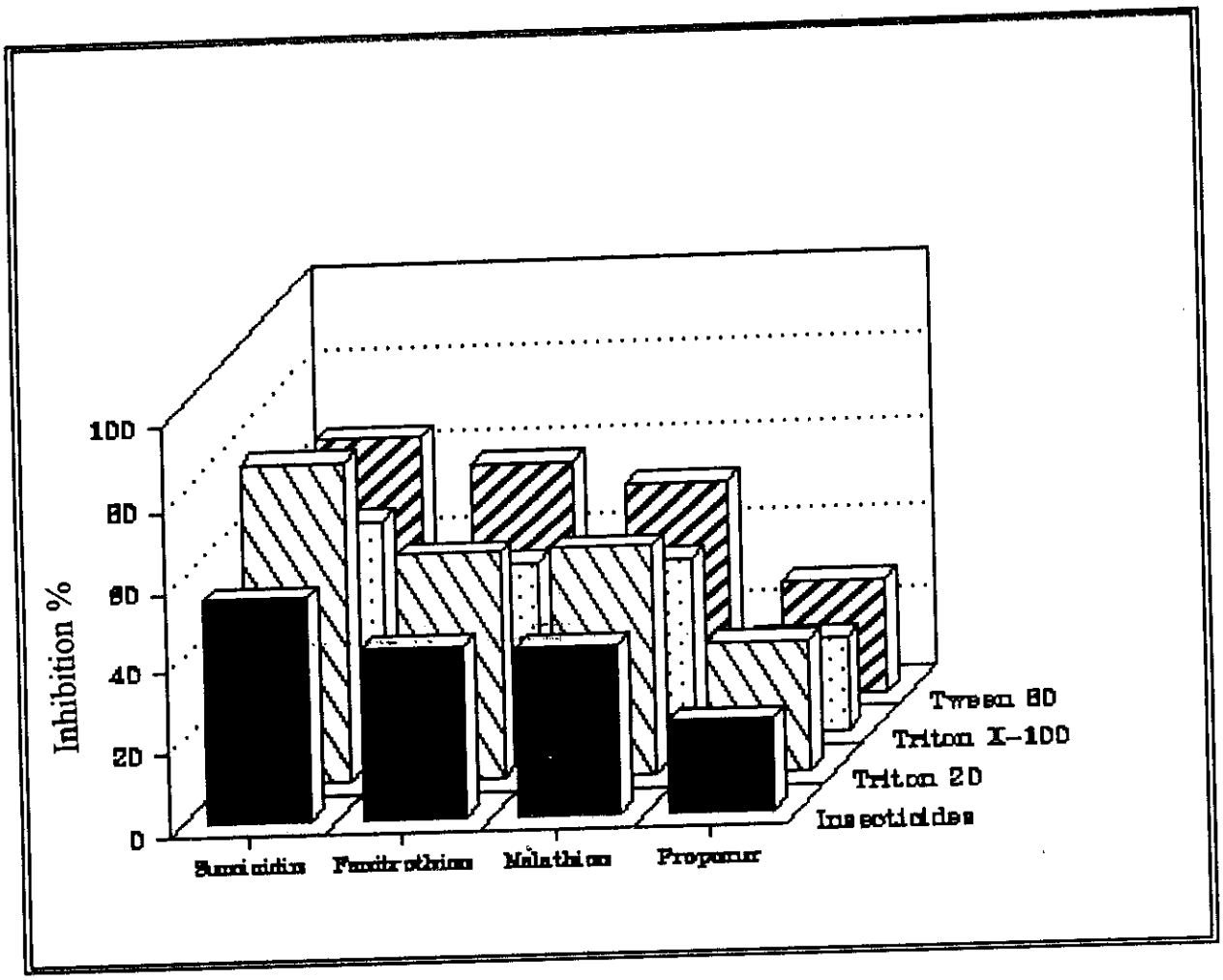

Figure (13):Persentages of the inhibitory action of the insecticides (Sumicidin , Fenitrothion , Malathion & Propoxur) and their mixtures with surfactants (Triton 20, Triton X-100 & Tween 80) on ATPase activity of *Culex pipiens* larvae

4.2. Acid phosphatase

Biochemical analysis was carried out to detect the level of free and total acid phosphatase activity in the early fourth instar larvae of ***Culex pipiens*** untreated, insecticide treated with LC_{50} separatly or in mixture with surfactant (at 10 ppm. concentration) and with that treated with the surfactants alone (control) . The specific activity of free and total acid phosphatase expressed as µg pi /µg protein / 45 minutes. Results are presented as the following :

4.2.1. Effect of Sumicidin and surfactants or both on acid phosphatase activity :

Data of Sumicidin and its combination with the surfactants (Triton 20 , Triton X-100 & Tween 80) are presented in Table (14) and illustrated in Fig. (14) . Data indicated that :

- The increase in the acid phosphatase activity of ***Culex pipiens*** larvae treated with Sumicidin was found to be significant in comparison with the untreated larvae . ($P<0.05$)
- Addition of Triton 20 to Sumicidin treated larvae causes significant stimulation in total and free acid phosphatase activity followed by Tween 80 . ($P<0.05$)
- Triton X-100 seem to have a negligable changes in acid phosphatase activity when added to Sumicidin in comparison with Triton 20 and Tween 80 effect .

Table (14) : Effect of Sumicidin (LC_{50}) on free and total acid phosphatase activity of ***Culex pipiens*** larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80). at concentration (10 ppm). Specific activity expressed as µg pi released/ µg protein / 45 minutes at $37^{o}C$.

Sumicidin LC_{50} (0.2 ppm) / Surfactants used	Specific activity of free acid phosphatase µg pi/µg protein/45 min.			Specific activity of total acid phosphatase µg pi/µg protein/45 min.		
	Treated	Control	% Difference	Treated	Control	% Difference
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.		$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	
	0.0280 ± 0.002	0.02520 ±0.0001	11.11	0.0422 ±0.0001	0.0365 ± 0.0002	15.62
Triton 20	0.06510 ± 0.004	0.02550 ±0.0001	155.29	0.0910 ± 0.0003	0.0310 ± 0.0002	193.55
Triton X-100	0.03120 ± 0.005	0.02525 ±0.0001	23.56	0.0491 ± 0.0001	0.0305 ± 0.0001	60.98
Tween 80	0.05220 ± 0.004	0.02540 ±0.0001	105.51	0.0900 ± 0.0003	0.0320 ± 0.0001	181.25

N.B. The mean is the average of three samples ± standard error .

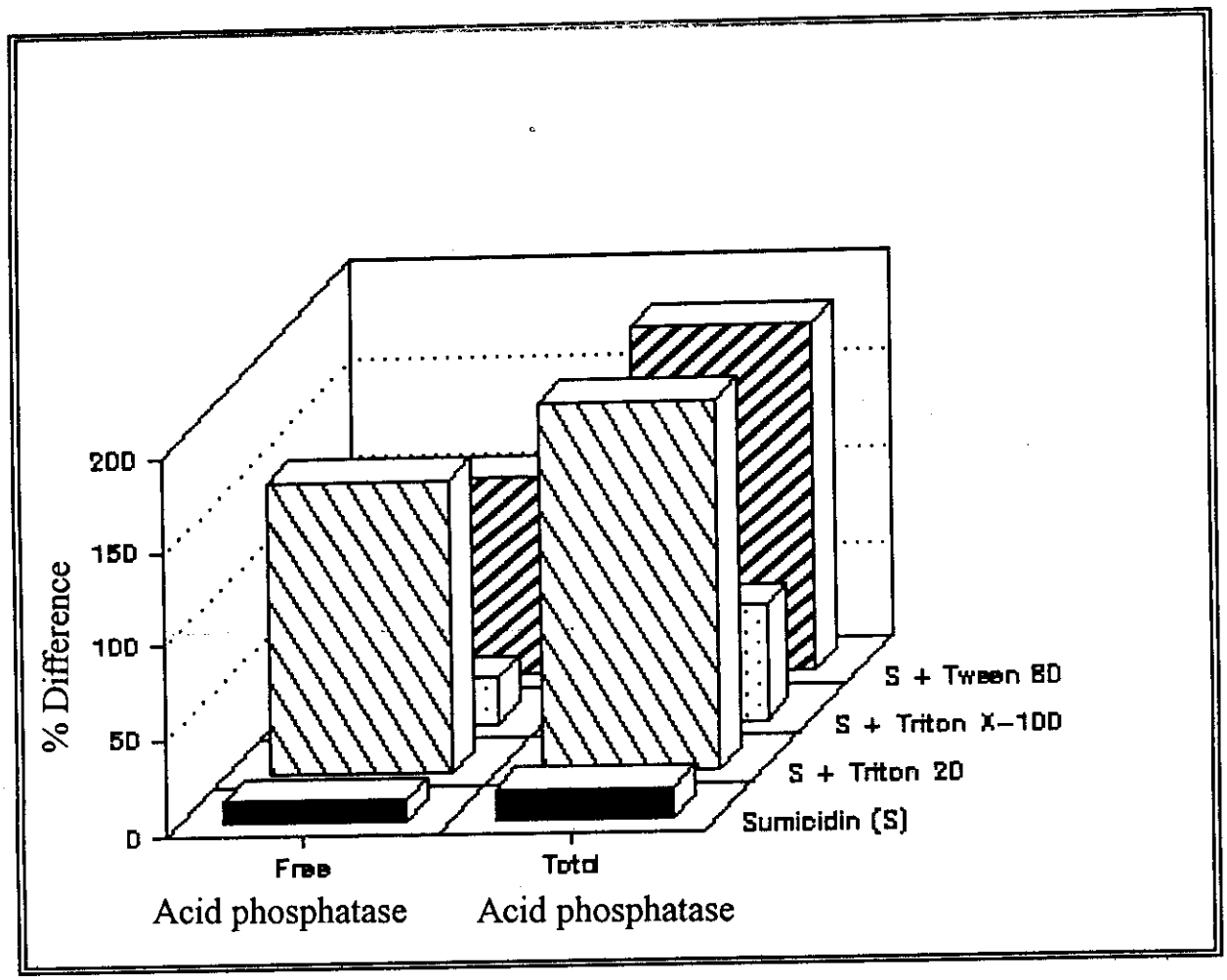

Figure (14) : Effect of Sumicidin and its mixture with surfactants on free and total acid phosphatase activity of *Culex pipiens* larvae.

4.2.2. Effect of organophosphorus and surfactants or both on acid phosphatase activity

Data concerning the effect of Fenitrothion and Malathion when used separatly or in combination with the surfactants (Triton 20, Triton X-100 & Tween 80) are tabulated in Tables (15 & 16) and graphically illustrated in Figs. (15 & 16) respectively. The obtainded data indicate:-

- The organophosphate insecticides (Fenitrothion and Malathion) showed a highly significant stimulation in the total and free acid phosphatase enzyme activity with in the treated ***Culex pipiens*** larvae . ($P<0.05$) .
- Addition of Tween 80 to ***Culex pipiens*** larvae treated with Fenitrothion resulted in a pronounced changes in acid phosphatase activity followed by Triton 20 addition then Triton X-100 .
- Triton 20 causes a significant stimulation in acid phosphatase activity to larvae treated by Malathion followed by Tween 80 addition then Triton X-100 .

Table (15) : Effect of Fenitrothion (LC_{50}) on free and total acid phosphatase activity of ***Culex pipiens*** larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) .at concentration (10 ppm). Specific activity expressed as µg pi released/ µg protein / 45 minutes at $37^{o}C$.

Fenitrothion LC_{50} (0.06 ppm) / Surfactants used	Specific activity of free acid phosphatase µg pi/µg protein/45 min.			Specific activity of total acid phosphatase µg pi/µg protein/45 min.		
	Treated	Control	% Difference	Treated	Control	% Difference
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.		$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	
	0.0501 ± 0.0001	0.02510 ±0.0001	99.60	0.0742 ± 0.0002	0.0355 ± 0.0002	109.01
Triton 20	0.0631 ± 0.0002	0.02524 ±0.0001	150.00	0.09550 ±0.0001	0.0364 ± 0.0002	162.36
Triton X-100	0.0541 ± 0.0001	0.02515 ±0.0001	115.10	0.0781 ± 0.0003	0.0360 ± 0.0001	116.94
Tween 80	0.0661 ± 0.0003	0.02520 ±0.0001	162.30	0.09980 ±0.0002	0.0365 ± 0.0002	173.42

N.B. The mean is the average of three samples ± standard error .

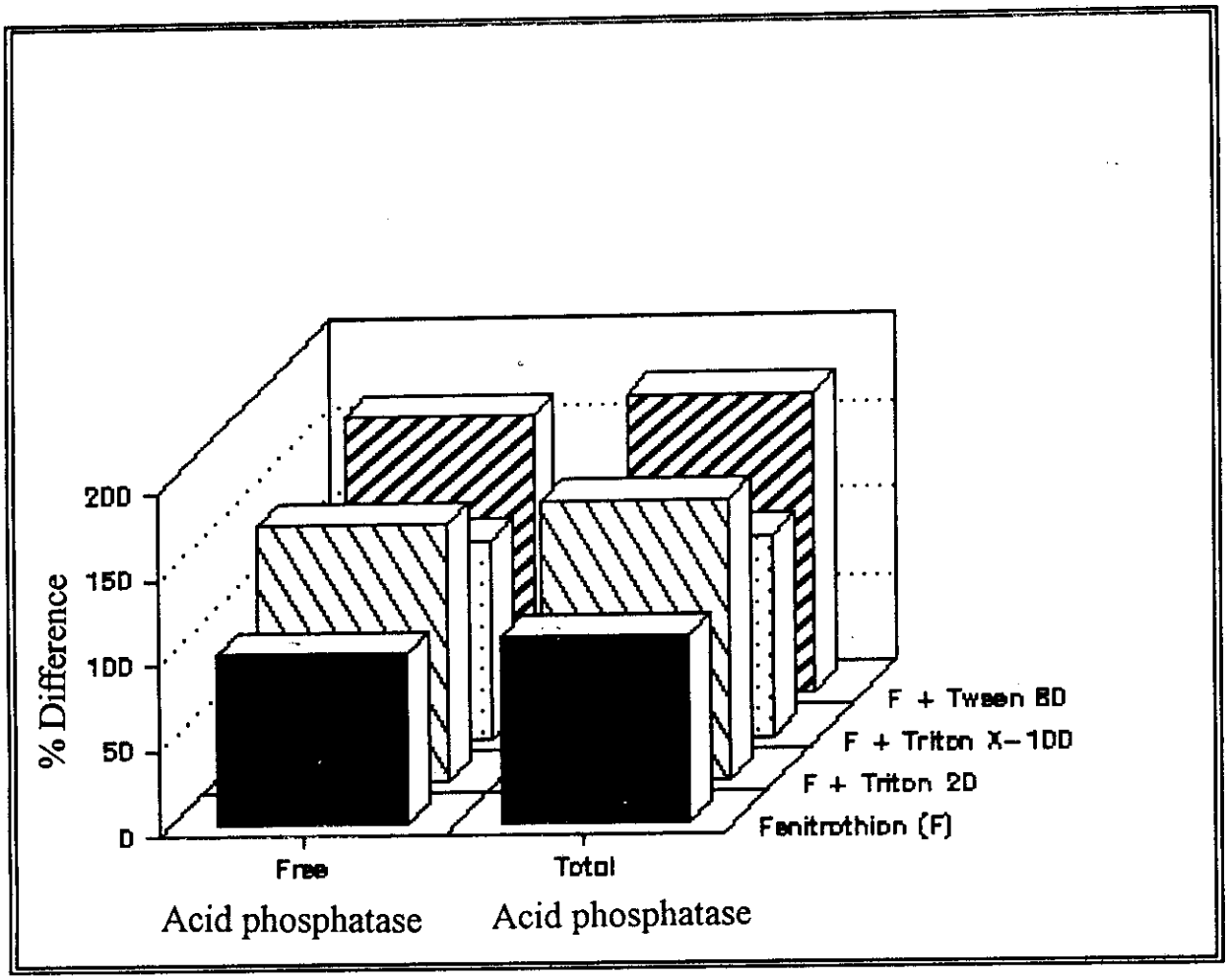

Figure (15) : Effect of Fenitrothion and its mixture with surfactants on free and total acid phosphatase activity of *Culex pipiens* larvae.

Table (16) : Effect of Malathion (LC_{50}) on free and total acid phosphatase activity of ***Culex pipiens*** larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) .at concentration (10 ppm). Specific activity expressed as µg pi released/ µg protein / 45 minutes at $37^{o}C$.

Malathion LC_{50} (1.49 ppm) / Surfactants used	Specific activity of free acid phosphatase µg pi/µg protein/45 min.			Specific activity of total acid phosphatase µg pi/µg protein/45 min.		
	Treated	Control	% Difference	Treated	Control	% Difference
	$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.		$\bar{X}$ ± S.E.	$\bar{X}$ ± S.E.	
	0.0491 ± 0.0004	0.02505 ±0.0002	96.01	0.0781 ± 0.0002	0.0360 ± 0.0001	116.94
Triton 20	0.0667 ± 0.0003	0.02510 ±0.0001	165.74	0.1040 ± 0.0003	0.0369 ± 0.0002	181.84
Triton X-100	0.0512 ± 0.0005	0.02508 ±0.0001	104.15	0.0791 ± 0.0004	0.0360 ± 0.0001	119.72
Tween 80	0.0610 ± 0.0003	0.02510 ±0.0002	143.03	0.0940 ± 0.0003	0.0365 ± 0.0001	157.53

N.B. The mean is the average of three samples ± standard error .

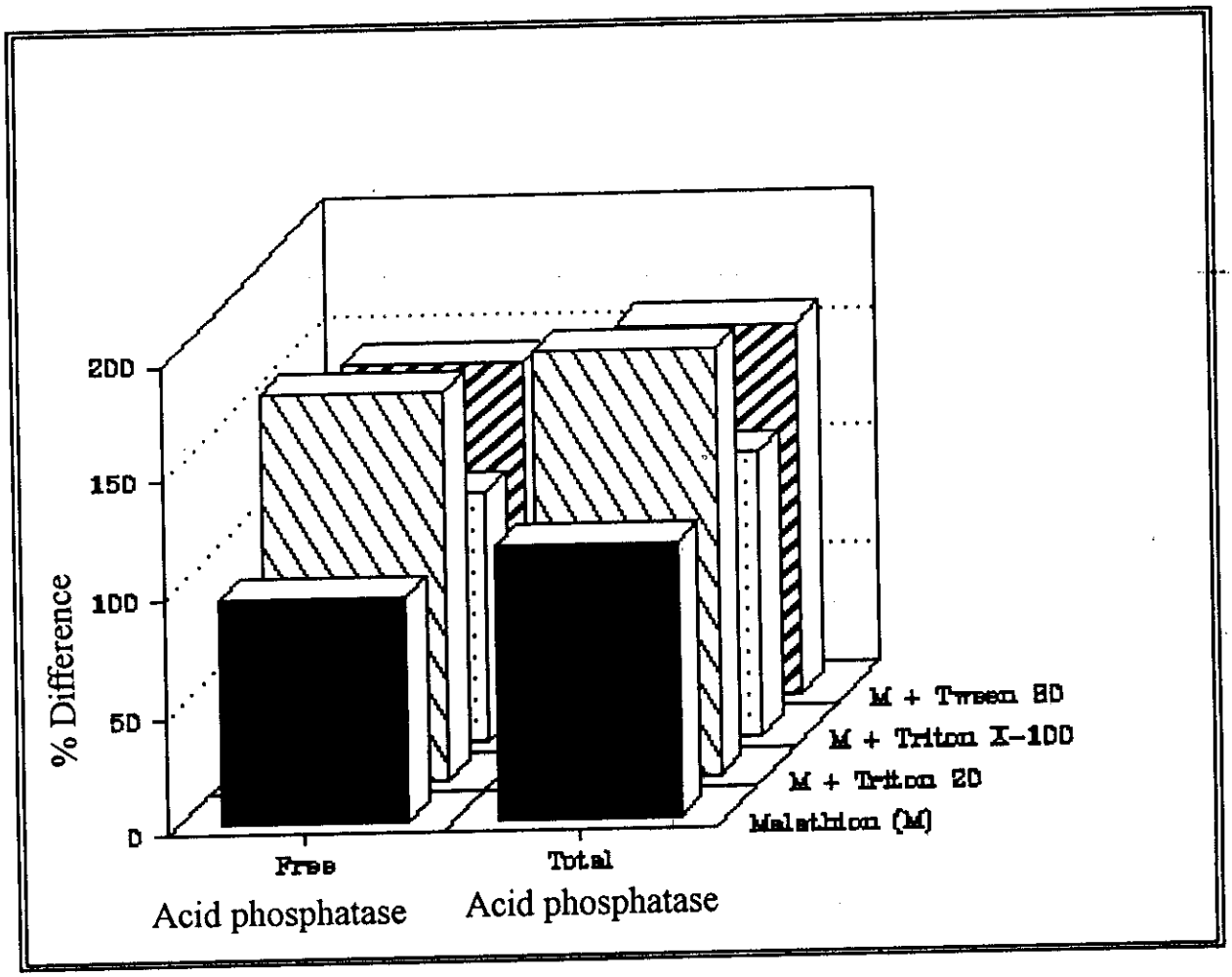

Figure (16) : Effect of Malathion and its mixture with surfactants on free and total acid phosphatase activity of *Culex pipiens* larvae.

4.2.3. Effect of carbamate and surfactant or both on acid phosphatase activity .

Data on the effect of Propoxur and its combination with the surfactants (Triton 20 , Triton X-100 & Tween 80) are presented in Table (17) and illustrated in Fig. (17) . Obtainded data indicated that :

- The increase in the acid phosphatase activity of ***Culex pipiens*** larvae treated with Propoxur was found to be significant in comparison with the untreated (control) larvae.
- Addition of surfactants to larvae treated with Propoxur causes a slight increase in the free and the total acid phosphatase activity. The descending order was as follows : Triton 20 >Tween 80 >TritonX-100

Table (17) : Effect of Propoxur (LC_{50}) on free and total acid phosphatase activity of *Culex pipiens* larvae after mixing with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) .at concentration (10 ppm). Specific activity expressed as µg pi released/ µg protein / 45 minutes at $37^{o}C$.

Propoxur LC_{50} (52.5 ppm) / Surfactants used	Specific activity of free acid phosphatase µg pi/µg protein/45 min.			Specific activity of total acid phosphatase µg pi/µg protein/45 min.		
	Treated $\bar{X}$ ± S.E.	Control $\bar{X}$ ± S.E.	% Difference	Treated $\bar{X}$ ± S.E.	Control $\bar{X}$ ± S.E.	% Difference
	0.0289 ± 0.0003	0.02518 ±0.0001	14.77	0.0493 ± 0.0001	0.0366 ± 0.0002	34.70
Triton 20	0.0300 ± 0.0002	0.02522 ±0.0001	18.95	0.0521 ± 0.0002	0.0368 ± 0.0002	41.58
Triton X-100	0.0294 ± 0.0004	0.02519 ±0.0001	16.71	0.0498 ± 0.0004	0.0365 ± 0.0001	36.44
Tween 80	0.0298 ± 0.0002	0.02520 ±0.0001	18.25	0.0514 ± 0.0003	0.0370 ± 0.0001	38.92

N.B. The mean is the average of three samples ± standard error .

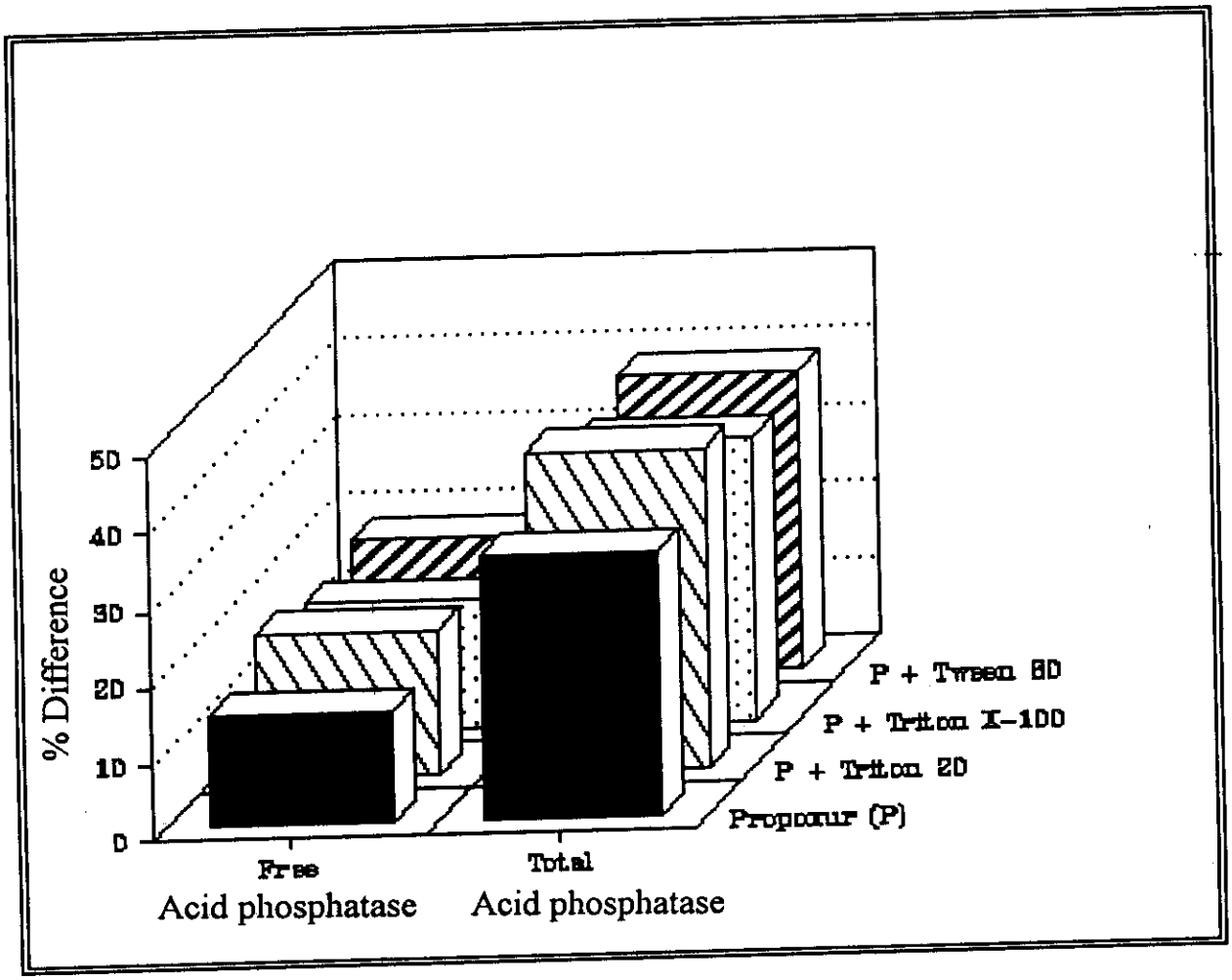

Figure (17) : Effect of Propoxur and its mixture with surfactants on free and total acid phosphatase activity of *Culex pipiens* larvae.

4.2.4. Comparative differences on free and total acid phosphatase activity of ***Culex pipiens*** larvae treated with the insecticides or with their mixture with the surfactants .

Percentages of differences on free and total acid phosphatase enzyme of ***Culex pipiens*** larvae treated with Sumicidin , Fenitrothion, Malathion and Propoxur insecticides separatly or in mixture with each of the surfactants (Triton 20 , Triton X-100 & Tween 80) were represented in Table (18) and graphically illustrated in Fig. (18) . Data indicated that :

- The organophosphate insecticide (Fenitrothion and Malathion) increases the stimulation of free and total acid phosphatase activity of ***Culex pipiens*** treated larvae; followed by the carbamate (Propoxur) and then the pyrethroid (Sumicidin) .
- Addition of Triton 20 causes a significant increases in acid phospha - tase of ***Culex pipiens*** larvae treated with Sumicidin as just as that added to the organophosphate insecticides .
- A slight change in the acid phosphatase activity following the addition of Triton X-100 to the tested insecticides in comparison with Triton 20 or Tween 80 addition .

Table (18) : Percentage difference on free and total acid phosphatase activity of ***Culex pipiens*** larvae treated with insecticides (Sumicidin, Fenitrothion, Malathion & Propoxur) or with the mixture of insecticides and surfactants (Triton 20 , Triton X-100 & Tween 80) at concentration (10 ppm).

Insecticides / Surfactants	% difference in acid phosphatase activity							
	Free				Total			
	Sumicidin	Fenitrothion	Malathion	Propoxur	Sumicidin	Fenitrothion	Malathion	Propoxur
	11.11	99.60	96.01	14.77	15.62	109.01	116.94	34.70
Triton 20	155.29	150.00	165.74	18.95	193.55	162.36	181.84	41.58
Triton X-100	23.56	115.10	104.15	16.71	60.98	116.94	119.72	36.44
Tween 80	105.51	162.30	143.03	18.25	181.25	173.42	157.53	38.92

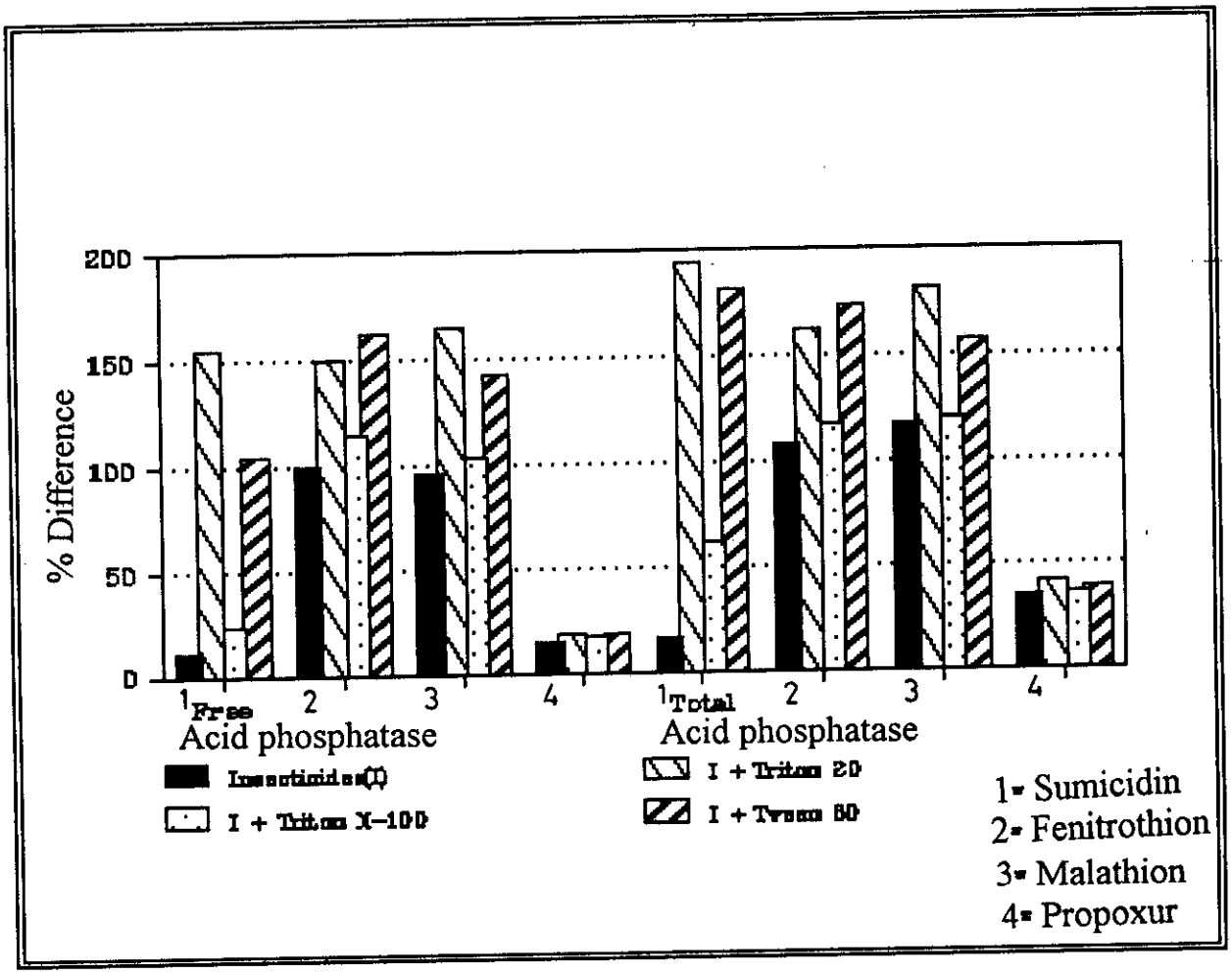

Figure (18) : Percentages of the specific activity of free and total acid phosphatase of *Culex pipiens* larvae treated with the insecticides (Sumicidin , Fenitrothion, Malathion & Propoxur) and their mixtures with surfactants (Triton 20, Triton X-100 & Tween 80) .

5. Histopathological studies :-

In the present investigation the normal histology of the non treated and insecticides treated ***Culex pipiens*** larvae, as well as the larvae treated with the mixture of insecticide and surfactant were considered for comparison to trace the synergistic effect of surfactants with any obtained histopathological consequence under the light microscope .

5.1.1. Sumicidin treated larvae :-

The photographs in Fig's (19-21) demonstrate a general view of transverse and longtidudinal sections of non treated (control) ***Culex pipiens*** larvae. The midgut epithelium monolayer structure and composed of somewhat cuboidall cells, which sometimes seen flattened. The cytoplasm is granulated in which the nuclei are located. The regenerative cells " nidi " are distinguished in between the epithelial cells. Some cells bear brush border.

The toxic effect of Sumicidin on the epithelial gut cells appeared as an enlargement of the nucleus and also the cytopalsm which become vacuolated Fig. (22) and Fig. (23) .

The cell size enlarged and the cell wall protrudes towards the gut lumen. The gut cells came enclose contact with the peritrophic membrane narrowing the endoperitrophic membrane space which start from the gut cells to the gut lumen in comparison with the normal Fig.(19) and Fig. (20) . However , the peritrophic membrane seems to

be not damaged by the treatment (Fig. 22) . The muscle bundle appeared to be slightly affected (Fig. 25). The fat tissues appeared destroyed Figs. (24 and 25), they became highly vacuolated and underwent necrosis. The gut epithelium lost their striated bruch borders and the cells became larger in size and some cell membrane become raptured liberating its own contents. The gastric caeca also showed the hypertrophy occur to the gut cells .

5.1.2. Larvae treated with (Sumicidin + Triton 20) mixture .

The transverse sections in Figs. (26-a and 27) in larvae treated with (Sumicidin + Triton 20) mixture showed the advanced destruction occurs to the alimentary canal cells in comparison with the Sumicidin treated larvae Fig. (22). With higher magnification in Fig. (26-b) in which the gut epithelia completely lost their boundary. Some nuclei are appeared with pyknotic features. In Figs. (27 and 29), it is so evident the complete destruction of the partial fat tissue beneath the cuticle . A clear area was found below the larva cuticle. The muscle bundle and the fat tissue became very loosely packed Fig.(28). The gastric caeca appeared to be extensivly damaged by such mixture (Fig.29) .

The longitudinal section in Fig. (30) revealed the destruction occurred to the larval integument .

5.1.3. Larvae treated with (Sumicidin + Tween 80) mixture.

The transverse section in Fig. (31) in a larva treated with (Sumicidin + Tween 80) mixture revealed the destruction occurred to

the epithelial cells of the midgut canal. The gut epithelia appeared swollen with vacuolated cytoplasm and pyknotic nuclei in comparison with the normal Fig. (19) or with that treated with sumicidin alone Fig. (22). The gastric caeca appeared to be affected also, the muscle bundle and fat tissue became to be very loosely packed .

A striking feature appeared in sections of the larvae treated with the mixture of the insecticide and the surface active agent Tween 80 was the complete destruction of the layers beneath the cuticle including the fat tissue in comparison with the normal (Fig.19) and that treated with the insecticide separatly. The longitudenal section in Fig.(32) revealed the complete destruction happend to some parts of the larval integument.

5.2.1. Fenitrothion treated larvae :-

Section in larvae that were treated with Fenitrothion showed to be affected in many features; the destruction of muscle bundle and the appearance of vacuoles in the fat tissue Fig's (33 and 34) . The longitudinal section in Fig. (37) displaying the destruction of the epithelial cells . The cytoplasm of these cells showed signs of a cloudy swelling . The gastric caeca was also affected Fig. (35). The transverse section in Figs. (36 and 38) revealed distinct deterioration of muscle layer . In another transverse section Fig. (39) and the longitudinal section Fig.(40), the layers beneath the cuticle are deteriotated but still present.

5.2.2 Larvae treated with (Fenitrothion + Triton 20) mixture.

Treated larvae section in Fig. (41) and Fig. (42) with the (Fenitrothion + Triton 20) mixture showed that the gut epithelial cells affected by this mixture . The cytoplasm was highly vacuolated . The gut epithelial cell became swollen. The peritrophic membrane was destroyed. The muscle and the fat tissue are affected.

The extensively damaged part in Fig. (42) indicated that the layers beneath the cuticle was completely detroyed in comparison with that treated with the Fenitrothion alone Fig. (33) .

A transverse section in Fig. (43) showed that the fat tissue was highly destroyed by such treatment with apparent sings of damage .

5.2.3. Larvae treated with (Fenitrothion + Tween 80) mixture :-

Examination of the sectioned material indicated that the larvae treated with Fenitrothion + Tween 80 mixture Fig. (44) demonstrated deterioration of peritrophic membrane depriving the gut from its lumen.

The gut epithelial cells appeared swollen with vacuolated cytoplasm and pyknotic nuclei the cellular hypertrophy was also clearly indicated. The basement membrane was detached from the epithelial cells .

The layers beneath the cuticle were also affected by such treatment with appearent signs of damage or complete lysis .

Figure (19) : A transverse section in a non treated larva at the midgut region.

Cu : Cuticle.

Ft : Fat tissue.

Mb. Muscle bundle.

N : Nucleus.

Gc : Gut contents.

Ec : Epithelium cell.

Pm. Peritrophic membrane.

Eps : Endo-peritrophic membrane space .

(X = 400) .

Figure (20) : Another part of the same section Fig. (19) .

Cl : Cortical layer.

Nm : Neuropile mass .

Ft : Fat tissue .

Ec : Epithelium cell.

N : Nucleus .

Gc : Gut Contents .

(X = 400) .

Figure (19)

Figure (20)

Figure (21) : Photomicrograph of a longitudinal section in a non treated larva.

Bm : Basement membrane .

Bb : Brush border .

Ec : Epithelium cell .

Cu : Cuticle .

(X = 200)

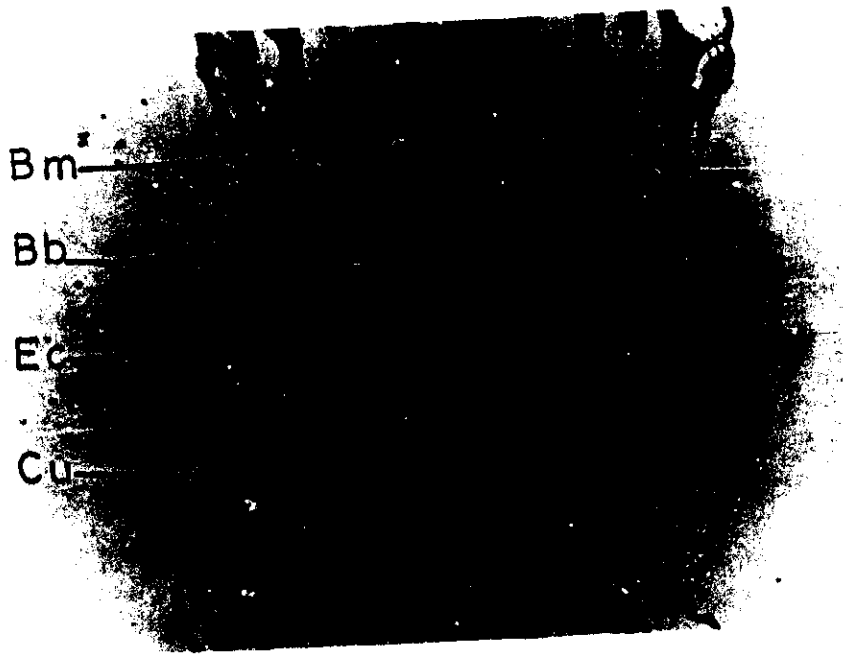

Figure (21)

Figure (22) : A transverse section of a larva treated with sumicidin insecticide .

Mb : Muscle bundle .

Cu : Cuticle.

Ft : fat tissue

Ec : Swollen epithelium cell .

Pm : Narrow peritrophic membrane .

Gc : Gut contents .

(X = 400) .

Figure (23) : A longitudinal section of a larva treated with Sumicidin insecticide .

Mb. Muscle bundle.

Pft : Partial fat tissue .

Cu : Cuticle .

(X = 300) .

Figure (22)

Figure (23)

Figure (24) : Photomicrograph of a transverse section in a larva treated with Sumicidin .

Cu : Cuticle .
Vft : Vacuoleated Fat tissue .
Dec : Destroyed epithelial cells .
Dmb : Destroyed muscle bundle .
(X = 100) .

Figure (25) : A longitudinal section in a larva treated with Sumicidin .

Cu : Cuticle .
Vft : Vacuoleated fat tissue .
Dmb : Destroyed muscle bundle .
(X = 200) .

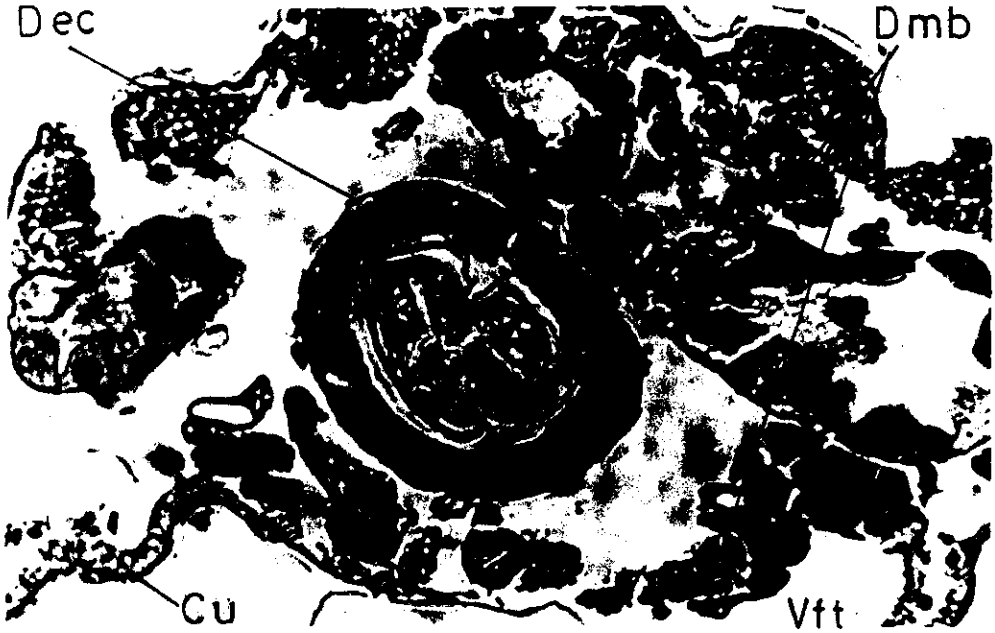

Figure (24)

Figure (25)

Figure (26-a) : A transverse section of a larva treated with (Sumicidin + Triton 20) Mixture showing the advanced destruction occur to the gut cells .

Dec : Destroyed epithelial cell .

Gc : Gut Contents .

Dml : Destroyed muscle layer .

F : Fissure in muscle bundle .

Nh : Nucleus hypertrophy .

Dc : Destroyed cytoplasm of epithelium cell .

(X = 400)

Figure (26-b) : Another part of the same section Fig. (26-a) , showing the destruction occur to the fat tissue .

Dft : Destoyed fat tissue .

Dml : Destoryed muscle layer.

Dec : Destroyed epithelial cells .

Gc : Gut Contents .

(X = 400) .

Figure (26-a)

Figure (26-b)

Figure (27) : A transverse section of a larva treated with (Sumicidin + Triton 20) mixture showing the complete destruction of the partial fat tissue beneath the cuticle .

Cu : Cuticle .
Gc : Gut contents .
Epms : Endo-perithrophic membrane space.
Dec: Destroyed epithelum cell .
Dm : Destroyed muscle layer.
Ft : Fat tissue .

(X = 300) .

Figure (28) A transverse section of a larva treated with (Sumicidin + Triton 20) mixture .

Epms : Endo peritrophic membrane space.
Gc : Gut contents .

(X=200).

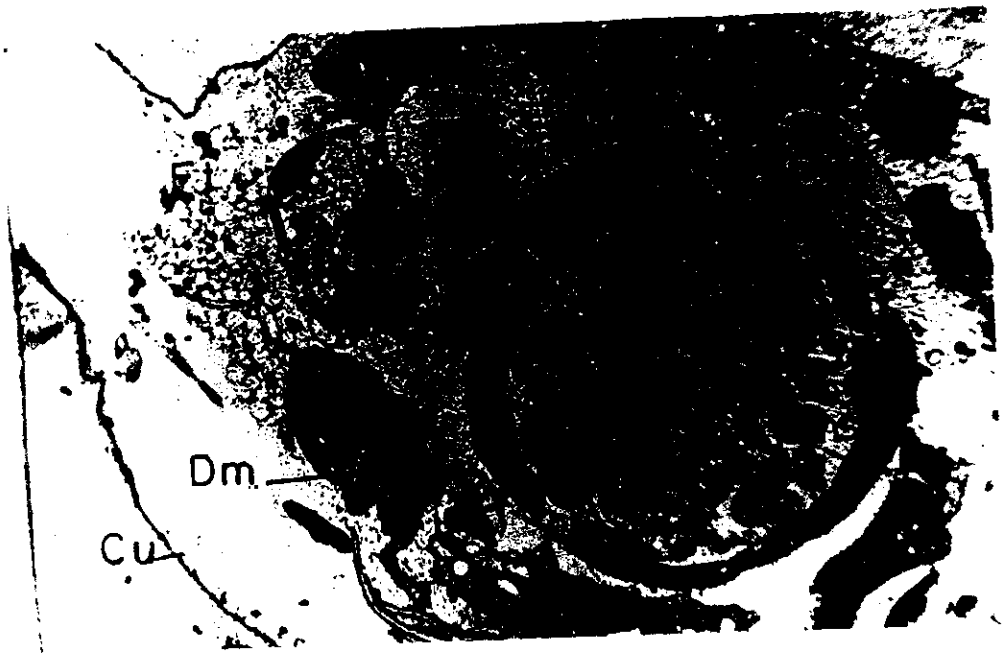

Figure (27)

Figure (28)

Figure (29) A transverse section of a larva treated with (Sumicidn + Triton 20) mixture . Midgut region.

Gaca : Gastric caecum.

Vec : Vacuoleated epithelial cells .

Cu : Cuticle.

Gc : Gut contents.

(X=100).

Figure (30) : A longitudinal section in a larva treated with (Sumicidin + Triton 20) mixture, revealing the destscution occur to the larva integument.

Gc : Gut contents .

Bm : Basement membrane .

Ml : Muscle layer .

Di : Destroyed integument

(X = 100).

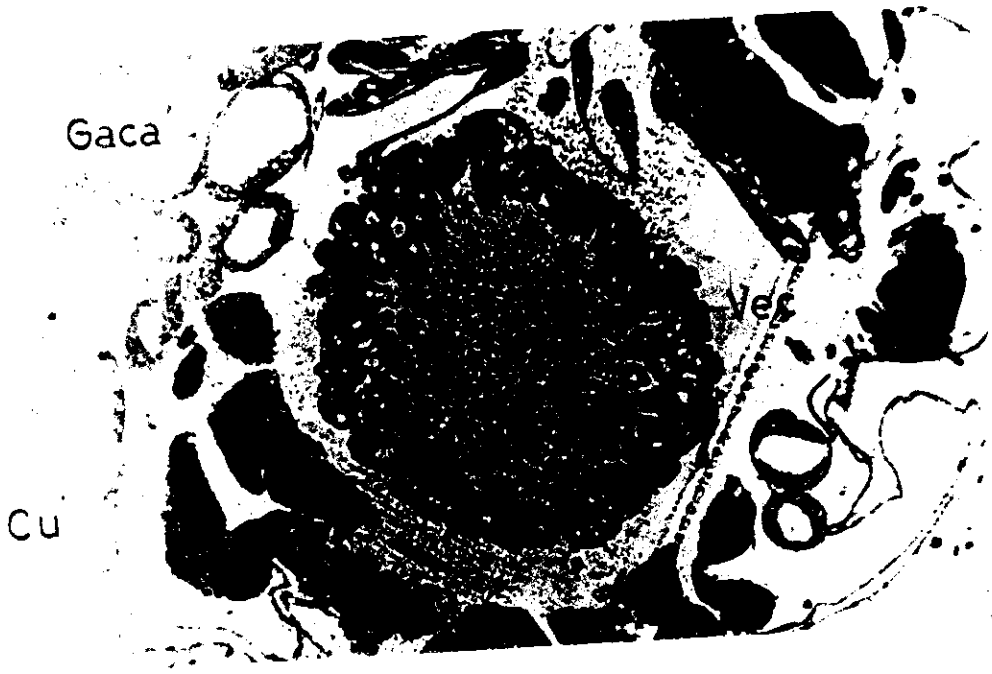

Figure (29)

Figure (30)

Figure (31) : Photomicrograph of a transverse section of a larva treated with the mixture of (Sumicidin and Tween 80) .
Hypertrophy of the gut epithelial cells are obvious .

Cu : Cuticle.
Gc : Gut cotents .
Eps : Endo peritrophic membrane space .
Ec : Epithelial cells .
Mb : Muscle bundle .
Gaca : Gastric caecum.

(X = 400) .

Figure (32) : Photomicrograph of a longitudinal seciton in a larva treated with (Sumicidin + Tween 80) mixture . Showing the destruction occur to the larval integument .

Di : Destroyed integument .
Ec : Epithelial cells .
Gl : Gut lumen .
Ml : Muscle layer .

(X = 100) .

Figure (31)

Figure (32)

Figure (33) : Part of a section from a larva treated with fenitrothion indicating the destruction of muscle bundle and the appearance of vacuoles in the fat tissue .

Mb : Muscle bundle .

Ft : Fat tissue .

Ec : Epithelial cells .

Cu : Cuticle.

(X = 400) .

Figure (34) : Photomicrograph of a longitudinal section in a larva treated with Fenitrothion displaying the destruction of the epithelial cells and muscle bundle .

Gc : Gut contents .

N : Nucleus .

Ec : Epithelial cell .

Ft : Fat tissue .

Mb : Muscle bundle .

Cu : Cuticle.

(X = 400) .

Figure (33)

Figure (34)

Figure (35) : Transverse section of a larva treated with Fenitrothion indicating the appearance of vacuoles in fat tissue .

Cu : Cuticle.

Ft : Fat tissue.

Ec : Epithelial cells. X = 200

Figure (36) : Photomicrograph of a transverse section of a larva treated with Fenitrothion displaying the destruction of the epithelial cells and degeneration of nucleus.

Cu : Cuticle.

Ec : Epithelial cells .

N : Nucleus .

Pm : Peritrophic membrane.

Ft : Fat tissue.

Mb : Muscle bundle.

Gaca : Gastric caecum.

Gc : Gut contents. X=200

Figure (35)

Figure (36)

Figure (37) : Part of a longitudinal section of a larva treated with Fenitrotheion indicating the destruction of epithelial cells .

Ec : Epithelial cells .

N : Nucleus .

Ft : Fat tissue .

Cu : Cuticle.

X=200

Figure (38) : Transverse section of a larva treated with Fenitrothion displaying the destruction of the epithelial cells and muscle bundle .

Ec : Epithelial cells .

Mb : Muscle bundle .

Cu : Cuticle.

Gc : Gut contents.

X=400

Figure (37)

Figure (38)

Figure (39) : Transverse section of a larva treated with Fenitrothion revealing distinct deterioration of muscle layer .

Dm : Destroyed muscle layer .

Cu : Cuticle .

Ft : Fat tissue .

X=200

Figure (40) : Photomicrograph of a longitudinal section in a larva treated with Fenitrothion illustrating destruction of muscle bundle and fat tissue .

Mb : Muscle bundle .

Ec : Epithelial cells .

Gc : Gut contents.

X=200

Figure (39)

Figure (40)

Figure (41) : Transverse section of a larva treated with the mixture of Fenitrothion and Triton 20 showing the vacuolation of epithelial cells and degeneration of nuclei .

Ec : Epithelial cell .

Dn : Degenerated nuclei.

Mb : Muscle bundle .

Cu : Cuticle.

X=200

Figure (42) : Transverse section of a larva treated with the mixtre of Fenitrothion and Triton 20 revealing the destruction of gastric caeca and epithelial cells .

Ga ca : Gastric caecum .

Ec : Epithelial cells .

Mb : Muscle bundle.

Cu : Cuticle.

X=200

Figure (41)

Figure (42)

Figure (43) : Transverse section of a larva reated with the mixture of Fenitrothion and triton 20 displaying destruction of fat tissue .

Cu : Cuticle .
Mb : Muscle bundle .
Ec : Epithelial cells .
Gc : Gut contents .
Pm : Peritrophic membrane .

X=200

Figure (44) : Transverse section of a larva treated with the mixture of Fenitrothion + Tween 80 showing deterioration of peritrophic membrane depriving the gut from its lumen .

Ec : Epithelium cell .
Pm : Peritrophic membrane
Ft : Fat tissue .
Cu : Cuticle .
Ch : Cell hypertrophy .

X=200

Figure (43)

Figure (44)

6- Ultrastructural Studies .

The aim of this study is to investigate the effect of the surfactant additive (Triton 20) on ***Culex pipeins*** larvae when used in combination with Sumicidin insecticide as well as control specimens. Untreated and that treated with Sumicidin alone were also prepared for ultrastructural examination. The obtained results revealed the followings :

6.1- Fine structure of non treated *Culex pipiens* larva .

6.1.1. Integument :-

Externally , there is an outer thin refractile membrane Fig. (45) . Sometimes darkely pigmented Fig. (46) called the " Epicuticle" (the'Gerenz-lamelle' or Grenzsaum of German writers) . Below this is a rigid layer called " Exocuticle ' or primary cuticula , Emailschicht, lackschicht or pigmentschicht, Finally there is a thick colourless elastic layer, the " Endocuticle " " or secondary cuticula " "Hauptlage " or "Inmenlage " .

The epidermis is seen in its full development only when the new cuticle is being laid down ; at other times it is exceedingly attenuated. Figures (46 and 47) demonstrated the epidermal cell (in insects often called the " Hypodermis "). As in most Arthropods the integument consists of a single layer of epidermal cells . The nucleus is relatively large in size with peripherally located chromatin. The plasma membrane is essentially permeable barrier that control the passage of molecuoles and ions between the cytoplasm and the surrounding

medium (Fig.48) . There are a few mitochondria scattered throughout the cytoplasm of the cells as in Fig. (47) . The mitochondria is bounded by two membranes , an external limiting membrane and an inner membrane from which arise the cristae. The mitrochondria of the hypodermis is slightly less electron dense.

Figure (49) showed the ***Culex pipiens*** integument in another larvae which seem to vary from the above mentioned Fig's. (45 and 46); this indicate that the epidermal products can vary with the time. However, it is known that the insect cuticles consist of chitin microfibriles embeded in a protein matrix . The three main types of cuticle could be detected and are laminated in construction. Each lamina consists of a sheet of microfibrils all of which are oriented in a single preferred direction. The microfibriles of successive cheets are oriented at a slight angle to each other. The angle changed progressively in one direction . Such architicture is referred to as "Helicoidal structure" .

6.1.2. Muscle cells :-

The muscles of ***Culex pipiens*** larva seem to be made up of striated fibres Fig. (50). Each fibre consists of a number of parallel fibrillae or sarcostyles. The fibrils are threads like with visible differentiation the Z line or **Zwischenscheibe** is detected , but the median disc or **Henson's** line (H-band) could not be detected . The section of Fig. (50) revealed the fine structure of myofibrils which

showing the presence of thick , apparently tubular, myosin filaments and fine actin filaments . In contractions these actin filaments slids between the myosin unites .

6.1.3. Fat tissues :-

The fat body is arranged as a loose meshwork of lobes , invested in delicate connective tissue membranes as previously mentioned in Fig. (19) under the optical microscopy and joined by connective tissue strands, so as to expose the maximum of surface to the blood . Often there is a peripheral layer beneath the cuticle and also joined with the muscles bundle .

The ultrathin section in Fig. (51) is showing the nucleus , of fat cell contains heavily electron-dense chromatin condensations also the cytoplasm contains numerous number of mitochondria. Pinocytosis site appears in sections as mitochondrial residues " as seen with the electron microscope " .

6.1.4. Midgut cells :-

The fine structure of the midgut epithelial cells of untreated ***Culex pipiens*** larva is presented in Fig. (52) .

The midgut is lined by a delicate peritrophic membrane . The lining epithelium is columnar cells with regular microvilli forming a striated border adjacent to the lumen. The basal membranes of the

cells. adjacent to the haemocoel, are infolded with few opening to the haemolymph so that the extra cellular spaces which they enclose are relatively isolated . The cell generally contain mitochondria and extensive endoplasmic reticulum with ribosomes assumed to be concerned with the synthesis of digestive enzymes . The nuclear chromatin is clumped into patches of varying denseties .

The Figure (53) is revealing the normal fine structure of gastric caeca in a non treated ***Culex pipiens*** larva . The luminal surface of the epithelial cells has a striated border constituted of long microvilli that are projecting inwards into the luminal cavity and acting principally to increase the surface of absorption of the cell .

The ground cytoplasm of these cells contains fine granulations dispersed in a less dense matrix . Within the cytoplasm lie the mitochondria, which are conspicously rather elongated or spherical in shape. However, the mitochondria show marked tendency towards grouping near the inner (luminal) and basal portions of these cells .

There is also an a bundance of lamellated rough endoplasmic reticulum. The majority of the elements of the endoplasmic reticulum in this case exist in the form of lamellar structure or flattened cisternal vesicles. These elements usually contain accumulation of material known as intracisternal inclusions . This type of endoplasmic reticulum is characterized by the presence of numerous minute granules bordering

the outer surface of the membranes of the reticulum. These particles are rich in RNA and proteins and hense they are known as the " ribonucleoprotein particles or ribosomes " . Similar particles are also dispersed in the cytoplasmic matrix .

The electron micrograph shows the existence of rounded bodies taking the form of sac-like structure, being surrounded by a single thin lipoprotein membrane. They breakdown cellular material such as protein, nucleic acid and polysacharides. The particles are, therefore known as lysosomes; that is bodies which can digest or lyse substances.

The Golgi dictyosomes appear as flattened curved sacs together with clusters of small vesicular bodies at their edges .

Figure (45) : Electron micrograph of a non treated ***Culex pipiens*** larva showing the fine structure of normal integument.

Ep. : Epicuticle

Ex. : Exocuticle .

En. : Endocuticle .

Hy. : Hypodermis .

Mb. : Muscle bundle.

X = 5 x 1000

Figure (46) : Electron micrograph of normal ***Culex pipiens*** larva illustrating the darkely pigmented epicuticle .

Ep. : Epicuticle .

Ex. : Exocuticle .

En. : Endocuticle.

Hy. : Hypodermis .

X = 140 x 1000 .

Figure (45)

Figure (46)

Figure (47) : Electron micrograph of untreated larva showing the normal composition of its integument, with higher magnification .

Cu. : Cuticle .

Ep. : Epdermis .

M. : Mitochondria .

Pm. : Plasma membrane

X = 140 x 1000

Figure (48) : Electron micrograph of normal ***Culex pipiens*** larva illustrating the epidermal layer.

N : Nucleus .

Ch : Chromatin .

Cy : Cytoplasm .

Bp : Basal plasma membrane .

Ne : Nuclear envelope .

Hy : Hypodermis.

Ep : Epicuticle.

Ex : Exocuticle.

En : Endocuticle.

X = 140 x 1000

Figure (47)

Figure (48)

Figure (49) : Electron micrograph of untreated ***Culex pipiens*** larva illustrating the integument .

Ep. : Epicuticle .

Ex. : Exocuticle .

En. : Endocuticle .

Hy. : Hypodermis .

X = 20 x 1000 .

Figure (50) : Electron micrograph of a non treated ***Culex pipeins*** larva , at high magnification illustrating the normal configuration of the muscle and the organization of bands .

Z : Z band .

Ct : Connective tissue X = 20 x 1000.

Figure (49)

Figure (50)

Figure (51) : Electron mcirograph of untreated ***Culex pipiens*** larva showing the nucleus; chromatin of fat cell and mitochondria.

P : Pinocytosis site " mitocondrial residues " .

Ch. : Chromatin .

N. : Nucleus .

X = 2.7 x 1000.

Figure (52) : Electron micrograph of untreated ***Culex pipiens*** larva showing the epithelial cell .

Bpm : Basal plasma membrane .

N : Nucleus .

Ch : Chromatin .

M : Mitochondria.

Pm : Peritrophic membrane .

Ne : Nuclear envelop .

X = 2.70 x 1000.

Figure (51)

Figure (52)

Figure (53) : Electron micrograph revealing the normal structure of gastric caeca in a non treated ***Culex pipiens*** larva .

N : Nucleus .

Ch : Chromatin .

M : Mitochondria.

Cy : Cytoplasm .

Mv : Microvilli .

Ly : Lysosomes.

X = 6.7 x 1000.

Figure (53)

6.2. Fine structure of Sumicidin treated *Culex pipiens* larva .

6.2.1. Integument :-

Ultrastructural examination of Sumicidin treated *Culex pipiens* larva Fig. (54) revealed an amorphous cuticular region instead of the normal lamellate cuticle deposition pattern in comparison with the non teated larva. The endo- and exo-cuticle are not distinguishable . The epidermal cells are not arranged in the form of a single layer epithelium, but rather are irregular distributed underneath the cuticle. Epidermal mitochondria showed variable morphological pictures . The mitochondria are swollen with irregular shape , partially lost or even totally its cristae .

The fat body underneath the cuticle could be detected with visible vacuolization as a resulte of the Sumicidin treatment .

6.2.2. Muscle cells :-

The ultrastructural examination of Sumicidin treated *Culex pipiens* larva Fig. (55) revealed degenerated muscles , resulting in diorganization of the components . The Z discs have irregular shape and are distorted . Gaps and vacuoles appeared in the sarcomere.

6.2.3. Fat tissues :-

The fat cells appeared destroyed Fig. (56) they became vacuolated and deteriorated .

6.2.4. Midgut cells :-

Ultrastructural examination of Sumicidin treated *Culex pipiens* larva midgut showed to be affected in many features .

Fig. (57) illustrate the appearance of cytoplasmic vacuoles . Some mictochondria are swallen with irregular shape and others are greatly elongated with prominent cristae . The two mitochondrial membranes are not demarkated with obvious increase in the mitochondrial granules Fig. (57) and Fig. (58) . Some mitochondria are seriously affected that ghosts of mitochondria and mitochondrial remnants are left . The Golgi bodies are fragmented into mall particles which are thinned out and gradually disappeared Fig. (58) .

Moreover, the rough endoplasmic reticulum is broken down into separate narrow and wide vacuolar structures without any clear connection with the nuclear membrane .

The lysosome were considerably increase in size and in number . The rupture of lysosomal membranes resulted in releasing of its containing enzyme causing destruction of the cellular constituent and complete dissolution of the cell .

The nucleus was also affected by the treatment . The nuclear chromatin is aggregated or clumped into numerous masses and later released by rupture. The nuclear envelope remains resonally intact but the contents are partially or completely lost. A condensation of the

chromatin occurs along or adjacent to the inner membrane of the nuclear envelope while chromatin had disappeared from other parts of the nucleus. Margination of chromatin appears to be an early change occurs in the nucleus after treatment leading to cell death Fig(59). The peritrophic membrane Fig. (60) was also affected .

The gastric caeca Fig . (61) of ***Culex pipiens*** larva treated with Sumicidin revealing the destruction of microvilli . The treatment induced cell vacuolization and cell wall rupture there is also evidence of autolysosomes and heterolysosomes. The gastric caeca in Fig. (62) with higher magnification was showing the deformed nucleus .

Figure (54) : Electron micrograph of a ***Culex pipiens*** larva treated with Sumicidin alone showing the destruction of the integument and fat body vacuolization .

Ep : Epicuticle .

Ex : Exocuticle .

En : Endocuticle .

H : Hypodermis

Fb : Fat body .

X = 10 x 1000.

Figure (55) : Ultrastructural changes in muscles of ***Culex pipiens*** larva treated with Sumicidin insecticide .

Mb : Muscles bundle

Z : Z. line.

X = 10 x 1000.

Figure (54)

Figure (55)

Figure (56) : Electron mcirograph revealing the destruction of fat tissue of ***Culex pipiens*** larva treated with Sumicidin insecticide .

L. : Lysosome.

Dm. : Deformed mictochondria.

X = 8 x 1000.

Figure (57) : Electron micrograph of ***Culex pipiens*** larva treated with Sumicidin showing the appearance of cytoplas - mic vacuoles and deformed rough endoplasmic reticulum .

V. : Cytoplasmic vacuoles.

Rer. : Rough endoplasmic reticulum.

X = 10 x 1000 .

Figure (56)

Figure (57)

Figure (58) : Electron mcirograph of ***Culex pipiens*** larva treated with Sumicidin showing the destruction occur to the gut cell and the deformed mitochondria .

N : Nucleus .

Mt : Mitochondria .

X = 6 x 1000.

Figure (59) : Electron micrograph of ***Culex pipiens*** larva treated with Sumicidin showing the lysed nucleus of the midgut.

Ne : Nuclear envelope .

Ch : Chromatin .

Mt : Mitochondria .

X = 8 x 1000.

Figure (58)

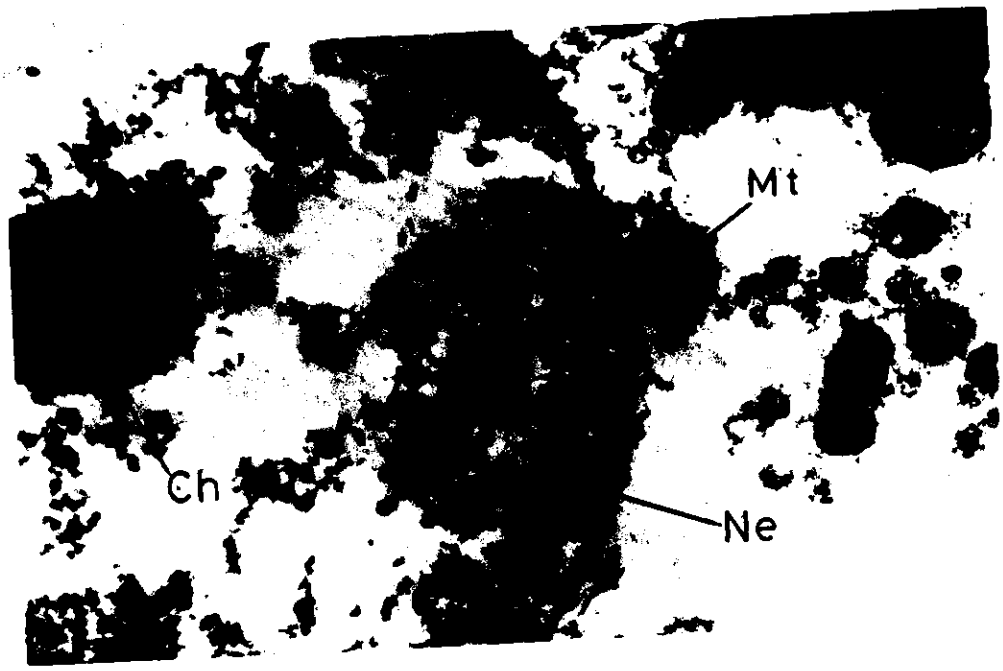

Figure (59)

Figure (60) : Electron micrograph of ***Culex pipiens*** larva treated with Sumicidin showing the affected peritrophic membrane .

Pm : Peritrophic membrane .

Mc : Midgut cell.

X = 10 x 1000 .

Figure (61) : Electron micrograph revealing the destruction of microvilli in the cells of gastric caeca in ***Culex pipiens*** larva treated with Sumicidin insecticide .

Mv. : Microvilli .

Ec. : Epithelial cell.

X = 5 x 1000

Figure (60)

Figure (61)

Figure (62) : Electron micrograph with higher magnification of gastric caeca of Sumicidin treated ***Culex pipiens*** larva showing the deformed nucleus .

N : Nucleus .

Ch : Chromatin.

Mv : Microvilli .

X = 6.7 x 1000 .

Figure (62)

6.3. Fine structure *Culex pipiens* larva treated with the mixture of Sumicidin and Triton 20 .

6.3.1. Integument :-

The ultrastructural effects of the ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 Fig. (63) revealed the presence of epicuticle and exocuticle identifing the outline of the larval integument. However, the Hypodermis and its ultrastrutural organelles were completely lost.

The fat body underneath the cuticle was also completely lost. The debris of tissues indicate the extensive damage occur to the integument.

6.3.2. Muscle cells :-

The ultrastructure examination of muscles bundle of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 Fig. (64) showed fissures and severe damage occur, this resulted in no visible differentiation for Z - line .

Another treated larva Fig. (65) with higher magnification showing affected sarcomere . The myofibrils shrunk and were grouped into masses separated by vacuoles and fissures .

6.3.3. Fat tissues :-

The fat cells seemed to be highly vacuolated with necrosis Fig. (66). Addition of Triton 20 to Sumicidin insecticide resulted in the lysis of these cell .

6.3.4. Midgut cells :-

Examination of the ultrathin section of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 revealed degeneration of the gut cells Fig. (67). The epithelial cells had lost their normal structure and cell membrane were destructed . The mitochondria were hypertrophed puffed, and severely deformed , their ridges were highely degenerated with damaged mitochondrial membranes. The rough endoplasmic reticulum was also markedly injured . There is also an obvious evidence of multivesicular bodies and autophagic vacuoles scattered in the cytoplasm. There are proposingly autoplagic lyosomes. The Golgi bodies were completly disappeared .

Figure (68) showed the destruction occur to the nucleus and its chromatin, also lysed cytoplasm. The cytoplasmic material was markedly deteriorated .

The epithelial cells Fig. (69) also displayed marked degeneration with pyknotized nuclei and with distinct vacuolar remnants. The same figure also showed mitochondrial deformation, marked coalescence and inner damage .

Figure (70) showed the destruction occur to gastric caeca cells with the increase in number of autofagic vacuoles and lysosomes . In addition Fig. (71) showed the extensive damage occur to the microvilli. The cells were filled with dense material also contain multivesicular bodies. The peritrophic membrane was seriously affected and degenerated Fig. (72) .

Figure (63) : Electron micrograph of a ***Culex pipiens*** larva treated with a mixture of surface active agent Tritron 20 and Sumicidin insecticide showing the extensive damage occured and the complete vacuolization .

Ep. Epicuticle .

Ex. Exocuticle .

En. Endocuticle.

Hy. Hypodermis.

X = 5 x 1000.

Figure (64) : Ultrastructural changes in muscles of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 showing damage occur without visible differentiation for Z-line .

Mb : Muscles bundle .

X = 10 x 1000.

Figure (63)

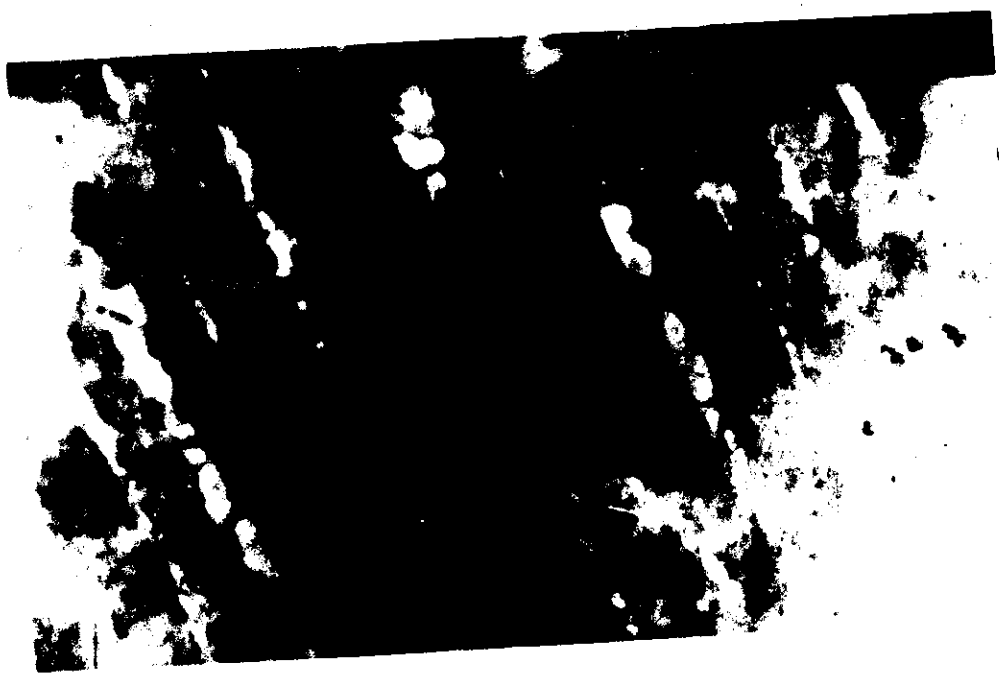

Figure (64)

Figure (65) : Magnified part of ultrastructural changes in muscles of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20.

Mb : Muscles bundle .

Z : Z -line .

X= 6.7 x 1000 .

Figure (66) : Electron micrograph revealing the destruction of fat tissue of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 .

Ft. : Fat tissue.

Mb. : Muscles bundle.

X = 4 x 1000.

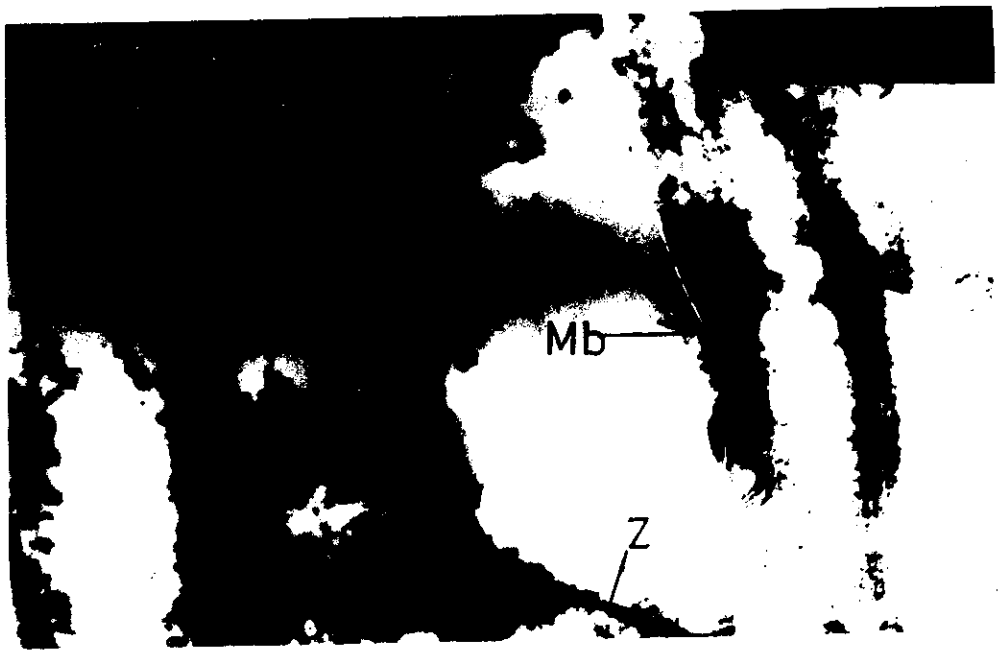

Figure (65)

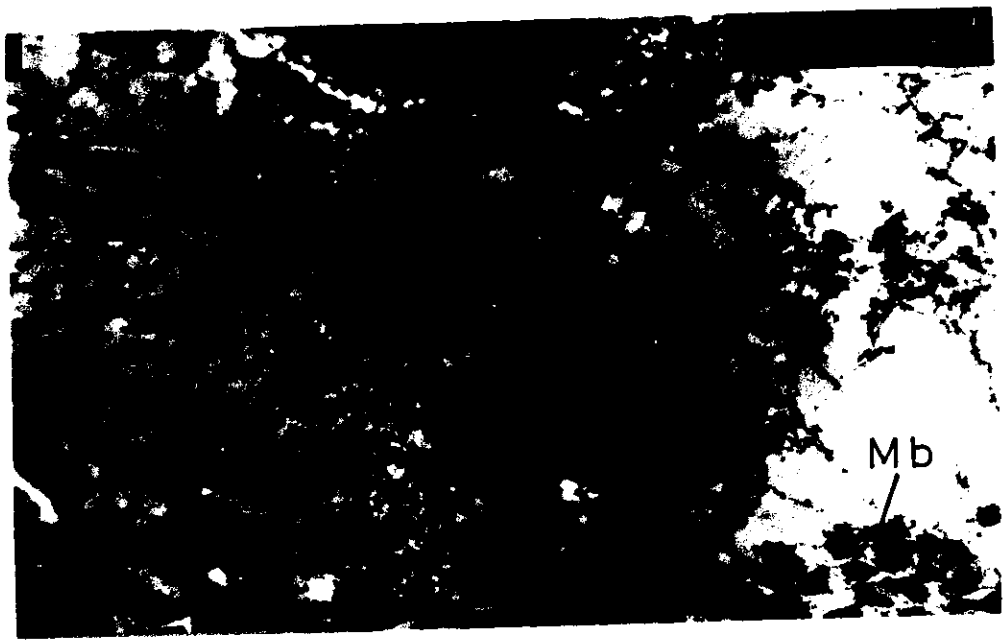

Figure (66)

Figure (67) : Electron micrograph of ***Culex pipiens*** larva treated with a mixture of (surface active agent Triton 20 and Sumcidin insecticide) showing the appearance of cytoplasmic vacuoles and deformed mitochondria .

Cv. : Cytoplasmic vacuoles.

Mt. : Mitochondria.

X = 10 x1000.

Figure (68) : Electron micrograph of ***Culex pipeins*** larva treated with a mixture of Sumicidin and Triton 20 showing the destruction of the nucleus and its chromatin. Note also the lysed cytoplasm.

Ne : Nuclear envelop.

N : Nucleus.

Mt : Mitochondria .

X = 8 x 1000

Figure (67)

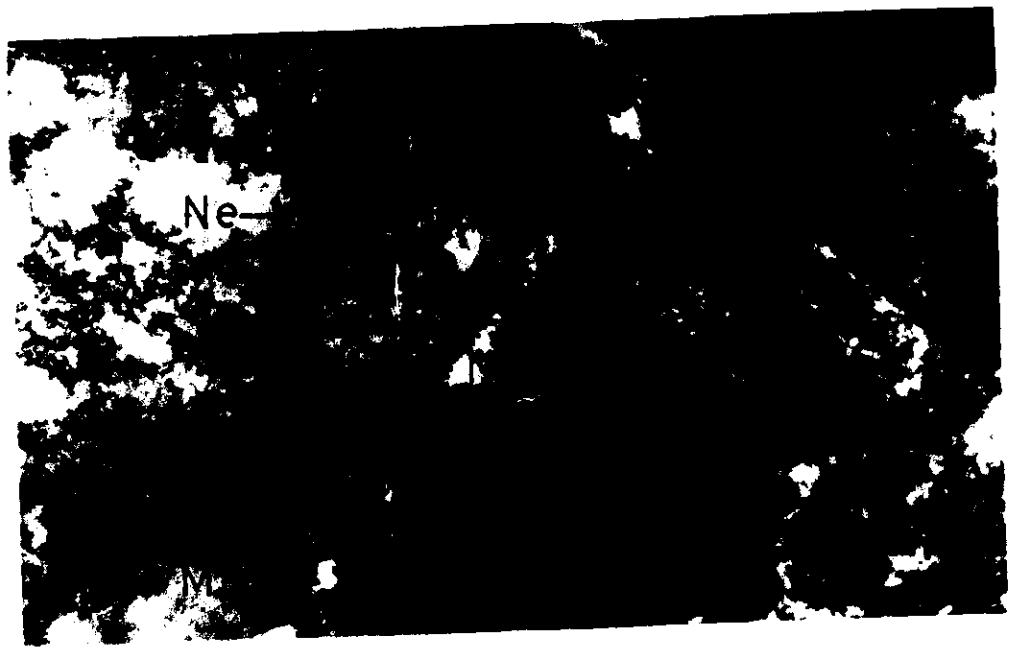

Figure (68)

Figure (69) : Electron micrograph of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 showing the destruction occur to the gut cell .

Bl : Basal lamellae.
Mt : Mitochondria .

X = 8 x 1000.

Figure (70) : Electron mcirograph of gastric caeca cells of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 Showing ruptured microvilli and lysed cytoplasm.

Mv : Microvilli.
Av : Autofagic vacuoles .
Ly : Lysosomes .
Lc : Lysed cytoplasm.

X = 2.7 x 1000.

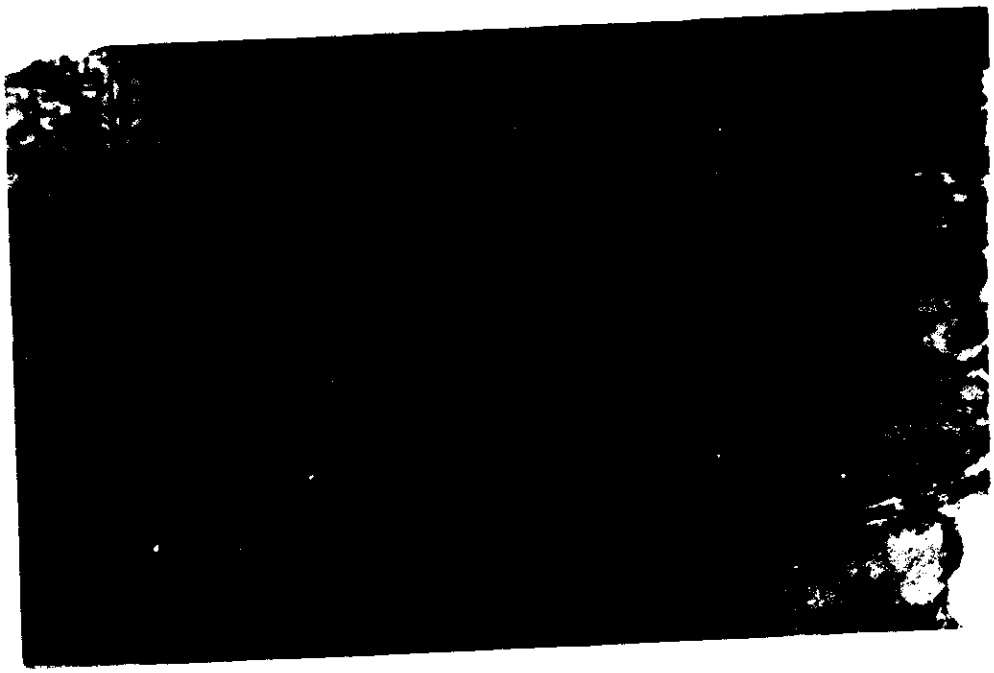

Figure (69)

Figure (70)

Figure (71) : Electron micrograph showing the extensive damage occur to the microvilli in the cells of gastric caeca in ***Culex pipiens*** larva treated with (Triton 20 + Sumicidin) mixture.

Mv. : Microvilli.
Mvb. : Multivesicular bodies.

X = 6.7 x 1000.

Figure (72) : Electron micrograph of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 showing the degeneration occur to the peritrophic membrane .

Pm : Peritrophic membrane.

X = 10 x 1000 .

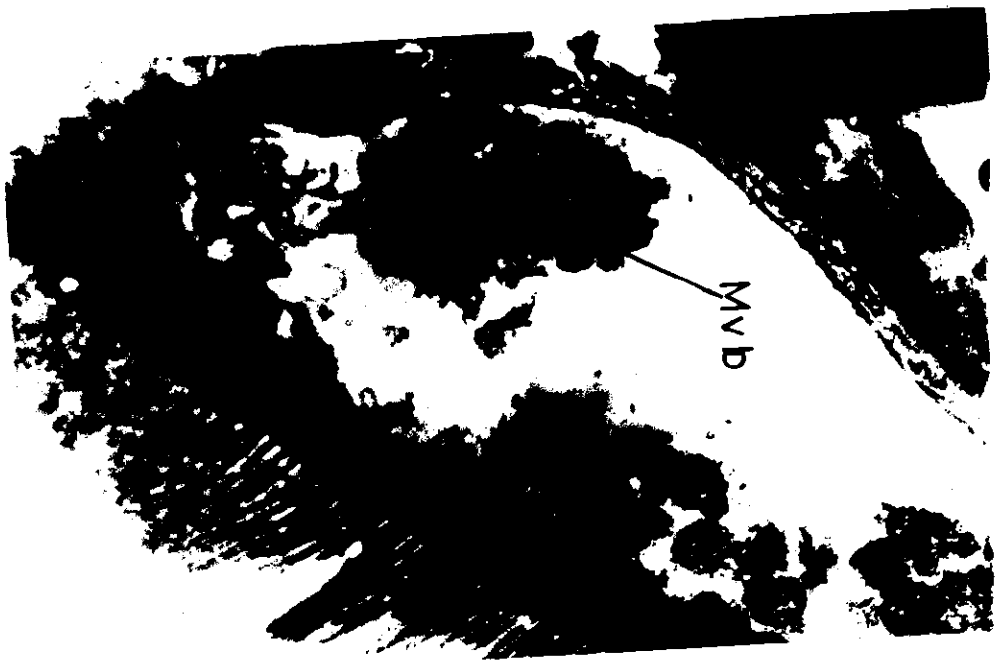

Figure (71)

Figure (72)

7- Ultrastructural localization of certain enzymes activity in mosquito larvae .

In order to elucidate the synergistic effect of the surfactant on the insecticides, the *Culex pipeins* larvae was tested for the fine structural localization of both ATPase and acid phosphatase . The untread larvae and that treated with either of the insecticide alone or with its mixture with the surface active agents were included in this investigation. Sumicidin was used as a representive for the insecticide and Triton 20 as a representive for surfactant. The ultrastructural observations for the tested enzymes were as the following :

7.1. Localization of ATPase in *Culex pipiens* fine structure :-

The normal distribution of ATPase activity of midgut cell is illustrated in Figs. (73) to (76) .

The reaction product of the ATPase enzyme was found to be in the mitochondrial matrix as a large deposits. ATPase was also present in the rough endoplasmic reticulum, in free accumulation near the nucleus, in between muscle cell and in the integument; Figs. (75 & 76).

The profile obtained after Sumicidin treatment in Fig. (77) indicated the mitochondria and the inhibition occurred to the ATPase activity. This is clear by the decrease in the reaction product of ATPase activity .

In case of the effect of the Triton 20 addition to Sumicidin in Fig. (78) which indicated the complete inhibition of the ATPase enzyme with the marked destruction of the mitochondrial cristae which may be partially lost or even totally.

Figure (79) illustrate a slight patchy reduction or inhibition of ATPase in muscle tissue after treatment with Sumicidin.

On the other hand, complete inhibition is observed in the muscle cells after treatment with the mixture of Sumicidin and Triton 20 with sever degeneration of the muscle fiber .

The integument of ***Culex pipeins*** larva treated with Sumicidin was showed the occurrence of slight inhibition of ATPase activity Fig. (81). However this inhibition is more pronounced in this region of a larva treated with the insecticide when combined with the surface active agent Triton 20 Fig. (82) .

Figure (73) : Electron micrograph showing the normal distribution of ATPase (Adenosine triphosphatase) in the mitochondrial matrix and in the rough endoplasmic reticulum of ***Culex pipiens*** larva .

M. : Mitochondrial matrix.

Rer. : Rough endoplasmic reticulum.

X = 8 x 1000.

Figure (74) : Electron micrograph showing normal free ATPase activity near the nucleus of ***Culex pipiens*** larva .

Rer. : Rough endoplasmic reticulum.

N. : Nucleus.

Figure (73)

Figure (74)

Figure (75) : Electron mcirograph showing the normal localization of ATPase in the cytoplasm of muscle cell and in the mitochondrial matrix as a large deposits .

M. : Muscle.

Mt. : Mitochondria. X = 2.7 x 1000.

Figure (76) : The normal distribution of ATPase in the ***Culex pipiens*** larval integument .

X = 40 x 1000 .

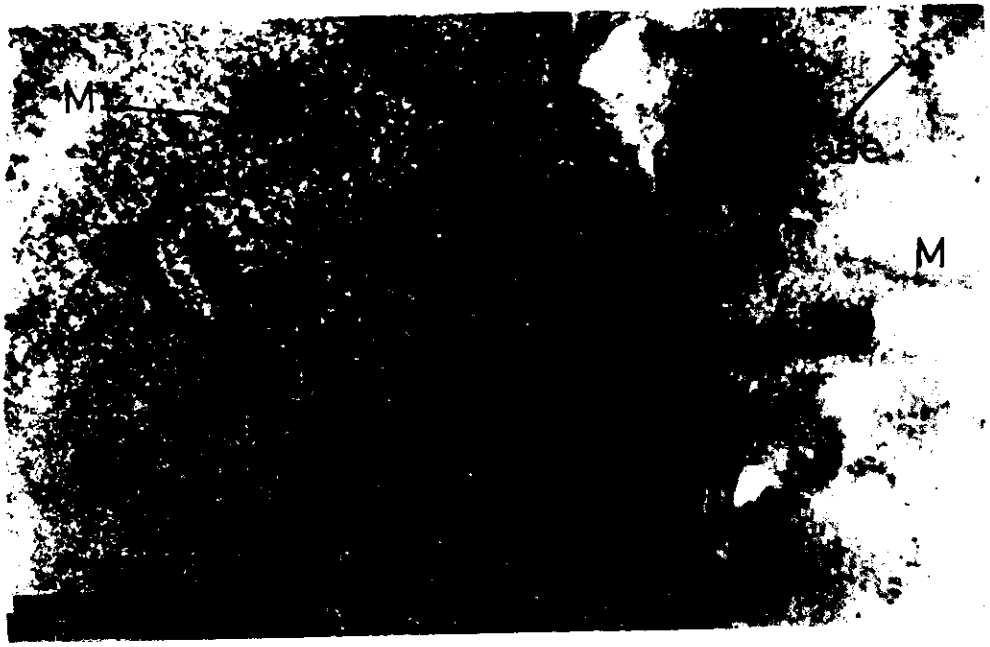

Figure (75)

Figure (76)

Figure (77) : Demonstration of inhibitory action of Sumicidin in the mitochondrial ATPase and in the cytoplasm.

Mt. : Mitochondria.

X = 2.7 x 1000.

Figure (78) : Demonstration of the complete inhibition of the ATPase on the mitochondria of the midgut cell due to the treatment with the mixture of Sumicidin and Triton 20 .

Mt. Mitochondria.

X = 14 x 1000

Figure (77)

Figure (78)

Figure (79) : Electron micrograph of ***Culex pipiens*** larva treated with Sumicidin showing the inhibition of ATPase in muscle cells.

Mb. : Muscle bundle.

Z. : Z-line.

X = 6 x 1000.

Figure (80) : Electron micrograph of ***Culex pipiens*** larva treated with the mixture of (Sumicidin + Triton 20) showing the inhibition of ATPase in the severly degenerated muscles .

Mb. : Muscle bundle.

Z. : Z-line.

X = 10 x 1000.

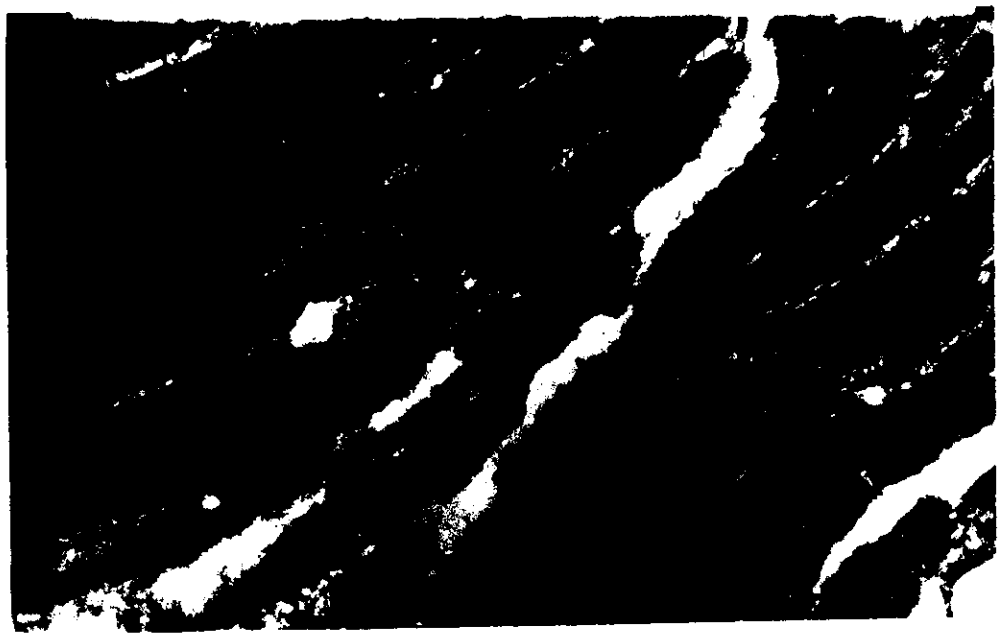

Figure (79)

Figure (80)

Figure (81) : A specimen obtained from ***Culex pipiens*** larval integument treated with Sumicidin showing the slight inhibition of ATPase .

Cu. : Cuticle.

Hy. : Hypodermis.

X = 20 x 1000

Figure (82) : Electron micrograph of ***Culex pipiens*** larval integument treated with the mixture of (Sumicidin + Triton 20) showing the complete inhibition of ATPase activity.

I. Integument.

X = 6.7 x 1000.

Figure (81)

Figure (82)

7.2. Localization of acid phosphatase in *Culex pipiens* larva fine structure.

The normal distribution of acid phosphatase activity of midgut cell is illustrated in Fig. (83) .

Lysosomes are the organelles which mainly demonstrate the acid phosphatase activity .

The primary and secondary lysosomes seem to be small in size with spherical shapes . Some of the secondary lysosomes are exhibiting large sizes . These are presumably autophagic . The ultrastructural localization of acid phosphatase was also seen to present in the rough endoplasmic reticulum. Also the mitochondria was found to be slightly less electron dense .

On higher magnification Fig. (84) free hydrolase can be seen in the non treated larva.

In figs. (85 , 86 to 87) in which the acid phosphatase activity were illustrated in ultrathin section of larvae treated with sumicidin alone. These profiles indicated some local membrane damage and limited leakage of enzyme, both in the lysosomes and Golgi complex. Autophagic lysosomes have shown to be enclosing lysed mitochondria. Multivesicular bodies " a vacuole containing vesicles set in a lucent or dense matrix " was found in profiles of a larva treated with Sumicidin Fig.(86). Since acid phosphatase has been demonstrated in multivesicular bodies they are considered to be a variety of lysosome.

Profiles obtained after treating the ***Culex pipiens*** larva with the mixture of Sumicidin and Triton 20 indicated the extensive damage

occur to the midgut cells with the leakage of acid phosphatase. All cells showed the presence of free acid phosphatase in the cytosol Fig. (87) .

The earliest signs of cell injury seen after the larval treatment with the mixture of Sumicidin and Triton 20 lie in the cytopalsm Fig. (88). Karyolysis of the nucleus was shown in Fig. (90) in which the nuclear envelope remains reasonably intact but the contents are partially or completely lost due to the release of acid phosphatase. Leakage of acid phosphatase at Golgi was evident.

The typical cell death in Fig. (91) illustrated the effect of the mixture of Sumicidin and Triton 20 which indicate the degeneration of the cytoplasmic organelles; only the cell membrane is present. There is a considerable accumulation of secondary lysosomes, some of which are obviously autophagic ,apparently engulfing lysed mitochondria . The presence of multivesicular bodies was evident with the leakage of lysosomes .

The microvilli Fig. (92) was found to be greatly swollen and distended with the accumulation of the free acid phosphatase as a result of the treatment with the mixture of Sumicidin and Triton 20 .

Figure (83) : Electron mcirograph showing the normal distribution of acid phosphatase activity in the primary lysosomes (ry Ly) and the secondary lysosomes (nd Ly) of the mid gut epithelial cells of ***Culex pipiens*** larva .

Rer. : Rough endoplasmic reticulum.
Ne. : Nuclear envelope .
Ch. : Chromatin.
Ly. : Lysosomes.

X = 5 x 1000 .

Figure (84) : Electron mcirograph showing normal free acid phosphatase activity with higher magnification .

Ap. : Acid phosphatase activity .
Rer. : Rough endoplasmic reticulum.

X = 6.7 x 1000.

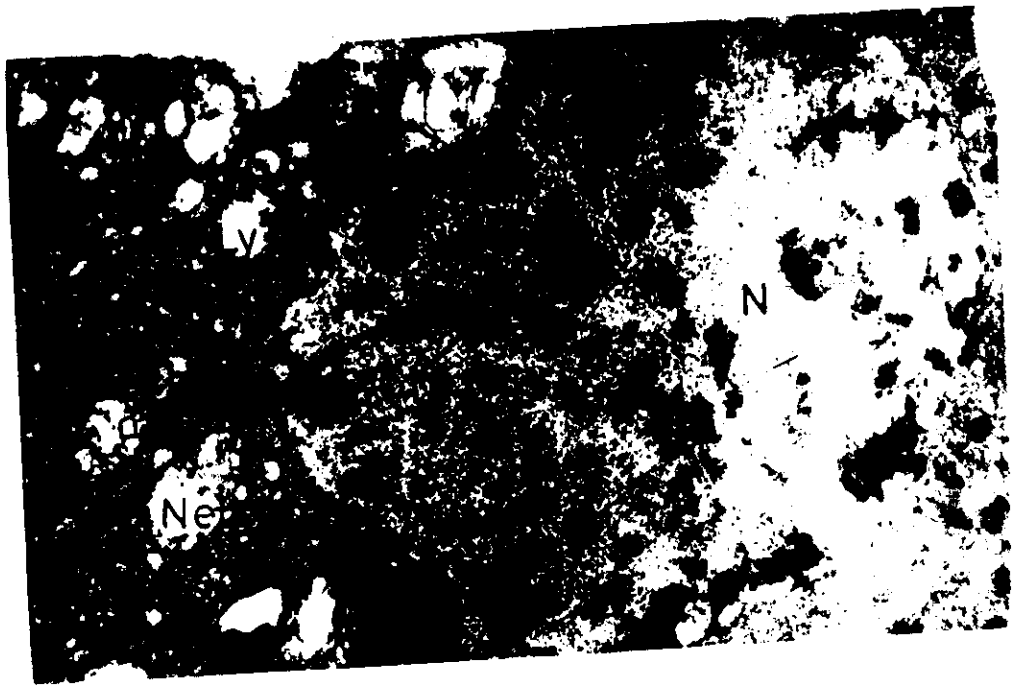

Figure (83)

Figure (84)

Figure (85) : Leakage of lysosomal acid phosphatase of ***Culex pipeins*** larva (midgut region) after Sumicidin treatment.

Ly. : Lysosome .

Der. : Destroyed endoplasmic reticulum.

N. : Nucleus .

Ne. : Nuclear envelope .

V. : Vacuoles.

Bl. : Basal Plasma .

Rer. : Rough endoplasmic reticulum.

Gc. Golgi complex.

X = 6.7 x 1000.

Figure (86) : A specimen obtained from ***Culex pipiens*** larva treated with Sumicidin displaying autofagic lysosomes enclosing lysed mitochondria and multivesicular bodies.

Af. : Autofagic lysosomes.

Mvb. : Multivesicular bodies.

X = 8 x 1000 .

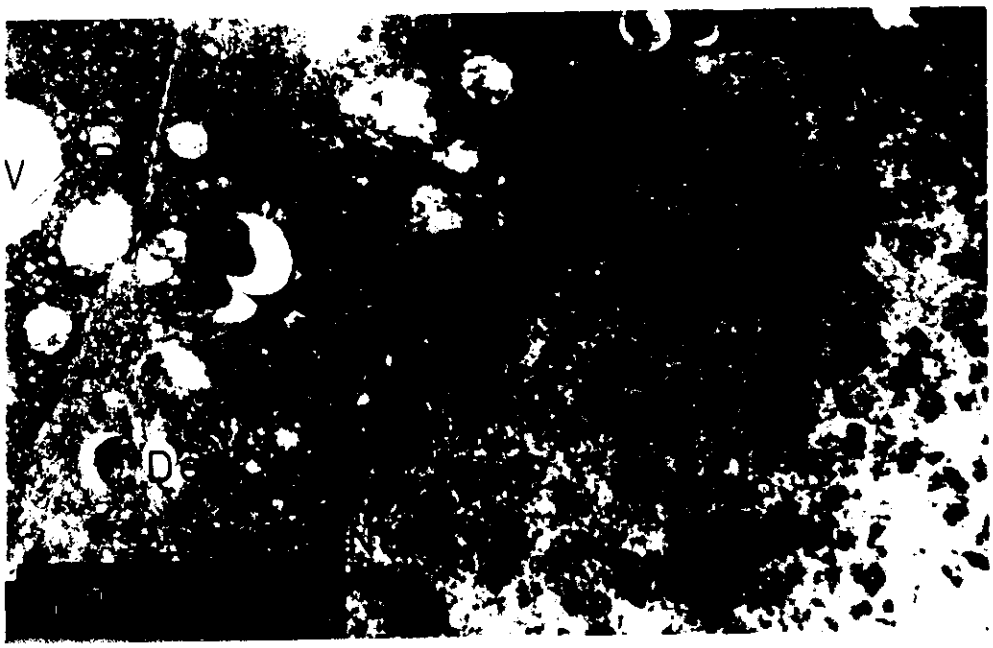

Figure (85)

Figure (86)

Figure (87) : Electron mcirograph of ***Culex pipiens*** larva treated with Sumicidin showing the release of acid phosphatase into cytosol and leakage of lysosomes .

Mv. : Microvilli .

Mvb. : Multivesicular bodies .

X = 6.7 x1000 .

Figure (88) : Electron mcirograph of midgut cells of ***Culex pipiens*** larva treated with mixture of Sumicidin and Triton 20 illustrating the earlient signs of cell injury lie in the cytoplasm. Note the activity of the acid phosphatase in the mitochondria .

Mt. : Mitochondria .

N. : Nucleus.

N. : Nuclear envelope.

X = 10 x 1000.

Figure (87)

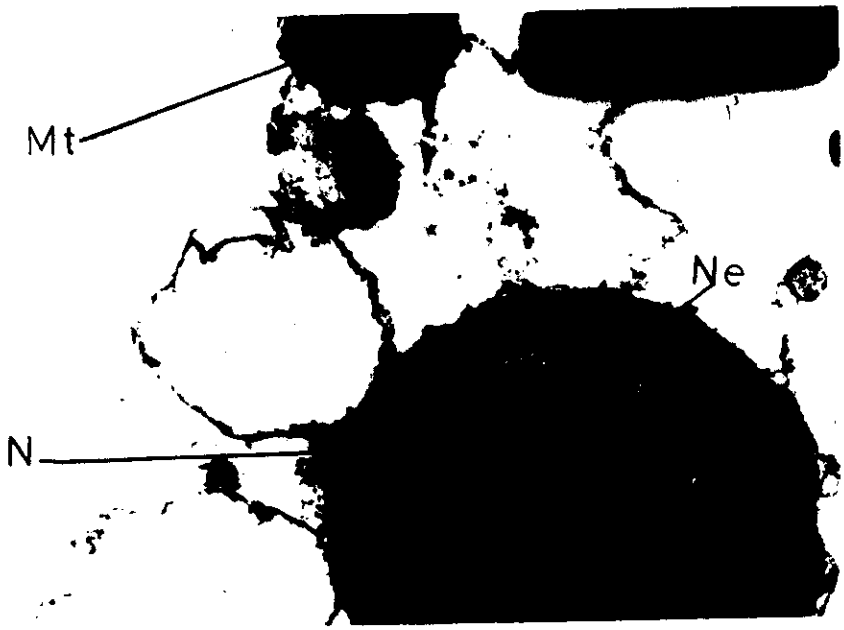

Figure (88)

Figure (89) : Electron micrograph of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 showing cell death of the midgut cells due to the release of acid phosphatase into cytosol.

2nd Ly : 2nd Lysosomes .

Mc. : Muscle cells .

X = 6.7 x 1000.

Figure (90) : Electron micrograph of the midgut cell showing karyolysis of the nucleus in which the nuclear envelope remains reasonably intact but the contents are partially or completely lost due to the release of acid phosphatase .

Mt. : Mitochondrial destruction .

Go. : Leakage of acid phosphatase at Golgi.

X = 10 x 1000.

Figure (89)

Figure (90)

Figure (91) : Electron micrograph of ***Culex pipiens*** larva midgut after treatment with the mixture of Sumicidin and Triton 20 illustrating the typical cell death and leakage of lysosomes.

Ly : Lysosomes .
Mvb : Multivesicular bodies .

X = 8 x 1000.

Figure (92) : Electron micrograph of ***Culex pipiens*** larva treated with the mixture of Sumicidin and Triton 20 showing the appearance of vacuoles the leakage of lysosomes and autofagic vacuoles . Note the diffuse of the acid phosphatase activity in the microvilli.
Ly. : Lysosomes.
Mv. : Microvilli.

X = 6.7 x 1000 .

Figure (91)

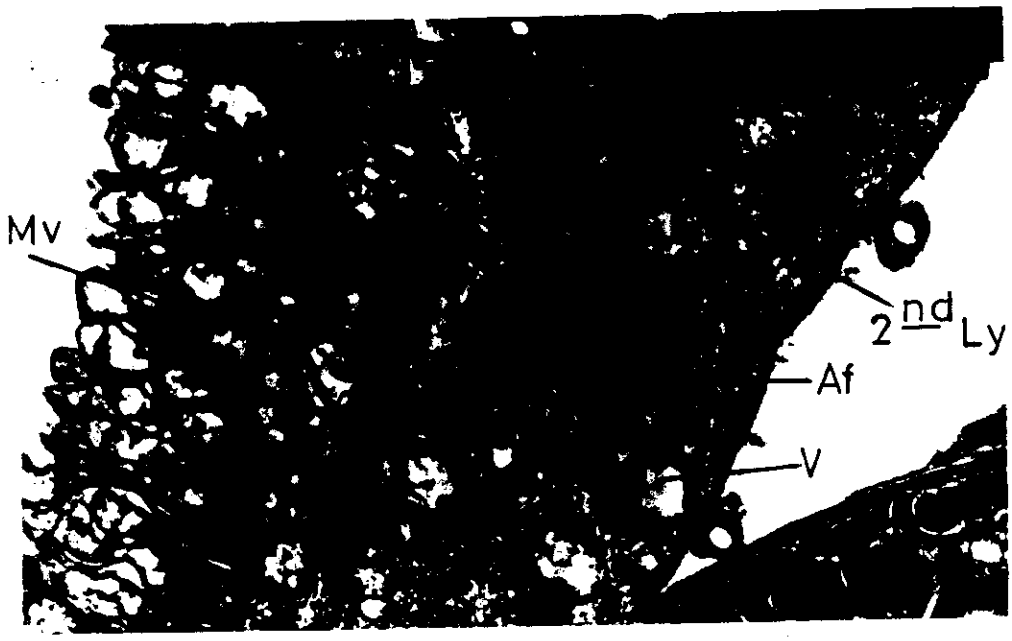

Figure (92)