RESULTS AND DISCUSSION

4.1.Toxicological effect of insecticides on *spodoptera littoralis* (Boisd)

4.1.1. Biological studies

4.1.1.1. Toxicological effect of *B. thuringiensis (Kurstaki)* on *S. littoralis*:

Data in table (3) and fig (1a) revealed that the LC_{50} of *B. thuringiensis* was $2.18x10^3$ IU/ ml which fell within a slightly large area further when the lower and upper fudical limits were taken into concentration, it was found that all the points of LC_{50} were within these limits. The obtained results also supported by the work of **Nasser (1999)** and **Hossani et al. (1998)** and **Mohamed(2003)** who found that *B. thuringiensis (Kurstaki)* exhibited good mortality against 4^{th} instar of *S. littoralis*. One formulation of *B. thuringiensis* was used to determine the toxicological effects of this biotic insecticide on the 4^{th} instar larvae of *S. littoralis*. The larvae were fed on castor-oil leaves previously treated with different concentrations of protecto for 24 hours, then transferred to untreated leaves till pupation. The toxicity of *B. thuringiensis* was investigated by **Abd El-Aziz (2000)** who classified lepidopteran larvae into three types based on their susceptibility to: endotoxin; which caused insects mortality by preparations of crystalline δ- endotoxin alone. He also, found that spores of bacterium are not responsible crystalline for the increase of toxicity, in some cases, mid gut pH may be closer to neutrality, allowing germination or the action of

endotoxin may cause a decrease in pH so that germination can occur. He also indicated that insects were susceptible to endotoxin but the effect was enhanced by the presence of spores. He also indicated that spore-endotoxin mixtures only killed insects. The mid gut pH of most susceptible larvae was too alkaline to allow spore germination but was suitable for dissolution and activation of protoxin. **Sokar (1995)** agree with the achieved results when reported that *S. littoralis* susceptibility was inversely proportional to age toward. Dipl 2X, also **Abd El Haleem (1997)** recorded that *B. thuringiensis* are toxic to larvae of Lepidoptera upon ingestion.

4.1.1.2. Toxicological effect of organophosphorus (chlorpyrifos) on *spodoptera littoralis* (Boisd):

It is one of organophosphorus compound (chlorpyrifos) was selected to determine the toxicological effect on 4^{th} instar larvae of *S. littoralis* and albino rat. The larvae fed on castor-oil leaves previously treated with different concentration of chlorpyrifos for 24 hours, then transferred to untreated leaves until pupation.

Data in table (3) and fig (1b) revealed that the LC_{50} of chlorpyrifos was 0.398 ppm which fell within a slightly large area further when the lower and upper fudical limits were taken into concentration it was found that all the points of LC_{50} were within these limits. The obtained results are confirmed with those obtained by **Eid et al. (1992)** and **Mohamed (2003**) who studied the effect of non-lethal doses of some of organophosphorus insecticides on some biological aspects of *S.*

Littoralis larvae i.e. chlorpyrifos and profenofos showed no effect on the acidosis 5th larvae instar.

4.1.1.3. Toxicological effect of insect growth regulators (IGRs) on *S. littoralis*:

The two insect growth regulators (IGRs), flufenoxuron and hexaflumuron were used to determine the toxicological effect on the 4th instar larvae of *Spedoptera littoralis.* The larvae were fed on castor-oil leaves previously treated with different concentration of (IGRs) for 24 hours, then transferred to untreated leaves until pupation.

Data in table (3) and fig (1c and 1d) revealed that the LC_{50} of flufenoxuron and hexaflumuron were (0.97 and1.93) ppm respectively. It was found that all the points of LC_{50} were within these limits. The obtained results are confirmed with those obtained by **Gad Allah et. al. (1990),** who worked on *Hiliothis armigra* larvae treated with juvenile hormone Pyriproxyfen. **Haga et. al (1984)** reported that Chlorfluazuron is very toxic to insects because it metabolizes slowly inside the insect body. The toxicity of Flufenoxuron against *S. littoralis* larvae was some what similar to that of the Chlorfluazuron against *H. armigra* (**Rao *et al*. 1994)** and *A. ipsilon* **(Shurab et. al. 1999)**. Also **Farag (2001)** reported that three chitin synthesis inhibitors caused high mortalities to 4th instars larval of *S. littoralis*.

Insecticides	LC_{50}	95 % fiducially limits		Slope
		Lower	upper	
B.t.	$2.18x10^3$ IU	0.0002	0.023	0.39 ± 0.10
CPF	0.398 ppm	0.145	0.67	0.975 ± 0.1
Flufenoxuron	0.247 ppm	0.086	1.22	2.12 ± 0.28
Hexaflumuron	1.93 ppm	1.67	2.245	2.116 ± 0.174

Table (3): Susceptibility of *S. littoralis* 4th instar larvae to B. t., CPF, Flufenoxuron and Hexaflumuron.

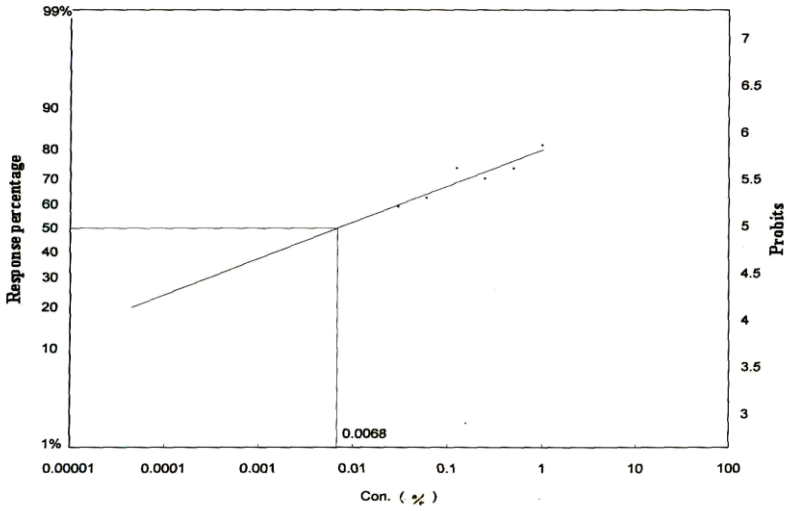


Fig. (1a): Toxicity regression lines post 48-h of feeding 4th instar larvae of *S. littoralis* on castor oil leaves treated with *Bacillus thuringiensis.*

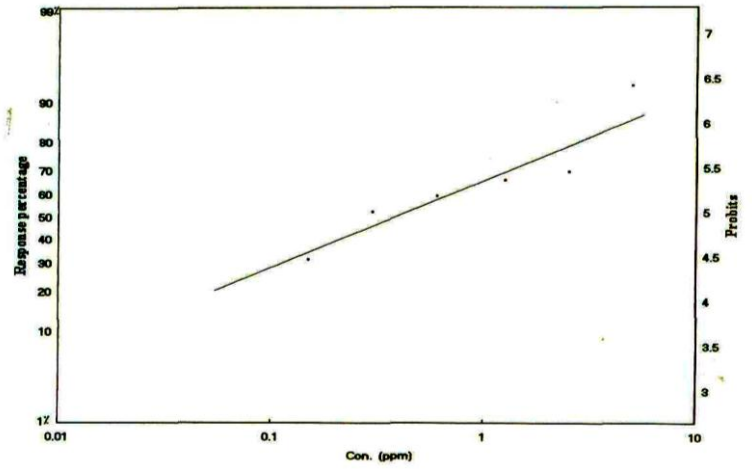


Fig. (1b): Toxicity regression lines post 48-h of feeding 4th instar larvae of *S. littoralis* on castor oil leaves treated with *Dursban.*

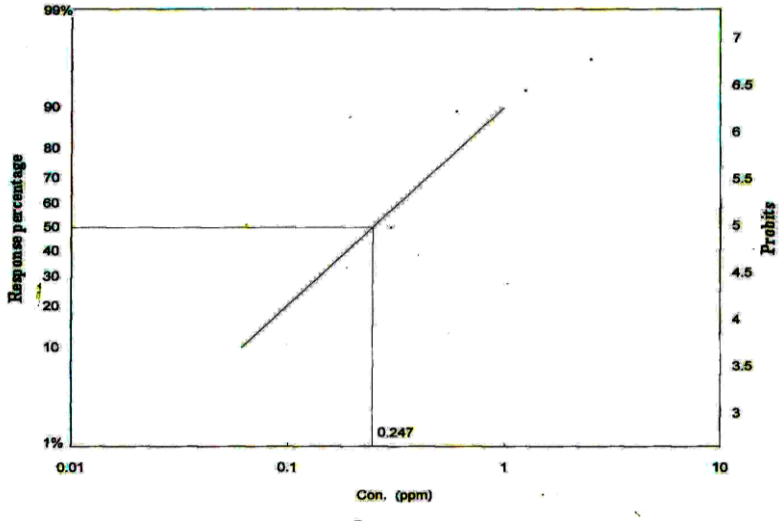


Fig. (1c): Toxicity regression lines post 48-h of feeding 4th instar larvae of *S. littoralis* on castor oil leaves treated with flufenoxuron.

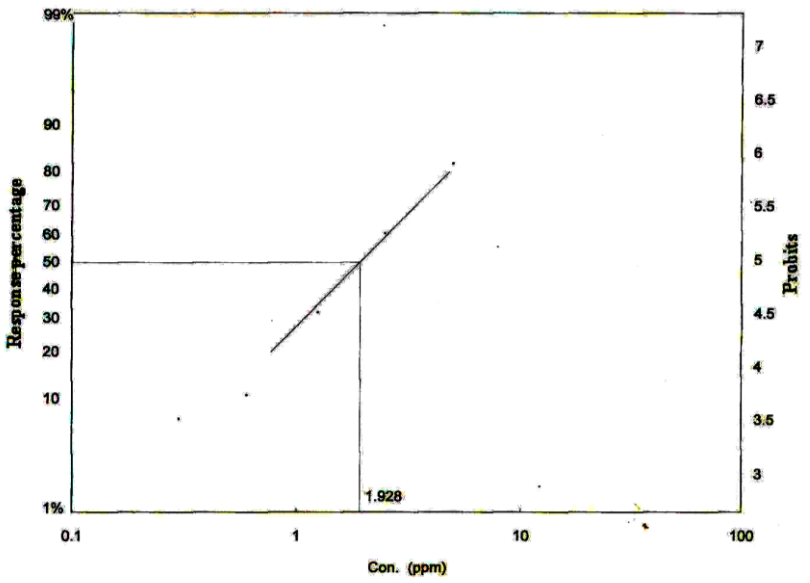


Fig. (1d): Toxicity regression lines post 48-h of feeding 4th instar larvae of *S. littoralis* on castor oil leaves treated with Hexaflumuron.

4.1.2. Latent effect:

The susceptibility of the 4th instar larvae of *S. littoralis* to the toxic action of the bacterial formulation is not the only phenomenon to determine its efficiency. The delayed action, i.e. the latent effect of *B. thuringiensis* which occurred during the developmental processes played an important role for the bacterial efficacy against 4th instar larvae of cotton leaf worn, *Spodoplera littoralis*. The main objective of this part of the study was to clarify and obtain information, through laboratory, about short-term biological and toxicological effects of *B. thuringiensis* on the different developmental processes of the cotton leaf worm, when 4th instar larvae were fed on castor oil

leaves dipped in different concentrations of the biotic insecticide under test.

a- Effect on larval duration

The data obtained (table 4 and fig 2a) shows that the treatment of 4th instar larvae of *Spodoptera Littoralis* with *Bacillus Thuringiensis* (protecto) resulted in a significant prolongation in the larval duration. This prolongation attained (17.6 days) as compared with control (12.3 days).The present results agree with the finding of **Mohamed (2003**), who shows that treatment with the of 4th and 6th instars larvae of *Spodoptera Littoralis* with *B. Thuringiensis* resulted in a significant prolongation in the larval duration. This prolongation attained (24.2, 21.5 days) as compared with control (12.1, 12.8 days) for 4th and 6th instars respectively.

Also the data obtained (table 4) show that the treatment of 4th instar larvae of S. *Littoralis* with Dursban resulted in a prolongation in the larval duration. The present results are similar to the finding of **Fouad (2004)**, who shows that treatment of 4th instar larvae of P. gosspiella with Chlorpyrifos resulted in insignificantly affect larvae duration compared to check treatment.

On the other hand table 4 shows that IGRs (Flufenoxuron and Hexaflumuron) resulted in a significant prolongation in the larval duration. This prolongation attained (14.3 days and 13.9 days) as compared with control (12.3 days) for 4th instar respectively. **Shurab *et al* (1999)** and **El-sheikh (2002)** working on *A .ipsilon* larvae treated with several IGRs. Also the present

investigation is similar to the finding of **Mohamed (2003)** who showed that treatment with Flufenoxuron resulted in a significant prolongation in larval duration of *S. Littoralis* as compared to control.

b- Effect on pupal duration:

The data obtained (table 4 and Fig 2a) shows that *B. thuringiensis* (protecto) a significantly increased the mean pupal duration of *Spodoptera Littoralis* (18.6 days) for 4th instar larvae, as compared to control (15 days) for 4th instar larvae. The present data were in a harmony with those of **Raslan (1998)** who studied the same aspects on *Agrotis ipsilon* treated as 2nd and 4th instar larvae with three formulations of *B. thringiensis* on larval and pupal durations and noted the connection of concentration and magnitude of increase. Also, such findings are in agreement with those reported by **Emara et. al. (1991**) on *Heliothis armigera*, **Abd El-Lateif (2001)** on *Spodoptera littoralis* 1st, 2nd instar larvae and **Mohamed (2003)** on *B. thuringiensis*. These findings resulted in a significant increment in mean pupal duration of *Spodoptera Littoralis* (18.4, 18.2 days) for 4th and 2nd instar larvae, as compared to control (14.2, 15.2 days) for 4th and 2nd instar larvae respectively.

Also, the data cited in table (4) shows that Chlorpyrifos led to a significant decrease in the mean pupal duration of *Spodoptera Littoralis* (8 days) for 4th instar larvae. It also, shows that IGRs Flufenoxuron and Hexaflumuron resulted in a significant decreasing the mean pupal duration (16.6 days and

15.5 days). The present data was in a harmony with those of **Mohamed (2003) and Sokar, (1995).**

c- Effect on pupal weight

The data in (table 4 and Fig 2b) concerning the effect of protecto on the mean weight of pupal ensuing from 4th instar larvae after feeding on castor oil leaves contaminated with LC_{50} of different concentrations of *B. thuringiesis* In general, the mean weight of pupae obtained from treated larvae was significantly less than those of the untreated one, the pupal weight of treated pupae were (280mg) while it was (350mg) for control one. The 4th instars larvae that successes to pupate and could escape death at any larval instar could proceed with their development until successful pupation. Also **Raslan (1998)** on *A. ipsilon* corroborated these findings, while on the other hand **Abdeen et. al. (1988**) elicited that *H. Armigera* larva which fed on high concentrations and successes to pupate may be equal or exceed in their pupal weight, the untreated ones. These results need reappraisal in the light of previous and later reports by **Emara et. al. (1991) and El- Sweirky (1994)** on the effect of *B. Thuringiensis* on the short-term biological activity on weight of pupae and reports by **Abd El-Latif (2001)** on *S. littoralis* 1st, 2nd instar larvae. The data in table (4) shows that chlorpyrifos decreased the pupal weight significantly. It was (270 mg) for treated one compared to 350 mg in case of control. The present data was in a harmony with those of **Fouad (2004)** of *P. gosspiella* with chlorpyrifos resulted in decreasing the pupal weight. Also the data in table (4) shows that IGRs (Flufenoxuron

and Hexaflumuron) resulted in a significant decrease in the mean pupal weight it were (290, 300 mg) for Flufenoxuron and Hexaflumuron respectively.

Insecticides	**Mean larval duration (days) ±S.E**	**Mean pupal duration(days) ±S.E**	**Mean pupal weight (mg) ±S.E**
B.t.	17.6*** ±1.2	18.6** ±1.2	280*** ±0.05
CPF	12 n s ±0.98	8*** ±0.6	270*** ±0.7
Flufenoxuron	14.3** ± 2.1	16.6** ±0.5	***290 ±0.032
Hexaflumuron	13.9* ±1.3	15.5 ns ±1.2	300*** ±0.02
Control	12.3 ±1.2	15 ±1.8	350 ±0.04

Table (4): Effect of LC_{50} of *Bacillus thuringiensis*, Chlorpyrifos, flufenoxuron and hexaflumuron on larval duration, pupal duration and pupal weight of *S. littoralis.*

fig (2a): Effect of B. t., CPF, flufenoxuron and hexaflumuron on mean larval and mean pupal duration.

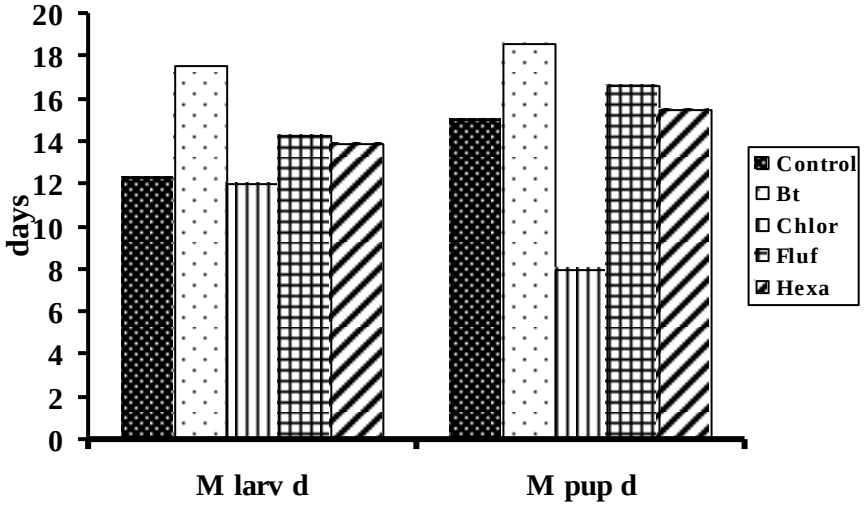
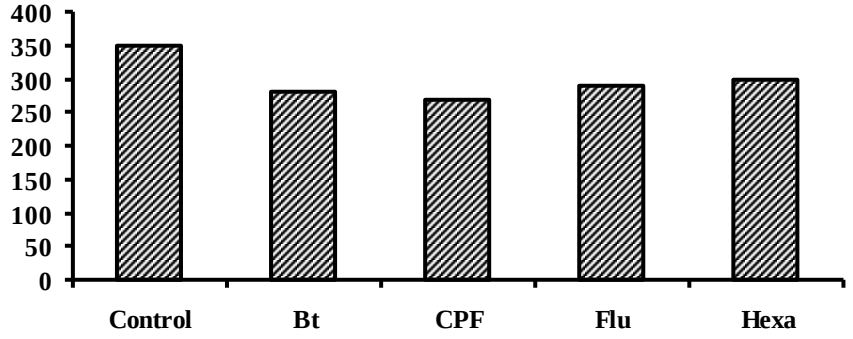


Fig (2b): Effect of B.t., CPF, flufenoxuron and hexaflumuron pupal weight of S. littoralis.



The obtained results are in full agreement with statements of **Clarke et. al. (1990) who** reported that IGRs Flufenoxuron significantly reduced the mean pupal weight of the resulting pupae from treated larvae.

d- The effect on pupation%, adult emergence % and adult longevity:

The results in (table5 and Fig 3a) show that all treatments caused reduction in the pupation % recorded (64, 34.6, 13 and 47.6%) for *B. Thuringiensis*, Chlorpyrifos, Flufenoxuron and Hexaflumuron respectively, compared with 96% for control .These results are in line with the findings of **Mohamed (2003)** who found that a significant reduction pupation % of *S. littoralis* as the result of treatment of 2nd, 4th instars larvae.

The adult emergence% have the same trend recorded (44.6, 84, 10.4 and 45.4%) for *B. Thuringiensis*, Chlorpyrifos, Flufenoxuron and Hexaflumuron respectively, compared with 92.8% for control. The achieved results are in agreement with those reported by **Sokar (1995**). Also all treatments show reduction in adult longevity for both sexes when *S. littoralis* treated as 4^{th} instars, the data was (13, 13, 11.5, 14 and 16 days) for male (12.5, 14, 12, 13 and 15.5 days) for females treatment with *B. Thuringiensis*, Chlorpyrifos, Flufenoxuron, Hexaflumuron and control respectively as shown in table (5 and Fig 3b).

Insecticides	Pupation %	Adult emerg. %	adult longevity(days) ±S.E	
			F	M
B.t.	64	44.6	13 ± 1.7	12.5 ± 0.5
CPF	34.6	84	13 ± 0.6	14 ± 0.3
Flufenoxuron	13	10.4	11.5 ±1.2	12 ± 0.8
Hexaflumuron	47.6	45.4	14 ± 1.1	13 ± 0.9
Control	96	92.8	16 ± 0.8	15.5 ±1.2

Table (٥): Effect of B.t., CPF, Flufenoxuron and Hexaflumuron on pupation %, adult emergence and adult longevity of *S. littoralis.*

fig(3a): Effect of B. t., CPF, Flufenoxuron and Hexaflumuron on pupation % and adult emergence% of *S. littoralis* .

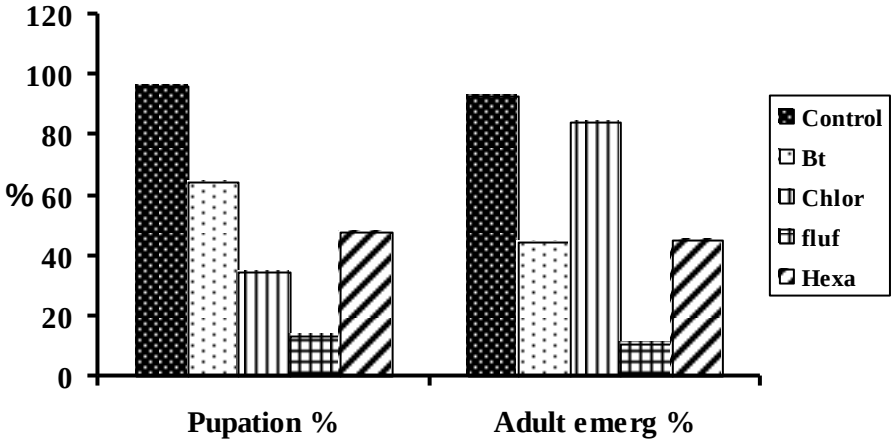
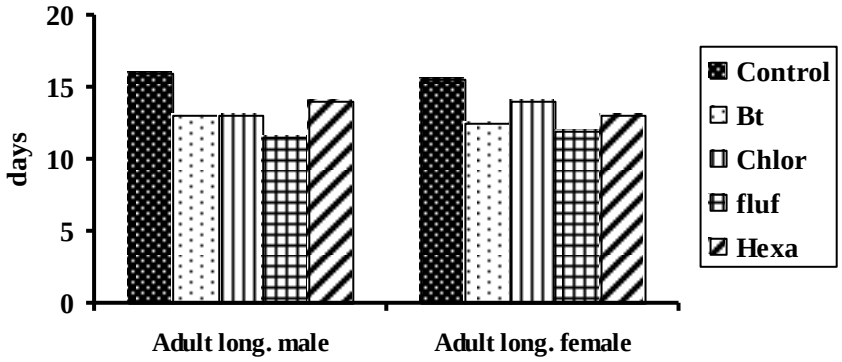


Fig (3b): Effect of B.t., CPF, Flufenoxuron and Hexaflumuron on adult longevity of S. littoralis.



e- Effect of IGRs on malformation of *S. littoralis*:

There is several larval malformation recorded when the 4th larval instar treated with Hexaflumuron and Flufenoxuron as shown in fig (4) the larvae showed shrinkage of the all body compared to control, fig (4). As the result of treatment the 4th instars of *S. littoralis* with Hexaflumuron and Flufenoxuron. The larvae failed to complete the pupation so that there is larval pupal intermediate shape as shown in fig (5). Also the abnormalities of adult appear as shorten of the wings and adult failed to get rid of its puparium as shown in fig (6). The obtained results are in agreement with those reported by (**Sokar (1995), Shurab et. al. (1999) and El- Sweirky (2002).**

Fig. (4): Larval malformation as the result of treatment the 4th instars of *S. littoralis* with Hexaflumuron and Flufenoxuron (B) compared to control (A).

Fig. (5): Pupal malformation as the result of treatment the 4th instars of *S. littoralis* with Hexaflumuron and Flufenoxuron.

Fig. (6): Adult malformation as the result of treatment the 4th instars of *S. littoralis* with Hexaflumuron and Flufenoxuron.

4.1.3 Biochemical effects of insecticides on *S. littoralis* Larvae.

a- Transaminases activity:

Data in table (6 and fig 7a) showed the effect of *Bacillus thuringiensis*, Chlorpyrifos, Flufenoxuron and Hexaflumuron on the activity of body tissue AST and ALT of late 6th instars of *S. littoralis* treated as 4th instars.

The results indicated that the activities of AST and ALT have no significance due to treatment with protecto, Chlorpyrifos, Flufenoxuron and Hexaflumuron as compared to control. The decrease in AST and ALT activities due to treatment with flufenoxuron is similar to the data obtained by **El-Sheikh (2002)** who found that treatment of *Agrotis ipsilon* larvae with flufenoxuron decreased amino transferase activity. Furthermore, the 6th instar larvae of *S. littoralis* treated with chlorfluazuron and flufenoxuron showed a decrease in amino transferase activity (**Abd El-Aal, 2003**). The decrease in AST and ALT in the present study may be attributed to the binding of the tested compounds with protein that leads to inhibition in amino transferases activity which is known to be intimately related to protein synthesis.

Treated	AST	ALT	Alk. Ph.	Acid ph.
	Mean specific activity# (IU/ml) ±S. E.			
B. t.	32.5 ^{ns} ±0.8	11.04 ^{ns} ±0.61	310.4* ±5.65	50.6*** ±2.1
Chlorpyrifos	15.3*** ±0.96	5.5*** ±0.6	352.8 *** ±6.12	32.4* ±1.2
Flufenoxuron	14.2*** ±0.5	7.3** ±0.8	292.3 * ±6.42	27.3** ±1.8
Hexaflumuron	12.4*** ±0.6	7.1** ±0.09	304.2 * ±3.35	61.93*** ±2.1
Control	34.5 ±0.3	11.9 ±0.98	288.4 ±6.24	38.5 ±1.2

Table (6): Effect of B. t., CPF., Flufenoxuron and Hexaflumuron on AST, ALT, alkaline phosphatase and acid phosphatase of S. littoralis.

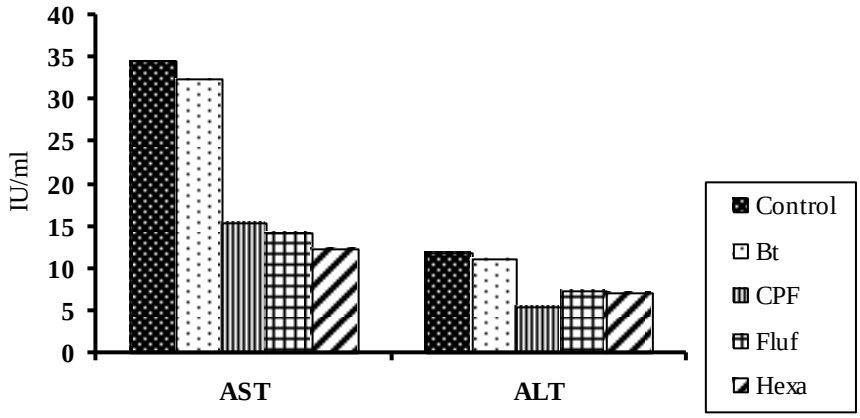


Fig (7a): Effect of B.t., CPF., Flufen. and Hexa. on AST and ALT of S. littoralis.

Fig (7b): Effect of B.t., CPF., Flufenoxuron and Hexaflumuron on Acid Ph. on S. littoralis.

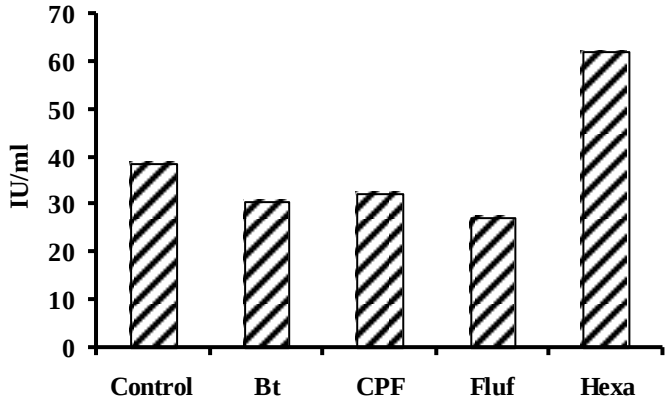
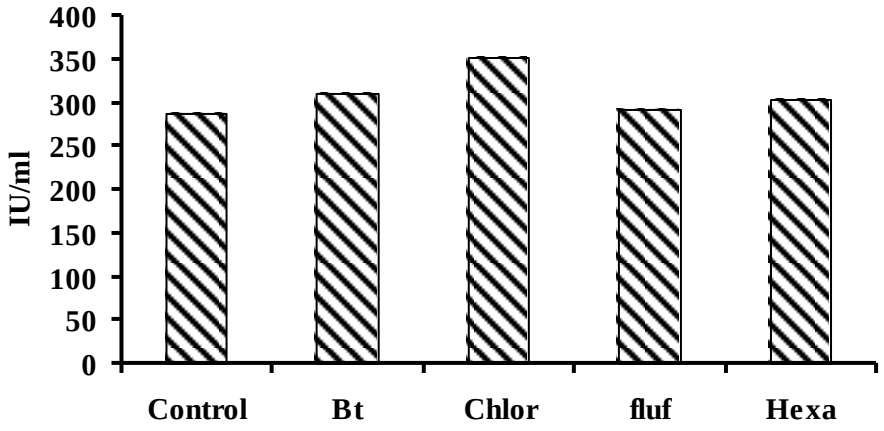


Fig (7c): Effects of B.t., CPF., Flufenoxuron and Hexaflumuron on alkaline ph. of S. littoralis.



b- Phosphatases activities

Data in t(able 6 and fig 7b, c) showed that the effect of *B. thuringiensis*, Chlorpyrifos, Flufenoxuron and Hexaflumuron on the activity of body tissue acid phosphatase of late 6th instars of *S. littoralis* treated as 4th instars. The results indicated that the activities of acid phosphatase were significantly increased due to treatment with protecto and hexaflumuron, as compared to control, but in case of treatment with flufenoxuron and Chlorpyrifos there was non significant decrease. On the other hand all treatment recorded a significant increase in alkaline phosphatases compared to control as shown in table (6) and fig (7 b, c). The same pattern was also obtained by **Abd El- Aziz (2000); also Bassel and Ismail (1985)** studied the activity of alkaline phosphatase in *S. littoralis* during the developmental stages. They found that alkaline phosphatase activity generally occurred in a high level in all stages compared with acid phosphatase. On the other hand, the two enzymes revealed a higher level of activity in the egg stage than in any other stage. In the larval stage, from hatching until the prepupal stage, a steady decrease was recorded in the activity of both enzymes.

Abd El Aal (2003) reported that the activity of haemolymph acid phosphatase of late 6th instars of *S. littoralis* was significantly increased by about 2.5, 2 and 1.5 times more than control in case of treated 4th instars with Chlorfluazuron, Flufenoxuron and Pyriproxyfen, respectively. Acid and alkaline phosphatases have been shown to be associated with insect development, especially in relation to nutrition and egg

maturation **Tsumuki and Kanehisa,** (**1984).** Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis. This latter process is appreciable at the metamorphic moults of holometabolous insects. It is known that acid phosphatase hydrolyzes a variety of ortho-phosphorylation reactions. Ecdysone is responsible for increase in the number of lysosomes and of the activity of acid phosphatase.

c- Total lipid

Data in table (7) and Fig (8a) showed that the effect of *B. thuringiensis*, Chlorpyrifos and Hexaflumuron on the body tissue total lipid of late 6^{th} instars of *S. littoralis* treated as 4^{th} instars. The results indicated that the total lipid was significantly decreased due to treatment with protecto, while, Flufenoxuron shows no significance. The results obtained coincident with that reported by **Abd El Aziz (2000*)*** on the same insect.

The results indicated that the total lipids were significantly increased due to treatment with Chlorpyrifos, Flufenoxuron and Hexaflumuron as compared to control. The same pattern was also obtained from **Abd El- Aal (2003)** who reported that the flufenoxuron and pyriproxyfen slightly increased the total content of haemolymph lipids of *S. littoralis* late 6^{th} instars. **Sokar (1995)** reported also an increment in the total content of haemolymph lipids of *S. littoralis* had been occurred when treated with Chlorpyrifos.

d- Total protein

Data in table (7) and fig (8a) showed that the effect of *Bacillus thuringiensis*, Chlorpyrifos, Flufenoxuron and Hexaflumuron on the activity of body tissue total protein of late 6^{th} instars of *S. littoralis* treated as 4^{th} instars.

The results indicated that the total protein was significantly decreased due to the treatment with protecto, chlorpyrifos, flufenoxuron and hexaflumuron as compared to control. The same pattern was also obtained from **Abd El Aziz (2000)** who reported that the protecto decreased the total content of proteins of *S. littoralis* late 6^{th} instars also **Abd El Aal (2003)** reported that the flufenoxuron and pyriproxyfen slightly decreased the total content of proteins of *S. littoralis* late 6^{th} instars.

e- Cholinesterase activity

Data in table (7) and fig (8b) showed that the effect of *B. thuringiensis*, Chlorpyrifos, Flufenoxuron and Hexaflumuron on the activity of body tissue cholinesterase of late 6^{th} instars of *S. littoralis* treated as 4^{th} instars.

The results indicated that the cholinesterase was significantly decreased due to treatment with protecto, Chlorpyrifos, Flufenoxuron and Hexaflumuron as compared to control. **Afifi (2001)** recorded insignificant decrease in the activity of Acetyl-cholinesterase of larval homogenate of *pectinophora gossypiella* when treated with LC_{50} of three commercial products of *B. thuringiensis*. **El-Nemaky (2000)** found that the activity of AchE in whole homogenates of the pink and spiny bollworms full-grown larvae treated with M-pede and MVPII was increased. On the other hand, **Abd El-Aziz (2000)** investigated the physiology of the cotton leafworm, *S. littoralis* larvae infected

with different concentrations of *B. thuringiensis* and recorded an inhibtion effect on AchE of *S. littoralis* haemolymph. . High haemolymph titers of juvenile hormone maintain the larval stage, while, low juvenile hormone titers initiate pupal and/or adult development **Sokar(1995).** Moreover, **Abd El-Fattah *et al.* (1986)** found that treatment of *S. littoralis* larvae with LC_{15}, LC_{30} and LC_{50} of diflubenzuron obviously increased non-specific esterases activity at initial time, and then markedly decreased to the lower level when compared to control. The reduction in the tested esterases by IGRs treatment indicated that these enzymes might play an important role in their resistance in *S. littoralis*.

f- Protease activity

Data in table (7) and fig (8b) showed the effect of *B. thuringiensis*, Chlorpyrifos, Flufenoxuron and Hexaflumuron on the activity of body tissue protease of late 6^{th} instars of *S. littoralis* treated as 4^{th} instars.

The results indicated that the protease activity was significantly decreased due to treatment with Chlorpyrifos, Flufenoxuron and Hexaflumuron as compared to control, except in case of treatment with protecto there was non significant increase. The obtained results agree with **Farag (2001)** who reported that protease activities were markedly decreased after treating with three IGRs

Treated	Total lipid mg/ml	Total protein gm/ml	Cholinesterase IU/ml	Protease IU/ml
	Mean total content ±S. E.		Mean specific activity±S. E.	
B. t.	21.9 ±0.97	16.59 * ±1.2	1490.5 ns ±18.6	310 ns ±10.1
CPF	54.2 *** ±1.8	12.58 ** ±0.98	850.2 *** ±6.8	72 *** ±1.3
Flufenoxuron	29.9 ns ±2.3	15.92 * ±0.8	1175 *** ±21.2	235 *** ±2.8
Hexaflumuron	56.7 *** ±1.4	16.29 * ±1.4	1001.3 *** ±15.8	249** ±1.4
Control	28.77 ±1.6	18.61 ±1.2	1509.5 ±25.3	306 ±2.5

Table (7): Effect of B. t., CPF, Flufenoxuron and Hexaflumuron on total lipid, total protein, ChE and Protease activities on *S. littoralis.*

Fig (8a): Effect of B. t., CPF, Flufenoxuron and Hexaflumuron on total lipid, total protein, of S. littoralis.

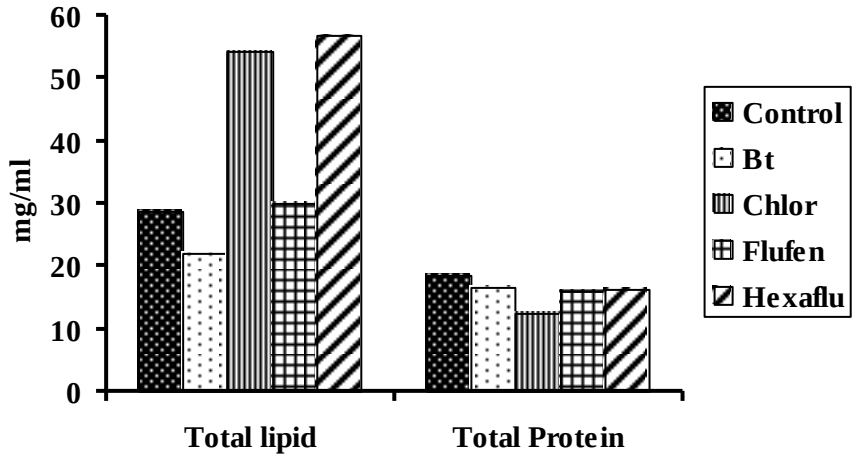
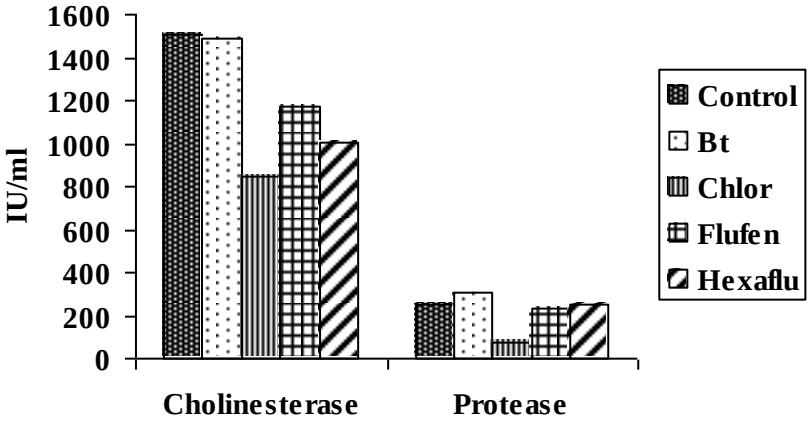


Fig (8b): Effect of B.t., CPF, Flufenoxuron and Hexaflumuron on cholinesterase and Protease activities on S. littoralis



4.2. Toxicological effect of insecticides on rats:

4.2.1. Toxicological and biochemical effects of *Bacillus thuringiensis* on rats:

Administration of *B. thuringiensis (Kurstaki)* for 12 weeks to rats at dosages of 10000 mg/ kg/ day after day did not produce toxic effects. No effects of *B. thuringiensis (Kurstaki)* on body weight showed an insignificant changes in body weight, liver, kidney weight and testicular weights of rats as compared to the level of the control group which are recorded in table (8), and fig (9a and 9b). Insignificance were observed in the values of serum liver enzymes (fig 10 a, b and c), triglycerides, cholesterol (fig

11 and b), total protein (fig 12 a,), kidney function tests (fig 12 b, c and d), serum cholinesterase activity (fig 13), hematological parameters as shown in (table 9). Such results are in agreement with that reported by **PIP (2006),** who reported that the LD_{50} is greater than 5000 mg/kg for the *B. thuringiensis (Kurstaki)* product Javelin in rats and greater than 13,000 mg/kg in rats exposed to the product Dipel 2x. Single oral dosages of up to 10,000 mg/kg did not produce toxicity in mice, rats, or dogs. The dermal LD_{50} for a formulated *B. thuringiensis (Kurstaki)* product in rabbits is 6280 mg/kg. A single dermal application of 7200 mg/kg of *B. thuringiensis* was not toxic to rabbits. **Ray, (1991),** reported that dietary administration of *B. thuringiensis* for 13 weeks to rats at dosages of 8400 mg/kg /day, did not produce toxic effects. Some reversible abnormal redness of the skin was observed when 1 mg/kg/day of formulated *B. thuringiensis* product were put on scratched skin for 21 days. No general, systemic poisoning was observed. There are no results that *B. thuringiensis* causes male fertility and reproductive system parameter changes (fig 14 a, b). **Abbott Laboratories (1982)** reported that no reproductive changes were observed at 9300 mg/kg/day for 15 weeks.

Also the above results are in agreement with the results of, **Tsai, et al. (1995)** who reported that no spore germination was observed in the tissues after administration of rats by *B. thuringiensis kurstaki*. The results confirmed the safety of *B. thuringiensis* (*kurstaki)* to rats. Also the results of **Roe, (1991)** and **Salamitou et. al. (2000),** seem on line with the present results of our study.

4.2.2. Effect of chlorpyrifos and flufenoxuron rats:

Rats treated with 1/10 of LD_{50} chlorpyrifos and flufenoxuron developed clinical symptoms, which were progressing by time marked distension of the abdomen was the only clinical symptoms observed in rats after the first two weeks of treatment. In the third week, rats lost their vitality and depression. Some rats developed nervous manifestation and were moved in circles. During the remaining weeks of the experiments, general weakness and cachexia were observed. The animals were reluctant to move and showed nervous manifestation and hurried respiration. The symptoms of toxicity of chlorpyrifos in rats were similar to that which reported by **Lemus, et al., (2000)**. Also the results of **US EPA (1998)** were in agreement with the obtained observations. They study the on chronic rat feeding of flufenoxuron which identified the following effects: seizures, including seizures resulting in death.

The effects of Flufenoxuron and Chlorpyrifos on body weight are recorded in table (8) and (fig 9 a, b), since it was observed a significant decrease in body weight of rats, increased liver but the kidney weight decreased, in addition to that there were slightly decrease in testicular weight as compared to control group. These results are in agreement with the results of **US EPA (1998)**. Also, **Blumbach et. et. (2000)** reported that organophosphorus in diet of rats depressed the body and kidney weights, increased liver weight in addition to that there were slightly decrease in testicular weight. They owned the kidney weights decrease in male rats dosed with organophosphorus

compounds dose-dependent by increase in the globulin content were observed in male. The increase of globulin accumulation was accompanied by the formation of protein droplets in the proximal tubules of male rats. Also the obtained results are in agreement with that reported by **El-Sherbiny et. al. (1995)**

Marty et al. (2004) and **Johnson (2005)** also studied the effect of IGRs on rats at different periods of feedings and different concentrations they reported that an absolute and relative increase liver weight adult rats at 25 mg/kg. Relative decrease in kidney weights, and body weights.

Weight in gm.		Control	Treated rats		
			Bt	Fluf	Chlor
Body weight	Mean	255	249	226.4**	198.3**
	SD	11.78	11.8	3.22	2.67
Liver weight	Mean	9.9	9.7	11.47	13.58
	SD	0.47	0.45	0.52	0.58
Kidney weight	Mean	2.95	2.75	2.16	2.24*
	SD	0.163	0.163	0.16	0.12
Testis weight	Mean	2.67	2.65	2.08	2.17
	SD	0.025	0.03	0.08	0.09

Table (8): Effect of B. t., CPF. and Flufenoxuron administration on body weight and some organs weight of rats in grams.

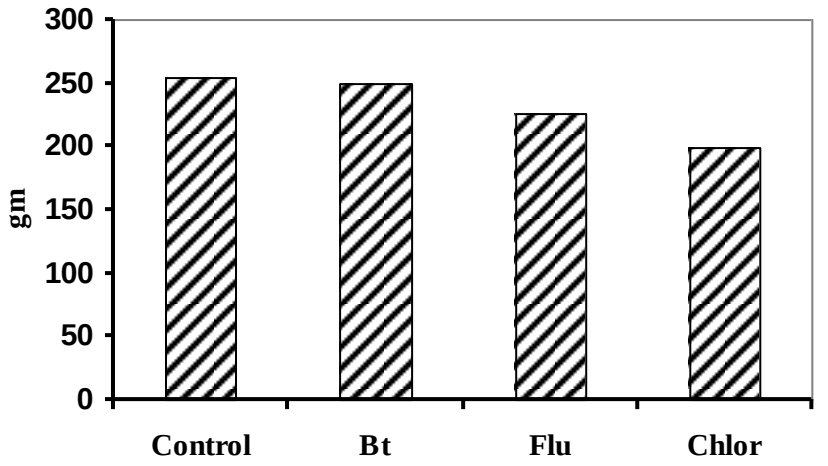


Fig (9a): Effect of B. t., CPF and Flufenoxuron administration on body weight.

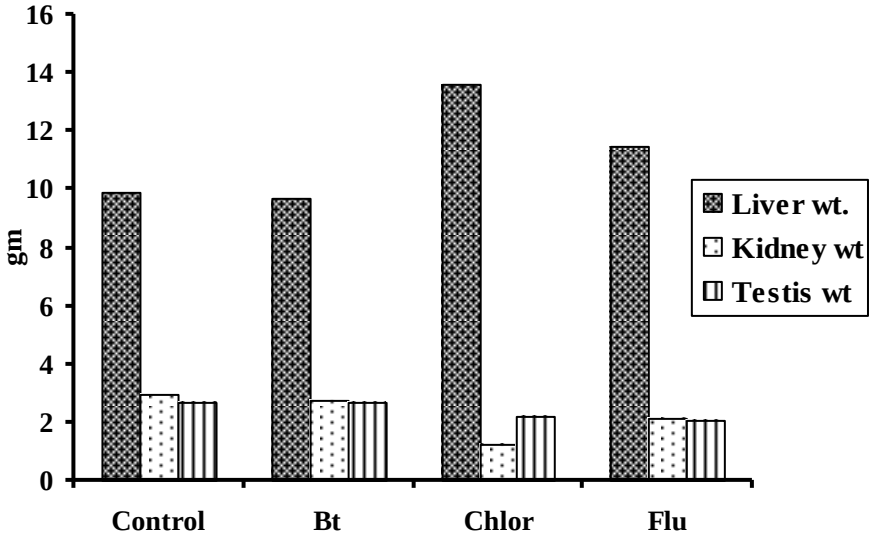


Fig (9b): Effect of B. t., CPF and Flufenoxuron administration on some organs weight of rats in grams.

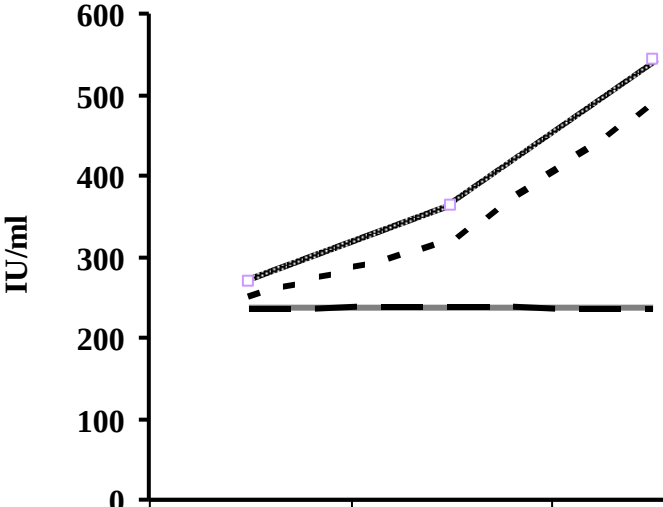
4.2.3. Biochemical effects of chlorpyrifos and flufenoxuron on rat:

4.2.3.1. Effect of chlorpyrifos and flufenoxuron on serum liver enzymes of rats:

The mean values of plasma transaminase activities of AST and ALT. (fig 10 a, b) showed slightly increase in the treatment of chlorpyrifos and flufenoxuron in comparing to control group in the 4 weeks and 8 weeks but there were highly significant increase in the 12 weeks. The data in (fig 10 c) showed significant increase in ALP at 12 weeks in comprising to control group. The obtained results are in agreement with the results obtained by **Jabbar et. al. (1994) and Gomes et. al. (1999)**. These findings were also in agreement with **El Kashory, (1999)**, **El Dosoky, (2000)** and **Armando et al., (2004),** they reported that chlorpyrifos and related insecticides is a major concern that the developmental toxicity of chlorpyrifos extends beyond the nervous system, to include cell signaling cascades that are vital to hepatic homeostasis. Also, **Firdaus et. al. (2003) Mohammed and Siddiqui (2003) and Johnson (2005)** the studied effect of sublethal dosage (120 mg/kg body weight/day) of the organophosphate pesticide, Chlorpyrifos, on the blood of adult male albino Sprague- Dawley rat under short-term conditions. They reported that the activities of ALP, AST and ALT were significantly increased. **US EPA (1998),** and **Sviatnyi, (1992)** were on line with the achieved results, They reported that the activities of ALP, AST and alanine transferase ALT are

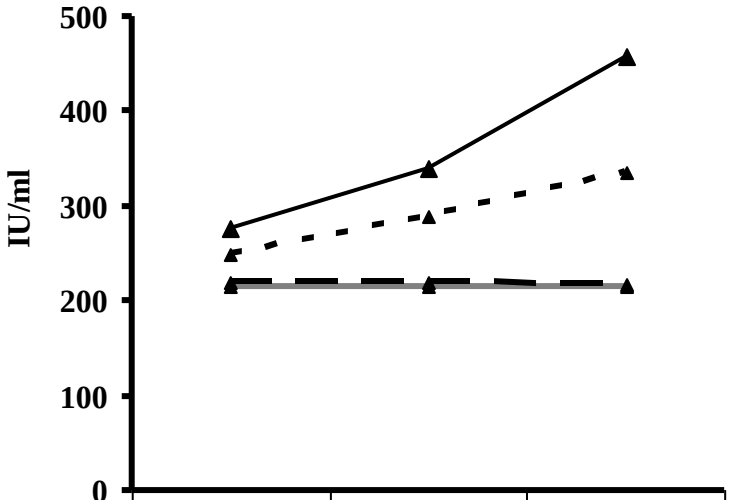
significantly increased**,** if flufenoxuron is introduce to rats at 1/10 of LD_{50} for 28 days.

Fig (10a): Effects of B. thuringeinsis, Flufenoxuron and Chlorpyrifos of the ALT activity of rats



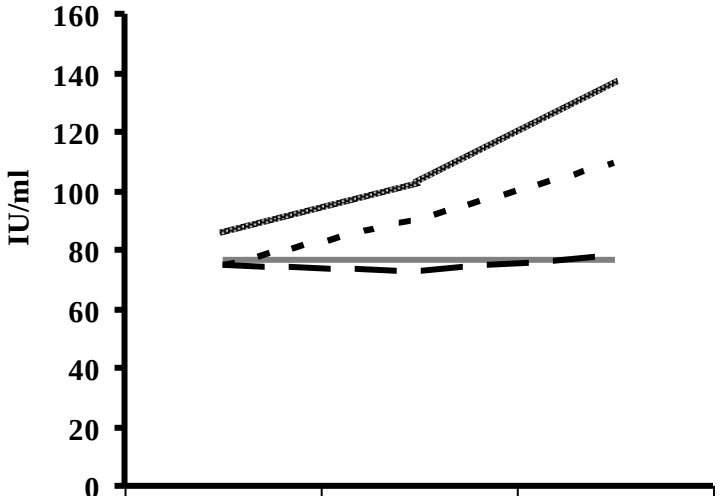
	4th. Week	8th. Week	12th. Week
Zero time	236.5	236.5	236.5
Bt	234.55	237.34	234.55
CFP	268.5	362	542.4
Flufen	250	318.5	489.3

Fig (10 b): Effects of B. t., Chlorpyrifos and Flufenoxuron of the AST activity of rats



	4th. week	8th. week	12th. week
Zero time	216	216	216
Bt	220.3	220.3	218.3
CPF	276	340.5	458.3
Flufen	248	289	334.5

Fig (10c): Effects of B. t., CPF and Flufenoxuron of the ALP activity of rats

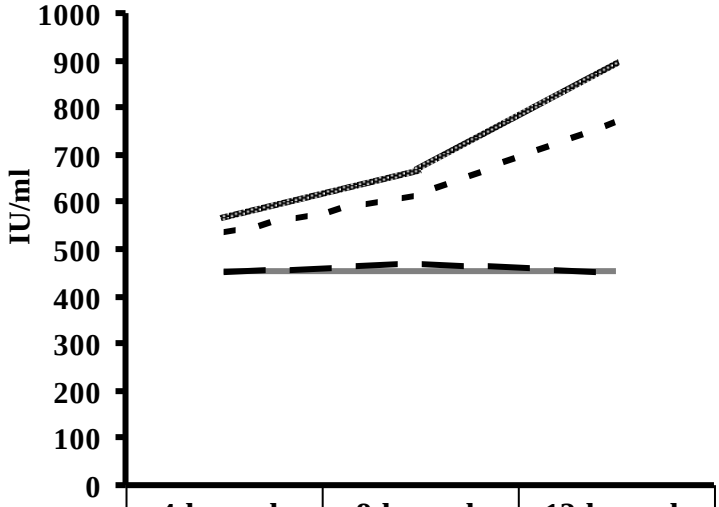


	4th. week	8th. week	12th. week
Zero time	76.8	76.8	76.8
Bt	74.5	72.6	77.8
CPF	85.4	102.3	137
Flufen	74.5	89.9	109

4.2.3.2. Triglycerides and cholesterol:

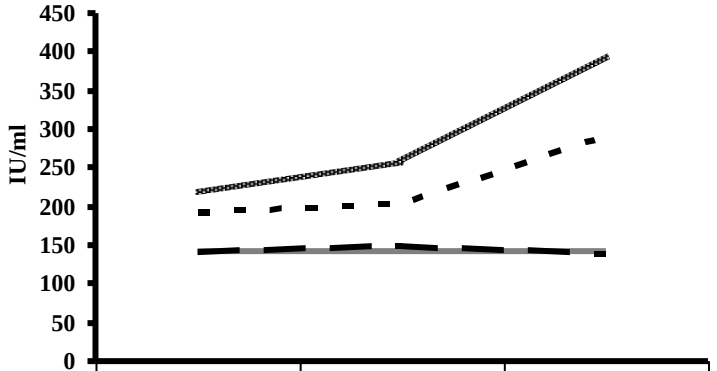
Values of total lipids showed significant increase throughout the experiment. Significant hypercholesterolemia was noticed, however, from the beginning to the end of the experiment since a sharp increase was observed during the 8^{th} and 12^{th} weeks of treatment as compared to the control levels (fig 11 a, b). The results of the present study are in agreement with the results of **El Kashory, (1999) and El Desoki, (2000)** who suggested that hyperlipidemia and hypercholesterolemia is expected to occur due to liver impairment of organophosphorus treated animals. **Also Firdaus et al. (2003) Mohammed and Siddiqui (2003)** studied the effect of sublethal dosage (120 mg/kg body weight/day) of the organophosphate pesticide, Chlorpyrifos, on the blood of adult male albino Sprague-Dawley rat under short-term conditions. Serum cholesterol showed increase level. **US EPA (1998), Tasheva and Hristeva, (1993), and Johnson (2005)** studies on the effects of benzoylphenylurea insecticides on lipid profile which was significantly increased in the Wister male rats who administered benzoylphenylurea insecticides 28 days at oral doses of 100 mg kg-1 each.

Fig (11a): Effects of B. t., CPF and Flufenoxuron of the triglycrides of rats



	4th. week	8th. week	12th. week
Zero time	454.6	454.6	454.6
Bt	447.1	467.4	449.8
CPF	562.5	663.7	894.5
Flufen	533.1	612.8	765.4

Fig (11b): Effects of B. t., CPF and Flufenoxuron of the cholesterol of rats



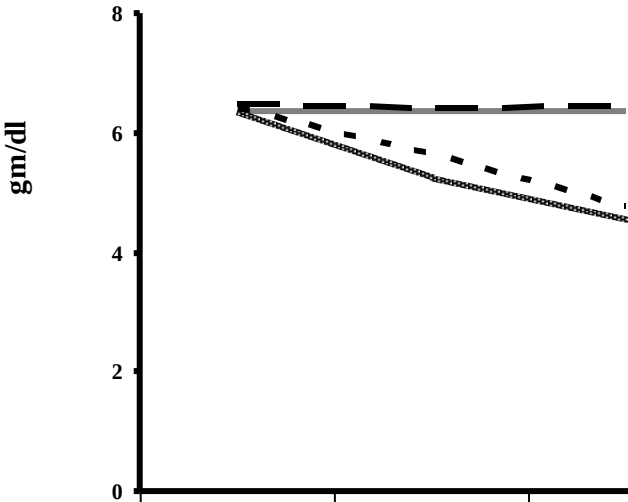
	4th. week	8th. week	12th. week
Zero time	143.5	143.5	143.5
Bt	139.8	147.1	137.8
CPF	217.5	257.1	394.3
Flufen	191.6	201.8	288.5

4.2.3.3. Total protein

Regarding the effect of Chlorpyrifos and Flufenoxuron on total protein, the results summarized in (fig 12 a) showed slight reduction in total protein after oral administration of Chlorpyrifos and Flufenoxuron after 4^{th}, 8^{th} and more decrease in the 12^{th} weeks in comprising to control group. Such results agreed with that reported by **El- Kashory (1999) and El-Dosoky (2000)** they noticed that serum total protein was significantly decreased in organophosphorus treated animals compared to control group. Also **Firdaus et al. (2003) and**

Mohammed and Siddiqui (2003) reported that effect of sublethal dosage (120 mg/kg body weight/day) of the organophosphate pesticide, chlorpyrifos, was studied on the blood of adult male albino Sprague-Dawley rat under short-term conditions, serum protein declined with a delay. The results of **US EPA (1998)** were in agreement with the acquired observations. They studied that on chronic rat feeding of Flufenoxuron which revealed slight decrease in the total protein level.

Fig (12a): Effects of B. t., CPF and Flufenoxuron of the total protein of rats



	4th. week	8th. week	12th. week
Zero time	6.37	6.37	6.37
Bt	6.45	6.38	6.44
CPF	6.31	5.21	4.53
Flufen	6.41	5.64	4.76

4.2.3.4. Effect on kidney function:

Serum urea and creatinine were determined as indicators to kidney functions, since the increase in these components means that the kidney is less active or abnormal case. Mean values of serum urea and creatinine were elevated throughout the experiment. The significant uremia was noticed during the 8th week and 12th week of treatment as compared to the normal level

of control group, (fig 12 b, c). The elevation of blood urea and creatinine in treated rats may be attributed to the toxic effect of chlorpyrifos and flufenoxuron which led to disorders of the kidney which reduced the glomerular filtration rate and consequently retention of urea in the blood The accomplished results closely resembled to those obtained by **El Kashory (1999) and El Dosoky (2000)** who found a significant increase of serum kidney function tests of organophosphorus treated animals.

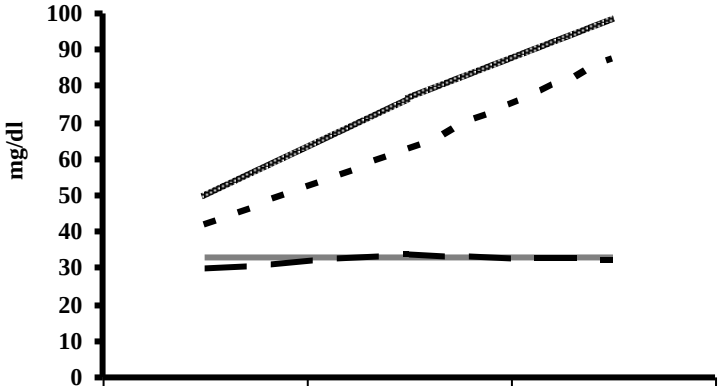
The acquired results are in harmony agreement with those of **Karim (1998)** who reported that administered flufenoxuron intragastrically by stomach intubation to pregnant rats at concentration levels 0 & 20 mg/kg b. wt. in saline solution every other day on gestation day 7 till parturition. A significant increase in blood creatinine levels and blood urea were observed in the pregnant rats.

Also**, Johnson (2005)** assessed subchronic toxicity after 90 days of treatment by flufenoxuron on adult albino rats at 0 and 1.0 mg/kg/day doses. A Significant increased in blood creatinine levels blood urea and serum uric acid were observed, these results were on line with the obtained results.

US EPA (2005) owned the elevated blood urea level may reflect an accelerated rate of protein catabolism rather than decreased urinary excretion of urea, while severe hepatic insufficiency causes decreased blood urea level apparently because of impaired urea synthesis. Since creatinine production is endogenous, being dependent on muscle mass its level in the

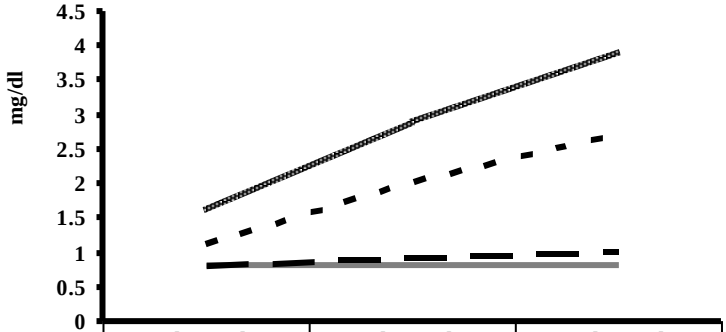
blood is usually in dependent of diet unlike urea.

Fig (12b): Effects of B. t., CPF and Flufenoxuron of the urea of rats



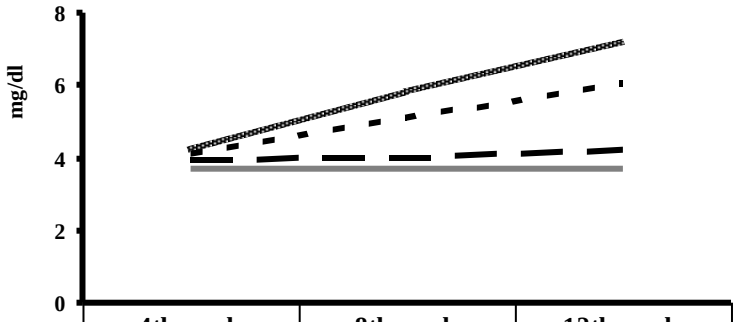
	4th. week	8th. week	12th. week
Zero time	32.9	32.9	32.9
Bt	29.8	33.4	31.7
CPF	49.7	76.6	98.3
Flufen	41.8	62.8	87.6

Fig (12c): Effects of B. t., CPF and Flufenoxuron on the serum creatinine of rats



	4th. week	8th. week	12th. week
Zero time	0.8	0.8	0.8
Bt	0.77	0.89	1
CPF	1.6	2.87	3.9
Flufen	1.1	2	2.7

Fig (12d): Effects of B. t., CPF and Flufenoxuron of the s. uric acid of rats.



	4th. week	8th. week	12th. week
Zero time	3.7	3.7	3.7
Bt	3.9	4	4.2
CPF	4.2	5.8	7.2
Flufen	4.1	5.1	6

4.2.3.5. Serum cholinesterase activity:

Serum cholinesterase (ChE) activity is composed of two distinct cholinesterase (Acetyl cholinesterase and butyryl cholinesterase). Acetyl cholinesterase is the true cholinesterase while the butryl cholinesterase is pseudo cholinesterase and present in the plasma. Both acetyl cholinesterase and butryl cholinesterase have similar inhibitors and activators. Therefore, inhibition butryl cholinesterase reflects inhibition of acetyl cholinesterase. The major substrate is acetylcholine, the neurotransmitter found at the myoneural junction**.**

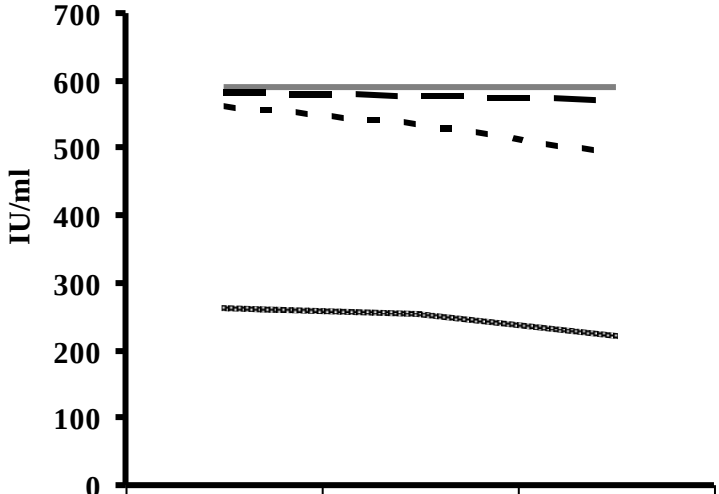
Data presented in (fig 13) indicated that Flufenoxuron administrated caused slight decrease in ChE activity at all time of treatment, but there were significant decrease in this enzyme activity in the treatment of chlorpyrifos in the 4^{th} week and became highly decreased in the wrest of time of treatment in comparison to control. These findings are in agreement with the results of **Vodela and Dalvi, (1995),** who studied the effect of Chlorpyrifos in rats. Rats were given single oral doses of 50 mg chlorpyrifos per kg. Oral administration resulted in a significantly higher inhibition of serum cholinesterase (82%).

El Kashory (1999) and El Dosoky (2000) reported that administration of sublethal doses of Profenofos resulted in significant inhibition of serum cholinesterase (ChE) activity, which the same of trend the present study results. And **Ashry et al (2002) and Verma et al. (2002)** who reported that Chlorpyrifos, exposure in rats caused significant inhibition of serum cholinesterase (ChE) activity. Following a single dose of

20 mg/kg chlorpyrifos in rats, the animals started exhibiting typical signs of organophosphate toxicity and the serum cholinesterase activity dropped by 52%. A further decrease of 62 and 72% was observed when the same rate of chlorpyrifos was given for two and three days.

The achieved results seem to be in agreement with **Goel et al (2000)** who explained that the toxic effects of chlorpyrifos on the liver of male rats and the protective potential of zinc in mediating its toxic effects were investigated. Chlorpyrifos (13.5 mg/kg body weight) treatment resulted in significant inhibition of serum and hepatic acetyl cholinesterase (AChE) activities after 8 weeks. However, zinc treatment (227 mg/ liter drinking water) resulted in significant normalization of the inhibited AChE activities. Similarly, a significant increase in the levels of various serum and liver marker enzymes (alkaline phosphatase, aspartate amino transferase (AST), and alanine amino transferase (ALT)) was observed following treatment with chlorpyrifos. However, coadministration of zinc to these animals restored these enzymes to within normal limits, even though some increase in the activity of serum ALT and hepatic alkaline phosphatase still persisted at the end of the study. Chlorpyrifos treatment diminished serum and hepatic zinc levels significantly compared to normal control animals.

Fig (13): Effects of B. t., CPF and Flufenoxuron on cholinesterase activity of rats



	4th. week	8th. week	12th. week
Zero time	588.9	588.9	588.9
Bt	581.6	576.4	569.8
CPF	261.2	251	218.1
Flufen	561.2	533.9	488.5

4.2.4. Effects of Chlorpyrifos and Flufenoxuron on Hematological parameters of rats:

Data presented in table (9) indicated that prolonged administration of chlorpyrifos in 4 weeks, 8 weeks, and 12 weeks caused a significant decrease in leukocyte count, erythrocyte count, hemoglobin concentration hematocrite value, platelet count and blood indices in treated rats. They showed significant decrease in treated rats in comparison to the control group. The results of the present study are in agreement with, **Firdaus et al. (2003) ;** and **Mohammed and Siddiqui (2003)** who reported that effect of sublethal dosage (120 mg/kg body weight/day) of the organophosphate pesticide, chlorpyrifos, was studied on the blood of adult male albino Sprague-Dawley rat under short-term conditions. Chlorpyrifos had no effect on hemoglobin (Hb). Red blood and white blood cell counts (RBCs and WBCs). Packed cell volume (PCV) showed a significant changes but transient decline. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) also increased transiently. Mean corpuscular hemoglobin concentrations (MCHC) showed persistent increase. **US EPA (1998) and Sviatnyi, (1992)** were in agreement with the obtained observations. They reported that the main manifestation of its toxic effect on the rats is its influence on the erythropoiesis. IGRs had a significant decrease of hemoglobin (Hb). Red blood (RBC) and packed cell volume (PCV) showed also a significant decline. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) also increased transiently, and

then decreased. Mean corpuscular hemoglobin concentrations (MCHC) showed significant decrease. Also **Tasheva and Hristeva, (1993),** studied on the effects of benzoylphenylurea insecticides on hematological parameters in rats. The benzoylphenylurea insecticides were administered to male Wister rats for 28 days at oral doses of 100 mg kg-1 each. Studies submitted were 28 day rat, 90 days rat/mouse/dog. In both the rat and dog, there was evidence of regenerative anemia with (rat 28- and 90- day). In rats; decreased erythrocyte parameters, was seen in most studies. These results were in agreement with the obtained results. Also **El-Sherbiny et. al. (1995)** results were in agreement with the achieved results. He studied in, 14-week feeding study technical grade diflubenzuron was administered in the diet to mice at dose levels of 0 (control), 12, 60, 300, 1500 or 7500 mg/kg/day). The study noted decreased erythrocyte counts, decreased packed cell volume.

Table (9) Effect of B. t., flufenoxuron and chlorpyrifos administration on hematological parameters of rats.

		control	4 weeks			8 weeks			12 weeks		
			Bt	Fluf	Chlor	Bt	Fluf	Chlor	Bt	Fluf	Chlor
Hb mg/dl	**Mean**	16.13	15.72	14.31	15.6	15.72	10.74	14.74	15.5	7.29	14.60
	SD	2.66	2.45	2.47	2.61	2.45	2.68	2.47	2.66	2.32	2.32
RBCs 10^6cell/mm	**Mean**	5.91	5.32	5.11	5.8	5.88	4.4	4.52**	5.23	2.8	3.24***
	SD	1.23	0.43	0.14	0.15	0.43	0.7	0.2	1.23	1.24	0.54
Hct %	**Mean**	48	46	43	40	46	30	28	48	21	19
	SD	2.3	2.1	2.3	1.8	2.1	1.9	2.32	2.3	2.41	1.6
MCV Fl	**Mean**	89	87	87	83	87	79	77	89	78	76
	SD	5.32	5.44	4.79	3.88	5.44	5.32	4.3	5.32	4.98	4.63
MCH pg	**Mean**	29.7	28.3	28.03	23.35	28.3	24	28.3	29.7	26.3	32
	SD	2.03	1.69	2.36	2.3	1.69	1.7	2.1	2.03	1.6	2.3
WBCs 10^3cell/mm	**Mean**	5.99	6.99	6.6	7.05*	5.78	5.75	4.55	7.34	7.33	6.04
	SD	1.98	1.55	1.25	1.12	2.55	1.28	1.36	3.29	1.27	1.29
Platelets 10^3cell/mm	**Mean**	450.2	453.2	430.36	420.15	453.2	340.2	322.2	450.2	280.66*	262*
	SD	98.33	96.8	115	123	97.9	152.3	115	94.78	96.3	99.6

4.2.5. Effects of chlorpyrifos and flufenoxuron on male fertility and reproductive system parameter of rats:

Chlorpyrifos and flufenoxuron studied from the reproductive toxicity aspect of view as male fertility effects. Albino rats were selected for application of this investigation as ideal models for male fertility according to **El- Sayyad et. al. (1995).** Data presented in (fig 14 a, b) indicated that Chlorpyrifos and Flufenoxuron administrated caused a significant decrease at testis weight at the end of 12^{th} week in comparison to that of control group. The concentration of sperm cells in Flufenoxuron administration showed significant decrease at 12^{th} week of the experiment, in comparison to the control group. However, it showed in Chlorpyrifos highly significant decrease at 12^{th} week of the experiment in comparison to the control.

Data presented in (fig 14 a, b), also indicated that the percentage of live sperms Flufenoxuron administration showed significant decrease live sperm and sperm mortality at 12^{th} week of the experiment, in comparison to the control group but showed in Chlorpyrifos highly significant decrease at 12^{th} week of the experiment in comparison to the control. The percentage of abnormality sperms Flufenoxuron administration showed slightly increase in abnormal sperm at 12^{th} week of the experiment, in comparison to the control group. However, it showed in Chlorpyrifos significant increase at 12^{th} week of the experiment in comparison to the control.

These results were in agreement with those **El Kashory (1999) and El Dosoky (2000)** who reported that multinucleated giant

cells and vanishing of sperms were observed in the majority of tubules of profenofos -treated animals in comparison with control. Epididymidis sperm of experimental group exhibited the presence of increased number of spermatic precursors as well as varieties of sperm anomalies. These results were in agreement with the results of **U.S. EPA, (1998)** concluded that the adverse effects on spermatocyte and/or spermatogonia in the dominant of lethal assay or the dose levels of 100 ppm in males Flufenoxuron in rats are consistent with the results of the 2-generation reproduction study in rats showing that Flufenoxuron is a reproductive toxicant, which appears to specifically target the germinal cells and/or tissues in the testis. Also, **Zhao et. al. (1995)** studied administration of water containing 100 mg/l and 20 mg/l Flufenoxuron to Wistar male rats. Rats were killed at the second, fourth and sixth weeks after experiment initiation. Results suggest that fluoride may have harmful effects on the reproductive system in male rats. These results are in agreement of the results of the present study.

These results were in agreement with **El-Sherbiny**, **et. al. (1995)**; **El-Sayyad and Karim (1998)** and **US EPA (1998)**.

Also, these results were in agreement with the results of **Marty et al. (2004)** who studied the effect of IGRs on rats at different periods of feedings and different concentrations, also they reported that there was an increased proportion of abnormal sperm at 25 mg/kg. Although not statistically significant, there were decreased fertility indices.

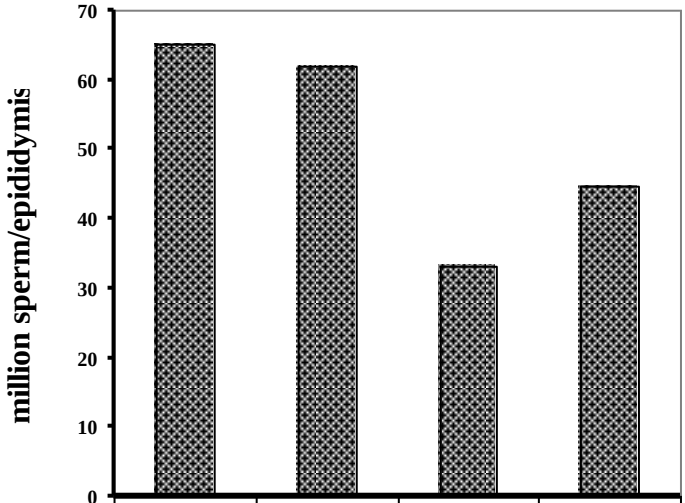
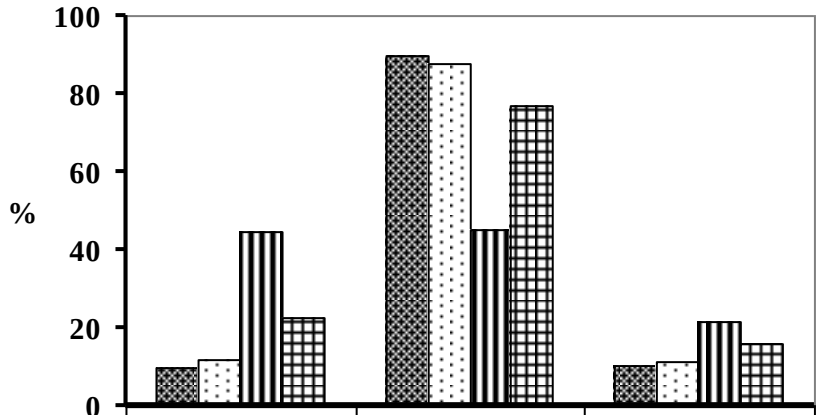


Fig (14a): Effect of B. thuringiensis, Chlorpyrifos and Flufenoxuron administration on Conc. sperm cells as a reproductive parameter of rats



	sperm mort.%	live sperm %	sperm abnorm%
Zero time	10	89.5	10.1
B.t.	12	87.6	11.2
CPF	44.5	45.23	21.3
Flufen	22.6	77.1	15.7

Fig (14b): Effect of B. thuringiensis, flufenoxuron and chlorpyrifos administration on reproductive parameters of rats

4.2.6. Effects of Chlorpyrifos and Flufenoxuron on histopathological changes of some organs of rats:

4.2.6.1. Effects of chlorpyrifos and flufenoxuron on testis:

The testis of the control rats showed normal histological pattern which is build up of seminiferous tubules and the interstitial cells were found in between the tubules. These tubules were rounded or oval in cross sections and contained different stages of spermatogenic cycle. The Lumina of

seminiferous tubules filled with spermatozoa. The interstitial cells were irregular polyhedral cells with large spherical nuclei, figure. (15). this picture is coincident with that described by **Russell et al (1996).** Flufenoxuron administration led to mild to moderate degree of degenerative changes in germinal layers of seminiferous tubules in the form of appearance of vacuolation moderate separation of basement membrane and occlusion of seminiferous tubules figure (16).

The picture of the testis of the Chlorpyifos treated rats shows severe degree of degenerative changes in germinal layers of seminiferous tubules with engorgement of blood vessels with absence of spermatic figure (17).

The findings of **Kumar and Susheela (1995)** were closed to the obtained results, they reported that Flufenoxuron treated rabbits 20 and 23 weeks showed significant decrease in the height of the pseudostratified columnar epithelium and significant increase in the diameter of both the caput and cauda ductus epididymis were observed only in the 23-weeks fluoride treated rabbits. The decreases in the epithelial cell height and the tubular diameter of the testis were significant only in 23-weeks treated animals. The histological finding of **EL-Sayyad, and Karim, (1998)**, were on line of the present study, where they administered flufenoxuron intragastrically by stomach tube at a dose of 20 mg/kg b.wt. to the Juvenile male albino rats (*Rattus norvegicus*) every other day for three weeks. Histological

examination of the testis of flufenoxuron-treated animals exhibited marked decrease of the thickness of tunica albuginea and atrophy of the seminiferous tubules. There was a marked increase of desquamated spermatogenic cells within the lumina of seminiferous tubules. Multinucleated giant cells and vanishing of sperms were observed in the majority of tubules of flufenoxuron-treated animals in comparison with control. Epididymis sperm of experimental group exhibited the presence of increased number of spermatic precursors as well as varieties of sperm anomalies. The observed gonadal dysfunction may be attributed to hepatic damage or decline of gonadal hormone involved in either processes.

Johnson (2005) assessed also subchronic toxicity of flufenoxuron after 90 days of treatment on adult albino rats at 0 and 1.0 mg/kg/day doses only. He assessed also chronic toxicity and he found that in males epididymal aspermia > 75 mg/kg/day, atrophy of seminiferous tubules. Severe degree of degenerative changes in germinal layers of seminiferous tubules with engorgement of blood vessels with absence of spermatic figure (3). These findings were in agreement with **Thomas (2001)**. He found that the organophosphorus compounds administered to rats in subchronic period Histopathological examination of the testis of profenofos-treated animals exhibited marked decrease of the thickness of tunica albuginea and atrophy of the seminiferous tubules. There was an increase of desquamated spermatogenic cells within the lumina of seminiferous tubules. Multinucleated giant cells and vanishing of sperms were

observed in the majority of tubules of the organophosphorus compounds -treated animals in comparison with control. Epididymides sperm of experimental group exhibited the presence of increased number of spermatic precursors as well as varieties of sperm anomalies.

These results are in agreement with the results of **El Dosoky (2000)** who reported that; the organophosphorus caused marked destruction of spermatogenic cell layer with appearance of vacuoles inside cytoplasm. They also, added that there were decrease in spermatogenesis and change in sperm function and fertility was impaired. The effect of organophosphorus on testis were not fully clear until now, however it might be attributed to a direct cytotoxic effect on seminiferous cells in view of degenerative and necrotic changes demonstrated in the spermatogenic tubules. Despite this explanation, it might be due to an indirect effect of organophosphorus on the blood vessels of testis, which cause vascular stasis, or it might be due to inhibition of overall hormonal control mechanism at either the gonadal or the hypothalamic pituitary levels. As a result of the obtained finding through this investigation, it can be recommend that careful handling and proper use of organophosphorus Also the application of organophosphorus must be predicted on selected quantities and manners of usage, which minimize the possibilities of exposure of animals to injurious hazards Chlorpyrifos.

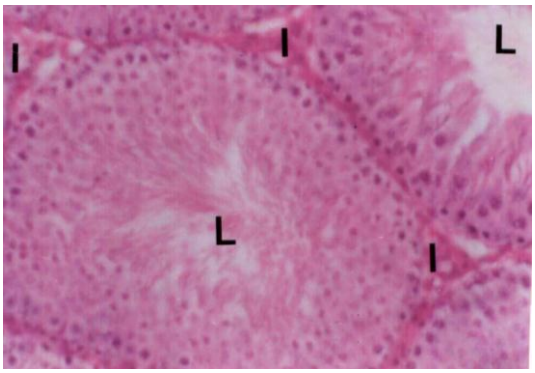


Figure (15): A photomicrograph of normal testis section of adult albino rat the control group showing: Normal histo-pathological pattern of seminiferous tubules and interstitial cells (I) in between tubules. The lumen of tubules (L) filled with spermatozoa.

(Haematoxylin & Eosin X 250)

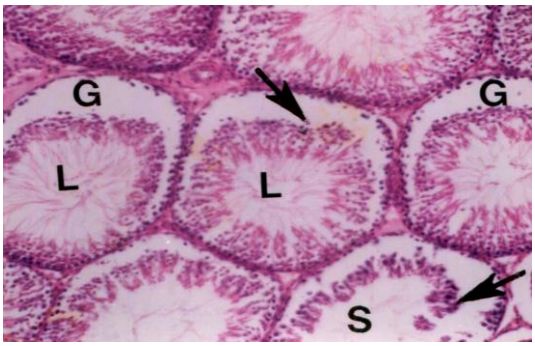


Figure (16): A photomicrograph of testis section of adult albino rat received 1/10 of LD50 of Flufenoxuron showing: marked degeneration of some seminiferous tubules (G)and interstitial cells in between tubules there were sperms in the lumen (L) & absence of sperms in other tubules (S). *(Haematoxylin & Eosin X 250*)

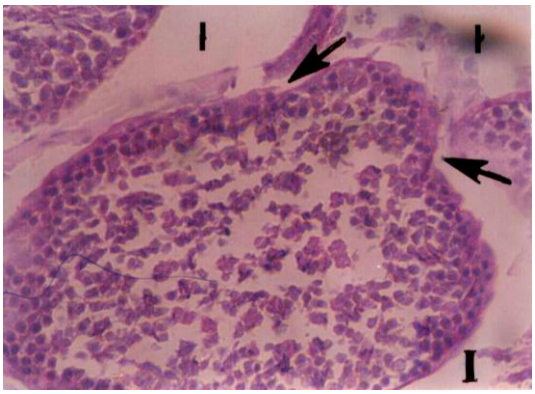


Figure (17): A photomicrograph of testis section of adult albino rat received 1/10 of LD50 of Chlorpyrifos showing: marked degeneration of seminiferous tubules and widening in interstitial cells in between tubules. (I) *(Haematoxylin & Eosin X 250*)

4.2.5.2. Effects of Chlorpyrifos and Flufenoxuron on kidneys:

The histological structure of the renal cortex of the control group showed the normal structure of both renal corpuscles and tubules. The renal corpuscles appeared as dense rounded structures known as glomeruli, which surrounded by narrow spaces called Bowman spaces figure (18). Bowman's capsule consisted of an inner or visceral layer covering the glomerulus and an outer layer or partial layer and the Bowman's space in between, in the Bowman's space, the partial layer become continuous with the wall of the proximal convoluted tubule, figure (18). This picture is the same as described in **Gartner and Hiatt (2001)**.

The capillary tuft appeared smaller with partial endothelial vacuolation. The Bowman's space was increased. The convoluted tubules were widely separated, narrowing of tubules due to cloudy swelling of their cells figure (19). **Johnson (2005)** assessed subchronic toxicity of flufenoxuron after 90 days of treatment on adult albino rats at 0 and 1.0 mg/kg/day doses only. He assessed also chronic toxicity and he found that in males, mineralization of renal pelvic epithelium > 75 mg/kg/day, hyperplasia of renal pelvic epithelium were observed.

Also **Karim, (1998) and US EPA (2005)** added that in the kidney, hypertrophy of the epithelial cells of the collecting duct was observed in rats. He added that hypertrophy of the epithelial cells correlated with elevated serum bicarbonate levels, urinary acidification, decreased urinary specific gravity and increased kidney weights. Other histopathological findings in the kidney

included degeneration with regeneration in the descending portion of the proximal tubules which was considered to be typical of acute necrosis with regeneration rather than a 90-days old lesion and multi-focal mineralization in the papilla and decreased kidney weights. These lesions did not appear to be reversible.

The glomeruli capillaries appear small with partial endothelial vacuolation. There were also widening in Bowman's spaces. There is an increase with marked distortion. The intertubular spaces were marked increased, fig (20). These results were in agreement with the results of **Revathi and kumar, (2000)** who reported that the injuries induced by the subacute dosage of 1/10th LC50 of chlopyrifos 40% E.C. in kidney were investigated. Kidney showed hyperemia of glomeruli and interstitial vessels.

Also **Oncu, et al (2002)** reported that histopathological changes were found in the kidney tissue of rats treated with chlorpyrifos for 90 days. These were infiltration in mononuclear cells at perivascular and peritubular areas, hydropic degenerations in tubule epithelium and glomerular sclerosis.

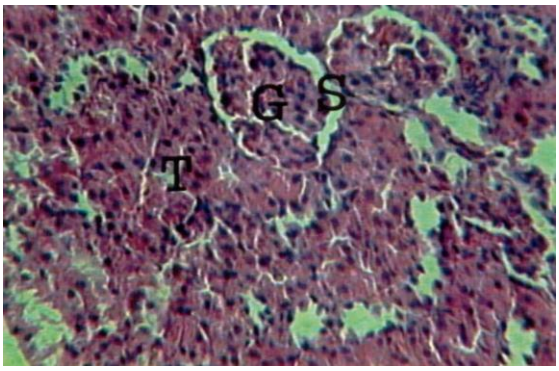


Figure (18): A photomicrograph of normal kidney section of adult albino rat the control group showing: Normal histopathological pattern of renal corpuscles, proximal & distal convoluted tubules (t).normal glomerulus (G) &Bowman space (S). *(Haematoxylin & EosinX 250)*

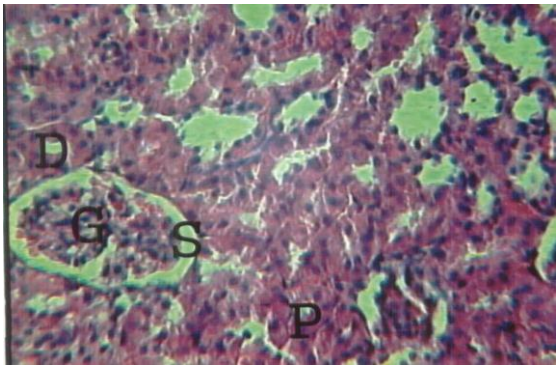


Figure (19): A photomicrograph of kidney section of adult albino rat received 1/10 of LD50 of Flufenoxuron showing: marked degeneration of some of renal corpuscles , proximal (P) & distal convoluted tubules (D).normal glomerulus (G) &increase in size of Bowman space (S) *(Haematoxylin & EosinX 250)*

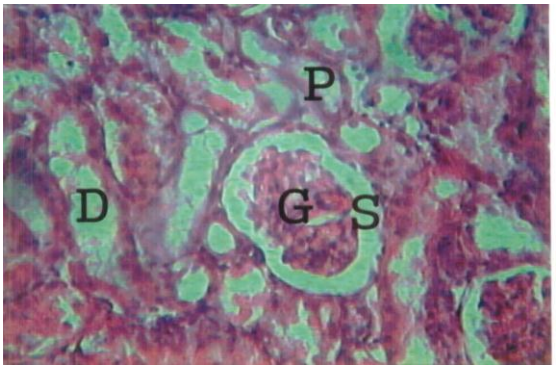


Figure (20): A photomicrograph of kidney section of adult albino rat received 1/10 of LD50 of CPF showing: marked de-generation of some of renal corpuscles , proximal (P) & distal convoluted tubules (D).decrease in size of glomerulus (G) &marked increase in size of Bowman space (S).

*(Haematoxylin & Eosi X 250***)**

4.2.6.3. Effects of Chlorpyrifos and Flufenoxuron on liver:

The normal structure of hepatic lobules and hepatocytes, hepatocytes form columns of cells adherent to each other by one or more surfaces. Bile canaculi were present in between two columns of hepatocytes. At least, one surface of any hepatocyte was on contact with liver sinusoids. The cytoplasm often appeared coarsely granular with empty vacuolated areas where lipid droplets have been dissolved during preparation of the section. The nuclei were spherical, centrally located and variable in size and with one nucleolus. Hepatocytes may be binucleated, the sinusoids were variable in diameter and lined by discontinuous sheet of endothelial cells with flat nuclei, Kupffer's cells also, located in the sinusoidal walls figure (21)**.** This picture is the same as described in **Ross and Pawlina (2005)**.

The hepatocytes showed cytomegalic changes in size and the cytoplasm was granular and vacuolar figure (22). Hepatic cell nuclei appeared fragmented chromatin along the hepatic sinusoids. Most of the hepatic blood sinusoides dilated. Intracytoplasmic vacuolation of different sizes Widening blood sinusoids denoting degenerating hepatic cells. The finding of **Karim** (**1998)** were in agreement with the obtained findings. He administered flufenoxuron intragastrically by stomach intubation to female rats at concentration levels 0 & 20 mg/kg b.wt. The observed defects in pregnant rats may be attributed to the histological abnormalities of liver and kidneys of as a result of the insecticide or its metabolites on the affected structures. Congestion of the liver, hepatocyte swelling and increased liver

weights. Increased relative liver weight was seen in rats fed 2,500 ppm (125 mg/kg/day) flufenoxuron for 90 days. Hepatic hyperplasia and hypertrophy and increased relative liver weight were noted in a 28-day feeding study in rats. Increased relative liver weight and diffuse and smooth granular livers were seen in a 28-day feeding study in mice. **EL-Sayyad, and Karim, (1998); and Johnson (2005)** found that the hepatic tissues toxicated with the insecticide showed nuclear disintegration, massive breakdown of hepatocytes and internal hemorrhage. These findings were coincident with the acheived observation. Microscopic examination revealed treatment-related hepatocellular hypertrophy (centrilobular) in males at 1000 mg/kg/day and in females at 500 and 1000 mg/kg/day.

The results of **Cox, (1997),** were also in agreement with the obtained results, who found that, in long-term (chronic) feeding tests, hexaflumuron increased the incidence and severity of a liver cell abnormality. A toxicological evaluation of hexaflumuron was realized. It was established that by its toxicity hexaflumuron belongs to hazardous substances of class III.

There were loss of cell architecture and increased degeneration of hepatic cells. Most of hepatic cells were necrotic showing most of hepatic cells were enlarged and cytoplasm were granular and vacules. Widening blood sinusoids denoting degenerating hepatic cells (has no nuclei) as shown in figure (23). These results are in agreement with the results of **Gomes et al.,(1999), Revathi and kumar, (2000)**.

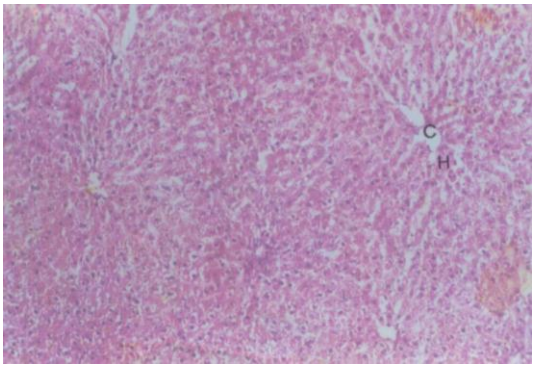


Figure (21): A photomicrograph of normal liver section of adult albino rat of the control group showing Central vein (C), hepatic cord (H), radiating from central vein.

(Haematoxylin & Eosin X 250).

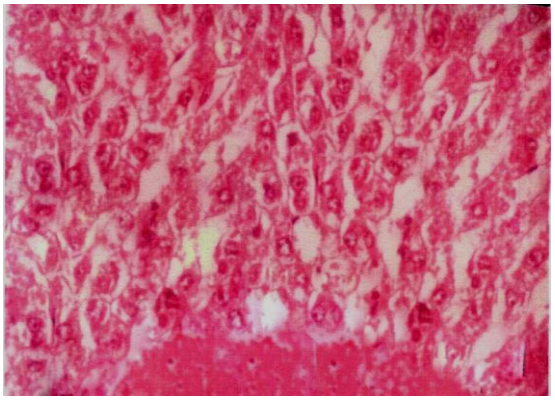


Figure (22): A photomicrograph of liver section of adult albino rat received 1/10 of LD50 of IGR showing:

a) Intracytoplasmic vacuolation of different sizes

b) Widening blood sinusoids denoting degenerating hepatic cells.

(Haematoxylin & Eosin X 400)

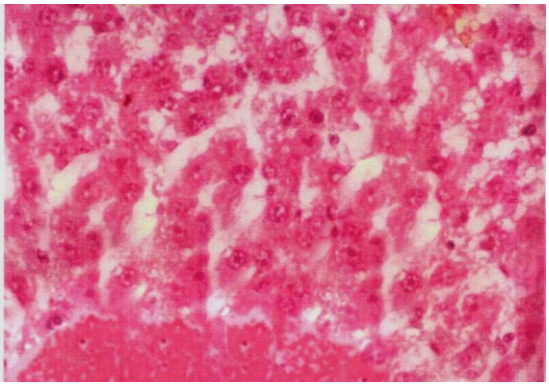


Figure (23): A photomicrograph of liver section of adult albino rat received 1/10 of LD_{50} of organophospherus showing most of hepatic cells were enlarged and cytoplasme were granular and vacules. Widening blood sinusoids denoting degenerating hepatic cells.

(Haematoxylin & Eosin X 400)