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Effect of Teflubenzuron and *Bacillus thuringiensis* on Some Haematolgical Parameters of Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.) and Albino Rats

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ABSTRACT

A study to evaluate the toxicological and hematological effects of the entomopathogen (*Bacillus thuringiensis kurstaki*) and the insect growth regulator (Teflubenzuron) on the cotton leaf worm, *Spodptera littoralis* (Boisd.) under laboratory conditions, as well their effects on the population % of the pest in Egyptian clover fields and also to evaluate their toxicity on some hematological parameters of the albino rats was carried out. Obtained results indicated that total haemocyte counts (THC) of *S. littoralis* 6th instar treated in 4th instar with the chitin synthesis inhibitor Teflubenzuron was significantly increased compared to the control. While in *Bt.*, the THC was slightly decreased (6.8 %) ($P<0.05$) compared to the control. Teflubenzuron affected some types of blood cells. It significantly decreased the number of oenocytoids, whereas plasmatocytes were significantly ($P<0.001$) increased. Meanwhile, *Bt.* increased insignificantly the number of prohaemocytes and spherulocytes, where plasmatocytes, granulocytes were slightly decreased compared to the control. Application of *Bt. kurstaki* for 12 weeks to rats at dosages of 10000mg/ kg/day didn't produce toxic effects. The effect of *Bt.* showed insignificant changes in body weight, liver, kidney and testicular weights, compared to the levels in the control. On the contrary, Teflubenzuron caused a significant decrease in body and kidney weight of rats and increased liver weight. In addition, there was a slightly decrease in testicular weight compared to the control. It was concluded that *Bt.* had not any segnificant effect on the haematological parameters.

Key words: *B. thuringiensis kurstaki,* IGR, Teflubenzuron, *Spodoptera littoralis,* Albino rats, Haematolgical parameters.

INTRODUCTION

Natural pesticides and biopesticides are the prospective solutions to avoid the deteriorating effects of synthetic pesticides. Recently, the wide use of biopesticides in agricultural and public health programs has adverse health effects on human and animal. Therefore, it is necessary to focus on studying the detrimental effects of such natural pesticides and biopesticides on mammals. Mammalian safety studies were carried out with *Bacillus thuringiensis kurstaki* orally administered to rats. The clearance and distribution of *B. thuringiensis* were evaluated. The results confirmed the safety of *Bt. kurstaki* to rats (Tsai *et al.*, 1995). Hazardous effects of the benzoyl-phenylurea on mammalian tissues are still under investigation and works are focused mainly on controlling insect production. Utilization of benzoyl-phenylurea resulted in altered enzyme activities of rat liver, renal damage, and reproductive disorders to experimental animals (Karim, 1998 and Hussi 2006).

The present study aimed to evaluate the toxicological and hematological effects of the entomopathogen (*Bt. kurstaki*) and the insect growth regulator (Teflubenzuron) on the cotton leaf worm, *Spodptera littoralis* (Boisd.) under laboratory conditions, as well their effects on the population % of the pest in Egyptian clover fields and also to evaluate their toxicity on some hematological parameters of the albino rats.

MATERIALS AND METHODS

I- Laboratory Experiments

1. Rearing technique

A stock culture of the cotton leaf worm, *S. littoralis* was obtained from the laboratory strain, maintained for several generations without any insecticidal pressure in the Cotton Pest Research Department, Plant Protection Research Institute (PPRI), Agriculture Research Center (ARC), Giza, Egypt. The insect was reared on castor-oil leaves *(Ricinus communis)* under the laboratory conditions at 25±2°C and 60±5% R.H. 4th instar larvae were used in the present study. The 4th instar larvae were fed on castor-oil leaves previously treated with different concentrations of both tested materials for 24 hours then transferred to untreated leaves until pupation.

2- Chemicals used

1- Teflubenzuron (NO Molt) chitin synthesis inhibitors IUPAC1- (3, 5-dichloro-2, 4-difluoro-pheny l)-3-(2, 6 difluorobenzoyl) urea.

2- *Bacillus thuringiensis* var. *kurstaki* (Protecto®) was obtained from PPRI. Biopesticide Production Unit.

3- Collection of haemolymph for total haemocyte counts

Late 6th instar larvae were submerged in a hot water bath at 56-58°C for 2 min., then removed and surfacely dried by a tissue paper. One of prolegs was

cut with a fine lancet and haemolymph was allowed to fall in clean test tubes, provided with phenylthiourea to prevent melanization. Three replicates of the haemolymph pool were obtained; each was obtained from 10 larvae. From each pool 10 films were prepared and counted.

3.1. Haemocyte counts and viability Reagents

1- Insect physiological saline solution: consisted of NaCl (8.8gm), KCl (0.2gm) and $CaCl_2$ (0.3gm) per liter: the pH was adjusted at 6.7-6.8.
2- Diluting solution consisting of trypan blue (0.4%) in insect physiological saline solution.

Procedure

Viability % was calculated using the formula given by Horhov and Dunn (1982) as follows:

$$\text{Viability \%} = \frac{\text{No. of viable cells}}{\text{Total No.of cells}} \text{ X } 100$$

3.2. Differential haemocyte counts

Fresh haemolymph from late 6 th instar larvae were smeared on a clean glass slide, air dried and then fixed for 2 minutes with ethanol. Blood films were stained with Giemsa stain freshly prepared by mixing stock Giemsa with distilled water (1:10 V/V) for 15 minutes. After short wash in distilled water, slides were dipped for about 30 seconds in tap water. Smears were air dried for 24 hours, mounted in Canada balsam and then examined. Differential haemocyte counts were accomplished by observing and differentiating at least 100 cells from random fields on each slide using light microscope. This procedure was repeated 10 times for each treatment.

3.3. Source and rearing of albino rats

In this study, a total of forty male albino rats (*Rattus norvegicus*), weighing 120-150 grams each, were used. The rats were obtained from the Farm of Central Organization of Serum and Vaccine (Abasia Farm, Egypt). Rats were allowed to be acclimatized to laboratory conditions for two weeks prior to the experiment. The rats were housed in plastic cage under hygienic condition in dry-bulb temperature 18-20°C and fed on a commercial pellet diet and barley (natural ingredient diet). The diet included protein, minerals, vitamins, energy resources and other beneficial dietary constituent as recommended by (National Research Council (NRC) 1995) and the diet of the rats of the present study was supported with Soya Bean. The water supply was evaluated as recommended as 1/4-1/3 of their body weight in water daily. Food and water were available all over the experimental period.

3.4. Experimental design of albino rats

The animals were arranged into three groups, each composed of 10 individuals as follows: control saline-treated rats group, *B. thuringiensis* treated rats group and Teflubenzuron treated rats group. After the end of each, blood samples were taken. *Bt. kurstaki* was suspended in saline solution and intra gastrically administered by stomach tube with different large doses, but it was observed that it had no effect on the studied rats. *Bt.* was supplied at the rate of 10000mg/ kg body weight from the commercial product to evaluate the sub chronic effect of *Bt.* at dose higher than that reported in previous studies. Teflubenzuron (benzoylphenyl-urea) was supplied at a concentration of 10%. The applied dose of Teflubenzuron was 105 mg/kg body weight (equivalent to 0.1 of LD_{50}). It was dissolved in saline solution, and then administered intra gastrically by stomach tube every other day for 3 months; the drug was freshly prepared prior to every treatment.

Blood samples were obtained from the retro-orbital plexus and the tail using 21-gauge needle of overnight fasted rats. Blood was collected into heparinized tube for assay of the complete blood picture.

II- Field Experiments

Field experiments were carried out in clover field, *Trifolium alexandrinum* L. cultivated at Kaha province, Qalyobia Governorate, Egypt, in 2009 and 2010 seasons. Teflubenzuron was sprayed by ULV motor sprayer (Kubota) at the recommended rate of 230 ml/ 40 litre /feddan. Dipel-2X (*Bt. kurstaki)* was applied at the recommended field rate 300 gm\feddan, while the control was sprayed with water. Efficacy of the tested compounds against *S. littoralis* was measured after 3, 5, 7 and 9 days post *Bt.* application and after 5, 10 and 15 days post Teflubenzuron treatment. Percentages of reduction in the population density of the pest were estimated and corrected according to Henderson and Tilton (1955).

RESULTS AND DISCUSSION

1- Bioassay on *Spodoptera littoralis*

Obtained data revealed that the LC_{50} of the *Bt.* was $2.18x10^3$IU/ ml, which falls within a slightly large area further when the lower (0.0002 IU/ml) and upper (0.023 IU/ ml) fudical limits were taken into consideration. All the points of LC_{50} were found within these limits (slope = 0.39 ± 0.10). Obtained results agree with that work of Mohamed (2003) who found that *Bt. kurstaki* exhibited good mortality against 4th instar of *S. littoralis*. Toxicity of *Bt.* was investigated by Abd El-Aziz (2000) who classified lepidopteran larvae into three types, based on their susceptibility to crystalline endotoxin. Abd El-Haleem (1997) and Abd El-Al *et al.* (2009) recorded that *Bt.* is toxic to larvae of lepidoptera upon ingestion.

For the LC_{50} of Teflubenzuron, it was 4.1 ppm. All the points of LC_{50} were found within these limits (slope = 1.8±0.174). Obtained results agree with those obtained by Haga *et al.* (1984) who reported that Chlorfluazuron is very toxic to insects because it metabolizes slowly inside the insect body. The toxicity of Teflubenzuron against *S. littoralis* larvae was somewhat similar to that of the Chlorfluazuron against *H. armigra* (Rao *et al.*, 1994) and *A. ipsilon* (Shurab *et. al.*, 1999). Also, Farag (2001) and Abd El-Al and El-Sheikh (2005) reported that chitin synthesis inhibitors caused high mortalities to 4th instar larvae of *S. littoralis*.

2. 1. Total haemocyte counts and viability

Total haemocyte counts (THC) of *S. littoralis* 6th instar treated in 4th instar with the chitin synthesis inhibitor Teflubenzuron was significantly increased as compared with the control. While in *Bt.*, the THC was slightly decreased (6.8 %) ($P<0.05$) compared to the control (Table 1). The viability of TVHC (Table 1) followed the same pattern of that of THC. Teflubenzuron significantly increased the viability of TVHC, as compared to the control. On the contrary, treatment with *Bt.* was slightly decreased the total number of viable haemocytes as compared to the control. The observed increase of total haemocyte counts (THC) in late 6th larval instar of *S. littoralis,* after treatment of 4th instar with the tested chitin synthesis inhibitors, agree with the findings of Osman *et al.*, (1984-1985) who recorded increase of the THC level for the 2nd larval instar of *S. littoralis,* when fed on diet containing 5ppm of diflubenzuron. Such increase in the THC gave an impression that blood cells may share in detoxifying these chemicals. Patton (1961) implicated that haemocytes may function in detoxification of insecticides. Nappi (1974) suggested that the brain endocrine complex was involved in haemocyte accumulation following an initial stimulus. In the present study, the cause of such stimulus might be the insect growth regulator. Also, in agreement with the obtained results; Salama (2007) reported that treatment of *S. littoralis* larvae with *Bt.* decreased the THC.

Table (1): Total haemocyte counts (T.H.C.) and total viable haemocyte counts (T.V.H.C.) (cell/mm^3x10^3) of late 6th instar *Spodoptera littoralis* treated with Teflubenzuron and *Bacillus thuringiensis* at LC_{50} level

Treatment	Mean T.H.C. ±S.E.	Mean T.V.H.C. ± S.E.
B. thuringiensis	$24.75^{*} \pm 0.23$	$17.20^{ns} \pm 0.29$
Teflubenzuron	$45.76^{***} \pm 0.53$	$25.30^{***} \pm 0.88$
Control	26.56 ± 0.30	17.33 ± 0.38

2.2. Differential haemocyte counts

There are five types of haemocytes in *S. littoralis* larvae, *i.e.* prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids. The effect of IGR on the change in the numbers of these cells in late 6th instar when treated as 4th instar is shown in table (2). Teflubenzuron significantly ($P<0.001$) increased plasmatocytes wheras decreased the number of spherulocytes, granulocytes and oenocytoids as compared to the control. *Bt.*, insignificantly increased the number of prohaemocytes and spherulocytes, as compared to the control whereas, plasmatocytes and granulocytes were slightly decreased.

The most obvious activity of haemocytes was the phagocytosis. In this context, the most active phagocytes are the plasmatocytes (Jones, 1962), which were significantly increased due to the treatment with Teflubenzuron. Plasmatocytes were implicated in encapsulation of necrotic tissues (Essawy, 1990). Thus, probably the increased numbers of plasmatocytes in the present study, particularly after treatment with Teflubenzuron was related to the detoxication of this IGR. This is concomitant to the higher toxicity of Teflubenzuron to insects. Teflubenzuron significantly decreased the number of spherulocytes, whereas *Bt.* insignificantly increased their number as compared to the untreated larvae in the present study. The function of these cells has not been fully described. These cells may store acid muco substances (Neuwirth, 1973). They are also implicated in secretion of some haemolymph proteins (Akai and Sato, 1979), control of cell adhesion, migration and regulation of clotting (Cook *et al.*, 1985).

It is generally believed that epidermal cells *in vivo* provide the chemical precursor for chitin synthesis in insects. Philogene and McFarlane (1967) had quite thoroughly established the evidence necessary to conclude that the process of chitin synthesis is extracellular. They reported that cuticle precursor forms layers on the surface or very close to the surface of secretory cells and that cuticular deposition may be completed at a distance from the secretory sites. They also linked oenocytoids and their secretions with cuticle formation *in vivo*. Moreover, Ritter and Bray (1968) indicated that blood can be cultivated in synthetic media for indefinite periods. Cultivated blood consists of a cell-fiber complex that exhibits biological growth. Birefingent, cellular objects identified as chitin were accumulated in these cultures. Thus, since oenocytoids are linked with cuticle formation, this leads to the suggestion that chitin synthesis inhibitors may affect oenocytoids by inhibiting their secretions rather than by reducing their numbers, as indicated particularly with Teflubenzuron treatment

Table (2): Differential haemocyte counts (D.H.C) of *S. littoralis* 6th instar larvae treated with Teflubenzuron and *B. thuringiensis* at LC_{50} level

Cell Types	Mean D.H.C. (%) ± S. E.		
	Teflubenzuron	*B. thuringiensis*	Control
Prohaemocytes	7.9* ±0.05	10.90^{ns} ± 0.06	9.50 ± 0.02
Plasmatocytes	32.2 * ± 0.04	24.8 * ± 0.02	26.70 ± 0.03
Granulocytes	39.5 * ± 0.06	35.91* ± 0.06	36.70 ± 0.05
Spherulocytes	22.3* ± 0.08	26.10^{ns} ± 0.04	25.20 ± 0.06
Oenocytoids	6.10^{ns} ± 0.03	7.10^{ns} ± 0.02	7.80 ± 0.03

Table (3): Effect of *B. thuringiensis* and Teflubenzuron on body weight and some organs weight of rats

Weight in gm.Mean ±SD	Control	*B. thuringiensis*	Teflubenzuron
Body weight	255±11.78	249±11.8	226.4±3.22
Liver weight	9.9±0.47	9.7±0.45	11.47±0.52
Kidney weight	2.95±0.163	2.75±0.163	2.16±0.16
Testis weight	2.67±0.025	2.65±0.03	2.08±0.08

in the present study. Also, chitin synthesis inhibitors interfere with the molting process by inhibiting the biosynthesis of chitin (Retnakaran *et al.* 1985).

3.1. Toxicological effect of Teflubenzuron on rats

Rats treated with Teflubenzuron at 1/10 of its LC_{50}, developed clinical symptoms, which were progressing by time marked distension of the abdomen. This was the only clinical symptom observed in rats after the first two weeks of treatment. In the 3rd week, rats lost their vitality and activity. Some rats developed nervous manifestation and 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 results of US EPA (1998) agree with the obtained observations concerning the study on chronic rat feeding with flufenoxuron, which identified the following effects: seizures, including seizures resulting in death.

3.2. Toxicological effects of *Bacillus thuringiensis* on rats

Application of *Bt. kurstaki* for 12 weeks to rats at dosages of 10000mg/kg/day did not produce toxic effects. The effects showed insignificant changes in body weight, liver, kidney weight and testicular weights of rats as compared to their levels in the control (Table 3). Results are in agreement with those reported by PIP (2006). The LD_{50} was greater than 5000 mg/kg for the *Bt.* product Javelin in rats and greater than 13,000 mg/kg in rats exposed to the product. Single oral dosages (up to 10,000 mg/kg) did not produce toxicity in mice, rats or dogs. The dermal LD_{50} for a formulated *Bt. kurstaki* product in rabbits was administrated by 6280 mg/kg. A single dermal application of 7200 mg/kg of *Bt.* was not toxic to rabbits. Ray (1991) reported that dietary administration of *Bt.* 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 *Bt.* product was placed on scratched skin for 21days.

Also, the above results are in agreement with the results of Tsai, *et al.* (1995) who reported that mammalian safety studies were carried out with *Bt.* orally administered to rats. Neither clinical symptoms nor histopathological changes were detected during the test. The total number of colony-forming units (CFU) recovered was less than in the initial inoculation. No spore germination was observed in the tissues after administration. The results confirmed the safety of *Bt. kurstaki* to rats. The results of Roe (1991), British Columbia Ministry of Health (1992), Washington State Department of Health (1993), Salamitou *et al.* (2000) seemed to be on line with the present results.

3.3 Hematological effects on rat

Data presented in table (4) indicated that prolonged aplication of IGRs affected hematological parameters in rats while *Bt.* recorded insignificant results on the hematological parameters. Teflbenzuron affected these parameters by decreasing Hb%, RBCs count, Hematocrite % (Hct%), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCH) and platelets except the leucocytes (WBCs) that showed significant increase of the result of the defense role of WBCs against toxic action of Teflubenzuron. These results are in agreement with El-Sherbiny *et al.*, (1995) who recorded decrease in erythrocyte counts and packed cell volume.

Field experiment

Results presented in table (5) indicated that the effect of the *Bt.* recorded 35, 55.4, 71.4 and 76.4% at 3rd, 5th, 7th and 9th days post treatment in the first season 2009. Respective values were 24.7, 44.4, 69 and 74.2% in the second 2010. According to the residual effect; percent of reduction in *S. littoralis* infestation caused 9 days post spray, recorded 76.4 and 72.2 % in the two successive seasons, respectively. These results agree with El-Sheakh (1998).

The effect of Teflubenzuron recorded 59, 751 and 87% after 5, 10 and 15 days pos treatment, respectively in the first season 2009. Respective, values were 61, 75.8, 90.6 % in the second season 2010 (Table 6). It is obvious that the tested IGR induced high reduction in the rate of *S. littoralis* infestation in both seasons up to15 days post spray. These findings are in agreement with Desuky *et al.*, (2005), El-Sheakh *et al.*, (2007) and Adel–al *et al.*, (2009).

Table (4): Effect of *B. thuringiensis* and Teflubenzuron on some hematological parameters of albino rats

Hematological parameters	Control	4 weeks		8 weeks		12 weeks	
		B .t.	Teflubenzuron	*B .t.*	Teflubenzuron	*B .t.*	Teflubenzuron
Hb g/dl	16.13±2.66	15.72±2.45	14.31±2.47	15.72±2.45	10.74±2.68	15.5±2.66	7.29±2.32
RBCs 10^6cellImm	5.91±1.23	5.32±0.043	5.11±0.014	5.88±0.043	4.4±0.07	5.23±1.23	2.8±1.24
Hct%	48±2.3	46±2.1	43±2.3	46±2.1	30±1.9	48±2.3	21±2.41
MCV *Fl*	89±5.32	87±5.44	87±4.79	87±5.44	79±5.32	89±5.32	78±4.98
MVH *pg*	29.7±2.03	28.3±1.69	28.03±2.36	28.3±1.69	24±1.7	29.7±2.03	26.3±1.6
WBCs 10^3cell/mm	5.99±1.98	6.99±1.55	6.6±1.25	5.78±2.55	5.75±1.28	7.34±3.29	7.33±1.27
Platletes10^3cell/mm	450.5±98.33	453.2±96.8	430.36±115	453.2±97.9	340.2±152.3	450.2±94.78	280.66±96.3

Table (5): Effectiveness of Dipel-2X *(B. thuringiensis)* on the cotton leaf worm, *S. littoralis,* expressed as % reduction of larval population and residual toxicity after spraying in Egyptian clover at Kaha province, Qaluobia Governorate, Egypt seasons 2009 and 2010

Season	Treatment	Rate/ Fadden	No of larvae before spray	No. of larvae and reduction % in larval population at indicated days after spray								Residual toxicity
				3 days		5 days		7 days		9 days		
				No.	Red %	No.	Red %	No.	Red %	No.	Red %	
2009	*B.thuringiensis*	300g\fed	325	205	35%	145	55.4%	92	71.4%	75	76.4%	62.5
	Control		346	334		357		342		332		
2010	*B.thuringiensis*		285	210	24.7%	150	44.4%	80	69%	65	74.2%	53.1
	Control		242	256		248		230		241		

Table (6): Effectiveness of Teflubenzuron on the cotton leaf worm, *S. littoralis,* expressed as % reduction of larval population and residual toxicity after spraying on Egyptian clover at Kaha province, Qaluobia Governorate, Egypt seasons 2009 and 2010

Season	IGRs 230ml\fed	No. before spraying	Initial effect after 5days		Residual effect after			
					10 days		15 days	
			No	Red %	No	Red %	No	Red %
2009	Teflubenzuron	256	100	59	68	75.1	32	87
	Control	346	321	-	325	-	332	-
2010	Teflubenzuron	275	102	61	64	75.8	25	90.6
	Control	242	232	-	234	-	231	-

REFERENCES

Abd El-al, A.E and T.A. El-Sheikh 2005. Effect of two Biocides and two insect growth regulators on esterase activity of cotton leafworm *Spodoptera littoralis* (Boisd), Egypt. J. Agric. Res., 2(1):401-409.

Abd El-al; T.A. El-Sheikh and A.M. Farag 2009. Effectiveness of insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd) population on Egyptian cotton in Menofia Governorate. Egypt. J. Agric. Res., 87 (2).

Abd El-Aziz, S. H. 2000. Physio-pathological studies on bacterial infection of cotton leaf worm, *Spodoptera littoralis*. M.Sc. Thesis, Fac. of Science,Ain shams Univ., 131pp.

Abd El-Haleem, M. S. 1997. Effect of certain bio-control agents against the cotton leaf worm, *Spodoptera littoralis* (Boisd.) in comparison with methomyl, Egypt J.Biol..Pest Control 7(1):35-39.

Akai, H. and S. Sato, 1979. Surface and internal ultra-structure of haemocytes of some insects. In A. P. Gupta (ed.), Insect Haemocytes: Development, Forms, Function and Technique. Cambridge University Press: 129-154.

British Columbia Ministry of Health 1992. *Bacillus thuringiensis* Report 1-46.

Cook, D.; D. B. Stoltz and C. Pauley 1985. Purification and preliminary characterization of insect spherulocytes. Insect Biochem., 15: 419-426.

Desuky, W.M.H.; M. M. Khedr; K.S.I. Yousef and S.M.A. EL-Shakaa 2005. Field and biochemical studies on same compounds against cotton leaf worm, *Spodoptera littoralis* (Boisd). Egypt J. Agric. Res., 83(3), pp 1087-1106.

El-Sheikh, A.A. 1998. Efficacy of Dipel-2x against the cotton leaf worm, *Spodoptera littoralis* (Boisd.) in cotton fields. J. Appl. Sci., 13(1): 228- 234.

El-Sheikh, T.A.A. and A.E. Abdel-al 2007. Field and laboratory studies on some compounds against cotton leaf worm, *Spodoptera littoralis* (Boisd). Bull. Ent. Soc. Egypt, Econ. Ser., 33 (175-190).

El-Sherbiny, S.A.; H.I. El Sayad; H.L. El-Gammal and E.M. El-Shershaby 1995. Studies on the toxic effects of Diflubenzuron and Chlorafluazuron on the testis of albino rat. Egypt. Ger. Soc. Zool., Vol. 17(C), 45-64.

Essawy, M.M. 1990. Changes in the differential haemocyte counts of the last larval instar of

Spodoptera littoralis (Biosd.) during wound healing. Alex. Sci. Exch, 2 (4): 151-169.

Farag, A.M. 2001. Biochemical studies on the effect of some insect growth regulators on the cotton leaf worm.M.Sc. Thesis, Fac. of Agric., Cairo Univ., 123 pp.

Haga, T.; T. Toki; T. Koyangio and R. Nishiyamo 1984. Structure activity relationships of benzoylphenylurea. Internat. Cong. Entomol., Hamburg (FRG).

Henderson, C.F. and E.W. Tilton 1955. Test with acaricides against the brown wheat mite. J. Econ. Ent.; 48: 157-161.

Hussein I.O. 2006. Biochemical studies on the effect of some pesticides on cotton leaf worm and experimental animals. Ph.D. Thesis, Fac. of Agric. Benha Univ., 164 pp.

Jones, J.C. 1962. Current concepts concerning insect haemocytes. Amer. Zool., 2: 209-246.

Karim, S.A. 1998. Patterns of developmental defects of rat fetus maternally treated with an environmental antimoulting insecticide Flufenoxuron. Egypt. Ger. Soc. Zool., Vol. 25(B), 65-81.

Mohamed, M.H. 2003. New approach for controlling the cotton leaf worm *Spodoptera littoralis* . M. Sc. Thesis, Fac. of Science, Mansoura Univ., 122pp.

Nappi, A. J. 1974. Insect haemocytes and the problem of host recognition of foreigners In: E. L. Cooper (ed): Contemporary Topics in Immunology. Vol. IV, Invertebrate Immunity. Plenum Press, New York and London, 207-221.

NRC (National Research Council) 1995. Nutrient Requirement of Laboratory Animals, 4th. Revised ed. National Academy Press. Washington, D.C.; 95-102.

Neuwirth, M. 1973. The structure of the haemocytes of *Galleria mellonella* (Lepidoptera). J. Morph., 139: 105-124.

Osman, S.E.; I. Rawash and M.M. El-Samadisi 1984-1985. Effect of antimoulting agent "Dimilin" on the blood picture and cuticle formation in *Spodoptera littoralis* (Boisd.) larvae. Bull. Ent. Soc. Egypt, Econ. Ser., 14: 37-46.

Patton, R.L. 1961. The detoxification functions of insect haemocytes. Ann. Ent. Soc. Amer., 54: 696-698.

Philogene, B. J. R. and J. E. McFarlane 1967. The formation of cuticle in the house cricket, *Acheta domestica* L. and the role of oenocytes. Can. J. Zool., 45: 181-190.

PIP (Pesticide Information Project) 2006. *Bacillus thuringiensis* Report: Cornell Univ., USDA/Extension Service/National Agric. Pesticide Impact Assessment Program. 1- 37.

Ray, D.E., (1991). Pesticides derived from plants and other organisms. In Handbook of Pesticide Toxicology. Hayes, W. J., Jr. and Laws, E. R., Jr., Eds.

Retnakaran, A.; J. Granett and T. Ennis 1985. Insect growth regulators. In Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 12 (ED. By Kerkut E. A. and Gilbert L. I.) pp. 529-603. Pergamon Press, New York.

Ritter, H. and M. Bray 1968. Chitin synthesis in cultivated cockroach blood. J. Insect Physiol., 14: 361-366.

Roe, R. M. 1991. Vertebrate toxicology of the solubilized parasporal crystalline proteins of *B. thuringiensis israelensis.* In Reviews in Pesticide Toxicology1: Toxicological Studies of Risks and Benefits. Hodgson, E., Roe, R. M. Eds. North Carolina State Univ., Raleigh NC, 10-148.

Salama Z.A. 2007. Biological, biochemical and histological studies of *Bacillus thuringiensis* and spinosad on cotton leafworm *Spodoptera littoralis* (Boisd) larvae with special comparison of pyrethroid compound. Ph.D. Thesis, Fac. of Science, Almansura Univ. Egypt, 183pp.

Salamitou, S.; F. Ramisse; M. Brehélin; D. Bourguet; N. Gilois; M. Gominet; E. Hernandez and D. Lereclus 2000. The plcR regulon is involved in the opportunistic properties of *B. thuringiensis* and *B. cereus* in mice and insects. Microbiol., 146: 2825- 32.

Shurab, E. H.; S. A. Emara; N. Z. Zohdy and A. E. Abdel-Aal 1999. Effect of four insect growth regulators on the black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae). The 2nd Int. Conf. of Pest Control, Mansoura, Egypt, Sept. PP: 773-776.

US EPA US Environmental Protection Agency 1998. Flufenoxuron. 738-R-98-023.

Tsai, S. F.; J. W. Liao and S. C. Ang 1995. Clearance and distribution of *Bacillus thuringiensis* subsp. *kurstaki* from rat by oral administration. Plant Protect. Bull. Taipei.; 37(3): 265-270.

Washington State Department of Health 1993. Report of health surveillance activities: Asian gypsy moth control program. Olympia, WA. 13-76.