EFFICACY OF LUFENURON, *BACILLUS THURINGIENSIS* AND SOYBEAN SEED OIL PLANT EXTRACT ON THE GENERAL BODY METABOLISM AND FAT BODY OF THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The effect of Lufenuron, *Bacillus thuringiensis* and Soybean seed oil plant extract on the proteins, lipids and glucose of the hemolymph and the fat body of the last larval instars of the cotton leafworm, *Spodoptera littoralis* were determined. The first larval instars were treated with *Bacillus thuringiensis var kurstaki* and Soybean seed oil plant extract but the second larval instars were treated with Lufenuron. In the untreated insects, the proteins and glucose levels were higher in the hemolymph than in the fat body. Moreover, the lipid level was lower in the hemolymph than in the fat body. In all treatments, a decrease in the proteins and glucose was observed with an increase in the lipid content of the hemolymph. On the other hand, the rate of biosynthesis of proteins, lipids and glucose content in the fat body was increased as compared to the untreated insects.

Electron microscopical observations of the fat body of treated last larval instars revealed that the fat body cells of larvae treated with Lufenuron and *Bacillus thuringiensis* possess autophagic vacuoles for protein storage with circular rough endoplasmic reticulum and mitochondria. The residual bodies and some protein granules were abundant in the vacuolated cytoplasm. However, the fat body cells of larvae treated with Soybean seed oil plant extract have multivesicular bodies for protein synthesis and secretion and less abundant autophagic vacuoles. The lipid droplets were observed. In all treated insects, disappearance of the cytoplasmic organelles and changes in the nuclear morphology were described.

INTRODUCTION

The polyphagous cotton leafworm, *Spodoptera littoralis* (Boisd.) was considered as the major cotton pest in Egypt, causing considerable damage to several important crops.

The extensive use of various conventional insecticides to control *Spodoptera littoralis* in Egypt has given rise to problems such as residual toxicity and pollution, development of pest resistance, rapid resurgence of target species, outbreaks of secondary pests and harmful effects on beneficial insects which are natural enemies of either target or non-target species. In order to avoid these hazards, there was great need to develop alternative safe control agents with new modes of action. Among these agents are the juvenile hormone analogues, as insect growth regulators, the plant extracts and different strains of *Bacillus thuringiensis*.

The efficacy of some new insect growth regulators against *Spodoptera littoralis* larvae was studied by numerous investigators Mesbah *et al.*, 1991; Ghoneim, 1994 and Shaurub *et al.*, 1998). Also, several plant extracts were used against a variety of Lepidopterous insects including *Spodoptera littoralis* by Abdalla and Sammour (1992) and Abdel- Aziz and El-Hawary (1997). The pathogenesis of *Bacillus thuringiensis* on Lepidopterous larvae were investigated by several authors (Dulmage and Martinez, 1973; El-Gemeiy, 1983 and Abdeen *et al.*, 1986).

Although it was well known that the insect fat body was the principal organ of intermediary metabolism as most hemolymph proteins were synthesized in the fat body and it also functions in the storage of proteins, lipids and carbohydrates (Chapman, 2003), studies on the fat body cells after the administration of insect growth regulators, bacteria or /and plant extract were very rare.

Consequently, the present work was undertaken to study the efficacy of insect growth regulator (Lufenuron), bacteria (*Bacillus thuringiensis var kurstaki)* and plant extract (Soybean seed oil plant) on the hemolymph and fat body of *Spodoptera littoralis*. Also, the ultrastructural changes in the fat body cells in accordance with the effect of the mentioned compounds and the changes in the main body metabolites were described.

MATERIAL AND METHODS

Insect culture

Spodoptera littoralis (*S. littoralis*) were provided from the Department of Plant Protection Research Institute, Dokki-Giza. Successive generations of *Spodoptera littoralis* colony were maintained at 25±1°C and 70±3%R.H. from the cotton leaf worm. The susceptible larvae were fed on castor leaves, *Ricinus communis*, while the adults were fed on 20% sucrose solution.

Treatment and bioassays

1. Lufenuron (Match- CGA-184699), a chitin synthesis inhibitor, was applied to determine the LC50 in a preliminary experiment, using the leaf dipping technique according to Ishaaya and Klein (1990). Meanwhile, the 2nd larval instars were allowed to feed on IGR-treated castor leaves according to El-Bermawy (2005) and the LC50 was calculated as 0.1ppm.

2. Newly moulted 1st instar larvae of *S. littoralis* taken from the culture were fed on a contaminated castor leaves containing 0.05% (LC50) of *Bacillus thuringiensis var kurstaki* (Diple-2x 32.000 units WP.) (*B.thuringiensis*) according to Mohamed *et al.* (2000).

3. Soybean seed oil plant extract was applied at the rate of 5% (LC50) to the newly moulted 1st instar larvae of *S.littoralis* according to Badr *et al.* (2000).

Physiological studies

Sample preparation

Samples were obtained from the untreated (control) and the treated last larval instars which were offered, Lufenuron, *B. thuringiensis* and Soybean seed oil plant extract. For this purpose hemolymph of the last larval instar was collected (after cutting a proleg, on ice) in apropylene microcentrifuge tube containing few crystals of phenylthiourea to prevent melanization. The hemolymph was centrifuged at 3000r.p.m. for 5min. at 4°C to remove hemocytes. The fat body was dissected out and washed in distilled water. All the hemolymph and the fat body samples were kept at -20°C until analysis.

Determination of the main metabolites

The protein content was determined from diluted hemolymph samples and fat body extracts using Lowry method (Lowry *et al.*, 1951).

The lipid extraction was carried out according to the technique of Folch *et al.* (1957) and the lipid estimation was calculated depending on Knight *et al.* (1972) calculation.

The glucose content in the hemolymph and fat body was measured by the glucose oxidase reagent described by Robyt and White (1987).

Statistical analysis

Data obtained for the test significance of difference between means were analyzed using the student's distribution, refined by Bessel correction (Moroney, 1956).

Ultrastructural studies

The three samples of the last larval instars resulting from the treated 1st and 2nd larval instars with the Lufenuron, *B.thuringiensis* and Soybean seed oil plant extract were inflated with 2.5% glutaraldhyde for 5min. The perivisceral fat body was dissected using the same fixative at 4°C. The tissues were prepared for the ultrastructural examination according to Mostafa *et al.* (2003). The examined treated samples were compared to the untreated ones which were previously studied by Mostafa *et al.* (2003).

RESULTS AND DISCUSSION

Effect of larval treatment on the protein content

Spectrophotometric analysis of the last larval instars of untreated individuals (control) showed an increase in the protein content of hemolymph than in the fat body (7.7± 0.88, 4.105 ± 0.41,respectively) (Fig.1). These results were in agreement with Tojo *et al.* (1978&1980); Rashad *et al.* (2003). The authors stated that the concentrations of storage proteins increased in the hemolymph of the final larval instars in case of *Hyalophora cecropia*, *Bombyx mori* and *Spodoptera littoralis*, reaching maximal levels at the time of spinning. They also reported that during larval-pupal transformation, these protein levels decreased in the hemolymph with simultaneous increase in the fat body. Similar results were reported by Rostom *et al.* (1992) in case of *Pectinophora gossypiella* where the concentrations of proteins in the hemolymph were higher as compared to that in the fat body.

When the last larval instars were treated with *B. thuringiensis* and Soybean seed oil plant extract, the hemolymph protein (6.745±0.97, 7.175±0.99) was higher than the fat body protein content (6.44 ± 0.6, 5.905± 0.42). Meanwhile, the hemolymph protein was lower than in the fat body when larvae were treated with Lufenuron (4.55 ± 0.86, 6.77± 0.63) (Fig.1). Analysis of the results showed that, Lufenuron and *B. thuringiensis* caused significant ($P<0.05$) reduction in the hemolymph protein of treated last larval instar as compared to the untreated ones. While Soybean seed oil plant extract caused a non significant ($P>0.05$) reduction in it.Lufenuron was more effective than *B. thuringiensis* for reducing protein in the last larval instar hemolymph. Moreover, Lufenuron, *B. thuringiensis* and Soybean seed oil plant extract caused significant ($P<0.05$) increment in the last larval fat body proteins as compared to the control (Fig.1). In this respect, Zeenath and Nair (1994) concluded that the treatment of *Spodoptera sp.* with JH analogues resulted in the

inhibition of accumulation of total protein in the hemolymph. Anita-Mane *et al.* (1998) also, recorded the reduction of hemolymph protein after the treatment of *Spodoptera littura* by Fenoxycarb and Methoprene. Ghoneim *et al.* (2003) when treated the prepupa of *Rhynchophorus ferrugineus* with different doses of Lufenuron and Dioflenolan found that both IGRs caused detrimental reduction of the total protein content, irrespective of the dose-level. El Bassiony *et al.* (2005) reporteded considerable reductions of hemolymph proteins of 3rd larval instar of *Cephalopina titillate* treated with Pyriproxyfen and Chlorfluazuron. El-Bermawy (2005) concluded that Cascade and Match affected the hemolymph protein pattern of *Spodoptera littoralis* larvae.

On the contrary, Amer (1990) detected an increase in hemolymph and fat body protein content in *Spodoptera littoralis* after larval treatment with Mevalonic acid. Ghoneim (1994) found that the same compound, separately or combined with the chitin inhibitor IKI-7899 caused, almost, similar effect on the same lepidopteran hemolymph and fat body. El-Sherif (1995) stated that these metabolic features were considered as indirect juvenilizing action of insect growth regulators. Ibrahim and Amin (2002) recorded a considerable increment of protein throughout different developmental stages in *Spodoptera littoralis* treated with Precocenes. Ibrahim (2006) found that treatment of the 2nd larval instar of *S. littoralis* with Flufenoxuron caused reduction in the total protein content of the resulting pupae.

Results concerning the efficacy of *B.thuringiensis* on the protein content were in agreement with those reported by Salama *et al.* (1983), Abou El Ela *et al.* (1991) in case of *Spodoptera littoralis* larvae and *Plodia interpunctella* after treatment with *B. thuringiensis*. Also, Sabbour (2001) proved the marked decrease in the hemolymph protein of larvae of *Earias insulana* when fed on a diet containing *B. thuringiensis*. Contradictory results were registered by Abdel-Razek *et al.* (2004) for *Rhynchophorus ferrugineus* larvae treated with nematode-bacteria complex resulting in a reduction in the fat body protein.

Larvae treated with Soybean seed oil plant extract showed a significant increment occurring in the fat body protein content. This may be an attempt by the larval protein to synthesize microsomal detoxifying enzyme which assist to detoxify the toxicants that entered into the animal body (Wilkinson, 1976). Similar results were observed by Shoukry and Hussien (1998), the authors found that two plant oil extract decreased the total protein content in the last larval instar of *Galleria mellonella*. Ibrahim (2006) detected increment in the total protein content of the

pupae of *S. littoralis* after treatment of the 2nd larval instar with seeds extracts of *Entrolobium cyclocarpum*.

Effect of larval treatment on the lipid content

Data depicted in Fig. (1) showed that the lipid content in the hemolymph (0.398 ± 0.11) was lower than the fat body (3.258 ± 0.74) of untreated individuals. Non significant (P>0.05) increase was observed in the hemolymph lipid content (0.925±0.10, 0.650 ±0.13, 0.438 ±0.13) when larvae were treated with Lufenuron, *B. thuringiensis* and Soybean seed oil plant extract, respectively as compared to the control. Meanwhile in the fat body, lipid content highly increased (P<0.01) (10.199 ± 0.94, 10.619 ± 0.98, 9.355 ± 0.92, respectively) as compared to the control. Comparison between the two treated tissues, showed that the lipid content in the fat body was highly significant (P<0.01) than in the hemolymph.

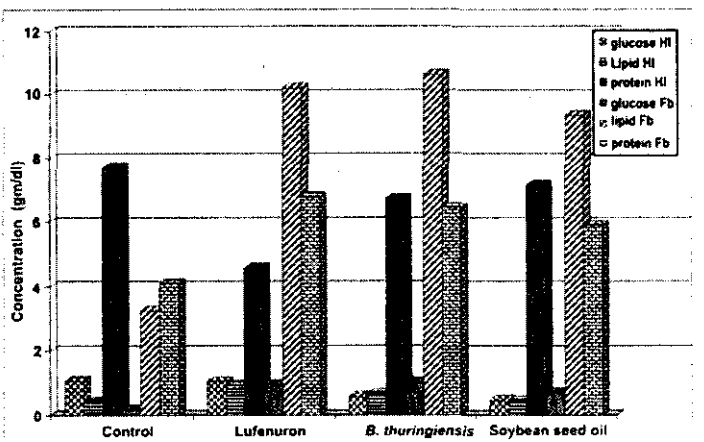

Fig.(1): The efficacy of Lufenuron, *B. thuringiensis* and Soybean seed oil plant extract on glucose, total lipid and total protein concentration (gm/dl) in the hemolymph (Hl) and fat body (Fb) of the last larval instars of *S. littoralis*.

In the present study, Lufenuron increased the lipids of the hemolymph and fat body. Another IGR, Fenoxycarb, affected the lipid synthesis in the fat body during the 6-day experimental period of *Choristoneura fumiferana* (Mulye and Gordan, 1993). Ghoneim (1994) reported a significant increments of lipid content throughout the pupal stage of *Spodoptera littoralis* by the larval treatments with Mevalonic acid or IKI-7899. Moreover, Ibrahim (2006) indicated that Flufenoxuron increased the total lipid content in the different ages of *S. littoralis*. Disagreement with our results, Ghoneim *et al.* (2003) found that Lufenuron and Diofenolan reduced the lipid content of the pupal stage of *Rhynchophorus ferrugineus*.

The decrease in the total lipid content in the hemolymph of *Spodoptera littoralis*, *Popillia japonica* and *Rhynchophorus ferrugineus* larvae after treatments with *Bacillus thuringiensis*, *Bacillus popilliae* and nematode-bacteria complex, respectively might be due to the fact that the infested larvae may produce enzymes that utilize lipids from the hemolymph in an effort to remove invading organisms (Boctor and Salama, 1983; Abdel-Razek *et al.*, 2004). Results obtained during this study on total hemolymph lipids disagreed with these findings. On the other hand, the increase in the total lipids of fat body of treated larvae might be due to the conversion of some proteins to fats (Raina, 1980).

The results obtained in lipid content due to Soybean seed oil plant extract were in agreement with those of Hill and Izatt (1974) who reported that lipid accumulation was more likely to be related directly to a lack of juvenile hormone. The tested treatment might cause degeneration in corpora allata which may allow elevation in the mean total lipids (El- Bokl *et al.*, 1998).

Effect of larval treatment on the glucose content

According to the results represented in Fig. (1), the hemolymph glucose (1.03 ± 0.23) was higher than the fat body glucose level (0.152 ± 0.06) in untreated last larval instar. Meanwhile, the glucose level in the hemolymph (1.025 ± 0.19) was higher than in the fat body (0.931 ± 0.14) if larvae were treated with Lufenuron. While it was lower in the hemolymph (0.544 ± 0.05, 0.45 ± 0.04) than the fat body (0.982 ± 0.2, 0.674 ± 0.16) when the larvae were treated with *B. thuringiensis* and Soybean seed oil plant extract, respectively. Thus the hemolymph glucose level decreased significantly ($P<0.05$) as the larvae was treated with *B. thuringiensis* and Soybean seed oil plant extract. Meanwhile, it decreased non significantly ($P>0.05$) in larvae treated with Lufenuron. The fat body glucose level increased as the larvae were treated with the three biopesticides.

The results obtained in the present study, on *S. littoralis*, evidently revealed the reducing action of Lufenuron, *B. thuringiensis* and Soybean seed oil plant extract on the hemolymph glucose content in the last larval instars. In contrast, each of these treatments promoted the fat body glucose content among the last larval instars. Literature cited various effects of several IGRs on this metabolite in different insect species, showing sometimes increasing content and sometimes decreasing one. The lowest dose of Lufenuron or Diofenolan caused a gradual decrease in carbohydrate content, but the highest and medium doses induced a reciprocal V-shaped trend in this metabolite throughout the pupal life of *R. ferrugineus* (Ghoneim *et al.*, 2003). Increased carbohydrate content in different times during the pupal stage of *S.*

littoralis was estimated by Ghoneim (1994) after larval treatment with the chitin inhibitor IKI-7899 and Mevalonic acid, separately or combined. As well as, significant increase of carbohydrate content were observed in larvae of *Spodoptera littoralis* by the JHA (Isopropyl 3, 7, 11-triethyl-2, 4-dodacadiote) and Kinorene (Fouda and Amer, 1990).

The changes in larval carbohydrates treated with *B. thuringiensis* have been studied in the hemolymph and fat body by many authors (Abou El-Ela *et al.*, 1991; Ghoneim, 1994; Abdel-Razek *et al.*, 2004). The decline in carbohydrate content may be due to utilization of carbohydrates stored as a reserve also, carbohydrate may contribute to protein synthesis (Abdel-Razek *et al.*, 2004).

Our findings concerning Soybean seed oil plant extract treatment were in agreement with Abou El-Ela *et al.* (1995) when larvae of *Musca domestica* were treated with water extracts of four plants. However, Abo El-GHar *et al.* (1995) showed that petroleum ether extract of two plant extracts fed to 6th instar larvae of *Agrotis ipsilon* greatly reduced the hemolymph carbohydrates.

Effect of larval treatment on the ultrastructure of the fat body

Ultrastructural studies of the fat body cells of untreated last larval instar showed a slightly reduced rough endoplasmic reticulum and mitochondria (Fig.2A). Early stages of autophagic vacuoles which contained rough endoplasmic reticulum and mitochondria were detectable (Fig.2B). The protein granules were mostly small (Fig.2C). The lipid droplets were large, with smooth contours and outlined by profiles of endoplasmic reticulum (Fig.2C). The cytoplasm became less dense around the protein granules and contained some glycogen granules (Fig.2A, C). The cytoplasm organelles, such as the endoplasmic reticulum and the mitochondria, became confined in a relatively small area of the cytoplasm. Along the cell membrane, the mitochondria and the rough endoplasmic reticulum disappeared (Fig.2C), but were restricted to regions between groups of protein granules and around the nucleus (Fig.2A, D).Golgi complexes were absent. The nucleus became elongated and contained large chromatin clumps (Fig.2D). Some lamellar bodies were observed in the cytoplasm (Fig. 2A) (Mostafa *et al.*, 2003).

The fat body vacuolar system can distinguish two kinds of vacuole function. These were vacuoles for digestion (autophagic vacuoles, multivesicular bodies, lamellate bodies, protein granules, phagocytic vacuoles and vacuoles digesting symbionts), and there were vacuoles for less drastic alteration of the contents, often in relation to storage (tyrosine storage and urate storage vacuoles,

urate granules, protein granules, secretory vesicle, vacuoles maintaining symbionts and vacuoles associated with glycogen) (Locke,1984).

When larvae were treated with Lufenuron, the mitochondria were absent or rarely observed as small and degenerated mitochondria. More pronounced late stages of autophagic vacuoles with circular rough endoplasmic reticulum and mitochondria were clear (Fig.3A). Figure (3B) showed the autophagic process of the rough endoplasmic reticulum by the autophagic vacuole and abundant residual bodies. However, *B. thuringiensis* induced highly reduction in the rough endoplasmic reticulum and the mitochondria in the fat body cells of the last larval instar. The mitochondria became swollen and did not display internal structural details. Late stages of autophagic vacuoles (with circular rough endoplasmic reticulum and mitochondria) and the residual bodies were prominent and widely distributed (Fig.4A). As in larvae treated with Lufenuron, autophagic process of the rough endoplasmic reticulum was clear and obvious (Fig.4A). On the other hand, Soybean seed oil plant extract did not affect the presence of the rough endoplasmic reticulum and the mitochondria, but, the autophagic vacuoles were absent or small and autolysed. The residual bodies were rare or evenly absent (Fig.5A).

The present study showed that larvae treated with Lufenuron possessed highly vacuolated cytoplasm with small protein granules. Moderate oval nucleus with sporadically clumps of chromatin was detectable and glycogen granules were deposited in the cytoplasm (Fig.3A). Golgi complexes were absent. Figure (3C) illustrated the highly vacuolated cytoplasm. Meanwhile, figure (3D) revealed the presence of large lipid droplets. Thus the existence of lipid vacuoles suggesting little activity (Chen *et al.*, 1975). Radwan (1995) studied the effect of Pyriproxyfen on the ultrastructure of the fat body cells of *Nezara viridula*. She detected fewer and larger lipid droplets with relatively changed nuclear shape. While, extreme effect of Pyriproyfen resulted in the nuclei elongation with condensed chromatin. The cells exhibited stages of organelles degeneration with increasing in vacuoles.

In the larvae treated with *B. thuringiensis*, there were many large lipid droplets which were outlined by profiles of endoplasmic reticulum (Fig.4B) as compared to the untreated individuals. In the cytoplasm, the nucleus was elliptoid with sporadically chromatin clumps (Fig.4C). Mitochondria were small, oval and abundant near the cell membrane and in between the numerous protein granules. The protein granules were non-crystalline with distorted shape (Fig. 4B). Golgi complexes were absent. Figure (4D) showed highly deposited glycogen granules as compared to the control. Abu-Hakima and Faye (1981) found that the fat body cells

from the *Hyalophora cecropia* injected with bacteria contained abundant rough endoplasmic reticulum and Golgi bodies, whereas those from wounded and untreated controls do not. They stated that the fat body was the only tissue that responds to bacterial injection by increased incorporation of 3H-uridine into RNA. These findings support the idea that the fat body was the main site of synthesis of the immune proteins.

The fat body of the larvae treated with Soybean seed oil plant extract contained lipid droplets which were outlined by rough endoplasmic reticulum as compared to the control (Fig.5B). They were larger and more abundant than in the untreated larvae. The cytoplasm was vacuolated in between the small protein granules. It contained moderate nucleus with chromatin surrounded by many small rounded mitochondria and rough endoplasmic reticulum. Granules of glycogen, degenerated mitochondria and lysis of rough endoplasmic reticulum were near the cell membrane with the presence of many ribosomes (Fig.5A). Multivesicular body was rare between the protein granules while, autophagic vacuoles were degenerated or absent (Fig.5B). The protein granules and the lipid droplets were present with different shapes (Fig.5C). Residual bodies were rare or absent.

The marked changes in the nuclear morphology in the present work were similar to those demonstrated by Nanya and Bicudo (1995) in the fat body of *Drosophila mullesi*. These authors detected that nuclear areas were larger in stages of high protein synthesis and smaller when that synthesis level decreased. Also, the change in nuclear size was correlated with the amount of DNA (Butterworth *et al.*, 1988). In addition, the enlargement of the nuclei in the treated fat body cells might be due to the increased transcription rates since this was associated with increased size (Locke, 1985). However, nuclear enlargement was also linked with increased poloidy levels (Kooman and Nair, 1982). Willlott *et al.* (1988) stated that acquisition of Golgi complex and rough endoplasmic reticulum concomitant with the time of major protein production. They noticed that loss of many cellular organelles such as Golgi complex and rough endoplasmic reticulum as protein production drastically decreased. The protein reductions suggested a hormonal activity of Lufenuron during insect development as mentioned by (Ueno *et al.*, 1983; Ghoneim *et al.*, 2003). Other IGRs also interfered with the insect endocrine system causing a hormonal imbalance (Richards, 1981). Yin *et al.* (1989) determined that the protein increments might be due to the failure in the insect tissues to uptake the produced and released proteins from fat body and hemolymph during the pupal or adult apolysis.

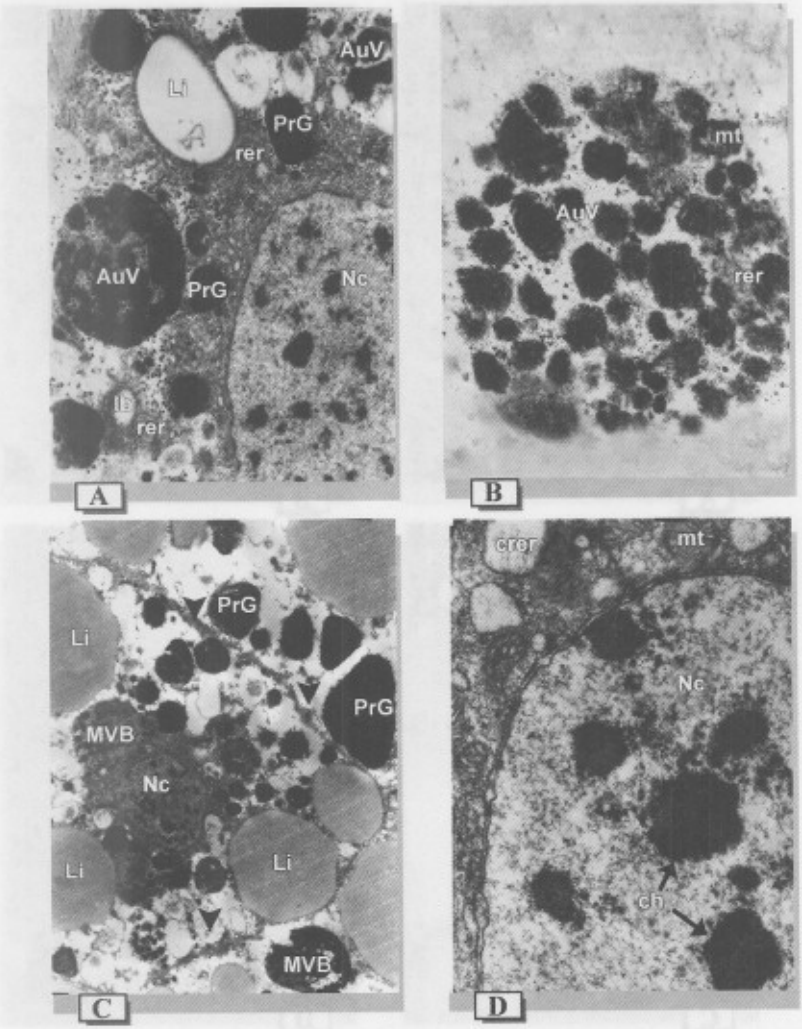

Fig. (2-A, B, C& D): Electron micrograph in the fat body of the last larval instar of *S. littoralis*: (A) Showing elongated nucleus (Nc) with chromatin clumps, non-crystalline protein granules (PrG) and rough endoplasmic reticulum (rer) and mitochondria (mt) around the nucleus and between protein granules. Autophagic vacuoles (AuV) appear. Lamellar bodies (lb) and Lipid droplets (Li) were also observed (X 7500). (B) Showing the main contents of an autophagic vacuole (AuV) which contains mitochondria (mt) and rough endoplasmic reticulum (rer) (X 20000). (C) Showing the nucleus (Nc) surrounded by multivesicular bodies (MVB), non-crystalline protein granules (PrG), Lipid droplets (Li). Cell membrane (arrow head) appears between fat cells (X 2500). (D) Showing the nucleus (Nc) with large chromatin clumps (ch) surrounded by many of mitochondria (mt) and circular rough endoplasmic reticulum (crer) (X15000).

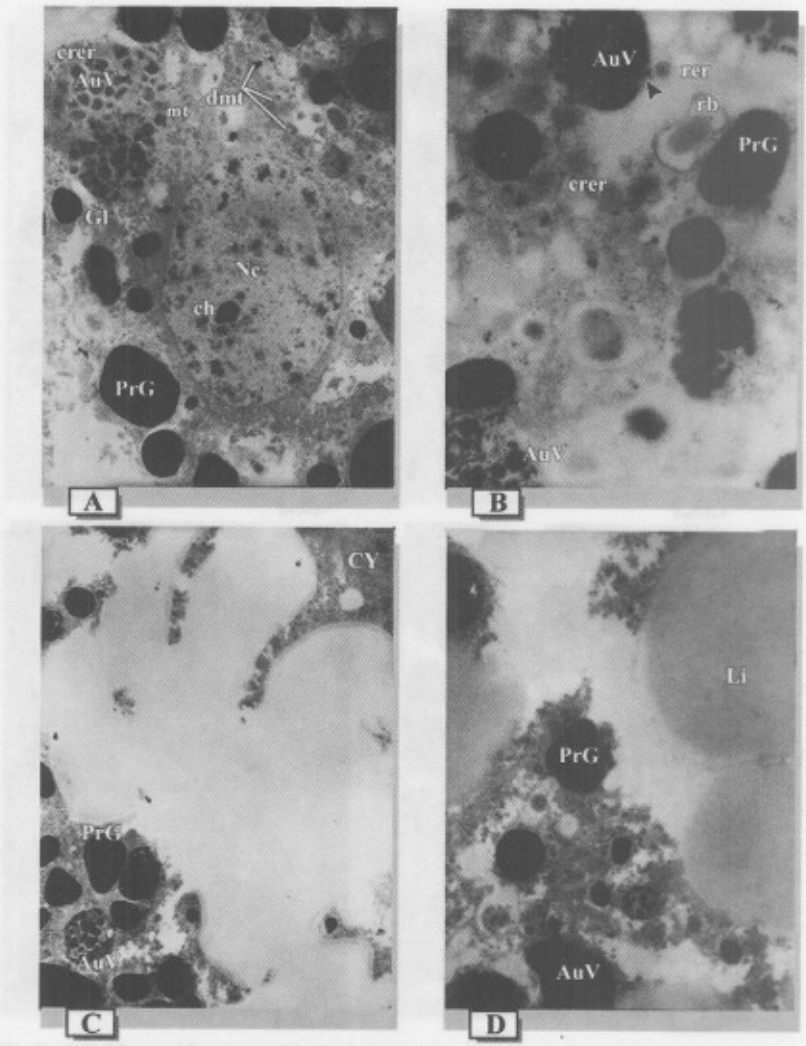

Fig. (3-A, B, C & D): Electron micrograph in fat body of the last larval instar of *S. littoralis* treated as 2nd instar with Lufenuron. (A) Showing degenerated mitochondria (dmt), autophagic vacuoles (AuV) with circular rough endoplsmic reticulum (crer) and mitochonria (mt). Oval nucleus (Nc) with sporadically clumps of chromatin (ch), glycogen granules (Gl) deposited in the cytoplasm and protein granules (PrG) were detected (X 4000). (B) Showing the autophagic process (arrow head) of the rough endoplasmic reticulum (rer) by the autophagic vacuole (AuV). Residual bodies (rb), circular rough endoplasmic reticulum (crer) and protein granules (PrG) were in the cytoplasm (X 7500). (C) Showing the vacuolated cytoplasm (CY) with many protein granules (PrG) and autophagic vacuole (AuV) (X 3000). (D) Showing large lipid droplet (Li) (X 3000).

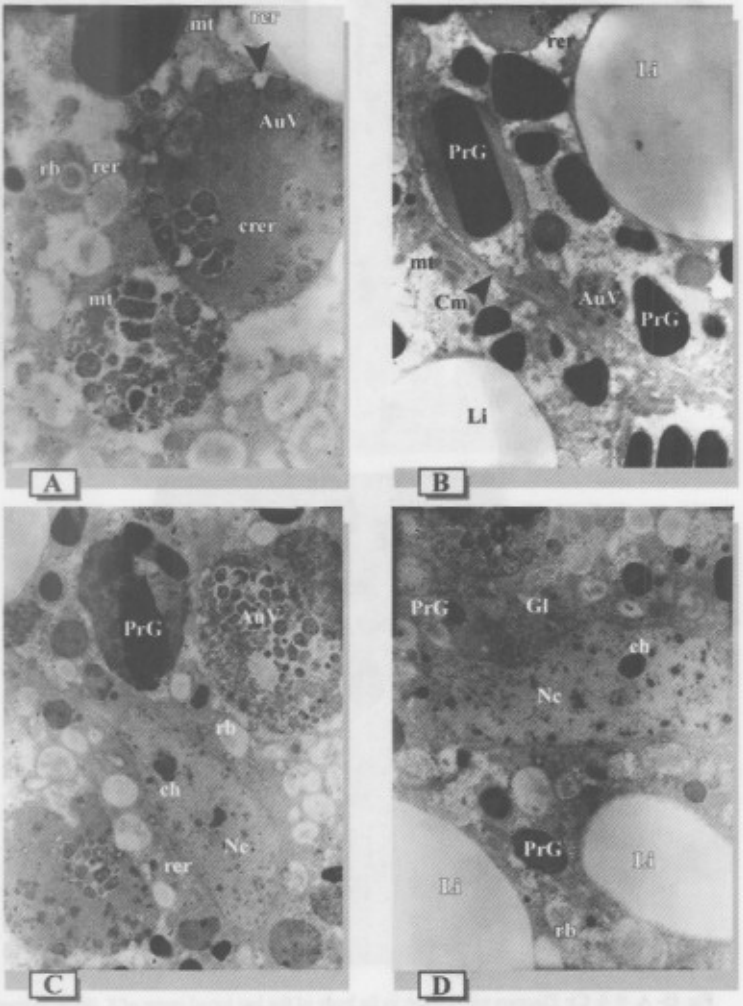

Fig. (4- A, B, C & D): Electron micrograph in fat body of the last larval instar of *S. littoralis* treated as 1st instar with *B. thuringiensis*. (A) Showing autophagic vacuoles (AuV) with circular rough endoplsmic reticulum (crer) and mitochondria (mt). The autophagic process (arrow head), the residual bodies (rb) and reduced number of mitochondria (mt) and rough endoplsmic reticulum (rer) (7500). (B) Showing many large lipid droplets (Li) outlined by rough endoplsmic reticulum (rer). Abundant crystalline and distorted protein granules (PrG), mitochondria (mt) and autophagic vacuole (AuV) near the cell membrane (Cm) (X 4000). (C) Showing elliptical nucleus (Nc) with sporadically chromtin clumps (ch) (X 4000). (D) Showing highly deposited glycogen granules (Gl) (X 5000).

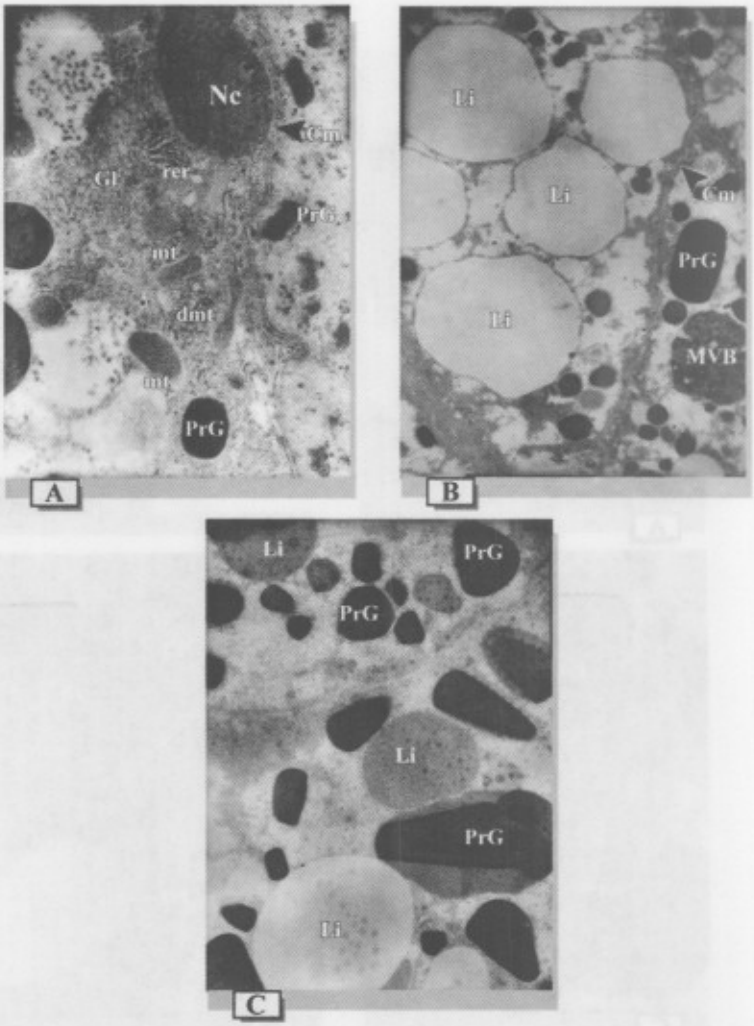

Fig. (5- A, B & C): Electron micrograph in fat body of the last larval instar of *S. littoralis* treated as 1st instar with Soybean seed oil plant extract. (A) Showing abundant rough endoplasmic reticulum (rer), mitochondria (mt) and nucleus (Nc) with chromtin (ch). Degenerated mitochondria (dmt) and glycogen granules (Gl) near the cell membrane (Cm) (X 15000). (B) Showing many lipid droplets (Li), protein granules (PrG) and multivesicular body (MVB) near the cell membrane (Cm) (X 3000). (C) Showing distorted protein granules (PrG) and lipid droplets (Li) (X 4000).

In many insects, the fat body contains only a single cell type, the trophocyte, adapted primarily for protein synthesis and secretion, lipid and glycogen metabolism, and intermittently for the storage and breakdown of protein and urate.

Reviewing the obtained results, it concluded that: the protein in the fat body was lower than the protein in the hemolymph of untreated last larvae of *S. littoralis* because, these proteins were synthesized in the fat body of insects in their last larval stage and secreted in the hemolymph shortly before pupation, storage proteins were taken up by fat body tissue and stored as protein granules (Levenbook, 1985; Mostafa *et al.*, 2003). Lufenuron and *B.thuringiensis* caused protein accumulation and autophagic activity in the fat body. This was confirmed by the presence of autophagic vacuoles, protein granules and deformed or disappearance of mitochondria. On the other hand, Soybean seed oil plant extract decreased the protein content in the fat body than in the hemolymph which induced it to synthesize protein and to secrete into the hemolymph. This was confirmed by the presence of multivesicular bodies and mitochondria.

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