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HISTOCHEMICAL EFFECTS OF ABAMECTIN AND *Bacillus thuringiensis* ON THE MIDGUT AND THE FAT BODIES OF THE COTTON LEAFWORM, *Spodoptera littoralis*(LEPIDOPTERA: NOCTUIDAE) LARVAE.

MAGDA MOHAMED ABUO-EL-MAHASEN
Department of Zoology, Faculty of Science
Menoufiya University , Shebin El-Kom, Egypt.

ABSTRACT

The histochemical effects of thuringiensin , B-extotoxin of *Bacillus thuringiensis* and abamectin (Avermectin B_1) upon the midgut and the fat bodies of the cotton leafworm, *Spodoptera littoralis* (Boisd) Larvae were investigated. Fourth-instar Larvae were fed for 3 days on castor bean leaves treated with two sublethal concentrations (0.0001 and 0.01ppm) of each compound. This study showed that the two tested compounds (biological agents) reduced the polysaccharides , the lipid , the protein content and the synthesis of RNA after 3 days from treatment. However no appreciable difference could be observed for the synthesis of DNA . Also , this study showed that *B. thuringiensis* was more effective than abamectin.

INTRODUCTION

The cotton leafworm, *S. littoralis* (Boisd) is a polyphagous noctuid that is a serious pest on several agriculturally important crops in Egypt. Control programmes of this insect mostly depend on chemical control methods by various insecticides. The increasingly serious problems of pest resistance to insecticides and of contamination of the biosphere associated with the large-scale use of broad spectrum synthetic pesticides have dicated the need for effective biodegradable insecticides with greater selectively . (Saxena, 1983).

Plant –derived natural products have been used for human health and plant protection. An additional source of natural materials, with an excellent history of success for human and animal health products, has been metabolites of soil microorganisms. Examples include erythromycin, streptomycin , tobamycin and tylosin. However, the development of such metabolites for crop protection has resulted in few products of commercial value. The most notable product to date is abamectin (avermectin) which is isolated from the fermentation of soil bacteria, *Streptomyces avermitilis* (Burg et al., 1979).

Abamectin showed high toxicity to a number of insect , mite and nematode pests (Putter et al., 1981 ; and Dybas et al ., 1989). The avermectins like many insect growth regulators that may exhibit growth- regulating activity (Wright, 1984). Putter et.al., 1981) described the formula for avermectin B_1 and reported that isolate, a though slow acting as a toxin at low dosages, adversely affected the reproduction of some insects. Physiologically, avermectin B_1 blocks post-synaptic potentials of neuromuscular junctions (Firstz et al., 1979). Campbell et al . , 1983) indicated that avermectin B_1 acts on the mediation of neurotransmission by gamma-amino butyric acid (GABA) leading to paralysis. In addition to its toxic and delayed activity, avermectin B_1 has been shown to inhibit feeding (Pienkowski and Mehring , 1983). Laboratory studies have shown that their modes of actions differ from those of currently used agricultural pesticides (Putter et al., 1981). Thus , these compounds could be useful efficacious in the control of pests that exhibit resistance to certain organophosphorus and carbamate pesticides.

Efforts for determining the delayed effects of abamectin on several insect speices are conducted currently.For example , in laboratory testes, exposure of insects to abamectin has resulted in increased mortality Bull, 1986), reduced feeding (Pienkowski and Mehring , 1983), disrupted development (Wright, 1984 , and Bull, 1986), blindness and torpidity (Agee, 1985) and impaired reproduction . Various reproductive) significant effects include damaged ovaries (Glancey et al., 1982 ,increased sterility, reduced pheromone production (Wright , 1984), reduced mating (Beach and Todd, 1985) and reduced fertility .

The biological effects of abamectin on *Spodoptera* sp. were studied by Anderson et al., (1986) on *Spodoptero. eridania,* Trumble et al. (1987) on *Spodoptero. exigua,* Christie and Wright (1990), Abo El-Ghar, et al ., (1995a) and Abdullah and Nassar (2001)on *S. littoralis* . The biochemical effects of abamectin on *S. littoralis* were studied by Christie and Wright (1991) and Abo El-Ghar et al., 1995b). The histopathological effects of abamectin on *S. littoralis* were studied by Abo El-Ghar et al., 1994).

Another such unconventional product is thuringiensin , the B- exotoxin produced by several varieties of *Bacillus thuringiensis* (Sebesta et al ., 1981). The toxin is an anolog of adenosine triphosphate (Benz, 1966)Thuringiensin inhibits DNA-dependent RNA polymerase , subsequently blocking cell mitosis(Sebesta et al.,1981). Sublethal doses of thuringiensin have produced deformities , particularly during developmental stages such as moulting, pupation and metamorphosis (Ignoffo and Gregory, 1972 ; Maciejewska et al., 1988).

Thuringiensin acts an inhibitor for protein synthesis through interference of DNA-dependent RNA polymerase by structurally mimicking ATP and competing for binding site (Sebesta et al., 1981) Toxic effects of B-exotoxin in insects include mortality , problems associated with moulting processes, teratological abnormalities and reduced fecundity (Ignoffo and Gregory, 1972). B-exotoxin may act as a feeding deterrent of larvae of several Lepidoptera species (Herbert and Harper , 1987).

The biological, biochemical and histopathological effects of thuringiensin on *S. littoralis Larvae* were studied by Abo El- Ghar et al., 1995 a,b and 1994 , respectively . Histochemical studies on insects are considered one of the most specific and interesting types of investigation . The histochemistry of the insect midgut and the fat bodies have received very little attention. However the very few investigations of histochemical effects of insecticides on insects have been carried out . Hamed et al.(1974) studied the histochemical effects of DDT on the Larvae of *Anopheles pharoensis* . Saleem and Shakoori (1985) studied the effect of permethrin and malathion on DNA, RNA and total protein content in *Tribolium castaneum* larvae. Paul et al. (1991) studied the effect of some insecticides on protein histochemistry of the testis of *Dracrisia oblique* Walker. Assar and Emara (1997) studied the histochemical effects of dimilin on the midgut and the integument of *S. exigua.* Shaurub et al., (1998) studied the histochemical effects of pyriproxyfen on localization and distribution of nucleic acids , protein , carbohydrate and lipid in the ovarioles and testis of *S. littoralis* treated as larvae. Assar and Abo-Shaeshae (2004) studied the histochemical effects of pyriproxyfen and hexaflumuron on the protein and nucleic acids in the midgut ,the fat bodies and the integument of *Musca domestica* larvae. Assar (2003) studied the histochemical effects of some botanical extracts on the midgut of *S. littoralis* larvae. Assar (2004a) studied the histochemical effects of abamectin on the house fly, *M. domestica.* (Assar (2004b) studied the histochemical and biochemical effects of abamectin and spinosad on the flesh fly, *Parasarcophaga aegyptiaca* Salem.

The present study was carried out to investigate the histochemical effects of abamectin and thuringiensin on the midgut and the fat bodies of *S. littoralis* larvae with reference to carbohydrates (polysaccharides) , proteins , lipids and nucleic acids.

MATERIALS AND METHODS

A: Insect culture:

The cotton leafworm *S. littoralis* (Boisd) used in this work was obtained from a susceptible laboratory strain reared at the Zoology Department Faculty of

Science Menoufiya University . The insects were maintained at 27±2 ^{0}c and 65±5%. R.H. The larvae were reared on castor bean leaves (*Ricinus communis*), while the adults were fed on sugar solution.

B- Chemicals :

The compounds used were formulations of thuringiensin (B-exotoxin of *Bacillus thuringiensis,* ABG 6162A; 1-5% wt/wt emulsifiable concentrate ; and abamectin as emulifiable concentrate (EC) (MK-936, 113M12; 1.8% wt/vol) . These compounds were obtained from the Ministry of Agriculture (Egypt). The test materials were diluted with water to stock solutions and appropriate water-diluted concentrations were prepared freshly before treatments.

3- Treatment procedure :

The treatment was done by dipping castor oil leaves in the appropriate concentrations (0.0001) and 0.01ppm). The treated leaves were allowed to dry at room temperature ; the leaves were offered to the 4th instar larvae for only 72 hours and then replaced by untreated leaves. The larvae fed on untreated leaves served as control .

4- Histochemical studies :

After 72 hr of the treatment of the larvae with *B. thuringiensis* and abamectin . Some larvae were dissected in Ringers saline solution. Parts of the midgut and the fat bodies were quickly removed and dropped in the appropriate fixatives. Carnoy's fluid was suitable for the fixation of proteins and nucleic acids. Total proteins were detected according to the mercury bromphenol blue method (Bonhag , 1955) . Proteins appear blue colour . Nucleic acids were demonstrated using Schiff-Feulugen methylene blue reaction (Garvin et al. , 1979). DNA stains red to purple while RNA stains blue.

Polysaccharide material was demonstrated following the application of periodic acid Schiff's technique (PAS) (Hotchkiss , 1948). Materials were fixed in alcoholic bouin. The PAS-positive material appears pink or red violet.

For the demonstration of lipids, good results were obtained from paraffin wax (preriously fixd in cobalt nitrate for one week and post-chromed in 3% postassium dichromate for 2 days (McManus, 1946). The hydrated sections stained in saturated soulution of sudan black B in 70% ethyl alcohol for 30 minutes, differentiated in 70% ethanol, washed in distilled water and mounted in glycerin jelly. Lipids aquiver a black or dark blue colour.

RESULTS

I-The carbohydrates (polysaccharides):-

A large amount of polysaccharide material was observed in the midgut cells (Fig 1) and in the fat bodies (Fig 4) of control larvae of *S. littoralis* , as indicated by the strong (marked) PAS-positive reaction given by these cells.

Following 72 hours of treatment, a noticeable reduction in polysaccharide content was observed in the midgut and the fat cells, as compared to the control . *B. thuringensis* at. 0.0001ppm induced a moderate reaction with PAS in the midgut cells(Fig 2a), while at 0.01ppm induced a marked decrease in polysaccharide (Fig 2b). Also , *B. thuringensin* at 0.0001ppm induced a moderate reaction with PAS in the fat bodies (Fig 5a), while at 0. 01ppm produced a marked decrease in polysaccharide (Fig 5b). Abamectin at 0.0001ppm induced a moderate reaction with PAS in the midgut (Fig 3a) and in the fat cells (Fig 6a), while at 0.01ppm produced a marked decrease in polysaccharide in the midgut and the fat cells (Fig 3b and 6b, respectively). The decreased level of polysuccharides on the midgut and the fat cells was more by *B. thuringensis* than that by abamectin.

2- The Lipids :

The histochemical findings after staining with sudan black B indicated the presence of lipid substance in the cytoplasm and the nuclei of midgut (Fig 7) and the fat cells (Fig 10). The lipid content in the midgut (Fig 8a) and in the fat cells (Fig 11a) highly effected by treatment with *B. thuringiensis* at 0.0001ppm. The effects were dlose dependent . The lipid content in the midgut (Fig 9a) and in the fat cells (Fig 12a) slightly affected by treatment with abamectin at 0.0001ppm, while the lipid content decreased in the midgut (Fig 9b) and in the fat cells (Fig 12b) by treatment with abamectin at 0.01ppm.

LIST OF ABBREVIATIONS

Cm: Circular muscles: Lu: Lumen
DNA: Deoxy ribnucleic acid: Nu: Nucleus
Ep: Epithielium: Po: Polysaccharides
Fc: Fat cell: Pr: Protein
Li: Lipid: RNA: Ribnucleic acid
Lm: Longitudinal muscles: Va: Va

Cm	Circular muscles	Lu	Lumen
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Ep	Epithielium	Po	Polysaccharides
Fc	Fat cell	Pr	Protein
Li	Lipid	RNA	Ribnucleic acid
Lm	Longitudinal muscles	Va	Va

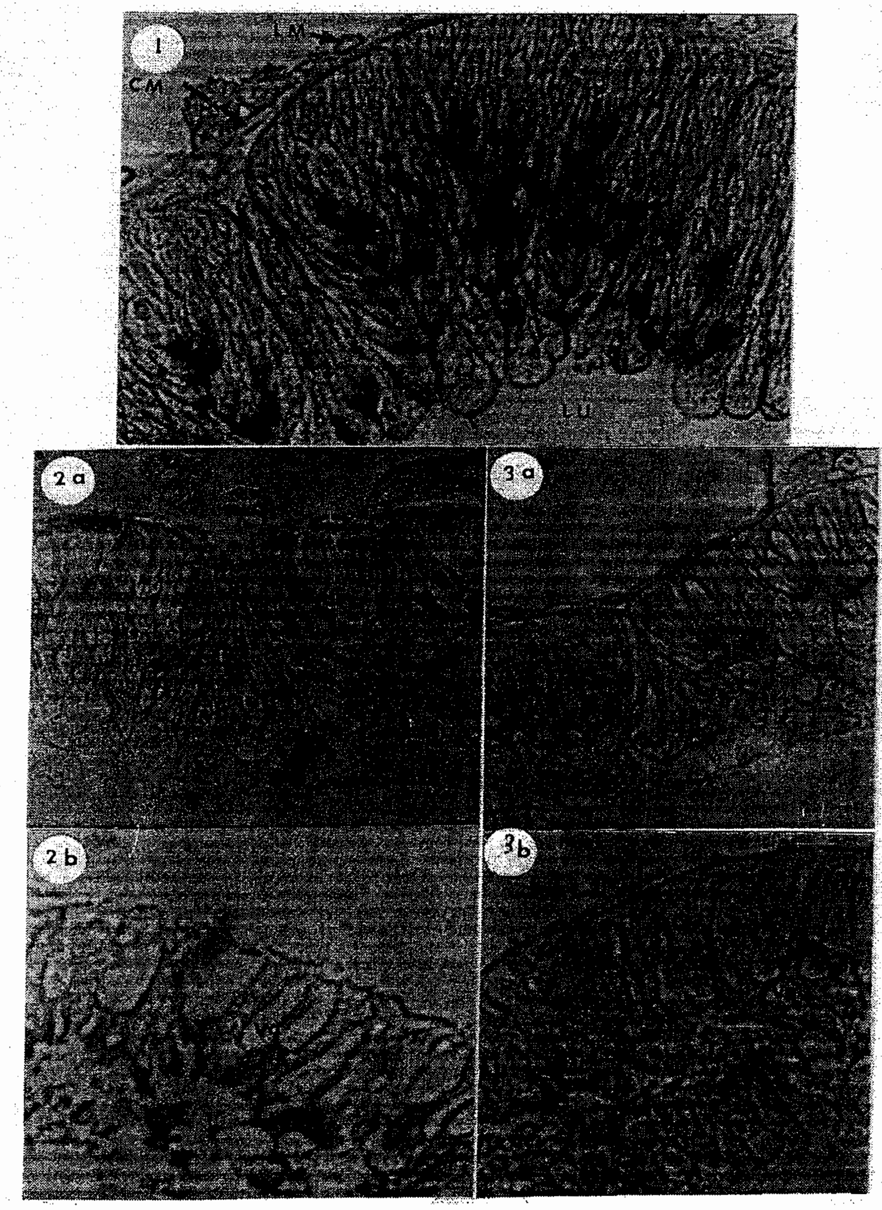

Fig. (1): Midgut section of control larvae stained by PAS showing polysaccharides particles (red colour).
Fig. (2): Midgut section of larvae treated with *B. thuringiensis.* (a): 0.0001ppm. (b):0.01ppm.
Fig. (3): Midgut section of larvae treated with abamectin. (a): 0.0001ppm. (b): 0.01ppm.

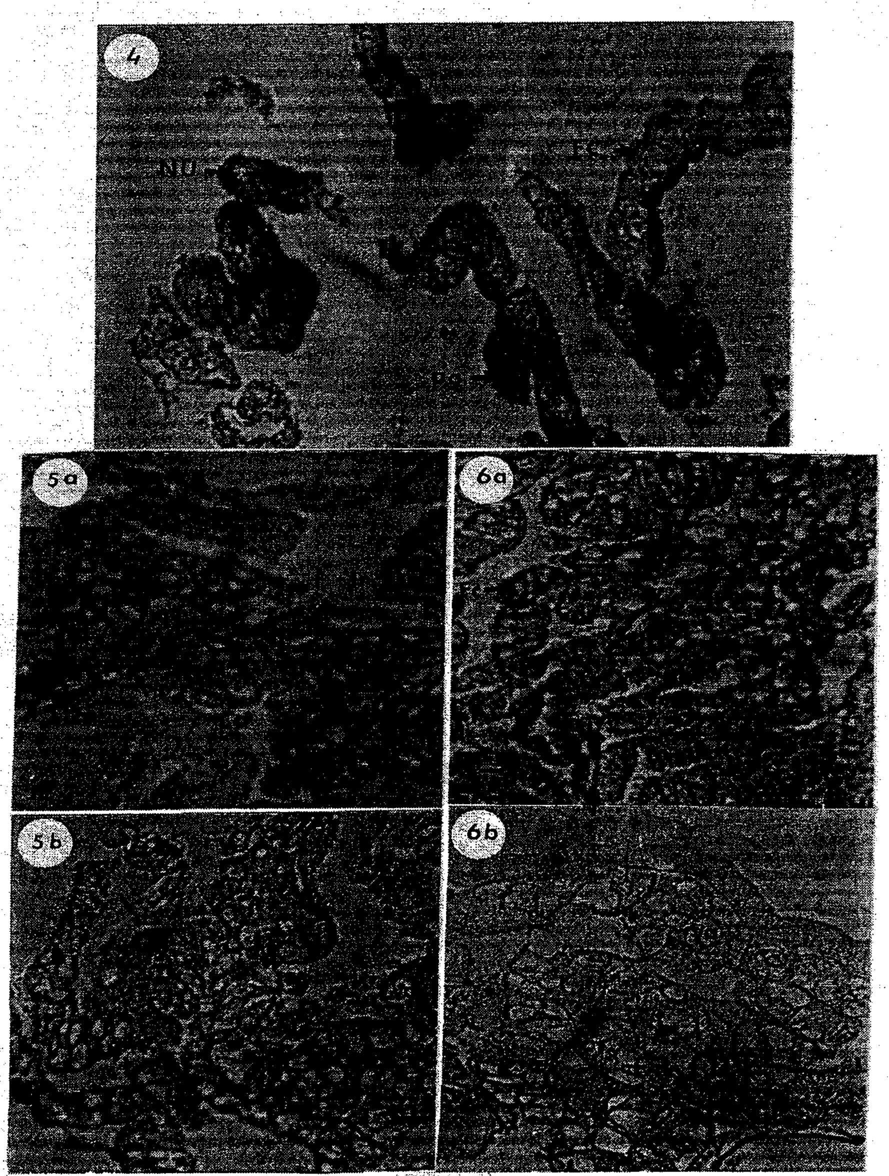

Fig. (4): Section in the fat body of control larvae stained by PAS showing polysaccharides particles (red colour).
Fig. (5): Section in the fat body of larvae treated with *B. thuringiensis.* (a): 0.0001ppm: (b): 0.01ppm.
Fig. (6): Section in the fat body of larvae treated with abamectin. (a): 0.0001ppm: (b): 0.01ppm.

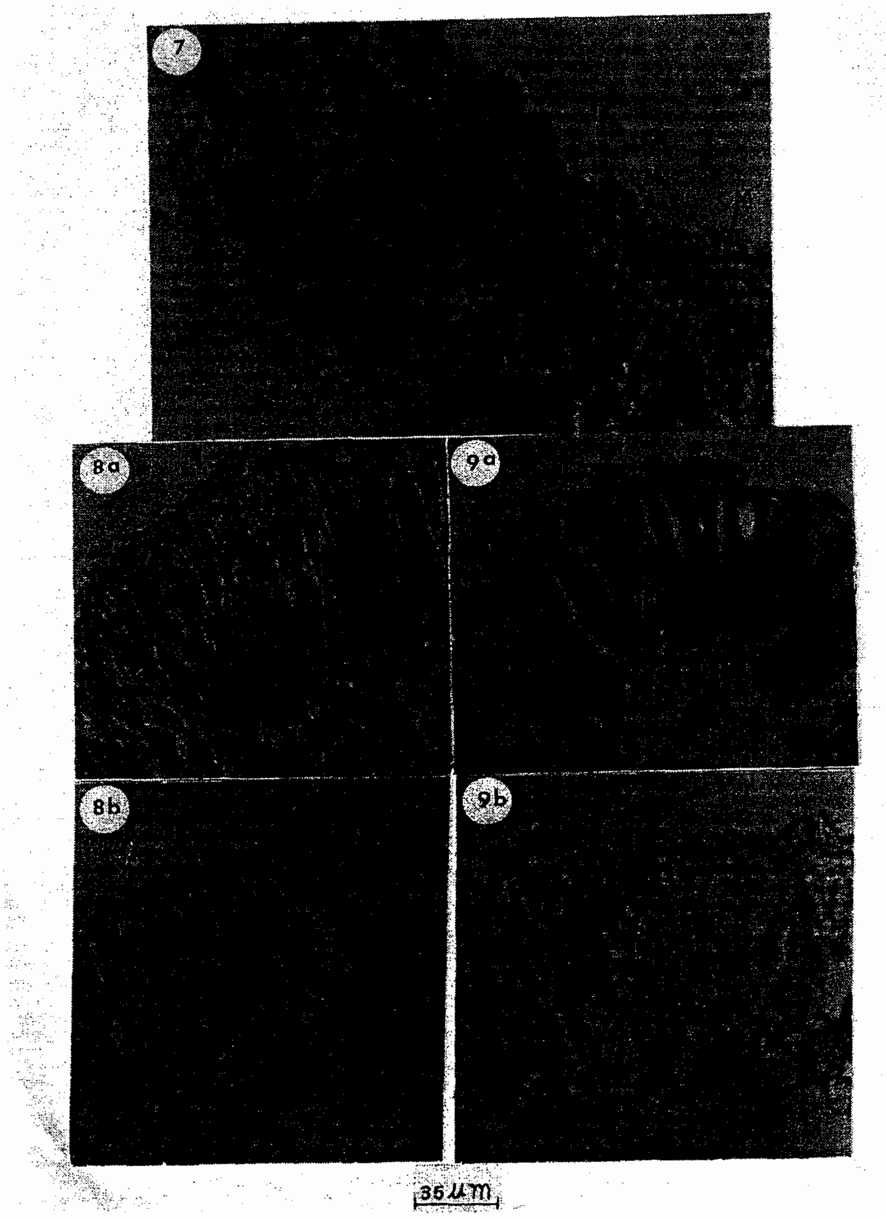

Fig. (7): Midgut section of control larvae stained by sudan black B showing the normal pattern of lipids (black colour).
Fig. (8): Midgut section of larvae treated with *B. thuringiensis* .
(a): 0.0001ppm: (b): 0.01ppm.
Fig. (9): Midgut section of larvae treated with abamectin.
(a): 0.0001ppm: (b): 0.01ppm.

3- The total proteins :-

The total proteins in the midgut and in the fat cells of *S.littoralis* larvae were reflected by the appearance of a positive affinity to mercury bromphenol blue visualized by the appearance of a bluish colouration. This was illustrated in the normal (control) midgut and fat cells (marked reaction (Fig 13, 16, respectively). As apparent in these figures, the cells contain bluish colour . Total proteins appeared as a great amount of dense blue particles.

The two tested biological agents *B. thuringiensis* and abamectin at 0.0001ppm gave a marked reaction in the midgut cells (Fig 14a , 15 a, respectively) and in the fat cells (Fig 17a , 18a, respectively) with mercury bromphenol blue . Also , the tesed compounds at 0.01ppm showed a marked decrease in protein content in the midgut cells (Fig 14b, 15b) and in the fat cells (Fig 17b and 18b). *B. thuringiensis* was more effective than that abamectin.

4- The nucleic acids :-

Midgut (Fig 19) and fat bodies (Fig 22) sections of the control larvae stained by Feulgin methylene blue method showed a normal pattern of RNA and DNA. RNA particles appeared as blue granules in the cytoplasm and in the nuclei. The nuclei exhibited a red colour indicating their DNA contents. Midgut cells of larvae treated with 0.0001ppm *B. thuringiensis* (Fig 20a) and abamectin (Fig 21a) showed a slight decrease of RNA only, while with 0.01ppm of the two tested compounds, the RNA content was highly decrease (Fig 20b and 21b). The fat cells of larvae treated with 0.0001ppm of *B.thuringiensis* (Fig 23a) and with 0.01ppm (Fig 23b) showed a high decrease of RNA. The fat cells of larvae treated with 0.0001ppm of abamectin showed a slight decrease of RNA, while the same compound at 0.01ppm elicited a high decrease of RNA. *B. thuringiensis* was more effective on RNA conten than that abamectin. DNA in the midgut and the fat cells not affected with the treatment by the two tested compounds.

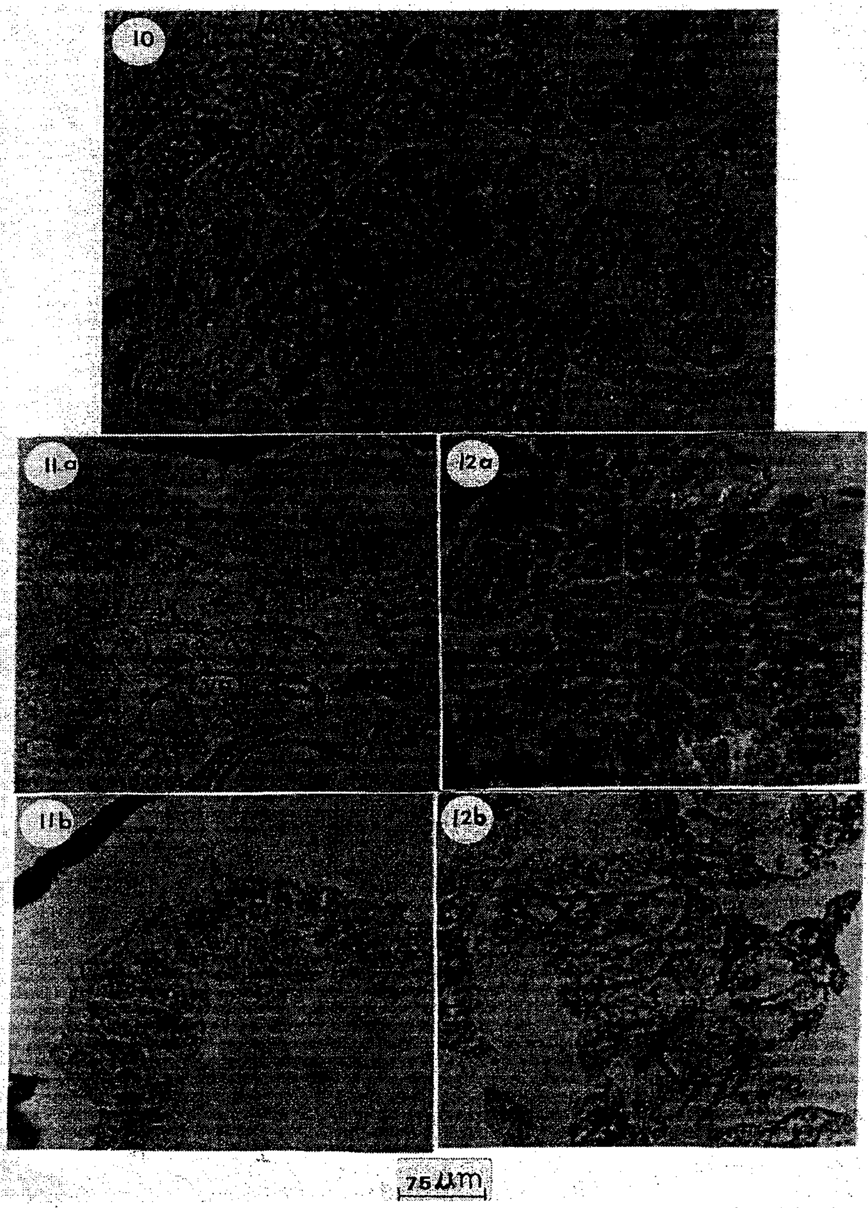

Fig. (10): Section in the fat body of control larvae stained by sudan black B showing the normal pattern of lipid (black colour).
Fig. (11): Section in the fat body of larvae treated with *B. thuringiensis* . (a): 0.0001ppm: (b): 0.01ppm.
Fig. (12): Section in the fat body of larvae treated with abamectin. (a): 0.0001ppm: (b): 0.01ppm.

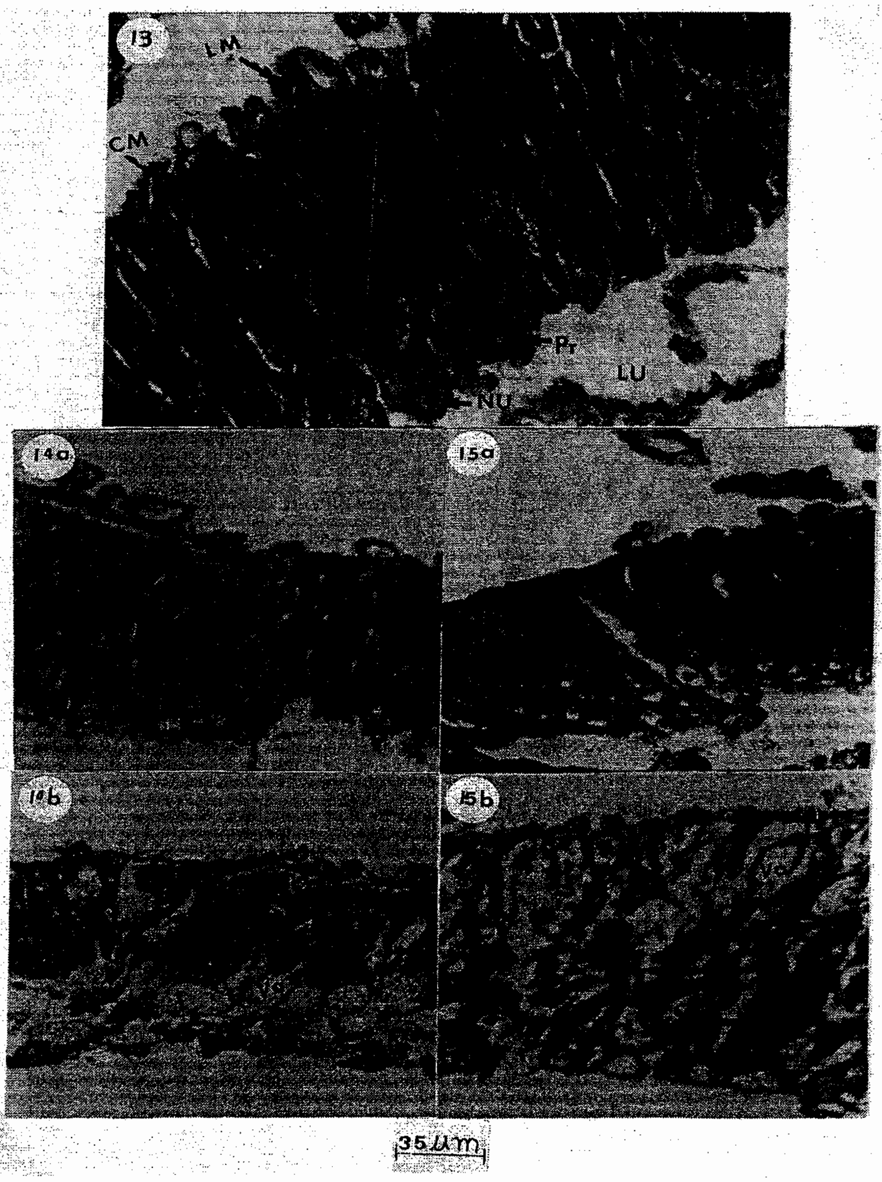

Fig. (13): Midgut section of control larvae stained with mercury bromphenol blue showing normal pattern and localization of total protein (blue colour).
Fig. (14): Midgut section of larvae treated with *B. thuringiensis*.
(a): 0.0001ppm: (b): 0.01ppm.
Fig. (15): Midgut section of larvae treated with abamectin.
(a): 0.0001ppm: (b): 0.01ppm.

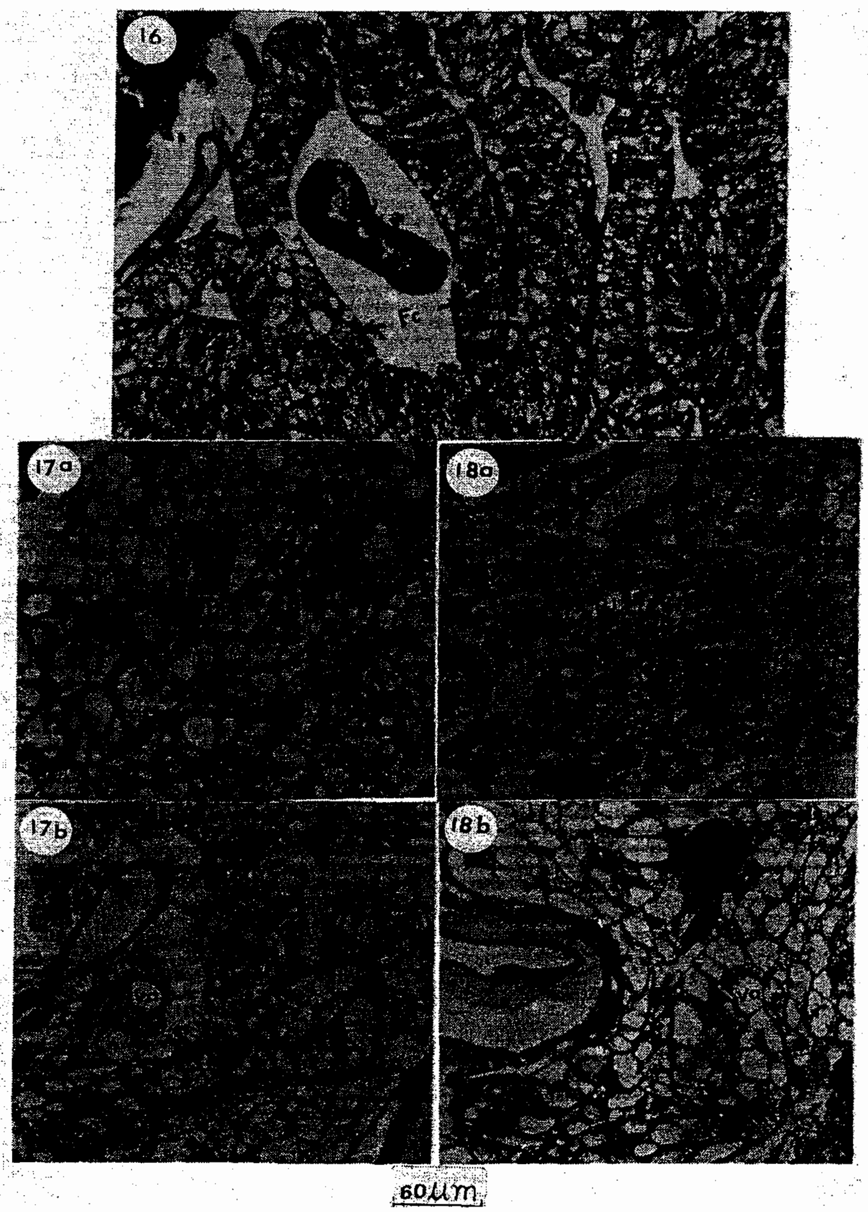

Fig. (16): Section in the fat body of control larvae stained with mercury bromphenol blue showing a marked readaction (blue colour).
Fig. (17): Section in the fat body of larvae treated with *B. thuringiensis* . (a): 0.0001ppm: (b): 0.01ppm.
Fig. (18): Section in the fat body of larvae treated with abamectin. (a): 0.0001ppm: (b): 0.01ppm.

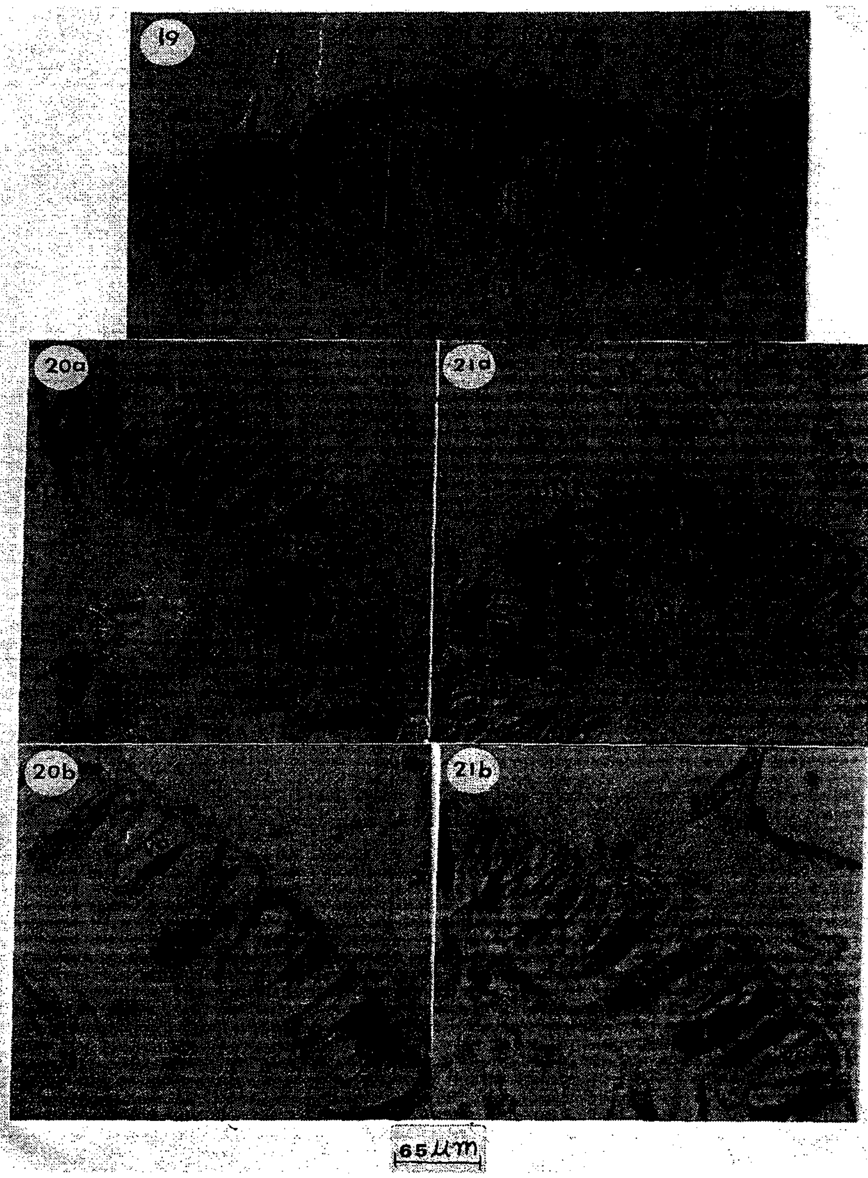

Fig. (19): Midgut section of control larvae stained by Schiff-Feulgen methylene blue showing RNA in the form of blue granules and nuclei exhibited a red colour indicating their DNA content.
Fig. (20): Midgut section of larvae treated with *B. thuringiensis*.
(a): 0.0001ppm: (b): 0.01ppm.
Fig. (21): Midgut section of larvae treated with abamectin.
(a): 0.0001ppm: (b): 0.01ppm.

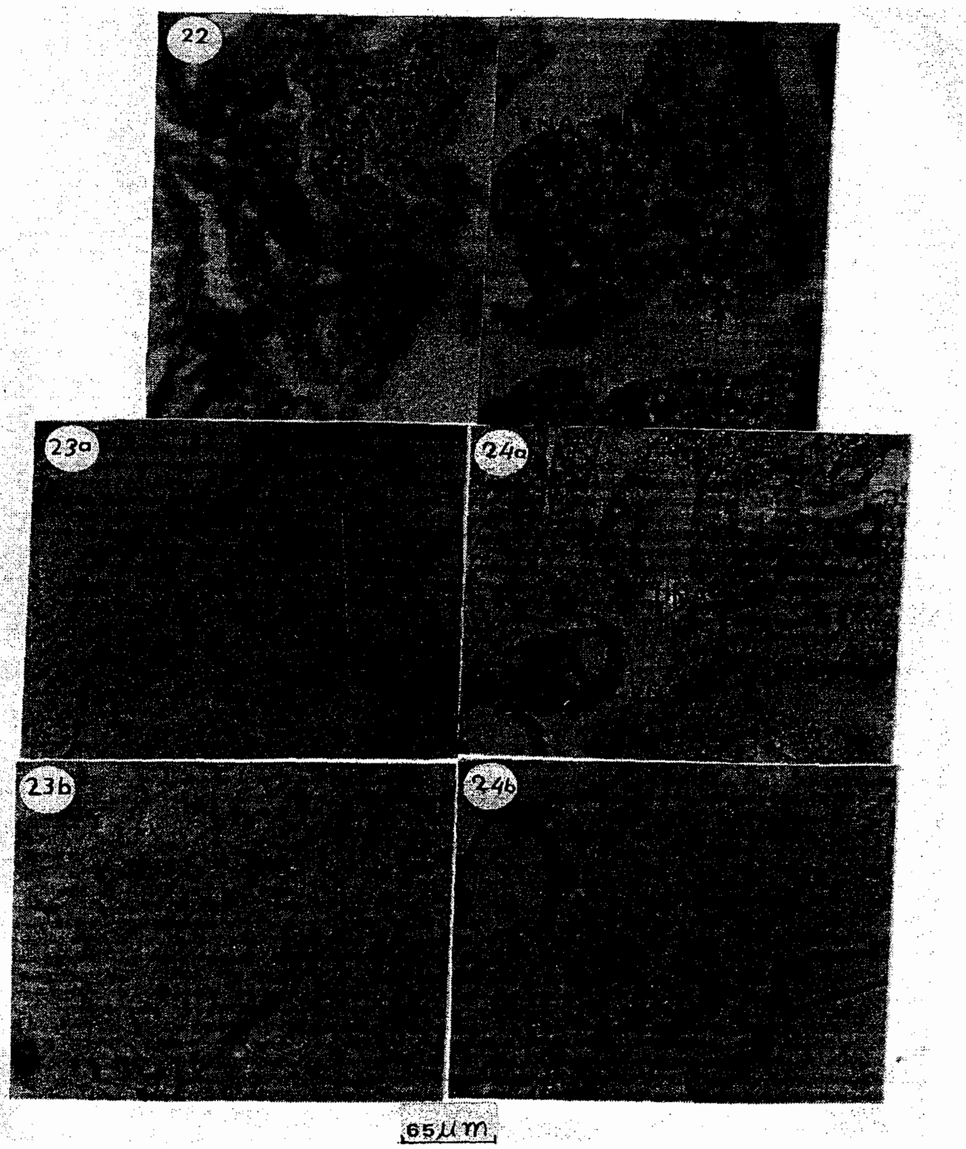

Fig. (22): section in fat body of control larvae stained by Schiff-Feulgen methylene blue showing normal pattern and localization of nucleic acids (RNA, blue) (DNA, red).

Fig. (23): Section in the fat body of larvae treated with *B. thuringiensis* . (a): 0.0001ppm: (b): 0.01ppm.

Fig. (24): Section in the fat body of larvae treated with abamectin. (a): 0.0001ppm: (b): 0.01ppm.

DISCUSSION

The fat body plays an especially important part in the conversion process known as intermediary metabolism , synthesis of fatty acids, protein and conversion of carbohydrates (glucose to trehalose)occur in the fat body. Which also stores energy reserves in the form of fats, glycogen and protein. These reserves can be converted rapidly into energy according to the needs of the insect. Parts of the midgut and other areas may also be storage sites for food reserves (Ross et al. , 1982) . Histochemical studies showed that the midgut and the fat cells of *S. littoralis* lost most of their polysaccharides content after treatment with *B. thuringiensis* and abamectin . *B. thuringiensis* was more effective than abamectin. These observations are in the agreement with the findings of ,Oakley and Kalmus (1987), Assar and Emara (1997), Shaurub et al. (1998), Assar (2003) and Assar (2004a) . Hamed et al . (1974) showed that the gut cells of *A. pharoensis* larvae lost most of their carbohydrate content following dieldrin and DDT treatment . Assar and Emara (1997) reported that , dimilin decreased the carbohydrates content in the midgut of *S. exigua.* Assar (2003) mentioned that the acetone and water extracts of *Artmisia monosperma* , *Zygophyllum coccineum, Lupinus termis* and *Brassica tournefortii* decreased the polysaccharide content in the midgut of *S. littoralis.* Assar (2004c) stated that the insect growth regulators, pyriproxyfen, hexaflumuron and methoxyfenozide reduced the polysaccharide content in the midgut and the fat bodies of *Parasarcophaga aegyptiaca* larvae

B. thuringiensis and abamectin decreased the lipid content in the midgut and the fat cells of *S. littoralis* larvae. Assar and Emara (1997) reported that the lipid decreased in the cytoplasm of the midgut of *S. exigua* larvae treated with LD_{50} of dimilin. On the other hand , no appreciable difference could be observed histochemically for the synthesis of lipid in the midgut and the fat cells of *P. aegyptiaca* larvae treated with hexaflumuron, methoxyfenozide and pyriproxyfen as compared with normal larvae (Assar , 2004c).

Protein substances are essential constituents of the general animal cells and also in the maintenance of different activities . All the two tested compounds decreased the total protein content in the midgut and the fat cells of the larvae of *S. littoralis* . *B. thuringiensis* was more effective than abamectin. This confirms the findings of Assar (2004a), who stated that abamectin reduced the protein content in the larvae of *M. domestica.* These changes may be due to certain defects in enzymes that are responsible for protein and lipid synthesis . On the other hand , Assar (2004b) stated that the total protein and amino acid contents in the larval midgut and the fat bodies of *P. aegyptiaca* increased after 5 days from treatment with the Lc_{25} of abamectin and spinosad. Fell et al (1982), Rajender (1990) and Shakoori and Saleem (1991), who attributed the greater protein synthesis in insecticidal treatment larvae to the synthesis of the proteinase needed for insecticide detoxification. In *Chrysomia albiceps* (Ismail and Fouad, 1985), on *M. domestica* (Bakr, 1986 ; Hamdy 1988 ; Assar and Abo- Sheashae (2004), on *Earis insulana* (Hewdy , 1990); on *Synthesiomyia nudiseta* (Abo El-Ela, et al 1993 a,b) and on *S. exigua*(Assar and Emara ,1997), who noticed a remarkable reduction in the total protein content after treatment with some IGR's. Prasad and Nath (1986) mentioned that endosulfan and malathion caused decline in total protein of *S. litura* larvae . Shaurub et al (1998) reported that pyriproxyfen reduced the proteins in the ovaries and testes of *S. littoralis* treated as 4th instar larvae. Assar (2003) mentioned that the four tested plant extracts decrased the level of protein in the midgut of *S. littoralis.*

B. thuringiensis and abamectin induced a marked reduction of RNA content in the midgut and the fat cells of *S. littoralis* larvae, while DNA not affected by these compounds . This confirms the findings of Assar (2004a), who stated that abamectin resuced the synthesis of RNA in the larvae of *M. domestica.* Assar and Emara (1997) reported that dimilin elicited a slight decrease of RNA and high decrease of DNA of *S. exigua* larvae. Shaurab et al. (1998) stated that pyriproxyfen reduced the synthesis of RNA and DNA in the ovaries and the testes of *S. littoralis* treated as 4th instar larvae. Assar and Abo-Shaeshae (2004) reported that pyriproxyfen and methoxyfenozide affected on RNA content in the midgut and the fat bodies of *M. domestica* larvae . Assar (2004c) stated that hexaflumuron and methoxyfenozide induced reduction of RNA content in the midgut and the fat cells of *P. aegyptiaca* larvae, while pyriproxyfen elicited a slight decrease . DNA not affected with all these IGR's.

CONCLUSION

In conclusion, it is obvious that the two tested biological agents, *B.thuringiensis* and abamectin decreased the polysaccharides, the lipid, the protein contents and the synthesis of RNA in the midgut and the fat cells of *S. littoralis* larvae. The polysaccharides (carbohydrates) and the lipid are essential to energy production. Because both glucose and protein are essential to chitin synthesis , the depletion of these metabolic macromolecules indicate that chitin production must be inhibited . Also, RNA is essential for protein synthesis . The protein is essential for energy production, the fertility and the fecundity of adult will affect. Also, the obtained results agree (confirmed) with the mode of action of these materials . *B. thuringiensis* inhibits protein synthesis through interference of DNA –dependent

RNA polymerase by structurally mimicking ATP and competing for the binding site (Sebesta, et al., 1981). Abamectin acts on the mediation of neurotransmission by α-amino butyric acid (GABA) leading to paralysis . In addition to its toxic ad delaying activity, abamectin (avermectin B_1) has been shown to exhibit growth-regulating activity (Wright, 1984) and inhibit feeding (Beach and Todd, 1985).

REFERENCES

Abdullah, M. A. and Nassar, M. M. I. (2001): Effect of some natural products (*Abamectin* , *Boxus chinensis* , *Nerium oleonder* and *Amaranthus viridis)* extracts against the cotton leafworm *Spodoptera littoralis* (Boisd) (Lepidoptera : Noctuidae) J. Egypt. Ger. Soc. Zool., 36(D) : 177-189.

Abo-El-Ghar, G. E. S. ; Radwan, H. S. A. ; El- Bermawy, Z. A. and Zidan , L.T. M. (1994) Histopathological effects of abamectin, thuringiensim and diflubenzuron on the midgut of *Spodoptera littoralis* (Lepidoptera : Noctuidae) larvae. M Sc. Thesis Fac. Agric. Menoufiya Uni Shebin El-Kom, Egypt.

Abo-El-Ghar, G. E. S. ; Radwan, H. S. A. ; El- Bermawy, Z. A. and Zidan , L.T. M. (1995a) Sublethal effects of avermectin B_1, B-exotxin of *Bacillus thuringiensis* and diflubenzuron against cotton leafworm (Lep., Noctuidae). J. Appl. Ent., 119: 309-313.

Abo-El-Ghar, G. E. S. ; Radwan, H. S. A. ; El- Bermawy, Z. A. and Zidan , L.T. M. (1995b) Inhibitory effect of thuringiensin and abamectin on digestive enzymes and non-specific esterases of *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) Larvae. J. Appl. Ent. , 119:355-359.

Abou-El-Ela, R.G.; AL-Zahaby, A.S.; Rahed, S.S; Shoukru, I. F. and Khalaf, A.A. (1993a): Effect of imilin, baysir and altosid treatment on the amino acids contents of *Synthesiomyia nudiseta* (Wulp.) pupae (Diptera : Muscidae). Egypt. J. Appl. Sci., 8(4): 538-46.

Abou-EL-Ela, R.G.; Taha, M. A.; Rashed, S. S.; Shoukry , I.F. and Khalaf, A.A. (1993b): Biochemical activity of three insect growth regulators on the fly *Synthesiomyia nudiseta* (Wulp) (Diptera : Muscidae). J. Egypt. Ger. Soc. Zool.,12(D) : 15-25.

Agee, H. R. (1985): Neurobiology of the bollworm moth : Effects of abamectin on neural activity and on sensory systems. J. Agric. Entomol. 2: 325-336.

Anderson, T. E. ; Babu, J. R.; Dybas, R. A. and Mehta, H. (1986): Avermectin B_1: Ingestion and contact toxicity against *Spodoptera eridania* and *Heliothis virescens* (Lepidoptera : Noctuidae) and potentiation by oil and piperonyl butoxide . J. Econ. Entomol. 79: 197-201.

Assar, A.A.(2003) : Histochemical Effects of some botanical extracts on the mid-gut of the cotton leafworm *Spodoptera_ littoralis* (Boisd) (Lepidoptera: Noctuidae). J. Fac. Sci, Menufiya Univ.,XVII:1-18.

Assar, A. A.(2004a): Biological and histochemical effects of abamectin on the house fly, *Musca domestica vicina* (Diptera : Muscidae. The Inter.cof. Brological sciences.,3 (1):216-234.

Assar, A.A.(2004b) : Effect of abamectin and spinosad on the protein and amino acid content in the larvae of the Flesh Fly *Parasarcophaga aegyptiaca* (Salem.) (Sarcophagidae : Diptera) J. of Union of Arab. Biologists , Cairo , 22(A): 75-98.

Assar, A.A.(2004 c): Histochemical effects of some insect growth regulators on the larvae of the flesh fly, *Parasarcophaga aegyptiaca* salem (Sarcophagidae: Diptera. J. Egypt. Acad. Soc. Environ. Develop. (A-Entomology): 5(2): 73-89.

Assar, A.A.and Abo-Shaeshe, A.A.(2004) : Effect of two insect growth regulators , methoxyfenozide and pyriproxyfen on the house fly, *Musca domestica* Vicina (Diptera: Muscidae) J. Egypt. Ger. Soc. Zool., 44(E) : 19-42.

Assar, A.A. and Emara, T. E.(1997) : Histochemical effects of dimilin on the lesser cotton leafworm, *Spodoptera exigua* (Lepidoptera : Noctuidae). J. Egypt. Ger. Soc. Zool. 24(E): 127-153.

Bakr, R.F. (1986): Morphogenic and physiological aberration induced by certain IGR's in the house fly *Musca domestica* Ph. D. Thesis Fac. Sci. Ain Shams Univ. Cairo.

Beach. R. M. and Todd, J. W. (1985): Toxicity of avermectin to larval and adult soybean looper (Lepidoptera : Noctuidae) and influence on larval feeding and adult fertility and fecundity . J. Econ. Entomol. 78 : 1125-1128.

Benz. G. (1966) : On the chemical nature of heat stable toxin of *Bacillus thuringiensis* Berliner in *Locusta migratoria*. J. Invertebr. Pathol., 3: 381-388.

Bonhag , P. F. (1955) : Histochemical studies of the ovarian nurse cell, tissues and oocytes of the milkweed bug *Oncopeltus fasciatus* Dallas. I. Cytolog-nucleic acids and carbohydrates. J.Morph.,96: 381-440.

Bull, D. L. (1986): Toxicity and pharmacodynamics of avermectin in the tobacco budworm, corn earworm , and fall armyworm (Noctuidae : Lepidoptera) . J. Agric. Food Chem. , 34: 74-78.

Burg, R. W. ; Miller, B. M.; Baker, E. E. ; Birnbaum , J.; Currie, J. A; Hartman; R; Kong, Y.L. ; Honaghan, R. L.; Olson, G.; Putter, I.; Tunae, J.P.; Wallick, H.; Stapley, E.O.; Oiwa, R. and Omura, S. (1979): Avermectins , new family of potent antelmintic agents : producting organism and fermentation (Antimicrob. Agents Chemother , 15: 361-367.

Campbell, W. C. ; Fisher , M. H. ; Stapley, E. O.; Albers – Schonberg, G. and Jacob, T.A. (1983) : Ivermectin : Apotent new Antiparasitic agent. Science 221: 823-828.

Christie . P. T. and Wright. D. J. (1990): Activity of avermectin B_1 against larval stages of *Spodoptera littoralis* Boisd and *Heliothis armigera* Hubner. (Lepidoptera : Noctuidae) and Possible mechanisms determing differential toxicity Pestic . Sci. 29, : 29-38.

Christie , P. T. and Wright , D. J. (1991): Distribution and metabolism of abamectin in larval stages of *Spodoptera littoralis* (Boisd) and *Heliothis armigera* HÜbner (Lepidoptera :Noctuidae) Pestic. Sci., 31, : 249-261.

Dybas , R. A. ; Hilton. N. J. ; Babu, J. R. ; Preiser , F. A. and Dolce, G. J. (1989): Novel second – generation avermectin insecticides and miticides for crop protection. In novel microbial productes for Medicine and Agriculture , ed. A. L. Demain , G. A. Somkuti, J. C. Hunter. Cevera, H. W. Rossmoore, PP. 203-12. New York: Elsevier.

Fell, D.; Ioneda, T..; Chiba, S. and Glannotti, O. (1982): Effect of disulfoton (Disyston) on the protein and carbohydrate content on the nervous system of *Periplaneta americana* L.Arquivos do-Instituto-Biologico-Sao-Paulo., 49(114): 31-36.

Fritz, L. C. ; Wang, C. C. and Gorio, A. (1979) : Avermectin Bl airreversibly blocks postsynaptic potentials at the lobster neuromuscular junction by reducing muscle membrane resistance . Proc. Nat. Acad. Sci. USR. 76: 2062-2066.

Garvin, A. J; Hall, B. J.; Brissie, R. M. and Spocer, S. S. (1979): Cytochemical differentiation of nucleic acids with a Schiffmethylene blue sequence. J. Histochem . Cytochem., 24(4) : 587-590.

Glancy, B. M. ; Lofgren, C. S. and Williamilliams, D. F. (1982): Avermectin Bla: Effects on the ovaries of red imported fire ant. *Solenopsis invicta* (Hymenoptera : formicida) queens . J. Med. Entomol . 19: 143-147.

Hamdy, A.M. (1988): Biochemical and histological studies of some antimoulting and antihormone compounds on insects . M.Sc. Thesis Fac. of Agric. AL-Azhar Univ.Cairo.

Hamed, M. S.; Guneidy, A. M. ; Riad, Z. M and Soliman, A. A. (1974): Histopathological and histochemical studies on *Anopheles pharoensis* larval treated with insecticides. Bull. Entomol. Soc. Egypt. Econ. Ser. VIII: 91-98.

Herbert, D.A. and Harper, J. D. (1987): Food consumption by *Heliothis Zea* (Lepidoptera : Noctuidae) larvae intoxicated with a B-exotoxin of *Bacillus thuriengiensis.* J. Econ. Entomol .80: 593-596.

Hewady, M. A. (1990): Response of spiny boll worm *Earias insulana* to certain novel insecticides with references to resistance development.Ph. D. Thesis Fac. of Sci. Ain-Shams Univ. Cairo.

Hotchkiss, R. D. (1948) : A micro chemical reaction resulting in the staining of polysaccharides structure in fixed preparation . Arch. Biochem., 16 : 113-141.

Ignoffo, C. M. and Gregory , B. (1972): Effects of *Bacillus thuringiensis* B-exotoxin on larval maturation , adult longevity, Fecundity and egg viability in several species of Lepidoptera. Environ. Entomol. 1: 269-272.

Ismail, I. E. and Fouad, M. A. (1985): Effect of a juvenile hormone analogue on the total carbohydrates, proteins and lipids of pupae of *Chrysomia albiceps* (Wiedman). J. Egypt. Soc. Parasitol., 15 (2): 645-49.

Maciejewska, J.; Chamberlain, W. F. and Temeyer , K. B. (1988): Toxic and morphological effects of *Bacillus thuringiensis* preparations on larval stages of the ental rat flea (Siphonaptera : Pulicidae). J. Econ. Entomol. 81: 1656-1661.

McManus, J. F. A. (1946): The demonstration of certain faty substances in paraffin sections. J. Path. Bact., 58-93.

Oakley, W.E and Kalmus, G.W.(1987): Effects of diflubenzuron and benzoyl peroxide on the development of 5th instar larvae of *Tribolium confusum.* J. Elisha Mitchell. Sci. Soc., 103: 88-93.

Paul, S.; Ghosh, M. R. and Sahu, C. R. (1991): Effects of insecticides on protein histochemistry of the testis of *Diacrisia oblique* Walker (Lepidoptera : Arctiidae). Environ . Ecol., 9: 167-169.

Pienkowski, R. L. and Mehring , P. R. (1983). Influence of avermectin $B_1$and carbofuran on feeding by alfalfa weevil, *Hypera postica,* larvae (Coleoptera : Curculaionidae). J. Econ. Entomol. 76: 1167-1169.

Prasad, C. S. and Nath, G. (1986): Effect of early toxication of insecticides on total haemolymph protein of *Spodoptera litura* (Fab.). Nat. Acad. of Sci. Ind. Sci. Letters, 9(4): 121-122.

Putter , I.; Macconnell, J. G.; Preiser, F. A.; Haidri, A. A.; Ristich, S.S. and Dybus, R. (1981): Avermectins : Novel insecticides , acaricides and nematicides from a soil microorganism . Experientia 37: 963-964.

Rajender, K. (1990): Effect of two different insecticides on the metabolism of cockroach, *Periplaneta americana.* J. Envi. Biol., II: 35-43.

Ross, H. H.; Ross, C. A. and Ross, J. P. R. (1982): A textbook of Entomology. 4th ed. By Jhon Willey and Sons . Inc.

Saleem, M. A. and Shakoori, A. R. (1985): Effect of permethrin and malathion on DNA- RNA and total protein contents in *Tribolium castaneum* larvae. Proc. 5th Pakistan Congr. Zool. : 61-69.

Saxena, R. C. (1983): Naturally occurring pesticides and their potential in : chemistry and world food supplies : the new frontiers . CHEMRAWN 11, Manila, Philippines , 1982, Pergamon Press, Oxford, New York, NY, : 143-161.

Sebesta, K.; Farkas, J. ; Horska, K. and Vankova, J. (1981): Thuringiensin , the beta-extoxin of *Bacillus thuringiensis,* pp.249-281. In Burges, H. D. [ed] . Microbial control of pests and plant diseses 1970-1980. Academic , London.

Shakoori, A. R. and Saleem, M. A. (1991): Comparative biochemical composition of a susceptible (Fss I I) and two malathion resistant (CTc 12 and Pakiston) of *Tribolium castaneum* (Coleoptera: Tenebrionidae) Pakistan J. Zool., 23: 1-16.

Shaurub, E. H. ; Ahmed, Z. A. and Samira , E. M. El-Nagar (1998): Impacts of pyriproxyfen and extracts of *Schinus terebinthiloolius* Radi on development, reproduction and reproductive organs in *Spodoptera littoralis* (Boisd) (Lepidoptera Noctuidae) J. Egypt. Ger. Soc. Zool., 27 (E): 57-82.

Trumble, J. T.; Moar, W.J. ; Babu, J. R. and Dybas , R. A. (1987): Laboratory bioassay of the acute and antifeedant effects of avermectin B_1. J. Agric. Entomol. 4: 21-28.

Wright, J. E. (1984): Biological activity of avermectin B1 aginst the boll weevil (Coleoptera: Curculionidae). J. Econ. Entomol. 77: 1029-1032.