Protein Patterns in Larvae of the Greater Wax Moth *Galleria mellonella* L. Induced by *Bacillus thuringiensis kurstaki*

Omar, Naglaa A. M.*, M. M. El-Husseini* and M. H. El-Bishry*
* Center of Biological Control, Faculty of Agriculture, Cairo University
** Plant Protection Research Institute, ARC, Dokki, Giza, Egypt
(Received, June 30, 2005; Accepted, August 8, 2005)

ABSTRACT

The SDS-PAGE analysis revealed large numbers of peptides ranging between 11-120kda. They were arranged in 22 peptide groups in descending order of 5 kda. Results showed 4 peptide groups, *i.e.*, P2 (111-115kda), P5 (96-100kda), P7 (86-90kda), and P9 (76-80kda) detected only in *B.t. kurstaki* treated larvae of *G. melonella* and were absent in the control, where P7 was detected also in the vegetative cells of the tested microbe. On the other hand, rest of the detected peptides was found in both treated and untreated larvae, but their amount and time of appearance varied irregularly. It is worth to state that, the peptides appeared only in treated larvae on the 1st day post treatment (but not in the control), is a result for alterations in the peritrophic membrane proteins caused by the *B. t.* endotoxin. This result could be supported by the architectural changes in the peritrophic membrane observed histologically in previous studies.

Key Words: *Bacillus thuringiensis kurstaki, Galleria mellonella,* Infection, Body proteins.

INTRODUCTION

Changes in protein picture of the diseased insect larvae infected with *Bacillus thuringiensis* were subjected to investigation, *e.g.*, in *Pieris brassicae* (Van Der Geest and Borgsteede, 1969), *Heliothis zea, H. virescens* and *H. subfkexa* (Vinson and Lewis, 1969), *H. armigera* (Abdeen *et al.*, 1986), *Mamestra brassicae* (Bai and Degheele, 1988), and in *Chilo agamemnon* and *Ostrinia nubilalis* (Salama *et al.*,1999). But in case of the greater wax moth larvae *Galleria mellonella* L. infected with *B. thuringiensis,* proteins were not well studied. Only one record was detected in the literature dealing with effect of *B.t.* on protein content in treated *G. mellonella* larvae published by Shen *et al.* (1994), who found that the content of soluble protein in the whole body of larvae treated with *B.t.* var. *galleriae* crystal proteins were decreased compared with normal larvae. Three other records were found dealing with protein but only in healthy wax moth larvae, *i.e.*, Croizier and Odier, 1974; Miller and Silhacek, 1982 and Halwani *et al.*, 2001).

The present investigation deals with the daily picture of such changes based on the detected peptides using SDS-PAGE technique, when larvae of *G. mellonella* were infected with spores and endotoxin crystals of *B. thuringiensis kurstaki* compared to healthy individuals as control.

MATERIALS AND METHODS

Larval treatment

G. mellonella was reared in the laboratory on the diet described by Ibrahim *et al.* (1984), but bee honey was excluded from diet prepared for present experiments because of its known antimicrobial effect (Omar, 2004). The LC_{50} of the *B.t.* formulation Dipel 2X (4.784 g/100 g diet) was used for infecting the larvae *per os.* to allow obtaining about 50% survived diseased larvae among a period of at least 5 days post treatment (Omar, 2004). About 1500 larvae (L_3) were fed on the treated diet, while another 700 ones were fed on untreated diet to serve as control. Daily samples each of 100 larvae were collected from the treatment as well as from the control and processed for protein determination.

Protein extraction

Following the method described by Khalil (1981), the larvae were ground in porcelain mortar and pestle with liquid nitrogen. Then, 1 ml of tris-buffer (0.0625 M, pH 6.8 contained 10% glycerol) was added to each 2 g larval weight. The clear supernatant containing the soluble proteins was recovered by centrifugation at 18,000 rpm for 30min. Protein content was adjusted to 2 mg/ml per sample and estimated according to the technique of Bradford (1976).

Preparation of samples and the standards

Sodium dodecyle sulphate was added to the sample at rate of 4mg SDS/1 mg protein, then 50 µl 2-6 mercaptoethanol were applied to each 950µl of the sample, and the mixture was heated to 100°C in a water bath for 2-5min.

To identify the protein bands resulting from the sampled larvae using the computerized program, the standard proteins β-galactocidase (*E.coli)* of 110 kda, bovine serum albumin (66 kda), and lactalbumin (bovine milk) of 15kda were used as protein marker references.

Preparing of gels

Preparation of the gels followed the method described by Laemmili (1970) for the resolving (separating) grading gel for 5-20% SDS-PAGE gel area (5.0 ml acrylamide solution 30% + 3.75 ml 3M Tris-HCl pH 8.8, 0.3ml 10% W/V SDS, 20.25 ml deionized water, 0.7 ml 1.5% W/V APS, and 10µl TEMED), and the stacking gel 3.5%) consists of 3.5ml acrylamide solution, 7.5ml stacking gel buffer (pH 6.8), 1.0 ml APS, 0.3ml SDS, 17.8 ml deionized water, and 0.25µl TEMED.

The resolving gel was poured between glass sandwich by gradient maker (Model XPO 77 Hoefer, USA) and covered with water. The gel polymerization started within 15-30min. After polymerization, the surface of the resolving gel was washed by deionized water, then the stacking gel was poured by pipette and the comb was inserted quickly in place after pouring. The stacking gel polymerization started within 15-30min.

Loading of samples and gel running

Twenty-five microleters of the prepared protein solution were applied to the wells of the stacking gel. The samples were covered with electrode buffer (39 tris-base, 14.4g glycine, and 1g sodium dodecyl sulphate dissolved in 1000 ml deionized water). Few drops of bromophenol blue (4 mg/100ml deionized water) were added to the electrode buffer (tracking dye).

Electrophoresis (gel running) was performed in a vertical slabs mold (Model LKB 2001 Hoefer, USA) measuring 16 x 14x0.15cm, and carried out at 30 mA at 10°C for 6 hours.

Staining, drying, and densitometric scanning of the gel

The silver staining method for protein bands followed the 10 steps described by Sammons *et al.* (1981). It is sensitive and detects as little as 2ng of protein in single band. The staining procedure takes place in as shown in Table (1).

The gel was dried using a gel dryer (Model SE 160 Hoefer, USA) and scanned with densitometer (GS Apparatus transmission Model GS 300 Hoefer, USA) attached to an EPS computer provided with the software for integrating of peak areas (GS data system Hoefer Scientific Instruments). Scans of the 1 lane were made sequentially negative to positive and took about 2min. The position of each band was recorded as rate of flow (R_f) which is calculated according to Gottlieb and Hepden (1966) by the formula:

$$R_f = \frac{\text{Distance of protein migration}}{\text{Distance of tracking dye migration}}$$

Cluster analysis (Dendrogram)

Grouping of the daily detected peptides by SDS-PAGE in sampled untreated (control) and *B.t.*var *kurstaki* treated larvae of *G. mellonella,* as well as a sample of the microbe vegetative cells were computerized using the 1-D Advanced – [Dendrogram Window Program]. They were clustered by the average linked technique (unweighed pair-group method) (Joseph *et al.*, 1992) to study the similarity levels (degrees %) among the resulted peptides. The results were expressed as Phenogram. In this analysis, clustering began with fusion of the two most similar fractions and proceeded until all fractions were used. The clustering process was presented in the form of a Phenogram (tree or dendrogram) in which the top branch indicated the highest fusion level, and so on for reference purposes, the fusion levels were designated 1, 2, from top to bottom, respectively.

RESULTS AND DISCUSSION

Results of SDS-PAGE for protein profiles in *B.t. kurstaki* treated and untreated larvae of *G. mellonella* are presented in Table (1).The resulted proteins (bands) of different molecular weights were categorized in 22 groups of peptides with descending ranges of 5 kda starting with P1 (protein no.1) ranging from 116-120 kda to P22 ranging from 11-15 kda.

Peptides of high molecular weight

They are of a slow mobility (76-120kda) and comprising 9 groups of peptides showing clear differences between *B. t.*-infected and healthy larvae of *G. mellonella* The peptides P2 (111-115kda), P5 (96-100 kda) and P9 (76-80kda) were found only in the *B.t.* infected larvae. P2 appeared in the diseased larvae at the 1st and 2nd days post ingestion of the *B. t.* treated diet, while P5 appeared on the 3rd, 5th and 6th days post treatment; and P9 at the 4th and 5th days.

On the other hand, P3 and P4 were specific to the control larvae, where P3 appeared on the 4th day and P4 on the 1st and 2nd days of the test. Also, a peptide of 86-90kda (P7), which was detected in the vegetative cells of *B. t. kurstaki,* appeared only in the treated larvae at the 4th and 5th days post treatment. It is believed that the appearance of this peptide at that time resulted from multiplication of the vegetative cells followed the germination of *B.t.* spores in the haemolymph of treated larvae. The absence of this peptide in treated larvae during the first three days post treatment explains the duration (three days), during which effect of the endotoxin crystals is progressing in midgut until a pathway between the damaged epithel cells allows the *B. t.* spores to enter the haemocoel.

Other peptides of high molecular weight like P1 (116-120) kda, P6 (91-95 kda) and P8 (81-85 kda) appeared in both treated and untreated larvae at different times showing quantitative differences. The amounts of P1 were always larger in the control (13.31-20.06%) than in the treated larvae (3.31-5.75%); while those of P8 were larger in the treatment (1.49-6.33%) than in the control larvae (3.57%) as shown in Table (1).

Accordingly, the absence of P3 and P4 in the treated larvae could be considered as an evidence for major protein alteration caused by the ingested *B. t.* toxin and resulted in the disappearance of all the peptides ranging from 101-105kda in the diseased larvae. Thus, the peptides found only in the treated larvae could be considered as new-formed peptides due to infection with the *B. t.*

Peptides of medium and low molecular weight

These peptides of medium (P10-P19) and fast mobility (P20-P21) were detected in *B. t.* vegetative cells as well as in treated and untreated larvae of *G. mellonella,* with the exception of P22 (11-15kda) which was absent in *B.t.* cells as shown in Table (1).

Table (1): Molecular weights (kda) and amounts (%) of peptides detected by SDS-PAGE in *B.t. kurstaki*, treated (T) and untreated larvae of *G. mellonella*.

Peptide Code	kda	*B.t.*	Days after treatment											
			1		2		3		4		5		6	
			T	C	T	C	T	C	T	C	T	C	T	C
P1	116-120	10.40					5.75	20.06	5.52		3.31	17.48	5.56	13.21
P2	111-115		7.12		7.07									
P3	106-110									14.87				
P4	101-105			12.94		10.68								
P5	96-100						6.72				7.04		5.23	
P6	91-95			1.73	5.66				5.32					
P7	86-90	3.10							3.11		1.83			
P8	81-85	2.68	6.33			3.57					1.49		2.30	
P9	76-80		3.03				2.95		1.70		1.68			
P10	71-75	3.26	5.02	5.52	7.60	4.22	5.20			3.25	5.82		4.76	2.79
P11	66-70	4.92		5.42		3.37			5.22	3.31				
P12	61-65	2.67	4.29		4.08	3.09	4.12	5.66		2.14	5.65		4.39	
P13	56-60	6.39		4.81		4.70		5.29	10.76	5.39	4.74	9.76	5.19	9.88
P14	51-55	2.41	9.28	2.92	8.02	3.54	10.14	2.02	3.84	3.02	3.99	2.94	3.34	3.39
P15	46-50	7.20	6.51	9.21	4.14	3.56	5.14	3.66	4.37	6.41	6.85	5.83	4.91	7.03
P16	41-45	10.38	8.35		9.92	6.47	10.30	7.10	10.93	4.19	8.77	5.10	9.99	5.44
P17	36-40	6.19	6.61	4.22	2.33	5.62	7.52	4.35	2.35	4.65	6.82	4.14	6.72	5.81
P18	31-35	4.78	4.40		9.21	9.28	4.99	4.58	9.65		7.55	8.59	7.82	9.06
P19	26-30	9.66	20.59	23.76	17.50	6.35	19.77	16.46	20.59	18.52	16.98	14.66	9.84	14.53
P20	21-25	4.00		7.68	5.55	14.22		5.57		12.14		7.76	9.06	7.06
P21	16-20	22.04	13.26	21.81	15.60	21.05	12.22	20.84	12.46	22.12	12.22	20.24	20.89	21.81
P22	11-15		5.32		3.31		5.17	4.14	4.17		5.25	3.50		

It is believed that peptides which appeared only in treated larvae on the 1st day post treatment, *i.e.*, P12 (61-65 kda), P16 (41-45 kda) and P22 (11-15 kda) as well as P2, P8 and P9 resulted from alteration of the peritrophic membrane proteins as stated by Rupp and Spence (1985) in case of the tobacco hornworm, *Manduca sexta*. Results of the present study are in agreement with such finding, especially that histopathological alterations were recorded in the peritrophic membrane in *B.t. kurstaki* treated larvae of *G. mellonella* at the 1st day post treatment (Omar, 2004; Omar *et al.*, 2005).

No other data on the peptide profile in *B.t.* treated *G. mellonella* were found in the available literature except that of Shen *et al.* (1994) who stated that the content of soluble protein in treated larvae decreased in comparison with the control. Similar studies were carried out on different lepidopteran larvae; *e.g.*, on *Bombyx mori* (Kato *et al.* 1978; Tojo *et al.*, 1980), *M.sexta* (Greene and Dahlman, 1973; Goodman *et al.*, 1978; Kramer, 1980; Peterson *et al.*, 1982), *Daphnis nerii* (Shukla and Parasher, 1978), *Plodia interpunctella* (Vanhaecke and Degheele, 1980), *P.rapae* (Kim and Seo, 1981), *P.brassicae* and *Mamestra brassicae* (Bai and Degheele, 1988), *Heliothis* spp. (Vinson and Lewis, 1969; Abdeen *et al.*, 1986), *Sitotroga cerealella* (Chippendale, 1971), *T.ni* (Smilowitz, 1971), *Pectinophora gossypiella* (Rostom *et al.*,1972), *Spodoptera littoralis* (Bai and Degheele, 1988), and on *C. agamemnon* and *O. nubilalis* (Salama *et al.*,1999).

Only five records for certain peptides in the normal (healthy) larvae of *G. mellonella* were available in the literature. Miller and Silhacek (1982) detected the polypeptides of 81-82 kda and 4-76 kda in larvae of the greater wax moth as the storage proteins, which were detected in the present study in the peptide categories P8, P9 and P10 (Table 1). A protein with an apparent molecular mass of 77 kda, possibly apolipophrin-II was found by Halwani *et al.* (2001) associated with, the apolipophrin-III in mature larvae of *G. mellonella*, which was detected also in the present study (P9: 76-80 kda). Lysenko (1972) separated 19 protein bands from the larval haemolymph, while Croizier and Odier (1974) detected 9 protein bands and considered the bands No5 and 6 as major haemolymph proteins. But Thomas (1979) isolated 3 fractions (LP1a, LP1b and LP2) as lipoproteins, which by further electrophoretic analysis revealed that the LP1a fraction contains three components, LP1b contains only one component; while LP2 contains two closely associated components. All of the three lipoprotein fractions are lipo-glycoproteins.

Clustering of peptides (Dendrogram)

Clustering the daily-detected peptides presented in Table (1) resulted the dendrogram illustrated in Fig. (1). The dendrogram placed the detected peptides in the treated larvae among the 4 days post treatment (T1-T4) in one cluster with high similarity of 96.38% between T2 and T3, and by 85.76% between them and T1; while the similarity between T1, T2 and T4 as one category and T3 decreased to 68.05%. Meanwhile, all the peptides detected in the untreated larvae along the 6 days of the test (C1-C6) formed categories of one cluster

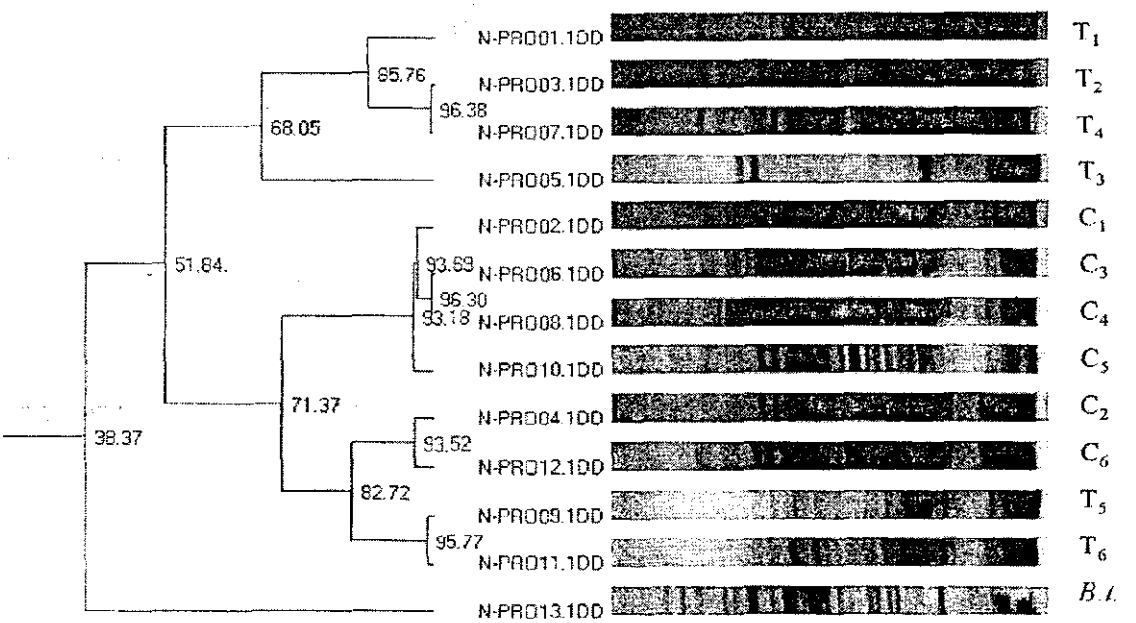

Fig.1. Dendrogram clusters the detected protein peptides among 6 days in *B.t. kurstaki* treated larvae of *G. mellonella* (T_1-T_2) and control (C_1-C_2) showing similarity% between the peptides.

with similarities between each other. Similarity levels recorded 93.3% between C1 and C3, 96.30% between C3 and C4, 93.18% between C3 and C5, and 93.52% between C2 and C6. Clustering C1, C3, C4 and C5 in one category and C2 and C6 in another category showed less similarity of 71.37%. The high similarity between T5 and T6 (95.77%) in relation to C6 by 82.72% may be attributed to the low applied *B.t.* concentration (LC_{50}), which enabled about half of the treated larval population to survive or resurge, and thus the treated larvae approach physiological conditions near to those of the control beginning from the 5th day post treatment and on the next days; a result clearly presented on the dendrogram.

A clear difference appeared between detected peptides clustered for each of the two larval groups of *G. mellonella* (treated and control), that the similarity level between them lowered to 51.84% due to the presence of certain and the same peptides in both treated and untreated larvae.

Although certain peptide (bands) of those detected in the vegetative cells of *B.t. kurstaki* were found in some cases in treated or untreated larvae as shown in Table (1), the a similarity (differences) between them placed those of *B.t.* in a sole category (Fig.1) showing the lowest similarity level (38.37%) between peptides of the *B.t.* vegetative cells from one side and both those of the treated and control larvae on the other side (Fig. 1).

Studying the available literature revealed no such studies for clustering the detected peptides of *B.t.* treated or untreated insect larvae; but records for identification and similarity levels of *B.t.* isolates or strains (Saleh, 1998). Accordingly, the present dendrogram study for peptides detected in *B.t. kurstaki* vegetative cells, *B. t.* treated and untreated larvae of the greater wax moth, *G. mellonella* could be considered as first record.

REFERENCES

Abdeen, S. A. O.; Gadallah, A. I.; Saleh, W. S.; Hossein, N. M. and Moawad, G. M., 1986. Some toxicological and biochemical effects of bacterium, *Bacillus thuringiensis* on the American bollowrm, *Heliothis armigera* Hbn. Ann. Agric. Sci. (Cairo), 31 (2): 1445-1462.

Bai, C. and Degheele, D., 1988. Characterisation of the gut juice and haemolymph proteins of *Mamestra brassicae* L., *Pieris brassicae* L., and *Spodoptera littoralis* (Boisd). larvae and susceptibility to *Bacillus thuringiensis var. thuringiensis* crystal toxins. Mededelinger Van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent., 53 (1): 255-259.

Bradford, M. M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein dye binding. Analytical Biochemistry, 72: 248-254.

Chippendale, G. M., 1971. Selective protein storage by the fat body of the angoumois grain moth, *Sitotroga cerealella*. Insect Biochem.1:122-124.

Croizier, G. and Odier, F., 1974. Etude des variations individuelles des proteins de l'hemolymph de lepidoptere *Galleria mellonella*: mise en évidence de groupes seriques. Experimentia, 30:189-190.

Goodman W.; O'Hern, P. A.; Zaugy, R. H. and Gilbert, L. J., 1978. Purification and characterization of a juvenile hormone binding protein from the haemolymph of the fourth instar tobacco hornworm, *Manduca sexta*. Mol. Cell. Endocrinol., 11: 225-242.

Greene, J. R. JR, and Dahlman, D. L., 1973. Haemolymph protein patterns in developing tobacco hornworm larvae, *Manduca sexta*. J. Insect Physiol., 19: 1241-1250.

Halwani, A. E.; Niven, D. F. and Dunphy, G. B., 2001. Apolipophorin-III in the greater wax moth, *Galleria mellonella* (Lepidopterta: Pyralidae). Arch.Insect Biochem. & Physiol., 48(3): 135-143.

Ibrahim, S. H.; Ibrahim, A. A. and Fayad, Y. H., 1984. Studies on mass rearing of the wax moth, *Galleria mellonella* L. and its parasite *Apanteles galleriae* W. with some biological notes on the parasite. Agric. Res. Rev. 62 (1): 349-353.

Joseph, F. H. H.; Anderson, R. E. and Tatham, R. L., 1992. Multivariate Data Analysis, McMillan Publishing Company, New York, 544 pp.

Kato, Y.; Nakayama, S. and Takeuchi, T. (1978). On PI value and amino acid composition of some protein fractions in the haemolymph of the silkworm, *Bombyx mori*. J. Sericult. Sci. Japan, 47: 445-446.

Khalil, M. S., 1981. Biochemical and Serological studies on flax rust. Ph. D. Thesis, Fac. Agric., Cairo Univ., 143 pp.

Kim, H. R. and Seo, E. W. (1981). A change of haemolymph proteins during metamorphosis of *Pieris rapae*. Korean J. Entomol., 11: 33-41.

Kramer, S. J., 1980. Purification and properties of manducin, an amino acid storage protein of the haemolymph of larvae and pupae of *Manduca sexta*. Insect Biochem. 10: 279-288.

Laemmili, U. K., 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T_4. Nature, 227: 680-685.

Lysenko O., 1972. Some characteristics of *Galleria mellonella* haemolymph proteins. J. Insect Pathol., 19: 335-341.

Miller, S. G. and Silhacek, D. L., 1982a. Identification and purification of storage proteins in the tissue of the greater wax moth, *Galleria mellonella*. Insect Biochem., 12: 277-292.

Omar, Naglaa A. M., 2004. Impact of *Bacillus thuringiensis* on some biological, histological , and physiological aspects of *Galleria mellonella* L. as a susceptible host. Ph.D. Thesis, Faculty of Agriculture, Cairo University; pp.148.

Omar, Naglaa A. M.; El-Husseini, M. M. and El-Bishry, M. H., 2005. histopathological changes in larvae of the greater wax moth, *galleria mellonella* l. caused by *bacillus thuringiensis* var. *kurstaki*. Egypt. .J .Biol. Pest Cntrol, 15(1):35-40.

Peterson, R. C.; Dunn, P. E.; Seballos, H. L.; Barbeaus, B. K.; Keim. P. S.; Riley, C.T.; Heinrikson, R.L. and Law, H., 1982. Juvenile hormone carrier protein of *Manduca sexta* haemolymph. Improved purification procedure, protein modification studies and sequence of the amino terminus of the protein. Insect Biochem. 12: 643-650.

Rostom, Z. M. F.; Algauhari, A. M. I. and Abdel Fattah, M. M., 1972. Free amino acids and total protein in the haemolymph of *Pectinophora gossypiella* during induction and termination of diapauses. Proc. Egypt. Acad. Sci., 25:71-79.

Rupp, R. A. and Spence, K. D., 1985. Protein alterations in *Manduca sexta* midgut and haemolymph following treatment with sub-lethal dose of *Bacillus thuringiensis* crystal endotoxin. Insect Biochem., 15 (2): 147-154.

Salama, H. S.; El-Moursy, A. and abdel-Rahman, A., 1999. Changes in the chemistry of the haemoloymph of the corn borers *Chilo agamemnon* (Crambidae) and *Ostrinia nubilalis* (Pyraustidae) after treatment with *Bacillus thuringiensis*. Archives of Phytopathology and Plant Protection, 32: (3) 249-256.

Saleh, W. D., 1998. Production of bioinsecticides by *Bacillus thuringiensis* strains isolated from Egyptian soil. Ph. D. Thesis, Faculty of Agriculture, Cairo University, 211pp.

Sammons, D. W.; L. D. Adams and E. E. Nishizawa, 1981. Ultrasensitive silver based colour staining of polypeptides in poly-acrylamide gels. Electrophoresis, 2: 135.

Shen, J. Z.; Qian, C. F.; and Zhang, S. F., 1994. Effect of sub-lethal dosages of *Bacillus thuringiensis galleriae* on the metabolism of substances in *Galleria mellonella* larvae. Acta Phytophylacica Sinica, 21 (4):373-377.

Shukla, R. N. and Parasher, G. S., 1978. Haemolymph proteins of the developing moth *Daphnis nerii*. Sci. Cult., 44: 173-174.

Smilowitz, Z., 1971. Haemolymph proteins in developing cabbage lopper larvae and pupae, *Trichoplusia ni*. Ann. Entomol. Soc. Am., 64: 340-343

Thomas, K. K., 1979. Isolation and partial characterisation of the haemolymph lipoproteins of the wax moth, *Galleria mellonella*. Insect Biochem., 9: 211-219.

Tojo, S. Nagata, M. and Kobayashi, M., 1980. Storage proteins in the silkworm, *Bombyx mori*, Insect Biochem., 10: 289-303.

Van Der Geest, L. P. S. and Borgesteede, F. H. M., 1969. Protein changes in the haemolymph of *Pieris brassicae* during the last larval instars and beginning of the pupal stage. J. Insect physiol., 15: 1687-1693.

Vanhaecke, M. and Degheele, D., 1980. Electro-phoretic characterisation of the haemolymph proteins, glycoproteins and lipoproteins of *Galleria mellonella*, *Plodia interpunctella* and *Ephestia kuehniella*. Meded. Fac. Landbouwet. Rijksuniv. Gent. 45 (5): 1287-1298.

Vinson, S. B. and Lewis, W. J., 1969. Electro-phoretic study of protein changes between several developmental stages of three species of *Heliothis*. Comp. Biochem. Physiol. 28:215-220.