Changes in Protein Profile of Cotton Leaf Worm, *Spodoptera Littoralis*, Induced by Bt-Formulations Stored at Cold and Hot Storage Conditions

Karima, H.E. Haggag

Department of Pest Rearing, Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt
karima.haggag@yahoo.com

Abstract: The insecticidal activity of six commercial Bt-formulations named Agerin, Agry,Delfin, Dipel 2X, Dipel DF and Protecto as well as the chemical insecticide of Selecron EC 72% were tested against 2^{ed} and 4th instars larvae of *Spodoptera littoralis* (Boisd) *in vitro* after stored at cold and hot condition. Results of bioassay revealed that the Bt-formulations caused the larval mortality against 2^{ed} instar insect larvae were in the range of 60.0 to 100.0% after cold storage conditions, while it was in the range of 60.0 to 96.7% after hot storage conditions, compared to the range of 83.3 – 100.0% at initial time. Results also revealed that the Bt-formulations caused the larval mortality was in the range of 66.7 to 100.0% and from 50.0 to 100.0% with 4th instar larvae after cold and hot storage conditions, respectively, compared to the rage of 76.7 to 100.0% at initial time. At initial time, the Dipel DF, Dipel 2X and Delfin were highly killed the insect larvae, followed by Agry, Prorcto and Agerin, respectively. These results showed that the storage of Bt-formulations reduced their insecticidal activity, except Dipel DF. Data of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis revealed that the protein profiles of larvae haemolymph of *S. littoralis* showed the different molecular weight proteins (bands) were categorized into 41 peptides groups with P_1 (Protein no.1) ranging from 121 – 226 KDa to P_{41} ranging from 21 – 25 KDa. Changes in protein profiles of treated larvae were found with all tested Bt-Formulations at cold and hot storage conditions. The dendrogram placed the detected peptides in the larvae treated with cold stored Bt-formulations in one cluster I, while other treated with hot stored conditions in cluster II.

[Karima, H.E. Haggag. **Changes in Protein Profile of Cotton Leaf Worm, *Spodoptera Littoralis*, Induced by Bt-Formulations Stored at Cold and Hot Storage Conditions**. *Nat Sci* 2013;11(7):77-85]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 13

Key words: Agerin, Agry, Delfin, Dipel 2X, Dipel DF, Protecto, Protein profiles, *Spodoptera littoralis*, Bioassay, Insecticidal activity.

1. Introduction

Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd), is a major one among the many serious pests of vegetable, field and ornamental crops in Egypt (**Mohamed *et al.*, 2005**). The problems associated with chemical insecticides application are resistant insect that may be due changes in genes, contamination the environment and their residues in agriculture and agrochemical industries. Therefore, application of alternative approach such as biological control (bio-pesticides) successfully controlled the serious pests (**Balasubramanian *et al.*, 2008**). The most widely used bio-pesticides are subspecies and strains of *Bacillus thuringiensis* (Bt). *B. thuringiensis* is a spore-forming bacterium well-known for its insecticidal properties due to its ability to produce crystal inclusions during sporulation. Each strain of this bacterium specifically kills one or a few related species of insect larvae such as Lepidopteran, Dipteran and Coleopteran.Because bio-pesticides often contain live organisms, they may have specialized storage instructions. It is not uncommon for labels to specify that a product can be stored for a several months if refrigerated but only a few weeks if stored at room temperature (**Gao *et al.*, 2008 and A´lvarez *et al.*, 2009).**

Changes in protein picture (profiles) of infected insect larvae with *B. thuringinsis* were studied in *Aeliothis zea* (**Vinson and Lewis, 1969**) and *Pieris brassicae* (**Bai and Degheele, 1988**).**Omar *et al* (2005)** reported that the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of greater wax moth (*Galleria mellonella*) infected with *B. thuringinsis* showed four peptide groups were in the range of 11- 120KDa. Information on changes in protein profiles of *S. littoralis* infected with Bt formulations is not enough. But **El-Shiekh *et al.* (2010)** showed that the protein profiles of *S. littoralis* treated with five profenofos formulations (Selecron, Ictacron, Sylian, Telaton and Cord), after stored at cold and hot conditions, were differently changed and distinguished into several separated protein bands were in the range of 3.6 to 195.5 KDa. The question that needs an answer in this study, does insecticidal activity and protein profile of *S. littoralis* larvae affected with cold and hot conditions stored Bt formulations.

Therefore, the goal of this present work is aimed to study the effect of cold and hot storage conditions

on the insecticidal activity of some commercial Bt-formulations named Agerin, Agry, Protecto, Delfin, Dipel 2X and Dipel DF, compared to Selecron against larvae of *S. littoralis in vitro* tests and study the relationship between these storage conditions and the changes in the protein profiles of treated larvae haemolymph using SDS-PAGE technique.

2. Materials and Methods

1-Insect:

The cotton leaf warm larvae of *S. littoralis* were reared on castor bean leaves in a rearing room, under laboratory conditions away from insecticidal contamination, Pest Rearing Department, Central Agricultural Pesticides Laboratory. The insect larvae were confined at environmental chamber conditions of temperature of 25 ± 2ºC, relative humidity (RH) of 70 ± 5% and photoperiod of 18 : 6 (light : dark) [**Adham *et al*.,2009 and Kamel *et al*.,2010**].

2-Bt-formulations:

Six of Bt-formulations named Agerin WP 6.5% [*Bacillus thuringiensis*], Agry WG 50% [*Bacillus thuringiensis*],Delfin WG 85% [*Bacillus thuringiensis*], Dipel 2X [*Bacillus thuringiensis* subsp.*kurstaki*], Dipel DF WP 6.4% [*Bacillus thuringiensis* subsp.*kurstaki*]and Protecto WP 9.4% [*Bacillus thuringiensis*] as well as the chemical insecticide of Selecron EC 72% [Perofenos] as control were obtained from Central Agricultural Pesticides Laboratory, Giza, Egypt, were used in this study.

3-Storage conditions:

The tested Bt-formulations of Agerin, Agriy, Protecto, Delfin, Dipel 2X and Dipel DF, as well as the chemical insecticide of Selecron (SN), were stored at cold conditions of 0 ºC ± 1 for 7 days in the refrigerator and at hot conditions in glass bottles in the oven at 54ºC ± 2 ºC for 14 days, according to method described by **F.A.O. (1988) and W.H.O. (1999).**

4-Bioassay:

The insecticidal activities of the tested Bt-formulations, compared to Selecron at the recommended dose were tested against *S. littoralis* larvae using the dipping leaf technique **(Ahmed, 2009)**. Castor leaves were thoroughly washed, dried under laminar flow and then cut into 5 cm leaf discs. Each castor leaf disc was dipped into each stored Bt-formulation suspension for 10 s with gentle agitation and then allowed to dry on towel on both sides. The castor discs were treated with sterile distilled water for control.After drying, one leaf disc was placed in a sterile 9-cm-diameter Petri dish. Then, ten larvae from each 2^{ed} /or 4th instars larvae were separately placed in each Petri dish for each treatment. Five Petri dishes were used as replicates for each treatment as well as the control treatment. All Petri dishes were kept at above environmental laboratory conditions. Larvae were considered dead if they showed no sign of the movement. The percentages of mortality levels were recorded at 24-hr intervals using Abbott`s formula **(Abbott, 1925).** The final mortality data were recorded up to 5 days.

5-Larvae treatment for protein analysis:

Larvae of *S. littoralis* are reared in the laboratory on castor leaves as mentioned before (**Ahmed, 2009**). The tested Bt-formations as well as Selecron was used at $LC_{50,}$ for infection the cotton leaf worm larvae; to allow obtaining about 50% survival diseased larvae at least 5days post treated **(Omar *et al*.,2005).** About 200 larvae (4th instar) were separately fed on cold and/or hot stored Bt-formations treated castor leaves as well as Selecron. Samples each 50 larvae were collected from each treatment and processed for protein analysis.

6- Protein analysis:

6.1-Protein extraction:

The haemolymph samples of healthy larvae treated with tested Bt-formations were obtained after 5 days of treatment by cutting the fore legs (**El-Sershaby *et al*., 2008**). The haemolymph was drawn into small serological test tubes and kept in a freezer at – 20ºC for analysis. Total protein concentration in the haemolymph of cotton leaf worm larvae was measured by Standard Bovine Albumin according to the method described by **Bradford (1976)**.

6.2-Preparation of gels:

Preparation of the gels followed the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by **Laemmli (1970).** The gel was prepared from monomer solution of 30% acrylamide and 0.8% N-N-bis-methylene-acrylamide. The denatured gels prepared as 12 % of separating gel in 1.5 M Tris-HCl buffers (pH 8.8) and 3 % of stacking gel in 0.5 M Tris-HCl buffer (pH 6.8).

The separating gel was poured between glass sandwiches. After polymerization, the surface of separating gel was washed by deionized water, and then the stacking gel was poured by pipette and the comb was inserted quickly in place after pouring for making the wells.

6.3-Loading of samples and gel running:

Ten micro liters of the haemolymph protein solution, from each treated larvae, were denatured by boiling in 5 ml of 5X sample buffer (60 mM Tris–HCl, pH 6.8, 2.0% SDS, 25% glycerol, 14.4 mM β-mercaptoethanol and 0.1% bromophenol blue) in water bath for 2 min and then quickly transferred to ice water and directly loading of the gel. About 10 µg of denatured protein for each haemolymph were put in each well of stacking gel, as well as, standard

protein marker. The Protein Molecular Weight Marker Mix ranged from 6.5 – 205 KDa (13 Proteins from 6.5 to 205 KDa), product No. (M-4038) from Sigma was used. Then, the samples were covered with electrode buffer.

Electrophoresis running was made in Vertical slab gel units.The lid was placed on the unit and run in the anode direction for 6 hr at 2 mA per each well. After electrophoresis was completed, the power supply was turned off and the cell lid was removes. The gel was removed from the other glass plate and proteins were visualized by staining with Coomassie blue R-250 with gentle shaking for an hour. Then,it was distained for over night in distaining solution I and then in distaining solution II until the protein bands become colorless.

6.4-Scanning the gel:

The gel was photographed and scanned. The molecular weight and percentages of the protein in profiles of treated *S. littoralis* larvae haemolymph were determined by scanning with Gel-Pro Analyzer V.3.0 Report Program (Mass Co., Cairo, Egypt).

6.5- Cluster analysis:

Grouping of daily detected peptides by SDS-PAGE in protein profiles of treated larvae of *S. littoralis* were computerized using the 1-D Advanced – [Dendrogram Window Program]. The results were expressed as dendrogram to study the similarity levels (degrees %) among the resulted peptides **(Omar *et al.*, 2005).**

7- Statistical analysis

Data of mortality were subjected to analysis of variance using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co., USA, and means compared with the Least Significant Difference (LSD) test at $P = 0.05$ **(Snedecor and Cochran, 1980).**

3. Results and Discussion

I-Bioassay against:

1- Second instar larvae:

Results of bioassay of tested Bt-formulations against the second instar larvae of *S. littoralis*, revealed that the insecticidal activity of Bt-formulations at initial time was in the range of 83.3 to 100.0% larval mortality, compared to Selecron (83.3%). Delfin, Dipel 2X and Dipel DF gave the highest mortality about 100.0%, followed by Agry, Agerin and Protecto, where the larvae mortalities were 90.0, 83.3 and 83.3%, respectively. The percentages of mortality of insect larvae treated with cold stored Bt-formulations were in the range of 60.0 to 100.0%, compared to 83.3% with Selecron. Dipel 2X as well as Dipel DF gave the highest mortality about 100.0%, followed by Delfin, Agry, Protecto and Agerin, where the mortalities were 90.0, 76.7, 70.0 and 60.0%, respectively. It is clear that the cold storage conditions signficantly reduced the insecticidal activity of tested Bt-formulations of Delfin, Agry, Protecto and Agerin in the range of 10.0 to 28.0%, while it not affected on Dipel 2X and Dipel DF, compared to results of larvae mortalities at initial time. The toxicity of Agerin was highly affected with these storage conditions, followed by Protecto, Agry, and Delfin, respectively (Table, 1).

The mortality of larvae treated with hot stored Bt-formulations was in the range of 60.0 to 100.0%, compared to 100.0 % with Selecron. Dipel DF gave the highest mortality about 96.7%, followed by Delfin (90.0%), Dipel 2X (86.7%), Protecto (73.3%), Agry (66.7%) and Agerin (60.0%), respectively.The hot storage conditions significantly reduced the insecticidal activity in the range of 3.3 to 28.0%, where the Agerin also was highly affected with these storage conditions, followed by Agry, Dipel 2X, Protecto, Delfin and Depel DF, respectively (Table, 1).

Table (1): Insecticidal activity of Bt-formulations at initial time and after cold and hot storage conditions against second and fourth instars larvae of *Spodoptera littoralis in vitro* tests.

Pesticides	Insecticidal activity (Mortality %)									
	2sd instar larvae					4th instar larvae				
	Initial	Cold storage at 0ºC for 7 days		Hot storage at 54ºC for 14 days		Initial	Cold storage at 0ºC for 7 days		Hot storage at 54ºC for 14 days	
		Mortality %	**Reduction %**	Mortality %	**Reduction %**		Mortality %	**Reduction %**	Mortality %	**Reduction %**
Agerin	83.3 Ac	60.0 Be	**28.0**	60.0 Bf	**28.0**	76.7Ad	66.7 Bf	**13.0**	50.0 Cf	**34.8**
Agry	90.0 Ab	76.7 Bd	**14.8**	66.7 Ce	**25.9**	80.0 Ac	80.0 Ad	**0.0**	56.7 Be	**29.1**
Delfin	100.0 Aa	90.0 Bb	**10.0**	90.0 Bb	**10.0**	96.7 Ab	83.3 Bc	**13.9**	80.0 Cb	**17.3**
Protecto	83.3 Ac	70.0 Cf	**16.0**	73.3 Bd	**12.0**	76.7 Ad	70.0 Be	**8.7**	60.0 Cd	**21.8**
Dipel 2X	100.0 Aa	100.0 Aa	**0.0**	86.7 Bc	**13.3**	100.0 Aa	93.3 Bb	**6.7**	80.0 Cb	**20.0**
Dipel DF	100.0 Aa	100.0 Aa	**0.0**	96.7 Ba	**3.3**	100.0 Aa	100.0 Aa	**0.0**	100.0 Aa	**0.0**
Selecron	83.3 Ac	83.3 Ac	-	70.0 Bg	-	100.0 Aa	70.0 Be	-	66.7 Cc	-
Control	6.7 Ad	0.0 Cg	-	3.3 Bh	-	3.3 Ae	0.0 Bg	-	0.0 Bg	-

1 –Percentage of died larvae ; 2-Reduction % of insecticidal activity according to initial time.
Means in each column and row followed by the same small and capital letter,respectively, are not significantly different according to LSD test (P = 0.05).

2- Fourth instar larvae:

At fourth instars larvae of *S. littoralis*, results bioassay showed that the insecticidal activity of Bt-formulations at initial time was in the range of 76.7 to 100.0% larval mortality, compared to Selecron (100.0%). Dipel 2X as well as Dipel DF gave the highest mortality about 100.0%, followed by Delfin (96.7%), Agry (80.0%), Agerin (76.7%) and Protecto (76.7%), respectively. The mortality caused by cold stored Bt-formulations was in the range of 66.7 to 100.0%, compared to 70.0% with Selecron. Dipel DF gave the highest about 100.0%, followed by Dipel 2X (93.3%), Delfin (83.3%), Agry (80.0%), Protecto (70.0%) and Agerin (66.7%), respectively. The insecticidal activity was significantly reduced in the range of 0.0 to 20.0%, where the insecticidal activity of Agry and Dipel DF was not affected with these storage conditions, compared to their effect at initial time. Delfin was highly affected, followed by Agerin, Protecto and Dipel 2X, respectively (Table, 1).

The Bt-formulations stored at hot storage conditions gave the larvae mortality was in the range of 50.0 to 100.0%, compared to Selecron (66.7%). Dipel DF gave the highest mortality was about 100.0%, followed by Dipel 2X (80.0%), Delfin (80.0%), Protecto (60.0%), Agry (56.7%) and Agerin (50.0%), respectively. The toxicity of Bt-formulations after hot storage conditions was reduced in the range of 0.0 to 34.8%, where Agerin was highly affected, followed by Agry, Proecto, Dipel 2X and Defin, respectively while Dipel DF was not affected (Table, 1).

Our results revealed that the tested Bt-formulations had insecticidal activity against 2^{ed} and 4th instars of *S. littorals* larvae, where Dipel DF highly killed the insect larvae, followed by Dipel 2X, Delfin, Agry, Protecto and Agerin, respectively. These results are agreement with those recorded by **Kaur (2000).** Who reported that *B. thueingiensis* applied for controlling of lepidopteran, dipteran and coleopteran insects for decades. *B. thueingiensis* produced more than 93% mortality on first instar larvae of *Spodoptera frugiperda* and *Peridroma saucia* (**A´ lvarez *et al*., 2009).** *B. thurigiensis* Berliner is promising agent for microbial control of agriculturally and medically important insects **(Souza *et al,* 2009).** Results of storage conditions are agreement with those reported that the Bt products tend to have a shorter shelf life than other insecticides and having reduced effectiveness after two to three years of storage. Liquid formulations are more perishable than dry formulations but shelf life is greatest when storage conditions are cool, dry and out of direct sunlight. Therefore, special formulation and storage procedures are necessary for some microbial pesticides. The best storage conditions of fungus, *Trichothecium rescum*, was at temperature - 4 ºC, where of rates in viability of the conidia were 74, 44 and 32% after 30, 60 and 90 days, respectively (**Blanford *et al*.,2012**). Our results answer on the part one of the above question, where the cold and hot storage conditions clearly affected on the insecticidal activity of tested Bt-formulations, especially at hot conditions.

II-Protein SDS-PAGE analysis:

1-Protein profiles:

The protein profiles of larval haemolymph of *S. littoralis* treated with Bt-formulations of Agerin, Agry, Protecto, Delfin, Dipel 2X and Dipel DF as well as Selecron, after cold and hot storage conditions, are shown in Table (2) and Fig (1). The protein profiles showed the several separated proteins (bands), with different molecular weight, were categorized into 41 peptides groups with descending ranges of 5 KDa with P_1 (Protein no.1) ranging from 222 – 226 KDa to P_{41} ranging from 21 – 25 KDa..

1.1-High molecular weight peptides:

These peptides are a slow mobility were in the range of 126 – 226 KDa and can be classified into 20 groups showing clear differences among the protein profiles of larvae treated with cold conditions stored Bt-formulations, compared to those in larvae treated with the same Bt-formulations after hot storage conditions (Table,2). The protein profiles of larvae treated with cold stored Bt-formulations showed 9 peptides were in the range of 162 to 226 KDa. The peptide of P_1 (222-226 KDa) was found in larvae treated with Bt-formulations of Agerin, Agry, Protecto and Delfin. While, P_2 (217-221 KDa) was found only in the larvae treated with Dipel 2X and Dipel DF, the P_6 (197-201 KDa) was detected in protein profiles of larvae treated with Agerin and Agry. Results also showed that the P_7 (192-196KDa) was found in larvae profiles treated with Protecto, Delfin, Dipel 2X and Dipel DF, while P_{12} (167 – 201 KDa) was found with Agerin, Protecto, Delfin and Dipel 2X treatments. The peptides of P_5 (202-206 KDa), P_{10} (177 – 181 KDa), P_{11} (172 176 KDa) and P_{13} (162 – 166 KDa) were found in profiles of larvae treated with Protecto, Agerin, Agry and Dipel DF, respectively. On other hand, the peptides of P_3 (212-216 KDa), P_6 (187 – 191 KDa) and P_{14} (157 – 161 KDa) were detected only in profiles of larvae treated with Selecron (Table, 2).

The protein profiles of larvae treated with hot stored Bt-formulations showing 9 peptides were in the range of 136 – 221 KDa. The peptide of P_2 was found with Agerin, Agry and Delfin treatments, while the peptides of P_3, P_4 (207-211 KDa) and P_6 were detected in protein profiles of the larvae treated with Protecto, Dipel DF and Dipel 2X and Agerin,

respectively. The peptides of P_9 (182-186 KDa) and P_{18} (136 - 140 KDa) were detected on in larvae profiles treated with Agerin. The peptide of P_{10} was found in larvae treated with Bt-formulations of Agry, Protecto, Delfin, Dipel 2 X and Dipel DF as well as Selecron. The peptide of P_5 was found only in larvae treated with Selecron. Results showed that while the P_{14} was found with Agry, Protecto and Delfin treatments, the P_{15} (152 – 156 KDa) was found in profiles of larvae treated with Dipel 2X and Dipel DF (Table, 2).

Table (2): Protein profiles of *Spodoptera littoralis* on the basis of protein molecular weight [KDa] and the percentages of protein amount in each band.

Band No.	Molecular weight (KDa)	Protein profiles of *Spodoptera littoralis* stored at													
		Cold stored at 0ºC for 7 days							Hot stored at 54ºC for 14 days						
		Agerin	Agry	Protacto	Delfin	Dipel 2X	Dipel DF	Selecron	Agerin	Agry	Protecto	Delfin	Dipel 2X	Dipel DF	Selecron
1	222-226	6.35	5.35	6.76	8.44	-	-	-	-	-	-	-	-	-	-
2	217-221	-	-	-	-	7.05	7.32	-	6.70	9.39	-	3.66	-	-	-
3	212-216	-	-	-	-	-	-	8.03	-	-	6.97	-	-	-	-
4	207-211	-	-	-	-	-		-	-	-	-	-	-	6.54	-
5	202-206	-	-	2.38	-	-	-	-	-	-	-	-	-	-	5.69
6	197-201	4.67	4.38	-	-	-	-	-	-	-	-	-	2.19	-	-
7	192-196	-	-	2.76	5.46	5.53	2.59	-	-	-	-	-	-	-	-
8	187-191	-	-	-	-	-	-	2.89	-	-	-	-	-	-	-
9	182-186	-	-	-	-	-	-	-	11.11	-	-				
10	177-181	-	1.52	-	-	-	-	-	-	10.18	9.52	9.73	10.12	9.52	6.57
11	172-176	3.82	-	-	-	-	-	-	-	-	-		-	-	-
12	167-171	-	1.53	1.84	10.8	2.96	-	-	-	-	-	-	-	-	-
13	162-166	-	-	-	-	-	2.88	-	-	-	-	-	-	-	-
14	157-161	-	-	-	-	-	-	3.69	-	3.31	2.67	1.97	-	-	-
15	152-156	-	-	-	-	-	-	-	-	-	-	-	1.74	2.05	-
16	147-151	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	141-146	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	136-140	-	-	-	-	-	-	-	3.02	-	-	-	-	-	-
19	131-135	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	126-130	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	121-125		-	-	-	-	-	-	-	-	-	-	-	-	-
22	116-120	17.13	12.92	-	-	-	-	-	-	-	-	-	-	-	-
23	111-115	-	-	10.31	5.00	12.97	-	-	-	-	-	-	-	-	-
24	106-110	-	-	-	-	-	14.39	12.30	7.33	8.05	6.63	-	-	-	-
25	101-105	23.20	-	-	-	-	-	-	-	-	-	12.10	-	-	-
26	96-100	-	24.14	24.26	-	-	-	-	17.32	21.30	-	-	30.75	-	31.60
27	91-95	-	-	-	17.08	23.33	17.51	16.71	-	-	17.10	20.04	-	28.98	-
28	86-90	-	-	-	4.99	10.0	3.50	4.20	8.44	-	-	-	-	-	-
29	81-85	8.55	7.52	2.85	-	-	-	-	-	-	-	-	-	-	-
30	76-80	-	-	5.12	7.90	7.69	7.68	7.44	5.43	4.91	5.51	3.98	4.76	6.60	4.83
31	71-75	6.56	-	-	-	-	-	-	-	-	-	-	-	-	-
32	66-70	-	6.72	6.35	6.41	4.59	5.95	-	5.38	3.85	5.21	-	3.00	2.06	8.12
33	61-65	4.33	-	-	-	-	-	6.07	2.90	5.02	2.66	7.26	5.43	5.10	-
34	56-60	-	5.50	-	-	-	-	-	5.62	7.32	8.74	8.11	7.67	7.05	7.99
35	51-55	12.03	12.00	11.08	7.55	8.36	11.23	7.57	10.00	2.50	2.58	7.91	5.83	1.69	2.65
36	46-50	-	-	-	-	-	-	-	-	-	-	2.65	-	-	-
37	41-45	-	-	-	-	6.75	3.26	3.38	7.76	6.55	5.97	7.22	5.91	7.50	5.02
38	36-40	-	-	-	-	-	-	-	-	-	-	-	-	-	2.44
39	31-35	13.36	9.40	9.56	7.74	7.34	7.84	9.38	-	-	-	-	-	-	-
40	26-30	-	5.38	6.49	14.42	4.06	7.50	8.53	11.31	10.28	14.96	12.60	15.09	14.98	16.39
41	21-25	-	-	-	-	3.18	-	-	6.12	7.34	7.79	2.77	7.50	8.93	8.75

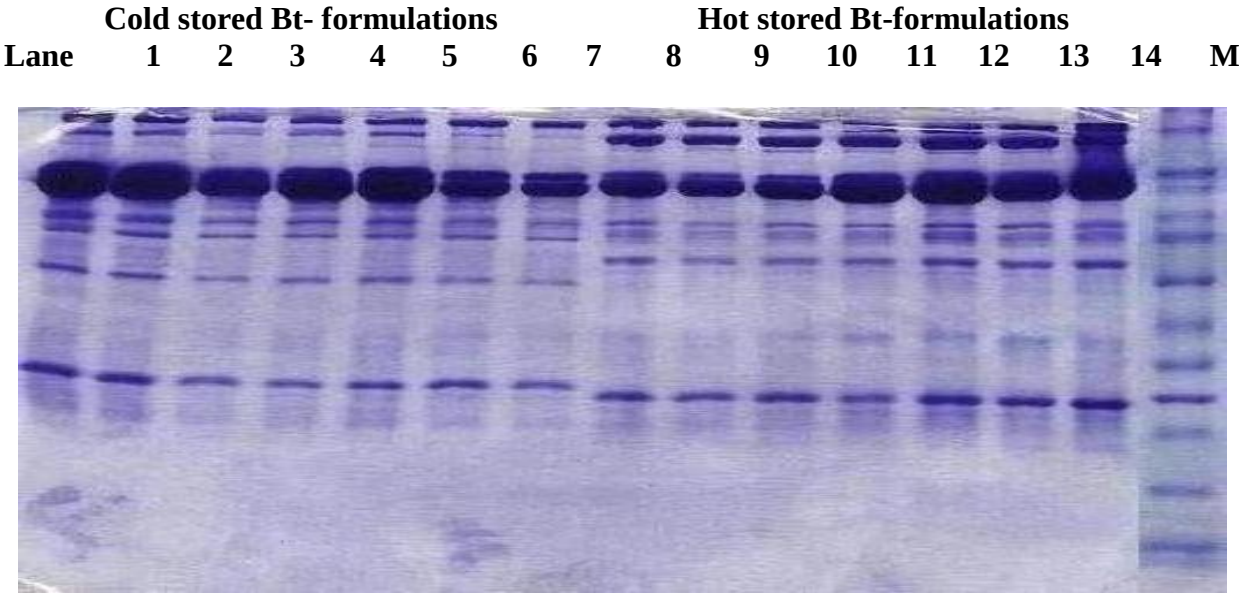

Fig (1): Protein profiles of whole cellular proteins of larvae hemolymph of *Spodoptera littoralis* treated with cold stored Bt-formulations of Agerin (lane 1), Agry (lane 2), Protecto (lane 3), Delfin (lane 4), Dipel 2X (lane 5), Dipel 2X (lane 6) and Selecron (lane 7) as well as hot stored Bt-formulations of Agerin (lane 8), Agry (lane 9), Protecto (lane 10), Delfin (lane 11), Dipel 2X (lane 12), Dipel DF (lane 13) and Selecron (lane 14) and protein marker (lane 15).Protein bands are identified from the top to the bottom according to molecular weight.

1.2-Medium molecular weight peptides:

These peptides are medium mobility in the range of 71-125 KDa and can be classified into 11 groups showing some differences among protein profiles of treated larvae (Table 2).The protein profiles larvae treated with cold stored Bt-formulations showed 10 peptides groups were in the range of 71 to 120 KDa. Results showed that while the P_{22} (116 – 120 KDa) was found with Agerin and Agry treatments, the P_{23} (111-115 KDa) was detected with Protecto, Delfin and Dipel 2X treatments, the P_{24} (106-110KDa) with Dipel DF as well as Selecron. The peptides of P_{25} (101-105 KDa) and P_{31} (71-75 KDa) was found only in Agerin treatment. The peptide of P_{26} (96-100 KDa) was found in larvae treated with Agry and Protecto, while both P_{27} (91-95 KDa) and P_{28} (86-90 KDa) were found in larvae treated with Delfin, Dipel 2X and Dipel DF as well as Selecron. The peptide of P_{29} (81-85 KDa) was found in larvae treated with Agerin, Agry and Protecto, while P_{30} (76-80 KDa) was detected with Protecto, Delfin, Dipel 2X and Dipel DF treatments as well as Selecron (Table, 1).

The protein profiles of larvae treated with hot stored Bt-formulations showed 6 peptide groups were in the range of 76 to 110 KDa. The peptide of P_{24} was found in larvae treated with Agrine, Agry and Protecto; the P_{26} was detected with Agerin, Agry and Dipel 2X treatments as well as Selecron, while the P_{27} was found in profiles of larvae treated with Protecto, Delfin and Dipel DF, respectively. The peptides of P_{25} and P_{28} were found in larvae treated with Delfin and Agerin. Results indicated that the P_{30} was found in protein profiles of larvae treated with all tested Bt-formulations as well as Selecron (Table,2).

1.3-Low molecular weight peptides:

These peptides are a fast mobility in the range of 21-70 KDa and can be classified into 10 peptide groups showing slight differences among Bt-Formulations (Table, 2). The protein profiles of larvae treated with cold stored Bt-formulations revealed 8 peptides were in the range of 21-70 KDa. The peptide of P_{32} (66-70 KDa) was found in profile of larvae treated with all tested Bt-formulations, except Agerin, as well as Selecron. The peptide of P_{35} (51-55 KDa), P_{39} (31-35 KDa) and P_{40} (26-30 KDa) [except, Agerin] were found in larvae treated with all Bt-formulations as well as Selecron. Results showed that the P_{33} (41-45 KDa) was detected in larvae treated with Agerin and Selecron, while P_{37} (41-45 KDa) was detected with Dipel 2X and Dipel DF and Selecron. The P_{34} (56-60 KDa) and P_{41} (21-25 KDa) were found only in protein profiles of larvae treated with Agerin and Dipel 2X, respectively.

The protein profiles of larvae treated with hot stored Bt-formulations showing 9 peptides were in the range of 21-70 KDa. The peptides of P_{32} (except, Delfin), P_{33} (except, Selecron), P_{34}, P_{35}, P_{37}, P_{40} and P_{41} were found in all protein profiles of larvae treated with all tested Bt-formulations as well as Selecron. The peptide of P_{36} and P_{38} were found only in profiles of larvae treated with Delfin and Selecron, respectively.

Clustering peptide (Dendrogarm):

Clustering the detected peptides presented in Table (2) and the dendrogram illustrated in Fig (2). The dendrogrem placed the detected peptides in protein profiles of *S. littoralis* larvae treated with cold stored Bt-formulations in one cluster (Cluster I) with high similarity of 85% among Protecto, Agerin and Agry and by 70% between them and Dipel 2X, while the similarity between Protecto, Agerin,Agry and Dipel 2X as one category and Delfin decreased to 60%.The dendrogom also placed the Dipel DF and Selecron in one category with the similarity was 75% and decreased to 50% between them and above category.

The detected peptides in profiles of insect larvae treated with hot stored Bt-formulations were placed in one cluster (Culster II) with high 90% between Dipel DF and Dipel 2X and by75% between them and Protecto.The similarity between Dipel Df, Dipel 2X and Protecto as one category and Agry decreased to 65% and by 60% between them and Delfin, while decreased to 55% between them and Selecron. A clear differences appeared between detected peptides cluster for each of two larvae groups of *S.littoralis* (treated with cold and hot storage conditions), that similarity level between them lowered to 40% due to the presence of certain and the same peptides in cold and hot stored Bt-formulations larvae. Results placed the Agrein when stored at hot storage conditions in sole category showing the lowest similarity level about 35% btween peptides of hot stored Bt-formulations from one side and those of cold stored Bt-formulations on the other side.

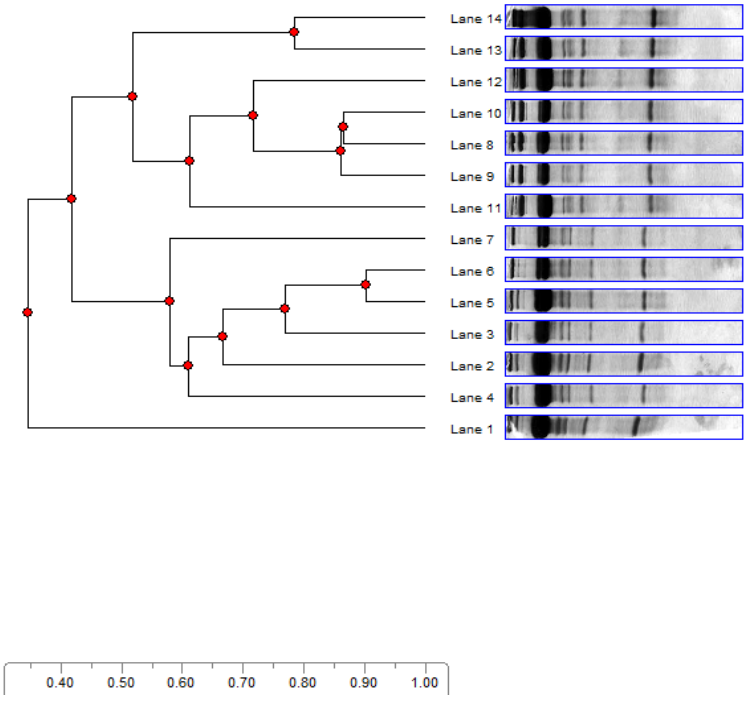

Fig (2): Dendrogram clusters the detected protein peptides in among 4th instar larvae of *Spodoptera littoralis* treated with cold stored Bt-formulations of Agerin (lane 1), Agry (lane 2), Protecto (lane 3), Delfin (lane 4), Dipel 2X (lane 5), Dipel DF (lane 6) and Selecron (lane 7) as well as hot stored Bt-formulations of Agerin (lane 8), Agry (lane 9), Protecto (lane 10), Delfin (lane 11), Dipel 2X (lane 12), Dipel DF (lane 13) and Selecron (lane 14).

Results of SDS-PAGE analysis revealed that the protein profiles of *S. littoralis* were changed when larvae treated with Bt-formulations after cold and hot storage conditions. These results may add some interpretations on the changes associated to the differences in insecticidal activity of tested Bt-formulations. These results confirm by the results obtained by **El-Sherhaby *et al*. (2008).** They reported that the total protein content in haemolymph of 4th instar larvae of *S.littoralis* was affected with Dipel 2X feeding, where positive relationship was found between post treatment period and changes in the percentage of total protein content. **El-Sherhaby *et al*. (2008)** also mentioned that toxins of *B. thuringensis* are responsible for the inhibition of protein synthesis by forming a protein complex. **Etebari *et al.* (2006**) showed that many insecticides decreased feeding efficiency and protein amount of an insect´s body. The infection of tobacco cutworm *Spodopetra litura* (Fab.) with *B. thuringiensis* var. *kurastaki* resulted in significant reduction in total lipid content of haemolymph in 3ed, 4th and 5th instars infected larvae **(Tripathi and Sinhg, 2002).** A significant reduction in total protein content in *S. littoralis* larvae after 120h of Agerin, Dipel 2X and Dipel DF treatments, compared with the control **(Kamel *et al*., 2010).** These results concluded that the insecticidal activity of tested Bt-formulations was affected by storage conditions as well as the protein profile of insect larvae. Results answer on the part second of the above question, where the cold and hot storage conditions clearly affected on protein profiles of insect larvae treated with tested Bt-formulations, especially at hot conditions. Therefore, the Bt – formulation must be store at good conditions, especially the bio-pesticides often stored for long time for marketing or the farmers kept it until used.

Corresponding author
Karima, H.E. Haggag
Department of Pest Rearing, Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt
karima.haggag@yahoo.com

References

1. Abbott, W. S. (1925). A method for computing the effectiveness of an insecticide. J. Econ. Entoml., 18(2) : 256-267.
2. Adham, K. Fatma ; Eman, M. Rashad; S. F. Ibrahim and Enas, E. Nasr (2009). Host plants shifting affects the biology and biochemistry of *Spodoptera littoralis* (Boisd.) [lepidoptera: noctuidae]. Egypt. Acad. J. biolog. Sci., 2(1): 63-71.
3. Ahmad, M. (2009). Observed potentiation between pyrethroid and organosphosphours insecticides for the management of *Spodopter litura* (Lepidoptera : Noctuide). Crop Protection, 28: 264 – 268.
4. A´ lvarez, A.; E.G. Virla ; L. M. Pera and M. D. Baigori (2009). Characterization of native *Bacillus thuringiensis* strains and selection of an isolate active against *Spodoptera frugiperda* and *Peridroma saucia*. Biotechnol. Lett., 31:1899–1903.
5. Bai, C. and D. Degheele (1988). Characterisation of the gut juice and haemplymph proteins of *Mamestra brassicae* L., *Pieris brassicae* L. and *Spodoptrea littoralis* (Boisd) larvae and susceptibility to *Bacillus thuringiensis* var. *thuringiesis* crystal toxins. Mededelinger van de Facultcit Landbouwetenschappen Rijksuniversiteit Gent, 52 (1): 255-259.
6. Balasubramanian, A.V.; S. Arumugasamy; K. Vijayalakshmi and S. Sridhar (2008). Plant Products as Biopesticides: Building on traditional knowledge of vrkshayurveda: Traditional Indian Plant Science. 16 th IFOAM Organic World Congress, Moderna, Italy, June 16-20, Archived http:// orgprints org/view/projects/conference. htmI.
7. Blanford, S. ; Nina, E. Jenkins ; R. Christian ; Brian, H.K. Chan ; Luisa Nardini ; M. Osae ; L. Koekemoer; M. Coetzee ; A. F. Read and B. Matthew (2012). Storage and persistence of a candidate fungal biopesticide for use against adult malaria vectors. Malaria Journal, 11:354.
8. Bradford, M. M. (1976).A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72: 248-254.
9. El-Shershaby, M.; N. A. Farag and A.A. I. Ahmed (2008). Impact of *Bacillus thuringiensis* on protein content and enzymes activity of *Spodoptera littoralis*. Research Journal of Agriculture and Biological Sciences, 4(6): 861-865.
10. El-Shiekh, Y. W. A.; Karima, H. E. Haggag and Olfat, A. Radwan (2010). Comparative studies among trade Profenofos insecticides using physical, chemical and biological parameters. American-Eurasian J. Agric. & Environ. Sci., 8(4): 370-282.
11. Etebari, K. (2006). Biochemical changes in haemolymph of silkworm larvae due to pyripyroxyfen residue. Pesticide Biochemistry and Physiology, Doi: 10.1016/j.pestbp. 2006.08.005.
12. F.A.O. (1988). Food Agricultural Organization (FAO) Specifications, Insecticides Emulsifiable

Concentrates, Storages Stability 54+1ºC for days, PP.19-20.
13. Gao, M.; R. Li; S. Dai, ; Y. Wu and D. Yi (2008). Diversity of *Bacillus thuringiensis* strains from soil in China and their pesticidal activities. Biological Control, 44: 380–388.
14. Kamel, S. Aida; Mona, F. Abd-EL Aziz and Nehad, M. EL-Barky (2010). Biochemical effects of three commercial formulations of *Bacillus thuringiensis* (Agerin, Dipel 2X and Dipel DF) on *Spodoptera littoralis* larvae. Egypt. Acad. J. biolog. Sci., 3 (1): 21- 29.
15. Kaur, S. (2000). Molecular approaches towards development of novel *Bacillus thuringiensis* biopesticides. World Journal of Microbiologgy and Biotechnology, 16:781 -793.
16. Laemmli,U. K. (1970). Cleavage of structural proteins during the assembly of the head of bateriophage T_4. Nature, 227: 680 – 685.
17. Mohamed, H. M. Eman ; Sawsan, M. El-haleam and M. M. El-Husseini (2005). Efficacy and residual effect of *Bacillus thuringiensis* against larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd) in Egyptian clover fields**.** Egyptian Journal of Biological Pest Control, 15(1): 81-83.
18. Omar, A. M. Naglaa ; M. M. El-Husseini and M. H. El-Bishry (2005). Protein patterns in larvae of the greater wax month, *Galleria mellonella* L., induced by *Bacillus thuringiensis kurstaki*. Egyptian Journal of Biological Pest Control, 15(1): 71-75.
19. Snedecor, G.W. and W. G. Cochran (1980). *Statistical methods*. 5th Ed., Iowa State Univ. Press, Ames, Iowa, USA, 593 pp
20. Souza, Jr, J. D. A.de ; S. Jain ; C.M.F. de Oliveira ; C. F. Ayres and W.A. Lucena (2009).Toxicity of a *Bacillus thuringiensis israelensis*-like strain against *Spodoptera frugiperda*. BioControl, 54:467–473.
21. Tripathi, R. and N. P. Singh (2002). Biochemical alterations in the haemolymph of *Bacillus thuringiensis* var. *kurstaki* (B.t.k.) infected larvae of *S. litura* (Fab). Asian J. Exp. Sci. 16 (1&2): 35-39.
22. Vison, S. B. and W. J. Lewis (1969). Electrophoretic study of protein changes between several developmental stages of three species of *Heliothis.* Comp. Biochem. Physiol., 28:215 – 220.
23. W.H.O. (1999).World Health Organization (WHO), Specification and Evaluation for Public Health Pesticides (Chloropyrifos), PP: 5.

3/2/2013