Toxicological and Biochemical Studies on Development of Resistance in *Spodoptera littoralis* (Boisd.) During Selection with *Bacillus thuringiensis* MVPII

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Abstract: The present investigation studied the toxicity of five bacterial formulations of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) (MVPII, Dipel -2X, Ecotech-Bio and Dipel Es) and *B. thuringiensis* var. *aizawai* (*Bta*) (Xentari) to the cotton leaf worm *Spodoptera littoralis*. The LC50s for such formulations were measured as 0.08, 0.11, 0.22, 0.27 and 0.00045 %, respectively. The larvae were selected for resistance to MVPII for twelve generations and the resistance increased to12.5 folds higher than the susceptible parent strain. No correlation (cross resistance) between MVPII-resistant strain and the other *Bt* formulations was indicated. The levels of resistance fell in the range of vigour tolerance to be 1.82, 1.3, 1.67 and 3.78 fold for Dipel-2X, Echotech-Bio, Dipel Es and Xentari, respectively. The possibility of using protein as biochemical parameters to detect the development of resistance was also studied. The effect of different bacterial formulations was studied at 2nd, 4th, 6th and 9th day post treatment of 4th larval instar. Most of bacterial formulations were reduced the total protein contents particularly Dipel–2X showed none increasing in protein for any different days post treatment comparing with untreated control. The 9th day post treatment showed not only highly significant increase in total protein contents for the rest four treatments (MVPII, Ecotech-Bio, Dipel Es and Xentari) but also with MVPII treatment on different generations during selection. The electrophoretic analysis of proteins by SDS-PAGE was carried out for untreated normal strain and different days post treatment with LC_{50} of MVPII during selection. Twenty four bands were separated and their molecular weight ranged between 5.9 and 187.96 KDa. The number of separated bands varied among different generations according to the tested strain. The appearance of new protein might be due to increasing of protein synthesis while the disappearance of other could be attributed to their breakdown as a result of *Bt* infection or the resistance.

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1. Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Bosid.) is one of the most important pests in Egypt. It attacks several host plants specially the cotton crop which considered one of the main sources for the economy. Resistance could arise if the insecticide fails to reach the site of biochemical action, or does so at a lower rate. This could happen as a result of excretion of the toxin, storage in insect fat bodies or increase in the rate of toxin detoxification by means of some enzymatic reactions. So, biochemical mechanisms encompass. The increased ability of an insect to metabolize or detoxify an insecticide is known as metabolic resistance **(Wilkinson, 1983)**. With the increasing uses of *B. thuringiensis* in the field, resistance of insects to the conventional *B. thuringiensis* products became a serious pest management problem **(Navon, 2000).** The tobacco budworm *Heliothis virescens* and the pink bollworm *Pectinophora gossypiella* have shown reduction in binding of *t* toxins as a mechanism of resistance **(Ferré and Van Rie, 2002).**

The bacterial toxins are active in insects; those toxins of *B. thuringiensis* are responsible for the inhibition of protein synthesis by forming a protein complex with an alkaline pH of haemolymph **(Hemipel and Angus, 1959; Angus and Norvis, 1968)** or pH of midgut **(Cooksey, 1971 and Burges, 1982)**. Not only the level of resistance is almost associated with pH but also with proteinase and mainly with reduced binding of toxins to the brush border membrane of the insect midgut. This type of resistance confers limitation of cross-resistance **(Knipple *et al.*, 1988; Sparks *et al.*, 1989 and Oppert *et al.*, 1994)**. There are several strains or varieties of *B. thuringiensis* that have been selected for the control of specific insects, like *B. thuringiensis* var. *kurstaki* (*Btk*) and *B. thuringiensis* var. *aizawai* (*Bta*) **(Cerda, 2003).** Each strain of this bacterium produces a different mixture of proteins causing the insect larvae to starve **(Nixon, 2004).**

Each protein is considered as reflect to the activity of specific gene through the production of enzyme which act as catalyst to produce protein responsible for specific biological character **(Cerda, 2003)**. *B. thuringiensis* toxins require extensive knowledge about the mechanisms, genetics, biochemistry and ecology of resistance genes.

The purpose of this investigation is studying the toxicity of five bacterial formulations of *B. thuringiensis* and the development of resistance in larvae of the cotton leaf worm *S. littoralis*. Changes in the total protein content and protein patterns were also studied to could serve as effective biological indicators for the *B. thuringiensis* resistance.

2. MATERIALS AND METHODS

Insects:

Spodoptera littoralis (Bosid.) was obtained from the Division of the Cotton Leaf worm, Plant Protection Research Institute and reared according to methods of **El- Defrawi *et al.* (1964),** on an artificial diet as described by **Navon (2000)**. All subsequent tests were made with larvae of the 4th instar.

Bacteria:

Five *Bacillus thuringiensis* formulations were used in the present study. They are MVPII 10%, Dipel-2X, Ecotech–Bio and Dipel Es which are *B. thuringiensis* var. *kurstaki* containing 32.000 international units /mg (IU/mg) and the type Xentari is *B. thuringiensis* var. a*izawai*, containing 35.000 Diamondback Moth units/mg of product. All *B. thuringiensis* varieties were obtained from Mycogen Corporation, U.S.A.

Susceptibility of *S. littorali*s larvae to bacterial formulations:

Different concentrations of *B. thuringiensis* toxins were mixed with the larval diet and the mortality was recorded after 7 days. Bacteria were discontinued after an exposure time of 48 hr and the survived larvae were transferred to other clean jars and supplied daily with untreated diet until pupation **(Moar *et al.,* 1995)**. A control experiment was performed using untreated artificial diet in the same manner. Four replicates were used and the LC_{50}s were recorded for each formulation.

Selection of *B. thuringiensis* resistance:

Selection for resistance was carried out by rearing the larvae firstly on treated diet with the *B. thuringiensis* var. *kurstaki* (MVPII) formulation. Large numbers were employed and the selection pressure was always sufficient to cause 75% mortality. According to the response of the treated insect to selection, a higher concentration of the toxicant was sometimes used in subsequent generations. A periodical test for resistance was carried out on the third generation after the release of *B. thuringiensis* pressure. The stability of resistance was taken as a sign of homogeneity of the strain **(Muller *et al.,* 1994).**

Resistance spectrum of the resistant strain to various *B. thuringiensis* formulations:

The MVPII selected resistant larvae of *S. littoralis* were tested against Dipel-2X, Echotech – Bio, Dipel Es and Xentari by using the same concentrations used to carry out the selection and the susceptibility was measured **(Moar *et al.,* 1995)**. In comparisons of the resistabilities as called resistance ratio (LC_{50} of the resistance strain / LC_{50} of susceptible strain), differences of five folds or more (true resistance differences) were considered as indicating positive correlation; those between 1 and 4 folds (tolerance differences) as indicating no correlation and any differences less than one as representing a probable negative correlation. The parent (normal) strain was used for comparison **(Litchfield and Wilcoxon, 1949).**

Determination of total protein concentration:

Larvae which were treated with LC_{50} of different bacterial formulations at the 2nd, 4th, 6th and 9th days post treatment, as well as those from the different generations (F0, F2, F4, F6, F8, F10 and F12), during the selection with *B. thuringiensis* var. *kurstaki* (MVPII) along with untreated control were used. The whole body tissues were homogenized in a cold porcelain mortar containing 2 ml of 0.9% saline solution and then centrifuged for 5 min at 12000 r.p.m. According to the method of **Henry (1964),** the total protein concentration was determined by using Total Protein Kit (Diamond-Diagnosis Co., U.S.A). Absorbance (optical density) of the resultant color of sample (A_{sample}) and standard ($A_{standard}$) was measured at 546 nm against reagent blank. Each sample was replicated three times.

Protein fractionation:

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (PAGE-SDS) 15% was performed for larval homogenate samples in the presence of a standard protein marker as described by **Smith (1976)**. Larvae which were treated with MVPII at the 2nd, 4th, 6th and 9th days post treatment during the selection with *B. thuringiensis* var. *kurstaki* (MVPII), as well as those from the different generations (F0, F6, F8, F10, F12), during the selection along with untreated control were used.

Statistical analysis:

The percentage of mortality was corrected according to the Abbott formula **(Abbott, 1925)** for correction wherever required. Probit analysis was determined to calculate LC_{50} **(Finney, 1971),** through software computer program. Fitness of the regression line was checked by (Chi) ^{2} test. Statistical significant differences between individual means were determined by one way analysis of variance (ANOVA). Protein patterns were analyzed using Phoretix ID Advanced version 5.1 Non linear Dynamics, UK program.

3. Results

Toxicity of *B. thuringiensis* formulations to *S. littoralis* larvae:

Susceptibility tests on a laboratory strain of the 4th larval instars of *S. littoralis* against different bacterial formulations of *B. thuringiensis* var. *kurstaki* (MVPII, Dipel-2X, Ecotech-Bio and Dipel Es) and Xentari of *B. thuringiensis* var. *aizawai* are indicated in Table 1. The highest mortality rate was achieved by Ecotech-Bio followed by MVPII, Dipel-2X, while the lowest effect was recorded by Dipel Es formulation. The LC_{50}s for the tested formulations were measured as 0.08, 0.11, 0.22 and 0.27 % for MVPII, Dipel-2X, Ecotech–Bio and Dipel Es, respectively. The mortality percentages for Xentari formulation were ranged between 28.88–100 % for the concentrations 0.00001 and 0.005 %, respectively. The LC_{50} for Xentari formulation was determined as 0.00045 % (Table 1).

Selection for *B. thuringiensis* resistance:

Selection for MVPII resistance in *S. littoralis* larvae was made by rearing the larvae first on treated diet contained 0.1% of MVPII for the first 3 generations and then the selected concentration was increased to 0.125 % for the next 3 generations, then to 0.25 % for another 3 generations and finally to 0.5 for the last 3 generations of selection. They measured 0.16, 0.21, 0.25, 0.39, 0.72 and 1 %, respectively (Table 2). The level of resistance was increased to 2 folds at F2 generation, then gradually increased as the generation of selection increased to 2.63, 3.13, and 4.88 folds more than the recorded in treated normal parent (F0) at F4, F6 and F8, respectively, which considered lied within the vigour tolerance range, then the resistance sharply increased to 9 folds at the tenth generation of selection (F10) while the selected generation in F12 was recorded the highest resistant ratio and measured 12.5 folds higher than the normal susceptible strain (Table 2). Table 2 also indicates the development of LC_{50s} of MVPII during selection. The selected strain after treatment was 12.5 times as resistant as the normal susceptible one during twelve successive generations.

Resistance spectrum of the resistant strain to various *B. thuringiensis* formulations:

Table 3 shows no correlation to all the other *B. thuringiensis* var. *kurstaki* and *B. thuringiensis* var. *aizawai* formulations. The LC_{50} of Dipel-2X, Echotech–Bio, Dipel Es and Xentari were increased to 1.82, 1.3, 1.67 and 3.78 fold, respectively more than that recorded with the normal susceptible parent (F0). These values fall in the range of vigour tolerance, and fall between 1- 4 folds or (tolerance differences) indicating no correlation.

Effect of bacterial formulations on the total protein concentrations of *S. littoralis* larvae:

All bacterial formulations decreased the total protein contents at different days post treatment, except Ecotech–Bio, which showed increase in protein content at the 4th and 9th days post treatment to 14.73 and 7.58 ug/ul, respectively. Dipel Es (17.98 and 20.58 ug/ul) and Xentari (12.77 and 19.71 ug/ul) showed high values in total protein after 6th and 9th days post treatment, respectively. The total protein content was increased to 17.32 ug/ul at the 9th days post treatment with MVPII (Table 4).

Effect of MVPII selection on the total protein concentrations for *S. littoralis* larvae:

The total protein concentration in untreated normal strain ranged between 6.28 and 9.31 µg/µl during the larval development at the tested days (Table 5) Total protein content as a result of treatment with MVPII showed highly significant reduction in all generations especially at the 2nd, 4th and 6th days post treatment except the resistant strain (F12) which have significant increase in protein contents comparing with untreated control. High significant increase in total protein was noticed at 9th day in all strains especially normal parent (F0) and resistant strain (F12) which were (17.33 and 17.33 µg/µl, respectively) comparing with untreated (6.28 µg/µl). Significant increases in total protein were recorded in F6, F8 and F10 compared with untreated control.

Fractionation of proteins during selection with *B. thuringiensis* var. *kurstaki* (MVPII):

The electrophoretic analysis of SDS–PAGE protein was carried out for untreated normal strain (control) and the treated 4th larval instar of *S. littoralis*. This instar was tested at the 2nd, 4th, 6th and 9th days post treatment with LC_{50} of MVPII (Fig. 1). The treatments were applied for treated normal parent and the other strains during selection.

The SDS-PAGE revealed that the maximum numbers of protein bands for the whole body tissues of the samples were separated into 24 bands. Twenty one of them were present in untreated samples. The protein bands were distributed as 23, 23, 24 and 24 at the 2nd, 4th, 6th and 9th days, respectively. There were 14 common bands of M.W. ranged between 153.77 and 5.9 KDa present in all tested days, these proteins may be characteristics for untreated control. Two common bands of M.W. 89.82 and 66.42 KDa are present in all tested days during all generations (Fig. 1). The protein electrophoresis showed differences between the untreated and treated samples at the different tested days and different generations. The average band number of treated samples ranged between 10 and 17 bands comparing with 15 and 16 bands for untreated control in the 2nd and 4th days post treatment, while the average number of protein bands (20-21) was detected in the last instars (6th and 9th days) of untreated control comparing with (10-22) bands in treated generations (Figs 1 & 2). Specific protein bands of M.W. 55.46, 31.05 and 29.62 KDa were recorded in treated normal parent (F0) and resistant strain (F12) in certain days (Figs 1 & 2). Also the disappearing of eight bands of M.W. 174.23, 153.77, 94.54, 81.96, 78.66, 69.89, 62.62 and 35.54 KDa was detected in the selected strain (F12).

Table 1: Susceptibility of the 4th larval instar of *S. littoralis* to different formulations of *B. thuringiensis* var. *kurstaki* and *aizawai*

Concentration (%)	Mortality % (corrected)					
	No. of tested larvae	MVPII	Dipel-2X	Ecotech-Bio	Dipel Es	Xentari
0.00001	100	-	-	-	-	28.88
0.0001	100	-	-	-	-	42.22
0.00125	100	-	-	-	-	56.67
0.0025	100	-	-	-	-	63.33
0.005	100	-	-	-	-	100
0.01	100	20	32.63	20	8.88	-
0.0625	100	44	45.26	24.44	22.22	-
0.125	100	58	52.63	42.22	33.33	-
0.25	100	70	62.11	44.66	50	-
0.5	100	80	78.95	68.88	63.33	-
1.0	100	88.88	83.16	100	72.22	-
LC_{50}		0.08	0.11	0.22	0.27	0.00045
Slope function		7.39	10.69	-	8.02	22.5
95% CL* Lower – upper		0.054-0.118	0.069-0.175	0.136-0.356	0.180-0.406	0.00024-0.00083

95% CL*: 95% Confidence limits

Table 2: Susceptibility of the 4th larval instar of *S. littoralis* during selection with *B. thuringiensis* var. *kurstaki* (MVPII)

Concentration (%)	Mortality % (corrected)						
	Parents (F0)	F2	F4	F6	F8	F10	F12
0.01	20	26	-	-	-	-	-
0.0625	44	46	42	20	20	14	-
0.125	58	52	50	46	33.33	26.67	10
0.25	70	60	54	60	44.44	30	20
0.5	80	68	56	70	53.33	46	30
1	88.88	74	66	70	62	52	55.6
1.5	100	100	80	76	70	64	60
2	-	-	82	80	80	70	75
LC_{50}	0.08	0.16	0.21	0.25	0.39	0.72	1
R R*	-	2	2.63	3.13	4.88	9	12.5
Slope function	7.39	11.47	11.07	6.97	10.44	10.4	4.9
95% CL* Lower - upper	0.054-0.118	0.099-0.258	0.131-0.336	0.171-0.366	0.246-0.617	0.455-1.14	0.73-1.369

95% CL*: 95% Confidence limits R R*: Resistance Ratio

Table 3: Susceptibility of the 4th larval instar of *S. littoralis* selected by (MVPII) *to B. thuringiensis* formulations

Concentration (%)	Mortality % (corrected)									
	MVPII		Dipel-2X		Ecto-Bio		Dipel Es		Xentari	
	Parent (F0)	Selected	Parent (F0)	Selected	Parent (F0)	Selected	Parent (F0)	Selected	Parent (F0)	Selected
0.00001	-	-	-	-	-	-	-	-	28.88	17.39
0.0001	-	-	-	-	-	-	-	-	42.22	20
0.00125	-	-	-	-	-	-	-	-	56.67	45.65
0.0025	-	-	-	-	-	-	-	-	63.33	45.65
0.005	-	-	-	-	-	-	-	-	100	72.83
0.0125	-	-	-	-	-	-	-		-	83.7
0.01	20	-	32.63	17.39	20	13.04	8.88	13.04	-	-
0.025	-	-	-	-	-	-	-	-	-	89.13
0.0625	44	-	45.26	23.91	24.44	19.57	22.22	15.22	-	-
0.05	-	-	-	-	-	-	-	-	-	100
0.125	58	10	52.63	41.3	42.22	36.96	33.33	23.91	-	-
0.25	70	20	62.11	65.22	44.66	50	50	34.78	-	-
0.5	80	30	78.95	69.57	68.88	65.22	63.33	51.09	-	-
1.0	88.88	55.6	83.16	76.09	100	78.26	72.22	67.39	-	-
1.5		60	-	-	-	-	-	-	-	-
2.0		75	-	-	-	-	-	-	-	-
LC50	0.08	1	0.11	0.2	0.22	0.26	0.27	0.45	0.00045	0.0017
R R*		12.5		1.82		1.3		1.67		3.78
Slope function	7.39	4.9	10.69	9.01	11.64	5	8.02	6.1	22.5	9.41
95% CL* Lower – upper	0.054-0.118	0.73-1.369	0.069-0.175	0.13-0.308	0.136-0.356	0.19-0.357	0.180-0.406	0.316-0.61	0.00024-0.00083	0.0011-0.0026

95% CL*: 95% Confidence limits R R*: Resistance Ratio

Table 4: Total protein concentrations of the 4th larval instar of *S. littoralis* at different days post treatment with LC_{50s} of different bacterial formulations

Days post treatment	Total protein content (mean± S.E) (µg/µl)					
	untreated normal control	MVPII	Dipel-2X	Ecotech-Bio	Dipel Es	Xentari
2nd	7.04 a (± 0.017)	1.52 e (±0.016)	2.16 d (±0.062)	6.28 b (±0.038)	5.85 c (±0.25)	1.52 e (±0.187)
4th	8.44 b (± 0.002)	1.3 d (±0.03)	3.68 c (±0.012)	14.73 a (±.249)	8.23 b (±0.125)	3.88 c (±0.031)
6th	9.31 c (±0.125)	4.55 d (±1.65)	3.90 e (±0.025)	6.50 d (±0.062)	17.98 a (±0.186)	12.77 b (±0.124)
9th	6.28 e (±0.17)	17.33 c (±0.037)	2.60 f (±0.037)	7.58 d (±0.038)	20.58 a (±0.12)	19.71 b (±0.187)

Means with the same letter are not significantly different.
S. E*: Standard Error

Table 5: Total protein concentrations of the 4th larval instar of *S littoralis* at different days post treatment with LC50s of MVPII during selection

Days post treatment	Total protein content (mean± S.E) (µg/µl)					
	untreated normal control	Treated parent (F0)	F6	F8	F 10	F 12
2nd	7.04 a (± 0.017)	1.52 e ± 0.016	1.3 f ± 0.12	2.17 d ± 0.012	2.38 c ± 0.026	3.47 b ± 0.012)
4th	8.44 b (± 0.002)	1.3 g ± 0.03	1.95 f ± 0.187	2.60 e ± 0.025	3.47 d ± 0.013	3.90 c ± 0.037
6th	9.31 c (± 0.125)	4.55 c ± 1.65	3.25 d ± 0.063	3.03 d ± 0.102	4.33 d ± 0.038	10.83 b ± 0.125
9th	6.28 e (± 0.17)	17.33 c (± 0.037)	8.66 d ± 0.124	10.76 b ± 0.037	9.10 c ± 0.125	17.33 a ± 0.174

Means with the same letter are not significantly different.
S. E*: Standard Erro

4. Discussion

With bacterial formulations of *B. thuringiensis* var. *kurstaki*, the highest mortality was achieved by Ecotech-Bio followed by MVPII, Dipel- 2X, while the lowest effect was recorded by Dipel Es. These results are in agreement with the finding of **El-Sweerki (1994); Raslan (1998); Abd El-Aziz (2000)** and **Huang *et al.* (2005).** In the same time, the LC_{50} for Xentari formulation was determined as 0.00045 %. The same results were reported by **Mascarenhas *et al.* (1998)**. Mortality of the infected larvae may be due to the undigestion of the ingested food, or due to paralysis and/or the physiological disturbance due to the toxicity of the haemolymph **(Lotfy, 1988)**. *B. thuringiensis* strains produce at least two endotoxins that affect insect larvae. One of the toxins is associated with endospore coat, while the other is found in parasporal bodies (crystals). The toxic material in parasporal bodies is a crystalline protein **(Chamblis and Boyd, 1988)**.

After continuous selection with MVPII formulation, from the 2nd generation to the 12th generation, the resistance ratio was raised from 2.63 to 12.5 fold compared with the normal strain. However, this resistance is relatively low if compared with chemical selection. It brings the ability of *S. littoralis* to developed resistance. The resistant organisms tolerate the effects of toxic elements either by developing ways of the preventing the toxins from reaching the target sites or by modifying the site so that its sensitivity to the toxin is decreased **(Cerda, 2003).**

The present data revealed a moderate resistance after selection pressure of S. *littoralis*. These results were similar with that recorded by **(Sneh *et al.*, 1983)** who found no development of true resistance in *S. littoralis* after 10 generations of selection by *Bt. entomocidus***.** On the other hand, unsuccessful attempts to select lepidopteran insects for *Bt* resistance has been reported by **Moar *et al.* (1995)** who found no significant increase in resistability in *S. littoralis* and S. *exigua* against *B. thuringiensis*.

The present results also showed that MVPII selected strain had no correlation to all the other *B. thuringiensis* var. *kurstaki* and *B. thuringiensis* var. *aizawai* formulations so; no cross resistance were occurred. **MacIntoch *et al.* (1991)** observed multiple changes in the receptor binding sites in resistant strain of *H. virescens* compared with susceptible one. Accordingly $CryIA_b$ and $CryIA_c$ bind equally to midgut of susceptible but differ in resistant strains, while CryIIIA did not bind in both indicating negative cross-resistance. Resistance to subsp. *kurstaki* caused relatively narrow-spectrum resistance to CrylA(b) and CrylA(c) toxins **(McGaughy and Johnson, 1994).** No or little cross-resistance toward CrylAa, CrylAb, Cry2Ab and Cry2Aa in resistant *strains* to CrylAc and Dipel was observed by **Sayed *et al.* (2000) and Tabashnik *et al.*(2002)**. On the other hand **McGaughy and Johnson (1987)** found that 36 out of 57 isolates of *B. thuringiensis* Dipel resistant strain caused cross-resistance to 16 other isolates from subsp. *kurstaki*. Also **Muller *et al.* (1994)** worked on *S. littoralis* resistant to CryIC exhibited positive cross-resistance to CrylE and CryID.

In the present investigation, all bacterial formulations caused highly significant inhibition on total protein concentration except with Ecotech-Bio which caused increasing in total protein comparing

with untreated control. This fluctuation in the protein may due to biochemical and physiological changes in the infected insect with pathogenic bacteria and considered the net result of produced toxins, which may inhibit totally some metabolic functions of the host, or host metabolic reactions in response to pathogenic infection. Also, this observation may be explained that bacterial infection inhibited the protein contents and its synthesis **(Lotfy, 1988 and Abd El-Aziz, 2000)**. The decrease in the total protein in treated larvae may reflect the decrease in the activity of various enzymes **(Kyung and Kim, 1990)**. The present results agree with that obtained by **El-Sweerki (1994)** and **Sokar (1995) Abd El-Aziz (2000)** on *S. littoralis*.

Selection with MVPII caused highly significant inhibition in protein synthesis in all generations. The most inhibition was in normal treated parent (F0) which recorded highly reduction percent (84.6 %) at the 4th day then gradual increase in the protein content was observed as larval resistance developed. Decreasing of protein in the early generations of selection may be due to bacterial infection which inhibited the protein contents and its synthesis, or the infected insect larvae quickly stop feeding due to paralysis of its mouth parts **(Abd- El- Aziz, 2000)**. *B. thuringiensis* could increase the larval protease activity leading to protein hydrolysis and hence its concentration was decreased **(Kamal and Abdel Hamid, 2005).**

The total protein increased as the resistance was increased specially at the 9th day in all selected generations. The most effected generation (F12) which was sharply increased, more or less similar to that of the normal treated parent strain, but still more than untreated strain. Increasing in the protein may be attributed to the protein crystals produced by bacterial cells which was toxic if ingested by larvae **(Kamel and Abdel Hamid, 2005)**. This result is in agreement with that of **Heimpel and Angus (1959)** where the mid gut pH of most susceptible larvae is too alkaline to allow spore germination but is suitable for dissolution and activation of protoxin.

In the present study, protein patterns of untreated control revealed that some bands appeared in certain days and disappeared in other days. There were 14 common bands of M.W. ranged between 153.77 and 5.9 KDa present in all tested days, these proteins may be characteristics for untreated control. Two common bands of M.W. 89.82 and 66.42 KDa are present in all tested days during all generations and may be indicating that these proteins are characteristics for *S. littoralis* larvae.

Since total proteins are markedly increased in the treated larvae, proteins electrophoresis was done to assess the possible change in protein bands in each treatment. Electrophoretic mobility of whole body proteins from *S. littoralis* larvae treated with MVPII revealed the appearance of a new protein band in the infected larvae and disappearance of other. The appearance of these new protein fractions might be due to the increase in the protein synthesis as a result of treatment while the disappearance of other fractions might be attributed to their breakdown**.**

The number of bands decreased as the resistance was increased. Changes in the protein patterns may be due to bacterial infection, larval development, or resistance increase. These observations agree with that reported in *Phylosamia ricini* where the number of the electrophoretically separated protein bands was reduced from 11 to 6 bands in the seventh day following infection **(Poonia, 1979)**.

The changes in the protein fractions translated by the detection of three extra specific protein bands of M.W. 55.46, 31.05 and 29.62 KDa were recorded in treated normal parent (F0) and resistant strain (F12) in certain days. Also the disappearing of eight bands of M.W. 174.23, 153.77, 94.54, 81.96, 78.66, 69.89, 62.62 and 35.54 KDa was detected in the selected strain (F12). These observations are in agreement with **Hughes *et al.* (1983)** and **Abou El-Seoud *et al.* (2005)** who demonstrated that the appearance of five specific proteins bands in larvae treated with the insecticide.

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