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The Entomocidal Crystal Proteins Cry1Ac and Cry2Ab and Their Relationship to Physiological Responses in Egyptian Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

Hassan F. Dahi[1], Ahmed M. A. Salman[2] and Asmaa M. A. Bedawi[1]
1- Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
2- Plant Protection Department, Faculty of Agriculture, Sohag University, Egypt
E. mail* hassandahi@yahoo.com

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ABSTRACT

The current work was carried out to evaluate the biochemical effects of LC_{50} of the *Bacillus thuringiensis* as spores against *Spodoptera littoralis* 2nd and 4th instars and Cry1Ac and Cry2Ab against newly hatched larvae to determine the effects of these compounds on total carbohydrates, proteins, lipids, acetylcholinesterase, chitinase, phenoloxidase, carbohydrates hydrolyzing enzymes, nonspecific esterases, phosphatases and transaminase enzymes. The obtained results indicated that total proteins and lipids content were highly significantly decreased and a highly significantly increased in total carbohydrates with *B. thuringiensis* spores for both instars. A highly significant decrease in total carbohydrates with Cry1Ac and increased with Cry2Ab. While total protein was highly significantly increased with Cry1Ac and decreased with Cry2Ab. The activity of invertase and trehalase were highly significantly decreased with *B. thuringiensis* spores for both instars. On the other side, A highly significantly increased in amylase and invertase enzyme activity with Cry1Ac and decreased with Cry2Ab. In contrast, Cry1Ac and Cry2Ab caused a highly significant decrease in trehalase activity. It is clearly noticed that *B. thuringiensis* spores significantly increased GOT for both instars larvae, in contrast, GPT was highly significantly increased in 2nd instar larvae only. While GOT activates were a highly significant dropped with Cry1Ac and Cry2Ab. Alpha esterase was highly significantly activated with *B. thuringiensis* spores in 2nd instar larvae and inhibition in 4th instar larvae while Beta esterase and Acetylcholinesterase enzymes activities were a highly significant increase in both instars larvae. On the other side, Alfa, beta esterase and acetylcholinesterase were highly significantly decreased with Cry1Ac and increased with Cry2Ab.

INTRODUCTION

The Egyptian cotton, *Gossypium barbadense* L., is considered a major economic crop in Egypt; it represents the first cash crop for national income. Thus, the governmental policy in Egypt is offering all facilities to encourage farmers to increase the production of cotton in order to meet the increasing requirements for local production and allow surplus for exportation. Several million pounds are paid every year for controlling the cotton

pests, the control of *S. littoralis* based mainly on foliage treatments with chemical synthetic insecticides. The widespread and intensive use of different synthetic insecticides for controlling this pest caused increasing environmental problems including insect resistance, excessive persistence of residues, human health hazards and harmful effects on non-target organisms (Dahi, 2012a). Therefore, there is a great need to develop alternative or additional techniques, such as bioinsecticides considered one of the safest methods for pest control (Dahi, *et al.*, 2016).

Bacillus thuringiensis is a Gram-positive, aerobic, or facultative anaerobic entomopathogenic bacterium found in soil, on plant surfaces, and in grain storage dust. *B. thuringiensis* forms δ-endotoxins as parasporal inclusions, during sporulation these inclusions dissolve in the insect midgut, releasing protoxins (Cry proteins) (Yu, 2014). Those proteins after hydrolysis have specific toxin activity for insects and other invertebrates, not causing harmful effects in other organisms (Silva-Werneck *et al.*, 2001). After being ingested in the midgut, those *Bt* protoxin is activated by the gut proteases into an active toxin, which binds to specific receptors like cadherin and amino peptidase-N or alkaline phosphates in the peritrophic membrane, forming pores in the midgut epithelial cells (Fortier *et al.*, 2007, Abdullah *et al.*, 2009, and Talaei-Hassanloui *et al.*, 2014). The *Bt* crystal inclusion, toxicity is dependent on a complex process that requires multiple steps, these include solubilization of the crystal proteins, proteolytic processing of the protoxin to the active form, high-affinity binding with the midgut receptor, and the irreversible insertion of the toxin into the gut membrane (Jenkins *et al.*, 2000). In Lepidoptera, the Cry-binding proteins include cadherin, APN, alkaline phosphatase (ALP), 270 glycoconjugate (a 270 kDa protein), and P252 (a 252 kDa protein) (Yu, 2014). Since 1996, some of these insecticidal *Bt* proteins (mainly Cry proteins) have been incorporated into transgenic crops (*Bt* crops) for control of several major pests of Lepidoptera and coleopteran. In 2015, more than 84 million hectares of *Bt* cotton, corn, and soybean were planted globally (James, 2015).

In cotton, the proteins expressed (Cry1Ac and Cry2Ab) confer protection from a broad array of lepidopteran herbivores, enabling the use of broad-spectrum insecticides to be greatly reduced (Dahi, 2013). The activities of enzymes such as alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are parameters widely used in the diagnosis of diseases as these could give indications of progressive toxicity long before the actual manifestation of the toxic effects (Hanley *et al.*, 1986). Enzyme assays with purified insect midgut microvilli membranes showed that aminopeptidase and alkaline phosphatase are enzyme markers of these membranes. Systematic digestive enzyme assays revealed that the physiological role of midgut microvilli membranes may change along the midgut and among insect taxa and should include surface (terminal) digestion, absorption, ion homeostasis, signaling and unique digestive secretory mechanisms (Terra and Ferreira, 1994, 2012).

The present work was carried out to evaluate the biochemical effects of LC_{50} of the *B. thuringiensis* against *Spodoptera littoralis* 2nd and 4th instars and Cry1Ac and Cry2Ab against newly hatched larvae.

MATERIALS AND METHODS

Insect Rearing:

Two laboratory strains of *S. littoralis* were obtained from Plant Protection Research Institute, Cotton Leafworm Department, Dokki, Giza. The first strain was fed on castor oil plants *Ricinus communis* and the rearing technique was according (Dahi, 1997) as follows:

Egg masses of *S. littoralis* were resulting from a maintained insect culture reared in the laboratory for at least four generations on the fresh leaves of castor oil plant as natural food which resemble the natural food in the field (i.e. cotton leaves). For strain establishment, the eggs were maintained at 27 ± 2°C and 65 ± 5 % R.H until hatching. Newly hatched larvae were transferred to clean 5-pound glass-Jars covered with muslin and secured with rubber bands. They were provided with fresh castor-oil leaves which were renewed daily until the larvae show the signs of pupation. A thin layer of fine saw-dust was spread on the bottom of every glass-Jars to help the successful pupation. Pupae were kept individually in a vial until moth emergence. Ten pairs of newly - emerged moths were confined into oviposition cages. Which consists of conventional mating glass bells (16cm. high and 8cm.diam.) opened at each end. Each mating-glass bell was supplied with a small fresh branch of *Nerium oleander* to serve as an oviposition site and placed on its wide end on a half petri-dish. Tops of the glass bells were covered with muslin and secured with rubber bands. Cages were examined daily to replace *N. oleander* branches with new ones and renew the adult feeding solution (a small piece of absorbent cotton wool previously soaked in 10% sucrose solution). The cages were maintained at the same conditions of temperature and % R.H. Deposited egg- masses were kept in petri-dishes, and they were available to achieve the different experiments for the toxicity of *Bt* spores (Protecto®) against 2nd and 4th instar larvae of *S. littoralis* laboratory strain. The second strain: the larvae were fed on an artificial diet according to (Abdel-Hafez *et al.,* 1982). The rearing technique was according (Dahi, 2005). Newly hatched larvae were fed individually in glass vials (20 x 7.5 cm) filled to one-third with the artificial diet, covered with absorbent cotton and held in the same conditions as mentioned above. The larvae pupate is usually on the diet's top or between its parts. At the beginning of pupation, the vials were examined daily for transferring the formed pupae individually to clean vials and incubated until moth emergence. Ten pairs of newly emerged cotton leafworm moths were confined in glass oviposition, which consists of a conventional mating glass bell (16 cm. high and 8cm. diam). opened at each end. Each mating - glass bell was supplied with a small fresh branch of *Nerima oleander* to serve as an oviposition site and placed on its wide end on a half petri-dish. Tops of the glass bells were covered with muslin and secured with rubber bands. Cages were examined daily to replace *N. oleander* branches with new ones and renew the adult feeding solution (a small piece of absorbent cotton wool previously soaked in 10% sucrose solution).

Tested Compounds:

1- **Bio-insecticide** (Protecto) used as a commercial product of *Bacillus thuringiensis* subsp. *Kurstaki* contains 32x10^3 I. U/mg). Active ingredient 9.4% was obtained from Plant Protection Research Institute, Agricultural Research Centre, Egypt.

2- **Cry1Ac and Cry2Ab toxins** were freeze-dried powders obtained from Monsanto Company, USA.

Cry1Ac and Cry2Ab protoxin is a crystal protein produced by the gram-positive bacterium, *Bacillus thuringiensis (Bt)* during sporulation. Transgenic *Bt* cotton expressed Two *Bt* gene, which codes for Cry1Ac and Cry2Ab. Transgenic crops expressing the insecticidal proteins Cry1Ac and Cry2Ab from *Bacillus thuringiensis (Bt)* are used worldwide to suppress damage by lepidopteran pests, often used in combination between Cry1Ac and Cry2Ab toxin to delay resistance evolution in *Bt* cotton.

Biochemical Studies:

Biochemical analyses were carried out on the 2nd and 4th instars for *Bacillus thuringiensis* as spores and newly hatched larvae for Cry1Ac and Cry2Ab, both treated with LC_{50} of each compound and compared with control.

Preparation of Homogenate Samples for Biochemical Analysis:

Homogenate was collected from pooled samples by homogenizing in insect physiological saline. Homogenate was collected in cold tubes (on ice). The samples were centrifuged at 4500 rpm for 5 minutes under cooling (4°C) to remove the remnant of tissues. After centrifugation, the supernatant fluid was collected and divided into small aliquots (0.5 ml) and stored at –20 °C until analysis.

Enzyme Assays:

1- Determination of Non-Specific Esterases Activities:

Alpha- and beta-esterases (α-E, β-E) activities were determined according to the method of **Van Asperen (1962).**

2- Determination of Acetylcholine Esterase Activity:

The activity of acetylcholine esterase (AChE) was measured according to the method described by **Simpson *et al.*, (1964)** using acetylcholine bromide (AChBr).

3- Determination of Phosphatases Activity:

Acid and alkaline phosphatase activity was measured according to the method of **Laufer and Schin (1971).**

4- Phenoloxidase activity determination

Phenoloxidase activity was determined according to a modification of Ishaaya (I971).

5- Determination of Transaminases Activity:

The level of both transaminases (GOT and GPT) was determined colorimetrically according to Reitman and Frankel (1957).

6- Determination of Total Protein Content:

The protein content of the Haemogenate samples was determined using folin phenol reagent according to the method of Lowry *et al.* (1951).

7- Determination of Total Lipid Content:

The total lipid content of the hemolymph was determined by the phosphovanillin method of Baronos and Blackstock (1973).

8- Determination of Total Carbohydrate Content:

The total carbohydrate content of the haemolymph was determined according to Singh and Sinha (1977).

9- Determination of Carbohydrases Activity:

Invertase, Trehalase and amylase activities were determined according to Ishaaya and Swirski (1970) and Isahaaya *et al.* (1971).

10- Determination of Chitinase activity:

Chitinase was assayed using a 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine liberated on chitin digestion according to the method described by Ishaaya and Casida (1974).

The aim of this work is investigation was carried out to evaluate the biochemical effects of LC_{50} of *B. thuringiensis* as spores on 2^{nd}, 4^{th} after 3 days from treatment and after 5 days of newly hatched larvae treated with Cry1Ac and Cry2Ab.

RESULTS AND DISCUSSION

Carbohydrates, proteins and total lipids are efficiently utilized by insects and most species derive the main part of their nourishment from these nutrients. The utilization of these nutrients depends on the digestive enzymes; amylase, trehalase and invertase. Hamama *et al.* (2015) reported that changes in enzymatic activities after treatment with bioinsecticides indicated that changes in the physiological balance of the midgut affect

these enzymes. After infection with bioinsecticides, the epithelial cells undergo excessive oxidation due to bacterial endotoxin.

Effect of *B. thuringiensis* as Spores, Cry1Ac and Cry2Ab on the Biochemical Parameters of Larvae of *S. littoralis*

1-The main metabolic components:

The main metabolic (total carbohydrates, total proteins and total lipids) are major biochemical components necessary for an organism to develop, grow and perform its vital activities (Abdel-Khalek, 2007).

Carbohydrates play a major role in insect development like metabolism, metamorphosis, development of flight muscles, reproduction and embryonic development (Chapman, 1998).

The total protein content is one of the major biochemical components necessary for an organism to develop, grow and perform its vital activities. Proteins are important for individual-level fitness associated traits such as body size, growth rate and fecundity and at higher levels of the organization. They have been linked to population dynamics, life histories and even biological diversification (Fagan *et al.*, 2002). Wilkinson (1976) stated that proteins are the most important components of the biochemical of insects that bind the foreign compounds.

Lipids are the most suitable reserves for the storage of energy. Compared to carbohydrates, lipids can supply as much as eight times more energy per unit weight (Beenakkers *et al.*, 1985).

1.1-Effect of LC_{50} of *B. thuringiensis* as Spores (commercial product) on the Total Carbohydrates, Proteins, and Lipids Against 2nd and 4th instar Larvae of *S. littoralis*:

Results in Table (1) indicated that total carbohydrates content of 2nd and 4th instar of *S. littoralis* was highly significantly increased with *B. thuringiensis* spores compared with control for 2nd and 4th instars. Total carbohydrates content were 166.38 and 150.21 (µg/g.b.wt) for 2nd and 4th instars, respectively. While it was 152.46 and 136.98 (µg/g.b.wt) with their control, respectively.

The total haemolymph protein content of 2nd and 4th instars of *S. littoralis* was highly significantly decreased with *B. thuringiensis* spores compared with control for 2nd and 4th instars. Total proteins were 260.31 and 289.34 (µg/g.b.wt) for 2nd and 4th instars, respectively. While it was 312.58 and 295.64 (µg/g.b.wt) with their control, respectively.

Also, data in Table (1) revealed that the total lipids were highly significantly decreased with *B. thuringiensis* spores compared with control for 2nd and 4th instars. Total lipids were 277.64 and 269.25 (µg/g.b.wt) for 2nd and 4th instars, respectively. While it was 292.36 and 291.35 (µg/g.b.wt) with control for 2nd and 4th instars, respectively.

These results agreed with those obtained by Salem (2011) who found a reduction in total soluble proteins of *Sesamia cretica* in treated larvae compared with control. Also, El-Kattan (1995) studied the effect of *B. thuringiensis* on the total protein and lipid contents in the haemolymph of *Plodia interpunctella* larvae after injection with LC_{50} concentration. Infection produced a gradual decrease in protein content of infected larvae at 12 and 18 hr intervals following injection. Zidan *et al.* (1998) found also that *B. thuringiensis* caused a significant reduction in protein content of the *S. littoralis*. Abd El-Aziz (2000) found that was a reduction in the lipid content after treating *S. littoralis* larvae with Dipel 2X (*B. thuringiensis*). Tripathi and Singh (2002) studied the effect of *B. thuringiensis* on the pathophysiology of *Spodoptera litura* larvae at 24-, 48- and 72-hours post-infection. These results showed that total carbohydrates contents of haemolymph were found significantly higher in diseased larvae than untreated ones. Also, they found a significant reduction in the total lipid content of larval haemolymph. They suggested that the reason for the lower fat content in larvae could be due to the extended larval period of the treated insects and

blocked food ingestion, and the fat reserves might have been utilized for maintenance during the extended larval period. The decrease in the total protein in treated larvae may reflect the decrease in the activity of various enzymes (Kyung and Kim, 1990). These results also are in accordance with that demonstrated by Hamama *et al.* (2015) on Agerin and Rashwan, (2013) and Abdel-Salam *et al*. (2018) on Protecto, which showed a significant decrease in total proteins of *S. littoralis* larvae compared with untreated larvae. Total proteins are major biochemical components necessary for an organism to develop, grow and perform its vital activities. The reduction of protein content may be due to inhibition of DNA and RNA synthesis (Elbarky *et al.*, 2008).

Table 1: Effect of LC_{50} of *B. thuringiensis* as spores (commercial product) on the total carbohydrates, proteins and lipids against 2nd and 4th instar larvae of *S. littoralis*

Compound	Total carbohydrate	Total protein	Total lipid
2nd instar			
***Bt* spores**	166.38±1.10**	260.31±1.0	277.64±0.33
Control	152.46±1.54	312.58±0.29**	292.36±0.54**
4th instar			
***Bt* spores**	150.21±0.92**	289.34±0.33	269.25±0.49
Control	136.98±0.26	295.64±0.28**	291.35±0.19**

**: highly significant (P<0.01)

1.2-Effect of LC_{50} of Cry1Ac and Cry2Ab on the Total Carbohydrates, Proteins and Lipids Against the Newly Hatched Larvae of *S. littoralis*:

Data in Table (2) revealed that, after treatment of newly hatched larvae of *S. littoralis* with LC_{50} concentrations of Cry1Ac and Cry2Ab, the total carbohydrates were a highly significantly decreased with Cry1Ac compared with control and Cry2Ab were a highly significantly increased compared with control. Total carbohydrates were 170.32, 177.39 and 175.45 (µg/g.b.wt) for Cry1Ac, Cry2Ab and control, respectively.

Also, data in Table (2) showed that total proteins were highly significantly increased with Cry1Ac compared with control and on the other side were a highly significant decrease with Cry2Ab compared with control. Total protein values were 290.36, 270.15 and 280.66 (µg/g.b.wt) for Cry1Ac, Cry2Ab and control, respectively. Same Table (2) showed that there is no significant difference between Cry1Ac, Cry2Ab and control in total lipids.

Many investigators studied the impact of bacterial toxins in insects. These toxins of *Bacillus thuringiensis* are responsible for the inhibition of protein synthesis by forming a protein complex. These data confirm the findings of (Angus and Norvis 1968) who demonstrated that d-endotoxin of *B. thuringiensis* forms a complex protein having an alkaline isoelectric pH. Also, (Lecadet and Martouret 1967a and 1967b) found that the crystal-like endotoxin of *B. thuringiensis* splits into protein and peptide. An obvious reduction in haemolymph protein concentration of 3rd instar *S. littoralis* larvae treated with *B. thuringiensis* var. *kurstaki* (Btk) combined with some chemical additives was found by (Latha *et al.*, 1996).

Table 2: Effect of LC_{50} of Cry1Ac and Cry2Ab on the total carbohydrates, proteins and lipids against the newly hatched larvae of *S. littoralis*

Compound	Total carbohydrate	Total protein	Total lipid
Cry1Ac	170.32±0.37 [c]	290.36 ±0.72[a]	312.56±0.49
Cry2Ab	177.39±0.73 [a]	270.15±0.38 [c]	299.58±5.60
Control	175.45±0.75 [b]	280.66±0.79 [b]	302.14±0.76
LSD	1.95	1.99	NS

Means with the same letter(s) is not significantly

2-The carbohydrates hydrolyzing enzymes:

Three digestive enzymes; amylase, trehalase and invertase were determined in 2nd and 4th instar larvae of *S. littoralis* which were treated with LC_{50} of *B. thuringiensis* as spores and newly hatched larvae treated with Cry1Ac and Cry2Ab.

2.1-Effect of LC_{50} of *B. thuringiensis* as Spores (Commercial Product) on the Carbohydrates Hydrolyzing Enzymes against 2nd and 4th Instar Larvae of *S. littoralis:*

Data in Table (3) showed that no significance in the amylase activity compared with control for the 2nd instar. While 4th instar exhibited a highly significant decrease the enzyme activity compared with control. Which was 39.14 and 44.86 (µg/g.b.wt), respectively.

The same Table (3) illustrated that the invertase activity was highly significantly decreased with *B. thuringiensis* spores compared with control for 2nd and 4th instars. The values were 36.47 and 36.12 (µg/g.b.wt) for 2nd and 4th instars, respectively. While it was 42.36 and 41.56 (µg/g.b.wt) with their control, respectively.

Also, Table (3) revealed that the trehalase activity was highly significantly decreased with *B. thuringiensis* spores compared with control for 2nd and 4th instars. The values were 21.34 and 21.10 (µg/g.b.wt) for 2nd and 4th instars, respectively. While it was 33.15 and 27.34 (µg/g.b.wt) with their control, respectively.

These results agreed with El-Ghar *et al*. (1995**)** who found that *B. thuringiensis*, caused a pronounced decrease in digestive enzyme activity, especially invertase.

Table 3: Effect of LC_{50} of *B. thuringiensis* as spores (commercial product) on the carbohydrates hydrolyzing enzymes against 2nd and 4th instar larvae of *S. littoralis*

Compound	Amylase	Invertase	Trehalase
2nd instar			
***Bt* spores**	44.15±1.28	36.47±0.60	21.34±0.42
Control	40.12±0.77	42.36±0.53**	33.15±0.87**
4th instar			
***Bt* spores**	39.14±0.43	36.12±0.26	21.10±0.29
Control	44.86±0.13**	41.56±0.81**	27.34±0.43**

**: highly significant (P<0.01)

2.2-Effect of LC_{50} of Cry1Ac and Cry2Ab on the Carbohydrates Hydrolyzing Enzymes Against the Newly Hatched Larvae of *S. littoralis*:

Data in Table (4) revealed that, after treatment of newly hatched larvae of *S. littoralis* with LC_{50} concentrations of Cry1Ac and Cry2Ab, the amylase activity was highly significantly increased with Cry1Ac compared with control and were highly significantly decreased compared with control. Total amylase were 52.45, 37.26 and 48.23 (µg/g.b.wt) for Cry1Ac, Cry2Ab and control, respectively. Also, data in Table (4) showed

that invertase was a highly significant increase with Cry1Ac compared with control and on the other side were a highly significant decrease with Cry2Ab compared with control. The invertase values were 35.26, 22.17 and 31.24 (µg/g.b.wt) for Cry1Ac, Cry2Ab and control, respectively.

Same Table (4) showed that the trehalase activity was highly significantly decreased with both Cry1Ac and Cry2Ab compared with control. Total trehalase values were 18.71, 11.28 and 25.39 (µg/g.b.wt) for Cry1Ac, Cry2Ab and control, respectively.

Table 4: Effect of LC_{50} of Cry1Ac and Cry2Ab on the carbohydrates hydrolyzing enzymes against the newly hatched larvae of *S. littoralis*

Compound	Amylase	Invertase	Trehalase
Cry1Ac	52.45±0.52 [a]	35.26±0.18[a]	18.71±0.38 [b]
Cry2Ab	37.26±0.61 [c]	22.17±0.55 [c]	11.28±0.42 [c]
Control	48.23±0.65 [b]	31.24±0.81 [b]	25.39±0.42 [a]
LSD	1.86	1.75	1.25

Means with the same letter(s) are not significantly different.

3-Effect on Phosphatase Enzymes:

Acid phosphatase (ACP) and alkaline phosphatase (ALP) are responsible for cytolysis of tissues during insect development (Dadd, 1970) and may act as hydrolases during the final stages of digestion (Cheung and Low, 1975), gonad maturation and the final stages of metamorphic moults (Tsumuki and kanehisa, 1984). ACP is responsible for synthesizing higher energy compounds (Hollander, 1971). ALP has the primary function to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes (Etebari *et al*., 2005). Also, ALP is an important synthesizing enzyme of tyrosine which is known to take part in the control of levels of insect developmental hormones (Rauschenbach *et al.*, 2007). Alkaline phosphatase is a brush border membrane marker enzyme and is especially active in tissues with active membrane transport, such as intestinal epithelial cells and Malpighian tubules (Ferreira and Terra, 1980). (Sridhara and Bhat 1963) stated that the increase or decrease of both phosphatase enzymes development is reflected in an increase or decrease in acid-soluble phosphorus content.

3.1-Effect of LC_{50} of *B. thuringiensis* as spores (commercial product) on the activity of phosphatase (ACP and ALP) against 2nd and 4th instar larvae of *S. littoralis*

Data in Table (5) showed that no significant difference between Acid phosphatase (ACP) and alkaline phosphatase (ALP) with *B. thuringiensis* spores compared with control for 2nd and 4th instars.

Table 5: Effect of LC_{50} of *B. thuringiensis* as spores (commercial product) on the activity of phosphatase (ACP and ALP) against 2nd and 4th instar larvae of *S. littoralis*

Compound	Acid phosphatase	Alkaline phosphatase
2nd instar		
***Bt* spores**	13.55±0.73	4.23±0.48
Control	9.39±0.60	5.12±0.81
4th instar		
***Bt* spores**	14.25±0.39	6.37±0.46
Control	13.24±0.38	5.79±0.18

**: highly significant (P<0.01)

3.2-Effect of LC_{50} of Cry1Ac and Cry2Ab on the Activity of Phosphatase (ACP and ALP) Against the Newly Hatched Larvae of *S. littoralis:*

Data in Table (6) indicated that after treatment of newly hatched larvae of *S. littoralis* with LC_{50} concentrations of Cry1Ac and Cry2Ab, Acid phosphatase (ACP) were no significant difference between Cry1Ac and control while it were a highly significantly increased compared with control. Acid phosphatase values were 10.27, 12.44 and 9.18 (µg/g.b.wt) for Cry1Ac, Cry2Ab and control, respectively.

Also, Data in Table (6) indicated that, after treatment of newly hatched larvae of *S. littoralis* with LC_{50} concentrations of Cry1Ac and Cry2Ab, Alkaline phosphatase (ALP) were no significant difference between Cry1Ac, Cry2Ab and control.

Table (6): Effect of LC_{50} of Cry1Ac and Cry2Ab on the activity of phosphatase (ACP and ALP) against the newly hatched larvae of *S. littoralis*

Compound	Acid phosphatase	Alkaline phosphatase
Cry1Ac	10.27± 0.69[b]	6.48± 0.67
Cry2Ab	12.44 ± 0.43 [a]	6.14± 0.21
Control	9.18± 0.031 [b]	4.59± 0.37
LSD	1.42	NS

Means with the same letter(s) are not significantly different.

4-Effect on Transaminases Enzymes:

Transaminases are a group of enzymes that catalyze the interconversion of amino acids and oxoacids by the transfer of amino groups. Aspartate aminotransferase (AST), formerly termed glutamate oxaloacetate transaminase (GOT), and alanine aminotransferase (ALT), formerly termed glutamate pyruvate transaminase (GPT). Transaminases (GPT and GOT) help in the production of energy (Azmi *et al.,* 1998), and serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions.

4.1-Effect of LC_{50} of *B. thuringiensis* as Spores (commercial product) on the Activity of Transaminases against 2nd and 4th Instar Larvae of *S. littoralis*:

Glutamate Oxaloacetate Transaminase (GOT) was tabulated in Table (7) exhibited a highly significant increase with *B. thuringiensis* spores compared with control for 2nd and 4th instars. Values were 42.59 and 34.77($Ux10^3$/g.b.wt) for 2nd and 4th instars, respectively. While it was 29.38 and 22.66 ($Ux10^3$/g.b. wt) with control for 2nd and 4th instars, respectively.

Data in Table (7) showed that Glutamate Pyruvate Transaminase (GPT) was significantly increased with *B. thuringiensis* spores compared with control for 2nd instar which was 46.23 and 38.49 ($Ux10^3$/g.b. wt), respectively. While there is no significant difference in GPT activities between treated 4th instar larvae and control.

This agreement with El-Shershaby *et al.,* (2008) found fluctuated changes in the activity of GPT and GOT of *S. littoralis* larvae infected with Dipel 2X. The GPT activity was clearly decreased after 48 and 72 hrs. of treatment than in the untreated. The post-treatment period increased to 120 hrs. GPT enzyme activity detected the highest positive change. They suggested that this may be attributed to the occurrence of reversible binding between pesticides and enzymatic site of action on the enzyme surface.

Table 7: Effect of LC_{50} of *B. thuringiensis* as spores (commercial product) on the activity of transaminases against 2nd and 4th instar larvae of *S. littoralis.*

Compound	GOT (µg oxaloacetate/min/ml)	GPT (µg pyruvate/min/ml)
2nd instar		
***Bt* spores**	42.59±0.56**	46.23±0.43**
Control	29.38±1.14	38.49±0.44
4th instar		
***Bt* spores**	34.77±0.66**	36.12±0.61
Control	22.66±0.32	37.89±0.84

**: highly significant (P<0.01)

4.2-Effect of LC_{50} of Cry1Ac and Cry2Ab on the Activity of Transaminases against the Newly Hatched Larvae of *S. littoralis:*

Data in Table (8) mentioned that GOT activates were a highly significant dropped with Cry1Ac and Cry2Ab compared with untreated control. While there is no significant change in GPT activities between Cry1Ac and control and a highly significant increase was found between Cry2Ab and control.

Table 8: Effect of LC_{50} of Cry1Ac and Cry2Ab on the activity of transaminases against the newly hatched larvae of *S. littoralis*

Compound	GOT (µg oxaloacetate/min/ml)	GPT (µg pyruvate/min/ml)
Cry1Ac	26.48±0.88 ^{b}	40.12±0.70 ^{b}
Cry2Ab	29.44±1.11 ^{b}	42.48±0.52 ^{a}
Control	37.44±1.17 ^{a}	38.59±0.93 ^{b}
LSD	3.22	2.23

Means with the same letter(s) is not significantly different

5-Non-Specific Esterases and Acetylcholine Esterase Activity:

Nonspecific esterases perform important functions in the insect organism: they perform catabolism of esters of higher fatty acids that proceeds actively in the flight muscles and enable insects to fly, mobilization of lipids, including those of the fat body (Roslavtseva *et al.*, 1993), and degradation of inert metabolic esters, including various xenobiotic (Terriere, 1984). Nonspecific esterases were found to participate in the metabolism and detoxication of organophosphates, pyrethroids, carbamate, and juvenoids (Small and Hemingway, 2000; Pasteur *et al.*, 2001).

Acetylcholinesterase (AChE) is a key enzyme catalyzing the hydrolysis of the neurotransmitter acetylcholine (Ach) in the nervous system of various organisms (Oehmichen and Besserer, 1982). AchE is also an important target site for insecticide action in the central nervous system of insects (Nathan *et al.*, 2008).

5.1-Effect of LC_{50} of *B. thuringiensis* as Spores (commercial product) on the Activity of α, β - Esterase and Acetyl-cholinesterase against 2nd and 4th Instar Larvae of *S. littoralis:*

Data in Table (9) mentioned that Alpha esterases were highly significantly activated with *B. thuringiensis* in treated 2nd instar larvae compared with the untreated control, as follows 102.060 and 77.350 (α-naphthol/min/g.b.wt), respectively. On the other

side, it found a highly significant inhibition in Alpha esterases with treated 4th instar larvae compared with control, as follows 215.124 and 229.385 (α-naphthol/min/g.b.wt), respectively.

Beta esterases were tabulated in Table (9) showed that a highly significantly increased with *B. thuringiensis* spores compared with control for both 2nd and 4th instars larvae. Values were 89.871 and 97.357 (β-naphthol/min/g.b.wt) for 2nd and 4th instars larvae, respectively. While it was 60.253 and 92.462 (β-naphthol/min/g.b.wt) with control for 2nd and 4th instars, respectively.

The same table showed that Acetylcholinesterase (AchE) was highly significantly increased with treated 2nd and 4th instar larvae compared with their control, as follow: 551.250 and 573.214 (AchBr/min/g.b.wt) for treated 2nd and 4th instar larvae, respectively and 161.317 and 483.964 (AchBr/min/g.b.wt) for untreated 2nd and 4th instar larvae, respectively. Similar results were obtained by Gaaboub *et al.* (2012) studied the effect of LC_{50} of *B. thuringiensis* on the 5th instar larvae of *S. littoralis,* and showed that, a moderate increase in activity of AChE.

Table 9: Effect of LC_{50} of *B. thuringiensis* as spores (commercial product) on the activity of α, β - Esterase and Acetyl-cholinesterase against 2nd and 4th instar larvae of *S. littoralis**;***

Compound	α-Esterase	β- Esterase	Acetylcholinesterase
	µg α-Naphthol/min/ml	µg β-Naphthol/min/ml	µg Acetyl-cholinebromide/min/ml
2nd instar			
***Bt* spores**	102.060±0.46**	89.871±0.38**	551.250±0.17**
Control	77.350±0.68	60.253±0.19	161.317±0.53
4th instar			
***Bt* spores**	215.124±0.82	97.357±0.56**	573.214±1.16**
Control	229.385±0.52**	92.462±0.37	483.964±0.86

**: highly significant (P<0.01)

5.2-Effect of LC_{50} of Cry1Ac and Cry2Ab on the activity ofα, β - Esterase and Acetylcholinesterase against the Newly Hatched Larvae of *S. littoralis*:

Data in Table (10) revealed that, after treatment of newly hatched larvae of *S. littoralis* with LC_{50} concentrations of Cry1Ac and Cry2Ab, the Alfa esterase was a highly significantly decreased with Cry1Ac and a highly significantly increased with Cry2Ab compared with control, as follow: 82.354,1 17.728 and 89.640 (α-naphthol/min/g.b.wt), respectively.

Also, Table (10) illustrated that β- esterase was a highly significantly decreased with Cry1Ac and a highly significantly increased with Cry2Ab compared with control, as follow: 59.364, 116.912 and 66.341 (β-naphthol/min/g.b. wt), respectively.

Acetylcholinesterase (AchE) was tabulated in Table (10) showed that was a highly significantly decreased with Cry1Ac and a highly significantly increased with Cry2Ab compared with control, as follows: 153.951, 526.360 and 168.149 (Ach Br/min/g.b.wt), respectively.

Table 10: Effect of LC_{50} of Cry1Ac and Cry2Ab on the activity ofα, β - Esterase and Acetylcholinesterase against the newly hatched larvae of *S. littoralis*

Compound	α-Esterase	β- Esterase	Acetylcholinesterase
	µg α- Naphthol/min/ml	µg β-Naphthol/min/ml	µg Acetyl-cholinebromide/min/ml
Cry1Ac	82.354±0.41 c	59.364± 0.59c	153.951±0.76 c
Cry2Ab	117.728±0.25 a	116.912± 0.74 a	526.360±0.28 a
Control	89.640±0.91b	66.341±0.76 b	168.149±0.13 b
LSD	1.81	2.13	1.45

Means with the same letter(s) are not significantly different.

6-Effect on Chitinase and Phenoloxidase Activities:

Phenoloxidase is an important component of insect immune systems. Phenoloxidase activity has been shown to correlate with resistance to some parasites/pathogens across species (Nigam *et al.,* 1997).

6.1-Effect of LC_{50} of *B. thuringiensis* as Spores (commercial product) on the Activity of Phenoloxidase and Chitinase Activities against 2nd and 4th instar Larvae of *S. littoralis*

Phenoloxidase activity showed no significant difference with *B. thuringiensis* as spores in both 2nd and 4th instar larvae as shown in Table (11) compared with their untreated control.

Data in Table (11) revealed that chitinase activity was a highly significant increase with *B. thuringiensis* spores compared with control for 2nd instars larvae. Chitinase activity were 13.22 and 7.15 (µg NAGA/min/g.b. wt) for 2nd instar and control, respectively. While treated 4th instar larvae showed no significant difference between treated and untreated control.

Table 11: Effect of LC_{50} of *B. thuringiensis* as spores (commercial product) on the activity of phenoloxidase and chitinase activities against 2nd and 4th instar larvae of *S. littoralis*

Compound	Phenoloxidase (O.D. units/min/ml)	Chitinase activity (µg NAGA/ min /ml)
2nd instar		
***Bt* spores**	6.58±0.46	13.22 ±0.35**
Control1	8.13±0.42	7.15±0.72
4th instar		
***Bt* spores**	9.73±0.29	11.78±0.44
Control2	10.46±0.32	12.34±0.51

**: highly significant (P<0.01)

6.2-Effect of LC_{50} of Cry1Ac and Cry2Ab on the activity of phenoloxidase and chitinase against the newly hatched larvae of *S. littoralis:*

Phenoloxidase showed no significant difference between Cry1Ac, Cry2Ab and Control as shown in Table (12).

Also, data in Table (12) illustrated that no significant difference was observed in chitinase activity in treated larvae with Cry1Ac and control. While a highly significant increase was found in treated larvae with Cry2Ab and control as follows: 15.36 and 7.56 (µg NAGA/min/g.b. wt), respectively.

Table 12: Effect of LC_{50} of Cry1Ac and Cry2Ab on the activity of phenoloxidase and chitinase against the newly hatched larvae of *S. littoralis*

Compound	Phenoloxidase (O.D. units/min/ml)	Chitinase activity (µg NAGA/ min /ml)
Cry1Ac	8.69±0.66	6.45±0.71 [b]
Cry2Ab	8.67±0.45	15.36±0.39 [a]
Control	7.99±0.60	7.56±0.29 [b]
LSD	NS	2.61

Means with the same letter(s) is not significantly different.

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ARABIC SUMMARY

البروتينات الحشرية السامة Cry1Ac و Cry2Ab وعلاقتها بالاستجابات الفسيولوجية في دودة ورق القطن المصرية

حسن فرج ضاحي [1] – احمد محمود علي سالمان [2] – اسماء محمود احمد بديوي [1]
1- قسم بحوث دودة ورق القطن- معهد بحوث وقاية النباتات – مركز البحوث الزراعية – مصر
2- قسم وقاية النبات – كلية الزراعة – جامعة سوهاج – مصر

أجريت الدراسة الحالية لتقييم التأثيرات البيوكيميائية للتركيز النصف مميت لكلا من جراثيم بكتيريا الباسليس ثورينجينسز على العمر اليرقي الثاني والرابع لدودة ورق القطن والسموم الحشرية البلورية Cry1Ac وCry2Ab على اليرقات حديثة الفقس لتحديد تأثير هذه المركبات على اجمالى الكربوهيدرات والبروتينات والدهون و على انزيمات التحليل المائي للكربوهيدرات وانزيمات الفوسفاتيز القلوية والحامضية وانزيمات الشايتينز والفينولكسيدز وانزيمات الاستريز الغير متخصصة وانزيم الاستيل كولين استريز وانزيمات نقل الأمين. أوضحت النتائج إجمالي البروتينات والدهون نقص بصورة معنوية وازداد إجمالي الكربوهيدرات بصورة معنوية في يرقات العمر الثاني والرابع لدودة ورق القطن بعد معاملتها بجراثيم الباسليس. انخفض إجمالي الكربوهيدرات بصورة معنوية في اليرقات المعاملة ب Cry1Ac وازداد بصورة معنوية بعد المعاملة ب Cry2Ab. في حين ازداد إجمالي البروتينات بصورة معنوية بعد معاملتها ب Cry1Ac ونقص بصورة معنوية بعد المعاملة ب Cry2Ab. أنخفض نشاط أنزيمي الانفرتيز والتريهاليز بصورة معنوية في العمري اليرقي الثانى والرابع بعد معاملتهم بجراثيم بكتريا الباسليس. وعلى الجهة الأخرى كان هناك زيادة معنوية في نشاط انزيمى الاميليز والاتفرتيز في اليرقات المعاملة ب Cry1Ac وانخفض بصورة معنوية بعد المعاملة ب Cry2Ab. كما أدت المعاملة ب Cry1Ac وCry2Ab الى انخفاض نشاط انزيم التريهاليز بصورة معنوية في اليرقات حديثة الفقس المعاملة. ومن الواضح ان المعاملة بجراثيم بكتريا الباسليس أدت الى زيادة نشاط انزيم ناقلة أمين الاسبارتات في كلا العمريين المعاملين وأدت الى انخفاض نشاط انزيم ناقلة أمين الألانين في العمر الثانى المعامل فقط. في حين انخفض نشاط انزيم ناقلة أمين الاسبارتات في اليرقات المعاملة ب Cry1Ac وCry2Ab. ازداد نشاط الالفا استريز بصورة معنوية في العمر الثاني وانخفض في العمر الرابع بعد المعاملة بجراثيم بكتريا الباسليس وأيضا ازداد نشاط انزيم البيتا استريز والاستيل كولين استريز بصورة معنوية في العمرين. في الجهة الأخرى انخفض نشاط انزيمات الالفا والبيتا استريز والاستيل كولين استريز في يرقات حديثة الفقس المعاملة ب Cry1Ac وازداد بصورة معنوية في اليرقات المعاملة بCry2Ab .