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Biochemical effects of three commercial formulations of *Bacillus thuringiensis* (Agerin, Dipel 2X and Dipel DF) on *Spodoptera littoralis* larvae

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ABSTRACT

Effectiveness of commercial formulations of *Bacillus thuringiensis* var Kurstaki "Agerin, Dipel 2X and Dipel DF" on total protein and lipid contents were studied. Activities of detoxification and metabolic enzymes were also measured in treated and control larvae of cotton leafworm *Spodoptera littoralis*. Collected samples from treated larvae at intervals of 48 and 120 hrs post treatment were analyzed to assess the total protein and lipid contents as well as the enzymes activities. Significant reductions were observed in total protein content at 120 hrs larvae in all treatments compared with control. In all larvae treated with Agerin and Dipel DF the lipid content was significantly elevated after 48 and 120 hrs of treatment while Dipel 2X showed no significant difference in lipid content. However, fluctuated changes in the enzymes activity of treated larvae were found. We conclude that there are differences in biochemical effects between various commercial formulations of *Bacillus thuringiensis* kurstaki. In most cases Agerin was found more effective on *S. littoralis* than others.

Key words: *Bacillus thuringiensis, Spodoptera littoralis*, protein, lipid, detoxification enzymes and metabolic enzymes.

INTRODUCTION

The Egyptian cotton leafworm, *S. littoralis*, is considered a destructive polyphagous pest to many agricultural crops specially cotton. *Bt* have shown high toxicity against *S. littoralis* (Salama & Foda, 1982). Exposure of sublethal doses of the Cry toxin(s) greatly affects the biological activity and reproduction of the treated insects (Oron *et al.,* 1985).

The most widely used microbial pesticides worldwide are those based on preparations of the bacterium *Bacillus thuringiensis* (*Bt*) (Lambert & Peferoen, 1992).

The toxicity of *Bt* is due to the production of crystalline protein protoxins, known as δ-endotoxins (Broderick *et al.*, 2006). Solubilized protoxins are activated by midgut proteases and bounded with the receptors of the epithelial cells (Pigott and Ellar, 2007). The toxins insert themselves into the cell, where they form pores that lead to cell lysis, subsequently causing insect death (de Maagd *et al.,* 2003). Commercial *Bt* products generally consist of a mixture of spores and crystals, produced in large fermenters and applied as foliar sprays, much like synthetic insecticides. (Sanchis *et al*., 1999).

The activities of enzymes such as alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) 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).

The goal of this study was to compare the effects of Egyptian *Bt* biopesticide with other commercial formulations, Agerin, Dipel 2Xand Dipel DF, against larvae of

S. littoralis with reference to their effects on enzyme activities as well as lipid and protein contents.

MATERIALS AND METHODS

1. Tested Insects:-

A laboratory strain of cotton leafworm *S. littoralis* (Lepidoptera- Noctuidae) was obtained from Plant Protection Research Institute, Agricultural Research Centre, Cairo, Egypt. Insects were reared on castor bean leaves in laboratory under constant conditions of 27 ± 2°C, photoperiod of 14 hrs light and 10 hrs dark and 65 ± 5 % R.H.

The culture of the cotton leaf worm, *Spodoptera littoralis* (Boisd) was initiated from freshly collected eggs masses supplied from the division of cotton leaf worm of plant protection research Institute, Dokki, Egypt. All stages of *Spodoptera littoralis* were cultured and tested at 27±2° C and 70± 5 % R.H. Larval stages were reared on caster bean leaves which were provided daily.

2. *Bacillus thuringiensis*:

The three commercial formulations were obtained from Plant Protection Research Institute, Agricultural Research Centre, Cairo, Egypt. Agerin 6.5%, Dipel 2X 6.4 % and Dipel DF 54 %.

3. Experiments:

3.1. Bioassay tests:

Leaf-dip bioassay method was followed as described by Tabashnik *et al.*, (1991) using castor leaves. The leaves were first washed with distilled water and dipped in solutions of different concentrations of *Bt* commercial formulations (Agerin, Dipel 2X and Dipel DF) prepared with distilled water. Each leaf was dipped for 5–10 second and allowed to air dry for a period of one hour. The leaves were then placed individually into Petri dishes (15 cm diameter). Newly hatched second instar larvae were released on each dish with three replications (twenty insects /replicate) including controls. There were eight concentrations ranging from 0.33 – 0.01 % to Agerin and Dipel 2X and 2.70 - 0.08 % to Dipel DF. Larvae were allowed to feed for 48 hr on treated leaves. Then these leaves were removed and replaced by another untreated ones. Larval mortality was recorded at 48 hrs. Probit analysis (Finney 1971) was used to estimate LC_{50} value.

3.1.2. Biochemical Analysis:

Sixty newly hatched second instar larvae were treated as previously described (twenty larvae / three replicates) with the LC_{50} of Agerin, Dipel 2X and Dipel DF for 48hrs. Then treated leaves were removed and replaced by another untreated ones for 120hrs. Twenty larvae per treatment and control were randomly selected for each time interval (48 and 120 hrs) to estimate lipid and protein contents. Activities of metabolic enzymes (GPT), (GOT) and (LDH) and detoxification enzymes [alkaline phosphatase (ALP), acid phosphatase (ACP) and phenoloxidase] were also estimated. Analysis of protein and lipid were carried out by methods described by Bradford (1976) and Knight *et al.* (1972), respectively. ALP and ACP activities were measured as described by (Powell & Smith, 1954) using disodium phenylphosphateas substrate. Phenoloxidase activity assayed according to a modification of Ishaaya (1971) using catechol as the substrate. GPT and GOT were determined according to the method of (Reitman & Frankle, 1957). The estimated LDH was done as described by Randox kit (Randox laboratories td., United Kingdom) using an optimized standard method according to the recommendation of Deutschen Gessellschaft fur Klinische chemie (DGCK 1970). Means were tested for

significance by the one way analysis of variance (ANOVA). When the ANOVA statistics were significant ($P \leq 0.01$), means were compared by the Duncan's multiple range test.

RESULTS

Toxicity of three commercial formulations of *Bt* was evaluated against second instar larvae of the *S. littoralis* by leaf dip bioassay (Table 1). 48 hrs. median lethal concentrations (LC_{50}) of Agerin, Dipel 2X and Dipel DF were 0.18, 0.069 and 0.10%, respectively.

Table (1): Toxicity of commercial formulations of *Bacillus thuringiensis* to *S. littoralis* after 48 h of exposure.

Bt commercial formulations	LC_{50} %
Agerin	0.18
Dipel 2X	0.069
Dipel DF	0.10

Results in Table (2) revealed no different values of protein contents between control and treated larvae after 48 hrs of exposure to different commercial formulations of *Bt.* A significant reduction was observed at 120 hrs larvae in all treatments compared with the control. In all larvae treated with Agerin and Dipel DF the lipid content was significantly elevated after 48 and 120 hrs of treatment. Dipel 2X showed no significant difference in lipid content from the control.

Table (2): Total protein and lipid contents of *S. littoralis* larvae after the treatment with different commercial formulation of *Bacillus thuringiensis*.

Bt commercial formulation*	Protein (mg/g. b.wt)** (Mean ± S.E)		Lipids (mg/g. b.wt) (Mean ± S.E)	
	48 h	120 h	48 h	120 h
Control	49.27 ± 0.61^{a}	34.23±0.67^{a}	16.54± 0.35^{b}	12.11±0.35^{b}
Agerin	49.67 ±1.17^{a}	27.05 ±0.15^{b}	20.27 ±1.15^{a}	16.77±0.64^{a}
Dipel 2X	48.9±0.56^{a}	28.50±0.29^{b}	14.48±0.44^{b}	13.35±0.44^{b}
Dipel DF	50.9 ± 0.3^{a}	27.43± 0.73^{b}	19.2 ±0.8^{a}	17.54±0.73^{a}

Vertically means bearing different letters are significantly different at $P \leq 0.01$.
*2^{nd} larval instars treated with LC_{50} of Agerin, Dipel 2X and Dipel DF. (0.18, 0.069 and 0.10 %, respectively)
** b.wt = body weight.

Differences in metabolic enzymes activities (GPT, GOT and LDH) between the control and treated larvae are shown in Table (3). Results revealed no different values in GPT and LDH activities between control and infected larvae after 48 h of exposure. At the same interval GOT activity was significantly higher in treated larvae with Agerin compared with the control and other treatments. But its activity significantly decreased in larvae treated with Dipel 2X and Dipel DF than those treated with Agerin. There were significant increases in GPT and GOT activities at 120 hrs, of larvae treated with Dipel 2X and Agerin. Whereas the activity of LDH was

significantly lower in larvae treated with Dipel 2X and Dipel DF than those treated with Agerin and control.

Table (3): Metabolic enzymes activities of *S. littoralis* larvae after the treatment with different commercial formulation of *Bacillus thuringiensis*

Bt commercial * formulations*	GPT (U** × 10^3 / g.b.wt.)*** (Mean ± S.E)		GOT (U × 10^3 / g.b.wt.) (Mean ± S.E)		LDH (U × 10^3 / g.b.wt.) (Mean ± S.E)	
	48 h	120 h	48 h	120 h	48 h	120 h
Control	2627±36.59[a]	1397.67±82.60[b]	1693±20.41[b]	1840±30.55[b]	15196. 7±347.4[a]	4750±145.1 [a]
Agerin	2775.33±132.9[a]	1927.67±76.33[a]	2142±74.66[a]	2277.3±90.81[a]	15546.7± 318.5 [a]	5377±210.1[a]
Dipel 2X	2626.33±63.60 [a]	2293.33 ±99.38[a]	1430±41.93[cb]	1678.33±26.91[bc]	14225. 7 ±243.5[a]	3428.7± 212.9[b]
Dipel DF	2590.67±92.4 [a]	1468.5 ±51.5[b]	1479.33±55.56[cb]	1635.0± 65.0[bc]	15730± 178.7 [a]	3036.5±83.5 [b]

Vertically means bearing different letters are significantly different at P≤ 0.01.
*2nd larval instars treated with LC_{50} of Agerin, Dipel 2X and Dipel DF. (0.18, 0.069 and 0.10 %, respectively)
U= unit of enzyme activity. * b.wt = body weight

Effects of the three commercial formulations of *Bt* on detoxification enzymes phenoloxidase, ALP and ACP are shown in. Table (4). Activity level of ALP enzyme in 48 hrs post-treatment decreased significantly in all treatments than the control. The highest activity was observed in case of larvae infected with Agerin and Dipel DF.after 120 hrs.

Table: (4): Detoxification enzymes activities of *S. littoralis* larvae after the treatment with different commercial formulation of *Bacillus thuringiensis*

Bt commercial * formulations*	ALP (U** x 10^3 / gm/b.wt)*** (Mean ± S.E)		ACP (U x 10^3 / gm/b.wt) (Mean ± S.E)		Phenoloxidase (U × 10^3 / g.b.wt.) (Mean ± S.E)	
	48h	120h	48h	120h	48h	120h
Control	576.3±10.0[a]	99.0±3.5[c]	143.67± 1.5[a]	48.32± 1.2[c]	21.64 ±0.39[c]	15.77 ± 0.34[a]
Agerin	436.33±3.7[b]	185.67±3.9[a]	122± 5.5[b]	82.54±2.3[a]	29.38±0.76[a]	6.02±0.32[c]
Dipel 2X	298.67±2.3[c]	79.33±2.7[c]	84.0 ±2.1[c]	53.74±2.8[bc]	25.95±0.30[b]	11.95 ±0.18[b]
Dipel DF	432.0 ±6·0[b]	140.5±7.5[b]	120.0± 2.[b]	61.41 ± 2.8[bc]	22.49±049[c]	5.34 ±0.49[c]

Vertically means bearing different letters are significantly different at P≤ 0.01.
*2nd larval instars treated with LC_{50} of Agerin, Dipel 2X and Dipel DF. (0.18, 0.069 and 0.10 %, respectively)
U= unit of enzyme activity. * b.wt = body weight

The activity of this enzyme reached its highest level in larvae treated with Agerin followed by Dipel DF, while Dipel 2 X did not show significant difference from control. ACP activity decreased significantly in all treated larvae after 48 hrs. of exposure than the control. No significant difference was observed between larvae infected with Agerin and Dipel DF. The highest activity of ACP was observed after 120 hrs of exposure to Agerin but no difference was observed in Dipel DF and Dipel

2X treated larvae than the control. After 48 hrs of infection phenoloxidase had a greater activity level in larvae infected with Agerin followed by Dipel 2X. No significant difference was observed in its activity in larvae treated with Dipel DF and control. In contrast, after 120 hrs the phenoloxidase activity decreased in larvae treated with Agerin and Dipel DF than those of control which recorded the highest activity than all treated larvae.

DISCUSSION

Treatment of *S. Littoralis* larvae with the LC_{50} of the commercial formulations of *Bt* (Agerin, Dipel 2X and Dipel DF) induced considerable changes in physiological and biochemical parameters which studied in this paper.

Protein has been shown to affect important individual-level fitness-associated traits such as body size, growth rate, and fecundity; and at higher levels of organization has been linked to population dynamics, life histories, and even biological diversification (Fagan *et al.*, 2002). Etebari (2006) showed that many insecticides decreased feeding efficiency and protein amount of an insect's body. Our results showed a significant reduction of protein after 120 hrs of exposure in all treatments than the control and this observation agreement with El-Shershaby *et al,.* (2008) who found that Dipel 2X gradually suppress protein synthesis as post treatment period increase and reached its maximum effect after120 hrs. Nath *et al.*, (1997) suggested that this could be due to the break down of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the krebs cycle by retaining free amino acid content in hemolymph. In contrast to this conclusion (Narayan & Jayaraj, 1974) observed that infected larvae of *S. littoralis* with *Bt* induced a significant hyperproteinia. This can be attributed to stimulate synthesis of protein producing factors in insects as the protein requirement was increased for of *Bt* spores.

Lipids are the most suitable materials for storage of energy reserves. Compared to carbohydrates, lipids can supply as much as eight times more energy per unit weight (Beenakkers *et al.*, 1985). In this study, Agerin and Dipel DF showed high significant elevation in lipid contents in infected larvae after 48 and 120 hrs of treatment than the control. Similarly lipid content in tobacco cutworm *Spodoptera litura* (fab.) larvae was studied by Tripathi & Singh (2002), the infection resulted in significant reduction in total lipid content of haemolymph in 3rd, 4th and 5th instars infected larvae. They suggested that the reason for the lower fat content in infected larvae could be the extended larval period of the treated insects and blocked food ingestion, and the fat reserves might have been utilized for the maintenance during extended larval period.

Transaminases (GPT and GOT) enzymes help in the production of energy (Azmi *et al.*, 1998). GOT and GPT serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions (Etebari *et al.,* 2005). The activity of both GPT and GOT increased in larvae infected with Agerin and Dipel 2X at 48 and 120 hrs of treatment. 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.

LDH is an important glycolytic enzyme that is present in virtually all tissues (Kaplan & Pesce, 1996). It is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Diamantino *et al.,* 2001). In this study the activity level of lactate LDH was significantly lower in larvae treated with Dipel 2X and Dipel DF than those treated with Agerin and control after 120h of infection. Nathan et al. (2005) showed that feeding of *Spodoptera litura* on *Ricinus communis* L. treated with azadirachtin (plant extraction) and nucleopolyhedrovirus decreases the amount of this enzyme in midgut that demonstrates low nutritional efficiency of the larvae. Similar results were also observed on effectiveness of *Melia azedarach* on rice leaffolder (Nathan, 2006). The higher enzyme activity in the midgut of control insects is most probably due to consumption as well as utilization of large quantities of food. Imbalance in enzyme–substrate complex and inhibition of peristaltic movement of the gut might have inhibited the enzyme activity in the treated insects (Nathan *et al.,* 2005).

ALP and ACP are hydrolytic enzymes, which hydrolyse phosphomonoesters under acid or alkaline conditions, respectively. ALP is mainly found in the intestinal epithelium of animals and its primary function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes. ALP is involved in the transphosphorylation reaction (Etebari *et al.,* 2005). The present study showed that ALP and ACP activities decreased significantly after 48 hrs. post-infection in all treatments compared to the control. In the same time the activity of these enzymes didn't have significant difference in larvae treated with Agerin and Dipel DF. After 120 hrs of *Bt* exposure invert changes were observed in the activity of ALP and ACP. The maximal elevation of activities of these enzymes was obtained by Agerin. Changes in ALP and ACP activities after treatment with *Bt* indicate that changing the physiological balance of the midgut might affect these enzymes.

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

Our results showed that after 48 hrs phenoloxidase had a greater activity level in larvae treated with Agerin followed by Dipel2X. In contrast, after 120 hrs. the phenoloxidase activity decreased in larvae treated with Agerin than those of control which recorded the highest activity than all treated larvae. Upon entry of bacteria into the insect hemolymph, the prophenoloxidase (proPO) system is activated by a cascade of serine proteases (Aspan & Soderhall, 1991) and proPO components are released from insect hemocytes into the hemolymph. In this system two enzymes are activated. One is the enzyme ppA (prophenoloxidase activating enzyme), which converts inactive prophenoloxidase into active phenoloxidase (PO). Active phenoloxidase in turn converts tyrosine to dihydroxyphenyalanine, which binds to bacterial cell surfaces and triggers production of melanin (melanization). (Chattopadhyay, 2004)

Conclusion and Recommendation:

It could be concluded that Agerin has more significant biochemical and physiological affects on larvae of *Spodoptera littoralis* than the other commercial formulations Dipel 2x and Dipel DF. The difference in activity might be due to the presence or absence of biologically active Cry toxins, their relative amounts and additive/synergistic effect of these toxins in the formulations, and batches of products Shelton *et al.,* (1993).

Agerin is the best using Bt biopesticide due to its efficacy and harmless to human health and environment.

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ARABIC SUMMERY

التغيرات البيوكيميائيه لثلاث مركبات تجاريه من باسيلاس ثيرينجنسيز (الاجرين و دايبل X 2 و دايبل DF) علي يرقات دوده ورق القطن

عايدة سعيد كامل – منى فوزى عبد العزيز – نهاد محمد البرقى
قسم علم الحشرات – كلية العلوم – جامعة بنها.

تناولت الدراسه التغيرات البيوكيمائيه التي حدثت ليرقات دوده ورق القطن نتيجه معاملتها بثلاث مركبات تجاريه مختلفه من بكتريا باسيلاس ثيرينجنسيز و هي الاجرين (عزلة مصرية) و دايبل X2 و دايبل DF و قد تمت معامله يرقات العمر الثاني حديثة الأنسلاخ بجرعات غير مميته و تقدير قيمه البروتين و الدهون و الانزيمات المضاده للاكسده و و الانزيمات الأيضية في اليرقات المعامله و الغير معامله و ذلك بعد 48 و 120 ساعه من المعامله . و قد اوضحت النتائج انخفاض شديد في المحتوي البروتيني بعد 120 ساعه من المعامله. كما أظهرت النتائج حدوث ارتفاع في محتوي الدهون بعد المعامله بالاجرين و دايبل DF بعد 48 و 120 ساعه، بينما لم يظهر تأثير علي المحتوي الدهني لليرقات المعامله بدايبل X2. كما أظهرت النتائج حدوث تغيرات في نشاط الانزيمات التي تم تقديرها. نستنتج من هده الدراسه وجود تغيرات بيوكيمائيه ليرقات دوده ورق القطن نتيجه معاملتها بثلاث تركيبات تجاريه مختلفه من بكتريا باسيلاس ثيرينجسينسز و بوجه عام كان الاجرين هو الاكثر فاعليه عن بقيه المركبات.