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EFFICACY OF PROTECTO, A COMMERCIAL PRODUCT OF *BACILLUS Thuringiensis kurstaki*, AGAINST *Spodoptera littoralis* (BOSID.) AND *Agrotis ipsilon* (HUFN.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Efficacy of the *Bacillus thuringiensis kurstaki* formulation Protecto 9.4% WP was tested against 2nd larval instar and some biological aspects of *Spodoptera littoralis* and *Agrotis ipsilon*. Also, a histological effect on mid-gut of 4th larval instar of *A. ipsilon* was done under laboratory conditions. Results showed that, *B.t.* was more effective against *S. littoralis* (LC50 11.94 x 10^6 IU/L) than *A. ipsilon* (LC50 19.87 x 10^6 IU/L). Also, it increased the larval and pupal durations while decreased pupation, pupal weight, adult longevity and fertility for the two insects. In addition, the histopathological examination showed that, the elongation of mid-gut epithelial cells with separations appeared between 4th larval instar of *A. ipsilon* after 3 days from treatment with LC50 of *B.t.*

INTRODUCTION

In Egypt, using *Bacillus thuringiensis (B.t.)* as an environmental safe biocontrol agent for controlling polyphagous lepidopterous larvae showed different efficacy levels on a certain target pest were investigated by many authors e.g. Emara *et al.* (1991) on *Helicoverpa armigera*; Abul-Nasr *et al.*, (1983) and Sakr *et al.* (2007) on *Pectinophora gossypiella* and *Earias insulana*; El-Husseini *et al.* (2000) and Marie *et al.* (2007) on *Spodoptera littoralis*; Nasr and Ibrahim (1997) on *Sesamia cretica*; Omar (2003) on *Galleria mellonella* and Hafez *et al.* (1993) and Mourad *et al.* (2002) on *Agrotis ipsilon*. With regard to the insecticidal hazards and their grave pollutional consequences, together with the pressing need to advocate satisfactory pest management, and safe utilization of *B. thuringiensis*, based on experiments in different countries according to Matter (1991), *B.t.* represents a good example for the new methods of biological control.

Therefore, the present investigation is carried out to evaluate the efficiency of *B.t. Kurstaki* against the 2nd larval instar of *S. littoralis* and *A. ipsilon*. As well as, its effect on some biological aspects of the two insects and histological effect on mid-gut of 4th instar *A.ipsilon* larvae.

MATERIALS AND METHODS

1- Rearing technique:

Larvae of *S. littoralis* and *A. ipsilon* were obtained from a laboratory culture of Plant Protection Research Institute, ARC. The cultures were reared in the laboratory under constant conditions of 26 ± 1°C and 70 ± 5% RH.

2- Toxicological and biological tests:

Effect of Protecto 9.4% WP contain 32000 IU/mg based on *Bacillus thuringiensis* subsp. *Kurstaki* was clarified against the two considered insects. Five concentrations; $8x10^6$, 16×10^6, 32×10^6, 64×10^6 and $96x10^6$ IU/L were tested against 2nd larval instar of both species. Castor oil leaves were dipped in each of the tested concentrations for 30 seconds then left to dry at room temperature. Similar leaves were dipped in distilled water for 30 seconds to be used as a check. Three replicates each was contained 12 larvae/glass jar (1L-volume) were used for each concentration as well as check. Mortality counts were recorded 2, 3, 5 and 7 days after treatment, the data were corrected by Abbot's formula (Abbott, 1925). Data of LC50 value at 5% confidence limits and slopes of regression lines were represented. The larval and pupal durations, percentage of pupation, pupal weight, adult longevity and number of egg hatching (fertility) were determined in each concentration for the two insects. Differences between means were analyzed by using analysis of variance (F-test and least significant difference at 5% level of probability).

3- Histological technique:

Ten *A. ipsilon* larvae 4th instar, treated with Protecto at LC50 and another ten of the check were separately fixed in Bouin's alcohol for 12h after 3 days of treatment. The larvae were dehydrated in 50% and 70% ethyl alcohol, then transferred to 80% and 90% ethyl alcohol. The specimen were embedded in soft paraffin wax and finally to hard wax to be ready for cutting at 10 microns thickness sections. These sections were mounted on clear slides, stained with hematoxylin and eosin and photographed to evaluate the effect of Protecto on the mid-gut.

RESULTS AND DISCUSSION

1- Toxicity effect:

As shown in Table (1) and Fig. (1), the percentage of larval mortality increased with the increase of *B.t.* concentration. The highest concentration of $96x16^6$ IU/L resulted the highest mortality among larvae after 7 days post feeding, being 70 and 66.7% for *S. littoralis* and *A. ipsilon*, respectively. The values of LC50 in case of *S. littoralis* and *A. ipsilon* were 11.94×10^6 and 19.87×10^6 IU/L, respectively. In general, *B.t.* was respectively more toxic against the larvae of *S. littoralis* than those of *A. ipsilon*. The toxicity of *B.t.* due to the crystalline protein must be consumed by a susceptible insect larvae, the protein crystal is actually a protoxin that is hydrolyzed by enzymes in the gut of susceptible insects, releasing the pure toxin, the toxin causes paralysis of the gut, the insect either starves to death, or the midgut epithelial cells are damaged (Lewis, 1985). These results are similar to that reported by Hafez *et al.*, 1993; Mourad *et al.*, 2002; Marie *et al.*, 2007 and Sakr *et al.*, 2007.

Table (1): Accummulated corrected larval mortality percentages of *S. littoralis* and *A. ipsilon*, second instar larvae treated with *B. t.*

Conc. IU/L	*S. littoralis*				*A. ipsilon*			
	Days after treatment				Days after treatment			
	2	3	5	7	2	3	5	7
$8x10^6$	12.5	20.0	32.5	47.5	10.0	14.6	31.3	43.8
$16 x10^6$	20.8	25.0	40.0	52.5	15.3	22.9	37.5	45.8
$32 x10^6$	25.0	33.3	47.9	57.5	20.0	29.5	45.0	54.2
$64 x10^6$	27.1	35.4	50.0	60.0	22.5	32.5	47.5	58.3
$96 x10^6$	32.5	43.8	58.3	70.0	22.5	35.0	50.0	66.7
LC50 (7 days after treatment)	$11.94 x10^6$				$19.87 x10^6$			
Lower limit	$2.46 x10^6$				$8.76 x10^6$			
Toxicity index (%)	100				60.06			
Resistance ratio (RR)	1.00				1.67			
Slope ± S.E.	0.48 ± 0.15				0.53 ± 0.15			

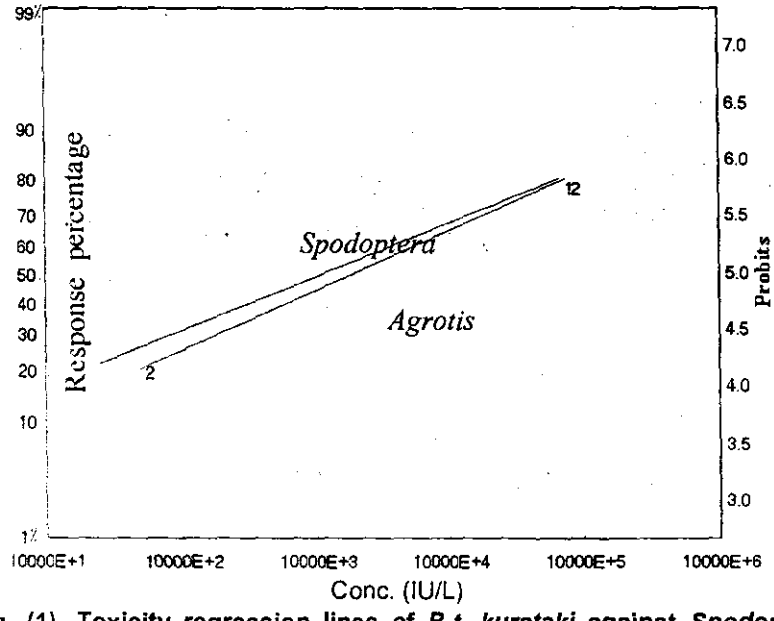

Fig. (1). Toxicity regression lines of *B.t. kurstaki* against *Spodoptera* and *Agrotis* larvae.

2- Effect on some biological aspects:

2.1- Larval and pupal durations:

As shown in Table (2), *B.t.* formulation in general, increases the larval and pupal durations up to 27.0 and 12.3 days for *S. littoralis* as compared to 22.8 and 9.5 days in the check, respectively. Also, up to 32.5 and 12.0 days for *A. ipsilon* as compared to 25.0 and 10.0 days in the check,

respectively. These results clear the superior role of Protecto in elongation the larval and pupal durations. The present data are in a harmony with those of Emara *et al.* (1991) and Ajanta *et al.* (1999) on *Helicoverpa armigera*, Omar (2003) on *Galleria mellonela* and Nasr (2005) on *S. littoralis*.

2.2- Pupation and pupal weight:

Data in table (2) showed that, the percentage of pupation in the treatments was greatly reduced comparing with the check, ranaged 25-50% compared with 90%, respectively in case of *S. littoralis*. In case of *A. ipsilon*, while the pupation ranged 29-54% in the treatments, it reached 88% in the check. On the other hand, pupal weight decreased gradually as the concentration was increased from 348 to 287mg for *S.* littoralis and from 370 to 244mg for *A. ipsilon* compared with 375 and 381 mg in the check, respectively.

This decrease may be, partially attributed to a decrease in total water content and/or a decrease in the intensity of protein synthesis needed for growth and development. These results are similar to those obtained by Emara *et al.* (1991) and Ajanta *et al.* (1999) on *H. armigera;* Salama and Sharaby (1988) on *A. ipsilon*; Aboel-Ghar *et al.* (1994) on *S. littoralis* and Nasr (2005) on *S. littoralis*.

2.3- Adult longevity and fertility:

The data given in Table (2) indicate also that, the treatment adult longevities reduced greatly for the two insects. From 7.5 to 5.5 days for *S. littoralis* and from 8.5 to 7.5 days for *A. ipsilon* thus may be due to the reduction in their weights and inhibition of proteins, lipids and carbohydrates. The percentages of egg hatching ranged 19-56 for *S. littoralis* compared to 96% in the check. This range in case of *A. ipsilon* was 24-60 compared to 89% in the check. These results agree with those obtained by Salama and Sharaby (1988) on *A. ipsilon* and Aboel-Ghar *et al.* (1994) on *S. littoralis*.

3- Effect on the mid-gut:

Cross sections in the mid-gut of untreated 4^{th} instar larvae of *A. ipsilon* (Fig. 2) showed the classical normal architecture of the epithelial cells surrounded by the basement membrane, circular and longitudinal muscles. Also, a well defined secreted peritrophic membrane is present, and the brush border membrane of the epithelial cells could be seen at different locations of the mid-gut according to the position of tissues by sectioning. Three days after feeding on leaves treated with *B.t.Kurstaki* (LC50), showing the elongation of mid-gut epithelial cells with separations appeared between them (Fig. 3). Similar results were observed by Sayed (2001) and Omar (2003).

This histopathological changes in the architecture of mid-gut when infected with *B.t.* endotoxin-spore-complex are launched by the action of the delta endotoxin crystals (parasporal bodies). The ingested crystals are dissolved in the alkaline mid gut lumen of the susceptible larvae and are cleaved by the trypsin like enzymes into trypsin-resistant toxin molecules. Then, the toxin diffuses across the peritrophic membrane and binds to specific proteins of the brush-border membrane, Wolfersberger (1992).

Table (2): Effect of *B.t. kurstaki* on some biological aspects of *A. ipsilon* and *S. Littoralis*.

Conc. IU/L	Larval duration (days)		% Pupation		Pupal duration (days)		Pupal weight (mg)		Adult longevity (days)		% Egg hatching (fertility)	
	S. littoralis	*A. ipsilon*	*S. littoralis*	*A. ipsilon*	*S. littoralis*	*A. ipsilon*	*S. littoralis*	*A. ipsilon*	*S. littoralis*	*A. ipsilon*	*S. littoralis*	*A. ipsilon*
$8x10^6$	24.5± 0.58 c	29.8± 0.50 c	50	54	10.3± 0.50 d	11.0± 0.81 ab	348± 0.01 ab	370± 0.20 ab	7.5± 0.58 a	8.5± 0.57 b	56	60
$16 x10^6$	25.3± 0.50 bc	30.3± 0.50 bc	46	51	10.8± 0.50 cd	11.3± 0.50 a	331± 0.01 bc	348± 0.01 abc	7.3± 0.50 ab	8.3± 0.50 bc	48	51
$32 x10^6$	25.3± 0.96 bc	30.5± 0.57 bc	38	42	11.3± 0.57 bc	11.8± 0.50 a	327± 0.01 bc	322±0.03 bc	6.5± 0.58 abc	7.8± 0.50 bcd	41	43
$64 x10^6$	26.4± 0.96 ab	31.5± 1.29 ab	35	38	11.8± 0.50 ab	11.8± 0.50 a	309± 0.01 cd	291± 0.06 bcd	6.0± 0.82 bc	7.5± 0.57 cd	29	36
$96 x10^6$	27.0± 0.95 a	32.5± 1.29 a	25	29	12.3± 0.50 a	12.0± 0.81 a	287± 0.02 d	244± 0.08 d	5.5± 0.58 c	7.3± 0.50 d	19	24
Untreated (check)	22.8± 0.96 d	25.0± 0.81 d	90	88	9.5± 0.58 e	10.0± 0.81 b	375± 0.01 a	381± 0.01 a	7.8± 0.50 a	12.0± 0.81 a	96	89
L.S.D. 0.05%	1.26	1.33	-	-	0.76	1.01	32	58	1.45	0.88	-	-

Means followed by the same letter(s) in each column are not significantly different.

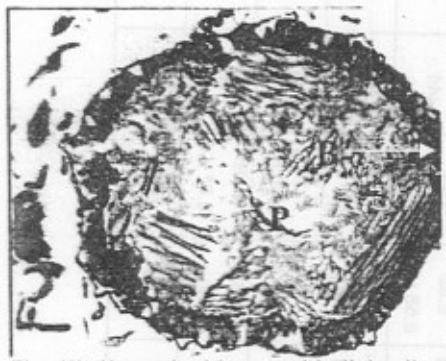

Fig. (2): Normal mid-gut epithelial cells of 4th larval instar of *A. ipsilon* showing a thin peritrophic membrane (P) and the brush membrane (B).

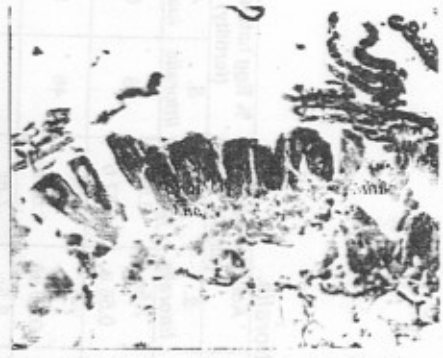

Fig. (3): Three days post infection showing the elongation of mid-gut epithelial cells with separations between them of 4th larval instar of *A. ipsilon.*

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فاعلية المبيد البكتيري برتكتو على كل من دودة ورق القطن والدودة القارضة

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يهدف هذا البحث إلى إلقاء الضوء على فاعلية المبيد البكتيري برتكتو WP ٩,٤ على العمر اليرقي الثاني وبعض المظاهر البيولوجية لكل من دودة ورق القطن والدودة القارضة بالإضافة إلى التأثير الهستولوجي على المعى الأوسط للعمر اليرقي الرابع لحشرة الدودة القارضة تحت ظروف المعمل.

حيث أوضحت النتائج أن هذا المبيد كان أكثر فاعلية لحشرة دودة ورق القطن عن الدودة القارضة حيث كانت قيم LC50 ١١,٩٤ × ١٠ ^٦ و ١٩,٨٧ × ١٠ ^٦ وحدة دولية / لتر على الترتيب. كذلك سبب المبيد زيادة عمر كل من اليرقة والعذراء وخفض النسبة المئوية للتعذير ووزن العذراء وفترة حياة الفراشات والنسبة المئوية لفقس البيض الناتج للحشرتين محل الدراسة. أظهرت نتائج الفحص المجهري للمعى الأوسط للعمر اليرقي الرابع لحشرة الدودة القارضة بعد ٣ أيام من المعاملة بالجرعة النصف مميتة بالبكتريا أستطالة الخلايا وبداية انفصالها عن بعضها البعض في عدة مناطق بسبب تحلل مادة الميوكوبولي سكاريد اللاحمة للخلايا مما يسمح بدخول جراثيم البكتريا من القناة الهضمية إلى سائل الدم ومكوناته الأخرى فيؤدي لموت اليرقة لاحقاً.