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TOXICOLOGICAL AND BIOCHEMICAL EFFECTS OF BIO-AGENT PRODUCTS ON THE COTTON LEAF WORM, *SPODOPTERA littoralis (BOISD.*) (LEPIDOPTERA: NOCTUIDAE)

[63]

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ABSTRACT

A comparison on the larvicidial activity of four commercial bacterial and viral bioagents, Profect®, Virotecto®, Viroset® and Protecto® were evaluated on the 2nd and 4th larval instars of *Spodoptera littoralis* (Biosd.). The LC_{50} values showed 1.35, 1.52, 1.57 and 1.61 mg/ ml against 2nd instar larvae, respectively. On the other hand, the LC_{50} values recorded 2.03, 2.5, 2.72 and 3.01 mg/L. on 4th instar larvae of *S. littoralis* using the above mentioned commercial bioagent products, respectively. The effect on four isozymes, i.e., α, β esterase (EST), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH) and alcohol dehydrogenase (ADH) were evaluated. The obtained results indicated differences in the activity of the isozymes in treated 4th instar larvae as compared to untreated larvae.

Key words: *Spodoptera littoralis,* Profect, Virotecto, Viroset, Protecto, Esterase (EST), Glutamate oxaloacetate transaminase (GOT), Malate dehydrogenase (MDH), Alcohol dehydrogenase (ADH)

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Biosd.) is considered as one of the major and important economic pests not only in Egypt, but also in many parts of the world attacking over 112 plant species belong to 44 families (**Gamil, 2004**). The cotton leaf worm has acquired resistance to many insecticides and the use of other control measures is essential to aid in an over all Integrated Pest Management Program. Many lepidopteran species have been successfully controlled by using microbial agents, e.g. by *Bacillus thuringinensis* **(Salem, 1995 and El-Gahr *et al* 1995)** and NPV **(Salama *et al* 1993).**

The present work was conducted to compare the effect of four commercial bioagent products of bacteria, *Bacillus thurigiensis* (Protecto), the nuclear polyhedrosis virus (Viroset), Bacteria

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with NPV (Profect) and granulosis virus (Virotecto) against 2nd and 4th instar larvae of *Spodoptera littoralis* (Biosd.).

Biochemical changes of isozymes activity as induced by the tested bacterial and viral bio-agents were also considered in both infected and untreated larvae. Isozymes activity was studied as biochemical detection in treated and untreated larvae trying to explain how the tested bioagents affected the 4th instar larvae comparing with non-treated larvae.

MATERIAL AND METHODS

1. Rearing of the cotton leaf worm *S. littoralis*

The cotton leaf worm, *S. littoralis* larvae were obtained from a well-established culture, maintained at the Department of Plant Protection, Faculty of Agriculture, Ain Shams University. Newly laid egg masses were placed in plastic cups covered by muslin held by elastic bands.

The cups were kept under laboratory conditions (25-27°C and 65-70% R.H). Egg masses were observed constantly and upon hatching, newly hatched larvae were transferred to much larger plastic cups measuring 40 x 30 cm.

Saw dust was placed at the base of each cup to absorb excess humidity. Fresh clean castor oil leaves were placed in appropriate quantities in each cup as a source of larval food. Daily, fresh castor oil leaves were offered and larval feces were removed.

2. Bioassay of commercial product bioagents

The larvicidal activity of four commercial bioagents, Profect (NPV + B.t), Virotecto, (GV), Viroset (NPV) and Protecto (B.t.) was evaluated on newly moulted 2nd and 4th instar larvae of *S. littoralis*. A range of concentration (1to 3 mg/ml) was prepared from each bioagent. Fresh castor oil leaves were cut in leaf discs, measuring 3 cm in diameter, these discs were immersed in each of the prepared concentrations of each tested bioagents and then left to dry at room temperature before being offered to the 2nd and 4th larval instars. Larvae were offered contaminated leaf discs for 3 days, subsequently. Each treatment comprised 60 larvae and each replicated 6 times. A similar number of larvae were considered as a control, these larvae were offered castor oil leaves immersed in distilled water. Mortality was calculated daily and accumulative larval mortality was determined at the end of the larval stage. Results were presented graphically as log/ probit regression lines and LC_{50} values were calculated by the program of Sigma plots.

3. Biochemical studies

Four isozyme systems were studied, i.e.α, β esterase (EST), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH) and alcohol dehydrogenase (ADH).

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3.1. Polyacrylamide gel electrophoresis of isozymes

Native-polyacrylamide gel electrophoresis (Native-PAGE) was used to identify isozymes fractions according to **Stegemann *et al* (1980)** using vertical Bio-Rad gel electrophoretic apparatus with slab diameter (180 x 200 mm) and 1.5 mm combs, for two slot formers for 15 wells.

3.2. Extraction of larval isozymes

Fourth instar larvae of *S. littoralis* were fed for 72 hours on castor oil leaf disc contaminated with the determined LC_{50} of each commercial bioagent products under consideration. Larvae of each treatment were homogenized in (1 ml) isozyme extraction buffer and then centrifuged at 10000 rpm / 10 min. The supernatants were transferred to new tubes and mixed with bromophenol blue as a tracking dye. The same procedures were carried out on untreated larvae of the same age as a control.

The preparation of gel buffer solutions of isozymes was described by **(Market & Faulhaber, 1965),** while gel stock solutions of isozymes were prepared according to **Ballve *et al* (1995).**

3.3. Samples application

60 µl from isozyme extractions were applied into gels. Gels were run at 110 v for the first 20 min. The volt was raised to 330 v until the, end of the run, lasting nearly 2-3 hrs.

3.4. Staining of isozymes

1- α, β-Esterase (α, β EST) according to **(Wendel & Weeden, 1989).**
2- Glutamate oxaloacetate transaminase (GOT) according to **(Ballve *et al* 1995).**
3- Alcohol dehydrogenase (ADH) according to **(Wendel & Weeden, 1989).**
4- Malate dehydrogenase (MDH) according to **(Wendel & Weeden, 1989).**

RESULTS

1. Bioassay experiments

Data in Table (1) showed that, second instar larvae were more susceptible than fourth instar ones as exhibited by the lower LC_{50} for the four tested bioagents. As shown in Table (1) and Figs. (1&2), Profect, a mixture of bacteria (*Bacillus thurigiensis*) and NPV proved to be the most potent as it's LC_{50} revealed 1.35 and 2.03 mg/ ml for 2nd and 4th instar larvae, respectively. Protecto (*Bacillus thurigiensis*) was the least effective showing LC_{50} of 1.61 and 3.01 mg/ ml, respectively. The viral bioagents Virotecto (GV) and Viroset (NPV) were relatively similar in their effect to both larval instars.

When Profect the most potent toxicant (=100) was considered as a base line for calculation, the toxicity index of Virotecto, Viroset and Protecto were 88.0, 85.0 and 83.0, respectively for tested 2 nd instar larvae. Meanwhile, this toxicity index showed, 80, 74 and 67 in 4th instar larvae infected with the above mentioned bioagents, respectively.

2. Biochemical studies

2.1. α, β esterase (EST)

According to **(WBC, 2005)** the cholesterol esterase catalyzes gives the following reaction:

Sterol ester ⟶ sterol + fatty acid

As shown in Figure (3-1) α, β esterase was activated in both treated and untreated larvae, the electrophoretic patterns ofα, β esterase isozyme exhibited a maximum number of six bands.

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Table 1. Susceptibility of *S. littoralis* 2nd and 4th instar larvae to infection by commercial bio-agent products

Commercial product	2nd instar larvae			4th instar larvae		
	LC_{50} (mg/ml)	Slope (b)	Toxicity index	LC_{50} (mg/ml)	Slope (b)	Toxicity index
Profect	1.35	0.96	100.0	2.03	0.91	100.0
Virotecto	1.52	0.91	88.0	2.52	0.87	80.0
Viroset	1.57	0.89	85.0	2.72	0.93	74.0
Protecto	1.61	0.90	83.0	3.01	0.79	67.0

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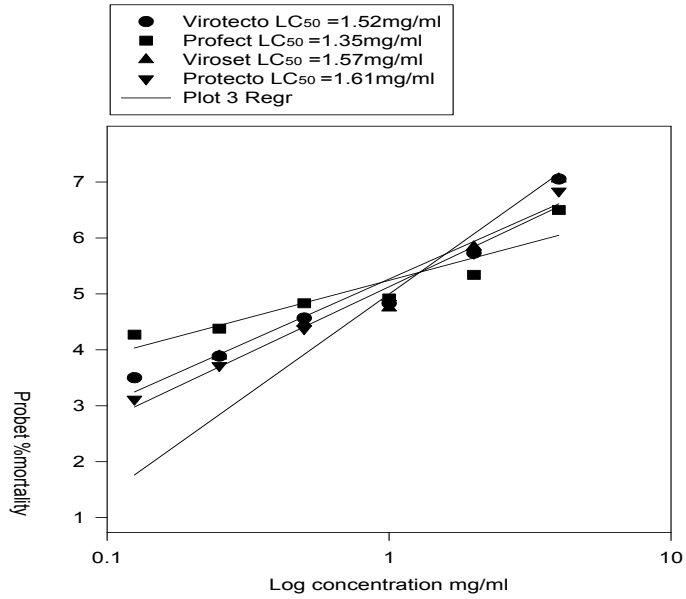

Fig. 1. Toxicity lines of tested bio-agents against 2nd instar larvae of S. *littoralis*

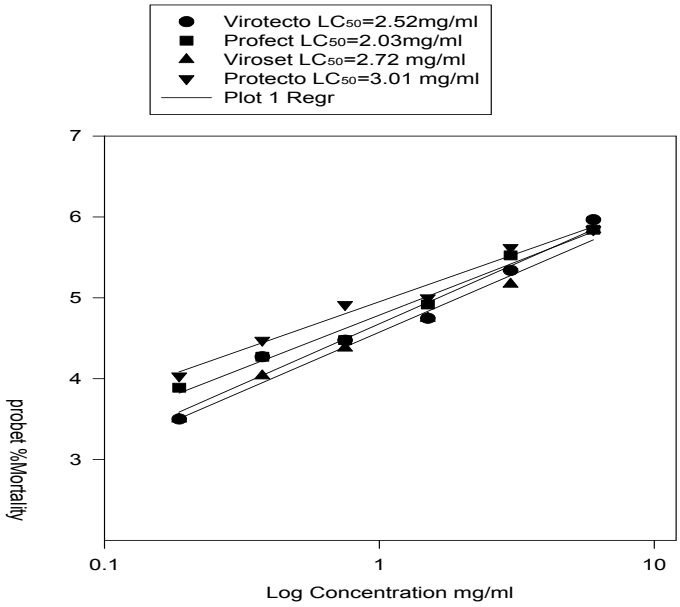

Fig. 2. Toxicity lines of tested bio-agents against 4th instar larvae of *S. littoralis*

As shown in Table (2), band Est. 2 was detected as a monomorphic band in both treated and untreated larvae, all treated larvae shared the presence of bands Est. 1 and Est. 4, meanwhile band Est. 5 was specific for control larvae and band Est. 6 was only exhibited in Protecto treated larvae

The values of similarity matrix were detected, as shown in Table (3). The similarity values between untreated larvae (control) and those treated with four bioagents (Profect, Virotecto, Protecto and Viroset) showed 33.0, 22.0, 25.0%., respectively. The Virotecto and Viroset were highly similar to each other (91.0%) although Virotecto was lower in similar Protecto (55.0%), while Profect was trended to the same similarity value with Protecto and Viroset (75.0%), although both Profect and Virotecto, recorded the similarity value of 67.0%.

2.2. Glutamate oxaloacetate transaminase or aspartate amino-transferase (GOT)

As described by **WBC, (2005)**, the enzymatic reaction of GOT isozyme is:

L-aspartate + 2 – oxoglutarate ⟶ Oxaloacetate + L-glutamate

GOT isozyme was expressed in both treated and untreated larvae in about three bands (Fig. 3 - 2). As the RF value of bands GOT.2 and GOT.3 were 0.31 and 0.32, respectively which proved

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Table 2. The presence and absence ofα, β esterase isozyme bands in treated and untreated larvae of *S.littoralis*

Bands	Total RF	Protecto	Profect	Virotecto	Viroset	Control
EST. 1	0.19	+	+	+	+	-
EST. 2	0.23	+	+	+	+	+
EST. 3	0.66	+	-	-	-	-
EST. 4	0.71	+	+	+	+	-
EST. 5	0.73	-	-	-	-	+
EST. 6	0.76	+	-	-	-	-

(+) Detected (-) Not detected

Table 3. Similarity matrix between the four bioagents based on α, β esterase isozyme analysis

Lanes	Protecto	Profect	Virotecto	Viroset	Control
Protecto	0				
Profect	0.75	0			
Virotecto	0.55	0.67	0		
Viroset	0.6	0.75	0.91	0	
Control	0.25	0.33	0.22	0.25	0

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no difference between the control and treated larvae by the four bioagent products. Band GOT.4 was exhibited in both Protecto and Profect treated larvae, whilest, band GOT.1was apparent only in untreated larvae as well as Viroset treated larvae, as shown in (Table, 4).

The similarity matrix in Table (5) shows that, only larvae treated by Profect and Viroset were related to control, with similarity value 40 and 80%, respectively. The Protecto was similar to the Virotecto with (67.0%). The Profect behaved the same trend of similarity with Protecto and Viroset with (50.0%). The same result was observed in α, β esterase isozyme analysis.

2.3. Alcohol dehydrogenase (ADH)

According to **WBC, (2005)** ADH isozyme follows this reaction:

$$RCH_2OH + NAD^+ \xrightarrow{\quad} RCHO + NADH + H^+$$

The ADH isozyme was expressed only in control larvae, while the enzymatic reaction was inactivated in all the treated larvae. Significant inhibition of this isozyme function was evident Fig. (3 - 4).

2.4. Malate dehdrogenase (MDH)

MDH isozyme reaction according to **WBC, (2005)** is as following:

$$\text{L-malate} + NAD^+ \xrightarrow{\quad} \text{Oxaloacetate} + NADH + H^+$$

MDH isozyme shows a reduction trend in the specific activity in all the treated larvae, in spite of the activity of MDH isozyme shown in untreated larvae in Fig. (3 - 3).

DISCUSSION

The use of bioagents (bacterial or viral) products have been successfully used for the control of many lepidopeterous insects; e.g**. Salama and Foda (1982), Navon (1989), Moawad *et al* (1992), Salama *et al* (1995), Nasr (1992) and Romeilah and Abdel Mageed (2002)**. with the spreading of the use of *Bacillus* sp. Or nuclear polyhedrosis (NPV) for the control of lepidopteren pests, a liability of resistance by these insects is possible. A mixture with virus has been proved to be quite successful. **Salama *et al* (1993)** obtained an additive toxicological effect after treating *S. littoralis* larvae with a combination of B. *thurigiensis* and NPV. **Hunter – Fujita *et al* (1997)** tested a mixture of granulosis virus and NPV.

Biochemical studies indicated differences in the expression of some isozymes. Alcohol dehydrogenase (ADH) isozyme, responsible for the reduction of acetaldehyde to ethanol **(WBC, 2005)**, clearly detected in untreated insects, was not expressed in larvae infected by the four tested bioagents. Also, malate

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dehydrogenase (MDH) is very important for lipid metabolism and enzymatic activity of mitochondria (**WBC, 2005)** was weakly expressed in treated insects. The absence or weak presence of these two isozymes (ADH and MDH) could be one of the causes of the toxic effect of bioagents. In the present investigation the product Profect which is a mixture of NPV and *Bacillus*, proved to be the most toxic to *S.littoralis* larvae.

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Table 4. The presence and absence of GOT isozyme bands in treated and untreated larvae of *S. littoralis*

Bands	Total RF	Protecto	Profect	Virotecto	Viroset	Control
GOT. 1	0.23	-	-	-	+	+
GOT. 2	0.31	+	-	+	-	-
GOT. 3	0.32	-	+	-	+	+
GOT. 4	0.39	+	+	-	-	-

(+) Detected (-) Not detected

Table 5. Similarity matrix between the four bioagents based on GOT isozyme analysis

Lanes	Protecto	Profect	Virotecto	Viroset	Control
Protecto	0				
Profect	0.5	0			
Virotecto	0.67	0	0		
Viroset	0.00	0.5	0	0	
Control	0.00	0.4	0	0.8	0

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Figure 3. Electrophoretic patterns of four isozymes of the four bioagents, Protecto (A), Profect (B), Virotecto (C) and Viroset (D)
(1) α, β esterase isozyme (2) Got isozyme
(3) MDH isozyme (4) ADH isozyme

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The expression of α, β esterase isozymes in treated larvae was different than their expression in untreated insects. The bands EST_1 and EST_4 were expressed in infected larvae but not detected in untreated insects; also EST_5 was inhibited in infected larvae. It is noteworthy that Protecto (B. *thurigiensis)* proved the least toxic among the tested bioagents against *S. littoralis* larvae was expressed by two new bands, EST_3 and EST_6 which not detected by the other bioagents or in the control.

Glutamate oxaloacetate transaminase (GOT) was vital for the intermolecular transfer of amino groups in metabolic process **(Ballve *et al* 1995)**, was expressed by different bands in infected larvae.

Treatments with bioagents have been reported to alter the activities of the enzymes of treated insects. In this respect **El ghar *et al* (1995)** reported a marked decrease of invertase, amylase and trehalase activities by 81.76 and 54% respectively, in *S. littoralis* larvae treated with *B. abamectin*. **Zidan *et al* (1996)**, found that treatments with *B. thurigiensis* caused a latent inhibition effect on acetylcholinesterase and a reduction in acid phosphatase activity. Recently, **Gamil (2004)** recorded biochemical changes in protein patterns and isozymes as induced by bacterial and viral bioagents.

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التأثير التسممي لبعض المنتجات الحيوية على يرقات دودة ورق القطن

[63]

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تمت دراسة تأثير أربعة مركبات تجارية حيوية (بروفيكي، تروفيك، بيوسبتين، بروتكتو) على العمر الثاني والعمر الرابع ليرقات دودة ورق القطن حيث لوحظ أن التركيز النصف قاتل LC_{50} قيمته (1.61، 1.57، 1.52، 1.35 مجم / مل) للمركبات بالترتيب وذلك ضد يرقات العمر الثاني لدودة ورق القطن بينما قيم LC_{50} على يرقات العمر الرابع لدودة ورق القطن 2.03 لمركب بروفيكي 2.52 لمركب ... 2.72 لمركب بيوسبتين و بروتكتو LC_{50} 3.01 (مجم / مل).

تمت دراسة تأثيرات المركبات الحيوية الأربعة على النشاط الإنزيمي ليرقات دودة ورق القطن أى أنزيمات الفا والبيتا استريز والترانس أمينيز وكذلك الوسيط الأمينى ... أسبرتات ... ولوحظ أن المركبات الأربعة أظهرت الدراسة ... تأثيرات على النشاط الإنزيمي مقارنة بالكنترول مجال الدراسة ... بالمعاملة.

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