CHANGES IN ENZYMATIC ACTIVITIES OF THE PINK BOLLWORM LARVAE TREATED WITH SOME BIOPESTICIDES

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(Manuscript received 24 July 2011)

Abstract

The present investigation aimed to clarify the changes in the enzymatic activities of the Pinkbollworm larvae treated with some biopesticides. Data showed that Abamectin(1.8%EC) is the most effective than Spinosad (24%SL) on the third instar larvae of the pink bollworm (*pectinophora gossypiella*) (Saund) followed by Esfenvalerate (20%EC) and Dipel 2x (6.4%WP) (*Bacillus thuringiensis*) respectively. The LC50 of Abamectin, Spinosad, Esfenvalerate and Dipel 2x caused increasing effect in protein concentration, reaching 28.8, 25, 48.9 and 39.1% respectively in gut tissue of larvae after 6 hrs. of treatment. After 24 and 48 hrs. of treatment this content was decreased in Dipel 2x and Abamectin by 33.1, 33.5% and 39, 26.3% respectively. But the treatment of Spinosad and Esfenvalerate increased the protein by 26.2, 13.2% and 18, 16.2% respectively. Great increase in activity of protease and glutathione-s-transferase enzymes was recorded in larvae after all intervals of treatment by the tested pesticides.

A high inhibitions in the activity of ATPase in larval tissues after all intervals of treatment except Abamectin and Esfenvalerate after 48 hrs. of treatment were occurred. A Significant increase in acid and alkaline phosphatase activities was detected in 6hrs. treatment of the four insecticides, but this response was changed after 24 and 48hrs. of treatments .

Key words: Pinkbollworm, *B.t.*, insecticide, Biochemical insecticides, enzymes activity.

INTRODUCTION

The pinkbollworm, *Pectinophora gossypiella* (Saund), is a serious and destructive insect pest of cotton bolls in Egypt. Many insecticides belongs to synthetic Pyrethroids, Organophosphorus and Carbamates are commonly used to control the pinkbollworm in fields. Such insecticides caused serious environmental and health problems (Hussein et. al., 2002). The biocontrol of insect pests using traditional microbial agents has been reported as a safe and economic method. One of the most promising biocontrol approach is *Bacillus thuringiensis* (B.t.) toxins as insecticides. The main mode of action of this crystalliferous bacterium was disrupting of the epithelial lining of the midgut-*B.t.* causes great physiological changes in the digestive system of infected insect (Cannan 1993). Another safer biocontrol agents are biochemical

insecticides such as Abamectin and Spinosad. Abamectin acts as electro-physiological binding agents, affect chloride uptake , acetyl choline release and stimulate gamma-amino butyric acid (GABA) (Cully et. al., 1999). Spinosad activates the nicotinic acetyl choline receptor and causes paralysis of insects (Waldron et al., 2000). Under insecticidal stress there are many adaptations in physiological mechanisms of treated insects (Nath et. al., 1997).

This work aims to study the toxicity of the four insecticide {Abamectin, Spinosad, Esfenvalerate (pyrethroid) and Dipel 2x (*B. t.*)} on the third instare larvae of Pinkbollworm under laboratory condition. Also to study the effect of (LC_{50}) of the tested insecticides on certain biochemical activities of the Pinkbollworm larvae such as:- Total protein, protease, ATPase, GST (glutathione-s-transferase), Acid and alkaline phosphatases.

MATERIALS AND METHODS

1.1- Insect

A laboratory strain of the pinkbollworm, ***pectinophora gossypiella*** (Saund) was reared on artificial diet as described by (Rashad and Ammer 1985), under constant conditions (27± 2°C and 70 ± 5% R.H.) for five years in centeral agriculture pesticides laboratory, with out any exposure to insecticides.

1.2- Insecticides used

1.2.1- The bacterial Formulation Dipel 2x (6.4%Wp – Valent Biosciences) a commercial product based on *Bacillus thuringiensis var kurstaki* used at concentration of 1500 , 1125 , 750 , 562.5 , 375 , 281.25 and 187.5 ppm .

1.2.2-Abamectin (Vertimec 1.8% EC – Syngenta) produced by an actinomycete bacterium, *Streptomyces avermitilis,* found in soil, used at 15.84, 11.52, 8.64, 5.76, 2.88, 1.44 and 0.72 ppm .

1.2.3- Spinosad (Spintor 24% SL - Dow Agrosciences) made up of two complex organic compounds, spinosyn A (right) and spinosyn D. These compounds are produced by certain microbes, used at 144, 108, 72, 54, 36, 18 and 9 ppm.

1.2.4- Esfenvalerate (20% EC Sumi –gold KZ- Kafr Elzayate) pyrethroid insecticide used at 150, 120, 90, 60, 45, 30 and 15 ppm.

Each concentration was mixed with artificial diet in twenty glass tubes (0.1ml / 5 gm wt.) then 3rd instar larvae were transferred individually to treated and untreated tubes (Five replicates of each treatment). Mortality was counted after 6, 24 and 48 hours of treatment. All data were analyzed using SAS probit (1997).

1.3- Biochemical assays

The untreated and treated 3rd instar larvae were dissected and the gut tissues were isolated from larvae. Hundred milligrams of gut tissues were homogenized in 3 ml of sodium phosphate buffer (pH 7) using a teflon homogenizer surrounded by a jacket of crushed ice and centrifuged for 30 min at 10000 rpm / min. Then supernatant was transferred to new tubes and was preserved at -20°C. The total protein content was measured based on Biuret's method (Henry 1964). In this method proteins made a complex of violet blue color with an alkaline copper solution , which absorption value at 545 nm has a direct relation to the amount of protein .

1.3.1- Adenosine triphosphatase assay

The activity of the enzyme was assayed according to the method described by (Shiosaka et al., 1971). In this method, the protein was precipitated with trichloroaceticacid.

The protein free filtrate was treated with acid molybdate solution and the phosphoric acid formed was reduced by the addition of 1-amino-2-naphthol-4-sulphoric acid (ANSA) reagent to produce blue color. The intensity of the color was proportional to the amount of phosphorous present.

1.3.2- Glutathione–s–transferase assay

CDNB (1–chloro–2, 4- dinitrobenzene) substrate was used to measure the enzyme activity. The conjugated glutathione was measured at 340 nm for 2 min and activity was calculated with an extinction coefficient of 9.6 mM / cm for CDNB (Kristensen, 2005).

1.3.3- Phosphatase enzymes assay

Disodiumphenylphosphate (Substrate) was hydrolyzed by the enzyme and the released phenol was reacted with 4- amino anti pyrine by which brown color was appeared by addition of potassium ferricyanide, this color was measured at 510 nm (Powell and Smith 1954).

1.3.4- Protease assay

Azocasein was used as substrate in carbonate buffer ($NaHCO_3$ – $NaCO_3$ pH 11) to measure the activity of protease enzyme at 450 nm after addition of 1M NaOH was described by (Brik et al., 1962) with some modification by (Wang et al., 2007).

RESULTS AND DISCUSSION

1. Effect of tested pesticides on 3rd larval instare of the Pinkbollworm

Data in table (1) showed that Abamectin was the most potent insecticide [LC_{50} = 7.21, 3.67 and 1.82 ppm with toxicity index (T.I.) 100%] Followed by

Spinosad (63.33 , 38.25 and 16.14 ppm with 11.39 , 10.00 and 11.28 % T.I.) and Esfenvalerate (79.80 , 41.00 and 18.55 ppm with 9.04 , 8.95 and 9.81 % T.I.) against 3rd instar larvae after 6 , 24 and 48 hours of treatment, respectively. *B. thuringensis var kurstaki* (Dipel 2x) had the lowest effect (896.42, 641.18 and 318.74 ppm and 0.08, 0.57 and 0.57 % T.I.) on these larvae at the same intervals, respectively. These results agree with those of Hussein *et* al., (2002) who mentioned that Vertimec 1.8% EC (LC_{50} = 0.02 ppm) was more effective on *p. gossypiella* larvae than neemazal-T5%SC (LC_{50} = 50 ppm). Radwan (2002) reported that toxicity of Spinosad 24% SL was high than Dipel-2x 6.4% WP and Agerin 6.5%WP (LC_{50} = 6.47, 8.58 and 43.99 ppm respectively) to the last instar larvae of spiny bollworm.

2. Total protein content and protease activity in gut tissue of 3rd instare larvae of the Pinkbollworm

Data in table (2) showed that the treatment of *p. gossypiella* with LC_{50} of Esfenvalerate, Dipel 2x, Abamectin and Spinosad pesticides caused the increasing values in gut protein, reaching 48.9 , 39.1 , 28.8 and 25% ,respectively after 6 hours. But after 24 and 48 hours of treatment the increasing effect was recorded in Spinosad at 26.2 and 13.2 % and Esfenvalerate of 18 and 16.2 %. On the other hand Dipel 2x (*B.t.*) of 33.1 and 33.5 % and Abamectin of 39 and 26.3 % decreased the gut protein. These results agree with Nath *et* al., 1997, who mentioned that 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 insect tissues.

Protease activity was stimulated with all tested insecticides after 6, 24 and 48 hrs. of treatments, especially Esfenvalerate which caused the highest stimulation 454.5 , 482.7 and 178.4 %, respectively. The higher activity of protease after 48 hrs. was pronounced with B.t. treatment (346.4 %), this due to the mode of action of the (B.t.) that produces crystals that contain insecticidal crystal proteins (ICPs). ICPs, or Cry proteins, are in the form of protoxins in the crystal. ICPs are solubilized and processed to toxic peptides by gut proteases in susceptible insects. Because proteases are important to toxicity, research into interactions of prteases with Bt proteins may lead to improved toxin efficacy. Insect gut proteases are involved in crystal dissolution and protoxin activation and contribute to toxin specificity.

Insect proteases can further degrade activated ICPs; they may also be involved in receptor-toxin interactions and post-binding events. (Oppert , 1999).

3. Activity of ATPase and GST (glutathione-s-transferase) in gut tissue of 3rd instare larvae of the Pinkbollworm

Data in table (3) revealed that all tested pesticides, in general, had inhibiting effects on the activity of ATPase of *p. gossypiella* gut after 6, 24, 48 hrs., except the treatments of Esfenvalerate and Spinosad after 48 hrs. which produced stimulating effect on the activity of enzyme (43.9 and 21.5 %) respectively. The insecticidal treatments disturb absorption of metabolites and nutrients (Zibae et al., 2008). GST activity was highly increased with Esfenvalerate (98.2, 81.2 and 111.2 %), *B.t.* Dipel 2x (88.2, 19.4, 82.9%), Spinosad (23.8, 63.5 and 21.7%) and Abamectin (11.3, 24.7 and 182.9%) after 6 .24 and 48 hrs. of treatment. Sivori et al., 1997 mentioned that pyrethroid insecticides showed a significant increase in GST activity in terms of specific enzyme activity.

4. Activity of acid and alkaline phosphatase in gut tissue of 3 rd larval Instare of the Pinkbollworm

Data in table (4) showed the significant stimulation in acid phosphatase enzyme with all treatment after 6 hrs. , and after 24 hrs. with Dipel 2x and Abamectin and after 48 hrs. with Abamectin only. The highly stimulation in enzyme activity was recorded in Esfenvalerate treatment after 6 hrs. (89%). Also a significant decreased value (38%) was recorded after 48 hrs. of the same insecticide.

The significant stimulation in alkaline phosphatase activity was occurred by Abamectin (74.1%) and Spinosad (64.3%) than the other two pesticides at 6hrs. treatments, and Esfenvalerate (86%) after 24 hrs. and Dipel 2x (83.6 %) and Spinosad (54.6%) after 48 hrs. treatments. There is a slight inhibition in enzyme activity after 24 hrs. treatments of Dipel 2x (11.4%) and Spinosad (8.3%) and after 48 hrs. of treatments with Esfenvalerate (24%) and Abamectin (3.8%) . Hussein et. al., (2002) recorded the high reduction in acid and alkaline phosphatase in treated *P. gossypiella* with Abamectin than the control ones.

Table 1. Effect of tested pesticides on 3rd larval instare of *P. gossypiella* (Saund)

Treatment intervals / Insecticide		Abamectin	Spinosad	Esfenvalerate	B.t.
6 hours	**LC_{50}(ppm)**	7.21	63.33	79.80	**896.42**
	Slope	1.60	1.71	1.48	**1.26**
	±S.D.	± 0.465	± 0.214	± 0.333	**±0.558**
	Toxicity index (%)	100	11.39	9.04	**0.80**
24 hours	**LC_{50} (ppm)**	3.67	38.25	41.00	**641.18**
	Slope	1.39	2.26	1.52	**1.22**
	±S.D.	± 0.348	± 0.119	± 0. 228	**± 0.356**
	Toxicity index (%)	100	10.00	8.95	**0.57**
48 hours	**LC_{50} (ppm)**	1.82	16.14	18.55	**318.74**
	Slope	1.55	1.50	1.36	**1.43**
	±S.D.	±0.174	± 0.283	± 0.347	**± 0.168**
	Toxicity index(%)	**100**	**11.28**	**9.81**	**0.57**

Toxicity index(%) = $\frac{LC_{50} \text{ of the most effective insecticide}}{LC_{50} \text{ of the other insecticide}} \times 100$

Table 2. Total protein content and protease activity in control and treated 3rd instare larvae in gut tissue of *p. gossypiella* (Saund)

Treatment	Conc. of protein Mean ± S.D. mg / 100 mg gut tissue						Activity of protease Mean ± S.D. O.D unit x10^3 / min / 100 mg gut tissue					
Intervals	**6 hrs.**	**Change %**	**24 hrs.**	**Change %**	**48 hrs.**	**Change %**	**6 hrs.**	**Change %**	**24 hrs.**	**Change %**	**48 hrs.**	**Change %**
Control	1.84 c ± 0.735	0. 0	1.72 a ±0.011	0. 0	1.67 a ±0.328	0. 0	277.14 c ±0.274	0.0	262.96 c ±1.602	0.0	258.22 c ±0.628	0.0
Dipel 2x	2.56 a ± 0.567	+ 39.1	1.15 b ±0.304	- 33.1	1.11 b ±1.271	- 33.5	420.26 a ±1.72	+ 51.6	152.82 a ±248.8	+ 338.4	693.40 b ±0.403	+ 168.5
Abamectin	2.37 b ± 0.394	+ 28.8	1.05 b ±0.105	- 39	1.23 b ±0.378	- 26.3	438.65 a ±9.88	+ 58.2	604.62 b 0.625±	+ 129.9	927.24 a ±0.417	+ 259
Spinosad	2.30 b ± 0.666	+ 25	2.17 a ±1.006	+ 26.2	1.89 a ±1.061	+ 13.2	364.80 b ±0.973	+ 31.6	625.28 b ±0.625	+ 137.7	864.77 a ±1.078	+ 234.8
Esfenvalerate	2.74 a ± 0.183	+ 48.9	2.03 a ±1.158	+ 18	1.94 a ±1.000	+ 16.2	336.63 b ±1.603	+ 21.4	642.34 b ±0.689	+ 144.2	818.97 a ±0.400	+ 217.15

Change% = $\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

Means followed by the same letter at the same column are not significantly different.

Table 3. Activity of ATPase and GST (glutathione-s-transferase) in gut tissue of control and treated 3rd instare larvae of p. gossypiella (Saund)

Treatment	ATPase (µmol / mg protein / min)						GST (µ mol / mg protein / min)					
Intervals	6 hrs.	Change %	24 hrs.	Change %	48 hrs.	Change %	6 hrs.	Change %	24 hrs.	Change %	48 hrs.	Change %
Control	48.35 a ±3.17	0.0	42.73 a ±3.22	0.0	37.60 a ±2.61	0.0	99.76 c ±10.25	0.0	c 90.28 ±5.42	0.0	83.17 d ± 6.50	0.0
Dipel 2x	35.51 b ±2.21	- 26.6	29.92 b ±2.04	- 30	29.33 b ±1.82	- 22.0	187.79 a ±15.80	+88.2	107.81 b ±4.15	+19.4	152.08 b ±5.11	+82.9
Abamectin	26.19 c ±2.65	- 45.8	19.24 c ±3.79	- 55	45.67 a ±1.64	+ 17.7	111.05 b ±9.41	+11.3	112.57 b ±7.24	+24.7	235.26 a ±8.36	+182.9
Spinosad	29.60 c ±2.73	- 38.8	22.13 c ±2.96	- 48.2	21.81 c ±3.11	- 42.0	123.50 b ±11.48	+23.8	147.62 a 6.18 ±	+63.5	101.22 c ± 4.71	+21.7
Esfenvalerate	20.83 d ±3.12	- 56.9	31.73 b ±1.64	- 25.7	42.21 a ±1.17	+ 12.3	197.73 a ±13.97	+98.2	163.55 a ±10.88	+81.2	175.68 b ±12.73	+111.2

Change% = Treatment –Control × 100
Control

Means followed by the same letter at the same column are not significantly different.

Table 4. Activity of acid and alkaline phosphatase in gut tissue of control and treated 3 rd larval Instare of p. Gossypiella (Saund)

Treatment	Activity of acid and alkaline phosphatase (µmol /mg protein /min)											
	Acid						Alkaline					
Intervals	6 hrs.	Change %	24 hrs.	Change %	48 hrs.	Change %	6 hrs.	Change %	24 hrs.	Change%	48 hrs.	Change %
Control	$^{1.01}$d 0.082±	0.0	$^{1.35}$c 0.056±	0.0	$^{1.42}$a 0.016±	0.0	$^{15.34}$c 0.341±	0.0	$^{14.23}$b 0.215±	0.0	$^{14.19}$ c 0.181±	0.0
Dipel 2x	$^{1.24}$c 0.036±	+22.8	$^{1.98}$a 0.011±	46.7+	$^{1.28}$b 0.042±	9.9 –	$^{20.64}$b 0.522±	+34.6	$^{12.61}$b 0.625±	-11.4	$^{26.05}$ a 0.317±	+83.6
Abamectin	$^{1.46}$b 0.027±	44.6+	$^{1.78}$b 0.046±	+31.9	$^{1.59}$ a 0.026±	+12	$^{26.70}$a 0.417±	+74.1	$^{15.44}$ b 0.818±	+8.5	$^{13.65}$ c 0.255±	-3.8
Spinosad	$^{1.59}$b 0.018±	57.4+	$^{1.15}$c 0.029±	14.8–	$^{1.31}$ b 0.033±	-7.8	$^{25.20}$a 0.354±	+64.3	$^{13.05}$ b 0.436±	-8.3	$^{21.93}$ b 0.542±	+54.6
Esfenvalerate	$^{1.91}$a 0.044±	89.1+	$^{0.95}$d ±0.051	-29.6	$^{0.88}$ c 0.023±	-38	$^{20.72}$b 0.762±	+35.1	$^{26.47}$a 0.717±	+86	$^{10.79}$ d 0.349±	-24

Change% = $\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

Means followed by the same letter at the same column are not significantly different.

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التغيرات فى الانشطة الانزيمية ليرقات ديدان اللوز القرنفلية المعاملة بالمبيدات الحيوية

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إستهدفت الدراسات البيوكيميائية إلى إيضاح التغيرات فى الانشطة الإنزيمية ليرقات ديدان اللوز القرنفلية المعاملة ببعض المبيدات الحيوية وتبين النتائج أن المبيد الحيوى أبامكتين (1.8EC)هو الاكثر فاعلية من مبيدات سبينوساد (24SL%) يليه مبيد إس فينفاليرات (20%EC) ثم دايبل 2 اكس (6.4 %WP).

وقد تسببت المعاملة بالتركيز النصفى المميت لل(أبامكتين –سبينوساد – إس فينفاليرات –دايبل 2 اكس) فى زيادة تركيز بروتين انسجة معى اليرقات بعد ستة ساعات من المعاملة بنسب (28.8، 25 ،48.9، 39.1%) على التوالى. كما حدث نقص فى ذلك المحتوى بعد 24 و48 ساعه من المعاملة بمبيدى دايبل 2اكس والابامكتين بنسب (33.1 ، 33.5 %) و (39، 26.3%) على التوالى . فى حين ان المعاملة بمبيدى سبينوساد وإس فينفاليرات احدثت زيادة فى البروتين بنسب (26.2، 13.2%) و (18، 16.2%) على التوالى.

وقد وجدت زيادة كبيرة فى نشاط انزيمى (البروتييز والجلوتاثيون– اس– ترانسفيريز) فى اليرقات بعد كل فترات المعاملة بالمبيدات المستخدمة .

كما حدث تثبيط مرتفع فى نشاط انزيم (ادينوسين تراى فوسفاتيز) فى انسجة اليرقات بعد كل فترات المعاملة ما عدا (الابامكتين و إس فينفاليرات) بعد 48 ساعة من المعاملة.

كما سجلت زيادة معنوية فى نشاط انزيمى الفوسفاتيز الحامضى والقاعدى بعد ستة ساعات من المعاملة بالمبيدات الاربع، لكن هذه الاستجابة تغيرت بعد 24، 48 ساعة من المعاملة.