Mansoura Journal of Biology, Vol. 35 (1), June 2008

BIOCHEMICAL STUDIES OF *BACILLUS THURINGIENSIS*, SPINOSAD AND CYPERMETHRIN ON THE CARBOHYDRATES HYDROLYZING ENZYMES AND PHOSPHATASE ENZYMES OF COTTON LEAF WORM, *SPODOPTERA LITTORALIS*

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ABSTRACT

The present investigation was carried out to evaluate the effect of the three tested compounds on the carbohydrate hydrolyzing enzymes and activity of haemolymph acid and alkaline phosphatase of 5th instar larvae of *S. littoralis* treated as 4th instar larvae with the LC_{50} levels of *Spinosad, B. thuringiensis* and cypermethrin. The results indicated that the acid phosphatase activity was insignificantly increased by about 2.55% more than the control in the case of treatment with *B. thuringiensis*. On the other hand, Spinosad and cypermethrin insignificantly decreased the activity of acid phosphatase.The application of the three tested insecticides to the 4th instar larvae of *s. littoralis* had changed the carbohydrate hydrolyzing enzymes as indicated that there was a large significant increase in amylase activity of 5th instar larvae of *S. littoralis* in case of spinosad treatment; and treatment with Cypermethrin significantly decreased the amylase activity, whereas treatment with *B. thuringiensis* significantly decreased in compared to control. Invertase activity was significantly decreased in case of all tested compound treatments compared to control. Trehalase activity was significantly decreased, compared to control for Spinosad treatments. Whereas, Cypermethrin and *B. thuringiensis* treatment insignificantly decreased trehalase activity compared to control.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) which is considered as one of the major and economic pests not only in Egypt, but also in many parts of the world, infests over 112 plant species, belonging to 44 families, including cotton *Gossypiurn*

hirsutum (L.). The larval stage is known as a notoriously leaf eater accepting almost all herbaceous plants **[Hill (1975)]**. The cotton leafworm larvae feed for about two weeks mostly on the leaves and occasionally on flowers and bolls. The use of insecticides for control of such pest proved to be the most accepted during the recent years. However, the practical application of different insecticides extensively has resulted in several problems such as development of resistance in field population of insects one of the safe and promising control methods having no adverse effects on human, domestic animals, plants and environment in the use of biological pest control agent. There is many lepidopterian species that have been successfully controlled by microbial agents. This work was carried out to evaluate the control of *S. littoralis* larvae by the bacterium *Bacillus thuringiensis* var. kurstaki, the bacterium *spinosad* and the pyrethroid compound (*cypermethrin).*

MATERIALS AND METHODS

1- Tested Compounds:

In the present studies two commercial formulations of entomopathogens which considered as bioinsecticides and one pyrethroid compound were selected to test their effects against the 4th instar larvae of *Spodoptera littoralis.*

These entomopathogens bioinsecticides were,

1.1- *Bacillus thuringiensis var. kurstaki Berliner*

Produced by Valent Biosciences Corporation – USA

Trade name	*Diple 2x*
Common name	*Bacillus thuringiensis var.kurstaki*

1.2- *Spinosad*

Trade name	Traceer
Common name	*spinosad* 24% (Fermentation product of B.T.)
Chemical formula	$C_{41}H_{65}NO_{10}$ (spinosyn A) + $C_{42}H_{67}NO_{10}$ (spinosyn D)

Spinosad (spinosyn A and spinosyn D) are a new chemical class of insecticides that are registered by the EPA to control a variety of insects. The active ingredient is derived from a naturally occurring soil dwelling bacterium called *Saccharopolyspora spinosa*, a rare actinomycete reportedly collected from soil in an abandoned rum distillery on a Caribbean Island in 1982 by a vacationing scientist. It has

not been found in nature since that time, and was subsequently described as a new species. The bacteria produce compounds (metabolites) while in a fermentation broth. The first novel fermentation-derived compound was formulated in 1988. Spinosad has since been formulated into insecticides that combine the efficacy of a synthetic insecticide with the benefiits of a biological pest control organism.

1.3- ***Cypermethrin***

Trade name Synthetic pyrethroid
Common name *cypermethrin*
Chemical formula $C_{22}H_{19}O_3NCl_2$

All of these tested compounds were obtained from the Plant Protection Research Institute, Ministry of Agriculture,dokki, Cairo, Egypt.

2.1- Rearing of the Egyptian cotton leaf worm *Spodoptera littoralis* (*Boisd.*)

- The stock culture of susceptible Egyptian cotton leaf worm *Spodoptera littoralis* was reared on castor leaves ***(Ricinus communis* L.)** for several generations at laboratory conditions of 25 ± 2°C and 70 ± 5% RH. Egg masses were placed on castor oil leaves in cylindrical glass jars. The jars were covered with muslin cloth and fastened with rubber band. First instar larvae hatched within 2-3 days. The newly hatched larvae were transferred into rearing jars bottomed with sheets of towel paper to absorb excess humidity. Castor bean leaves were provided daily to the larvae in sufficient amounts. The accumulated feces and debris were cleaned out daily. After pupation, pupae were collected and placed in wide clean jars till adult emergence. Then, the emerged adults were supplied with a piece of cotton wetted with 5-10% sugar solution and branches of Tafla ***(Nerium oleander)*** as suitable site for oviposition **[El-Defrawi *et al.*, (1964)]**. Newly laid egg masses were collected daily and transferred into the rearing jars.

3- Preparation of samples for biochemical assay.

For biochemical analysis purposes, haemolymph was collected from 3 pooled samples, each from 8-10 late 5^{th} instar larvae fed as 4^{th} instar for 24 hours on castor-oil leaves treated with the LC_{50} values of each tested compound. One of the prolegs was removed and the haemolymph was collected in cold tubes (on ice) previously coated with

crystals of phenylthiourea to prevent melanization. The samples were centrifuged at 2500 rpm for 5 minutes under cooling (4°C) to remove the blood cells. After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at –20°C until analysis.
Acid phosphatase and alkaline phosphatase were determined according to the method described by **Laufer and Schin (1971)**.

The methods used to determine the digestion of trehalose, starch and sucrose by trehalase, amylase and invertase enzymes respectively, were similar to those described by **Ishaaya and Swiriski (1976)**.

4- Susceptibility tests

A series of concentrations (in water) for each insecticide were prepared on the active ingredient (a.i) in ppm by diluting the commercial formulation. Castor-been leaves were dipped for 30 seconds in each concentration then left to dry for one hour. The 4th instars larvae with each tested strain were confined with treated leaves in glass jars covered with muslin for 24 hrs. Test also included a non treated leaves control in which leaves were dipped in water (as a check). Treated leaves were then removed and the fresh untreated leaves provided for another day. Three replicates (each of 30 larvae) were tested for each concentration. Daily inspection was carried out for all treatments and mortality percentages were recorded after treatment. The average of mortality percentage was corrected using **Abbott's formula (1925).** The corrected mortality percentage of each compound was statistically computed according to **Finney (1971).** From which the corresponding concentration probit lines (lc-p lines) were estimated in addition to determine 50 and 90% mortalities, slope values of tested compounds were also estimated.

RESULTS

1- Susceptibility test:

Data presented in **Table(1)** summarized the susceptibility of the three tested compounds. The two bioinsecticides (*Bacillus thuringiensis*, *spinosad*) and pyrethroid compound (*cypermethrin*) used in the present work caused considerable toxic effects against the 4th larval instar of *Spodoptera littoralis, however ,it is quite clear in the case of treatments* with cypermethrin and *Bacillus thuringiensis*. *Cypermethrin* was the most toxic to *S. littoralis larvae* and one can say that the toxicity of the toxicity of the bioinsecticide was significantly lower than that of the

chemical insecticide. The LC_{50} of *cypermethrin* was 1.675 followed by 7.59 and 28.868 ppm agianst 4th instar larvae with *Bacillus thuringiensis* and spinosad. respectively.

2-Effects of LC_{50} of *Bacillus thuringiensis*, spinosad and cypermethrin on carbohydrates hydrolyzing enzymes of 4th instar larvae of *Spodoptera littoralis*.

2.1- Amylase enzyme

Amylase activity through the present study indicated that there was a large significant increase in amylase activity of 5th instar larvae of *S. littoralis* in case of Spinosad treatment: however treatment with Cypermethrin significantly decreased the amylase activity, whereas treatment with *B. thuringiensis* insignificantly decreased amylase activity in compared to the untreated one as follow (1.92,1.15 and 1.24 with spinosad , cypermethrin and Bacillus thuringiensis respectively compared to 1.32 of the control as shown in **Fig.(2)** and **Table (3)**

2.2-Invertase enzyme

In the present study all the tested compound had a remarked effect on Invertase activity in the haemolymph of 5th instar larvae of Spodoptera littoralis. Spinosad. cypermethrin and *B. thuringiensis* were significantly decreased the invertase activity compared to the untreated larvae as follow (72.37. 67.19 and 72.58% respectively. compared to 100% for the untreated one (control group).

2.3.Trehalase enzyme

The Change % in trehalase enzyme decreased after the 4th day post- treatment in all treated larvae. however it was higher than the untreated larvae. *Spinosad* showed the highest level of enzyme activity followed by *cypermethrin* and *B.thuringiensis*, respectively. However the decrease was significant only with spinosad and there is no significant effect with larvae treated with *cypermethrin* and *B.thuringiensis*. Generally, change % in trehalase activity reached its maximum increase with *spinosad*.

3- Effects of LC_{50} of *Bacillus thuringiensis*, spinosad and cypermethrin on acid and alkaline Phosphatase in the haemolymph of 4th instar larvae of *Spodoptera littoralis*.

Phosphatase activity:

Data obtained in **Table (3)** and in **Fig. (2)** show the effect of the three tested compounds on the activity of haemolymph acid and alkaline phosphatase of 5th instar larvae of *S. littoralis* treated as 4th instar larvae with the LC_{50} levels of Spinosad, *B. thuringiensis* and Cypermethrin. The results indicated that the acid phosphatase activity was slight significantly increased by about 2.55% more than the control in case of treatment with *B. thuringiensis*. On the other hand, Spinosad and Cypermethrin insignificantly decreased the activity of acid phosphatase. It is quite clear from our result that the tested compound caused a significant decrease in the activity of haemolymph alkaline phosphatase of *S. littoralis* by70.37, 75.2 and 77.61% with spinosad, Cypermethrin and *B. thuringiensis*, respectively in respect to 100% in case of untreated larvae. Acid phosphatase activity predominated that of alkaline phosphatase in either non-treated or treated larvae **Fig. (2).**

Table (1): Susceptibility of *Spodoptera littoralis* 4th instar larvae to Spinosad, Cypermethrin and B. thuringiensis

Treatments	LC_{50}	95% Fiducial limits		Slope ± SE	X^2
		Lower	Upper		
Spinosad	28.868	24.727	33.59	3.598 ± 0.576	2.026
Cypermethrin	1.675	1.441	1.99	3.509 ± 0.606	6.883
B. thuringiensis	7.59	6.332	9.29	2.583 ± 0.422	1.258

Table (2): Effects of LC_{50} of *Bacillus thuringiensis*, spinosad and cypermethrin on Phosphatase in the haemolymph in 5th instar larvae treated as 4th larval instar of S.littoralis

Treatments	Acid phosphatase activity	% change from the control	Alkaline phosphatase activity	%change from the control
Spinosad	4.30	99.53	6.13	70.37*
Cypermethrin	4.12	95.37	6.55	75.2*
B. thuringiensis	4.43	102.55	6.76	77.61*
Control	4.32	100	8.71	100

- =significant at P =0.05

Table (3): Effects of LC50 of Spinosad, Bacillus thuringiensis and Cypermethrin on carbohydrates hydrolysing enzymes of 5th instar larvae treated as 4th larval instar of S.littoralis.

Treatment	Amylase	% change from the control	Invertase	% change from the control	Trehalase	% change from the control
Spinosad	1.92	145.46*	3.22	72.37*	2.52	89.67*
Cypermethrin	1.15	87.12*	2.99	67.19*	2.65	94.3
B. thuringiensis	1.24	93.93	3.23	72.58*	2.68	95.37
Control	1.32	100	4.45	100	2.81	100

- =significant at P =0.05

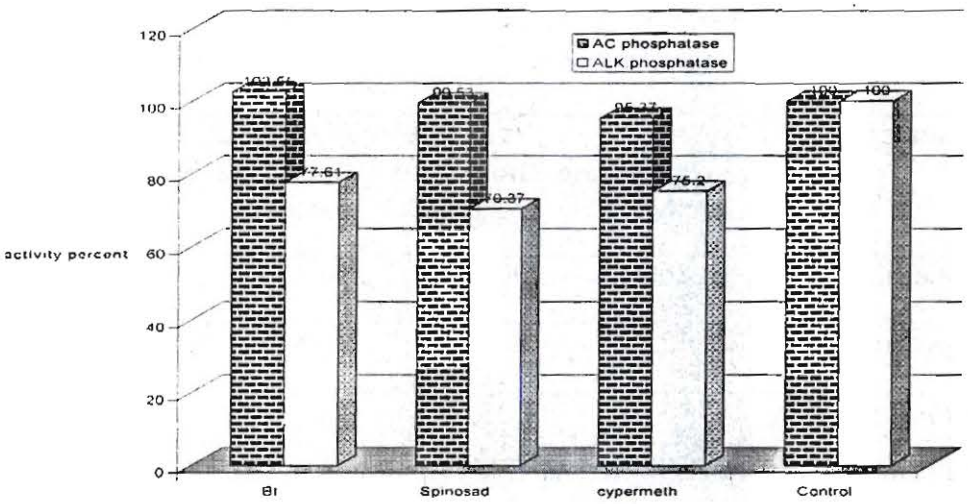

Fig. (2): Effects of LC_{50} of *Bacillus thuringiensis* , *Spinosad* and *Cypermethrin* on phosphatases enzymes in 5th instar larvae of *Spodoptera littoralis treated as 4th larval instar.*

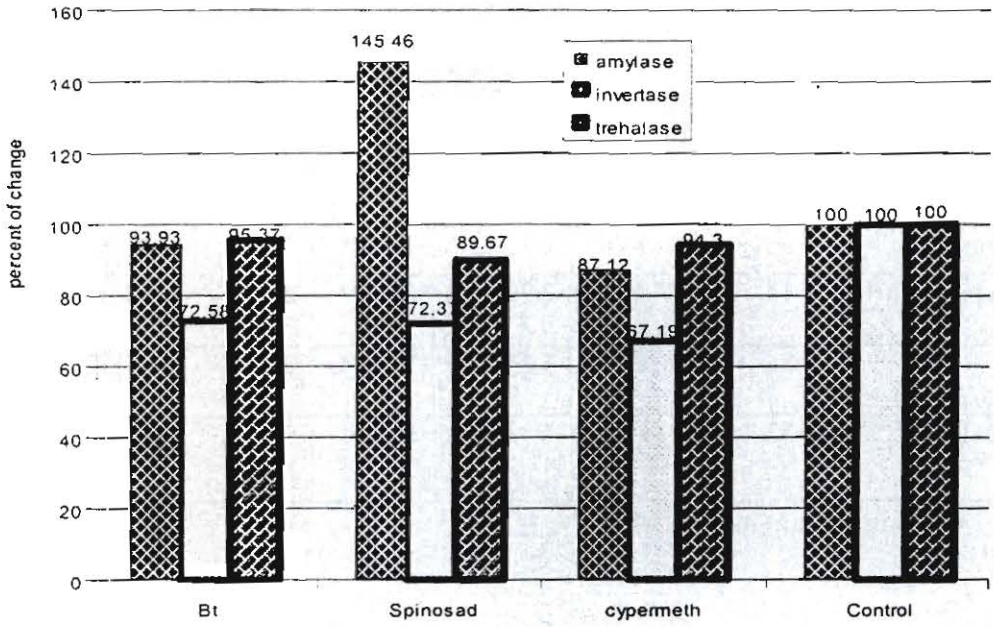

Fig. (1): Effects of LC_{50} of *Bacillus thuringiensis*, *Spinosad* and *Cypermethrin* on carbohydrates hydrolyzing enzymes in 5th instar larvae of *Spodoptera littoralis treated as 4th larval instar.*

DISCUSION

1-Carbohydrate hydrolyzing enzymes

Carbohydrates are one of vital importantance since the insect body can utilize them for producing energy or conversion to lipids or proteins. Metabolism of carbohydrates controlled mainly by trehalase, amylase and invertase that play aprinciple role in the digestion and utilization of carbohydrate by insects (Wyatt, 1967 and Wiggles worth, 1972). Our findings showed that both spinosad and cypermethrin caused decrease of haemolymph amylase and invertase activities. These results agree with those obtained by **El-Mageed *and Elgohry* (2006)** who observed that Spinosad decreased amylase and invertase activity in 4th instar larvae of *S. littoralis*.

1.1-Trehalase enzyme

The fat body is generally regarded as the principal site of trehalose biosynthesis in insects. Trehalase is activated for the production of glucose needed for chitin build-up in the newly synthesized cuticle; it is generally present in large amounts in the haemolymph of most insects and it has the important function of energy supply to insect; and its activity might be an indicator of energy reserves resulting from availability of carbohydrate nutrient **(Wyatt, 1967).**

The Change % in trehalase enzyme decreased after the 4^{th} day post- treatment in all treated larvae, but it was higher than the untreated larvae. *Spinosad* showed the highest level of enzyme activity followed by *cypermethrin* and *B.thuringiensis*, respectively. But the decrease was significant only with *spinosad*, however it was insignificant with larvae treated with cypermethrin and *B.thuringiensis* generally, change % in trehalase activity reached its maximum increase with *spinosad*. Our results are in harmony with **El-Ghar *et al.* (1995)** who stated that *B. thuringiensis* at concentration of 200 p.p.m. reduced trehalase activity by 53% after 2 days of treatment. **Mordue and Blackwell (1993)** reported that disrupted mid gut tissues would function abnormally at which the enzymes secretion and nutrient absorption would be disrupted. Rapid decrease of glucose concentration at the end of last instar larvae of *S. littoralis* was probably caused by high metabolic activity of the epidermis, which is known as a tissue with low trehalase, so it is unable to utilize trehalose **[Florkin & Jeauiux, (1964)].** Therefore, as a result of treatment with spinosad, the activity of haemolymph trehalase of

treated insects in the present study lowers than that of non-treated ones. The disturbance of trehalase activity might prevent the supply of glucose needed for chitin build up.

1.2- Amylase enzyme

Amylase activity through indicated that there was a large significant increase in amylase activity of 5th instar larvae of S. littoralis in case of *Spinosad* treatment; however treatment with *Cypermethrin* significantly decreased the amylase activity, whereas treatment with *B. thuringiensis* induced insignificantly decreased when compared to the untreated one. This is parallel with the finding of **El-Ghar *et al.* (1995)** who found that *B. thuringiensis* at (5 p.p.m.) caused a remarkable decrease in amylase activity at which maximum inhibition, about 77% was reduced 3 days after treatment also. at concentration 200 p.p.m. reduced amylase activity by 53% after 2 days of treatment.

1.3-Invertase enzyme

Generally, all the treatments decreased the activity of invertase enzyme in the 5th larval instar treated as 4th larval instar than control at all tested expirments .

2-Effect on phosphatases enzymes

Acid and alkaline phosphatases:

Acid and alkaline phosphatases have been shown to be associated with insect development especially in relation to nutrition and egg maturation **[Tsumuki & Kanehisa (1984)].** Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis. This latter process is appreciable at the metamorphic moults of holometabolous species to which *S.littoralis* belongs. Show the effect of the three tested compounds on the activity of haemolymph acid and alkaline phosphatase of 5th instar larvae of *S. littoralis* treated as 4th instar larvae with the LC_{50} levels of *Spinosad, B. thuringiensis* and Cypermethrin. The results presented in this study indicated that the acid phosphatase activity was insignificantly increased by about 2.55% more than the control in case of treatment with *B. thuringiensis*. On the other hand, *Spinosad* and *Cypermethrin* insignificantly decreased the activity of acid phosphatase these resembles are in agreement with **Abdel Hafez *et al.* (1988)** who tested the effects of diflubenzuron and triflumuron on *S. littoralis* larvae treated with.

The present work showed insignificant decline in the activities of acid but indicated a significant decrease in alkaline phosphatase as a result of treatment with all tested compounds. **Ayyangar and Rao (1990)** reported that injection of azadirachtin into 6th instar larvae of *S. littoralis* resulted in decreasing activities of alkaline phosphatase. This results are supported by the works of **El Ghar *et al* (1995).**

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دراسات كيميائية حيوية للباسيليس ثيورينجينسيز و سبينوساد والثيبرميثرين على دودة ورق القطن سبودوبتيراليتوراليس

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التأثيرات البيوكيميائية إنزيمات الفوسفاتيز: أدت المعاملة بالباسيليس ثيورجينسيز Diple2x إلى زيادة غير معنوية في نشاط إنزيم الفوسفاتيز الحامضي بينما أدت المعاملة بسبينوساد (*Spinosad*) إلى نقص معنوي في نشاط إنزيم الفوسفاتيز القلوي. ومن ناحية أخري أدت المعاملة بالثيبرميثرين (*Cypermethrin*) إلى نقص معنوي فى نشاط هذين الإنزيمين.

الإنزيمات المحللة للكربوهيدرات: وجدت زيادة معنوية لإنزيم الأميليز في حالة سبينوساد (*Spinosad*) بينما وجدت نقص غير معنوية لنشاط هذا الإنزيم فى حالة المعاملة بكل من بالباسيليس ثيورجينسيز Diple2x والثيبرميثرين (*Cypermethrin*) . أدت المعاملة بالباسيليس ثيورجينسيز Diple2x وبالثيبرميثرين (*Cypermethrin*) إلى نقص غير معنوية في نشاط إنزيم التريهاليز وعلي العكس أدي استخدام سبينوساد (*Spinosad*) إلى نقص معنوي في نشاط ذلك الإنزيم. والواضح ايضا من هذة الدراسة أن جميع المواد الثلاثة أدت إلى حدوث نقص معنوى في نشاط إنزيم الإنفرتيز.