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# The Effect of Some Bio-Insecticides on the Cotton Leaf Worm *Spodoptera littoralis* (Boisd.) Larvae

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## ABSTRACT

This study was conducted to investigate the effect of $LC_{50}$concentration of three commercial bio insecticides; viruset, *spodopetra littoralis* nuclear polyhedrosis virus, protecto *Bacillus thuringensis*var. *kurstaki*and their mixture profect on the protein profile and enzyme activity of three carbohydrate digestive enzymes (amylase, invertase and trehalase).The digestive enzymes were determined in 6th larva linstar after treatment of 3rd larval instar with the $LC_{50}$ concentration. Results showed that amylase activity was relatively un affected in treated larvae with viruset and protecto,but there was asignificant decrease when treated with profect compound.On the other hand the activity trehalase was significantly decreased in all samples. Also protein profile of the treated and untreated larvae was studied by SDS –PAGE technique. Results showed that the protein pattern of larvae treated with the profect compound leads to inhibition and appearance of some bands followed by protecto compound, while viruset has a slight change in the pattern. Thus, our findings indicated that among the three tested compounds, profect compound showed a highest significant effect on both protein profile and enzyme activity of the larvae.

## INTRODUCTION

The cotton leaf worm, *Spodoptera littorals* (Bosid.) causes severe damage for cotton plants and subsequently leads to economic losses in cotton industry in Egypt. This pest develops a resistance to the majority of chemical insecticides due to uncontrolled intensive use of these insecticides.To avoid unfavorable side effects of the intensive use of chemical insecticides on non-target organisms and environment, alternative materials have been initiated using safe and effective insect pathogens such as microbial insecticides (Crickmore 2006).The most widely used microbial insecticides are entomopathogenic bacteria especially Bacillus thuringiensis Berliner (B.t), which its insecticidal activity was attributed to proteinaceous crystals and vegetative insecticidal protein contents of this bacterium that produced during sporulation and vegetative growth phase, respectively(Fang *et al.* 2007; Sanahuja *et al.* 2011).The second most common microbial insecticide is Baculovirus, which has been used as a safe bioinsecticide due to its efficiency and specificity as well as its contents of protein crystals containing infectious particles (Groner 1986). Carbohydrates are important sources of energy for insect, and so their metabolic enzymes, including trehalase, amylase and invertase, are essential for insect survival (Wyatt, 1967 and Wigglesworth, 1972).

Therefore the present investigation was planned to study the efficacy of some bio insecticides on the total protein pattern and digestive enzymes of the *spodopteralittoralis* larvae.

## MATERIALS AND METHODS

**Insect**

The used insect is a laboratory strain of the cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) which had been reared in the laboratory without any exposure to chemicals and was obtained as egg masses from the Research Division of the cotton leaf worm, Plant Protection Research Institute, Dokki- Giza, Egypt.

**Compounds:**

Three commercial bio insecticides were used in this investigation. All tested bio-agents were obtained as wettable powder produced by the Plant Protection Research Institute, Bio pesticide Production Unit, Dokki- Giza, Egypt. They were as follows:

**a. Protecto® WP 9.4% (*Bacillus thuringiensis* var. *kurstaki*)**

*Bacillus thuringiensis* (Kingdom: Eubacteria; Order: Bacillales; Family: Bacillaceae) is a gram-positive spore-forming bacterium that produces crystalline proteins called deltaendotoxins during its stationary phase of growth. This product is used at rate of 300 gm/feddan.

**b. Viruset® WP 4% (*Spodoptera littoralis* nucleopolyhedrosis virus [*Spli*NPV])**

Nucleopolyhedrosis virus (NPV) is a double stranded DNA virus that belongs to baculoviruses subgroup (Family: Baculoviridae). *Spodoptera littoralis* NPV was isolated from diseased larvae and formulated to be used at rate of 300 gm/feddan.

**c. Profect® WP 5% + 2% (*Btk*+ *Spli*NPV)**

This product is a mixture of 5% of *Bacillus thuringiensis* var. *kurstaki* and 2% of *Spli*NPV. The product is used at rate of 400 gm/feddan.

**Biochemical studies**

The activity of three carbohydrate enzymes amylase, invertase and trehalase was determined in 6th instar larvae surviving treatment of 3rdinstar larvae with $LC_{50}$ of each the three tested compounds.

***Preparation of insects for analysis***

Theinsects were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 rpm for 15 min at 2 ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants, were stored for one week without appreciable loss of activity when stored at 5ºCAmin, (1998).Digestive enzymes were determined according to Ishaaya and Swirski, (1970) and Ishaaya *et al.,* (1971).

***Analysis of total soluble proteinby SDS polyacrylamide electrophoresis***

For determination of total protein, the 4th instar larvae treated with some bio insecticides were collected and stored at –20°C. One gram of each group of larvae was grind in 1ml homogenate buffer (0.02 M tris-Hcl pH~7.5) (w/v) using a mortar. The contents were transferred to a new Eppendorf tube, centrifuged at 10.000 rpm for 10 min at 4°C, and then the supernatant was kept frozen at –20°C until required. Following measuring protein concentration, the obtained protein was separated using 10% SDS-PAGE which was then stained by Comassie blue as previously described by Lamelli (1970).

## RESULTS

**Biochemical studies**

The effect of $LC_{50}$ concentrations of Viruset, Protecto and Profect on enzyme activity of three carbohydrate digestive enzymes in *S. littoralis* 6th larval instar treated as 3rdlarval instar was estimated. In untreated larvae amylase activity was found to be 217µg glucose / min / g.b.w. Following treatment with $LC_{50}$ of Viruset and Protecto the activity of amylase was relatively un affected in treated larvae with $LC_{50}$ of Viruset and Protecto i.e.210 and 210.33 µg glucose / min / g.b.w., respectively. Meanwhile, when Profect were used at $LC_{50}$ values amylase activity was significantly decreased to198 µg glucose / min / g.b.w.In untreated larvae invertase activity was found to be 610µg glucose/min /g.b.w. Following treatment with $LC_{50}$ of Viruset, Protecto and Profect the activity of invertase was significantly decreased to 525.66, 561.66, 209.8 and552µg glucose/min /g.b.w., respectively .Meanwhile, trehalase activity was significantly decreased from 423.33 in untreated larvae to 403, 415.6 and 391.33 µg glucose/min /gm. in the treated larvae with Viruset, Protecto and Profect, respectively.

**Table 1. Effect of the bioinsecticides on digestive enzymes activity (amylase, invertase and trehalase) in 6thlarval instar of *S. littoralis*.**

| Tested Compounds | Mean(µg glucose / min / g.b.w) ±S.E | | |
|---|---|---|---|
| | Amylase | Invertase | Trehalase |
| Viruset | 210 ± 1.55 ab | 525.66 ±12.24 c | 403 ± 4.7 bc |
| Protecto | 210.33 ± 5.3 ab | 561.66 ± 4.49 b | 415.6 ± 4.3 ab |
| Profect | 198 ± 1.55 b | 552 ± 10.25 bc | 391.33 ± 4.17 c |
| Control | 217 ± 6.55 a | 610 ± 6.5 a | 423.33 ± 3 a |
| F values | 3.422ns | 16.36*** | 12.35** |
| L.S.D | 13.942 | 28.52 | 13.07 |

**b.w. = body weight.**

**SDS polyacrylamide gel electrophoresis**

One dimensional denaturing SDS-PAGE was used to detect change in protein patterns of whole body tissue homogenate of *S. littoralis* 4th instar larvae after treatments with protecto, profect, Virusetas compared to control (non-treated).SDS-PAGE of total soluble proteins showed 10, 8, 7 and 9 polypeptide bands of control, viruset, protecto and profect, respectively. The molecular weight of these bands ranged from15 to 240 KDa. In addition, these bands varied inintensity and molecular weight. There were two monomorphic (common) bands(50.6 and 46.1 KDa), 8 polymorphic bands (15 - 70 KDa), and 7 unique bands (4 bands in the control larvae,240,100,73and 68KDa and 3 bands in the profect treated larvae; 90,65 and 33KDa).

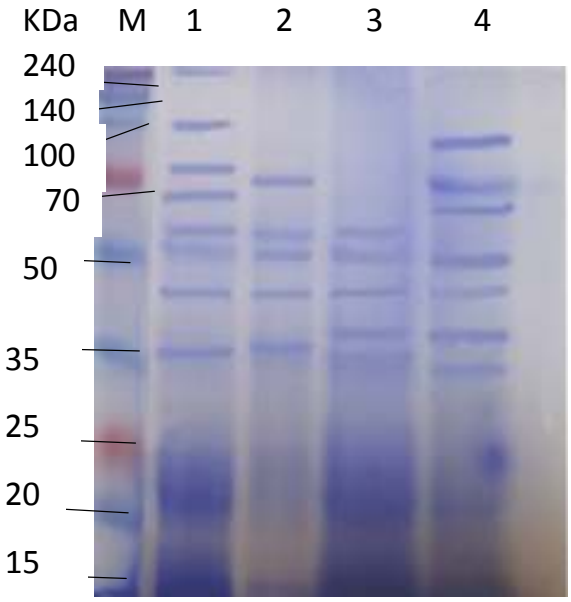


**Fig. 1. SDS-Polyacrylamide gel of protein pattern of 4thlarval instar of *S. littoralis*treated with bio insecticides.**

**M=Marker, Lane 1 = Control, Lane 2 = Viruset, Lane 3 = protecto,Lane 4 = Profect.**

## DISCUSSION

In the present study, a general disturbance in three carbohydrates enzymes was detected in *S. littoralis* 6th larval instar treated as 4thlarval instar with $LC_{50}$ of any of the three tested bio-agentsviruset, protecto and profect. This agrees with the results of El-Sheikh (2012)who showed that the tested compounds have a remarked effect on invertase activity. *B. thuringiensis* significantly decreased the invertase activity compared to the untreated larvae. In addition, El-Ghar*et al.* (1995) found that *B. thuringiensis,* caused a pronounced decrease in digestive enzyme activity especially amylase. Furthermore, Al Shanna f*et al.,* (2012) reported adecrease in invertase activity in *H. armigera* following all treatments. Assar, *et al.* (2016)also found a significant decrease in the activity of trehalase and amylase activity following application of bioinsecticides.

In consistant with our results,Abd El-Aziz (2012)reported down regulation for total protein of the worm by *B. thuringiensis*(MVPII, dipel-2X, and dipel Es). This down regulation may be attributed to the adverse effect of the pathogens (bacteria) themselves (Lotfy, 1988 and Abd El-Aziz, 2000), and/or their toxins(El-Bermawy and Abulyazid, 1998), or due to utilization of carbohydrates for energy production (Saleem *et al.* 1998). This down regulation in larval protein may be responsible for the reduction in the

activities of digestive enzymes (Kyung and Kim, 1990). Moreover, Bakr *et al.* (2013) found that during treatment of S. *littoralis* with flufenoxron and *Spli* NPV some proteins were missed or expressed at different stages which may be responsible for all obtained deformities indicating formation of new proteins responsible for stimulation of the immune system of the insect as result of entering foreign objects inside the body of the insects.The same findings were also obtained by Mokbel (2013) who revealed that neonicotinoids insecticides inhibited formation of some proteins and induced some others within the insect body. The synthesis of such new protein may be induced by microsomal enzymes in the insect liver (Radwan, 2001). New protein bands were also recognized in insect protein by other studies (Bakr*et al.* 2013; Mokbel2013). This may be attributed to the formation of immune proteins as a result of the presence of foreign molecules in the larval bodies (Dunn, 1986 and Dimarcq *et al.,* 1990).

The changes in the protein content of the haemolymph reflect the specialization and adaptation in the organisms to the tested compoud, this type of produced protein may be responsible for a specific biological character (Abdel Azi*et al*, 2013). Similarly,Hamouda (2002)reported that treatment of the third instar larvae of *S.littoralis* by admiral, *Spli*NPV alone and their mixture caused changes in protein content of the treated larvae. Lower concentration of protein might have resulted from DNA damage causing shut down of some essential genes responsible for production of this protein after treatment (El-Bermawy and Abulyazid, 1998).

## CONCLUSION

Disturbance in the enzyme activity and protein pattern caused by the three compounds may lead to synthesis of different compounds and disruption of many physiological functions of the insect.This effect helps the bioinsecticides to exhibit their potential as a new source of insecticidal materials due to their harmless to human and invironment.So we can conclude that pathogenic bacteria and virus can be used as an effective insecticide either in combination with each other or with other insecticides. Also pathogenic bacteria may be effective pathogen but with some modifications and activations.

## REFERENCES


Abd El-Aziz, H. S. (2000). Physiopathological studies on bacterial infection of cotton leaf worm *Spodoptera littoralis*. M. Sc. Thesis, Fac. Sci., Ain Shams Univ., Egypt.

Abd El-Aziz, H. S; Osman, H. H; Sayed, S. Z. and El-Gohary, E. E. (2013). Effect of certain plant oils on some biological and biochemical aspects on the cotton leaf worm *Spodoptera littoralis*. Egypt. Acad. J. Biolog. Sci., 6 (3): 69-80.

Abd El-Aziz, M. M. (2012). Evaluating efficacy of certain bioagents and insect growth regulators against the cotton leafworm, *Spodoptera littoralis* (Boisd.), using molecular technique. Ph. D. Thesis, Institute of Environ. Stud. Res., Ain Shams Univ., 166 Pp.

Al-Shannaf, H. M.; H. M. Mead, and A. K. Hassan Sabry (2012).Toxic and Biochemical Effects of Some Bioinsecticides and IGRs on American Bollworm, *Helicoverpaarmigera* (hüb.) (Noctuidae : Lepidoptera) in Cotton.

Amin, T. R. (1998). Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd.) .Ph.D. Thesis, Faculty of Science, Cairo Univ. Egypt. Fields. J Biofertil,Biopestici 3.118- 122.

Assar,A.A.; M.M.Abo El-Mahasen; H.F.Dahi; H.S. Amin(2016) Biochemical effects of some insect growth regulators and bioinsecticides against cotton leafworm, *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae). J. of Biosc.and Appl. Res. ,2, (8):587-594.

Bakr, R. A.; Hafez, J. A.; Khamiss, O. A. and Zyaan, O. H. (2013). Biochemical studies on the effect of Chitin synthesis inhibitor, (flufenoxron) and Spli-NPV on the cotton leafworm *Spodoptera littoralis* Bosid (Lepidoptera: Noctuidae). Adv. Cancer Res., 29: 175-274.

Crickmore N (2006).Beyond the spore—past and future developmentsof Bacillus thuringiensis as a biopesticide. J ApplMicrobiol101:616–619.

Dimarcq, J. L. ; Zachary, D. ; Hoffmann, J. A. ; Hoffmann, D. and Reichart, J. M. (1990): Insect immunity: Experession of the two major inducible antibacterial peptides, defensin and diptericin in *Ph- ormia terranovae*. EMBO J., 9: 2507-2515.

Dunn, P. E. (1986).Biochemical aspects of insect immunity. Annu. Rev. Entomol., 31:321-329.

El-Bermawy, S. M. and Abulyazid, I. (1998).Genomic DNA polymorphism and biochemical assay in the pupal stage of Medfly *Ceratitis capitata* (Wied). Proc. Inter. Cong. Molec. Gen., 1:165-180.

El-Ghar, G.E.; S.A.Radwan; H.S.A. El- Bermawy and L.T.M. Zidan (1995). Inhibitory effect of *B.thuringiensis* and abamectin on digestive enzymes and non-specific esterases of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae. J. App. Entomol., 119(5): 355-359.

El- Sheikh, T. A. A. (2012).Biological, biochemical and histological effects of spinosad, *Bacillus thuringiensis* var. *kurstaki* and cypermethrin on the Cotton leaf worm, *Spodoptera littoralis* (Boisd), Egypt.Acad. J. biolog. Sci.,4 (1) 113-124.

Fang J, Xiaoli X, Ping W, Zhou ZJ, Anthony SM, Jiaan C, Ming FG,Zhicheng S (2007) Characterization of chimeric Bacillus thuringiensisVip3 toxins. Appl Environ Microbiol 73:956–961

Groner A. (1986): Specificity and safety of baculoviruses. In *The biology ofbaculoviruses Volume 1*. Edited by: Granados RR, Federici BA. Boca Raton:CRC; 1986:177-202.

Hamouda, L. S. (2002). Toxicological and biochemical studies on the effect of admiral (IGR) and nuclear- polyhedrosis virus (SNPV) on *Spodoptera littoralis* (Boisd.) larvae. J. Egypt. Acad. Soc. Envron. Deve- lop., 2(1):15-29.

Ishaaya, I. and Swirski, E. (1970). Invertase and amylase activity in the armoured scales *Chrysomphalusaonidium* and *Aonidellaaurantii*. J. Insect Physiol., 16: 1599-1606

Ishaaya, I.; Moore, I. and Joseph, D. (1971). Protease and amylase activity in larvae of the Egyptian cotton leaf worm, *S. littoralis*. J. Insect Physiol., 17: 945-953.

Kyung, Y. H. and Kim, H. R. (1990). Characterization of M-haemolymph protein from *Hyphantria cunea* Dr-ury. Korean J. Entomol., 20 (4): 239 – 246.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685. Lowry OH, Rosenbought NJ, Ferr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 165–175.

Lotfy, M. N. (1988). Pathogenesis of *Bacillus spaericus* as an entomopathogenic bacterium on certain mosquito species in Egypt. Ph. D. Thesis, Fac. Sci., Ain Shams Univ., Egypt.

Mokbel, E. M. S. (2013). Further studies on insecticides resistance in the cowpea aphid. Ph. D. Thesis, Fac. Agri., Cairo Univ., Egypt, Pp180.

Radwan, E. M. M. and Taha H. S. (2012). Toxic and biochemical effects of different insecticides on the tomato leafminer, *Tutaabsoluta* (Meyrick) (Lepido- ptera: Gelechiidae). Egypt. Acad. J. Biol. Sci., 4: 1-10.

Saleem, M. A.; Shakoori, A. R. and Mantle, D. (1998). Macromolecular and enzymatic abnormalities induced by a synthetic pyrethroid, ripcord (Cypermethrin), in adult beetles of a stored grain pest, *Triboliumcastaneum* (Herbst.) (Coleoptera: Tenebrionidae) Mushtaq. Arch. Insect Biochem. Physiol., 39:144–154.

Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P (2011). Bacillus thuringiensis: a century of research, development andcommercial applications. Plant Biotechnol J 9:283–300

Wigglessworth, V. B. (1972): The Principles of Insect Physiology. *(7th Ed. Chapman and Hall Ltd. 827pp.)*

Wuatt, G. R. (1967): The biochemistry of sugars and polysaccharides ininsects. *(Adv. Insect Physiol., 4: 287-360).*

## تأثير بعض المركبات الحيوية على دودة ورق القطن الكبرى *Spodoptera littoralis*

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تم دراسة تأثير ثلاثة من المركبات الحيوية التجارية على نشاط ثلاثة من انزيمات الهضم الخاصة بالحشرة وكذلك تأثيرها على النمط البروتينى الخاص بها. اوضحت النتائج انخفاض معنوى فى نشاط انزيم الاميليزعند معاملة اليرقات بالمبيد الحيوى بروفيكت ، بينما لم يظهر تأثير واضح عند معاملتها بالفيروست او البروتكتو. من ناحية اخرى ادى معاملة اليرقات بالمركبات الثلاثة الى انخفاض واضح فى انزيم التريهاليز.كذلك تم دراسة تأثير المركبات الثلاثة على النمط البروتينى الخاص باليرقه وذلك باستخدم تقنية التفريد الكهربى لنمط البروتين الذى تم تكسيرة باستخدام SDS واتضح ان هناك اختلاف فى عدد الشرائط البروتينة وتركيزها، حيث اختفى بعضها وظهر البعض الاخر. وكان هذا الاختلاف واضحا عند استخدام المركبين بروفيكت وبروتكتو على التوالى.