Egypt. Acad. J. Biolog. Sci., 6(2): 29 -38 (2013) **A. Entomology**
Email: egyptianacademic@yahoo.com **ISSN: 1687–8809**
Received: 25 / 4 /2013 **www.eajbs.eg.net**

Biochemical studies on the effect of Chitin synthesis inhibitor, (flufenoxuron) and SpliNPV on the cotton leaf worm *Spodopteralittoralis* Bosid (Lepidoptera:Noctuidae)

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ABSTRACT

In this studyflufenoxuron as insect growth regualtor and SpliNPV were used to investigate their toxicity on *Spodopteralittoralis.* Both 2nd and 4th larval instars of *S. littoralis* were exposed to the different concentrations of flufenoxuron and SpliNPV. The biochemical effect of IGR and SpliNPV on both 2nd and 4th larvae instar show changes in protein electrophoretic pattern in the current study reveals differences between the untreated samples and treated one, some proteins were missed or expressed at different stages which may be responsible for all the obtained deformation. The effect of these compounds on biochemical activities were revealed after studying electrophoretic protein (fractionated protein) using PAGE in order to determine their effects on the vital system of *S. littoralis*, which may indicate the formation of new proteins responsible for stimulating the immune system of the insect as a result of entering foreign objects inside the body of the insect.

Keywords: *Spodopteralittoralis*, flufenoxuron, SpliNPV, biochemical, protein.

INTRODUCTION

The cotton leafworm, *Spodopteralittoralis* (Boisd.) is an extremely polyphagous insect causing damage to a wide variety of crops. The cotton leafworm, *Spodopteralittoralis* (Boisd.) is an extremely polyphagous insect causing damage to a wide variety of crops. Moussa*et al.* (1960) listed some 112 plant species belonging to 44 families as food plants of *Spodopteralittoralis* in tropical and temperate zones of the old world. Abdel - Hafez (1978) found that from 112 plant species, 45 used as food for *S. littoralis*, 16 hosts for ovipositional sites and 12 for both. In Egypt, the cotton area sprayed with insecticides against leafworm varies from 10 to 65 percent of the total area; in 1991 some 69000 ha (hectares) were treated (Moawad, 1997).

In recent years, many of the conventional methods of insect control by broad spectrum synthetic chemicals have come under scrutiny because of their undesirable effects on human health and the environment (Perry, *et al.*, 1998).Furthermore, resistance has been recorded for most conventional insecticides. As a consequence, it provides impetus to study new alternatives and more ecologically acceptable methods of insect control. The use of insect growth regulator (IGRs) offer considerable potential as agents for the suppression of many species of pest insects (Mena and Beroza 1972) and for the enhancement of the productivity of beneficial species (Murakoshi*et al.*, 1972). Most of the compounds which have been tested show low gross toxicity to non-target organisms (Schaefer and Wilder 1972). One of the

benefits of using benzoyl phenyl ureas (BPUs) is that they do not impact parasitoid as much as chemical pesticides (Wilkinson *et al*. 1978, Brown 1996 and Webb *et al.* 1998). Insect growth regulators have captured worldwide attention in the development of novel compounds capable of interfering with the process of growth, development and metamorphosis of the target insects (Ishaaya and Horowitz, 1997). The use of insect growth regulators (IGRs) against a variety of insect species including *Spodopteralittoralis* may replace some of the compounds now used for pest control which have unwanted ecological side effects or high toxicity of mammals (Abo El-Ghar*et. al.* 1994).

Chitin is an essential component in insect cuticle (Neville, 1975). Furthermore, chitin is important because it provides the cuticle with strength and a certain degree of flexibility. It protects insects against the entry of harmful substances and the exit of useful ones (notably water). The formation and decomposition of chitin are therefore carefully controlled at each stage of the life cycle (Hassall, 1990). In the absence of chitin, the cuticle becomes thin and brittle. It is also unable to support the insect or to withstand the rigors of molting compounds which interfere in any way with chitin biosynthesis. The damaged newly formed cuticle cannot withstand haemolymph pressure and muscular traction (Cohen, 1987). Benzoylphenylurea (BPU's) are insecticides acting on various insect orders by reducing chitin deposition into insect's cuticle, which in turn disrupts normal molting (Mülder&Gijswijt, 1973; Retnakaran*et al.*, 1985).

The moulting processes allow immature insects and other arthropods to shed the old cuticle, which is rigid and limits growth to replace it with a new one which is sufficient flexible to permit expansion and growth (Hajjar, 1985), and it was found that, the use of IGRs interfere with cuticle deposition, leading to abnormal endocuticular depositions as well as abortive moulting (Van Daalen*et al*., 1972; Post and Vincent, 1973).

Viruses from several taxonomic groups have been used in pest control programs. Insect viruses have been used and are in use in forestry, horticulture and agriculture; Entwhistle (1983) listed 31 lepidopterous, 6 hymenopterous and 1 coleopterous pest species for which baculoviruses control has been considered likely. The viruses that are used in pest control programmes are produced exclusively in whole insects reared in the laboratory in large scale production or purpose-built breeding facilities (Stockdal, 1984), various pesticide-NPV combinations have been evaluated for control of lepidopteran pests on cotton (Chapman and Ignoffo, 1972; Morris *et al*., 1974), generally with promising results. Although there have been some studies in the literature that have evaluated the impact of chemical insecticides on insect cell cultures (Mitsuhashi*et al.*, 1970; Stipanovic*et al.,* 1990; Yoshida *et al.*, 1979).

In the work presented here, we select flufenoxuron; as chitin synthesis inhibitors and SpliNPV which is considered as one of the most famous occluded virus as belonging to Baculovirus group since its discovery in 1956. The mix of each IGR was also used with the virus to evaluate their biochemical effect on the 2nd and 4th instar larvae of *Spodopteralittoralis*.

MATERIALS AND METHODS

1- Insect used

Insect used in the present study was the Egyptian cotton leaf worm *Spodopteralittoralis* (Boisd.) (Lepidoptera: Noctuidae). It was obtained from the Plant protection, Research institute, Agriculture, Research center (Giza), and was reared in

the laboratory under constant laboratory conditions of 27 ± 2°C and 70 ± 5 % RH to be used in our investigations.

2- Chemicals used

Flufenoxuron belongs to insect growth regulators (IGRs) group. It was delivered from the Plant protection, Research institute, Agriculture, Research center (Giza).

The virus used in this study was delivered from EntomovirologyCenter in the Faculty of Agriculture, CairoUniversity (Giza). Virus (nucleopolyhedrovirus) was naturally collected from recently dead larvae of *Spodopteralittoralis* (Boisd.). Multiplication of virus was applied by infecting 4th instar larvae through the surface contamination of the larval diet. The infected larvae were incubated at 25°C for 7 days before collecting cadavers. The dead larvae were stored at -20°C to obtain the virus from them. To obtain and purify virus particles (virions), polyhedra were dissolved in an alkaline solution of sodium thioglycolate buffer 0.25 Mol. 10.5 PH for 1.5-3 min. according to the concentration of polyhedra in the suspension. The dissolution of polyhedra was indicated by the clear aspect of the suspension which means the liberation of virus particles. This suspension was centrifuged at 5000 r.p.m for 5 minutes and the pellet was discarded. The supernatant which contained virion was placed on sucrose solution with a concentration graded from 20 to 60 % and centrifuged at 18000 rpm at 8°C for 45 min. using a rotor. The virions were sedimented as net bands on the correspondent sucrose concentrations graded from 30% to 45% approximately. The virion bands were delicately aspired with the help of a peristaltic pump and transferred to a new centrifuge tube with about15 ml distilled water and then centrifuged at 20000 r.p.m at 8°C for 45 min. The pellet should contain highly purified virions.

3- Samples preparation:

Untreated and treated larvae by flufenoxuron and virus from different stages were killed by freezing and stored at - 20°C until used. Larva of each group was homogenized in a small cold mortar containing 0.1 ml of distilled water. The contents were transferred to a new Eppendorf tube, and centrifuged at 10,000 r.p.m. for 10 min at room temp. The supernatant was transferred to a new Eppendorf tube and kept frozen at -20°C till further application. Protein was separated by PAGE according to the method of Laemmli (1970).

RESULTS

Separation of protein bands by electrophoresis

SDS-PAGE of protein pattern for both control and 3rd instar of *S. littoralis* larvae treated as 2nd instar with three different conc. of flufenoxuron alone or combined with SpliNPV were separated into 12 and 11 different bands, respectively.

Data demonstrated in figure (1) showed that, the total number of bands in control sample, and treated samples by $0.5X10^{-2}$, $0.5X10^{-5}$& $0.5X10^{-9}$ ppmflufenoxuron were 3, 5, 8 & 6, respectively. The molecular weight of bands in all samples ranged from 14 to 69 K Da. Two common bands 3 & 5 were appeared between the control sample (C) and the other treated samples by three different conc. of flufenoxuron.

The data given in figure (2) screened that, the total number of bands in control samples (C), virus $3.5X10^4$ PIB (V) and three treated samples by virus flufenoxuron combination ($0.5X10^{-2}/3.5X10^4$, $0.5X10^{-5}/3.5X10^4$& $0.5X10^{-9}/3.5X10^4$ ppm/PIB) were 3, 6, 5, 6 and 6 respectively.

The molecular weight of bands in all samples ranged from 16.97 to 68.7 K Da. Two common bands were appeared between the control sample and the other four treated samples by virus and virus flufenoxuron combination.

SDS-PAGE of protein pattern for both control and 4th larval instar of *S. littoralis* from treated 2nd instar with three different conc. of flufenoxuron alone and flufenoxuron combined with SpliNPV were separated into 14 and 16 different bands, respectively. From figure (2) it is obvious that, the total number of bands in control (C), $0.5X10^{-2}$ (1), $0.5X10^{-5}$ (2) & $0.5X10^{-9}$ (3) ppmflufenoxuron were 8, 6, 4 and 5 bands, respectively. They were ranged from 13.7 to 67.9 K Da. in molecular weight value.

The data presented in figure (2) showed that the total number of bands in control sample (C), virus $3.5X10^{4}$ PIB (V) and three treated samples by virus flufenoxuron combination ($0.5X10^{-2}/3.5X10^{4}$, $0.5X10^{-5}/3.5X10^{4}$& $0.5X10^{-9}/3.5X10^{4}$ ppm/PIB) were 8, 7, 5, 5 and 6 respectively.

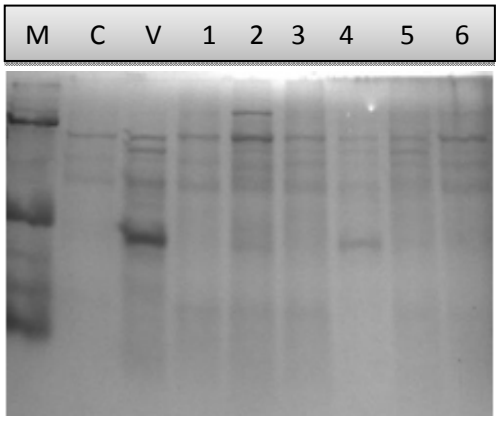

Fig. 1

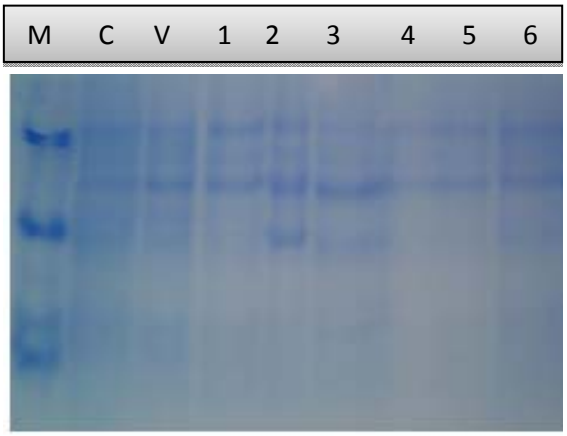

Fig. 2

SDS-PAGE of protein pattern for both control and 5th larval instar of *S. littoralis* from treated 2nd instar with three different conc. of flufenoxuron alone and flufenoxuron combined with SpliNPV were separated into 16 and 19 different bands, respectively.

The characteristic pattern of protein bands were illustrated in figure (3). Generally it was obvious that no common bands between the control sample (C) and the other three treated samples by flufenoxuron.

SDS-PAGE of protein pattern for both control and 6th larvae instar of *S. littoralis* treated at 2nd instar with three different conc. of flufenoxuron and flufenoxuron combined with SpliNPV were separated into 17 and 16 different bands, respectively.

The characteristic patterns of protein were illustrated in figure (4) the band No. 1, 2 & 5 were common bands between the control sample and other treated samples by flufenoxuron.

Data recorded in figure (4) represent fractionated protein produced from treated *S. littoralis* larvae by virus & virus flufenoxuron combination. The result showed that, bands No. 6, 8 & 10 with RF 0.21, 0.30 & 0.39 and MW 67, 61.6 & 56.4 K Da. were characteristic bands between the control sample and the other four treated samples by virus and virus flufenoxuron combination. Band No. 6 appear in sample (V) treated by virus alone with % amount twice than in control sample.

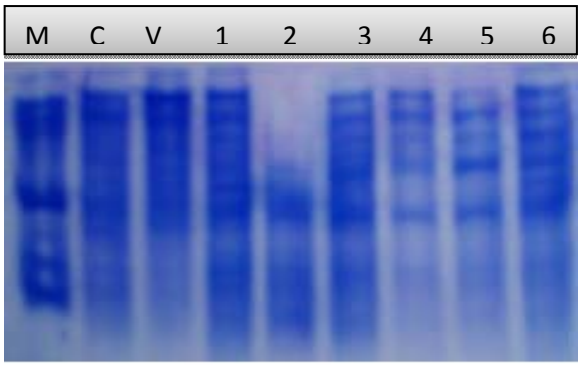

Fig. 3

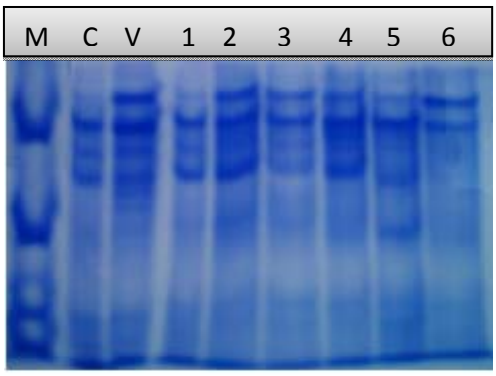

Fig. 4

SDS-PAGE of protein pattern for both control and 5th larval instar of *S. littoralis* from treated 4th instar with three different conc. of flufenoxuron and flufenoxuron combined with SpliNPV were separated into 16 and 17 different bands, respectively.

Data illustrated in figure (5) represent fractionated protein produced from treated *S. littoralis* larvae by virus & virus flufenoxuron combination. The result showed that, bands No. 16 & 17 with RF 0.77 & 0.84 and MW 13.2 & 8.7 K Da. were characteristic bands between the control sample and the other four treated samples by virus and virus flufenoxuron.

combination. But band No. 17 appear in the four treated samples treated by virus & virus flufenoxuron combination by amount twice than in control sample.

Data shown in figure (6) revealed that, bands No. 1, 2, 3 & 12 were common bands between the control sample and the other three treated samples by flufenoxuron.

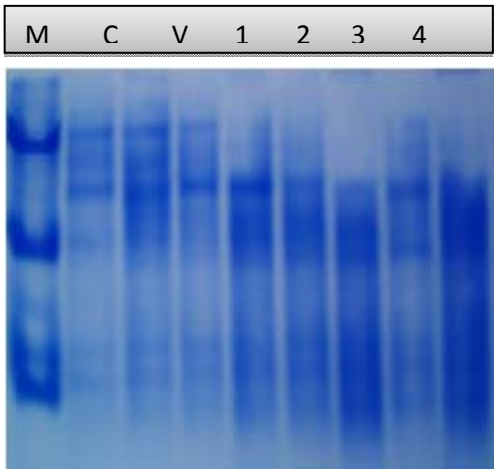

Fig. 5

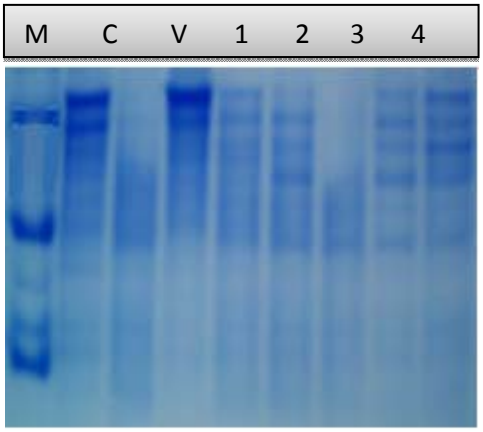

Fig. 6

SDS- Polyacrylamide gel of protein patterns of a control and 3rd larval instar resulted from treated 2nd instar with SpliNPV virus and insect growth regulator Flufenoxuron fig (1), SDS- Polyacrylamide gel of protein patterns of a control and 4th larval instar resulted from treated 2nd instar larvae with SpliNPV virus and insect growth regulator Flufenoxuron fig (2). Where: M = Marker (K Da.), C = Sample of control, V = Sample treated by virus, 1 = Sample treated by $0.5X10^{-2}$ ppm flufenoxuron, 2 = Sample treated by $0.5X10^{-5}$ ppm flufenoxuron, 3 = Sample treated by $0.5X10^{-9}$ ppm flufenoxuron, 4 = Sample treated by $0.5X10^{-2}$ ppm flufenoxuron combined with $3.5X10^{4}$ PIB virus, 5 = Sample treated by $0.5X10^{-5}$ ppm flufenoxuron combined with $3.5X10^{4}$ PIB virus, 6 = Sample treated by $0.5X10^{-9}$ ppm flufenoxuron combined with $3.5X10^{4}$ PIB virus.

SDS- Polyacrylamide gel of protein patterns of a control and 5th larval instar resulted from treated 2nd instar with SpliNPV virus and insect growth regulator Flufenoxuron fig (3) and SDS- Polyacrylamide gel of protein patterns of a control and 6th larval instar resulted from treated 2nd instar with SpliNPV virus and insect growth regulator Flufenoxuron fig (4). Where: M = Marker (K Da.), C = Sample of control, V = Sample treated by virus, 1 = Sample treated by 0.5X10-2 ppm flufenoxuron, 2 = Sample treated by 0.5X10-5 ppm flufenoxuron, 3 = Sample treated by 0.5X10-9 ppm flufenoxuron, 4 = Sample treated by 0.5X10-2 ppm flufenoxuron combined with 3.5X104 PIB virus, 5 = Sample treated by 0.5X10-5 ppm flufenoxuron combined with 3.5X104 PIB virus, 6 = Sample treated by 0.5X10-9 ppm flufenoxuron combined with 3.5X104 PIB virus.

SDS- Polyacrylamide gel of protein patterns of a control and 5th larval instar resulted from treated 4th instar with SpliNPV virus and insect growth regulator Flufenoxuron fig (5), and SDS- Polyacrylamide gel of protein patterns of as control and 6th larval instar resulted from treated 4th instar with SpliNPV virus and insect growth regulator Flufenoxuron fig (6). Where: M = Marker (K Da.), C = Sample of control, V = Sample treated by virus, 1 = Sample treated by 0.5X10-2 ppm flufenoxuron, 2 = Sample treated by 0.5X10-5 ppm flufenoxuron, 3 = Sample treated by 0.5X10-9 ppm flufenoxuron, 4 = Sample treated by 0.5X10-2 ppm flufenoxuron combined with 3.5X104 PIB virus, 5 = Sample treated by 0.5X10-5 ppm flufenoxuron combined with 3.5X104 PIB virus, 6 = Sample treated by 0.5X10-9 ppm flufenoxuron combined with 3.5X104 PIB virus.

DISCUSSION

It is worthy to mention that each protein is considered as reflect to the activity of specific gene through the production of the enzyme which act as catalyst to produce the demanded protein, this type of produced protein is responsible for a specific biological character.

The present study was carried out to clarify some biochemical effect of three concentration-levels of insect growth regulators and combination between flufenoxuron (which was more effective against *S. littoralis* than pyriproxyfen) and SpliNPV on the *S. littoralis* larvae. The data of biochemical test discussed as follow:

In the present work the general proteins of the whole body tissue of 3rd, 4th, 5th& 6th instar of *S. littoralis* larvae treated by flufenoxuron at 2nd instar were separated into 12, 14, 16 & 17 bands respectively. When the same instar treated by flufenoxuron combined with SpliNPV the protein bands were 11, 16, 19 & 16 bands at 3rd, 4th, 5th& 6th instar respectively.

The 5th& 6th instar of *S. littoralis* larvae treated by flufenoxuron at 4th instar whole body protein were separated into 16 & 14 bands respectively.

In case of 5th& 6th instar of *S. littoralis* larvae treated by flufenoxuron combined with SpliNPV, 17 & 15 bands respectively, were separated. Among those bands some were common between control and all treated samples while others were characteristic to certain concentration of treatment.

In comparing the bands appeared after treatment of 2nd instar with control, it was found that, 2 bands (No. 3 & 5) share between control and larvae treated by flufenoxuron or combined with virus, the two common bands appear about two times as percentage amount in the control sample than in the treated samples. The similar results obtained by Hamouda (2002) who studied the treatment of the third instar larvae of *Spodopteralittoralis* by admiral alone or SNPV alone or a mixture of them caused some changes in the protein content in the treated larvae, and also, said that the decrease in the concentration of the protein in the treated larvae may reflect the inhibition of DNA synthesis and the decrease in the activity of various enzymes is related to insect growth regulators mechanism. El-Bermawy and Abulyazid 1998

stated that, the lower concentration of protein may be resulted from DNA damaged causing shut off of some essential gene responsible for production of this protein after treatment.

The 4th instar samples resulted from treated 2nd instar larvae by flufenoxuron, two common bands share between them and control sample. In comparing the % amount in the common bands between the control sample (C) and the other treated samples, it was obvious that band No. 1 in treated samples had % amount about two times of the control sample (C), 6th instar resulted from 4th instar treated larvae by flufenoxuron combined with virus had a common band No. 14 with % amount in treated samples about twice than in control, which reveal that, the higher concentration of protein may has an antagonistic effect on the population.

In general new protein bands were appeared in treated samples by the used IGRs, which may be attributed to the formation of immune protein as a result of the presence of foreign molecules (IGRs) in the larval bodies (Dunn, 1986 &Dimarcq*et al*., 1990).

From these investigations, six protein bands which present in control sample were disappeared from samples of the 4th larval instar resulted from treated as 2nd instar by flufenoxuron. Also five bands were disappeared in samples treated by flufenoxuron combined with virus. These findings are in agreement withBakr*et al.* (2005) who studied the effect of pyriproxyfen on fractionation protein pattern of *Spodopteralittoralis*and found that new and specific protein were observed in all treated samples by chlorfluazuron and pyriproxyfen, which may be responsible for all the obtained deformation. Amer and Ghoneim, (1996) who studied the effect of pyriproxyfen (IGRs) on fractionation protein pattern of *Shistocercagregaria*, they found that six protein bands of control were disappeared in treated samples. While Hussein *et al*.(1993) studied the biochemical effects of pyriproxyfen on the pink bollworm larvae, they found that, treatment with juvenoide resulted in a considerable reduction in the protein bands which reflect inactivation of the genes responsible for production of mRNA synthesize these proteins.

The protein difference may act as taxonomic tool to identify the similarity index and genetic differences between control and other treated samples as concluded by Reddy *et al.* (1993); Krishnayya and Rao (1995).

The increase in the concentration of protein due to virus treatment of *Spodopteralittoralis*larvae may explain in terms of virus replications as follows: the first and most dramatic changes caused by the NPV are the turning off the host cell protein synthesis early in the infection cycle of the virus. The second is the temporal control of viral protein synthesis evident in the appearance and disappearance of specific viral protein during this period. Both transcriptional modifications of two virus proteins were also detected during this period. Also, a highly glycosylated intracellular protein is deglycosylated giving rise to another protein. The late stage is characterized by maturations of the virus (Ignoffo*et al*., 1971).

In this study, the protein pattern monitors number of bands as a difference between the control and the treated samples. This difference is translated as genetic distance, which recorded a great value ranged from 0.35 to 0.85 between the control sample and the samples treated by flufenoxuron as 2nd instar, and reach value 1 between the control sample and the samples 4th instar treated by flufenoxuron combined with virus as 2nd instar This great genetic distance may refers to a new taxonomic position for the treated samples apart genetically from control.

El-Bermawy (2005) treated 2nd larval instar of *S. littoralis*with different concentrations of cascade (flufenoxuron) and match and found that new and specific

proteins were obtained in all examined tissue and they were probably responsible for all the obtained deformation. The untreated larval tissue recorded 9 types of protein while the cascade treated tissue had 10 types of protein meanwhile the match treated tissue had 9 types of protein. Different characteristic types of protein were recorded for each treatment.

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ARABIC SUMMERY

دراسات كيموحيوية على تأثير مثبط تخليق الكيتين (فلوفينوكسورون) و فيروس (ان. بي. في.) على دودة ورق القطن *سبودوبترا ليتتوراليس*

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تمت دراسة تأثير سمية كلا منالفلوفينوكسورون من منظما تنمو الحشرات وفيروس ال ن.بي.في. على ورق القطن. وكلا من العمر اليرقي الثاني والعمر اليرقي الرابع مندودة ورق القطن قد تعرضوا لتركيزات مختلفة من الفلوفينوكسورونوفيروس ال ان. بي. في. والتأثير الكيموحيوي لمنظم النمو الحشري والفيروس علي كلا من العمر اليرقي الثاني والعمر اليرقي الرابع أظهر تغيرات في البروتين المجزأ الكترونيا وأوضح تغييرات بين العينات المرجعية والعينات المعالجة. بعض البروتينات اختفت اوظهرت في أعمار مختلفة ومن الممكن أن يكون مسئولا عن حدوث تشوه الحشرة. تأثير هذة المركبات علي الأنشطة الكيموحيوية اتضح بعد دراسة البروتين المجزأ الكترونيا بإستخدام الفصل الكهربي للبروتين من أجل تعيين التأثيرات المختلفه علي الجهاز الحيوي لدودة ورق القطن.

وبعد تحليل البروتين المفصول كهربياً منال عينات المعالجة ومقارنته بالبروتين المفصول كهربياً من العينات المرجعية سجل ظهور حزمبروتينية جديدة وذلك فى العينات المعالجة مما قد يشير الى تكوين بروتينات جديدة مسئولة عن تحفيزالجهازالمناعي للحشرة نتيجة دخولا جسام غريبة داخل جسم الحشرة.