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EFFECT OF FLUFENOXURON AND ABAMECTIN ON SOME BIOCHEMICAL CONTENTS IN THE AMERICAN BOLLWORM HELICOVERPA ARMIGERA (HUBNER)

A. Abd-El Halim[1]; M. A. Asfoor[1] and Khaled A. M. H. Elkhawas[2]
1-Plant Protection Research Institute, Agric. Res. Center, Dokki-Giza, Egypt
2-Faculty of Agriculture, Alazhar University, Cairo, Egypt.

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ABSTRACT: ***This laboratory study was conducted to evaluate the effect of two compounds , i.e Abamectin (Vertemic) as bio–compound agent and Flufenoxuron (Cascade) as chitin inhibitor on the total protein , Acetyl choline esterase (A.ch.E.) and transamin group enzymes, group of transamin protein enzymes (G.O.T) and glycol protein transamin (G.P.T) enzymes activity .***

Obtained results revealed that significant reduction in protein level in treatment as compared with chich larvael 19.550, 38.64, 44.12 and 39.45 to Flufenoxuron after 48,72,96 and 120 hrs , respectively . The results also refer to the highest reduction in protein quantity (-93.45%) after 120 hrs from treatment with Vertemic, where as after 96 hrs from treatment with Flufenoxyron this reduction was (-37.15%) .

The results indicated that significant relation between effect of two compounds on protein quantity of treatment larvae as compared with chich larvae to all periods after treatment . Also, the results show that percent reduction in (A.C.E) -22.14, 13.43, 17.86 and – 18.80 after 48,72,96 and 120hrs, respectively .

On the other hand the results clarified that there was fluctuation to G.O.T's activity after different periods treatment from treatments with Vertmic . It's activity was increase with the rate of + 47.96 , + 18.64 after treatment by 48and 72 hrs , respectively and decrease to -24.89 and 32.18 % after 96 and 120 hrs , respectively .

Generally , the obtained data show that significant decrease on G.P.T 's activity after treatment by 48 and 72 hrs , the decreasing due to treatment with Vertmic was – 48 and – 78% in contrast of -53.15 and - 43.06 for Flufenoxuron after 48 and 72 hrs , respectively . Also , the results revealed that increasing in enzyme activity after 96 and 120 hrs after treatment .

Key words: Flufenoxuron, Abamectin and Helicoverpa armigera.

INTRODUCTION

The Cotton Bollworm *Helicoverpa armigera* (Hubner) has been recognized as serious insect pest of cotton and other crops in Egypt. Recently, the juvenoids such as chitin biosynthesis disruptor belonging to

phenylbenzoylurea group have been considered as promising alternatives to conventional insecticides for combating *Spodoptra littoralis* (Radwan et al., 1985). Also, the bio-insecticides have emerged as feasible alternatives to conventional chemical insecticides. Such insecticides are avermectins that may inhibit growth-regulation activity (Wright, 1984). These chemicals affect the nervous system of arthropods by increasing chloride-ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis (Macconnell et al., 1989; Jansson and Dybas, 1998). It was previously noted that abamectin (Vertimec) caused great physiological changes in vital systems during the insect development (Deecher et al., 1989). It affected total protein content and interfered with the activity of the enzymes having an important role in the insect metabolism (Abdel-Hafez et al., 1988; Abou-Bakr, 1997; Abo-El-Ghar, 1994; Agee, 1985b; Gadallah et al., 1990; and Mamdouh et al., 1999).

MATERIALS AND METHODS

Treatments and preparing samples :

The 4th instar larvae of field strain of *H. amigera* were fed on castor-oil bean plant leaves previously treated with the LC_{50} values of Abamectin and flufenoxuron. Exposure and feeding on treated leaves was 2 days after which larvae were fed for additional three days on untreated leaves and haemolymph samples were collected after 48, 72 , 96 and 120 hrs intervals. Haemolymph was obtained by removing one of the prolegs by forceps and applying gentle pressure on the larva with the fingers. The haemolymph was collected in cold tubes previously coated with crystals of phenylthiourea to prevent melanization. The sample was centrifuged at 2500 rpm for 10 min. at low temperature (4°C) to remove the blood cells. After centrifugation, the haemolymph was divided into small portions (0.5 ml) and stored at -20°C until analysis.

Biochemical studies :

Determination of protein: The total protein was determined in the haemolymph samples, according to the method of Lowery et al. (1951). This method is principally based on using crystallized bovine serum (sigma) as the reference protein.

Determination of acetylcholinesterase activity : The method of Hestrin (1949) modified by Simpson et al. (1964) was used to determine AChE activity in the haemolymph of the 4th instar larvae of *Helicoverpa armigera* (Hubner) previously treated with LC_{50} value of abamectin and flufenoxuron and untreated (control). The larvae were homogenized in 0.1 M sucrose, the homogenate was left for half an hour and then centrifuged at 1500 rpm for 10 min. at low temperature (4°C), the supernatant was made up to 9 ml with sucrose and stored at 20°C until required. The reaction mixture contained 0.2

ml enzyme solution and 0.5 ml of 6 x 10^{-3} M acetylcholine bromide (ACh.Br) was incubated at 37°C for 30 min. At the end of the incubation period, 1 ml alkaline hydroxylamine (prepared by mixing 1 part of 3.5 M NaOH with 1 part of 2 M hydrochloride was added to each tube and shaken vigorously for 2 min. One-half ml of HCl (prepared by mixing 1 part of concentrated HCl with 2 parts of distilled water) was added and shaken, then one-half ml of 0.094 M ferric chloride was added and shaken for 1 min. The resulting mixture was centrifuged at 2500 rpm for 3 min. and the supernatant was measured spectrophotometrically at 515 nm.

The activity of AChE was expressed as mg of ACh.Br hydrolyzed per mg protein per 30 min.

Determination of transaminase activities :

a- Determination of glutamic oxaloacetic transaminase activity (GOT) :

Determination of GOT activity was carried out according to Reitman and Frankel (1957), using kits purchased from Bio-Meriux, France. The method of Reitman and Frankel (1957) depends upon the fact that plasma oxaloacetic transaminase accelerates the simultaneous transformation of alpha ketoglutaric acid to glutamic acid and aspartic acid to oxaloacetic acid as shown by the formula :

Aspartic + ketoglutarate $\xrightarrow{GOT}$ oxaloacetic acid + glutamic acid.

Measured by using spectrophotometer at a wavelength of 505 nm.

Calculation :

The number of GOT units/ml of sample was calculated using the standard curve for aspartate as the substrate for GOT. The curve shows a relationship between number of GOT units/ml and optical density (OD).

b- Determination of glutamic pyruvic transaminase activity (GPT) :

The activity of GPT enzyme in the plasma was measured by using the method of Reitman and Frankel (1957), which depends upon the fact that plasma glutamic pyruvate transaminase accelerates the transformation of alpha ketoglutaric acid and alanine to pyruvic acid and glutamic acid as follows :

Alanine + ketoglutarate $\xrightarrow{GPT}$ Pyruvic acid + glutamic acid.

Measured by using spectrophotometer at a wavelength of 505 nm.

Calculation :

The number of GPT units/ml of sample was calculated using the standard curve for aspartate as the substrate for ketoglutaric acid.

Statistical analysis :

The means and standard deviations were calculated for each experiment and the data were compared (using the ANOVA test) according to Snedecor (1971).

RESULTS AND DISCUSSION

The effect of the tested compound on the total protein :

Spectrophotometric analysis of proteins are of valuable use in ascertaining its purity, in clarifying the genetic interrelationships among proteins, in observing changes in its contents and enzyme activities in the developing organism. Insect haemolymph, as the only extracellular fluid, might be a good indicator of metabolic changes using spectrophotometric technique. Feeding the 4th instar larvae of *Helicoverpa armigera* (Hubner) for 2 days on castor oil plant leaves previously treated with the LC_{50} of abamectin and flufenoxuron caused, in general, an obvious significant decrease in the level of protein as shown in Table (1). The reduction percent in protein content than the check at intervals of 48, 72, 96 and 120 hrs were -19.55, -38.64, -44.12 and 39.45 % for abamectin versus -27.79, -38.64, -37.15 and-41.1 % for flufenoxuron, respectively. The results indicated that the highest percentages of reduction in protein level were (-39.45 % at 96 hrs) for abamectin, and -37.15 % for flufenoxuron at 96 hrs. On the other hand, the results showed that there were significant differences between the effects of the two tested compounds and check at all time intervals and also between abamectin and flufenoxuron at 48 hr and 72 hr, while the effectiveness between the two tested compounds was insignificant at 96 hr and 120 hr time intervals. It is clear that both compounds suppressed protein synthesis gradually at time intervals and reached its maximum effect after 96 hrs. Generally, it was obvious that flufenoxuron was more active than abamectin in reducing total protein content in the treated larvae. In agreement with those of Ahmed and Mostafa (1989). They found that treatment of the larval instar of cotton leafworm with two benzoylphenylurea; namely triflumuron and chlorfluazuron reduced remarkably the total protein. Besides, glutamic acid was decreased in the larvae treated with cholrfluazuron. Bakr *et al.* (1991) indicated that the total protein of treated larvae and pupae of *Musca domestica* treated with diflubenzuron and BAY-SIR was lower than the normal one.

Table (1) Change in protein contents of the 4th instar larvae ofHelicoverpa armigera following feeding for 48hrs on leaves treated with the LC50 values of abamectin and flufenoxuron.

Kind of treatment	µg protein / µl haemolymph at indicated intervals post-treatment							
	48-hrs	% Change	72-hrs	% Change	96-hrs	% Change	120-hrs	% Change
Flufenoxuron	25.15± 2.6 b	-27.79	219.60± 2.3 b	-38.64	30.18± 1.8 a	-42.17	33.09± 1.4 a	-41.10
Abamectin	30.12± 1.7 a	-219.550	219.56± 1.1 a	-38.64	29.16± 1.4 a	-44.12	31.20± 1.2 a	-39.45
Check (control)	41.32± 3.5 d		44.17± 3.6 d		52.19± 2.8 d		56.18± 3.6 e	

Effect of the tested compounds on the activities of some enzymes :

a- Acetylcholinesterase (AChE) :

Data in Table (2) showed acetylcholinesterase activity in the haemolymph of the 4th instar larvae of *Helicoverpa armigera* (Hubner) at different time intervals when the larvae were fed on castor bean oil plant leaves treated with LC_{50} of both abamectin and flufenoxuron. The usual activity of AChE in normal larvae tended to increase gradually by the progress in larval development and growth. The results also indicated that AChE activity was significantly reduced at all time intervals compared with untreated check for the two tested compounds. The reduction percent varied according to the type of toxicant used and time post treatment. The percentage of reduction at 48 hr, 72 hr, 96 hr and 120 hr time intervals were -22.14, -13.43, -17.68 and -18.80 % for abamectin and -27.89, -38.70, -35.27 and -27.79 % for flufenoxuron at the four mentioned intervals, respectively. The percentage of reduction reached its maximum level at 48 hr time interval for both tested toxicants, then less reduction was achieved at 72-, 96- and 120-hrs times intervals. Abdel-Hafez *et al.* (1993) who found that diflubenzuron caused a remarkably high reduction in activity of AChE in *Helicoverpa armigera* (Hubner) larvae.

Table (2) Activities of acetylcholinesterase in haemolymph of the 4th instar larvae ofHelicoverpa armigera following feeding for 48hrs on leaves treated with the LC50 values of abamectin and flufenoxuron.

Kind of treatment	Activities after different intervals of treatment (hrs) x 10-2 µm/min./mg protein							
	48-hrs	% Change	72-hrs	% Change	96-hrs	% Change	120-hrs	% Change
Flufenoxuron	41.12± 2.3 c	-327.89	43.12± 2.1 c	-38.70	48.12± 1.7ab	-35.27	55.13± 1.4 a	-27.79
Abamectin	53.19± 1.2 a	-22.14	59.41± 1.9ab	-13.43	61.32± 1.9 b	-17.68	61.99± 1.8 b	-18.80
Check (control)	68.32± 3.6 d		70.35± 3.9 d		74.49± 3.8 d		76.35± 3.7 d	

b- Amino acid transverases :

Glutamic oxaloacetic transaminase (GOT) :

Data in Table (3) showed the effects of the tested compounds, abamectin and flufenoxuron on the activity of glutamic oxaloacetic transaminase (GOT)

of the 4th instar larvae of Helicoverpa armigera (Hubner). The results indicated the occurrence of considerable gradual increase in GOT activity by the progression of time in normal larvae (check) where it reached its maximum activity at 120 hrs time interval. The data revealed also that there was a significant increase in GOT activities for flufenoxuron at 48, 72, 96 and 120 hrs time intervals by +93.93, -82.73, +58.92 and +25.66 % of the check. It was clear that the percentage of increase in the enzyme activity was generally negatively correlated with time increase. On the other hand, abamectin treatment caused increase in GOT activity at 48 hr and 72 hr time intervals reached +47.97 % and +18.94 % of check followed by drop in the enzyme activity (-24.89 % of check) at 96-hrs, then a highly pronounced reduction in GOT activity reached -32.18 % of check, occurred at 120 hrs time interval. The enzyme activity significantly increased for both toxicants compared with the untreated check at 48, 96 and 120-hrs post-treatment.

Table (3) Change in GOT activities in haemolymph of the 4th instar larvae ofHelicoverpa armigera following feeding for 48hrs on leaves treated with the LC50 values of abamectin and flufenoxuron

Kind of treatment	GOT activities ± S.E. mm pyretic/min./mg protein x 10-3							
	48-hrs	% Change	72-hrs	% Change	96-hrs	% Change	120-hrs	% Change
Flufenoxuron	20.13± 1.3 a	+93.93	28.16± 1.6 b	+82.73	3319.56± 1.5c	+58.92	44.17± 1.8 d	+25.66
Abamectin	15.36± 1.5 a	+47.97	18.33± 2.1 a	+18.94	16.62± 1.6 c	-24.89	19.11± 1.9 d	-432.18
Check (control)	10.38± 2.3 e		15.41± 2.5 a		22.13± 1.3 b		35.15± 2.3 e	

Glutamic pyruvic transaminase (GPT) :

The data presented in Table (4), showed different trend in the larvae treated with the two tested compounds. At the first two time intervals; 48-hrs and 72-hrs, the GPT activity was significantly reduced compared with untreated check. The percentages of reduction in the enzyme activity were 48.78 and 47.65 % for abamectin and -53.15 and -43.06 % for flufenoxuron at the two time intervals, respectively. At 96-hrs and 120-hrs time intervals, the enzyme activity increased significantly compared to the check. The increase in enzyme activity was +6.25 and +37.31 % for abamectin and +28.08 and +51.61 % for flufenoxuron relative to check at the two time intervals, respectively. It is evident GPT enzyme activity occurred at 48-hrs time intervals with the two toxicants followed by slight decrease in enzyme inhibition at 72-hrs. However, with time elapse to 96- hrs and 120 hrs post-

treatment an obvious recovery of the enzyme activity, occurred to reach 6.25 % and 28.08 % as normal for abamectin and flufenoxuron at 96-hr time interval, and reached the higher increase in the enzyme activation after 120-hrs post-treatment to be 37.71 % for abamectin and 51.61 % for flufenoxuron.

Although the activity of GPT enzyme decreased gradually by the elapse of time in normal larvae due to the larval growth and development, another trend was observed in the enzyme activity post-treatment with both toxicants. The results recorded herein were in agreement with Abdel-Hafez et al. (1988) in the 4th instar larvae of *S. littoralis* treated with diflubenzuron and triflumuron. Also, the same findings were reported by Mostafa (1993), Ishaaya and Swirski (1976) on the same insect.

Table (4) Change in GPT activities in haemolymph of the 4th instar larvae ofHelicoverpa armigera following feeding for 48 hrs on leaves treated with the LC50 values of abamectin and flufenoxuron

Kind of treatment	GOT activities ± S.E. mm pyretic/min./mg protein x 10-2							
	48-hrs	% Change	72-hrs	% Change	96-hrs	% Change	120-hrs	% Change
Flufenoxuron	59.16± 2.2 a	-53.15	31.961± 3.3 b	-43.06	42.41± 3.3 b	+28.08	44.18± 3.1 b	+51.61
Abamectin	419.56± 2.3 a	-48.78	34.12± 3.2 b	-47.65	35.18± 3.2 b	+6.25	40.13± 3.2 b	+37.71
Check(control)	92.11± 4.6 f		65.18± 2.6 a		33.11± 1.7 a		29.14± 1.2 a	

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تأثير كل من مركب الفلوفينوكسيرون ومركب الأبامكتين على بعض المحتويات البيوكيميائية لدودة اللوز الأمريكية

أحمد عبد الحليم عبد العال[١] – محمد عبد المنعم عصفور[١] – خالد أحمد الخواص[٢]

١- معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقى -الجيزة، مصر.

٢- كلية الزراعة – جامعة الأزهر – القاهرة – مصر.

الملخص العربى

أجريت هذه التجارب معملياً على العمر الرابع لدودة اللوز الامريكيه وذلك لدراسة تأثير مركب الابامكتين (فيرتيميك) كمركب حيوي ، ومركب الفلوفينوكسيرون (كاسكيد) كمانع انسلاخ على :-

كمية البروتين وتخليقه ، إنزيم الاسيتيل كولين استيريز و الإنزيمات الناقلة لمجموعة الأمين (G.O.P.) هذا بالأضافه إلى دراسة تأثير هذين المركبين على نشاط الإنزيم GPT.

أظهرت النتائج انخفاضا معنويا في مستوى البروتين بعد المعاملة وذلك يتوقف على نوع المركب المستخدم في المعاملة حيث بلغت نسبة الانخفاض في كمية البروتين مقارنة باليرقات الغير معاملة 19.550و 38.64- و 44.12- و % 39.45- لمركب الأبامكتين (فيرتيميك) (٢٧.٧٩-)و 38.64 و 37.15- و (% 41.1- لمركب فلوفينوكسيرون وذلك بعد 48، 72، 96، 120ساعة من المعاملة .كما تشير النتائج أن أقصى خفض فى كمية البروتين 39.45-) (% نتج بعد 120ساعة من المعاملة بمركب أبامكتين (الفيرتيميك) (، بينما بعد 96 ساعة من المعاملة بمركب الفلوفينوكسيرون. (% 37.15-) كما أظهرت النتائج وجود علاقة معنوية بين تأثير المركبين على كمية وتخليق البروتين لليرقات المعاملة مقارنة بالغير معاملة لكل الفترات المختبرة بعد المعاملة.

كما تشير النتائج أيضا أن نسبة الخفض في نشاط إنزيم الأسيتيل كولين استيريز - 22.14و 13.43- و 17.68- و18.80- بعد نفس الفترات 48، 72، 96، 120ساعة بعد المعاملة بمركب الأبامكتين (الفيرتيميك) ، في حين كانت نسبة الخفض لمركب الفلوفينوكسيرون - 27.89و 38.70-و 35.27- و (27.79- بعد نفس الفترات السابقة على الترتيب.

أما بالنسبة للإنزيمات الناقلة لمجموعة الأمين فقد لوحظ من النتائج تذبذب وعدم انتظام نشاط إنزيم GOT بعد الفترات المختلفة من المعاملة بمركب أبامكتين فقد زاد نشاطه بنسبة 47.97+، % 18.94+1 بعد 48، 72 ساعة من المعاملة ثم انخفض النشاط بنسبة - 24.89 و % 32.18- بعد 96، 120ساعة. أما مركب الفلوفينوكسيرون فقد سجل زيادة واضحة الارتفاع (% 93.93+) بعد 48ساعة تقل هذه الزيادة بمرور الوقت لتصل إلى % 25.66+ بعد 120ساعة من المعاملة.

أظهرت النتائج انخفاضا واضحا ومحسوسا على نشاط الإنزيم GPT بعد 48 و 72 ساعة من المعاملة، حيث بلغ الانخفاض % 48.78-، % 47.65- لمركب أبامكتين (فيرتيميك) بينما سجل انخفاض في النشاط مقداره % 53.15-، % 43.06- بعد 48، 72 ساعة من المعاملة بمركب فلوفينوكسيرون . هذا وقد سجل كلا المركبين زيادة في نشاط الإنزيم بعد فترة 96، 120ساعة من المعاملة.