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BIOCHEMICAL ASPECTS OF FLUFENOXURON AND ABAMECTIN ON THE 4*th* INSTAR LARVAE OF COTTON LEAFWORM *Spodoptera littoralis* (BOISD.)

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ABSTRACT

Biochemical results revealed that both flufenoxuron and abamectin caused, in general, reduction in total protein content of the treated larvae. The reduction percent than check in protein persisted for 120 hrs without signs of recovery. The results indicated that the highest percentage of reduction in protein level was -44.46 % at 120 hrs for abamectin, and -42.17 % for flufenoxuron at 96 hrs, while the lowest percentages were -27.1 % and -39.13 % at 48 hrs for the two tested compounds, respectively. The results showed that there was significant differences between the effects of the two tested compounds and the check at all time intervals. Both compounds resulted in remarkable percentages of reduction in acetylcholinesterase activity which was more pronounced for flufenoxuron than for abamectin. However, the maximum reduction occurred was -39.81 % at 48 hrs time interval for flufenoxuron, while the minimum was 15.55 % and occurred at 72 hrs for abamectin. The results revealed the occurrence of gradual increase in GOT activity by the progression of time in normal larvae. On the other hand, flufenoxuron exhibited similar trend of increase in GOT activity but of higher magnitude. In contrast, abamectin exhibited reduction in GOT, reached its maximum (45.63 %) at 120 hrs after treatment. The results showed significant reduction in GPT activity following treatment by abamectin and flufenoxuron after 48 and 72 hrs of post-treatment. Whereas at 96 and 120 hrs time intervals, the enzyme activity significantly increased relative to check at the two time intervals.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) has long been recognized as the most 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 *S. littoralis* (Radwan *et al.*, 1985). Also, the bio-insecticides have emerged as feasible alternatives to conventional chemical insecticides. Such insecticides are avermectins that may exhibit growth-regulation activity (Wright, 1984). It affects 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) causes 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 of significant role in 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 were fed on castor-oil 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 hrs, 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 *S. littoralis* 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 6×10^{-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 :

GOT

Aspartic + α-ketoglutarate ⟶ 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 as follows :

GPT

Alanine + α-ketoglutarate ⟶ 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 4*th* instar larvae of cotton leafworm, *S. littoralis* 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 hrs, 72 hrs, 96 hrs and 120 hrs were -27.1, -38.64, -44.12 and 44.46 % for abamectin versus -39.13, -38.64, -42.17 and-41.1 % for flufenoxuron, respectively. The results indicated that the highest percentages of reduction in protein level was achieved during 120 hrs (-44.46 % of check) for abamectin, and at 96 hr (-42.17 % of check) for flufenoxuron.

Table (1): Change in protein contents of the 4*th* instar larvae of *S. littoralis* following feeding for 48 hours on leaves treated with LC_{50} values of abamectin and flufenoxuron.

Kind of	µg protein / µl haemolymph at indicated intervals post-treatment							
treatment	48-hrs	% Change	72-hrs	% Change	96-hrs	% Change	120-hrs	% Change
Flufenoxuron	25.15±2.6 b	-39.13	27.10±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	-27.10	27.15±1.1 a	-38.64	29.16±1.4 a	-44.12	31.20±1.2 a	-44.46
Check(control)	41.32±3.5 d		44.17±3.6 d		52.19±2.8 d		56.18±3.6 e	

Table (2). Activities of acetylcholinesterase in haemolymph of the 4*th* instar larvae of *S. littoralis* following feeding for 48 hours on leaves treated with LC_{50} values of abamectin and flufenoxuron.

Kind of	Activities after different intervals of treatment (hrs) x 10^{-2} µm/min./mg protein							
treatment	48-hrs	% Change	72-hrs	% Change	96-hrs	% Change	120-hrs	% Change
Flufenoxuron	41.12±2.3 c	-39.81	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	-15.55	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	

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 abamectin suppressed protein synthesis gradually at time intervals and reached its maximum effect after 120 hrs. Also, it was obvious that flufenoxuron was more active than abamectin especially at the three first time intervals and reached its maximum reduction percent at 96 hr then slight recovery occurred lately at 120-hr. In agreement, Ahmed and Mostafa (1989) found that treatment of the larval instar of cotton leafworm with two benzoylphenylurea (triflumuron and chlorfluazuron) reduced remarkably the total protein. Besides, glutamic acid in chlorfluazuron treated larvae were also highly decreased. Likewise, 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.

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 4*th* instar larvae of *S. littoralis* at different time intervals when the larvae were fed on castor 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, -15.55, -17.68 and -18.80 % for abamectin and -39.81, -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 time intervals. Abdel-Hafez *et al.* (1993) who found that diflubenzuron caused a remarkably high reduction in activity of AChE in *S. littoralis* larvae.

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 4*th* instar larvae of *S. littoralis*. 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 hr, 72 hr, 96 hr and 120 hr time intervals by +93.93, -82.73, +58.92 and +25.66 % of the check.

Table (3). Change in GOT activities in haemolymph of the 4*th* instar larvae of *S. littoralis* following feeding for 48 hours on leaves treated with LC_{50} values of abamectin and flufenoxuron.

Kind of	GOT activities ± S.E. mm pyretic/min./mg protein x 10^{-3}							
treatment	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	35.17±1.5 c	+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	-45.63
Check(control)	10.38±2.3 e		15.41±2.5 a		22.13±1.3 b		35.15±2.3 e	

Table (4). Change in GPT activities in haemolymph of the 4*th* instar larvae of *S. littoralis* following feeding for 48 hours on leaves treated with LC_{50} values of abamectin and flufenoxuron.

Kind of	GOT activities ± S.E. mm pyretic/min./mg protein x 10^{-2}							
treatment	48-hrs	% Change	72-hrs	% Change	96-hrs	% Change	120-hrs	% Change
Flufenoxuron	59.16±2.2 a	-53.15	37.11±3.3 b	-43.06	42.41±3.3 b	+28.08	44.18±3.1 b	+51.61
Abamectin	47.17±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	

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 -45.63 % 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.

Glutamic pyruvic transaminase (GPT) :

The data presented in Table (4), concerning GPT activity, 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 were (-48.78 and 47.65 % for the check) and (-53.15 and -43.06 % for the check) for abamectin and flufenoxuron at the two pre-mentioned 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 clearly evident that the highest reduction of 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 1200hrs 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 maximum of enzyme activation after 120-hrs post-treatment with 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 4*th* 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.

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دراسات بيوكيميائية على مركب الفلوفينوكسيرون ومركب الأبامكتين على العمر الرابع لدودة ورق القطن

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أظهرت النتائج إنخفاضا معنويا في مستوى البروتين بعد المعاملة وذلك يتوقف على نوع المركب المستخدم فى المعاملة حيث بلغت نسبة الإنخفاض فى كمية البروتين مقارنة بالبرقات الغير معاملة (-٢٧,١٠ و -٣٨,٦٤ و -٤٤,١٢ و -٤٤,٤٦ %) لمركب الأبامكتين (فيرتيميك) و (-٣٩,١٣ و ٣٨,٦٤ و -٤٢,١٧ و -٤١,١ %) لمركب فلوفينوكسيرون وذلك بعد ٤٨، ٧٢، ٩٦، ١٢٠ ساعة من المعاملة. كما تشير النتائج أن أقصى خفض فى كمية البروتين (-٤٤,٤٦ %) نتج بعد ١٢٠ ساعة من المعاملة بمركب أبامكتين (الفيرتيميك)، بينما بعد ٩٦ ساعة من المعاملة بمركب الفلوفينوكسيرون (-٤٢,١٧ %). كما أظهرت النتائج وجود علاقة معنوية بين تأثير المركبين على كمية وتخليق البروتين للبرقات المعاملة مقارنة بالغير معاملة لكل الفترات المختبرة بعد المعاملة.

كما تشير النتائج أيضا أن نسبة الخفض فى نشاط إنزيم الأسيتيل كولين استيريز (-٢٢,١٤ و -١٥,٥٥ و -١٧,٦٨ و -١٨,٨٠) بعد نفس الفترات ٤٨، ٧٢، ٩٦، ١٢٠ ساعة بعد المعاملة بمركب الأبامكتين (الفيرتيميك)، فى حين كانت نسبة الخفض لمركب الفلوفينوكسيرون (-٣٩,٨١ و -٣٨,٧٠ و -٣٥,٢٧ و -٢٧,٧٩) بعد نفس الفترات السابقة على الترتيب.

أما بالنسبة للإنزيمات الناقلة لمجموعة الأمين فقد لوحظ من النتائج تذبذب وعدم إنتظام نشاط إنزيم GOT بعد الفترات المختلفة من المعاملة بمركب أبامكتين فقد زاد نشاطه بنسبة +٤٧,٩٧، +١٨,٩٤ % بعد ٤٨، ٧٢ ساعة من المعاملة ثم إنخفض النشاط بنسبة -٢٤,٨٩ و -٤٥,٦٣ % بعد ٩٦، ١٢٠ ساعة. أما مركب الفلوفينوكسيرون فقد سجل زيادة واضحة الارتفاع (+٩٣,٩٣ %) بعد ٤٨ ساعة تقل هذه الزيادة بمرور الوقت لتصل إلى +٢٥,٦٦ % بعد ١٢٠ ساعة من المعاملة.

أظهرت النتائج إنخفاضا واضحا ومحسوسا على نشاط الإنزيم GPT بعد ٤٨ و ٧٢ ساعة من المعاملة، حيث بلغ الإنخفاض -٤٨,٧٨ %، -٤٧,٦٥ % لمركب أبامكتين (فيرتيميك) بينما سجل إنخفاض فى النشاط مقداره -٥٣,١٥ %، -٤٣,٠٦ % بعد ٤٨، ٧٢ ساعة من المعاملة بمركب فلوفينوكسيرون. هذا وقد سجل كلا المركبين زيادة فى نشاط الإنزيم بعد فترة ٩٦، ١٢٠ ساعة من المعاملة.