# Triflumuron Effects as Insect Growth Inhibitor (IGI) on Amino-Acids Contents on Adults Reproductive Tracts of *Spodoptera littoralis* (Boisd.)

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**ABSTRACT:** The present study was designed to evaluate the physiological-insecticidal activity of triflumuron as IGI on amino acids content in the testes and ovaries of both sexes reproductive tracts of *Spodoptera littoralis*. The lethal dose 50% of those individuals subjected to triflumuron ($LD_{50}$=0.006 µl/larva) was topically applied on newly molted fifth instar larvae of the cotton leafworm *S. littoralis*. The treatment decreased the amounts of amino acids content of testes of adult males (2 days-old) obtained from treated larvae. This content was highly noticed and were ranged between 0.00 to 9.65% in 9 amino acids from 16 total amino acids examined i.e. DL-2-Amino-N-butyric Acid, Tryptophan(TRP), Isoleucine(ILE), DL-Threonine(THR), L-Arginine(ARG), L- Leucine(LEU), L- Alanine(ALA), DL-Serine(SER) and L- Lysine(LYS), while these amino acids amount percentages were ranged between 3.45 to 16.94%. The present results showed strong effects of $LD_{50}$ of triflumuron on the major amino acids that may stimulate and activate certain physiological functions in spermatogenesis in the testes of male reproductive tract i.e. SER and LYS. These latter amino acids in control males (2days-old) were 5.51 and 3.45%, respectively, but they were zero% in treated males. Both amino acids SER and LYS may play many important roles in the structure of both types of spermatozoa of the cotton leafworm. Moreover, the greatest decrease in amino acid content was observed especially in case of LYS (zero %) with triflumuron ($LD_{50}$) , while it was 19.92% in control female. LYS may play a specific role in the structure of egg of the cotton leafworm. The greatest increase of amino acid content was noticed in case of ASPARTIC ACID (ASP) (17.40%) with triflumuron ($LD_{50}$), while it was zero% in control female. The average weight of ovaries and testicles were recorded (32.21, 1.08 mg) with treated female and male by triflumuron in respect, while it was 78.65, 3.74 mg in control female and male, respectively. The results indicated that the biochemical composition of both sexes reproductive tracts were reduced. Triflumuron caused reproductive suppression, also, it affects the amino acids amount and it had its function on adults reproductive tracts, ultimately leading to reduce sperm transfer from the treated male insect to female.

**KEY WORDS:** Triflumuron, *Spodoptera littoralis*, Amino acids, reproductive tract and physiological functions.

## INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a polyphagous caterpillar damaging plants of economic importance in Southern Europe, Africa and the Middle East (Abo-El-Ghar *et al.,* 1986). Ecdysteroid hormones are important not only in initiating the development of the adult reproductive system, but they are also involved in controlling reproductive physiology in the adult insect; ecdysteroids regulate many developmental and physiological processes in insects (El-Sabrout, 2013; Friedländer and Reynolds, 1988; Gäde *et al*., 1997 and Seth *et al.,* 2004). In many insects, oviposition requires the development of the ovary, egg

maturation, mating, and in some cases feeding of the female on special meals (i.e blood). Ovarian development, which includes oocytes growth and vitellogenesis, is known to be under hormonal control (Engelmann, 1979). Also, the endogenous *H. armigera* proteins present in the male reproductive tract are responsible for stimulating oviposition and suppressing female receptivity. Males may have exploited females' endocrine system by hijacking the very molecules (e.g. JH, ecdysteriods, neuropeptides) involved in regulating key reproductive processes in order to manipulate females' reproductive physiology (Wedell, 2005). However, ecdysteroids are considered as potential specific target sites for pest control (Dinan, 1989). Reproductive inhibition induced by Benzoyl Phenyl Ureas (BPUs) has been reported most widely when applied on adults or eggs of insects (Wright and Spates, 1976).

The present study on triflumuron (Baycidal® 25%) (2-chloro-N-[[4 (trifluoromethoxy)phenyl]carbamoyl]benzamide) examined its effects as a chitin-synthesis inhibitor (i.e. chlorfluazuron) on reproductive potential of *S. littoralis* due to the similarity chemical structure and its mode of action when applied on *S.litura* by Perveen and Miyata (2000).The insect growth inhibitors (IGIs) induces an incomplete molt in several insect orders, while IGRs mimic the physiological activity in the normal insect molting hormone 20-hydroxyecdysone (20E) by binding to the ecdysteroid receptor complex (Wing, 1988). Although this non-steroidal ecdysteroid agonist was developed with an aim of disturbing the larval development, substantial effects were noted on Lepidoptera reproduction (Sun *et al*., 2000 and Khebbeb *et al*., 2008).

The present study was designed to evaluate the insecticidal activity of triflumuron applied topically on the newly fifth larval instars of *S. littoralis*. Sobeiha *et al.* (2000) reported that IGIs might have their own effects on the amino acids contents in adults (male and female) reproductive tracts. Triflumuron as one of IGIs was applied on the newly fifth larval instars of cotton leafworm. Moreover, the effects of triflumuron on amino acids contents of the two sexes were investigated.

## MATERIALS AND METHODS

### 1. Insect rearing

A susceptible strain of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) was reared under the laboratory conditions of 25 ± 2 ºC and 70 ± 5% R.H. on castor oil leaves, *Ricinus communis* L., (Family: Euphorbiaceae) according to El-Zoghby (1980) and El-Sabrout (2013). Egg-masses were confined in sterilized jars and tapped with muslin covers. Upon hatching, fresh and clean castor oil leaves were provided as food. Jars were daily cleaned out where fresh leaves were substituted for the used ones. Upon pupation, pupae were sexed prior to moth emergence. Adult moths were supplied with 10 % sugar solution in which a cotton wick was immersed for feeding. In addition, two leaves of *Nerium oleander* were provided as oviposition sites. Deposited egg-masses were daily collected and the hatched larvae were reared again for another generation.

**2. Triflumuron as Insect Growth Inhibitor (IGI)**

The tested chemical compound is triflumuron, dispersable concentrate WP 25% (w/v) and acts as (chitin synthesis inhibitor). The chemical name is 2-chloro-N-[[4-(trifluoromethoxy) phenyl]carbonyl]benzamide (IUPAC Name), with Molecular Formula: $C_{15}H_{10}ClF_3N_2O_3$. The commercial name is Baycidal® 25%, this product were magnification and provided by Bayer Company.

**3. Bioassay**

The product was applied topically. Preliminary tests were carried out to determine the lethal dose 50% ($LD_{50}$ =0.006 µl/larva) for this compound. $LD_{50}$ =0.006 µl/larva was then applied on the dorsal segment of mesothorax of the newly molted fifth instar larvae of cotton leafworm at a rate of 1 µl/larva. In triflumuron tests, the lethal dose 50% was prepared in water and 180 larvae were used for $LD_{50}$ =0.006 µl/larva and control. Control was set up using the water.

$LD_{50}$ treatment and control were tested, 18 replicates were carried out; in each replicate 10 larvae were released in a plastic dish (10 cm in diameter). The treated larvae were allowed to feed on untreated castor bean leaves, which changed every 24 hrs.

**4. Weight of gonads**

The lethal dose 50% ($LD_{50}$ =0.006 µl/larva) of triflumuron was topically applied on the newly fifth intsar larvae of *S. littoralis*. By dissection the testicles and ovaries of treated and untreated adult (2days-old) were removed and then they were weighted freshly.

**5. Analysis of Amino Acids by HPLC**

**Tissue preparation**

tissue was homogenized in 1:10 (w/v) phosphate buffer of pH=7, using Polytron Kinemetica homogenizer. The homogenate was centrifuged at 5000 rpm for 30 min at 4ºC using IEC-CRU 5000 cooling centrifuge. Supernatant was used as the source for the determination of free amino acids.

**Amino acids Standards**

L-Aspartic acid; L-Glutamic acid; L-Asparagine; L-Glutamine; L-Glycine; L-Tyrosine; γ-aminobutyric acid (GABA); L-Histidine; L-Tryptophan; L-Valine; L-Phenylalanine and L-Isoleucine all as standard amino acids were dissolved in deionised water (2.5 µ mole/ml), where all these standard acids were obtained from Loba Chemie Pvt. Ltd.

**Deproteinization of samples**

The whole supernatant contains soluble peptides and proteins that should be removed from the sample. Otherwise, these substances will clog the chromatographic column, increase instrumental backpressure and interfere with separation (Deyl *et al*., 1986). Methanol was used to deprotein (1:1) supernatant of sample (v/v) and

centrifuged at 10,000 rpm (HERMLE Labor Technik Z 306 ) for 5 min and the supernatant was collected to analyse the samples using HPLC and left for 30 min at room temperature to achieve derivatization well.

**The derivatization reagents included**

water and methanol of HPLC grade. Other chemicals used were analytical grade including sodium acetate, glacial acetic acid, boric acid, sodium hydroxide, *o*-Phthaldialdehyde and 3-mercaptopropionic acid (all were supplied by Sigma Chemical Company, St. Louis, MO, USA).

**OPA derivatization reagent**

The OPA (O-Phthaldialdehyde Solution) derivatization reagent was prepared by dissolving 3 mg of OPA in 50 µl of methanol, adding 450 ml of sodium borate buffer (0.5 mol/l, pH 10.2) and 5 µl of 3-MPA. (Borate buffer was prepared from 0.5 M boric acid solution adjusted to pH of 10.2 with 5 M sodium hydroxide solution). This OPA solution was placed in an amber crimp top vial with a silicone rubber PTFE-coated cap and kept in the dark at –20°C. Fresh solution was prepared each week.

**HPLC separation and evaluation of amino acids standards**

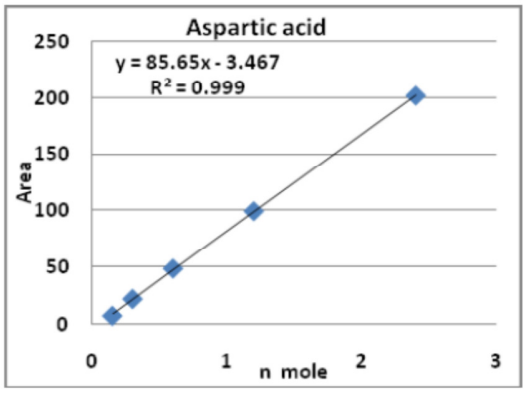
L-Asp; L-Glu; L-Asn; L-Gln; L-Gly; L-Tyr; GABA; L-His; L-Val; L-Try; L-Phe; L-Ile; was mixed (50 µl of each) well, except His was add by 10 folds; The standard curve for each amino acid was plotted.

**Mobile phase solution**

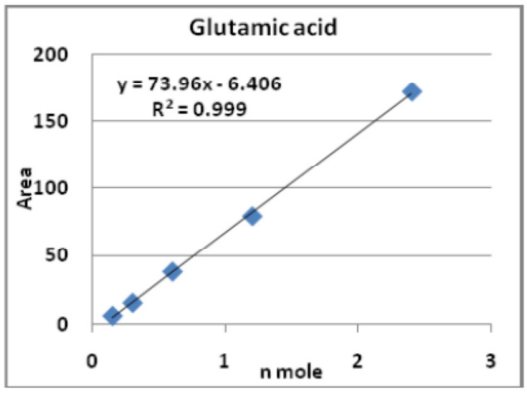
The sodium acetate buffer (0.015 M) in mobile phase was prepared by dissolving sodium acetate in HPLC-grade water and titrating to pH (6.8 for solvent A) with glacial acetic acid and methanol (solvent B). The mobile phases were filtered by passing through a 0.45-µm Durapore membrane filter (Millipore Inc., Milford, MA). Agilent Hewlett-Packard 1200 series HPLC system with solvent degasser system, quaternary pump, and autosampler fitted with a diode array and fluorescence detector was used. The system was controlled by a Hewlett-Packard Vectra Xm series 4 data analysis work station. A 250 mm. × 4.6 mm. I.D. stainless steel Zorbax SB C18 column was used. The mobile phase for isocratic elution was pumped at 1 ml/min, at 40$^{o}$C; detection was done at excitation 230 and emission of 450 nm. Standard curves for GABA and Glutamic acid were carried out.

**Standard curves of amino acids**

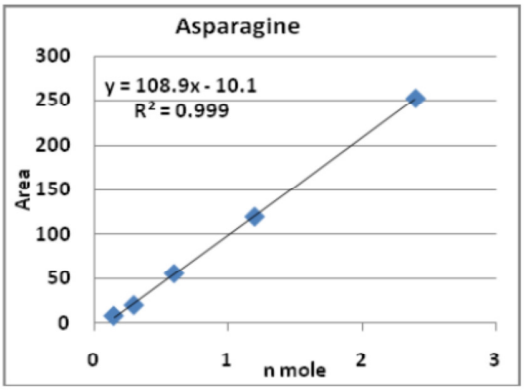
The standard curves for the amino acids were plotted as shown in figures (1&2) according to procedure Limit of Detection (LOD), for each standard Limit of Quantitation (LOQ) and the corresponding optical density were calculated and considered**.**



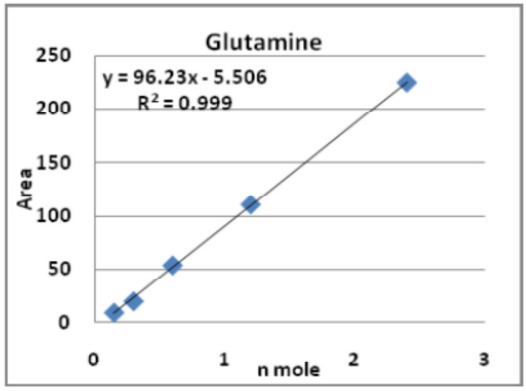

LOD: 0.041 n*M* LOQ: 0.123 n*M*



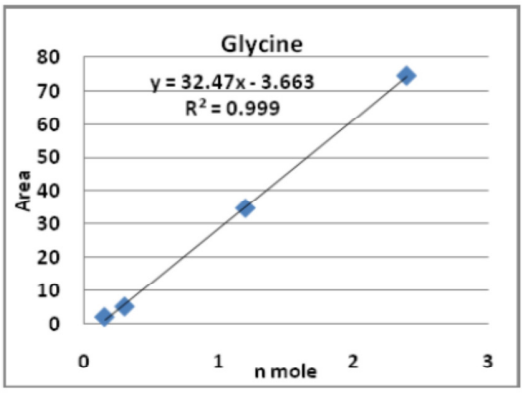

LOD: 0.085 n*M* LOQ: 0.257 n*M*



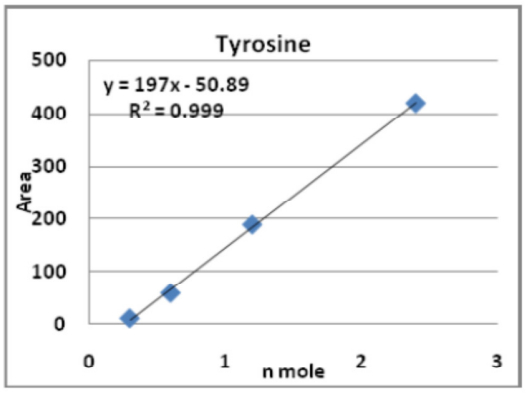

LOD: 0.058 n*M* LOQ: 0.175 n*M*




LOD: 0.080 n*M* LOQ: 0.241 n*M*

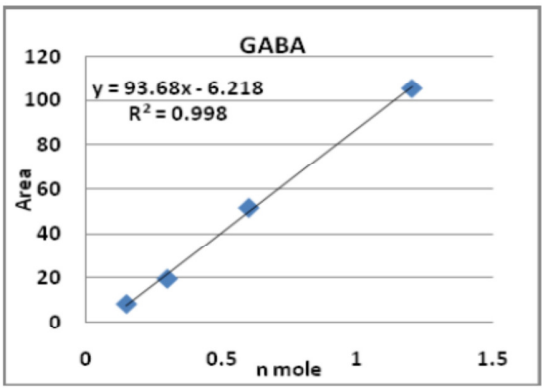



LOD: 0.096 n*M* LOQ: 0.292 n*M*

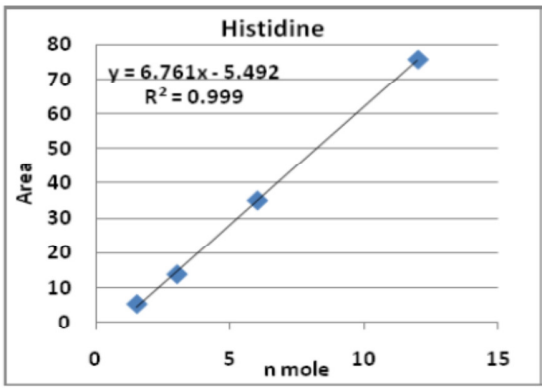



LOD: 0.111n*M* LOQ: 0.337 n*M*

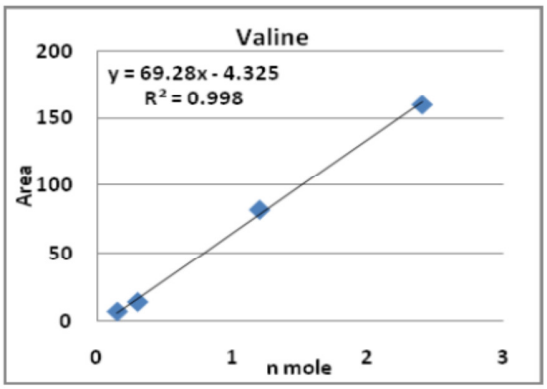
**Figure (1). Standard curves of amino acids**



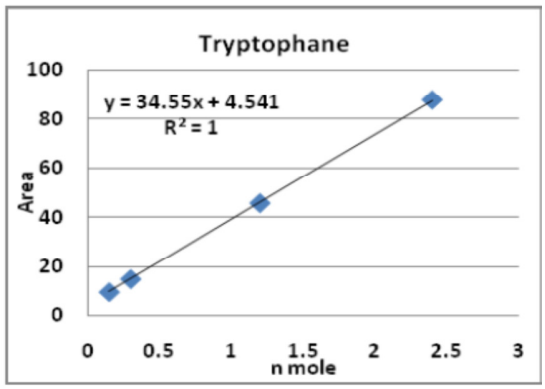

LOD: 0.073n*M* LOQ: 0.220 n*M*



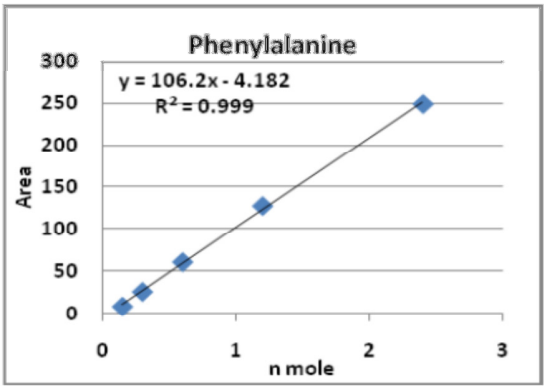

LOD: 0.402 n*M* LOQ: 1.218 n*M*



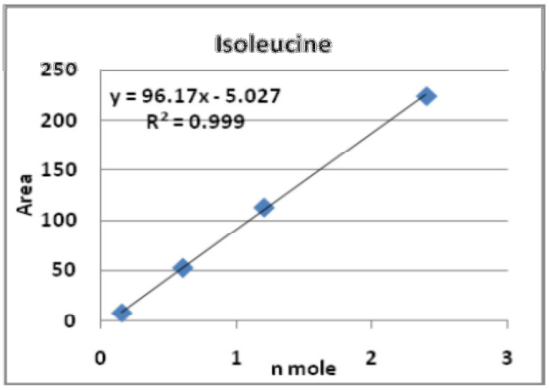

LOD: 0.156 n*M* LOQ: 0.474 n*M*




LOD: 0.025n*M* LOQ: 0.075 n*M*




LOD: 0.105 n*M* LOQ: 0.317 n*M*




LOD: 0.079 n*M* LOQ: 0.240 n*M*

**Fig. (1 cont.). standard curves of amino acids**

## RESULTS AND DISCUSSION

Certain physiological effects of triflumuron as IGI on amino acids content in the testes and ovaries of both sexes reproductive tracts of *Spodoptera littoralis* were investigated by topical application method with the median lethal dose ($LD_{50}$ =0.006 µl/larva).

**1- Amino acids content in testes of *S. littoralis* male**

The tested lethal dose 50% ($LD_{50}$ =0.006 µl/larva) of triflumuron was found to affect the contents of amino acids in both sexes (males and females) reproductive tracts, when it was applied on the thorax of newly fifth instar larvae of *S. littoralis*. The present results of amino acids content (µM/ml) in the control and treated males were calculated as percentages. The percentages of 16.94& 9.65% were recorded for DL-2-amino-N-butyric acid, 16.31&6.67% for TRP, 12.51& zero% for ILE, 10.94&7.27% for THR, 7.08& zero% for ARG, 5.57&3.17% for LEU, 5.51&3.85% for ALA, 5.51& zero% for SER, 3.45& zero% for LYS, 4.65&10.97% for GLN, 4.14 &9.38% for GLY, 3.85&16.08% for GLU, 3.54&13.65% for TYR, zero&6.20% for PHE, zero&3.58% for ASN and zero & 9.53% for the amino acid ASP (Table 1).

**Table (1). Comparison between control and treated males according to amino acids content (%).**

| Amino-Acids | Amino acids content (%) | |
|---|---|---|
| | Control male | Treated male |
| DL-2-Amino-N-Butyric Acid | 16.94 | 9.65 |
| Tryptophan (TRP) | 16.31 | 6.67 |
| Isoleucine (ILE) | 12.51 | 0.00 |
| DL-Threonine (THR) | 10.94 | 7.27 |
| L- Arginine (ARG) | 7.08 | 0.00 |
| L- Leucine (LEU) | 5.57 | 3.17 |
| L- Alanine (ALA) | 5.51 | 3.85 |
| DL- Serine (SER) | 5.51 | 0.00 |
| L- Lysine (LYS) | 3.45 | 0.00 |
| Glutamine (GLN) | 4.65 | 10.97 |
| Glycine (GLY) | 4.14 | 9.38 |
| Glutamic Acid (GLU) | 3.85 | 16.08 |
| Tyrosine (TYR) | 3.54 | 13.65 |
| Phenyl Alanine (PHE) | 0.00 | 6.20 |
| Asparagine (ASN) | 0.00 | 3.58 |
| Aspartic Acid (ASP) | 0.00 | 9.53 |
| **Total** | 100% | 100% |

The lethal dose 50% of triflumuron decreased the content of amino acid percentages detected in testes of the treated males (2 days-old). This content was highly decreased and was ranged between 0 to 9.65% in 9 amino acids from the total of 16 amino acids examined.

DL-2-amino-N-butyric acid, TRP, ILE, THR, ARG, LEU, ALA, SER and LYS, while these nine amino acids percentages were ranged between 3.45 to 16.94% in the control males. The amino acids percentages (content) of testes obtained from treated males increased and they were ranged between 3.58 to 16.08% for the other 7 amino acids (GLN, GLY, GLU, TYR, PHE, ASN and ASP), while they were ranged between 0 to 4.65% in control. However, the results showed the strong effects of the application of $LD_{50}$ (0.006 µl/larva) of triflumuron on the major amino acids which may play many physiological functions in spermatogenesis in the testes of male reproductive tract the amino acids.

SER and LYS in control males (2days-old) were recorded as 5.51 and 3.45%, respectively, but the same two amino acids in treated males were recorded as zero%. These two important amino acids (SER and LYS) may play many important roles in the structure of both types of spermatozoa of the cotton leafworm. Friedländer (1997) found the lepidoptera males bear concomitantly nucleate (eupyrene) and anucleate (apyrene) spermatozoa. Both kinds of spermatozoa derive from the same kind of bipotential spermatocytes. The shift of spermatocyte involvement from eupyrene to apyrene spermatogenesis is stimulated by a haemolymph factor that becomes active just before or after pupation, depending on species. Accordingly, eupyrene spermatogenesis begins during larval instars and stops after pupation, while apyrene spermatogenesis starts just before or after pupation, depending on species and persists in the imago. The shift is related to shorting of meiotic prophases and blocking synthesis of a meiotic lysine-rich protein fraction in apyrene cells.

The preparatory processes of spermatogenesis for nuclear elongation extend for four days and the elongation cannot be induced prematurely during this period by solely lowering the juvenile hormone titer experimentally. This period of preparation is expressed, subsequently, both in the morphogenetic events, during reshaping and elongation of the nucleus, and in the concomitant nuclear transition from lysine-rich to arginine-rich nucleoproteins. This is indicated by the distinctive and characteristic dynamic pattern of the cytoplasmic lysine-rich proteins displayed by the head cyst cell in the eupyrene line of spermatogenesis but not in the corresponding apyrene line of spermatogenesis.

Male of the silk worm *B. mori* has an endopeptidase, called initiatorin, in secretions of the posterior segment of the ejaculatory duct that is important in activation of both apyrene and eupyrene sperm and in maturation of the eupyrene. Initiatorin is a serine endoprotease that is active at pH 9.2. It digests the surface coat of apyrene sperm most easily, and these sperm become motile before the eupyrene

sperm are completely freed from their bundles (Nation, 2001). The present results of *Spodoptera littoralis* are in accordance with these of Nation (2001).

**2- Amino acids content in ovaries of *S. littoralis* female**

In Table (2), the amino acids content in the control and treated female resulted from treated larva with $LD_{50}$ of triflumuron were recorded as 19.92 & zero% for LYS , 12.55&9.94% for DL-2-amino-N-butyric acid, 10.20&8.84% for ILE, 9.45&7.40% for PHE, 3.82&2.90% for ARG, 6.40 &5.64% for GLY, 5.50&3.58% for LEU, 3.15&2.44% for ALA, 3.10&2.02% for GLU, 5.27&9.82% for THR, 6.97&7.84% for GLN, 3.55&4.72% for TRP, 10.12&17.46% for TYR, zero&17.40% for ASP and zero&zero% for ASP and SER.

**Table (2). Comparison between control and treated females according to amino acids (%).**

| Amino-Acids | Amino acids content (%) | |
|---|---|---|
| | Control female | Treated female |
| L- Lysine (LYS) | 19.92 | 0.00 |
| DL-2-Amino-N-Butyric Acid | 12.55 | 9.94 |
| Isoleucine (ILE) | 10.20 | 8.84 |
| Phenyl Alanine(PHE) | 9.45 | 7.40 |
| L- Arginine (ARG) | 3.82 | 2.90 |
| Glycine (GLY) | 6.40 | 5.64 |
| L- Leucine (LEU) | 5.50 | 3.58 |
| L- Alanine (ALA) | 3.15 | 2.44 |
| Glutamic Acid (GLU) | 3.10 | 2.02 |
| DL- Threonine (THR) | 5.27 | 9.82 |
| Glutamine(GLN) | 6.97 | 7.84 |
| Tryptophan(TRP) | 3.55 | 4.72 |
| Tyrosine (TYR) | 10.12 | 17.46 |
| Aspartic Acid (ASP) | 0.00 | 17.40 |
| Asparagine (ASN) | 0.00 | 0.00 |
| DL- Serine (SER) | 0.00 | 0.00 |
| **Total** | 100% | 100% |

The greatest decrease in amino acid content was observed in the case of the amino acid LYS since it was vanished was (zero%) due to the application of triflumuron, while it was 19.92% in control female. LYS amino acid may play a specific role in the structure of egg of the cotton leafworm. In the other hand, the greatest increase in amino acid content was noticed in case of ASP (17.40%), while it was absent (zero%) in control female. Figure (2) is illustrating the identification of the different amino acids content in treated and control adults (2days- old) of both sexes (male and female) of the cotton leafworm *S. littoralis*.

**3-Weight of gonads**

The ovaries of *S. littoralis* start to differentiate and develop at the pupal stage. By dissection, *S. littoralis* female was found to have paired ovaries that branch into four polytrophic meroistic ovarioles located on the ventral side of the body cavity. Ovarioles included basal oocytes developing simultaneously. Each ovariole is differentiated into three parts according to the developmental stages of the oocytes: A-the yellowish green pedicle, where fully matured ova are stored, B-the reddish orange vitellarium, which contains the developing oocyte and trophocyte follicles which bear accumulation of yolk proteins, and choriogenesis, and C-the whitish germarium, which contains oogonia, from which germ cells proliferate and follicles are formed. All stages of oocytes of treated and untreated (control) females (2days-old) were weighted freshly.

**Table (3). Effect of $LD_{50}$ (0.006 µl/larva) of triflumuron on testes and ovaries weight (mg) of 2 days-old emerged adults after topical application to newly molted fifth instars larvae of *S. littoralis***

| Treatments | No. of female used | Average weight of Fresh ovaries (mg) | No. of male used | Average weight of Fresh testes (mg) |
|---|---|---|---|---|
| **Control** | 10 | 78.65 | 10 | 3.74 |
| **$LD_{50}$** | 10 | 32.21 | 10 | 1.08 |

The average weight of ovaries was found to be 32.21 mg for the treated female with triflumuron, while it was 78.65 mg in control female. In other hand, the average weight of testicles was recorded as 1.08 mg for treated male with triflumuron, while it was 3.74 mg in control male. This indicated the biochemical composition of the testicles was reduced (Table 3).

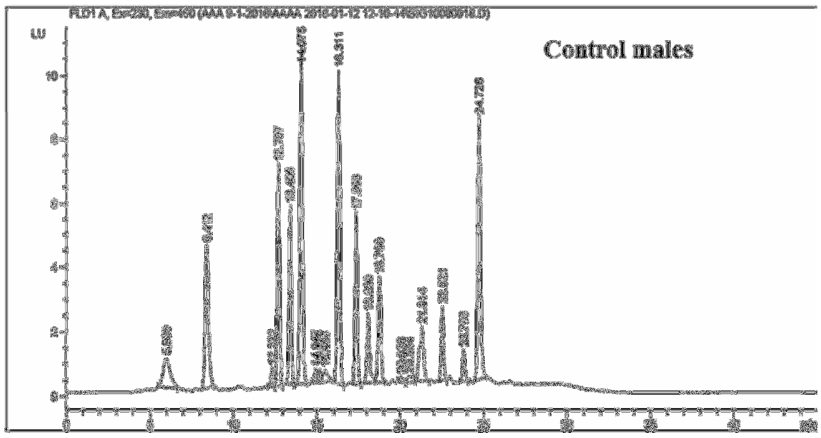
The treated adult females (2days-old) were found to have retarded ovarian development, caused by a delay of oocytes development. This caused a decrease in fecundity and egg viability of the females. Also, the maturation of oocytes was delayed in treated adult females as compared with the controls.

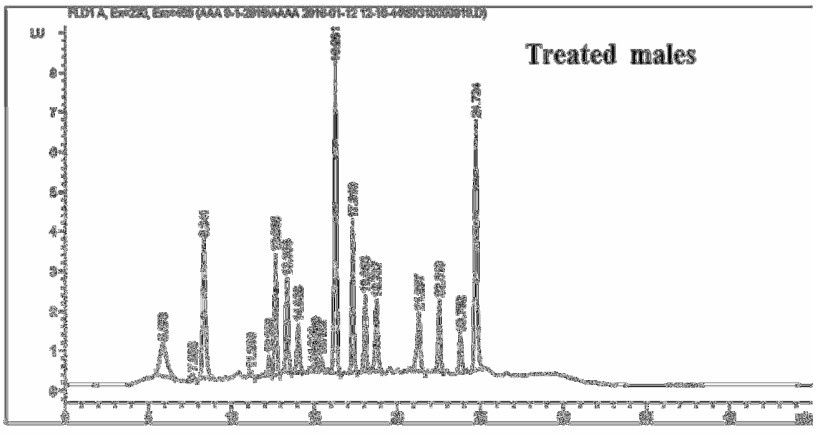
The maturation of insect eggs is dependent, among other factors, on the materials taken up from the surrounding hemolymph, and by materials synthesized by the ovary in situ (Indrasith *et al.* 1988). These materials include proteins, lipids, and carbohydrates, all of which are required for the embryogenesis ( Kanost *et al.,* 1990).

Decrease in the ovarian protein content suggests an interference of BPUs with vitellogenesis. Also, the decreased ovarian protein content was presumed to have several causes, such as the lack of protein in the ovarioles or interference of triflumuron with the mechanism controlling yolk deposition. It has been reported that triflumuron could affect ecdysteroid secretion from other organs, such as the

epidermis, according to Soltani and Mazouni (1992) who found the similar results in *T. molitor* and ovaries of *C. pomonella* (Soltani *et al.,* 1989), and the concentration of hemolymph constituents in *T. molitor* (Soltani, 1990). Investigation by Perveen and Miyata (2000) demonstrated the significant decrease in ovarian protein content in chlorfluazuron-treated females.

It could conclude that the larval exposure to triflumuron can cause significant reproductive suppression of this insect, and that these effects arise through delayed effects. Also, the effect on amino acids content in both sexes reproductive tracts and its function, ultimately leading to reduce sperm transfer from the treated male insect to the female.



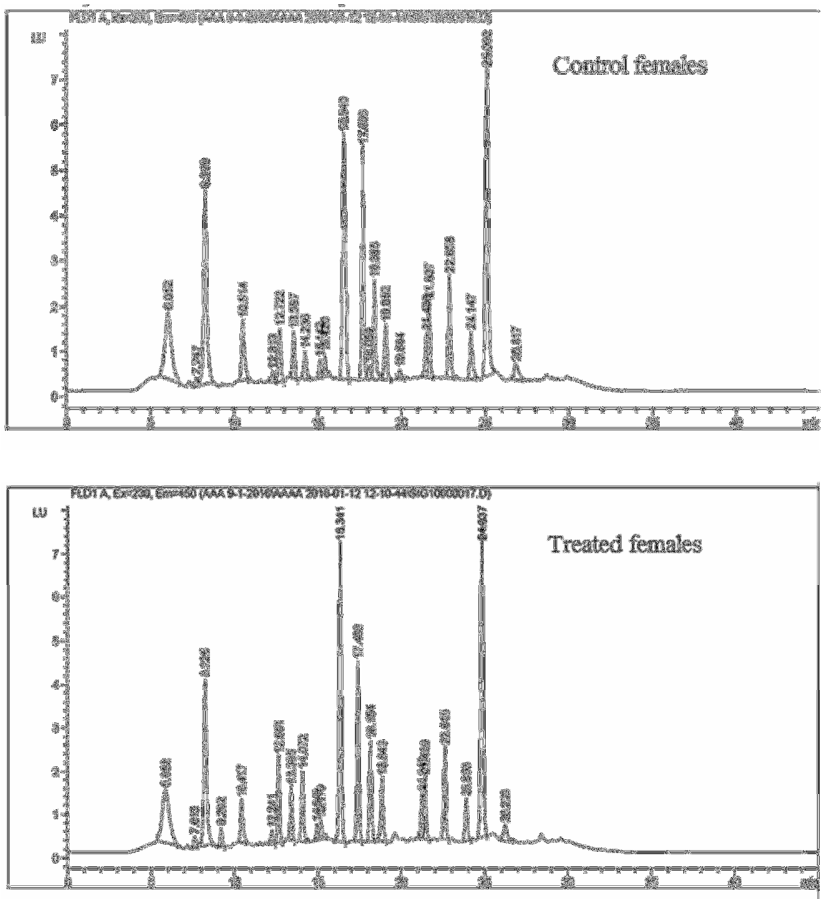



**Fig.(2). Identification of the different amino acids content HPLC (chromatograms) using in treated and control of both sexes of *S.littoralis.***

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**الملخص العربي**

# تأثيرات الترای فلوميورون كمثبط نمو حشري على محـتوى الأحماض الأمينيـة في الجهاز التناسلي للحشرات الكاملة لدودة ورق القطن

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تم تصميم هذة الدراسة لتقييم النشاط الإبادي الحشري الفيسيولوجي للترای فلوميورون كمثبط نمو حشري و ذلك على محتوى الأحماض الأمينية في خصيتين و مبيضين الجهاز التناسلي لكلا جنسي الحشرات الكاملة لدودة ورق القطن. حيث وجد عند تطبيق الجرعة التي تسبب موت ٥٠% ($LD_{50}$) لمادة الترای فلوميورون (الـ$LD_{50}$ = ٠,٠٠٦ ميكرولتر/يرقة )عند بداية انسلاخ العمر اليرقي الخامس ليرقات دودة ورق القطن إنخفاض النسبة المئوية للأحماض الأمينية المتواجدة في خصيتين ذكور الفراشات (عمر يومين) الناتجة من المعاملة. تراوحت نسبة الإنخفاض ما بين ٠–٩,٦٥ % في تسعة أحماض أمينية من أصل ١٦ حمض أميني تم إختبارهم (د إ ل–٢–أمينو– ن– بيوتريك أسيد , تربتوفان(TRP), أيزوليسين(ILE), د ل

ثيريونين(THR) , ل- أرجينين(ARG), ل- ليسين(LEU), إ ل- ألانين(ALA), د إ ل سيرين (SER) و إ ل- ليسين(LYS) بينما تراوحت النسب المئوية لكمية الأحماض الأمينية ما بين ٣,٤٥ – ١٦,٩٤%.

أوضحت النتائج أن المعاملة بالتراى فلوميورون كان لها تأثير واضح على الاحماض الأمينية التي تلعب دور هام في الوظائف الفيسيولوچية المسئولة عن عملية تكوين الحيوانات المنوية داخل الخصى بالجهاز التناسلي لذكور الفراشات مثل السيرين (SER) و الليسين (LYS) و التي كانت نسبتهما في ذكور الفراشات (عمر يومين) الناتجة من اليرقات غير معاملة (الكنترول)٥,٥١ – ٣,٤٥ % على الترتيب و لكن إنخفضت نسبتهما إلى صفر% في ذكور الفراشات الناتجة من اليرقات المعاملة. قد يلعب الحامضان الأمنييان السيرين (SER) و الليسين (LYS) أدوار هامة في تكوين و تركيب الحيوانات المنوية في ذكور فراشات دودة ورق القطن. و في حالة الإناث، لوحظ إنخفاضاً كبيراً في كمية الأحماض الأمينية خاصة حمض الليسين (LYS) حتى وصلت إلى صفر % عند المعاملة بالتراى فلوميورون($LD_{50}$)، بينما كان الحمض الأميني LYS ١٩,٩٢% في إناث الحشرات الكاملة الكنترول. حيث يلعب الليسين دور هام في تكوين و تركيب بيض إناث فراشات دودة ورق القطن ، بينما وصلت نسبة كمية الأحماض الأمينية إلى أقصاها في حالة الأسبارتك أسيد (ASP) و الذي بلغ ١٧,٤% في الأفراد المعاملة. بينما إنخفض إلى صفر% في حالة الإناث الكنترول(غير المعاملة).

كان متوسط أوزان المبايض و الخصيتان ٣٢,٢١ ، ١,٠٨ ملليجرام في حالة الإناث و الذكور الناتجة من المعاملة على الترتيب، بينما كانت متوسط أوزان المبايض و الخصيتان ٧٨,٦٥ ، ٣,٧٤ ملليجرام في حالة الإناث و الذكور الكنترول على الترتيب هذا يدل على حدوث إنخفاض و خلل في التكوين البيولوجي الكيميائي للأجهزة التناسلية في كلا الجنسين.

وعلى ما سبق نجد أن المعاملة بالمبيد الحشري التراي فلوميورون المستخدم كمثبط نمو حشري قد أدت إلى حدوث خلل في الجهاز التناسلي و أيضاً تأثرها على كمية الأحماض الأمينية ووظائفها في الجهاز التناسلي للحشرات الكاملة الناتجة من المعاملة مما أدى في النهاية إلى الإنخفاض في إنتقال الحيوانات المنوية من الذكورالمعاملة إلى الإناث.