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The Biological and Histopathological Aspects of The Black Cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) Treated with Flufenoxuron

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ABSTRACT

The biological and histological effect of insect growth regulator (flufenoxuron) evaluated on 2nd larval instar of *Agrotis ipsilon* as a chitin synthesis inhibitor. The effect of sublethal dose LC_{50} was used to investigate its effect on some developmental and reproductive parameters of *Agrotis ipsilon* that survived treatment of newly molted second instars. Results indicated that the flufenoxuron significantly enhanced the mean larval and pupal durations, whereas they significantly declined the mean percentage pupation, adult emergence, adult longevity, fecundity, and fertility compared to untreated insects. Also, our studies recorded many histological aberrations to the ovary of *Agrotis ipsilon* female moth.

INTRODUCTION

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is one of the major polyphagous and underground destructive worldwide pests. This species causes a high level of economic loss to a wide range of crops through the damage of roots, which consumes corn, cotton, wheat and many vegetables Wang *et al.*, (2021). They feed during the early stages of the plant by cutting down leaves, and at the base of the stems Mesbah *et al.*, (2020). In integrated pest management, a basic problem is needed highly effective insecticides and appropriate methods of application to control the black cutworm Falin *et al.*, (2019).

Insect growth regulators (IGRs) have effects on certain physiological regulatory processes essential to the normal development of insects.

The present study aimed to evaluate the insect growth regulator Flufenoxuron, it is an insecticide that belongs to the benzoylurea chitin synthesis inhibitor group, for controlling *Agrotis ipsilon* larvae. Determining their effect on the biological aspects and histopathological and effect on the ovaries.

MATERIALS AND METHODS

1-Insect Rearing:

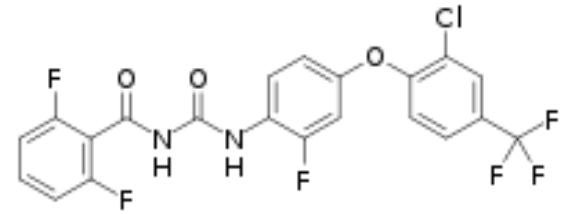
In the laboratory, the culture of *A. ipsilon* was reared according to Moustafa *et al.*, (2021). The culture was obtained from the Plant Protection Research Institute, Agricultural Research Center. They were incubated under constant conditions at 25 ± 2 °C, 70 ± 5% RH

and 12:12 h (L:D) photoperiod. To avoid larval cannibalism, larvae were reared individually in small cups (7.0 cm in diameter, 3.5 cm in height) with sawdust to reduce moisture He *et al.*, (2019), fed daily on fresh castor oil bean leaves (*Ricinus communis* L.) until pupation and moths were transferred to glass jars (5L), fed on 10% sucrose solution Kandil *et al.*, (2020).

2-Tested Compound:

Chemical group: Chitin synthesis inhibitors.

Common name: Flufenoxuron



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Trade name: Novo 10% DC

Rate of application: 200 cm / Fedden

3-Bioassay Studies and Sublethal Calculations:

The toxic effect of Flufenoxuron was evaluated against the 2nd larval instars of *A. ipsilon* fed on castor bean leaves dipped in different five concentrations, one hundred larvae were divided into four replicates; every 25 larvae were used for each concentration (3, 1.5, 0.75, 0.37 and 0.18 ppm. A control experiment was dipping castor bean oil leaves in water. The tested larvae were fed on treated castor bean oil leaves for 48 hrs. The Concentration-mortality percentages were recorded daily. Mortality in treatments was corrected with the corresponding mortality in the untreated check according to Abbott's formula (Abbott, 1925) and LC_{50} & LC_{90} values were calculated by using the probit- analysis method of (Finney, 1971).

4-Biological Aspects Investigation:

Treatment of the newly hatched 2nd instars larvae with the LC_{50} concentration of Flufenoxuron was replicated four times (25 larvae/replicate). Control was run with four replicated times (25 larvae/replicate). Determine data daily of surviving insects Awad *et al.*, (2022) to detect the post-treatment effects, such as (e.g., the larval and pupal duration and pupation %).

Virgin *A. ipsilon* females that survived treatment of newly molted second instars with the LC_{50} of flufenoxuron were transferred to glass jars with males treated also, fed on 10% sucrose solution, to determine adult emergence%, pre-oviposition, oviposition periods and post-oviposition and adult longevity, the number of laid egg per female and percent of hatchability, fecundity and fertility compared with the untreated control. The deterrent index was calculated according to Lundgren (1975) as:

$$\textbf{Deterrent index} = \{(A- B) / (A+B)\} \times 100$$

Where A: the total number of eggs per female in control.

B: the total number of eggs per female in treatment.

5-Histopathological Studies:

The histology of the ovaries was obtained from the adult female pretreated second instars larvae with the LC_{50} of Flufenoxuron was studied. The surviving virgin treated and untreated females were dissected in ringer's solution on the first day of emergence, during the pre-oviposition and oviposition period. The ovaries were fixed in Carnoy's solution, embedded in paraffin wax, and stained with hematoxylin and eosin.

6-Statistical Analysis:

Statistical analysis using a student t-test of obtained data was performed by using the COSTAT program, for windows.

RESULTS AND DISCUSSION

Bioassay Studies:

The lethal toxicity value of the insect growth regulator (IGR) Flufenoxuron was evaluated on the 2nd instar larvae of *A. ipsilon*, results are shown in Table (1). LC_{50} and LC_{95} values of Flufenoxuron were determined as 0.309, and 0.027 ppm respectively. The Slope of LC_{50} and LC_{95} were 2.6387±0.246 and1.981±0.203 respectively, showing the homogeneity of the larvae. Generally, treated larvae were observed to be less active in their movement with obvious muscle contractions and prior to their death larvae exhibited severe tremors followed by paralysis. Shaurub E. H. *et al.*, (2018) the toxicity of flufenoxuron to *A. ipsilon* larvae was about 16 times less toxic than *S. littoralis* larvae, where flufenoxuron showed LC_{50} of 4.68 mg/L to *A. ipsilon* larvae.

Table 1: Susceptibility of *Agrotis ipsilon* 2nd instar larvae to Flufenoxuron.

		Confidential limits for (95%)		Slope±S.E.	Accumulative % mortality (at the end of larval stage)
		Lower	Upper		
LC_{50} (ppm)	0.3091	0.1034	0.2568	2.6387±0.246	51.3
LC_{90} (ppm)	0.0272	0.0102	0.0312	1.981±0.203	90.0

Biological Aspects:

The effect of sublethal concentration of Flufenoxuron on the biological aspects of 2nd instar larvae of *A. ipsilon* in Table (2), demonstrated the significantly prolonged larval and pupal durations compared to the control where, the larval duration was 18.5 days but in control was 16.4 days, i.e., an increase of 12.8% than the control. The pupal duration was13.5 days, in the control, was 11.3 days so the increase in pupal duration was 19.5% more than that of the control. On the contrary, we found pupation% recorded (66.7%) which was a reduction by nearly half its value in untreated insects, i.e. a decrease of 28.53% than the control.

Table 2: Larval duration from initial instar treated, pupation percentage and duration of pupae of *Agrotis ipsilon* treated as 2nd instar larvae with LC_{50} of Flufenoxuron.

Treated Instar	Mean larval duration post-treatment (days ± S.E.)	Mean pupal stage duration (days ± S.E.)	Pupation%
Flufenoxuron	18.5** ± 0.29 (-12.8)	13.5** ± 0.17 (-19.5)	66.7 (28.53)
Control	16.4 ± 0.24	11.3 ± 0.14	93.33

Numbers between brackets present percentages of reduction than the control.
**: moderately significant ($p < 0.01$), (student-t-test).

The effects of the Flufenoxuron which occurred during the development of *A. ipsilon* attributed to the metamorphic disruption and the slower metabolic rate of these larvae as a direct effect of chitin synthesis inhibitors application. The results agreed with Moustafa Z. H. and Salem M.S. (2019), which showed the prolonged duration of the larval stage and

pupal period after treated *P. gossypiella* newly hatched larvae with LC_{50} of flufenoxuron compared with control. Also, Shaurub E.H. *et al.,* (2018) treated *A. ipsilon* larvae with chlorfluazuron and flufenoxuron and found significantly prolonged larval and pupal durations compared to the control. El-Sayed *et al.,* (2017) reported that significant reduction in the pupation% as the result of treatment of the 2nd instar larvae of *S. littoralis* with sublethal concentrations of lufenuron and flufenoxuron.

Table (3) showed the percentage of adult emergence, adult longevity and total oviposition periods of *A. ipsilon* treated as 2nd instar larvae with LC_{50} of Flufenoxuron, the percentage of adult eclosion was 88.24% which was a significant reduction from their control. We found the life span of these emerged male and female moths of *A. ipsilon* that survived larval treatment with LC_{50} of flufenoxuron was 9.5 days as compared to 14.5 days in the control, i.e. a decrease in their life span by 34.48%. Pre-oviposition and oviposition periods of treated survived larvae of *A. ipsilon* females with flufenoxuron recorded 3.5 and 2.5 days to 5.0 and 8.0 days of control respectively. Where pre-oviposition and oviposition periods significantly decreased by 30.0 and 68.75 days respectively compared to the control. However, the post-oviposition period was significantly increased by (1.33%) compared to the control. Where pre-oviposition period was recorded as 3.5 days to 5.0 days for the control, and in oviposition period was 2.5 days and 8.0 days for the control. The post-oviposition period was 3.5 days of 1.5 days of control.

Table 3: Adult emergence%, adult longevity and total oviposition periods of *Agrotis ipsilon* treated as 2nd instar larvae with LC_{50} of Flufenoxuron.

Treated Instar	Adult emergence %	Mean of adult life span (days ± S.E.)	Total oviposition periods (days)		
			Pre	Ovi	Post
Flufenoxuron	88.24 (11.76)	9.5^{***} ± 0.3 (34.48)	3.5^{**}±0.19 (30.0)	2.5^{***}±0.18 (68.75)	3.5^{**}± 0.12 (1.33)
Control	100	14.5 ± 0.89	5.0±0.58	8.0±0.38	1.5±0.18

Numbers between brackets present percentages of reduction than the control.
***: highly significant ($p < 0.001$) and **: moderately significant ($p < 0.01$), (student-t test).

That decrease in the percentage of adult eclosion may be due to the block of the maturation of imaginal discs by the toxin which is the primal integumentary structure in insects Suh *et al.,* (2000). The obtained data agreed with Shaurub E.H. *et al.,* (2018) treatment of *A. ipsilon* larvae with chlorfluazuron and flufenoxu and recorded decreased longevity of moths of *A. ipsilon*. The reduction in the pre-oviposition period indicates that the time of developing the first batch of eggs was a disturbance by treatment with flufenoxuron, while the reduction in the oviposition period may be due to the decrease in the number of developed oocytes in the ovaries. Also agreed with Abd EL Mageed E. N. I. (2022) study of the efficiency of spinosad, methoxyfenozide and extreme against the 4th instar larvae of *S. littoralis* and found the moths have a lower rate of adult longevity than the control. Our results also agree with Abdel-Aal A. E. (2012), who treated the fourth instars of *S. littoralis* with the LC_{50} of chlorfluazuron significantly, reducing the pre-oviposition and oviposition period of the surviving female moths.

The reproductive potential of mated moths emerging from larvae treated as 2nd instar of *A. ipsilon* with Flufenoxuron at LC_{50} value showed that their reproductive potential was significantly affected (Table 4). The number of the laid egg was 211.89 eggs per female, compared to 2076.0 eggs per female in the control. However, egg fertility was impaired as percentage hatchability was 25.01% in moths emerging from treated 2nd instar larvae, but lower than their control by 74.32%. The Deterrent index was recorded at 81.48%.

Table 4: Reproductive potential of *Agrotis ipsilon* moths treated as 2nd instar larvae with LC_{50} of Flufenoxuron.

Treated Instar	Mean No. of eggs/ female ± S.E.	Fecundity %	Mean No. of egg hatch ± S.E.	Deterrent index	Hatchability %
Flufenoxuron	211.89***±9.59 (89.79)	10.207	53.0***±1.76 (97.38)	81.48	25.01 (74.32)
Control	2076.0***±316.7		2022***±183		97.4

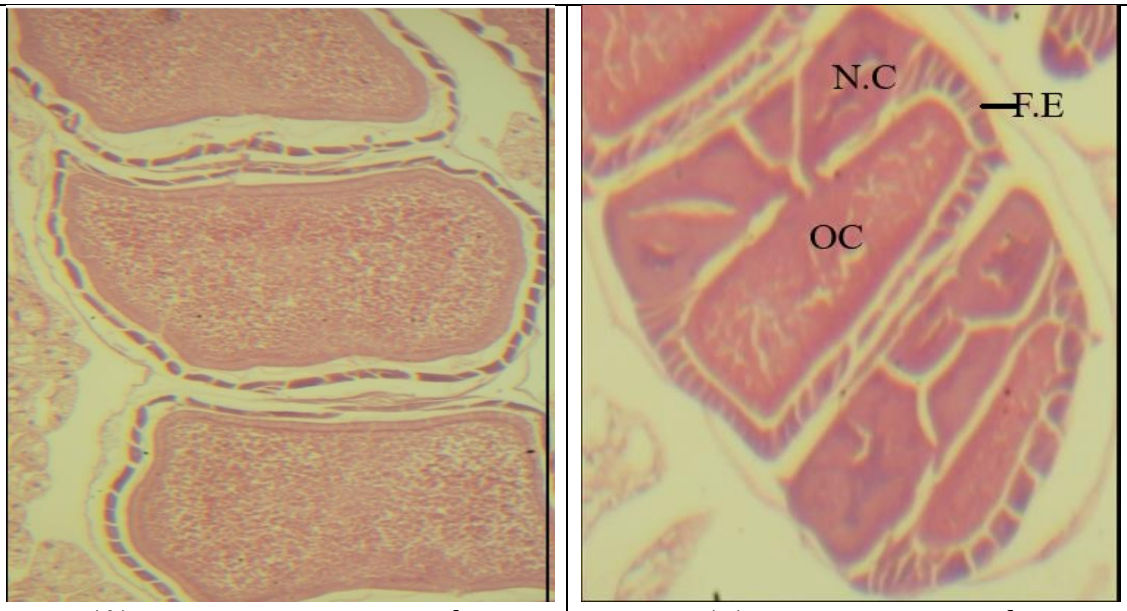
Numbers between brackets present percentages of reduction than the control.
***: highly significant ($p < 0.001$) and **: moderately significant ($p < 0.01$), (student-t test).

The suppression of reproductive potential of mated moths emerging from larvae treated as 2nd instar of *A. ipsilon* with Flufenoxuron at LC_{50} may be due to interference of flufenoxuron with oogenesis. In lepidopterans, moths provide their eggs with high concentrations of ecdysteroids to developing embryos and pre-hatching larvae Lafont *et al*., (2005). The treatment by Flufenoxuron causes interference with the ecdysteroid hormone which may lead to abnormal oocyte growth, egg formation, and embryogenesis, which may lead to a loss of progeny Dhadialla *et al.,* (2005). The reduction in the percentage of egg-hatch obtained in the present study may be attributed to the sterilization of either egg and or/sperms Abdel-Aal A. E. (2012).

Histopathological Studies:

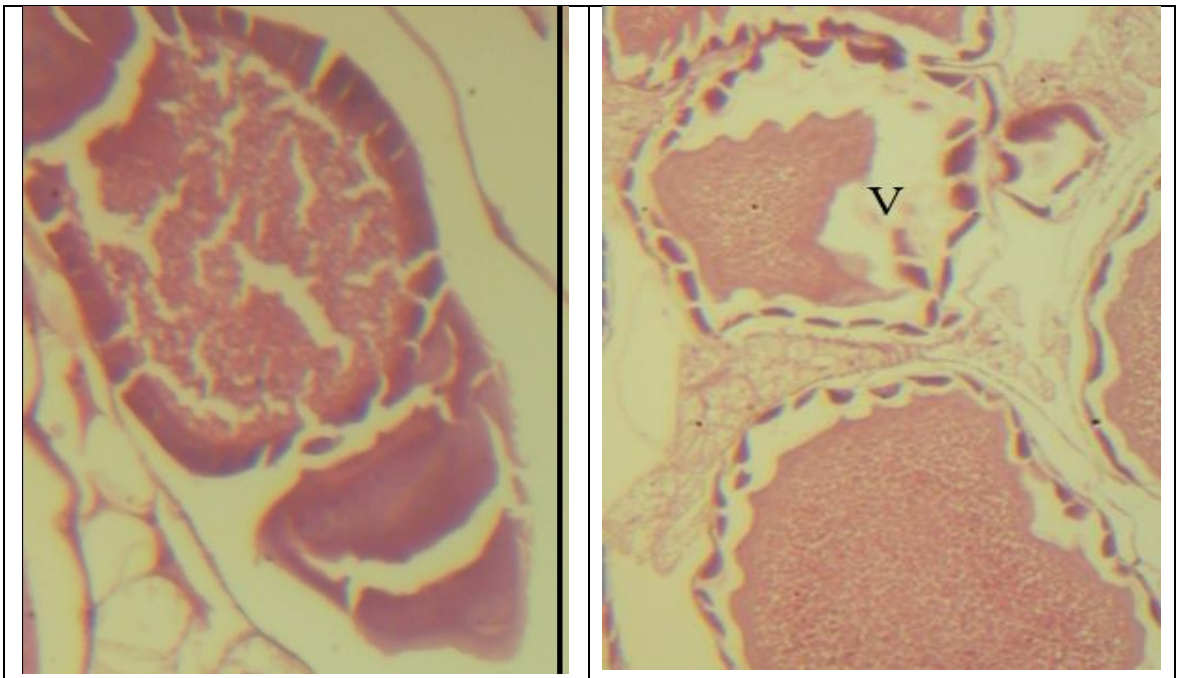
In normal female moths Fig (1) the four convoluted ovarioles in each ovary are "8" polytrophic ovarioles and each ovariole consists of a chain of developing egg follicles in case of both pre and oviposition periods. Histologically each follicle in the case of the oviposition period consists of a growing oocyte accompanied interiorly by a few numbers of nurse cells. The oocyte is surrounded by somewhat columnar or cuboidal follicular epithelium, while the nurse cells are surrounded by squamous follicular epithelium.

Fig (2) showed the effect of flufenoxuron treatment on the female ovary in the pre and oviposition period and b respectively. The histological abnormalities fig (2a) was in the form of clumping of chromatin materials leaving space near the epithelial cells, the absence of some nurse cells and also the oocytes being semi-absorbed. Fig (2b) showed that treatment was because of slight oocytes shrinkage which left space around it and others were semi-absorbed. The follicular epithelium became thin and vacuolated. The histological aberration in this study agrees with Abdel Aal and Abdel Wahab (2007) when they recorded histological aberration and the ovicidal effects of lufenuron on the cotton leafworm, Shurab E.H. *et al*. (2018) also reported complete damage for *A. ipsilon* female ovarian cells when treated as fourth instars with chlorofluazuron.



(A) In pre-oviposition period (B) In ovipositon period

Fig 1: L.S Through normal ovariole of *Agrotis ipsilon* female.
F.E: Follicular
N.C: Nurse cell
OC: Oocyte



(A) In pre-ovipositon period (B) In ovipositon period

Fig 2: L.S Through ovariole of *Agrotis ipsilon* female treated as 2nd instar larvae with LC_{50} of Flufenoxuron.
V: Vacule

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