

## Research Article

## Lethal and sublethal effects of a chitin synthesis inhibitor, lufenuron, against *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae)

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**Abstract:** *Glyphodes pyloalis* Walker is a serious pest of mulberry trees in Iran. In this study, lethal ( $LC_{50} = 19$  ppm) and sublethal effects ( $LC_{10} = 3.74$  and  $LC_{30} = 9.77$  ppm) of lufenuron were evaluated against 4<sup>th</sup> instar larvae of *G. pyloalis*. After treating the larvae at  $LC_{30}$  and  $LC_{50}$  level, most of them died during the molting process and only a few individuals developed to the 5<sup>th</sup> larval instar. The highest rate of mortality was observed in 5<sup>th</sup> larval (68.42%) and pre-pupal (59.57%) stages at  $LC_{50}$  concentration. Also, lufenuron caused an increase in larval, pre-pupal and pupal developmental periods. The successful pupation decreased to 53.64% at the  $LC_{30}$ . Also, no prepupa molted to pupation after treatment of larvae at the  $LC_{50}$ . Larval weight was significantly reduced at 48, 72 and 96 h after treatment, compared to the control. Adult emergence and female longevity were also reduced at  $LC_{30}$ . The  $LC_{30}$  of lufenuron negatively affected reproduction of *G. pyloalis*. Larval content of carbohydrate and protein were decreased significantly 48 and 72 h after treatment, however, lipid and glycogen content were decreased significantly only 72 h after treatment at  $LC_{10}$ ,  $LC_{30}$  or  $LC_{50}$ . Findings indicated adverse effects on some biological and biochemical parameters at lethal and sublethal concentrations of lufenuron which necessitate further investigations for its application in an integrated management of *G. pyloalis*.

**Keywords:** *Glyphodes pyloalis*; lufenuron; fecundity; sub-lethal effects; IGR

### Introduction

The lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae) is a serious leaf-damaging pest of mulberry trees in Iran (Jaafari Khaljiri *et al.*, 2006), eastern Georgia (Kanchaveli *et al.*, 2009), USA, Mexico, India, Japan and the Republics of Central Asia and Azerbaijan (Madyarov *et al.*, 2006; Mikaia, 2011). This insect has five larval instars. The

larvae feed on basal epidermis and mesophyll of mulberry leaves. The amount of food eaten by the first and second instar larvae is negligible, but feeding increases in later instars. Fourth and fifth instar larvae secrete fine threads to fold the leaf and feed on the mesophyll inside the folds. Fifth instar larvae feed on the whole leaf until only the ribs remain. In Iran this pest is active in late spring, summer and early fall and its damage to mulberry leaves, have bad impacts on silk farming (Khosravi and Jalali Sendi, 2010; Aruga, 1994).

The use of Insect growth regulator (IGRs) compounds in insect control is known as insect

Handling Editor: Khalil Talebi Jahromi

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Received: 22 May 2015, Accepted: 16 November 2015  
Published online: 13 January 2016

development inhibition, which inhibits or prevents normal metamorphosis of immature stages to the adult stages. IGRs are analogs of insect hormones those disrupt normal activity of the endocrine system, and affect development, reproduction, and metamorphosis. IGRs include juvenile hormone (JH) mimics, agonist ecdysone and chitin synthesis inhibitors (CSIs). CSIs, such as lufenuron inhibit production of chitin, an essential component of insect exoskeleton. The treated insects with CSIs cannot successfully molt to the next life stage (Hoffman and Lorenz, 1998). Compared with conventional insecticides, lufenuron exhibits excellent action against lepidopteran insects and shows a lower toxicity against vertebrates (Whiting *et al.*, 2000). This compound exhibits lethal and sublethal effects on some pests species such as *Ephestia figulilella* Gregson (Lepidoptera: Pyralidae) (Khajepour *et al.*, 2012), *Spodoptera littoralis* Bosid (Lepidoptera: Noctuidae) (Reda *et al.*, 2010), *Spodoptera litura* Fabricious (Lepidoptera: Noctuidae) (Zhu *et al.*, 2011) and *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) (Mervat *et al.*, 2012).

The concern specifically addressed in the present study relates to the possible lethal and sublethal effects of lufenuron on the lesser mulberry pyralid, an important pest of mulberry leaves in northern Iran. Many authors have reported that CSIs disrupt normal activity of the endocrine system and cause prolongation of larval and pupal periods. Also, the longevity, fecundity and fertility of adult lepidopteran insects can be disrupted by these compounds (Mervat *et al.*, 2012; Zhu *et al.*, 2012; Reda *et al.*, 2010).

Some biochemical studies have focused on evaluation of possible adverse effects of chemicals on insect storage of energy. In insects, carbohydrates, glycogen and lipids are closely related to physiological processes such as the flight, molting and reproduction (Ramdev and Rao, 1979). The content of carbohydrates and lipids in the hemolymph is an important indicator of the level of metabolism in insects, and a dynamic balance of the absorption,

metabolism, and utilization by different tissues (Nath, 2003; Etebari *et al.*, 2007). IGRs exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system (Oberlander *et al.*, 1997). LC<sub>50</sub> dose of lufenuron caused decrease total soluble protein and total lipid content of *P. gossypiella* (Mervat *et al.*, 2012). Application of essential oil of summer savory, *Satureja hortensis* L. (Family: Lamiaceae) decreased the amounts of protein, lipids and carbohydrates in *G. pyloalis* (Yazdani *et al.*, 2012). The total protein content decreased in the house fly treated with lufenuron (Assar *et al.*, 2010).

The objectives of this research were to evaluate the susceptibility of 4<sup>th</sup> instars of *G. pyloalis* to lufenuron. Then, the lethal and sublethal effects were studied on development, mortality, reproduction and larval biochemical composition (carbohydrate, lipid, protein and glycogen) at LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> levels.

## Materials and Methods

### Insect rearing

Larvae of *G. pyloalis* were collected from infested mulberry trees (*Morus* sp) in June and July 2013 from Guilan Province, Iran. They were reared on mulberry (var. Shin Ichinoise) leaves in laboratory conditions (25 ± 1 °C, 75 ± 10% RH, 16:8 h L: D photoperiod) in plastic boxes (18 × 15 × 7 cm). Emerged adults of moth were identified by sex and kept in plastic containers (18 × 15 × 7 cm) provisioned with 10% honey solution. Fresh mulberry leaves, petioles of which were placed into vials containing water, were used as an oviposition substrate. After egg hatching, larvae were transferred onto fresh leaves (using a soft camel-hair brush) and leaves were replaced daily.

### Toxicity tests

Leaf-dip method (Memarizadeh *et al.*, 2011) was used to determine the susceptibility of newly molted 4<sup>th</sup> instars to lufenuron. Five

concentrations (5, 8.12, 13.48, 22.38, 40 mg/L) of a commercial formulation of lufenuron (Match<sup>®</sup> 50 EC, Syngenta; chemical formula: N-[[[2,5 dichloro-4-(1,1,2,3,3,3 hexafluoropropoxy)-phenyl]carbonyl]-2,6-difluorobenzamide (CA)) were prepared in distilled water. Leaf disks (3.5 cm in diameter) were dipped in each concentration for 40 seconds. After drying at room temperature, the treated leaves were used to feed the 4<sup>th</sup> instar larvae. Ten 4<sup>th</sup> instars were placed in a plastic container (14 × 5 × 12 cm) containing two treated leaf disks with the same concentration and considered as one replication. Also, leaves dipped in water only were considered as controls. Six replicates were considered for each lufenuron concentration. Mortality was recorded 48 h after treatment, and LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> were calculated using probit analysis by Polo-PC software (Leora Software, 1987).

#### **Effects of lufenuron on biological parameters**

Four hundred newly molted 4<sup>th</sup> instars in 20 replicates for each concentration (20 larvae/replicate) from laboratory colony were selected randomly and exposed to LC<sub>30</sub> and LC<sub>50</sub> concentrations of lufenuron. Larvae were monitored after 48-h and surviving larvae were fed with untreated mulberry leaves and observed daily to determine larval duration, the pupation, numbers of malformed pupae and emerged adults. Pupae were placed individually into a small plastic container (7 × 8 × 3.5 cm). More than 20 pupae were used for each treatment. One female and one male of resulting adults were placed together in a plastic container (14 × 5 × 12 cm) with a piece of cotton soaked in a 10% honey solution for food and allowed to mate. Fresh mulberry leaf was provided for oviposition that petioles of which were inserted into vials containing water and the top of the vial was sealed with cotton. Eggs laid on leaves were counted daily, and male and female moths were transferred to new plastic containers (14 × 5 × 12 cm) daily until their death. Larval weight at 48, 72 and 96 h after treatment, larval malformation, larval, pre-pupal and pupal developmental

time and mortality rate, pupation rate, adult emergence, longevity and fecundity were also determined.

#### **Biochemical studies**

For these studies, 4<sup>th</sup> instars were treated with LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> concentrations. After 48 and 72 h, the alive larvae were randomly selected and their bodies were homogenized in either sodium sulphate buffer solution (Na<sub>2</sub>SO<sub>4</sub> 2%) for detection of carbohydrate lipid and glycogen contents or in phosphate buffer for detection of protein content. The homogenized solution was centrifuged at 8000 × g, for 10 min at 4 °C and the supernatant was used for determination of amounts of carbohydrate, lipid and protein. The pellet was used for determination of glycogen content.

#### **Determination of carbohydrate, lipid, protein and glycogen**

Carbohydrate, lipid and glycogen contents were extracted according to the Van Handel and Day (1988) method. Protein content was determined according to the Bradford (1976) method.

#### **Data analysis**

Data obtained from the experiments were analyzed using analysis of variance (Proc ANOVA; SAS Software 2003) and T test. Means were compared by Tukey's Studentized, accepting significant differences at P < 0.05. The corrected mortality of larvae was carried out using Abbott's formula (Abbott, 1925).

#### **Results**

##### **Toxicity of lufenuron on 4<sup>th</sup> instars**

The LC<sub>10</sub>, LC<sub>30</sub> and median lethal concentrations (LC<sub>50</sub>) of lufenuron with their confidence limits on 4<sup>th</sup> instars are shown in Table 1. The LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> were estimated as 3.74, 9.77 and 19 ppm, respectively.

### Mortality rate during the larval, pre-pupal and pupal stages

Percentage mortality of larvae, pre-pupae and pupae stages of *G. pyloalis* exposed to LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron or water controls are listed in Table 2. The highest rate of 5<sup>th</sup> instars mortality was found after exposure to the LC<sub>50</sub> concentration, compared with 5.88% mortality in controls. There was significant difference among treatments with control in the mortality rate of the 5<sup>th</sup> larval instar ( $F = 14.31$ ,  $df = 2, 16$ ,  $P = 0.0004$ ). Mortality rate in pre-pupae in LC<sub>50</sub> concentration was higher than that of LC<sub>30</sub> and control ( $F = 8.08$ ,  $df = 2, 8$ ,  $P = 0.0199$ ). All pre-pupae in LC<sub>50</sub> concentration died and no pupa emerged from them.

### Effects on larval weight

The larval weight in different concentrations of lufenuron and control are shown in Fig. 1. Sublethal (LC<sub>30</sub>) and lethal (LC<sub>50</sub>) concentrations decreased larval weight 48, 72 and 96 h after treatment compared with controls. The larval weight was 13.25, 10.81 and 9.68 mg/larvae in LC<sub>30</sub> concentration, 9.75, 7.97 and 6.01 mg/larvae in LC<sub>50</sub> concentration after 48, 72 and 96 h, respectively. Analysis of variance showed significant difference among concentrations and control after 48 h ( $F = 27.80$ ,  $df = 2, 19$ ,  $P < 0.0001$ ), after 72 h ( $F = 126.97$ ,  $df = 2, 20$ ,  $P < 0.0001$ ) and 96 h ( $F = 168.17$ ,  $df = 2, 20$ ,  $P < 0.0001$ ).

### Developmental periods

The effect of different concentrations of lufenuron on developmental durations of *G. pyloalis* is shown in Table 2. The larval developmental period was increased with increasing concentrations of lufenuron as

compared with control. Analysis of variance showed a significant differences between LC<sub>30</sub> or LC<sub>50</sub> concentrations compared with control ( $F = 47.49$ ,  $df = 2, 36$ ,  $P < 0.0001$ ).

The pre-pupal developmental durations were increased in treatments and pupal durations was increased in LC<sub>30</sub> compared with controls (pre-pupal:  $F = 60.65$ ,  $df = 2, 24$ ,  $P < 0.0001$ , pupal:  $t = 7.69$ ,  $df = 16$ ,  $P < 0.0001$ ).

### Percentage of pupation, adult emergence, longevity, and fecundity

All of the pre-pupae emerging from larvae treated with the higher concentration (LC<sub>50</sub>) died and only 53.64% of them developed to pupa at the lower concentration (LC<sub>30</sub>) (Table 2). There was a significant difference in the percentage of pupation between concentrations and control ( $F = 105.78$ ,  $df = 2, 7$ ,  $P < 0.0001$ ).

The percentage of the adult emergence was decreased in LC<sub>30</sub> concentration as compared with control (Table 3). The adult emergence at LC<sub>30</sub> (42.85%) was significantly lower than control (92.30%). ( $t = 2.51$ ,  $df = 19$ ,  $P = 0.0214$ ). The LC<sub>50</sub> concentration caused 100% inhibition of adult emergence.

The female longevity was reduced to 4.5 days in LC<sub>30</sub> concentration and, there was significant difference between LC<sub>30</sub> concentration and control ( $t = 10.62$ ,  $df = 9$ ,  $P < 0.0001$ ). No significant difference was found in the male longevity between LC<sub>30</sub> concentration and control ( $t = 1.99$ ,  $df = 7$ ,  $P = 0.0875$ ).

There was a significant difference in fecundity between LC<sub>30</sub> concentration and control ( $t = 14$ ,  $df = 8$ ,  $P < 0.0001$ ) and lufenuron negatively affected the reproduction of *G. pyloalis* causing (100%) sterility at LC<sub>30</sub> concentration.

**Table 1** Susceptibility of 4<sup>th</sup> larval instar of *Glyphodes pyloalis* to lufenuron after 48 h.

N <sup>1</sup>	LC <sub>10</sub> (95% CL) <sup>2</sup>	LC <sub>30</sub> (95% CL) <sup>2</sup>	LC <sub>50</sub> (95% CL) <sup>2</sup>	Slop ± SE	χ <sup>2</sup>	P
300	3.74 (1.22 - 6.30)	9.77 (6.30 - 12.98)	19 (14.48 - 25.28)	1.81 ± 0.36	0.87	0.0001

<sup>1</sup> Number of treated larvae.

<sup>2</sup> 95% confidence limits (CL) in parenthesis.

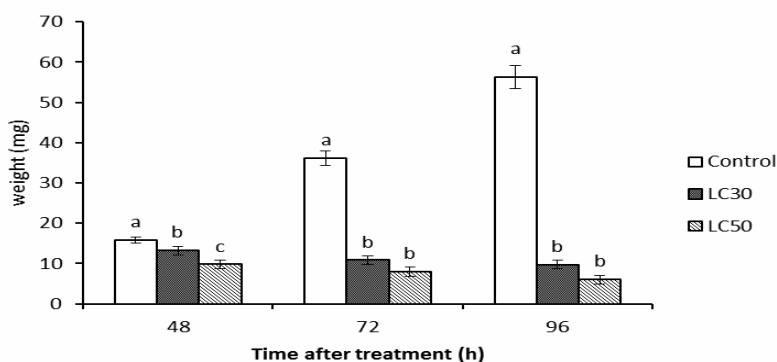
**Table 2** Effects of LC<sub>30</sub> and LC<sub>50</sub> concentrations of lufenuron on developmental time and mortality of *Glyphodes pyloalis*.

Treatment	Larval period ± SE (day) <sup>1</sup>	Larval mortality ± SE (%) <sup>1</sup>	Pre-pupal period ± SE (day) <sup>1</sup>	Pre-pupal mortality ± SE (%) <sup>1</sup>	Pupation ± SE (%) <sup>1</sup>	Pupal period ± SE (day) <sup>2</sup>
Control	5.62 ± 0.22 c	5.88 ± 0.25 b	2.23 ± 0.12 c	6.25 ± 0.25 b	93.66 ± 0.33 a	8.45 ± 0.15 b
LC <sub>30</sub>	8.38 ± 0.34 b	56.84 ± 0.42 a	3.40 ± 0.29 b	42.55 ± 0.57 a	53.64 ± 0.00 b	11.40 ± 0.50 a
LC <sub>50</sub>	10.87 ± 0.66 a	68.42 ± 0.31 a	10.00 ± 0.29 a	59.57 ± 0.5 a	0 c	
F	47.49	14.31	60.65	8.08	105.78	7.69
df	2, 36	2, 16	2, 24	2, 8	2, 7	16
P	< 0.0001	0.0004	< 0.0001	0.0199	< 0.0001	< 0.0001

<sup>1</sup> Means followed by different letters in a column are significantly different (Tukey's test  $P < 0.05$ ).

<sup>2</sup> Means followed by different letters in a column are significantly different (t-student test).

<sup>3</sup> Number of died pre-pupae were considered for 10 days.



**Figure 1** Mean fresh weight of 4<sup>th</sup> instars of *Glyphodes pyloalis* exposed to LC<sub>30</sub> and LC<sub>50</sub> concentrations of lufenuron at 48 h ( $F = 27.80$ ,  $df = 2, 19$ ,  $P < 0.0001$ ), 72 h ( $F = 126.97$ ,  $df = 2, 20$ ,  $P < 0.0001$ ) and 96 h ( $F = 168.17$ ,  $df = 2, 20$ ,  $P < 0.0001$ ) after treatment. Means within the same time after treatment marked with different letters are significantly different ( $P < 0.05$ ).

**Table 3** Effect of LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron on adult emergence, total inhibition of adult emergence, adult longevity, and fecundity of *Glyphodes pyloalis* treated at 4<sup>th</sup> larval instar.

Treatment	Adult emergence ± SE (%)	Longevity (day ± SE)		Fecundity ± SE
		F	M	
Control	92.30 ± 0.09	8.85 ± 0.4	5.14 ± 0.50 <sup>ns</sup>	87 ± 6.88
LC <sub>30</sub>	42.85 ± 0.19	4.5 ± 0.5	4.25 ± 0.25	0 ± 0
t	2.51	10.62	1.99	14
df	19	9	7	8
P	0.0214	< 0.0001	0.0875	< 0.0001

ns, \* and \*\*\* indicate non significant, and significant at 0.05 and 0.001 using t-student test, respectively.

### Effect on energy reserves

#### Carbohydrate

Carbohydrate content in 4<sup>th</sup> instars treated with LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron are shown in Fig. 2. Total carbohydrate level in controls was 5.36 and 11.81 mg/ gr larva after 48 and 72 h, respectively. The total carbohydrate was significantly reduced 48 and 72 h after exposure to sublethal concentrations of lufenuron. The highest reduction was observed at LC<sub>50</sub> concentration, 72 h after treatment ( $0.24 \pm 0.15$  mg/g larvae). Analysis of variance showed a significant difference among the concentrations and control 48 h ( $F = 12.60$ ;  $df = 3, 11$ ;  $P < 0.0021$ ) and 72 h ( $F = 3946.53$ ;  $df = 3, 11$   $P < 0.0001$ ) after treatment.

#### Lipid

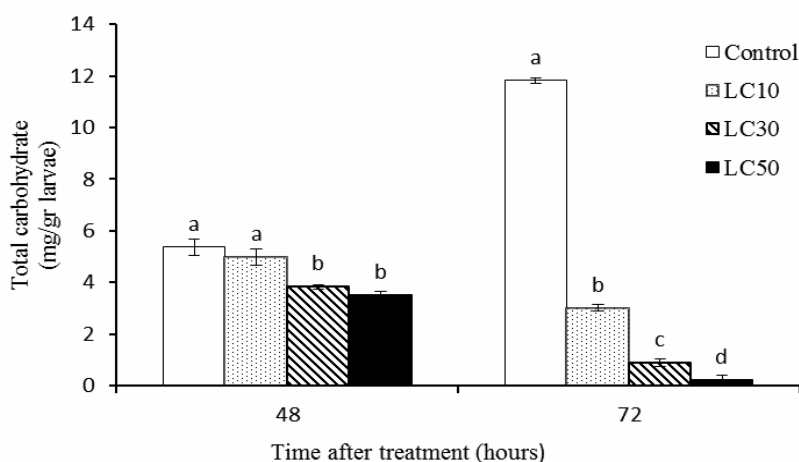
Treatment of 4<sup>th</sup> instars with LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron caused reduction in lipid content after 72 h comparing to controls (Fig. 3). Analysis of variance showed no significant differences among treatments compared with control after 48 h ( $F = 2.06$ ;  $df = 3, 11$ ;  $P = 0.1834$ ). But significant differences were observed between LC<sub>30</sub> and LC<sub>50</sub> concentrations and control after 72 h ( $F = 4.14$ ;  $df = 3, 11$ ;  $P = 0.0479$ ).

#### Protein

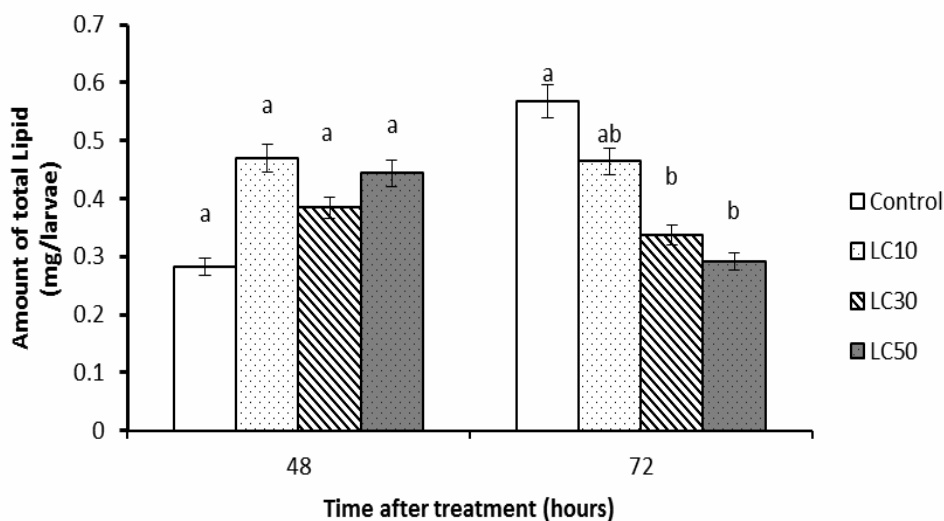
The protein content was reduced in larvae treated with LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron after 48 and 72 h as compared with control (Fig. 4). The amount of protein content after 72 h was 0.06 mg/larvae in LC<sub>50</sub> compared with 0.09 mg/larvae in control. Analysis of variance showed a significant difference among LC<sub>50</sub> and control after 48 h ( $F = 5.51$ ,  $df = 3, 11$ ,  $P = 0.0240$ ) and 72 h ( $F = 10.77$ ,  $df = 3, 11$ ,  $P = 0.0035$ ).

#### Glycogen

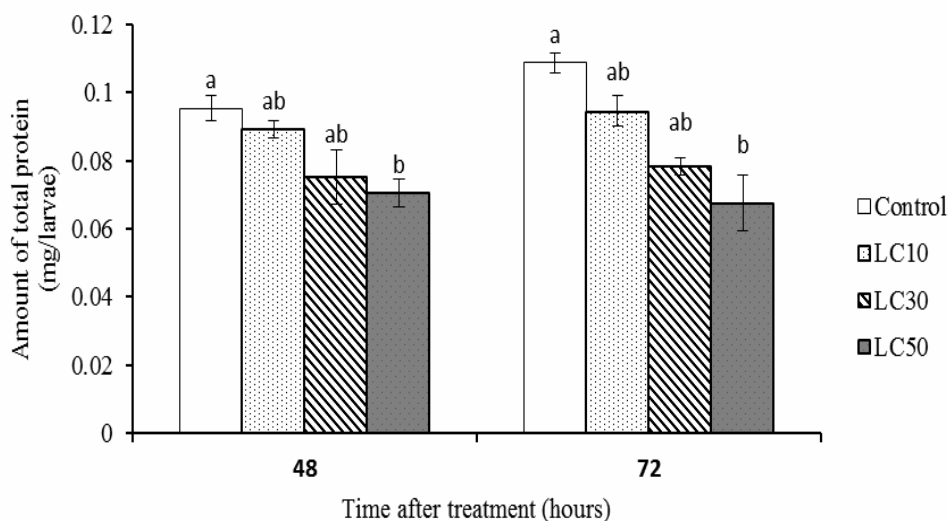
Treatment of 4<sup>th</sup> instar *G. pyloalis* with LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> lufenuron concentrations showed that glycogen content decreased 72 h after treatment (Fig. 5). The glycogen content was 0.02 mg/ larvae in LC<sub>50</sub> and 0.04 mg/larvae in control after 72 h. Analysis of variance showed no significant differences among concentrations compared with control after 48 h ( $F = 3.97$ ,  $df = 3, 11$ ,  $P = 0.0528$ ). However, there was a significant difference among LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> treatments compared with control after 72 h ( $F = 5.34$ ,  $df = 3, 11$ ,  $P = 0.0260$ ).



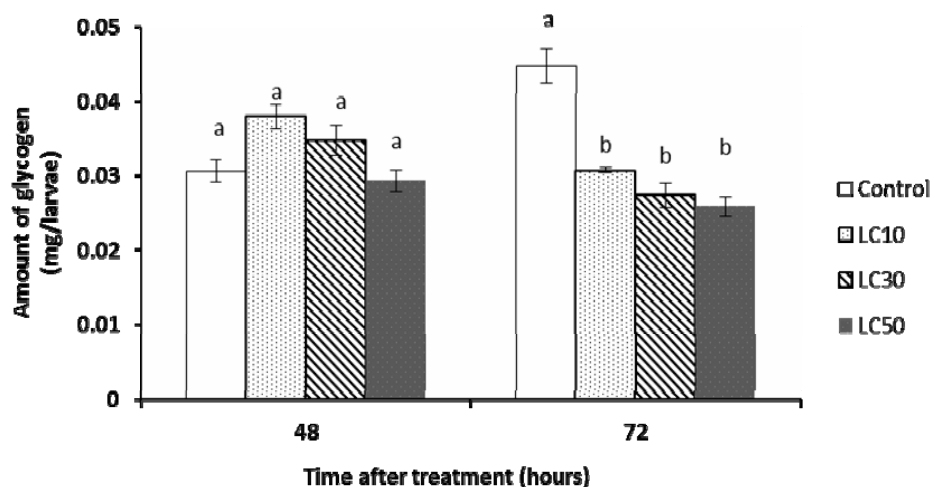
**Figure 2** Effect of LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron on mean total carbohydrate ( $\pm$  SE) in 4<sup>th</sup> instars of *Glyphodes pyloalis* at 48 h ( $F = 12.60$ ;  $df = 3, 11$ ;  $P < 0.0021$ ) and 72 h ( $F = 3946.53$ ;  $df = 3, 11$ ;  $P < 0.0001$ ) after treatment. Means within the same time after treatment marked with different letters are significantly different (Tukey's test;  $P < 0.05$ ).



**Figure 3** Effect of LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron on mean total lipid ( $\pm$  SE) in 4th instars of *Glyphodes pyloalis* at 48 h ( $F = 2.06$ ,  $df = 3, 11$ ,  $P = 0.1834$ ) and 72 h ( $F = 4.14$ ,  $df = 3, 11$ ,  $P = 0.0479$ ) after treatment. Means within the same time after treatment marked with different letters are significantly different (Tukey's test;  $P < 0.05$ ).



**Figure 4** Effect of LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> concentrations of lufenuron on mean total protein ( $\pm$  SE) in 4<sup>th</sup> larval instar of *Glyphodes pyloalis* at 48 h ( $F = 5.51$ ,  $df = 3, 11$ ,  $P = 0.0240$ ) and 72 h ( $F = 10.77$ ,  $df = 3, 11$ ,  $P = 0.0035$ ) after treatment. Means within the same time after treatment marked with different letters are significantly different (Tukey's test;  $P < 0.05$ ).



**Figure 5** Effect of LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> concentrations of lufenuron on mean total glycogen ( $\pm$  SE) in 4<sup>th</sup> larval instars of *Glyphodes pyloalis* at 48 h ( $F = 3.97$ ,  $df = 3, 11$ ,  $P = 0.0528$ ) and 72 h ( $F = 5.34$ ,  $df = 3, 11$ ,  $P = 0.0260$ ) after treatment. Means within the same time after treatment marked with different letters are significantly different (Tukey's test;  $P < 0.05$ ).

## Discussion

In this study, the effect of lufenuron was investigated on some biological and biochemical traits of *G. pyloalis*. Lufenuron showed highly toxic effects against *G. pyloalis* larvae in the present study. The lethal and sublethal concentrations of lufenuron caused mortality and malformation in 4<sup>th</sup> instar larvae of *G. pyloalis*. Malformed larvae unable to molt to 5<sup>th</sup> larval instar eventually died. Larval and pre-pupal mortality percentage increased with increasing the lufenuron concentrations. These results are consistent with results of Khajepour *et al.* (2012) who observed that lufenuron caused mortality in the last larval stage, defects in pupal and adult stages, and in some cases produced larval-pupal intermediates of *E. figulilella*. Reda *et al.* (2010) also reported mortalities in 2<sup>nd</sup> and 4<sup>th</sup> instars, morphological abnormalities of larvae, pupae and adults that emerged from 2<sup>nd</sup> and 4<sup>th</sup> instars of *S. littoralis* treated with an IGR, flufenoxuron (chitin synthesis inhibitor) the chemical structure and effects of which are similar to lufenuron.

The sublethal and lethal concentrations of lufenuron caused decreases in larval weight at

48, 72 and 96 h after treatment compared with control. The LC<sub>30</sub> and LC<sub>50</sub> concentrations reduced the body weight of *G. pyloalis*. Nevertheless these larvae survived for a longer duration before death or became pre-pupal.

Our results showed that lufenuron caused prolongation of larval, pre-pupal, and pupal developmental durations. Larval developmental duration was increased 1.49 and 1.93 times in LC<sub>30</sub> and LC<sub>50</sub> when compared with controls, respectively. Pre-pupal duration in LC<sub>30</sub> and LC<sub>50</sub> concentration treatment and pupal duration in LC<sub>30</sub> concentration treatment increased significantly. Mervat *et al.* (2012) found that the LC<sub>50</sub> concentration of lufenuron significantly prolonged duration of larval and pupal stages and significantly decreased larval weight of *P. gossypiella* (Saunders). Also, Zhu *et al.* (2012) reported that sublethal concentrations of hexaflumuron on the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars of *S. litura* caused prolongation of developmental time and a decrease in larval body weight. Also, their results showed that third instars treated with  $0.5 \mu\text{g ml}^{-1}$  hexaflumuron, died at 6<sup>th</sup> instar, while  $1.2 \mu\text{g ml}^{-1}$  hexaflumuron caused death of the 5<sup>th</sup> instars. Therefore no pupae formed. Khajepour



et al. (2012) reported that developmental time of the last instar larvae of *E. figulilella* were increased, when treated with lufenuron. Reda et al. (2010) reported the larval and pupal durations of *S. littoralis* were increased with increasing concentrations of flufenoxuron as compared with control. Butter et al. (2003) showed that 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instar larvae of *Helicoverpa armigera* (Huber) (Lepidoptera: Noctuidae) treated with lufenuron had swollen heads and were significantly, smaller than the controls. Also, their results showed that larval weight was reduced significantly and pupal duration was significantly extended in LC<sub>90</sub>, LC<sub>50</sub> and LC<sub>10</sub> concentrations of lufenuron as compared with control.

Our results showed that no pupa emerged from larval stages after treatments with LC<sub>50</sub> concentration of lufenuron. Treatment of 4<sup>th</sup> instar larvae of *G. pyloalis* with lufenuron decreased significantly rate of the adult emergence and longevity of female adults in LC<sub>30</sub> concentration as compared with control. The LC<sub>50</sub> concentration inhibited 100% of adult emergence. These findings are in agreement with results of Reda et al. (2010) who reported that flufenoxuron decreased percentages of the adult emergence of *S. littoralis* and Butter et al. (2003) that reported same reduction in adult emergence of *H. armigera* treated with lufenuron. In contrast to our results, Mervat et al. (2012) reported that LC<sub>50</sub> of lufenuron increased female and male longevity compared with controls.

Our result showed significant decrease in fecundity of *G. pyloalis* in LC<sub>30</sub> concentration of lufenuron. *P. gossypiella* larvae treated with LC<sub>50</sub> concentration of lufenuron had a high reduction in number of eggs laid by females (Mervat et al., 2010). Reda et al. (2010) reported a dose-dependent reduction in fecundity of *S. littoralis* after treating with flufenoxuron. Saenz-de-Cabezón et al. (2006) showed that lufenuron negatively affected the reproduction of *Lobesia botrana*, causing high percentage of sterility (90.2%). Also, lufenuron caused significant decline in fecundity of *H. armigera* (Butter et al., 2003). In these studies,

lufenuron showed morphological changes to the ovipositor, interfered with vitellogenesis, caused testicular reduction, and sperm transport incapacity.

The content of macromolecules (i.e. protein, carbohydrates and lipids) is good indicator of the level of metabolism in insects treated with chemicals (Zhu et al., 2012). In our study, treatment of 4<sup>th</sup> larval instar of *G. pyloalis* with LC<sub>10</sub>, LC<sub>30</sub> or LC<sub>50</sub> of lufenuron, affected the content of carbohydrate, lipid, protein and glycogen at 48 and 72 h after treatment. Carbohydrate content was reduced at 48 and 72 h after treatment. Decrease in carbohydrate content 72 h after treatment was much greater than that in controls. Treatments of *S. litura* with 0.1, 0.5, and 1.2 µg mL<sup>-1</sup> hexaflumuron showed that total carbohydrate slightly decreased in hemoplasm at 24 and 96 h after treatment (Zhu et al., 2012). Some of the reports indicated the possibility that benzoylphenyl ureas might affect the insect hormonal sites; thereby resulting in physiological disturbances such as inhibition of DNA synthesis (Soltani et al., 1984), altered carbohydrase and phenoloxidase activities (Ishaaya and Casida, 1974; Ishaaya and Ascher, 1977).

Our results showed that the lipid and glycogen content reduced 72- h after treatment and protein content reduced at 48 and 72 h after treatment compared with controls. This reduction could be due to the breaking the proteins into amino acids and their entry into the TCA cycle as keto acid (Schoonhoven, 1982) to compensate for lower energy caused by lufenuron stress. The present results are in agreement with those of Mervat et al. (2012) who reported LC<sub>50</sub> of lufenuron caused reduction in the total soluble protein and total lipid content as compared with control. Yazdani et al. (2012) reported a significant decrease in amounts of protein, lipids and carbohydrates of *G. pyloalis* treated with essential oil of Summer Savory, *S. hortensis* L. (Family: Lamiaceae). Assar et al. (2010) also found that the total protein content decreased in the house fly treated with lufenuron. Khosravi et al. (2010)

observed a significant decrease in the amount of lipid and total protein in larvae of *G. pyloalis* treated with *Artemisia annua*.

Recently, sublethal effects of insecticides on pest arthropod populations have attracted much attention and our results show that lethal and sublethal concentrations of lufenuron not only inhibited larval growth and extended the duration of development of *G. pyloalis*, but also affected the metabolism of carbohydrates, lipids, proteins, and glycogen. According to our results, because of its toxic effects on larvae and sublethal effects on pre-pupal, pupal, and adult stages of *G. pyloalis*, lufenuron may provide more benefits to an integrated pest management program for *G. pyloalis*.

## References

- Abbott, M. S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.
- Assar, A. A., Abo El-Mahase, M. M., Khalil, M. E. and Mahmoud, S. H. 2010. Biochemical effects of some insect growth regulators on the house fly, *Musca domestica* (Diptera: Muscidae). *Egyptian Academic Journal of Biological Sciences*, 2: 33-44.
- Aruga, H. 1994. *Principles of Sericulture*. CRC Press, 1<sup>st</sup> edition. Pp: 266.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, 72: 248-254.
- Butter, N. S., Gurmeet, S. and Dhawan, A. K. 2003. Laboratory evaluation of the insect growth regulator lufenuron against *Helicoverpa armigera* on cotton. *Phytoparasitica*, 31: 200-203.
- Etebari, K., Bizhannia, A. R., Sorati, R. and Matindoost, L. 2007. Biochemical changes in hemolymph of silkworm larval due to pyriproxyfen residue. *Pesticide Biochemistry and Physiology*, 88: 14-19.
- Hoffman, K. H. and Lorenz, M. W. 1998. Recent Advances in hormones in insect pest control. *Phytoparasitica*, 26: 323-330.
- Ishaaya, I. and Ascher, K. R. S. 1977. Effect of diflubenzuron on growth and carbohydrate hydrolases of *Tribolium castaneum*. *Phytoparasitica*, 5: 484-490.
- Ishaaya, I. and Casida, J. E. 1974. Dietary TH-6040 alters cuticle composition and enzyme activity of housefly larval cuticle. *Pesticide Biochemistry and Physiology*, 4: 484-490.
- Jaafari Khaljiri, Y., Rezaei, V. and Zargarpour Kazemian, P. 2006. Biological study of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae), a new pest of mulberry in Guilan Province, Iran. *Proceedings of the 17<sup>th</sup> Iranian Plant Protection Congress*, Karaj, Iran. P. 257.
- Kanchaveli, Sh., Partsvania, M., Tskvitaridze, O. and Lobzhanidze, M. 2009. *Proceedings of L.Kanchaveli Institute of Plant Protection, Plant Protection Problems*, 39: 159-166, Tbilisi.
- Khajepour, S., Izadi, H. and Asari, M. J. 2012. Evaluation of two formulated chitin synthesis inhibitors, hexaflumuron and lufenuron against the raisin moth, *Ephesia figulilella*. *Journal of Insect Science*, 12: 102. doi: 10.1673/031.012.10201.
- Khosravi, R. and Jalali Sendi, J. 2010. Biology and demography of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae) on mulberry. *Journal of Asia-Pacific Entomology*, 13: 273-276.
- Khosravi, R., Jalali Sendi, J. and Ghadamyari, M. 2010. Effect of *Artemisia annua* L. on deterrence and nutritional efficiency of lesser mulberry pyralid (*Glyphodes pyloalis* Walker) (Lepidoptera: Pyralidae). *Journal of Plant Protection Research*, 50: 423-428.
- LeOra Software. 1987. *POLO-PC A User's Guide to Probit or Logit Analysis*. LeOra Software, Berkeley, CA. USA.
- Madyarov, Sh. R., Khamraev, A. Sh., Otarbaev, D. O., Kamita, S. G. and Hammock, B. D. 2006. Comparative effects of wild and recombinant baculoviral insecticides on mulberry pyralid *Glyphodes pyloalis* Walker and mulberry silkworm *Bombyx mori*. *International Congress "Biotechnology"*. March 12-16, 2006, Moscow, Russia, pp. 230-231.

- Memarizadeh, N., Ghadamyari, M., Sajedi, R. H. and Sendi, J. 2011. Characterization of esterase from abamectin-resistant and susceptible strains of *Tetranychus urticae* (Acari: Tetranychidae). *International Journal of Acarology*, 37: 271-281.
- Mervat, A. K., Ahmed, A. F. and Hemat, Z. M. 2012. Toxicological and biochemical studies of lufenuron, chlorfluazuron and chromafenozide against *Pectinophora gossypiella* (Saunders). *Egyptian Journal of Biological Sciences*, 4: 37-47.
- Mikaia, N. 2011. The susceptibility of entomoparasitic nematode *Steinernema feltiae* on the mulberry moth larvae (*Glyphodes pyloalis* Walker). *Bulletin of The Georgian National Academy of Sciences*, 5: 95-98.
- Nation, J. L. 2008. *Insect Physiology and Biochemistry*, 2<sup>nd</sup> edition. CRC Press. 544 pp.
- Nath, B. S. 2003. Shifts in glycogen metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. *Pesticide Biochemistry and Physiology*, 74: 73-84.
- Oberlander, H., Silhacek, D. L., Shaaya, E. and Ishaaya, I. 1997. Current status and future perspectives of the use of insect growth regulators for the control of stored product pests. *Journal of Stored Products Research*, 33: 1-6.
- Reda, F. A. B., Mona, F. A. E., Mohamad H. A. and Hisham. M. E. A. 2010. Effect of chitin synthesis inhibitors (flufenoxuron) on some biological and biochemical aspects of the cotton leaf worm *Spodoptera littoralis* Bosid (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Sciences*, 2: 43-56.
- Ramdev, Y. P. and Rao, P. J. 1979. Consumption and utilization of castor, *Ricinus communis* Linn, by castor semilooper, *Achaea janata* Linn. *Indian Journal of Entomology*, 41: 260-266.
- Saenz-deCabezón, I. F. J., Perez-Moreno, I., Zalom, F. G. and Marco, V. 2006. Effects of lufenuron on *Lobesia botrana* (Lepidoptera: Tortricidae) egg, larval, and adult stages. *Journal of Economic Entomology*, 99: 427-431.
- SAS Institute. SAS users guide: Statistics, version 9, SAS Institute, Cary, NC. 2001.
- Soltani, N. and Mazouni, A. 1992. Diflubenzuron and oogenesis in the codling moth, *Cydia pomonella*. *Pesticide Science*, 34: 257-261.
- Schoonhoven, L. M. 1982. Biological aspects of antifeedants. *Entomologia Experimentalis et Applicata*, 31: 57-89.
- Yazdani, E., Jalali Sendi, J., Khosravi, R., Hajizadeh, J. and Ghadamyari, M. 2012. Effect of *Satureja hortensis* L. essential oil on feeding efficiency and biochemical properties of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). *Archives of Phytopathology and Plant Protection*, 46: 328-339.
- Van Handel, E. and Day, J. F. 1988. Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in field collected *Aedes vexans*. *Journal of the American Mosquito Control Association*, 4: 549-550.
- Whiting, D. C., Jamieson, L. E. and Conolly, P. G. 2000. Pre and postharvest effects of lufenuron on *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Journal of Economic Entomology*, 93: 673-679.
- Zhu, Q., He, Y., Yao, J., Liu, Y., Tao, L. and Huang, Q. 2012. Effects of sublethal concentrations of the chitin synthesis inhibitor, hexaflumuron, on the development and hemolymph physiology of the cutworm, *Spodoptera litura*. *Journal of Insect Science*, 12: 27. doi: 10.1673/031.012.2701.

## اثرات کشنده و زیرکشنده لوفنورون، بازدارنده‌ی سنتز کیتین، روی *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae)

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دریافت: ۱ خرداد ۱۳۹۴؛ پذیرش: ۲۵ آبان ۱۳۹۴

**چکیده:** پروانه برگ‌خوار توت *Glyphodes pyloalis* Walker یکی از آفات جدی درختان توت در ایران است. در این مطالعه، اثر غلظت‌های کشنده ( $LC_{50} = 19$  ppm) و زیرکشنده ( $LC_{10}$  و  $LC_{30} = 9.77$  ppm) لوفنورون روی لاروهای سن چهارم این آفت ارزیابی شد. بعد از تیمار لاروهای سن چهارم، بیش‌تر لاروها طی فرآیند پوست‌اندازی مردند و فقط تعداد اندکی از آنها توانستند به لاروهای سن ۵ تبدیل شوند. بالاترین میزان مرگ‌ومیر در مراحل لارو سن پنجم (۶۸،۴۲٪) و پیش‌شفیرگی (۵۹،۵۷٪) در غلظت  $LC_{50}$  مشاهده شد. طول دوره‌ی لاروی، پیش‌شفیرگی و شفیرگی افزایش یافت. درصد شفیرگی در غلظت  $LC_{30}$  به ۵۳/۶۴ درصد کاهش یافت. هم‌چنین، هیچ‌یک از پیش‌شفیره‌های غلظت  $LC_{50}$  به شفیره تبدیل نشدند. در مقایسه با شاهد، وزن لاروها به‌طور معنی‌داری ۷۲،۴۸ و ۹۶ ساعت بعد از تیمار کاهش یافت. ظهور حشرات کامل و طول عمر حشرات ماده نیز در غلظت  $LC_{30}$  کاهش یافت. غلظت  $LC_{30}$  لوفنورون به‌طور منفی تولید مثل *G. pyloalis* را تحت تأثیر قرار داد. محتوای کربوهیدرات و پروتئین لاروها ۴۸ و ۷۲ ساعت پس از تیمار و محتوای چربی و گلیکوژن ۷۲ ساعت پس از تیمار، با غلظت‌های  $LC_{10}$ ،  $LC_{30}$  و  $LC_{50}$  به‌طور معنی‌داری کاهش یافت. نتایج نشان‌دهنده اثرات مضر غلظت‌های کشنده و زیرکشنده لوفنورون روی برخی پارامترهای زیستی و بیوشیمیایی است که لازم به بررسی بیش‌تر برای کاربرد آن در مدیریت تلفیقی پروانه برگ‌خوار توت است.

**واژگان کلیدی:** *Glyphodes pyloalis*، لوفنورون، باروری، اثرات زیرکشنده، تنظیم‌کننده‌های رشد