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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 4th 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 5th larval instar. The highest rate of mortality was observed in 5th 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

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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 lepidopteron 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_{50} 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 carbohydratesin *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^{th} 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_{10}, LC_{30} and LC_{50} 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^{th} instars to lufenuron. Five

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concentrations (5, 8.12, 13.48, 22.38, 40 mg/L) of a commercial formulation of lufenuron (Match® 50 EC, Syngenta; chemical formula: N-[[[2,5 dichloro-4-(1,1,2,3,3,3 hexafluoro-propoxy)- phenyl]carbonyl]-2,6-difluoro-benzamide (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 4th instar larvae. Ten 4th 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_{10}, LC_{30} and LC_{50} were calculated using probit analysis by Polo-PC software (Leora Software, 1987).

Effects of lufenuron on biological parameters

Four hundred newly molted 4th instars in 20 replicates for each concentration (20 larvae/ replicate) from laboratory colony were selected randomly and exposed to LC_{30} and LC_{50} 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, 4th instars were treated with LC_{10}, LC_{30} or LC_{50} concentrations. After 48 and 72 h, the alive larvae were randomly selected and their bodies were homogenized in either sodium sulphate buffer solution (Na_2SO_4 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 4th instars

The LC_{10}, LC_{30} and median lethal concentrations (LC_{50}) of lufenuron with their confidence limits on 4th instars are shown in Table 1. The LC_{10}, LC_{30} and LC_{50} were estimated as 3.74, 9.77 and 19 ppm, respectively.

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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_{30} or LC_{50} concentrations of lufenuron or water controls are listed in Table 2. The highest rate of 5th instars mortality was found after exposure to the LC_{50} concentration, compared with 5.88% mortality in controls. There was significant difference among treatments with control in the mortality rate of the 5th larval instar ($F = 14.31$, df = 2, 16, $P = 0.0004$). Mortality rate in pre-pupae in LC_{50} concentration was higher than that of LC_{30} and control ($F = 8.08$, df = 2, 8, $P = 0.0199$). All pre-pupae in LC_{50} 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_{30}) and lethal (LC_{50}) 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_{30} concentration, 9.75, 7.97 and 6.01 mg/ larvae in LC_{50} 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_{30} or LC_{50} 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_{30} 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_{50}) died and only 53.64% of them developed to pupa at the lower concentration (LC_{30}) (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_{30} concentration as compared with control (Table 3). The adult emergence at LC_{30} (42.85%) was significantly lower than control (92.30%). ($t = 2.51$, df = 19, $P = 0.0214$). The LC_{50} concentration caused 100% inhibition of adult emergence.

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

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

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

N[1]	LC_{10} (95% CL)[2]	LC_{30} (95% CL)[2]	LC_{50} (95% CL)[2]	Slop ± SE	χ^2	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

[1] Number of treated larvae.
[2] 95% confidence limits (CL) in parenthesis.

Table 2 Effects of LC_{30} and LC_{50} concentrations of lufenuron on developmental time and mortality of *Glyphodes pyloalis*.

Treatment	Larval period ± SE (day)[1]	Larval mortality ± SE (%)[1]	Pre-pupal period ± SE (day)[1]	Pre-pupal mortality ± SE (%)[1]	Pupation ± SE (%)[1]	Pupal period ± SE (day)[2]
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_{30}	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_{50}	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

[1] Means followed by different letters in a column are significantly different (Tukey's test P < 0.05).
[2] Means followed by different letters in a column are significantly different (t-student test).
[3] Number of died pre-pupae were considered for 10 days.

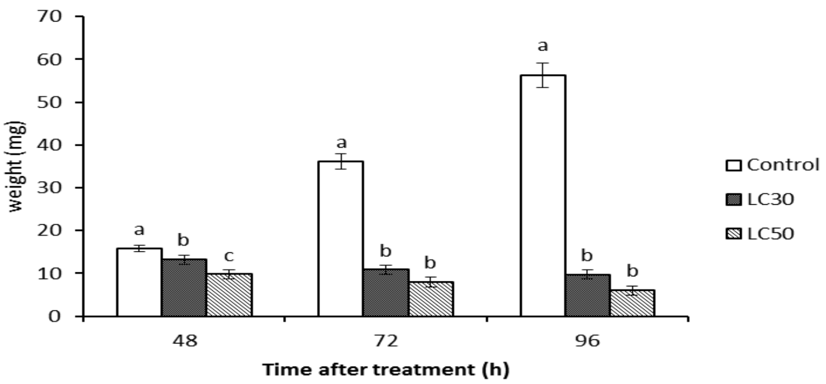

Figure 1 Mean fresh weight of 4th instars of *Glyphodes pyloalis* exposed to LC_{30} and LC_{50} concentrations of lufenuron at 48 h (F = 27.80, df = 2, 19, $P < 0.0001$), 72 h (F = 126.97, df = 2, 20, $P < 0.0001$) and 96 h (F = 168.17, df = 2, 20, $P < 0.0001$) after treatment. Means within the same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

Table 3 Effect of LC_{30} or LC_{50} concentrations of lufenuron on adult emergence, total inhibition of adult emergence, adult longevity, and fecundity of *Glyphodes pyloalis* treated at 4th laraval 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[ns]	87 ± 6.88
LC_{30}	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.

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Effect on energy reserves

Carbohydrate

Carbohydrate content in 4th instars treated with LC_{10}, LC_{30} or LC_{50} 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_{50} concentration, 72 h after treatment (0.24 ± 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 4th instars with LC_{10}, LC_{30} or LC_{50} 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_{30} and LC_{50} 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_{10}, LC_{30} or LC_{50} 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_{50} compared with 0.09 mg/larvae in control. Analysis of variance showed a significant difference among LC_{50} 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 4th instar *G. pyloalis* with LC_{10}, LC_{30} or LC_{50} lufenuron concentrations showed that glycogen content decreased 72 h after treatment (Fig. 5). The glycogen content was 0.02 mg/ larvae in LC_{50} 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_{10}, LC_{30} and LC_{50} treatments compared with control after 72 h (F = 5.34, df = 3, 11, P = 0.0260).

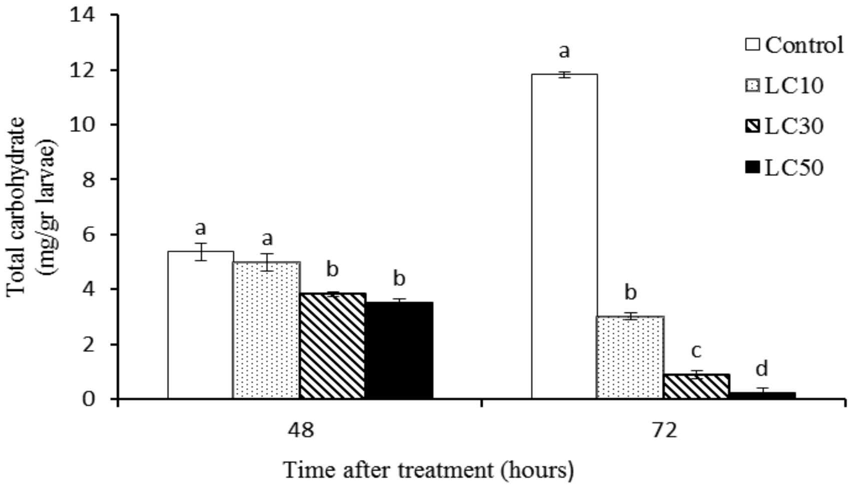

Figure 2 Effect of LC_{10}, LC_{30} or LC_{50} concentrations of lufenuron on mean total carbohydrate (± SE) in 4th instars of *Glyphodes pyloalis* at 48 h (F = 12.60; df = 3,11; $P < 0.0021$) and 72 h (F = 3946.53; df = 3, 11; $P <$ 0.0001) after treatment. Means within the same time after treatment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

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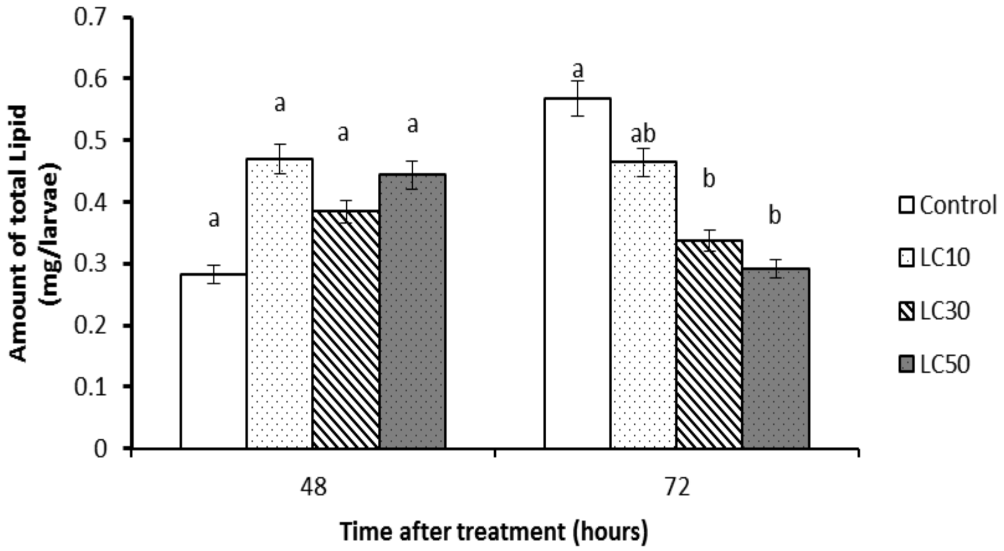

Figure 3 Effect of LC_{10}, LC_{30} or LC_{50} concentrations of lufenuron on mean total lipid (± SE) in 4th instars of *Glyphodes pyloalis* at 48 h (F = 2.06, df = 3, 11, $P = 0.1834$) and 72 h (F = 4.14, df = 3, 11, $P = 0.0479$) after treatment. Means within the same time after treatment marked with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

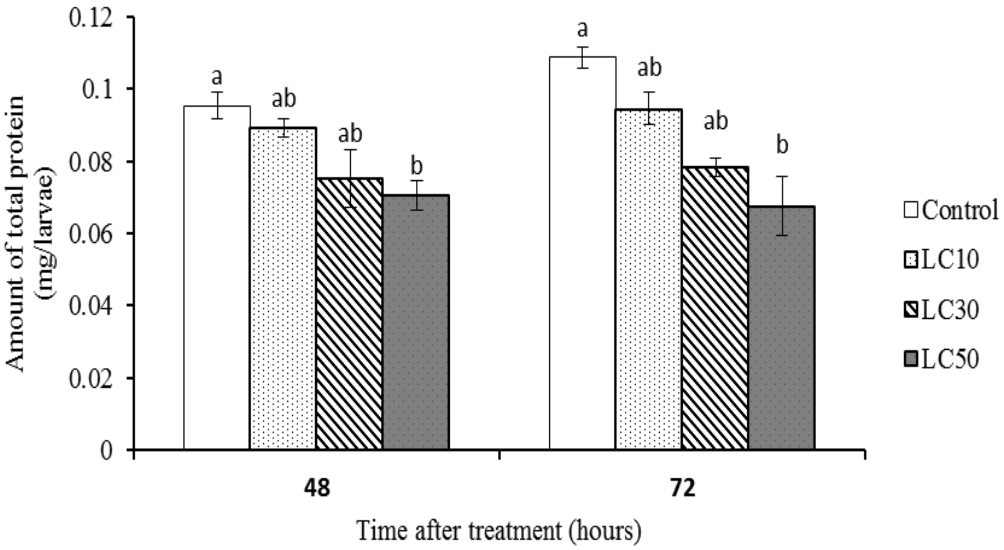

Figure 4 Effect of LC_{10}, LC_{30} or LC_{50} concentrations of lufenuron on mean total protein (± SE) in 4^{th} larval instar of *Glyphodes pyloalis* at 48 h (F = 5.51, df = 3, 11, $P = 0.0240$) and 72 h (F = 10.77, df = 3, 11, P = 0.0035) after treatment. Means within the same time after treatment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

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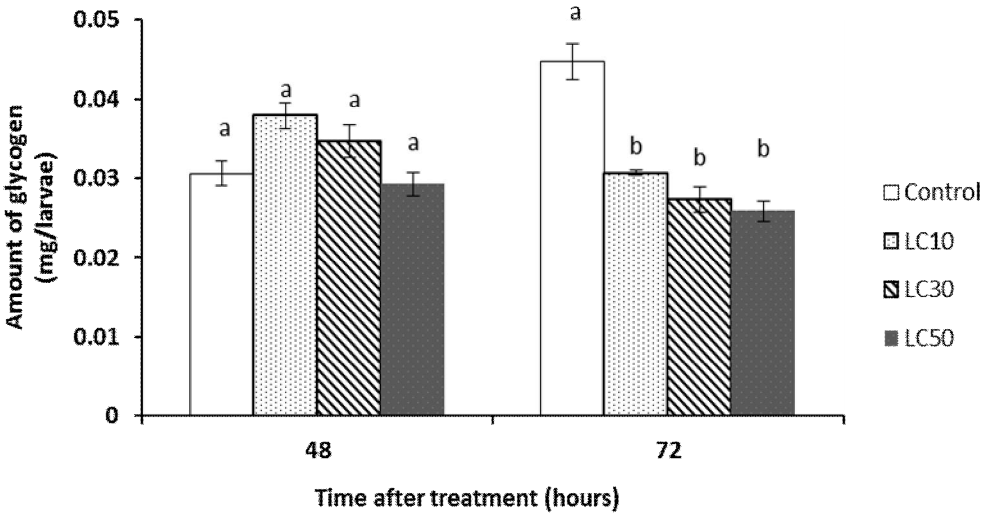

Figure 5 Effect of LC_{10}, LC_{30} and LC_{50} concentrations of lufenuron on mean total glycogen (± SE) in 4th larval instars of *Glyphodes pyloalis* at 48 h (F = 3.97, df = 3, 11, *P* = 0.0528) and 72 h (F = 5.34, df = 3, 11, *P* = 0.0260) after treatment. Means within the same time after treatment 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 to mortality and malformation in 4th instar larvae of *G. pyloalis*. Malformed larvae unable to molt to 5th 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 2nd and 4th instars, morphological abnormalities of larvae, pupae and adults that emerged from 2nd and 4th 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_{30} and LC_{50} 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_{30} and LC_{50} when compared with controls, respectively. Pre-pupal duration in LC_{30} and LC_{50} concentration treatment and pupal duration in LC_{30} concentration treatment increased significantly. Mervat *et al.* (2012) found that the LC_{50} 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 3rd, 4th and 5th 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 μg ml^{-1} hexaflumuron, died at 6th instar,while 1.2 μg ml^{-1} hexaflumuron caused death of the 5th instars. Therefore no pupae formed. Khajepour

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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 1st, 2nd, 3rd, 4th and 5th 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_{90}, LC_{50} and LC_{10} concentrations of lufenuron as compared with control.

Our results showed that no pupa emerged from larval stages after treatments with LC_{50} concentration of lufenuron. Treatment of 4th instar larvae of *G. pyloalis* with lufenuron decreased significantly rate of the adult emergence and longevity of female adults in LC_{30} concentration as compared with control. The LC_{50} 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_{50} of lufenuron increased female and male longevity compared with controls.

Our result showed significant decrease in fecundity of *G. pyloalis* in LC_{30} concentration of lufenuron. *P. gossypiella* larvae treated with LC_{50} 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-Cabezon *et al.* (2006) showed that lufenuron negatively affected the reproduction of *Lobesia botrana,* causing high percentage of sterility (90.2%). Also, lufenuron caused significant declinein 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 4th larval instar of *G. pyloalis* with LC_{10}, LC_{30} or LC_{50} 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^{-1} hexaflumuron showed that total carbohydrate slightly decreased in hematoplasma 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_{50} 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)

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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*.

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

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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_{30} = 9.77 ppm و LC_{10} = 3.74) 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*، لوفنورن، باروری، اثرات زیرکشنده، تنظیم کننده‌های رشد

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