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Biological and biochemical effects of lufenuron on *Xanthogaleruca luteola* (Muller, 1766) (Coleoptera: Chrysomelidae)

Bahareh MOHAMMADZADEH TAMAM [1], Mohammad GHADAMYARI [1, 2], Elaheh SHAFIEI ALAVIJEH [1]

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Biological and biochemical effects of lufenuron on *Xanthogaleruca luteola* (Muller, 1766) (Coleoptera: Chrysomelidae)

Abstract: *Xanthogaleruca luteola* (Mull., 1766) is the major defoliator pest of elm trees in urban area. In this study the effect of lufenuron on some biochemical and biological characteristics was investigated on *X. luteola*. The LC_{30} and LC_{50} of lufenuron were determined on the second instar larvae as 20.22 and 36.65 mg l^{-1}, respectively. Effects of LC_{30} and LC_{50} concentrations of lufenuron on some biological parameters showed that lufenuron caused an increase in larval, pre-pupal and pupal developmental periods. Also, none of the female insects that emerged from the treated larvae did not spawn during their life. The LC_{50} concentration of lufenuron decreased carbohydrate, lipid and protein content and increased glycogen content. But there was not a significant difference in glycogen, and protein contents following the exposure to LC_{30} concentration. However, glutathione-s-transferase (GST) and esterase activities were significantly increased at LC_{50}. In conclusion, due to lethal and sublethal effect of lufenuron on biochemical and biological traits of *X. luteola*, it can be recommended for control this pest in IPM program.

Key words: *Xanthogaleruca luteola*; lufenuron; developmental periods; sublethal effects; biochemical parameters

Biološki in biokemični učinki lufenurona na hrošča *Xanthogaleruca luteola* (Muller, 1766) (Coleoptera: Chrysomelidae)

Izvleček: Hrošč *Xanthogaleruca luteola* Mull je najpomembnejši defoliator brestov v urbanem okolju. V raziskavi so bili preučevani učinki lufenurona na nekatere biokemične in biološke lastnosti tega hrošča. LC_{30} in LC_{50} lufenurona sta bili določeni na drugem razvojnem štadiju ličink in sicer 20,22 in 36,65 mg l^{-1}. Učinki LC_{30} in LC_{50} koncentracij lufenurona na nekatere biološke parametre so pokazali, da je lufenuron povzročil povečanje razvojnih obdobij ličinke, obdobja pred zabubljenjem in obdobja bube. Nobena od samic, ki so se izlegle iz obravnavanih ličink v celotnem življenskem obdobju ni odlegla jajčec. Koncentracija LC_{50} je zmanjšala vsebnost ogljikovih hidratov, maščob in beljakovin ter povečala vsebnost glikogena, ni pa bilo značilnih razlik v vsebnosti glikogena in beljakovin pri izpostavitvi. LC_{30} koncentraciji. Aktivnosti glutation-s-transferaze (GST) in esterase sta se pri izpostavitvi LC_{50} značilno povečali. Zaključujemo, da bi zaradi letalnih in subletalnih učinkov lufenurona na biokemične in biološke lastnosti tega hrošča to sredstvo lahko priporočili za uravnavanje škodljivcev in v programih integriranega uravnavanja škodljivcev.

Ključne besede: *Xanthogaleruca luteola*; lufenuron; razvojna obdobja; subletalni učinki; biokemični parametri

1 Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran
2 Corresponding author, e-mail: ghadamyari@guilan.ac.ir

1 INTRODUCTION

The elm leaf beetle, *Xanthogaleruca luteola* (Muller, 1766) (Coleoptera: Chrysomelidae), is one the most destructive pests of elm trees in Iran. This beetle in both larval and adult stages by feeding on the elm leaves (**Ulmus** spp.) causes severe injurious to trees. In addition to defoliation and morphological changes, this pest causes physiological stress which increases the elm susceptibility to secondary pests and diseases. Additionally, *X. luteola* transmits the fungi spores of Dutch elm disease, *Ophiostoma* (*Ceratocystis*) *novo-ulmi* Brasier, that assumption the serious menace to these trees (Huerta et al., 2010). Due to the widespread plantation of elm trees in urban areas, the application of pesticides against *X. luteola* poses some problematic side-effects on human societies. Therefore, the application of pesticides with high selectivity to this pest and low toxicity to humans and environment is highly appreciated (Defagó et al., 2006).

The estimation of insecticide effects are accessed by lethal and sublethal studies through mortality assays and observation of biology, physiology, behavior and demographic aspects of insect pests and natural enemies(De França et al., 2017). Among the insecticides, insect growth regulators (IGRs) seem to have most adverse effects on insect pests. IGRs may affect the development of insect pests by the interruption of the molting process and cuticle formation, as well as disruption in the endocrine system of insects (Desneux et al., 2007). IGR compounds play a crucial role in control of insect pests, especially pests associated in urban area. Because of specificity in their mode of action and safety to humans, wild life and the environment, these compounds are suitable for pest control than other synthetic insecticides (Tunaz & Uygun, 2004).

Chitin synthesis inhibitors is categorized as IGR which have been detected for controlling of wide variety of immature insect pests (Tunaz and Uygun, 2004). Lufenuron (IRAC group 15) is a benzoylureas that classified as an inhibitors of chitin biosynthesis affecting chitin synthase 1 on insects (Dhadialla et al., 2009; IRAC, 2020) which has been successfully effective against pest species from Lepidoptera, Coleoptera, Hemiptera, Diptera and Thysanoptera (FAO, 2008) due to larvicidal effect along with transovarial–ovicidal and ovicidal effects (Yasir et al., 2019; Abdel Rahman, 2017). Based on results of Arruda et al. (2020), resistance inheritance to lufenuron was incompletely recessive, autosomal, and monogenic in *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae). Due to autosomal and recessive nature, resistance to this compound spreads at low rate lufenuron was registered against lepidopteran and psylla in Iran (Nourbakhsh, 2019).

The increasing in larval, pre-pupal and pupal developmental periods were reported in lufenuron affection in sublethal treatment of *Glyphodes pyloalis* Walker, 1859; which was associated reduction in fecundity and fertility of female adults (Piri Aliabadi et al., 2016). The reduction of glucose, protein and carbohydrate contents has been reported in IGRS treatments of *Pectinophora gossypiella* (Saunders, 1844) (Lepidoptera: Gelechiidae) (Kandi et al., 2012).

Toxicity and sublethal effects of lufenuron on elm leaf beetle has not been studied; besides, based on the mode of action of this pesticide, it seems that this compound is appropriate for control of this pest. The objective of this research was evaluation of the toxicity of lufenuron on *X. luteola*. Subsequently, some biological and biochemical parameters on 2th instar larvae were directed at LC_{30} and LC_{50} levels.

2 MATERIAL AND METHODS

2.1 CHEMICALS

Lufenuron (Match®, EC 50) was prepared from Syngenta Crop Protection (Iran, https://www.syngenta.ir/product/crop-protection/insecticide/match). Other chemical materials were purchased from Merck (Darmstadt, Germany), Wako (Tokyo, Japan) and Fluka (Buchs, Switzerland).

2.2 LABORATORY MASS CULTURE OF *X. LUTEOLA*

The elm leaf beetle adults were collected from University of Guilan campus (Rasht, Guilan province, Iran) without history of pesticide application. Insects were reared under laboratory conditions on the elm leaves at 25 ± 2 °C, 16:8 photoperiod (L:D) and 75 % relative humidity (RH). Transparent plastic jars (10 cm × 7 cm × 5 cm) containing holes in the lids were used for the rearing of larvae and adults. In order to obtain larvae in the same age for bioassay tests, each pair of male and female adults was kept in similar plastic jars and the laid eggs were put in a new container (14 cm × 12 cm × 5 cm) in laboratory condition as mentioned above, daily.

2.3 BIOASSAY

Bioassay tests were carried out on 2th instar larvae based on leaf-dip method (Memarizadeh et al., 2011). Five concentrations (10, 17.78, 31.62, 56.23 and 100 mg

l^{-1}) of lufenuron were used for determination of LC_{30} and LC_{50} which were diluted in distillated water. Elm leaf discs (3 cm × 3 cm) were dipped in the desired concentrations for 30 seconds and dried at room temperature for 30 min before being offered to *X. luteola* larvae. The distillated water was used as control. Ten 2th instars were transferred to each plastic container containing treated leaf. Five replications were used for each treatment. Mortality was recorded 72 h after treatment. The LC_{30} and LC_{50} values were calculated using the Polo-PC software (Software, 1987).

2.3.1 Sublethal and lethal assays

The evolution of sublethal and lethal effects of lufenuron in LC_{30} (20.22 mg l^{-1}) and LC_{50} concentrations (36.65 mg l^{-1}) were studied on 2th instar larvae with leaf-dip method. Larvae were fed on treated leaves for 48 h. Then, alive larvae were transferred to plastic jars which were nourished with fresh leaves up to adult emergence. During this test, mortality, larvae duration, pre-pupal duration, pupal duration, and fecundity of female adults were recorded.

2.3.2 Biochemical assay

2.3.2.1 Amounts of carbohydrate, lipid, and glycogen

Biochemical assays were carried out on treated 2th instars with LC_{30} or LC_{50} doses of lufenuron. After 48 h, the whole body of surviving larvae were homogenized in sodium sulphate buffer solution (Na_2SO_4 2 %) for determination of carbohydrate (Singh & Sinha, 1977), lipid and glycogen contents (Yuval et al., 1998).

2.3.2.2 Esterase activity measurement

Esterase activity (Van Asperen, 1962) and glutathione-s-transferase (GST) activity (Habig et al., 1974) were measured based on using α- and β-naphthyl acetate (NA) and 1-choloro-2,4-dinitrobenzene (CDNB) as substrates, respectively. Three to four replicates were conducted for all previously mentioned enzyme assays.

Protein concentration was measured according to Bradford (1976) method with bovine serum albumin as standard.

Table 1: Determination of sublethal and lethal concentrations of lufenuron on 2th instar larvae of *Xanthogaleruca luteola*

	Concentration (mg l^{-1})	CL*
LC_{30}	20.22	14,71-25,93
LC_{50}	36.65	29,45-46,38

*CL (confidence limits) which has been calculated with 95 % confidence

2.4 DATA ANALYSIS

All statistical analyses were performed using SAS software ($p \leq 0.05$). Tukey's test statistic was used as comparison means (Rodenhouse et al., 2004).

3 RESULTS

The LC_{30} and LC_{50} values were determined as 20.22 and 36.65 mg l^{-1}, 72 h after treatment, respectively which presented in Table 1. These concentrations were used as lethal and sublethal concentrations for the following experiments.

3.1 SUBLETHAL EFFECTS OF LUFENURON ON BIOLOGICAL PARAMETERS

3.1.1 Developmental periods

The larval developmental duration was increased by 13.38 % and 27.06 %, when the larvae treated with LC_{30} and LC_{50} concentrations, respectively. Also, LC_{30} and LC_{50} concentrations were increased the pre-pupal by 13.2 % and 17.5 % and pupal by 16.65 % and 26.74 %, respectively. Totally developmental periods were significantly increased at LC_{30} and LC_{50} concentrations in comparison to the control which have been reported in Table 2. The investigation on female fecundity showed that emerged females from the treated 2th instar larvae did not oviposit any eggs during their lifetime have been lasted 10 days.

3.2 SUBLETHAL EFFECTS OF LUFENURON ON ENERGY RESERVES

Significant differences were observed in carbohydrate and lipid contents of the larvae in LC_{30} concentration of lufenuron in comparison to the control which was significantly decreased 29.1 % and 45.44 %, respectively. However, protein and glycogen contents in this sublethal concentration showed non-significant differences in comparison controls. The protein content was decreased by 12.38 % and glycogen content was increased significantly by 17.79 % (Table 3).

Table 2: Life stages duration of *Xanthogaleruca luteola* after treatment with lufenuron

Treatment	larval developmental duration (3^{th} instar larvae) (day) ± SE*	Pre-pupal duration (day) ± SE**	Pupal duration (day) ± SE	Fecundity (%)
Control	6.93 ± 0.06[c]	2.17 ± 0.06[c]	6.96 ± 0.08[c]	41.01
LC_{30}	8± 0,08[b]	2.5 ± 0.12[a]	8.35 ± 0.19[b]	0
LC_{50}	9.5 ± 0.12[a]	2.63 ± 0.15[a]	9.5 ± 0.18[a]	0

*Means followed by the same letter do not differ significantly ($p \leq 0.05$).
**SE: Standard Error
Larval developmental duration (F = 421.86, df = 2, 66, p value < 0.0001)
Pre-pupal duration (F = 9.88, df = 2,53, p value = 0.0002)
Pupal duration (F = 62.54, df = 2,45, p value < 0.0001)

The LC_{50} treatment was associated with significantly decreasing carbohydrate, lipid, and protein contents in comparison with untreated larvae, 27.8 %, 60.37 %, and 24.9 %, respectively, while glycogen content was significantly increased by 45.56 % (Table 3).

3.3 SUBLETHAL EFFECTS ON DETOXIFICATION ENZYME

3.3.1 Total esterase activity

The esterase activity was increased by LC_{50} concentration 52.16 % and 62.75 %, respectively; when α-NA and β-NA used as substrates. Whereas, there were no significant differences between LC_{30} concentration and control (Table 4)

3.3.2 GST activity

The GST activity were increased by 14.64 % and 69.71 %, when treated by LC_{30} and LC_{50} concentrations, respectively, which was significant at LC_{50} (Table 4).

4 DISCUSSION

Investigation on sublethal effects of insecticides might be associated with variations in life history characteristics as growth developmental stages, fecundity, fertility (Stark & Banks, 2003; Saber et al., 2013; Rehan & Freed, 2015; Su et al., 2022), in addition to behavioral and physiological disturbances (Desneux et al., 2007). In this study, bioassay results showed that lufenuron is effective against *X. luteola* and LC_{50} was determined as 36.6 mg l^{-1}. The results of present study showed that LC_{50} and LC_{30} concentrations had the considerable effects on the developmental stages and fecundity of emerged female adults of *X. luteola*. Sublethal concentrations of lufenuron increased developmental stages in larvae, pre-pupal and pupal after the 2^{th} instar larvae treated with LC_{30} and LC_{50} concentrations which were longer in LC_{50} concentration.

This result is in consistent with the results of Kandi et al. (2012) which showed that lufenuron in LC_{50} concentration increased the larval and pupal durations in *Pectinophora gossypiella*. Besides, the reducing in the adult longevity, fertility and pupal weight of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) was reported in the sublethal treatment of lufenuron, methoxyfenozide, spinosad, endosulfan, novaluron and

Table 3: Effects of lufenuron on energy reserve of 2^{th} instar larvae resulting from treated second instar larvae of *Xanthogaleruca luteola*

Treatment	Carbohydrate (mg/Larvae) ± SE*	Protein (mg/Larvae) ± SE**	Glycogen (mg/Larvae) ± SE	Lipid (mg/Larvae) ± SE
Control	77.7 ±2.2[a]	71.9 ± 2.1[a]	66.1 ± 6.8[b]	603 ± 2.8[a]
LC_{30}	55.1 ± 1.6[b]	63 ± 0.8[ab]	80.4 ± 1.6[b]	329 ± 14.2[b]
LC_{50}	56.1 ±1.6[b]	54 ± 2.4[b]	121.4 ± 3.6[a]	239 ± 12.8[c]

*Means followed by the same letter do not differ significantly ($p \leq 0.05$).
**SE: Standard Error
The carbohydrate contents (F = 19.61, df = 2,6, p value = 0.0023)
The lipid contents (F = 286.11, df = 2, p value = 0.0004)
The protein content (F = 6.93, df = 2,6, 11, p value = 0.028)
The glycogen content (F = 1.24, df = 2,6, p value = 0.354)

Table 4: Effects of lufenuron on enzyme activities of 2th instar larvae resulting from treated second instar larvae of *Xanthogaleruca luteola*

Treatment	α-Esterase (μmol.min^{-1}.mg protein^{-1}) ± SE*	β-Esterase (μmol.min^{-1}.mg protein^{-1}) ± SE**	GST (μmol.min^{-1}.mg protein^{-1}) ± SE
Control	367.537 ± 14.496^{b}	36.703 ± 0.903^{b}	6.876 ± 1.593^{b}
LC_{30}	442.370 ± 84.631^{b}	55.084 ± 3.915^{b}	8.055 ± 1.004^{b}
LC_{50}	768.221 ± 34.693^{a}	98.506 ± 10.387^{a}	22.7 ± 2.9^{a}

*Means followed by the same letter do not differ significantly ($p \leq 0.05$).
**SE: Standard Error
The α-NA activity (F = 15.88, df = 2.6, *p* value = 0.004)
The β-NA activity (F = 26.55, df = 2.6, *p* value = 0.0124)
The GST activity (F = 19.41, df =2 , *p* value = 0.0192)

tebufenozide (Storch et al., 2007). Also, the delay in the developmental duration of *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) in parasitizing of *Diatraea flavipennella* (Box, 1931) (Lepidoptera: Crambidae) was observed in sublethal affection of lufenuron (Fonseca et al., 2015). Evaluation of flufenoxuron on biological parameters was associated with increasing the larval and pupal periods and morphogenic abnormalities in developmental stages of *Spodoptera littoralis* (Boisduval, 1833) (Reda et al., 2010).

In this study, the fecundity and reproduction of *X. luteola* was influenced by lufenuron. Females that emerged from the treated larvae with LC_{30} and LC_{50} doses of lufenuron did not lay any eggs during their lifetime. The effect of lufenuron on fertility has been attributed to morphological changes in the ovipositor, interference with vitellogenesis, testicular size reduction, and sperm transport incapacity (Sáenz-de-Cabezón et al., 2006). Decreased fertility in IGR-treated insects may be associated with IGR intervention in egg protein accumulation, vitellogenesis synthesis, uptake, and ovariole growth (Pineda et al., 2007). The results are accordance with the reduction of oviposition period in treatment with lufenuron (Josan & Singh, 2000), and cantharidin, selective inhibitor of protein phosphatase 2A (Zhang et al., 2003), has been reported on *Plutella xylostella* (L., 1758) (Huang et al., 2015). Hexaflumuron decreased the oviposition period, egg numbers, and adult emergence of *P. xylostella* (Mahmoodvand *et al*., 2012). Embryonic development changes have been reported in azadirachtin, lufenuron and deltamethrin sublethal treatments on *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) (Correia et al., 2013). In addition, fecundity declined in LC_{50} value of lufenuron on *Heticoverpa armigera* (Hubner, (1808)) (Butter et al., 2003). The percentage of egg hatching was reduced as a dose-dependent manner when *S. littoralis* treated with flufenoxuron (Reda et al., 2010).

On the other hand, the exposure to insecticide sublethal concentrations could influence the biological, physiological and biochemical parameters such as carbohydrates, lipids, and proteins content (Klowden, 2013). When an insect is treated with insecticide, high level energy consumption occurs during detoxification of insecticides. This phenomenon may be leads to lower or higher larval duration or a reduction in reproductive performance (Boivin et al., 2001) which is evidence also in present results. Carbohydrates are assumed as the basic source of energy and a starting material in chitin synthetase (Genc et al., 2002; Nation, 2008). Furthermore, lipids have major roles in preparation of energy, metamorphosis, exoskeleton substrates and biosynthesis of pheromones (Nation, 2008). Proteins are involved in structural and enzymatic functions as hormones and enzymes biosynthesis which are able to convert as an energy source (Klowden, 2013; Wigglesworth, 2012). Nutritional deficiencies along with the increase in metabolic activities for detoxification process during the exposure to pesticides are among the main reasons for the reduced energy level (De Coen & Janssen, 1997; Verslycke et al., 2003). Our results showed that a decrease in the level of energy sources, carbohydrate, lipid and protein, of the larvae following their treatment with LC_{30} and LC_{50} values of lufenuron. According to these results, the protein contents of *X. luteola* larvae decreased at both LC_{30} and LC_{50} treatments, however there was only significant difference in LC_{50} concentration compared to the control. This reduction could be due to the breaking the proteins into amino acids and their entry into the tricarboxylic acid (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 Kandi et al. (2012) who reported LC_{50} of lufenuron caused reduction in the total soluble protein. Piri Aliabadi et al. (2016) showed a reduction in the protein content of *Glyphodes pyloalis* Walker, 1859 larvae when treated with LC_{30} and LC_{50} of lufenuron. The exposure to pesticides can affect carbohydrate metabolism in different species of insects by either decreasing or increasing its content

(Mansingh, 1972). In this study, carbohydrate content of the elm leaf beetle larvae dropped when they were treated with lufenuron. Kandi et al. (2012) had observed the same results by using lufenuron against *Pectinophora gossypiella* (Saunders). Results of this study demonstrated a significant decrease in the lipid content of the larvae of the elm leaf beetle following their exposure to LC_{30} and LC_{50} concentrations of lufenuron. According to some studies, the exposure to pesticides affects synthesis and storage of lipids more than their breakdown (Ali et al., 2011; He et al., 2020). A similar result was reported for *G. pyloalis* larvae treated with lufenuron (Piri Aliabadi et al., 2016). Bashari et al. (2014) also showed a decrease in the lipid content of *X. luteola* larvae treated with hexaflumuron. Glycogen is one of the essential nutrient reserves in insect which can also be affected by pesticide treatment (Fahmy & Dahi, 2009). Our results revealed a significant increase in the glycogen level of the *X. luteola* when treated with LC_{50} concentration of lufenuron. Changes in glycogen level may be due to disruption of the homeostasis mechanism in insects (Nath, 2002; Oguri & Steele, 2007). Lufenuron significantly reduced larval and pupal mass and extended duration of both developmental stages of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in LC_{90}, LC_{50} and LC_{10} concentrations (Butter et al., 2003).

The detoxification enzymes including monooxygenases, GST and hydrolases play the important roles in insecticide metabolisms (Yu, 2014). Additionally, improvement of insecticide tolerance in short time and insecticide resistance after a long period of time exposure may even incorporated by increasing in specific activities and much expression of metabolic enzymes (Yin et al., 2008). Results of our study showed an increase in α- and β-esterase activities of the treated larvae which was significant at LC_{50} and not significant at LC_{30} compared to the control. In lufenuron treatment on *H. armigera,* the esterase activity was reduced significantly with correlation to dose- and time-dependent manner compared with control. It has been recommended the modification in esterase enzyme activities could have important role in fecundity and fertility reduction and inhibition of metamorphosis (Reda et al., 2010).

GSTs are another group of detoxifying enzymes which play an important role in the physiology of stress and intracellular transport and biosynthesis pathways of different cycles (Wilce & Parker, 1994). Results of this study showed that GST activity was increased after the treatment of the larvae with LC_{50}. This result demonstrates that GST activity of the elm leaf beetle maybe are involved in the detoxification of lufenuron as detoxification enzyme for conjugating pesticides and their metabolite with glutathione. Bashari et al. (2014) also reported enhanced activities of GST in *X. luteola* after hexaflumuron treatment.

5 CONCLUSION

Lufenuron in lethal and sublethal concentrations (LC_{50} and LC_{30}) caused affections on the developmental, survival and fecundity of the second instar larvae of *X. luteola* that has been significant influences. In practical approach, these impacts could modify the offspring numbers and maintain population under economic threshold level (ETL). Considering the effect of sublethal concentrations of lufenuron on energy reserves, enzyme activities and spawning rate of elm leaf beetle, it can be concluded that this compound has good potential to control this pest.

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