JOURNAL OF ENTOMOLOGICAL
SOCIETY OF IRAN
2014, 33(4): 59-70

Sublethal effects of pyriproxyfen on some biological and biochemical properties of elm leaf beetle, *Xanthogaleruca luteola* (Col.: Chrysomelidae)

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Abstract

The lethal and sublethal effects of pyriproxyfen (a juvenile hormone analogue) were investigated on feeding deterrence, growth and larval weight as well as enzymatic and non-enzymatic activities of the third instar larvae of elm leaf beetle, *Xanthogaleruca luteola* Müller. The LC_{30} and LC_{50} values were estimated to be 133 and 343 ppm per larvae after 72 hours, respectively. Due to the treatment with 133 and 343 ppm of pyriproxyfen, feeding deterrence and prolongation in larval duration were observed. Also, the amounts of total protein, glucose, glycogen, urea, acid phosphatase and α-amylase activities were significantly decreased in the treated specimens compared with the controls. On the other hand, alanine aminotransferase activity and cholesterol levels were increased in the specimens 72 hours after the treatment compared with the controls. There were significant differences in the amount of uric acid and the activity of alkaline phosphatase, glutathione S-transferase and esterases compared with the controls. Present results showed that pyriproxyfen caused some biological and physiological discrepancies in the survived insects after treating with LC_{50} concentration of the insecticide which indicates changes in the metabolism of these compounds. Hence, it seems that pyriproxyfen has the potential to be used as a biorational insecticide in the integrated pest management (IPM) program for *X. luteolla*.

Key words: *Xanthogaleruca luteola*, pyriproxyfen, sublethal effects, enzymatic and non-enzymatic changes, juvenile hormone mimic

چکیده

اثـرات زیرکشـندگی پـیری‌پروکسـی‌فـن روی بعضـی خصوصـیات بیولـوژیکی و بیوشـیمیایی سوسـک بـرگ‌خـوار نـارون، *Xanthogaleruca luteola* (Col.: Chrysomelidae)

بیتا والی‌زاده و جلال جلالی سندی

اثرات کشندگی و زیرکشندگی پیری‌پروکسی‌فن (شبه‌هورمون جوانی) روی مرگ و میر، رشد و بازدارندگی تغذیـه و همچنـین فعالیت‌های آنزیمی و مقادیر ماکرومولکول‌های لارو سن سوم سوسک برگ‌خوار نارون، *Xanthogaleruca luteola* Müller، بررسی شد. مقادیر LC_{50} و LC_{30} روی لارو سن سوم، پس از ۷۲ ساعت تیمار، به‌ترتیب ۳۴۳ و ۱۳۳ پی‌پی‌ام محاسبه شد. لاروهای تیمارشده با غلظت‌های LC_{50} و LC_{30}، مرگ و میر بالا، افزایش طول دوره‌ی لاروی، عدم ظهور حشره‌ی کامل، کاهش وزن و دورکنندگی تغذیه از خود نشان دادند. همچنین، مقدار پروتئین کل، گلوکز، گلیکوژن، اوره، فعالیت آنزیم‌های اسید فسفاتاز و آلفاآمیلاز تحت تیمار هر دو غلظت در مقایسه با شاهد به‌طور معنی‌داری کاهش یافت. از طرف دیگر، ۷۲ ساعت پس از تیمار، فعالیت آلانین آمینوترانسفراز و مقدار کلسترول افزایش معنی‌داری نسبت به شاهد نشان داد. میزان اسیداوریک و فعالیت آنزیم‌های آلکالین فسفاتاز، گلوتاتیون اس- ترانسفراز و استراز در لاروهای تیمارشده تفاوت معنی‌داری با شاهد نشان دادند. نتایج نشان داد که پیری‌پروکسی‌فن علاوه‌بر کشتن حشـرات در غلظت LC_{50}، منجر به ایجاد برخی تغییرهای بیولوژیکی و آنزیمی تحت اثرات زیرکشندگی این غلظت شده که نشان‌دهنده تغییـر در متابولیسم این ترکیبات است. ازاین‌رو، به‌نظر می‌رسد که پیری‌پروکسی‌فن که ترکیب شبه‌هورمون جوانی است، می‌تواند به‌عنوان یکی از حشره‌کش‌های طبیعی برای استفاده‌ی بالقوه در مدیریت تلفیقی آفات (IPM) علیه سوسک برگ‌خوار نارون مصرف شود.

واژگان کلیدی: *Xanthogaleruca luteola*، پیری‌پروکسی‌فن، اثرات زیرکشندگی، تغییرات آنزیمی و غیرآنزیمی، شبه‌هورمون جوانی

Introduction

The elm leaf beetle, *Xanthogaleruca luteola* Müller, is one of the important pests of elm (*Ulmus* spp.) in some parts of the central and southern Europe, north Africa, west and central Asia, southern Australia and temperate areas in the North and South America (Romanyk & Cadahía 2002; Borowiec & Sekerka 2010). Both the larvae and adults of *X. luteola* feed on the emergent leaves. Repeated infestations make the tree susceptible to different pests and diseases, (Pérez, 2003).

The unselective use of chemical pesticides is harmful to the environment, beneficial species and the human health (Perry *et al*., 1998). Insect growth regulators (IGRs), interfere with cuticle formation in a numerous insect groups (Cohen, 2001). They are also regarded as larvicides and ovicides (Lee *et al*., 1996). The sublethal doses of pesticides are considered as environmentally friendly alternatives to improve existing pest management strategies (Sial & Brunner, 2010). The sublethal doses influence the biology and physiology of insects that are survived after pesticide usage (Desneux *et al*., 2004). Certain studies have shown that the sublethal concentrations of pyriproxyfen affect a number of physiological parameters including feeding activity and larval duration (Yin *et al*., 2008; Nasr *et al*., 2010). The sublethal doses of pyriproxyfen also affect biochemistry of tested insects including protein, cholesterol, glucose and uric acid levels, etc. (Zibaee *et al*., 2011; Bemani *et al*., 2013). The objective of the present study was to investigate the effects of this biorationale product on an urban pest, *X. luteola*., also to probe in particular the effects of sublethal doses on the possible irreversible biochemical changes on *X. luteola* by this insecticide.

Materials and methods

Source of the insects

Xanthogaleruca luteola eggs and larvae were collected from elm trees in the city park of Rasht, north of Iran. The larvae were reared in plastic boxes (10-20 cm) in a rearing chamber set at 25 ± 2 °C; 14: 10 L: D; 65% RH. Fresh elm leaves were provided daily for feeding. Adults were reared similarly and their eggs were used to maintain the culture. Newly emerged (< 24 hrs) third instar larvae of the same age were used for bioassay.

Toxicity tests

Oral toxicity tests were performed on newly emerged 3rd instar larvae of *X. luteola* with different concentrations of pyriproxyfen (Admiral® 10% EC, Arista life Science France). In each experiment, 10 larvae were tested with 5 replicates in each concentration. The leaf discs (10 × 20 cm) were dipped in desired concentrations for 30 s and dried at room temperature for 30 min. Water dipped leaf discs were used as control. Mortality was recorded after 72 h and the LC_{50} and LC_{30} were estimated using POLO-PC software (LeOra, 1987).

Deterrent effects

This experiment was performed as described by Xie & Isman (1992).Two leaves of elm tree with the same size (10 × 20 cm) were selected, dipped in the desired concentrations (343 and 133 ppm) for 30 s and air dried for 30 min. Control leaf discs were dipped in distilled water. The leaves were placed 7 cm far from each other. Then the third instar larvae (< 24 hrs) were placed in the centre of each jar (three replicates and 10 larvae in each group). The number of larvae attracted to the control or treated leaf discs were recorded after 24 and 48 hrs. Deterrence index (DI) was calculated from the following formula of Xie & Isman (1992):

$$DI = (C - T) / (C + T) \times 100$$

where C is the number of larvae on the control leaf and T is the number of larvae on the treated leaves.

Effect of pyriproxyfen on the development of *X. luteola* larvae

The duration of larval life were evaluated after the treatment with two concentrations of the tested insecticide (343 and 133 ppm). Distilled water was used as the control. Larval duration was recorded in days after the treatment.

Preparation of the whole body homogenates for biochemical analysis

The pyriproxyfen treated third instar larvae (concentrations 343 and 133 ppm) were killed by freezing after 24, 48 and 72 hours of the treatment. The whole body was homogenized in 1 ml of universal buffer and were centrifuged for 10 min at 13000 g. The supernatant was transferred to new tubes and stored at -20 °C until used. Each biochemical analysis was repeated three times.

Determination of protein concentration

Protein concentration was determined by the method of Bradford (1976). First, each larva (whole body) was homogenized in 350 µl of distilled water and samples were centrifuged at10000 g for 5 min at 4 °C. Then, 10 µl of the supernatant was mixed with 90 µl of distilled water and 2500 µl dye (dissolving 10 mg powder of Coomassie Brilliant Blue G-250 (Bio-Red, Munchen, Germany) in 5 ml ethanol 96% and 10 ml phosphoric acid 85% (w/w) and the volume was brought to 100 ml with distilled water). The absorbance was read at 595 nm using a microplate reader (Stat Fax 3200, Awareness Technology, USA).

Determination of glucose and glycogen levels

Glucose level was analyzed by Siegert's (1987) protocol and glycogen amount was determined by a photometric method using anthrone reagent as described by Van Handel (1965). The anthrone reagent was prepared by dissolving 0.15 g in 100 ml of diluted sulphuric acid (76 ml sulphuric acid, d =1.84, in 30 ml water while stirring and cooling). One ml of homogenized body was stirred with 0.05 ml of saturated solution of Na_2So_4 in a centrifuge tube, followed by adding 3 ml of ethanol. The tube was placed in a boiling water-bath for 3 min and cooled in an ice-bath for at least 1 hour and then centrifuged. The glycogen pellet ($+Na_2SO_4$) was dissolved in 0.05 ml water after drying. 3 ml of freshly prepared anthrone reagent was added and the tube was heated at 90 °C for 20 min, cooled in ice water and the samples absorbance was read at 620 nm.

Determination of uric acid and urea values

Uric acid contents were determined using uricase as described by Valovage & Brooks (1979); uricase produces a purplish color which has a direct correlation (at 545 nm) with uric acid concentration. Urea was measured with urease-GDH kit (Biochem. Co, Iran) at 340 nm following the manufacturer’s protocol.

Determination of cholesterol level and α-amylase specific activity

Cholesterol level was determined by Richmond's (1973) method. This method is based on hydrolysis of cholesterol esters by cholesterol oxidase, cholesterol esterase and peroxidase.

The α-amylase specific activity was measured by the procedure of Bernfeld (1955), using 1% soluble starch as substrate. The reaction was performed by sodium phosphate buffer at 35 °C with 10 µl of the enzyme, 40 µl substrate in 40 µl sodium phosphate buffer (pH 6) for 30 min. To stop the reaction, 100 µl dinitro salicylic acid (DNS) was added and heated in boiling water for 10 min. Absorbance was read at 540 nm.

Determination of acid phosphatase and alkaline phosphatase specific activities

These enzymes specific activities were determined by Bessey assay (Bessey *et al*., 1946). The substrate in phosphate buffer (0.02 M, pH 7.2) was incubated with samples for 30 min. Alkaline was added to stop the reaction and adjusted pH for the determination of product concentration. The spectral absorbance of *p*-nitrophenolate was maximal at 310 nm. On converting the *p*-nitrophenolate into *p*-nitrophenol by acidification, the absorption maximum is shifted to about 320 nm.

Determination of aspartate and alanine aminotransferases specific activities

Alanine aminotransferase and aspartate aminotransferase activities were measured using Thomas' (1998) procedure. This assay was done by AST and ALT kit (Biochem Co., Iran). Absorbance was read at 340 nm.

Determination of esterases and glutathione S-transferase specific activities

The specific activity of α and β esterases were determined according to Van Asperen's (1962) method. Alpha-naphtylacetate (α-NA) and β-naphtylacetate (β-NA) (10 mM) were used as substrates. One gut was homogenized with 1000 µl 0.1 M phosphate buffer (pH 7) containing 0.01% Triton x-100, then the homogenized solution was centrifuged at 10000 g for 10 minutes at 4 °C. The supernatant was transferred to a new microtube and was diluted with phosphate buffer. This solution reacted with the substrate and by using dye indicator (Fast Blue RR salt) (1 mM) a colored solution was formed and the absorbance was read at 630 nm. For determination of the glutathione S-transferase (GST) specific activity, the method of Oppenorth (1979) was used. 1-chloro-2, 4-dinitrobenzene (CDNB) (20 mM) was used as the substrate. Initially a larva was homogenized in 20 µl distilled water, then the homogenate was centrifuged at 12500 g for 10 minutes at 4 °C. Fifteen µl of the supernatant was mixed with 135 µl of phosphate buffer (pH 7) and 50 µl of CDNB. The absorbance was read at 340 nm.

Statistical analysis

For the determination of mortality and lethal concentrations, POLO-PC software (LeOra, 1987) was used. The other data were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute, 1997). The least significant among treatments were compared using Tukey's multiple range test (SAS Institute, 1997). Differences among means were considered significant at $P \leq 0.05$.

Results

The corresponding LC_{50} and LC_{30} values, confidence limit (95%) and regression slope at 72 hours after exposure to pyriproxyfen are depicted in (table 1). The LC_{50} and LC_{30} values for pyriproxyfen were estimated as 343 and 133 ppm, respectively.

Deterrence effects of pyriproxyfen on third-instar larvae was calculated after 24 and 48 hours (fig. 1). With the increase in concentrations and time, the percentage of deterrence activity increased and the maximum deterrent activity (i.e. 86.6%) occurred at 48 hours after treatment with LC_{50} ($F = 150.72$; df = 3, 8; $P < 0.0001$). The duration of the larval stage significantly increased in the treated larvae ($F = 65.82$; df = 2, 6; $P < 0.0001$) compared with the controls (table 2).

A significant reduction was observed in the amount of total protein in the treated larvae with all the concentrations of pyriproxyfen tested ($F = 15.30$; df = 2, 6; $P = 0.0044$) (fig. 2). No significant changes were observed in cholesterol level after 24 and 48 hours. However, the amount of cholesterol increased in both the concentrations used after 72 hours of the treatment ($F = 9.43$; df = 2, 6; $P < 0.0144$) (fig. 2). The amount of glucose was highly decreased in the treated samples by pyriproxyfen after 24 and 72 hours but there were no significant differences at 48 hours of the treatment ($F = 7.33$; df = 2; $P = 0.0245$) (fig. 2). The amount of glycogen was highly decreased after the treatment by pyriproxyfen at three intervals ($F = 32.72$; df = 2; $P = 0.0006$) (after 24, 48 and72 hours) (fig. 2). The amount of urea was decreased in treated specimens compared with the controls at three intervals. While the amount of uric acid significantly increased with LC_{30} (133 ppm) but its concentration decreased at LC_{50} (343 ppm) after 48 hours ($F = 31.97$; df = 2; $P = 0.0006$) (fig. 2).

On the other hand, pyriproxyfen caused significant decrease in the α-amylase activity with both the concentrations after 24 hours ($F = 19.39$; df = 2; $P = 0.0024$), but there were no significant differences after 48 and 72 hours between the treated insects and the controls (fig. 3). Alkaline and acid phosphatase activities (U/mg protein) of larvae were significantly affected by different concentrations of pyriproxyfen (fig. 4). Also, the results showed that alkaline phosphatase activity increased significantly after 24 hours of the treatment and then decreased at 48 and 72 hours ($F = 7.58$; df = 2; $P = 0.0228$). Acid phosphatase activity decreased in the treated samples in comparison

Table 1. The LC_{50} and LC_{30} values confidence limit (%95) and regression slope after 72 hours of exposure to pyriproxyfen in larvae of *Xanthogaleruca luteola*.

Toxic material	Slope ± SE	X^2 (df)	LC_{50} (95% CI) (ppm)	LC_{50} (95% CI) (ppm)
Pyriproxyfen	1.194 ± 0.167	0.581 (3)	343 (218-428)	133 (66-168)

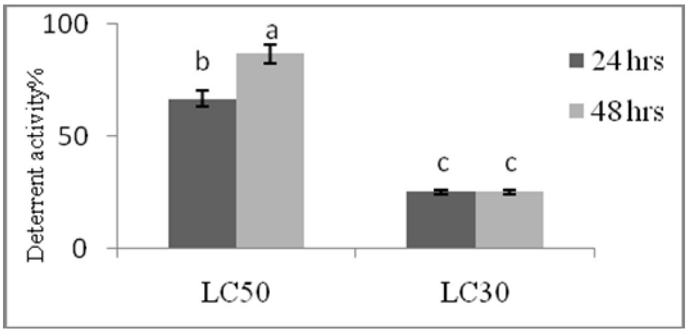

Fig. 1. Deterrent activity of pyriproxyfen against the 3rd instar larvae of *Xanthogaleruca luteola*.

with the controls after 24 hours and then an increase was observed after 48 hours (F = 7.77; df = 2; P = 0.0216). Significant effects on alanine (ALT) and aspartate (AST) aminotransferases activities were observed by two different concentrations of pyriproxyfen (fig. 5). Both concentrations increased the activity of ALT at various time intervals (F = 25.65; df = 2; P = 0.0011). In the case of AST, the enzyme activity was decreased due to the treatment with both concentrations after 48 and 72 hours, but on the contrary no difference was observed after 24 hours (F = 10.65; df = 2; P = 0.0106) (this amount of P indicate that it was significant). By measuring two detoxifying enzymes activities, general esterases and glutathione S-transferase, it was found that glutathione S-transferase is the main detoxifying enzyme of pyriproxyfen in *X. luteola*. The LC_{50} and LC_{30} of pyriproxyfen increased glutathione S-transferase activity significantly (fig. 6). The two concentrations of pyriproxyfen decreased general esterase activity after 48 hours of the treatment especially when α-naphtyl was used as substrate (F = 23.30; df = 2; P = 0.0015) but no differences were observed after 24 and 72 hours (fig. 7).

Table 2. Life cycle of the 3rd instar larvae of *Xanthogaleruca luteola* after the treatment with pyriproxyfen.

Treatments	3rd Instar larval duration (day)	Pupal and adult emergence
LC_{50}	15.66 ± 0.66^{a}	0
LC_{30}	13.00 ± 0.55^{a}	0
Control	5.666 ± 0.759^{b}	96.667 ± 2.402

Means followed by the same letter within a column are not significantly different from each other at P < 0.05, Tukey's studentized range test.

Discussion

Pyriproxyfen has attracted considerable attention recently for its inclusion in the integrated pest management (IPM) programs (Boina *et al.*, 2009). This compound affects insects by being toxic and causing a delay in larval growth and can act as an antifeedant agent (Nasr *et al.*, 2010). The results show its strong insecticidal, feeding deterrence, insect growth regulatory activities with some irreversible effect on the biochemistry of elm leaf beetle. Zapata *et al*. (2009) stated that the deterrent activity of any chemical used for insect control programs could be attributed to their effects on chemical sensila located in mouthparts via central nervous system. The results showed that pyriproxyfen had deterrent activity against *X. luteola* larvae. The deterrence increased as the concentrations of the insecticide and the treatment time increased.

A similar effect by pyriproxyfen was observed against *Tribolium confusum* Duval (in choice and no choice experiments) by Loni & Farazmand (2010). Nasr *et al*. (2010) reported that by increasing pyriproxyfen concentration and the treatment time, the feeding inhibition index was increased in the larvae of *Spodoptera littoralis* (Boisd.).

In this study, despite the increase in larval duration, growth retardation was reflected by lower larval weight. Pyriproxyfen as a juvenile hormone analogue, by interrupting hormonal balance, caused prolonged larval period. A majority of reports show an increase in larval period (Mojaver & Bandani, 2010; Naggar & Jehan, 2013) by using IGRs.

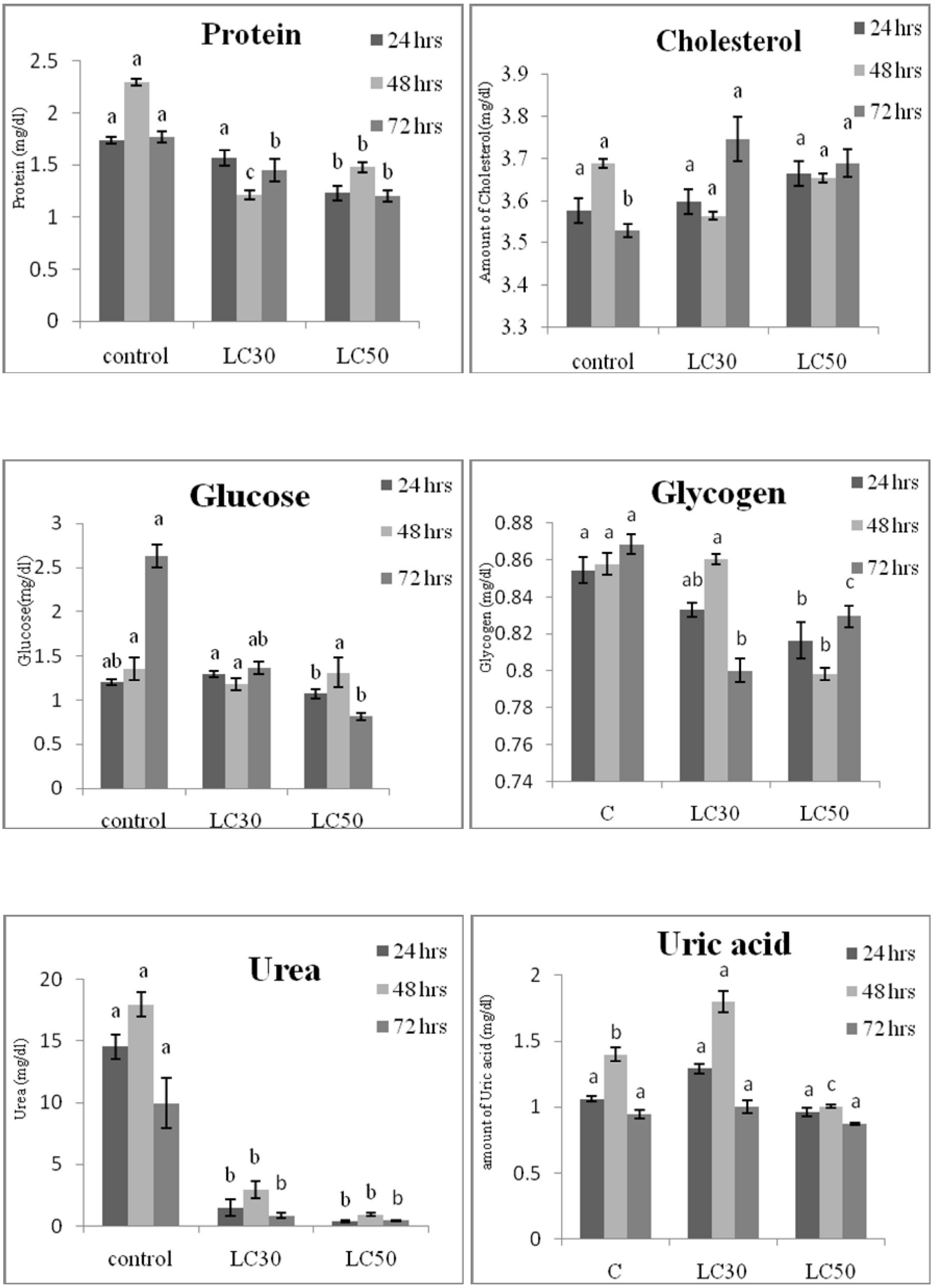

Fig. 2. Changes of some non-enzymatic compounds caused by two concentrations of pyriproxyfen in various time intervals (hours). Statistical differences are shown by different letter in each interval (Tukey's test; $P \leq 0.05$)

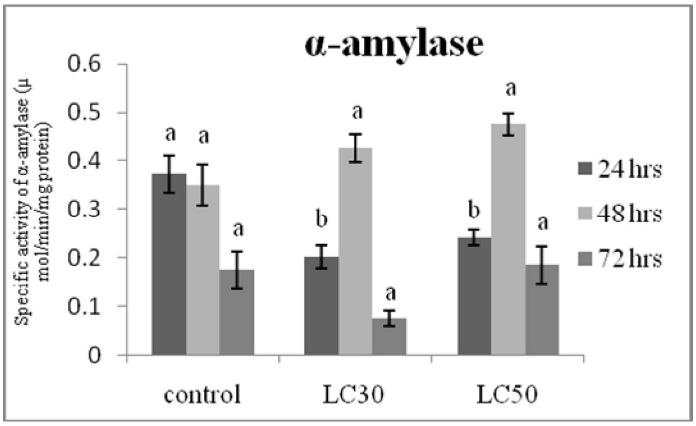

Fig. 3. The specific activity of α-amylase (μ mol/min/mg protein) in the 3rd instar larvae of *Xanthogaleruca luteola* after the treatment with pyriproxyfen. Statistical differences are shown by different letter in each interval (Tukey's test; $P \leq 0.05$).

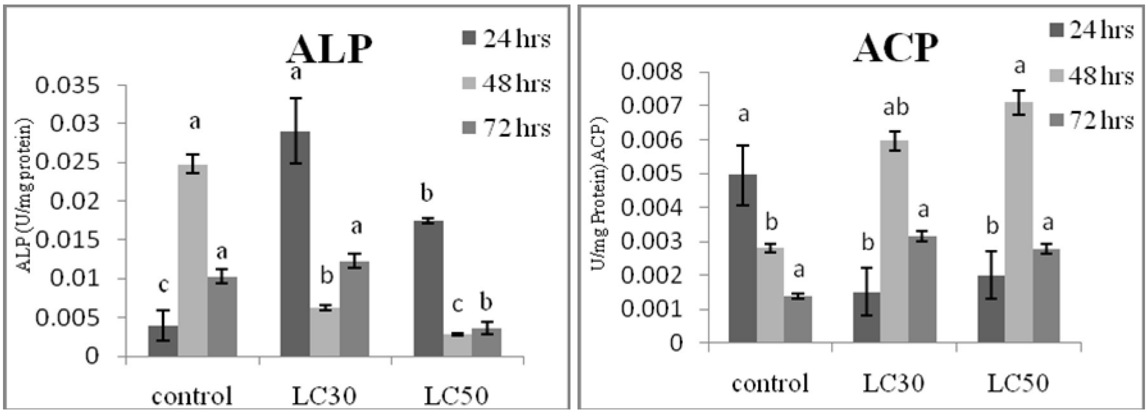

Fig. 4. Effect of pyriproxyfen on alkaline phosphatase and acid phosphatase specific activities in *Xanthogaleruca luteola* larvae (3rd instar). Statistical differences are shown by different letter in each interval (Tukey's test; $P \leq 0.05$).

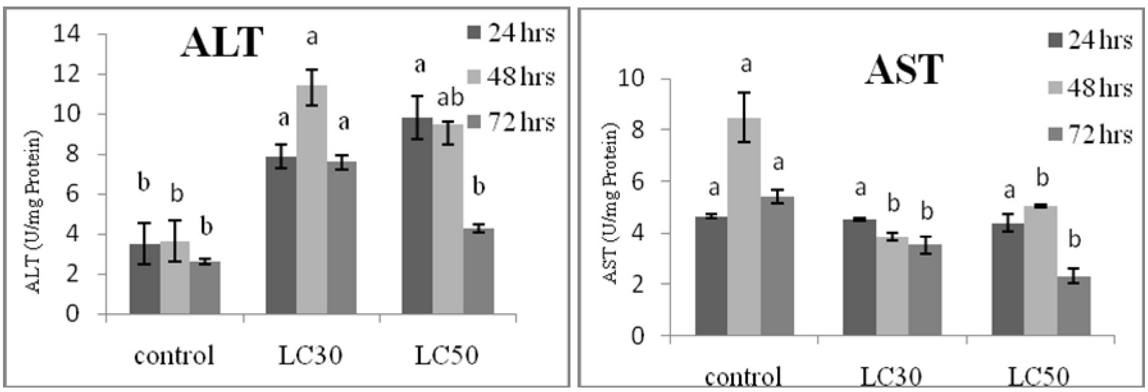

Fig. 5. Effect of pyriproxyfen on alanine aminotransferase and aspartate aminotransferase specific activities in *Xanthogaleruca luteola* larvae (3rd instar). Statistical differences are shown by different letter in each interval (Tukey's test; $P \leq 0.05$).

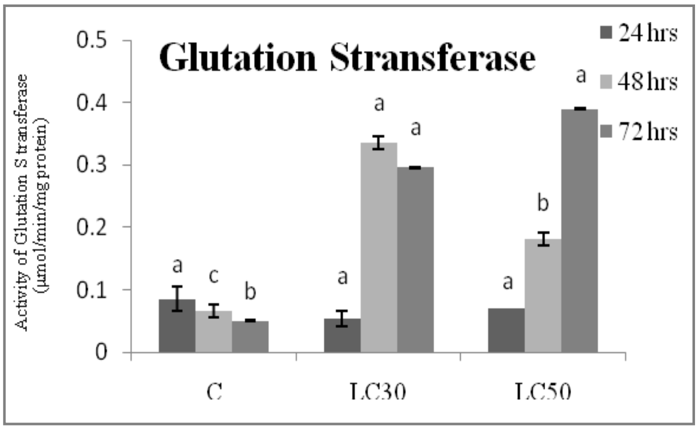

Fig. 6. The specific activities of glutathione S-transferase (µmol/min/mg protein) in the 3rd instar larvae of *Xanthogaleruca luteola* after the treatment with pyriproxyfen. Statistical differences are shown by different letter in each interval (Tukey's test; $P \leq 0.05$).

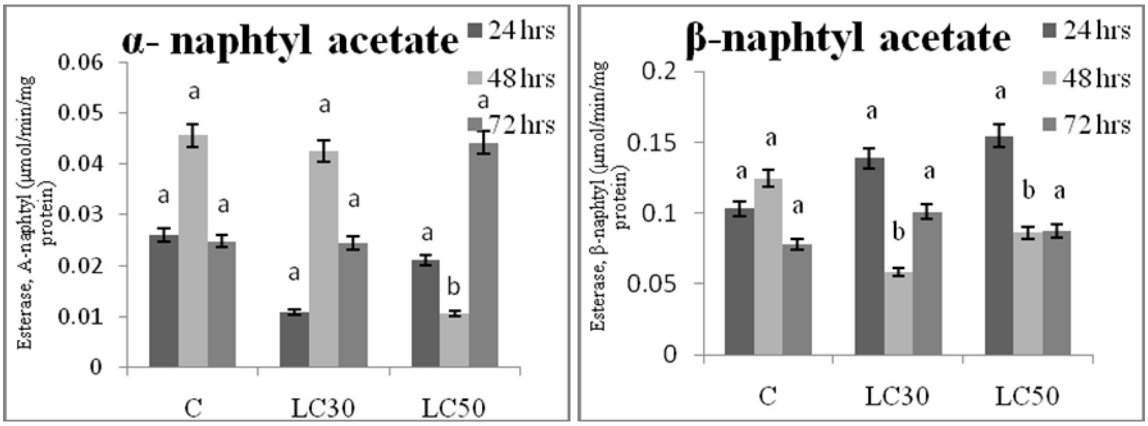

Fig. 7. The specific activities of esterases (µmol/min/mg protein) in the 3rd instar larvae of *Xanthogaleruca luteola* after the treatment with pyriproxyfen. Statistical differences are shown by different letter in each interval (Tukey's test; $P \leq 0.05$).

Many insecticides have antifeedant effect and decrease insect feeding efficiency which affects protein biosynthesis and accumulation (Etebari *et al*., 2007). The results showed that the amount of total protein decreased in the treated larvae after all the time intervals. This phenomenon could be due to break down of proteins into free amino acids which are transferred into the Kreb's cycle (Schoonhoven, 1982).

Lipids are significant molecules in biological systems and have different roles in the physiology of insects such as synthesis of phospholipids that are considered as an integral part of a cell membrane. They are also considered as part of various hormones and serve as sources of metabolic energy that can be mobilized to meet the energy requirements of the insect (Klowden, 2007). Increase in the amount of cholesterol after 24 hours could be due to the stress caused by the chemical treatment. This finding is in consistent with the reports of Cox *et al*. (1989) who found a significant increase in total cholesterol concentration after the treatment of female mice with pyriproxyfen.

The results showed a sharp decrease in the glycogen and glucose values after the larval treatment of elm leaf beetle with pyriproxyfen. The decrease in the amounts of certain compounds such as glycogen and glucose which was caused by pyriproxyfen as a physiological stress could also be attributed to an interruption in the absorption system (Etebari & Matindoost, 2004). These results are in agreement with the works of other researchers (Sharma *et al*., 2011; Bemani *et al*., 2013; Yazdani *et al*., 2013).

Amounts of urea and uric acid in the body depend on the reactions taking place after protein metabolism; in fact they are the excreted end product of insects. Hence, their amount is correlated with the amount of protein in the insect's body (Shekari *et al*., 2008). One of the important reasons for measuring uric acid and urea is to know about the nitrogen catabolism, because it has been reported that pyriproxyfen has a direct effect on nitrogen metabolism (Hirayama & Nakamura, 2002). After 48 hours of the treatment with pyriproxyfen, the amount of uric acid increased in the treated larvae with LC_{30} concentration. This result indicates that the increase in uric acid level is probably due to the altered metabolic pathway caused by the treatment which prevented the natural excretion of uric acid from the insect body (Shekari *et al*., 2008).

Alpha-amylase is an enzyme hydrolyzing starch to maltose and glycogen to glucose. The present study clearly depicts the enzyme's lower activity following the treatment with pyriproxyfen, which is in agreement with other reports (Lee *et al*., 1996; Abd EL-Mageed & Shalaby, 2011).

Alkaline phosphatase and acid phosphatase are hydrolytic enzymes, which hydrolyze phosphomonoesters under alkaline or acid conditions, respectively. The results showed that there were significant changes in the activities of ALP and ACP following the treatment with pyriproxyfen. There are different reports in this regard; as per the results of Zibaee *et al*. (2011) the treatment with pyriproxyfen in *Eurygaster integriceps* Puton increased ALP and ACP activities. While Adel *et al*. (2010) reported reduction in their activities in *S. littoralis* under the effect of *Artemisia monosperma* Del. wild plant hexane extract.

Alanine aminotransferase and aspartate aminotransferase have important role in Kreb's cycle for acquiring energy necessary in growth and reproduction. In the present investigation, a potent inhibitory effect on AST and increasing effect on ALT have been observed. Zibaee *et al*. (2011) and Etebari *et al*. (2007) also have shown an increase in ALT activity in *E. integriceps* adult and a decrease in AST activity in silkworm larvae, respectively.

Esterases (ESTs) are important detoxifying enzymes which hydrolyse the esteric bond in synthetic chemicals (Hemingway & Karunatne, 1998). The activity of glutathione S-transferase in detoxification process is well known, too (Devorshak & Roe, 1998). Pyriproxyfen caused a decrease in the esterases activity with both the concentrations after 48 hours of the treatment. In addition, glutathione S-transferase activity was increased significantly by both the concentrations of pyriproxyfen after 48 and 72 hours of the treatment. These results are similar to Fahmy (2012) investigations about the effect of pyriproxyfen on *S. littoralis*. Detoxification and the activity of detoxifying enzymes consume lots of energy which lead to the increase in larval life duration (Devorshak & Roe, 1998).

The present study shows that pyriproxyfen not only causes direct mortality (lethal effects) on larvae but also its sublethal doses affect various physiological processes in elm leaf beetle. These activities include deterrence, delay in larval development, reduction of the larval weight and irreversible changes in biochemical components. Hence, it can be concluded that pyriproxyfen can be considered in the integrated pest management program of elm leaf beetle, where classical insecticides are not considered.

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Received: 27 December 2013
Accepted: 15 March 2014