Effect of Neem based insecticide Achook® on mortality, biological and biochemical parameters of elm leaf beetle *Xanthogaleruca luteola* (Col.: Chrysomelidae)

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Abstract: The elm leaf beetle *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), is a serious pest of elm (*Ulmus* spp.) throughout Iran. In the present study, Lethal and sublethal effects of neem, Achook® containing 0.03% azadirachtin, were assessed on mortality, growth, larval weight and feeding deterrence as well as enzymatic and non-enzymatic activities of third instar larvae of the elm leaf beetle. LC_{50} and LC_{30} values of the third instar larvae 48 h post treatment were estimated to be 3.3 and 2.25 ppm respectively. Observations showed higher mortality, increase in larval duration, sterilization of adults, reduction in weight and feeding deterrence after LC_{30} and LC_{50} treatments. Biochemical analysis showed changes in the amounts of biochemical components in the treated larvae after 48 h. In the treated larvae, activity level of alanine aminotransferase, alkaline phosphatase, acid phosphatase and α-amylase as enzymatic components and urea and cholesterol as non-enzymatic ones changed significantly in LC_{50} and LC_{30} treatments. Aspartate aminotransferase, lactate dehydrogenase, protein, glycogen, and glucose levels decreased in these treatments. The activity level of detoxifying enzymes such as esterase A, esterase B and glutathione S-transferase were significantly affected. Hence, neem is suggested as a safe product that may have the potential for use as a bioinsecticide in integrated pest management of urban elms where use of chemical insecticides are discouraged.

Keywords: Biochemical parameters, Mortality, Achook®, Neem, *Xanthogaleruca luteola*

Introduction

Botanical insecticides have long been thought as alternatives to synthetic insecticides for pest management because botanicals reputedly pose little threat to the environment and to human health. The body of scientific literature documenting bioactivity of plant derivatives on arthropod pests continues to

expand, yet only a handful of botanicals are currently used in agriculture in the industrialized world (Isman 2000). Neem is one of the botanical products used as insecticide. Various neem products have been extensively studied in pest control programmes. A number of bioactive components have been isolated from various parts of the neem tree (*Azadirachta indica*; Meliaceae). These chemical compounds have different designations, among which Azadirachtin is the major component which is, the predominant insecticidal active ingredient of seeds, leaves and other parts of

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the neem tree. Neem components show multiple effects against different insects (Rehimi *et al*., 2011). Azadirachtin is known as repellent, natural products from seeds of the neem tree *Azadirachta indica* A. Juss (Meliaceae), impede the development of insects larvae and sterilize adults (Hummel, *et al.,* 2011).

Among a series of urban pests, the elm leaf beetle *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae), as a defoliating insect, causes important damage on elm (*Ulmus* spp.) in farms and urban areas (Chiffelle *et al*., 2013 and Huerta *et al.,* 2011). The elm leaf beetle is distributed mainly in central and southern Europe, North Africa, west and central Asia, southern Australia, and temperate areas in North and South America (Romanyk and Cadahía 2002 and Borowiec and Sekerka 2010). Both the adults and larvae feed on the emergent leaves of the elm. Repeated heavy infestation does not kill the tree outright, rather it usually weakens it, rendering it vulnerable to attack by insects and diseases. However, the beetle does transmit Dutch elm disease (Huerta *et al.,* 2010).

Botanical insecticides or their derivatives affect insect physiology in different ways. We examined the biological and biochemical effects of *Azadirachta indica* on the elm leaf beetle looking at aspartate amino transferase and alanine amino transferase as strategic link between the carbohydrates and protein metabolism. These enzymes are altered during various physiological and pathological conditions (Etebari *et al.*, 2005). Alpha amylase is a midgut enzyme, that is involved in starch and other carbohydrates metabolism and the enzyme's activity can be affected by chemicals (Saleem and Shakoori, 1987). Understanding of these biological and biochemical effects on feeding processes of incsects may definitely help to provide a safe procedure to control the population of *X. luteola* in laboratory bioassays and contribute to the development of integrated pest management (IPM).

Materials and Methods

Laboratory mass culture of *X. luteola*
Individuals of *Xanthogaleruca luteola* eggs and larvae were collected from the elm tree in the City Park of Rasht, in north of Iran where no pesticides were used. Insect colonies were maintained in the laboratory set at 25 ± 2 °C; 14:10 LD and 65% RH. Larvae were reared in plastic jars 10-20 cm in which the lid contained holes covered with muslin. In order to maintain humidity, the bottom of the jar was covered with compressed wet sponges and the leaves bearing the larvae were vertically placed over the sponges. Fresh leaves were daily provided for feeding. Adults were similarly reared and their eggs were used to maintain the culture.

Insecticide bioassay
Toxicity tests
Oral toxicity tests were performed on third instar larvae of *X. luteola* with five different concentrations of Achook®. This experiment was repeated 5 times with 10 larvae for each treatment. Distilled water was used as control. Mortality was recorded at 48 h and the LC_{50} and LC_{30} was estimated using Polo-PC software LeOra (1987)

Deterrent effects
Deterrent effects of Achook® on the elm leaf beetle were measured using the method described by Xie and Isman (1992). Two leaves of elm tree of the same size were selected, dipped in the desired concentrations (3.3 and 2.25 ppm) of Achook® for 30 s and air dried for 30 min. Control leaves were dipped in distilled water. Treated and control leaves were placed in a jar (10-20 cm). The leaves were placed at 7 cm spacing from each other. Third instar larvae were released in the centre of the jar. The number of larvae attracted to control or treated leaves were recorded after 24 h and 48 h. Deterrence index was calculated using the following formula): $DI = (C - T)/(C + T) \times 100$ where C is the number of larvae on control leaf and T is the number of insects on treated leaves

(Xie and Isman 1992). This experiment was repeated 3 times with groups of 10 larvae.

Effect of Achook® on the weight and development of *Xanthogaleruca luteola* larvae
Two concentrations of the tested insecticide (3.3 and 2.25 ppm) were used. The leaf discs were dipped in desired concentrations for 30 s. The newly emerged 3rd instar larvae (3 groups of 10 larvae each) were provided with treated leaves. Distilled water was used as control. Larval weight was recorded 2, 4 and 6 days after treatment. Larval duration was also determined.

Preparation of whole body homogenates for biochemical analysis
Third instar larvae after treatment with Achook® at concentrations of LC_{50} and LC_{30} were freeze-killed 48 h post treatment. The whole body was homogenized and samples from each treatment were centrifuged for 10 min in 13,000 rpm. The supernatant was transferred to new tubes and stored at -20 °C until used.

Estimation of aspartate (EC 2.6.1.1) and alanin aminotransferases (EC 2.6.1.1)
Alanine aminotrasferase and aspartate aminotransferase were measured using Thomas (1998) procedure. This assay was done by AST and ALT kit (Biochem Co., Iran). On this basis, reagents 1 and 2 were mixed (4:1). Then, samples were added and absorption was read at 340 nm.

Estimation of acid phosphatases (EC 3.1.3.2) and alkaline phosphatases (EC 3.1.3.1)
The enzyme assays were carried out as described by Bessey *et al.,* (1946). The buffered substrate (phosphate buffer, 0.02 m, pH 7.2) was incubated with samples for 30 min. Alkali were added to stop the reaction and adjust the pH for the determination of concentration of the product formed. The spectral absorbance of p-nitrophenolate was maximal at 310 nm. The molar absorbance of p-nitrophenolate at 400 nm is almost double that of p-nitrophenyl phosphate at 310 nm. On converting the *p*-nitrophenolate to

p-nitrophenol by acidification, the absorption maximum is shifted to about 320 nm with no detectable absorption at 400 nm.

Estimation of lactate dehydrogenase (EC 1.1.1.27)
For evaluating lactate dehydrogenase (LDH), King (1965) method was used. To standardize volumes, 0.2 ml NAD + solution was added to the test tubes and 0.2 ml of water was added to control test tubes, each containing 1 ml of the buffered substrate and 0.01 ml of the sample was also added to the test tubes. Test tube samples were incubated for exactly 15 min at 37 °C and then the reaction was arrested by adding 1 ml of coloring reagent (2,4-dinitrophenyl hydrazine) to each tube and the incubation continued for an additional 15 min. Then, the contents were cooled in room temperature, 10 µl of 0.4 N NaOH was added to each tube to make the solutions strongly alkaline. At exactly 60 s after the addition of alkali to each tube, the intensity of color was measured at 440 nm.

α-Amylase activity (EC 3.2.1.1)
The α-amylase 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 and 40 µl sodium phosphate buffer (pH = 9) for 30 min. To stop the reaction, 100 µl dinitrosalicylic acid (DNS) was added and heated in boiling water for 10 min. Absorbance was read at 540 nm after cooling. One unit of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35 °C. In this test, maltose was used to generate standard curve.

Estimation of Glucose
Whole body (100 µl) mixed with 500 µl 0.3 N perchloric acid and the precipitate was removed by centrifugation (10 min, 12000g). The supernatants were used for the determination of glucose concentrations (Siegert, 1987).

Estimation of urea

Urea was measured with urease-GDH kit . (Chemenzyme Co., Iran). In this method, ammonia ion is produced by urease enzyme and second reaction was catalyzed by glutamate dehydrogenase. Finally, reducing absorption rate was calculated at 340 nm.

Estimation of protein

Protein was measured based on Bradford's method (Bradford 1976) and by utilizing a total protein assay kit (Biochem Co., Iran). In this method, proteins made a complex purplish blue with an alkaline copper solution, which with its absorption value at 540 nm has a direct relation to the amount of the whole body protein.

Estimation of cholesterol

To measure the haemolymph cholesterol, Richmond (1973) method was used. The principles of this method are based on hydrolysis of cholesterol esters by cholesterol oxidase, cholesterol esterase and peroxidase.

Estimation of glycogen

Estimation was based on 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, poured into 30 ml water while stirring and cooling). A 1-ml sample was measured into a centrifuge tube and stirred with 0.05 ml of a saturated solution of NasSO4, followed by 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 ethanol was carefully decanted and the glycogen pellet (+ Na_2SO_4) after drying was dissolved in 0.05 ml water. Three ml of freshly prepared anthrone reagent was added and the tube was heated at 90 °C for 20 min, cooled in ice water and measured at 620 nm.

Assay of general esterase activity

The activity of general esterases were determined according to Van Asperen (1962) method. α-naphtylacetate (α -NA) and β-naphtylacetate (β-NA)(10 mM) were used as substrates. Initially one insect was homogenized in 1000 µl 0.1 M phosphate (pH = 7) and Triton X-100 in the ratio of 0.01%, and centrifuged at 10,000 g for 10 minutes at 48 °C. The supernatant was transferred to new micro tube and was diluted with phosphate buffer. Fast Blue RRsalt (1 mM) was added and the absorbance was read at 630 nm.

Glutation S-transferase activity

For determining the glutathion S-transferase (GST) activity, the method of Habing *et al.,* (1974) was used. 1-chloro-2, 4-dinitrobenzene (CDNB) (20 mM) was used as the substrate. Each larva was homogenized in 20 µl distilled water and centrifuged at 12,500 g for 10 min at 48 °C. Fifteen µl of supernatant was mixed with 135 µl of phosphate buffer (pH = 7), 50 µl of CDNB and 100 µl of GST. The absorbance was read at 340 nm.

Statistical analysis

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

Results

The LC_{30} and LC_{50} values measured 48 h after treatments were 0.001 and 0.003 ppm, respectively, and these concentrations were selected as sublethal doses for the rest of the experiments (Table 1).

The mean deterrence of 3rd instar larvae treated with LC_{50} was 100% after 24 h. This rate was 86% in larvae treated with LC_{30} after 48 h of treatment (F = 3.47, df = 2, 6, p = 0.0709). The duration of the larval stage sharply increased in treated larvae (F = 26.17, df = 2, 6, p = 0.0011) with LC_{30} compared with the controls. However, the larvae treated

with LC_{50} did not survive (Table 2).Weight gain of the larvae was dramatically reduced in the larvae treated with LC_{50} and LC_{30} values (Table 3).

The effect of Achook® on biochemical and metabolic activities in *X. luteola* are depicted in (Table 4). As illustrated in the table, the activity level of ALT was significantly increased in both LC_{30} and LC_{50} as compared with control insects (F = 127.03, df = 2, 6, p < 0.001). The effect of Achook® on AST showed a non significant decrease following treatment (F = 3.77, df = 2,6, p = 0.0870). Activity of ALP increased sharply after treatment with both concentrations of Achook®, so that the highest activity was observed in concentration of LC_{50} (F = 217.11, df = 2,6, p = < 0.001) and ACP activity level increased significantly after treating the larvae with LC_{50}. However, no significant differences were observed in LC_{30} concentration compared with the control (F = 12.50, df = 2,6, p = 0.0072). Concentrations of LC_{50} and LC_{30} decreased the activity of lactate dehydrogenase (LDH), however, this effect was non-significant (F = 0.23, df = 2,6, p = 0.23). Alpha amylase decreased significantly (F = 26.22, df = 2,6, p = 0.0011). In addition to enzymatic compounds, concentrations of Achook® significantly changed the amount of non-enzymatic compounds so that the amount of glucose was decreased in both treatments (F = 4.92, df = 2,6 p = 0.0544). Urea was increased significantly with concentration of LC_{50} but no significant difference was observed in LC_{30} concentration compared to control (F = 22.69, df = 2,6, p = 0.0016). Total proteins decreased sharply in both cases (F = 19.61, df = 2,6, p = 0.0023). The amount of cholesterol in LC_{30} concentration decreased but increased in LC_{50} concentration (F = 3.59, df = 2,6, p = 0.0942). Amount of glycogen was significantly reduced by both concentrations of Achook® (F = 902.42, df = 2,6, p < 0.001). By measuring two detoxifying enzymes, general esterase and glutathione S-transferase, it was found that general esterase is the main detoxifying enzyme of neem based Achook insecticide in *X. luteola*. The LC_{50} and LC_{30} of Achook® significantly increased general esterase activity especially when α-naphtyl was used as substrate (F = 9.33, df = 2, p = 0.0144) (F = 1.64, df = 2,6, p = 0.2702). In the case of glutathione S-transferase, this bioinsecticide increased activity of GST in larvae fed on treated leaves (F = 3.41, df = 2,6, p = 0.1024).

Table 1 The LC_{50} and LC_{30} values, confidence limit (%95) and regression slope after 48 h exposure to neem in larvae of *X. luteola.*

Toxic material	Slope ± SE	X^2 (df)	LC_{50} (ppm)	LC_{30} (ppm)
Achook®	3.147 ± 0.484	1.38 (3)	3.3	2.25

Table 2 Life cycle of *X. luteola* after treatment with Achook®.

Treatments	3rd Instar larval duration (day)	Pupal and adult emergence %
LC_{50}	7.333 ± 1.074[*]	0
LC_{30}	10.667 ± 1.074	0
Control	5.666 ± 0.759	96.667 ± 2.402

* larvae used for this treatment were dead after seven days.

Table 3 Mean weight (g) of *X.luteola* larvae after treatment with Achook[®].

Treatments	Weight (g)at 2 DAT	Weight (g) at 4 DAT	Weight (g) at 6 DAT
LC_{50}	0.0066 ± 0.025^b	0.0088 ± 0.020^b	0.0186 ± 0.027^b
LC_{30}	0.0072 ± 0.020^b	0.0098 ± 0.033^{ab}	0.0153 ± 0.039^{ab}
Control	0.0098 ± 0.022^a	0.0144 ± 0.055^a	0.0186 ± 0.053^a

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.
DAT = days after treatment.

Table 4 Effects of Achook[®] on some biochemical compounds of *X. luteola* third instar larvae.

Compounds	Control	LC_{30}	LC_{50}
Alkalinaminotransferase (IU/L)	508.33 ± 4.00^a	566.67 ± 8.00^a	104.38 ± 3.30^b
Aspartate aminotransferase (IU/L)	401.8 ± 4.00^b	413.7 ± 8.00^b	652.1 ± 3.30^a
Alkaline phosphatase (IU/L)	0.065 ± 0.08^c	0.126 ± 0.13^b	0.299 ± 0.39^a
Acid phosphatase (IU/L)	0.095 ± 0.133^b	0.093 ± 0.06^b	0.194 ± 0.21^a
α-Amylase(IU/L)	0.220 ± 0.13^a	0.088 ± 0.18^b	0.085 ± 0.15^b
Protein (mg/dl)	1.235 ± 0.21^a	1.079 ± 0.21^b	1.015 ± 0.19^b
Glucose (mg/dl)	0.082 ± 0.10^a	0.039 ± 0.12^b	0.053 ± 0.14^{ab}
Urea (mg/dl)	0.243 ± 0.77^b	0.247 ± 0.28^c	0.400 ± 1.18^a
Cholesterol (mg/dl)	0.073 ± 0.15^a	0.033 ± 0.07^a	0.094 ± 0.20^a
Glycogen (mg/dl)	0.575 ± 0.10^a	0.230 ± 0.13^b	0.169 ± 0.07^c
Lactate dehydrogenase (U/L)	0.029 ± 0.16^a	0.025 ± 0.16^a	0.016 ± 0.12^a
Esterase, α-naphtyl (μmol/min/mh protein)	0.027 ± 0.08^b	0.028 ± 0.19^b	0.076 ± 0.16^a
Esterase, β-naphtyl (μmol/min/mh protein)	0.002 ± 0.12^b	0.034 ± 0.10^a	0.009 ± 0.14^b
glutathione S-transferase (μmol/min/mh protein)	0.075 ± 0.1^a	0.033 ± 0.09^a	0.030 ± 0.05^a

Means followed by the same letter in a row are not significantly different at P < 0.05 using Tukey's Studentized Range Test.

Discussion

In terms of integrative pest control and environmental protection, secondary plant metabolites possessing insecticidal, repellent and/or antifeedant properties are very desirable as a possible means of plant protection (Kosti, *et al*., 2013). Our results on Achook[®] proved its strong insecticidal, feeding deterrence, insect growth regulatory and biochemical effects.

Feeding deterrence could be attributed to the effect of the extract on chemical sensilla located

on mouthparts. The chemicals may block the stimulant effect of glucose, sucrose and inositol on chemoreceptors (Zapata, *et al.*, 2009); however, after 2 days the chemicals may evaporate from the leaf or lose its potency and this may explain the loss of deterrence. At that time the sensilla returns to its normal condition and the insects resume their feeding (Sadek 2003). Our results showed that Achook® has deterrence activity against *X. luteola* larvae. Our findings confirm earlier reports of repellent and antifeedant effects of neem on some insect species (Ruther and Thiemann 1997; Isman 2000; Koul 2005; Pavela *et al.*, 2010). Similarly, essential oils obtained from many plants including *Carum carvi* and *Thymus vulgaris* have found application as mosquito repellents (Pavela *et al.*, 2009); Nerio *et al.,* 2010) as well as Shekari *et al.*, (2008) showed the growth inhibitory effects of methanolic extract of *Artemisia annua* on *X. luteola*.

A majority of reports have shown an increase in larval duration by using plant products (Senthil Nathan 2005 and 2006). Here the increase in larval duration agrees with the effects of *Achillea millefolium* L. on *Pieris rapae* (Hasheminia *et al.,* 2011). Alouani *et al.*, (2009) reported that treatment of *Culex pipiens* larvae also prolonged the duration of larval stage.

The results of our study clearly manifested that neem based insecticide Achook® significantly reduced the weight of larvae of *X. luteola* as compared to control. This is supported by Wondafrash, *et al.*, (2012) who reported a significant reduction in weight of larvae for *Helicoverpa armigera* after treatment with *Azadirachtin indica*.

The aminotransferases are enzymes that catalyze the reaction between amino acid and α-keto acid. This reaction removes the amino group from the amino acid, leaving α-keto acid and converting it into an amino acid. These enzymes serve as a link between the carbohydrates and protein metabolism and are altered during various physiological processes (Etebari *et al.,* 2005 and Shekari *et al.*, 2008). Our results showed that the activity level of

ALT sharply increased at 48 h of treatment, that is corresponding with previous reports. For example Ender *et al.*, (2005) reported that diet with high level of methyl parathion significantly increased the activities of ALT in greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. Zibaee, *et al.*, (2011) showed that activity of these enzymes sharply increased after 48 h of pyriproxifen treatment on *Eurygaster integriceps.* The present study showed a sharp decrease in AST on treatment with Achook®, which is similar to reports by Hasheminia *et al.*, (2011) in which *Artemisia annua* L. and *Achillea millefolium* L. led to a reduction in the activity of AST in *Pieris rapae* L.

Acid (ACP) and alkaline phosphatases (ALP) are the hydrolase enzymes responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions, respectively under the name of dephosphorylation (Zibaee *et al.*, 2011). Our study showed that ACP and ALP are increased by Achook®, these results are in consistence with observations of Hasheminia *et al.*, (2011). The results are indicative of their involvement in the detoxification processes.

LDH is an important glycolytic enzyme involved in carbohydrate metabolism in many tissues (Shekari *et al.*, 2008). In the current study, LC_{50} and LC_{30} concentrations decreased the activity of lactate dehydrogenase.

α-Amylases catalyze the endohydrolysis of long α-1,4-glucan chains such as starch and glycogen (Terra and Ferriera 2005). In this study, concentrations of Achook® reduced α-amylase activity in larvae of *X. luteola*. Reduction of α-amylase activity may be due to defense compounds of plant that include inhibitors that act on insect digestive enzymes such as hydrolyses, α-amylases and proteinases. Some plant extracts and molecules of plant origin inhibited the activity of the α-amylase in vitro (Yazdani *et al.*, 2012)

In the present study, the amounts of seven non enzymatic compounds on the larvae fed on Achook® treated leaves were measured. The

amount of total protein decreased as neem based Achook® concentration increased, this could be due to the break-down of proteins into amino acids and their entrance into TCA cycle as keto acid. So, protein depletion in tissues might play a role in compensatory mechanisms under insecticidal stress, to provide intermediates in the Krebs cycle, by retaining free amino acid content in hemolymph (Shekari *et al*., 2008 and Zibaee *et al*., 2008). These results agree with previous results, for example Khosravi *et al*., (2011) who observed a significant decrease in the amount of total protein in larvae of *Glyphodes pyloalis* treated with *A. annua* methanolic extract.

Glucose concentration decreased following treatment with Achook®. Earlier studies had also shown a decrease following treatment with chemicals such as pyriproxyfen and Fenitrothion (Ethion Nath 2003 and Etebari *et al*., 2005).

Urea is the excreted end product of insects and its amount is correlated with the amount of protein in the insect's body. Dungern and Briegel (2001) reported that the activity level of xanthin dehydrogenase increased due to presence of higher protein in the hemolymph. At 48 h post treatment, the amount of urea increased in treated larvae. This result indicates that the increase in urea is probably due to altered metabolic pathway after treatment that prevents the natural excretion of urea from the insect body (Etebari *et al*., 2004).

An increase in cholesterol after treatment with plant extracts was observed. Etebari and Matindoost (2004a) reported that if the feeding activity is normal, cholesterol amount in the hemolymph of silkworms increases. When the feeding of larvae, however, is interrupted the amount of these metabolites are severely decreased (Etebari and Matindoost 2004b).

Glycogen is a polymer of several glucose residues existing as a branched chain storage form (Klowden 2007 and Lide 1998). Concentrations of Achook® decreased the amount of glycogen of *X. luteola*. On the other hand, changes in amount of glycogen as observed in the present and previous studies

could also be due to upsetting the homeostatic mechanism in insects by insecticides (Zibaee *et al*., 2011; Oguri and Steele 2007, Surendra-Nath, 2003).

Esterase (EST) is an important detoxifying enzyme in vivo which hydrolyses the esteric bond in synthetic chemicals (Hemingway and Karunatne 1998). GSTs play an important role in insecticide resistance and are involved in the metabolism of organophosphorus and organochlorine compounds. Other xenobiotics such as plant defence allelochemicals against phytophagous insects induce GST activity (Yu 1982; Vanhaelen *et al*., 2001). In this study, the activities of esterase and glutathione –S transferase were increased in treated larvae. This shows that these enzymes have a role in detoxification of toxic compound. These results are consistent with observations of Khosravi *et al*., (2011) who reported that GST and general esterase activity increased in the larvae of *G. pylolais* treated with *A. annua* methanolic extract. Also Vanhaelen *et al*., (2001) showed that Brassicaceae secondary metabolites induced GST activity in *Myzus persicae* and several lepidopterous species such as *Heliothis virescens* Fabricius, *Trichoplusia ni* Hubner and *Anticarsia gemmatalis* Hubner.

In conclusion, the present results indicate that neem based insecticides have antifeedant and toxic effects on *X. luteola* and can inhibit growth through various metabolic processes. Therefore, this bioinsecticide could be applied to the third instar larvae when it may be more toxic to larvae and may therefore serve as an effective and safer method in the control of *X. lutola*.

Acknowledgments

References

Alouani, A., Rehimi, N. and Soltani, N. 2009. Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in

the republic of Algeria. Jordan Journal of Biological Sciences.

Bernfeld, P. 1955. α-Amylases. Methods in Enzymology, 1: 149-151.

Bessey, O. A., Lowry, O. H. and Brock, M. J. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. Journal of Biological Chemistry, 164: 321-329.

Borowiec, L. and Sekerka, L. 2010. Cassidinae. In: Löbl, I., Smetana, A. (Eds.), Catalogue of Palearctic Coleoptera, vol. 6, Chrysomeloidea. Apollo Books, Wroclaw, pp. 64-65, 368-390.

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.

Chiffelle, I., Huerta, A., Celis, M. and Araya, J. 2013. Proximal analysis and insecticidal effects of extracts from pepper tree (Schinusmolle) leaves on elm leaf beetle (*Xanthogaleruca luteola*) larvae. Industrial Crops and Products, 43: 523-528.

Dungern, P. and Briegel, H. 2001. Protein catabolism in mosquitoes: ureotely and uricotely in larval and imaginal *Aedes aegypti*. Journal of Insect Physiology, 74: 131-141.

Ender, I., Ferah, A., Kemal, B. and Ahmet, G. 2005. Biochemical stress indicators of greater wax moth exposure to organophosphorus insecticides, Journal of Economic Entomology, 98: 358-366.

Etebari, K., Bizhannia, A. R., Sorati, R. and Matindoost, L. 2005. Biochemical changes in hemolymph of silkworm larvae due to pyripyroxyfen residue. Pesticide Biochemistry and Physiology, 88: 14-19.

Etebari, K. and Matindoost, L. 2004a. A study on the effects of larval age on biochemical macromolecules abundance of haemolymph in silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae). Journal of Entomological Society of Iran, 24: 1-16.

Etebari, K. and Matindoost, L. 2004b. The study on effects of larval age and starvation stress on biochemical macromolecules abundance of hemolymph in silkworm *Bombyx mori*. Proceedings of the 16th Iranian Plant Protection Congress, August 28th-September 1st, 2004, The University of Tabriz, Iran, p. 435.

Habing, W. H., Pabst, M. J. and Jakoby, W. B. 1974. Glutathione S-transferases. The first step in mercapturic acid formation. Journal of Biological Chemistry, 249: 7130-7139

Hasheminia, S. M., Jalali Sendi, J., Talebi Jahromi, K H. and Moharramipour, S. 2011. The effects of *Artemisia annua* L. and *Achillea millefolium* L. crude leaf extracts on the toxicity, development, feeding efficiency and chemical activities of small cabbage *Pieris rapae* L. (Lepidoptera: Pieridae). Pesticide Biochemistry and Physiology, 99: 244-249.

Hemingway, J. and Karunatne, S.H.P. 1998. Mosquito carboxylesterases: A review of the molecularbiology and biochemistry of a major insecticide resistance mechanism. Medical and Veterinary Entomology, 12: 1-12.

Huerta, A., Chiffelle, I., Puga, K., Azua, F., Jimenez, R. and Araya, J. E. 2011. Life cycle of *Xanthogaleruca luteola* Mull (Col.: Chrysomelidae) in Santiago, Chile, and sex fenotype differentiation of adults. Boletín de Sanidad Vegetal Plagas, 37: 57-64.

Huerta, A., Chiffelle, I., Puga, K., Azúa, F. and Araya, J. 2010. Toxicity and repellence of aqueous and ethanolic extracts from Schinusmolle on elm leaf beetle *Xanthogaleruca luteola*. Crop Protection, 29: 1118-1123.

Hummel, H., Hein, D., Ley, S., Morgan, E., Kraus, W. and Schmutterer, H. 2011. Twenty five years of azadirachtins(1986-2011). Pestycydy/Pesticides Publication Type, 1 (4): 49-56.

Isman, M. B. 2000. Plant essential oils for pest and disease management. Crop Protection,19: 603 608.

Khosravi, R., Jalali Sendi, J., Ghadamyari, M. and Yezdani, E. 2011. Effect of sweet worm wood *Artemisia annua* crude leaf extracts on some biological and physiological

characteristics of the lesser mulberry pyralid, *Glyphodes pyloalis*. Journal of Insect Science, 11: 156.

King, J. 1965. The dehydrogenases or oxidoreductases. Lactate dehydrogenase, in: Practical Clinical Enzymology, Van Nostrand D, London, pp. 83-93.

Klowden, M.J. 2007. Physiological systems in insects. Academic Press, 697 p.

Kostic, I., Petrovic, O., Milanovic, S., Popovic, Z., Stankovic, S.,Todorovic, G. and Kostic, M. 2013. Biological activity of essential oils of *Athamanta haynaldii* and *Myristica fragransto* gypsy moth larvae. Industrial Crops and Products, 41: 17-21.

Koul, O. 2005. Insect Antifeedants. CRC Press, Boca Raton, FL.

LeOra software, 1987. Polo-Pc: a user guide to probit or logit analysis. LeOra software, Berkeley, California.

Lide, D. R. 1998. Handbook of Chemistry and Physics, 87 ed., CRC Press, Boca Raton, 589 FL, 3-534.

Nath, B. S. 2003. Shifts in glycogen metabolism in hemolymph and fat bodyof the silkworm, *Bombyxmori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticide toxicity. Pesticide Biochemistry and Physiology. 74: 73-84.

Nerio, L. S., Jesus, O. V. and Stashenko, E. 2010. Repellent activity of essential oils: A review. Bioresource Technology, 101: 372-378.

Oguri, E. and Steele, J. E. 2007. A comparative study of the metabolic effects of hyper trehalosemic hormone and 1,2,3,4,5,6-hexachlorocyclohexane (c-HCH) in the American cockroach, *Periplaneta Americana*. Pesticide Biochemistry and Physiology, 87: 196-203.

Pavela R 2010. Antifeedant activity of plant extracts on *Leptinotarsa decemlineata* Say. And *Spodoptera littoralis* Bois. larvae. Industrial Crops and Products, 32: 213-219

Pavela, R., Kazda, J. and Herda, G. 2009. Effectiveness of Neem (*Azadirachtaindica*) insecticides against Brassica pod midge (*Dasineura brassicae* Winn.). Journal of Pest Science, 82 (3): 235-240.

Rehimi, N., Alouani, A. and Soltani, N. 2011. Efficacy of Azadirachtin against Mosquito Larvae *CulexPipiens* (Diptera: Culicidae) under Laboratory Conditions. European Journal of Scientific Research, 57: 223-229.

Reinhold, J. G. 1953. in: M. Reiner (Ed.), Standard methods in clinical chemistry, Academic Press, NewYork. 1: 88.

Romanyk, N. and Cadahía, D. 2002. Plagas de Insectos en las Mas as Forestales. Ediciones Mundi-Prensa, Madrid.

Ruther, J. and Thiemann, K. 1997. Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. Entomologia Experimentaliset Applicata, 84: 183-185.

Sadek, M. M. 2003. Antifeedant and toxic activity of *Adhatodavesica* leaf extract against *Spodoptera littura* (Lepidoptera: Noctuidae). Journal of Applied Entomology. 127: 364-404.

Saleem, M. A. and Shakoori, A.R. 1987. Point effects of Dimilin and Ambush on enzyme activities of *Tribolium castaneum* larvae. Pesticide Biochemistry and Physiology, 29: 127-137.

SAS institute 1997. SAS/STAT User's Guide for Personal Computers. SAS Institute, Cary, Nc.

Senthil Nathan, S. and Sehoon, K. 2006. Effect of *Meliaazadarach* L. extract on the teak defoliator *Hyblae apuera* Cramar (Lep: Hyblaeidae). Crop Protection, 25 (3): 287-291.

Senthil Nathan, S. and Kalaivani, K. 2005. Efficacy of nucleopolyhedrovirus (NPV) and azadirachtin on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Biological Control, 34: 93-98.

Shekari, M., Jalali Sendi, J., Etebari, K., Zibaee, A. and Shadparvar, A. 2008. Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). Pesticide Biochemistry and Physiology, 91; 66-74.

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Siegert, K. J. 1987. Carbohydrate metabolism in Manducasexta during late larval development. Journal of Insect Physiology, 33: 421-427.

Terra, W. R., and Ferriera, C. 2005. Biochemistry of digestion. In: Comprehensive molecular insect science by Lawrence IG, Kostas I, Sarjeet SG, editors. Vol. 3. Oxford: Elsevier. p. 171-224.

Van Asperen K. 1962. A study of housefly esterases by mean of sensitive colorimetric method. Journal of Insect Physiology, 8: 401-416.

Van, H. E. 1965. Microseperation of glycogen,sugars and lipids. Analytical Biochemistry, 11: 26-271.

Vanhaelen, N., Haubruge, E., Lognay, G. and Francis, F. 2001. Hoverfly glutathione S-transferases and effect of Brassicaceae secondary metabolites. Pesticide Biochemistry and Physiology, 71: 170-177.

Wondafrash, M., Getu, E. and Terefe, G. 2012. Neem, *Azadirachta indica* (A. Juss) Extracts Negatively Influenced Growth and Development of African Bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Academic Journal of Entomology, 5 (1): 22-27.

Xie, Y. S. and Isman, M. B. 1992. Antifeeding and repellent effect of Meliaceous plants to some insect pest, South China Agricultural University, 4: 1-7.

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, 1-12

Yu, S. J. 1982. Host plant induction of glutathione S-transferase in the fall army worm. Acta Entomologica Sinica, 17: 52-60.

Zapata, N. Budia, F. Vinuela, E. and Medina, P. 2009. Antifeedant and growth inhibitory effects of extracts and drimanes of Drimyswinteri stem bark against *Spodoptera littoralis* (Lep: Noctuidae), Industrial Crops and Products, 30: 119-1255.

Zibaee, A., Zibaee, I. and JalaliSendi, J. 2011. A juvenile hormone analog, pyriproxifen, affects some biochemical components in the hemolymph and fat bodies of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). Pesticide Biochemistry and Physiology, 100: 289-298.

Zibaee, A., Jalali Sendi, J., Etebari, K., Alinia, F. and Ghadamyari, M. 2008. The effect of diazinon on some biochemical characteristics of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae), rice striped stem borer, Mun. Applied Entomology and Zoology, 3: 255-264.

تأثیر آزادیراختین روی مرگ و میر، ویژگی‌های زیستی و بیوشیمیایی سوسک برگ‌خوار نارون

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چکیده: سوسک برگ‌خوار نارون یکی از آفات مهم نارون در ایران می‌باشد. در مطالعه حاضر، اثرات کشندگی و زیرکشندگی چریش (Achook ®) حاوی ۰/۰۳ درصد آزادیراختین روی مرگ و میر، رشد، وزن لاروها و بازدارندگی تغذیه و همچنین فعالیت‌های آنزیمی و غیرآنزیمی لارو سن سوم، سوسک برگ‌خوار نارون بررسی شد. مقدار LC_{50} و LC_{30} روی لارو سن سوم، پس از ۴۸ ساعت تیمار، به‌ترتیب ۳/۳ و ۲/۲۵ پی‌پی‌ام محاسبه شد. نتایج، مرگ و میر بالا، افزایش در طول دوره لاروی، عدم ظهور حشره کامل، کاهش وزن و دورکنندگی تغذیه در لاروهای تیمار شده با غلظت‌های LC_{50} و LC_{30} را نشان می‌دهد. به‌علاوه، تجزیه و تحلیل بیوشیمیایی، اختلاف قابل‌توجهی در میزان ترکیبات بیوشیمیایی در لاروهای تیمار شده پس از ۴۸ ساعت نشان می‌دهد، به‌طوری که. در لاروهای تیمار شده، سطح فعالیت آلانین آمینوترانسفراز، آلکالین فسفاتاز، اسید فسفاتاز و α- آمیلاز به‌عنوان ترکیبات آنزیمی و اوره، و کلسترول به‌عنوان ترکیبات غیرآنزیمی افزایش یافته است، اما آسپارتات آمینوترانسفراز، لاکتات دهیدروژناز، پروتئین، گلیکوژن، گلوکز نتایج برعکسی را نشان می‌دهند و سطح فعالیت آنزیم‌های سم‌زدا مانند استراز و گلوتاتیون اس-ترانسفراز به‌طور قابل‌توجهی تحت تأثیر قرار گرفتند. از این‌رو، چریش یک ترکیب بی‌خطرممکن است به‌عنوان حشره‌کش طبیعی برای استفاده بالقوه در مدیریت تلفیقی آفات در نارون‌های شهری که در آن استفاده از حشره کش‌های شیمیایی به‌ندرت صورت می‌گیرد، استفاده شود.

واژگان کلیدی: آزادیراختین، ترکیبات بیوشیمیایی، سوسک برگ‌خوار نارون، کشندگی