Journal of Medicinal Plants Research Vol. 5(14), pp. 3229-3235, 18 July, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Artemisia annua L. (Asterasea) changes some biochemical compounds in the hemolymph of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae)

Idin Zibaee[1], Arash zibaee[2]*, Ali Reza Bandani[1], Jalal Jalali Sendi[2] and Reza Talaei-Hassanloei[1]

[1]Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj 31584, Iran.
[2]Department of Plant Protection, College of Agriculture, University of Guilan, Rasht, 41635-1314, Iran.

Accepted 31 March, 2011

Laboratory assays were carried out to evaluate the effect of methanolic extract of *Artemisia annua* L. on some biochemical compounds in the hemolymph of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). It was found that the activity levels of two aminotransferases and phosphatases increased after extract exposure. Activity of lactatae dehydrogenase, as an enzyme showing tissue damage, significantly increased in all time intervals. Non-enzymatic parameters including, trehalose, protein and urea decreased after treatment by plant extract but the amount of total lipid showed no significant differences. These results indicate that *A. annua* L. extract contains inhibitors of key metabolic pathways that may be useful in future control of the fall webworm.

Key words: *Artemisia annua*, biochemical compounds, *Hyphantria cunea*.

INTRODUCTION

The fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) is an insect native of North America, which has been accidentally introduced to various areas of Europe and Asia (Warren and Tadic, 1970). *H. cunea* Drury is an important pest of the forest, orchards and crop plants in the world and its current introduction and damage in the north of Iran cause extensive problems on the agriculture products especially orchards (Zibaee et al., 2010). The feeding activity of this pest makes trees without leaves and significantly decreases the photosynthesis leading to low quality of the fruits, as well as several deficiencies in wood processing in northern Iran.

Considering the negative effects of synthetic pesticides, especially, on non-target organisms caused a general perception that natural compounds are better products or Generally Regarded As Safe (GRAS) (Scott et al., 2003). So, researches has been concentrated on the plant kingdom for solutions leading to the production of a myriad of secondary compounds that can have toxic, growth reducing, and antifeedant properties against insects (Isman, 2006). The genus *Artemisia* is a member of a large plant family Asteracea (Compositae) encompassing more than 300 different species. Several isolated compounds from this species have shown anti-malarial, antibacterial, anti- inflammatory, plant growth regulatory and cytotoxicity (antitumor) activities (Akhtar and Isman, 2004). The species *A. annua* known as sweet worm wood grows wild in Europe and America and is planted widely in China, Turkey, Vietnam, Afghanistan and Australia (Bhakuni et al., 2001). The plant also grows wild in the northern parts of Iran around paddy fields and could be a potential source of plant extract production in the region.

Different control procedures have been conducted to decrease the population of *H. cunea*, but minor success has been achieved by the researchers. In addition, extensive usage of synthetic insecticides is troublesome because of pest prevalence in the urban areas and arising environmental pollution. In our previous study, an experiment was conducted to examine the potential effects of medicinal plants, *A. annua* and *Lavandula stoechas* in addition to the insect pathogenic bacterium, *Bacillus thuringiensis* var. *kurstaki* on activities of digestive enzymes (*α*-amylase, *α*- and *β*-glucosidase, lipase and proteases) and lactate dehydrogenase (LDH)

*Corresponding author. E-mail: arash.zibaee@gmx.com Tel: +98-0131- 6690264. Fax: +98 131 6690281.

in *H. cunea* in the presence of two hosts, mulberry and sycamore.

Results showed that *B. thuringiensis* var. *kurstaki* and plant extracts when administered orally, affected the digestive enzyme profiles of *H. cunea*. Combined effect of *B. thuringiensis*, *A. annua* and *L. stoechas* extracts on mulberry decreased the activities of digestive enzymes in a dose-related manner, except for *β*-glucosidase and lipase. When larvae were treated by different concentrations of the mentioned insecticides, LDH activity increased, that is, the higher activity was obtained by *B. thurengiensis* alone and *B. thurengiensis* and *L. stoechas* extracts together. The least activity was observed in the case of *L. stoechas* extracts alone on both hosts.

Physiological analysis would be particularly informative when using combination of biopesticides to enhance the efficiency of a safe management process. To continue our study and confirm previous one, we conducted the current one to find possible changes of the hemolymph components after treatment by *A. annua* extract. These findings help us to reach a safe and efficient control against *H. cunea*.

MATERIALS AND METHODS

Insect

H. cunea larvae were collected from plane trees (*Platanus orientalis*) in Rasht (Agricaltural university) Guilan, Iran. They were maintained under laboratory conditions at 27±2°C under 14 h light: 10 h dark photoperiod in cages 30×30×40 cm. The larvae fed by plane leaves so that the leaves changed daily to prevent any starvation stress on the larvae. These larvae were used to initiate the experiments.

Methanolic extract from leaves of *A. annua*

Leaves of *A. annua* were collected in June around paddy fields in Rasht, Guilan province of Iran. Leaves were washed with distilled water and dried at room temperature in the shade. Methanolic extraction was carried out according to the procedure described by Shekari et al. (2008). Briefly, 30 g of dried leaves were stirred with 300 ml of 85% methanol in a flask for 1 h.

The methanolic solution was incubated for 48 h at 4°C and then stirred for an additional hour and then filtered through Whatman No.4 filter paper. The solvent was removed by vacuum in a rotary evaporator and the dark green residue was dissolved in 10 ml acetone and used as a starting stock solution. Further dilutions with either acetone or distilled water were used to prepare different concentrations.

Bioassays and treatment

Bioassay experiments were performed by fourth instar larvae of *H. cunea* using three concentrations of 0.09, 0.22 and 0.44% from *A. annua* on plane tree. Control leaves were treated only with distilled water. For each treatment, 30 larvae were used in each concentration and whole experiments were replicated twice (180 larvae were used for bioassay). Fresh plane tree were sprayed with different concentrations of the *A. annua* and allowed to air dry. Fourth instar larva were starved for 4 h and then fed on leaves treated with the different concentrations of *A. annua* and larvae collected for biochemical tests after 24, 48 and 72 h.

Hemolymph extraction

The hemolymph was collected by capillary tubes and poured in Eppendorf tubes containing several crystals of phenylthiourea to prevent melanization. The hemocytes were sedimented by centrifuging at 4°C, 12000 rpm for 5 min. Supernatant was kept at -20°C and used in the subsequent experiments.

Biochemical assessments

Estimation of aspartate (EC 2.6.1.1) and alanin aminotransferases (EC 2.6.1.1)

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

Assay of estimation of acid (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 of that of p-nitrophenyl phosphate at 310 nm. On converting the p-nitrophenolate into 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), the 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. The sample containing 0.01 ml was also added to the test tubes. Test tube samples were incubated for exactly 15 min at 37°C and then arrested by adding 1 ml of color reagent (2,4- dinitrophenyl hydrazine) to each tube and the incubation continued for an additional 15 min. After cooling at room temperature, 10 ml of 0.4N 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.

Estimation of trehalose and Lipid

Trehalose and lipid content were assessed using the method of Van Handel (1965). Lipids were further processed to remove phospholipids by running the lipid containing extract through a column containing 0.2 g of 100-mesh silicic acid, which was subsequently washed eight times with 1 ml of pure chloroform each time to elute neutral lipids. Lipids were spectrophotometrically assayed against a range of known standards using the vannilin–phosphoric acid method. Trehalose was similarly assayed using the

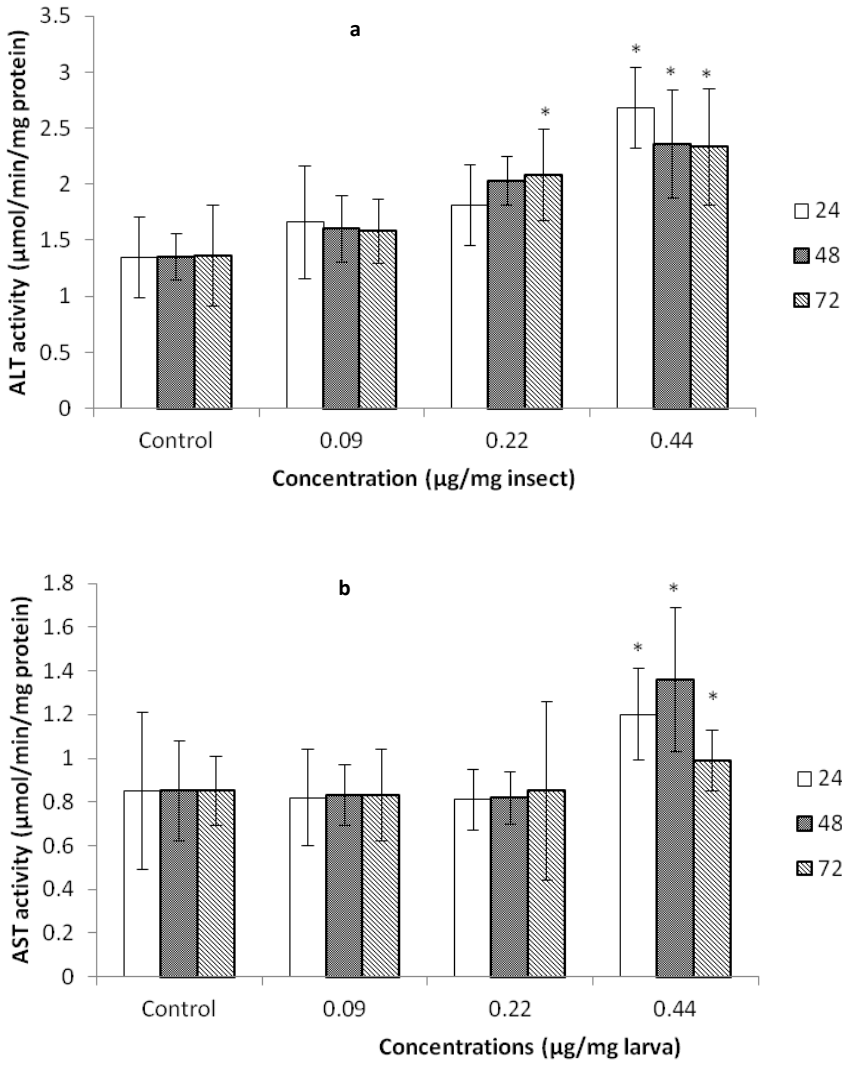

Figure 1. Effect of *A. annua* extract on alanine aminotransferase (a) and aspartate aminotransferase (b) in the hemolymph of *H. cuneas* larvae (4th instar). Asterisks showed significant differences in two way analysis (Factorial test) at $p \leq 0.05$).

anthrone–sulfuric acid method.

Estimation of protein and urea

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

Statistical analysis

For determination of mortality and lethal concentration, POLO-PC software (LeOra 1987) was used. All data were compared by two-way analysis of variance (ANOVA) followed by factorial test and significant differences were found at $p \leq 0.05$ (SAS 1997).

RESULTS AND DISCUSSION

A. annua extract significantly changed the activity of two assayed aminotransferase after all time intervals when concentration of 0.44 µg/mg larva were used (Figures 1a and b). The effect on these two enzymes was different because the activity of ALT increased after 24 h, then decreased after two other time intervals (Figure 1a). Although, activity of AST increased in all time intervals when 0.44 µg/mg larva were used but the highest activity was observed after 48 h (Figure 1b). ALT is a transaminase enzyme that is found in the various tissues

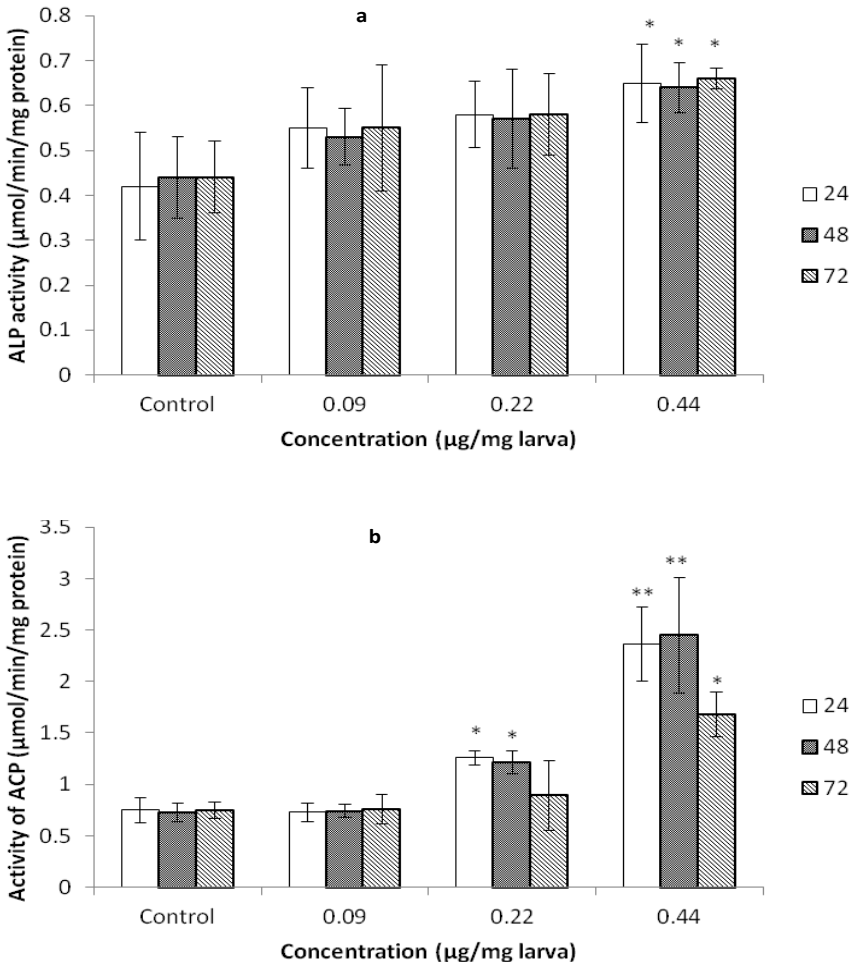

Figure 2. Effect of *A. annua* extract on alkaline phosphatase (a) and acid phosphatase (b) in the hemolymph of *H. cuneas* larvae (4th instar). Asterisks showed significant differences in two way analysis (Factorial test) at $p \leq 0.05$).

associated with the liver in vertebrates and fat bodies in insects (Thomas, 1998). The enzyme catalyzes two parts of the alanine cycle in transamination process. Elevation of ALT activity often suggest the existence of a physiological challenge in body such as microorganism infections, damage to some tissues or being a toxic material (Giboney, 2005). AST is similar to ALT, which is another enzyme associated in transamination process. It facilitates the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate, and vice-versa as an important activity during transamanation in insects (Klowden, 2007). The results of the current study were similar to previous studies on the effect of plant extracts on ALT and AST activity. Ender et al. (2005) reported that high level of methyl parathion significantly increased the activities of ALT and AST in greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. Shekari et al. (2008) demonstrated that different concentrations of *A. annua* on *Xanthogaleruca luteola* Muller (Coleoptera: Chrysomellidae) affected the activity of these two aminotrabnsferase by decreasing ALT activity after 24 h and increasing after 48 h for 5 and 10% concentrations. In the case of AST, concentrations of mentioned extract increased the enzyme activity after both 24 and 48 h.

Our results showed that different concentrations of *A. annua* increased the activity of two assayed phosphatases (ALP and ACP) in a time- and dose-manner (Figure 2 a, b). Although, the concentration of 0.44 µg/mg larva showed significant differences compared with control, but the activity of both enzymes decreased after 72 h of post-treatment. Alkaline phosphatase (ALP*, E.C.3.1.3.1*) and acid phosphatase (ACP, *E.C.3.1.3.2*) hydrolyze phosphomonoesters under alkaline or acid conditions, respectively. ALP was primarily found in the intestinal epithelium of animals and its first role is digestion of phospholipids in the food components. So, these phosphatases involve in the transphosphorylation reaction and hence, midgut must have the highest ALP and ACP activity as compared to

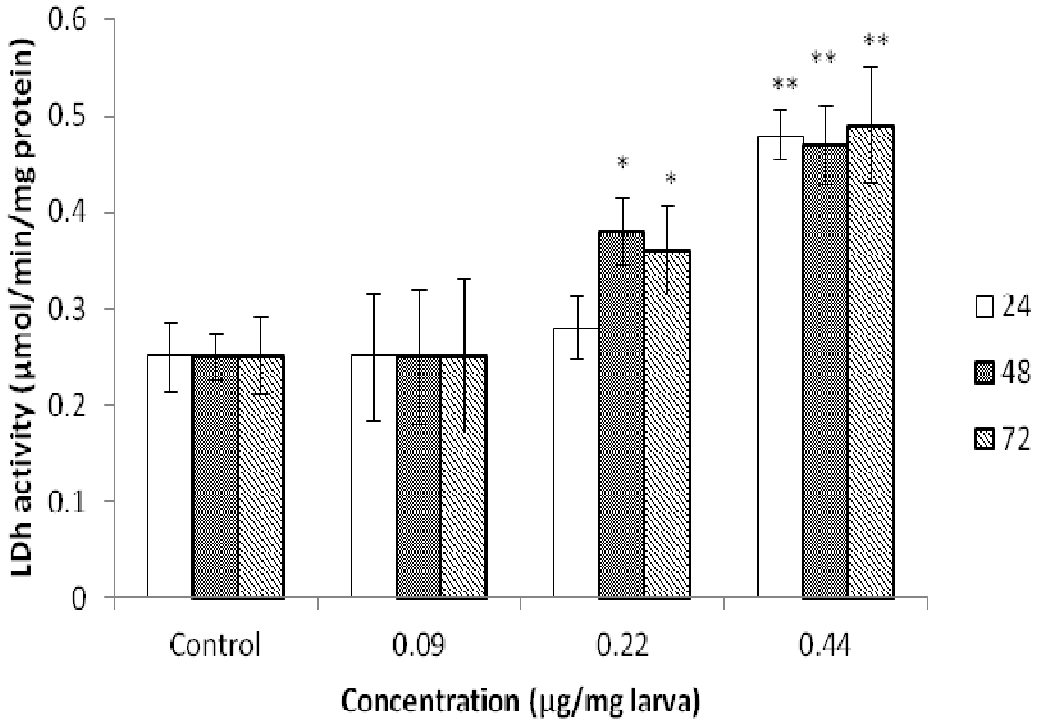

Figure 3. Effect of *A. annua* extract on lactate dehydrogenase in the hemolymph of *H. cuneas* larvae (4th instar). Asterisks showed significant differences in two way analysis (Factorial test) at $p \leq 0.05$).

other tissues (Sakharov et al., 1989). For example, Senthil Nathan (2006) showed that treatment of rice plants with *Melia azedarach* Juss (Meliaceae) extracts decreased the activity level of ALP in *Cnaphalcrocis medinalis* (Guenee). These authors reported that feeding *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on *Ricinus communis* L. treated with azadirachtin decreases the amount of this enzyme in the midgut (Senthil Nathan and Kalaivani, 2005). Zibaee and Bandani (2010) after treating *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) adults by *A. annua* extraction, found the overall reduction in the activity of ALP and ACP due to the increase in plant extract concentrations so that there were significant differences among control and three treatments. These findings indicated changing the physiological balance of the midgut and disruption in digestion and absorption of food in the treated larvae of *H. cunea*.

Feeding of *H. cunea* larvae by different concentrations of *A. annua* extract caused significant increase in LDH activity especially after using concentrations of 0.22 and 0.44 µg/mg larva at all-time intervals (Figure 3). LDH is an important glycolytic enzyme involved in carbohydrate metabolism in many tissues (Shekari et al., 2010). It has been used as an indicative criterion of exposure to chemical stress (Wu and Lam, 1997; Diamantino et al., 2001) and as an index of anaerobic metabolism (Chamberlin and King, 1998). Increasing the activity of LDH in organisms shows negative effects of toxic materials on tissue extraction and death of cells.

In the present study, the amounts of four non-enzymatic compounds were measured on the larvae fed on different concentrations of *A. annua* (Figure 4a to d). Results showed that the amount of all non-enzymatic components decreased in the hemolymph of fed larvae on all concentrations except for total lipid. The aim of this study was to show the correlation of these compounds with some enzymatic compounds that has been reported in Zibaee et al. (2010) and in this current study. The amount of these components depends on balanced physiological status in insects and their decreased amounts show any disruption in digestive enzymes activity and transaminase one. Etebari et al. (2006) showed that many insecticides decreased feeding efficiency and protein amount of an insect's body. Schmidt et al. (1998) showed that treatment of *Spodoptera littoralis* Fabricius (Lepidoptera: Noctuidae) and *Agrotis ipsilon* Stroll (Lepidoptera: Noctuidae) with azadirachtin decreased protein of hemolymph. This could be due to the break-down of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect. Therefore, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the krebs cycle by retaining free

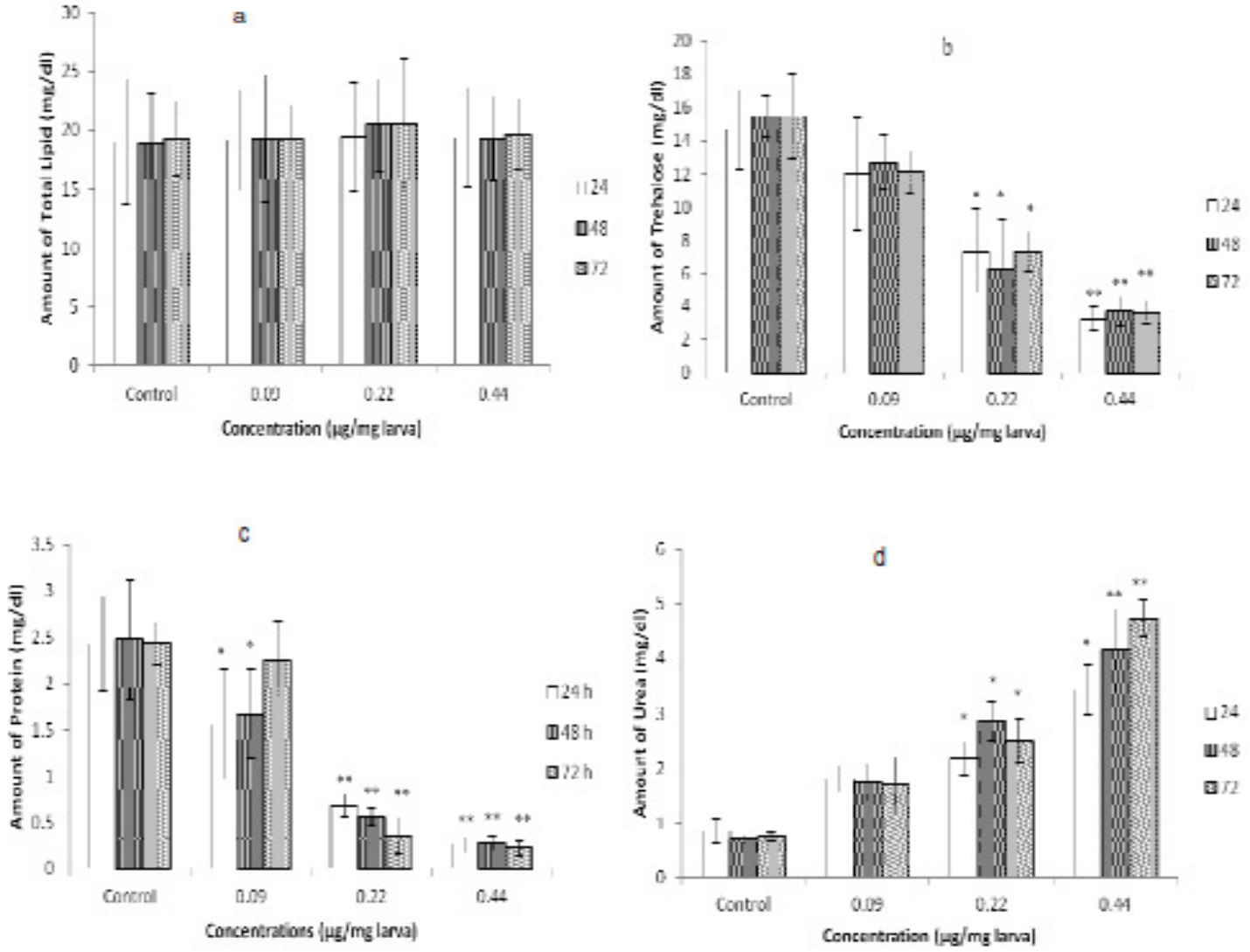

Figure 4. Effect of *A. annua* extract on the some non-enzymatic compounds in the hemolymph of *H. cuneas* larvae (4th instar). Asterisks showed significant differences in two way analysis (Factorial test) at $p \leq 0.05$).

amino acid content in hemolymph (Etebari et al., 2006). Shekari (2006) also showed similar results on *G. luteola* in the amount of glucose.

Overall results of this study revealed that feeding of *H. cunea* larvae by different concentrations of *A. annua* extract changed the activity of some enzymatic and non-enzymatic components. These changes decreased the survival of larvae in a subsequent life cycle and could be an alternative way to decrease destructive population of this pest in the north of Iran.

ACKNOWLEDGEMENTS

Authors would like to thanks B. Kouchaki for his assistance in collecting and rearing of the insects. Also, thanks to the laboratory staffs of the universities of Guilan and Tehran.

REFERENCES

Akhtar Y, Isman MB (2004). Comparative growth inhibitory and antifeedant effects of plant extract and pure allelo- chemicals on some phytophagous insect species. J. Appl. Entomol., 128: 32-38.

Bessey OA, Lowry OH, Brock MJ (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem., 164: 321-329.

Bhakuni RS, Jain DC, Sharma RP, Kumar S (2001). Secondary metabolites of *Artemisia annua* and their biological activity. Curr. Sci., 80: 35-49.

Bradford M (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Diamantino TC, Amadeu E, Soaresa MVM, Guilherminoc L (2001). Lactate dehydrogenase activity as an effect criterion in toxicity tests with *Daphnia magna*. Chemosphere, 45: 553-560.

Ender I, Ferah A, Kemal B, Ahmet G (2005). Biochemial stress indicators of greater wax moth, *Galleria mellonella* exposure to organophosphorus insecticides. J. Econ. Entomol. 98: 358-366.
Etebari K, Bizhannia AR, Sorati R, Matindoost L (2005). Biochemical changes in hemolymph of silkworm larvae due to pyripyroxyfen residue. Pestic. Biochem. Physiol., 88: 14-19.
Giboney PT (2005). Mildly Elevated Liver Transaminase Levels in the Asymptomatic Patient. Am. Fam. Physician, 71: 1105-1110.
Isman MB (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol., 51: 45-66.
King J (1965). The dehydrogenases or oxidoreductases. Lactate dehydrogenase, in: Practical Clinical Enzymology, Van Nostrand D, London, pp. 83-93.
Klowden MJ (2007). Physiological systems in insects. Academic Press. 697 p.
LeOra software Polo-Pc (1987). a user guide to probit or logit analysis. LeOra software, Berkeley, California.
Sakharov IY, Makarova IE, Ermolin GA (1989). Chemical modification and composition of tetrameric isozyme K of alkaline phosphatase from harp seal intestinal mucosa. Comp. Biochem. Physiol. Part B, 92: 119-122.
SAS institute (1997). SAS/STAT User's Guide for Personal Computers. SAS Institute, Cary, Nc.
Schmidt GH, Rembold H, Ahmed AII, Breuer AM (1998). Effect of *Melia azedirach* fruit extract on juvenile hormone titer and protein content in the hemolymph of two species of noctuid lepidopteran larvae (Insecta: Lepidoptera: Noctuidae). Phytoparas, 26: 283-291.
Scott IM, Jensen H, Scott JG, Isman MB, Arnason JT, Philogène BJR (2003). Botanical Insecticides for Controlling Agricultural Pests: Piperamides and the Colorado Potato Beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Arch. Insect. Biochem. Physiol., 54: 212-225.
Senthil NS (2006). Effects of *Melia azedarach* on nutritional physiology and enzyme activities of the rice leaffolder *Cnaphalocrocis medinalis* (Gnenee) (Lepidoptera: Pyralidae). Pestic. Biochem. Physiol., 84: 98-108.
Senthil NS, Kalaivani K (2005). Efficacy of nucleopolyhedrovirus (NPV) and azadirachtin on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Biol. Control, 34: 93-98.
Shekari M, Jalali J, Etebari K, Zibaee A, Shadparvar A (2008). Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). Pestic. Biochem. Physiol., 91: 66-74.
Thomas L (1998). Clinical Laboratory Diagnostic. 1st ed. TH Books Leaving society, Frankfurt, pp. 89-94.
Van HE (1965). Microseperation of glycogen,sugars and lipids. Anal. Biochem., 11: 26 271.
Warren LO, Tadic M (1970). The Fall Webworm, *Hyphantria cunea*(Drury). Arkansas. Agri. Exp. Station Bull., 759: 1-106.
Wu RSS, Lam PKS (1997). Glucose-6-phosphate dehydrogenase and lactate dehydrogenase in the green-lipped mussel (Perna viridis). Possible biomarker for hypoxia in the marine environment. Water Res., 31: 138-142.
Zibaee I, Bandani AR, Jalali Sendi J, Talaei HR, Kouchaki B (2010). Effects of *Bacillus thuringiensis* var. *kurstaki* and medicinal plants on *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). Invertebrate Surviv. J., 7: 251-261.
Zibaee A, Bandani AR (2010). A study on the toxicity of the medicinal plant, *Artemisia annua* L. (AStracea) extracts the Sunn pest, *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae). J. Plant Protection Res., 50: 48-54.