

ORIGINAL ARTICLE

## Effects of *Artemisia annua* methanolic extract on the enzymatic components of intermediary metabolism and the antioxidant system of *Pseudococcus viburni* Signoret

Samar Ramzi<sup>1\*</sup>, Ali Seraji<sup>1</sup>, Reza Azadi Gonbad<sup>1</sup>, Kimia Mirhaghparast<sup>2</sup>, Zahra Mojib-Haghghadam<sup>3</sup>

<sup>1</sup>Tea Research Center, Horticulture Science Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Lahijan, Iran

<sup>2</sup>Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

<sup>3</sup>Plant Protection Department, Research Center for Agriculture and Natural Resources, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran

Vol. 58, No. 3: 289–296, 2018

DOI: 10.24425/jppr.2018.124637

Received: February 4, 2018

Accepted: September 14, 2018

\*Corresponding address:  
s.ramzi@areeo.ac.ir

### Abstract

Toxicity and physiological alterations were determined in *Pseudococcus viburni* nymphs treated with *Artemisia annua* methanolic extract. The leaf dipping bioassay showed  $LC_{50}$  values of 0.287% and 0.194% 24 and 48 hours post-exposure. Activities of general esterases were significantly higher in the control nymphs than in those which had been treated except for the 48 h time interval using  $\alpha$ -naphthyl acetate. The activity of glutathione S-transferase using CDNB (1-chloro-2,4-dinitrobenzene) in the control nymphs, was significantly higher than in the control at both time intervals while no significant difference was observed after 24 h in addition to the higher enzymatic activity in the treated nymphs after 48 h. All three aminotransferases were significantly more active in the control nymphs except for time intervals of 24 h for  $\gamma$ -glutamyl transferase and 48 h for alanine aminotransferase. Higher activities of lactate dehydrogenase, acid- and alkaline phosphatase were found in the control nymphs than in treated nymphs for all time intervals. Activities of the enzymes involved in the antioxidant system including catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and glucose-6-phosphate dehydrogenase was increased in the treated nymphs compared to the control. Results of the current study demonstrated toxic effects of *A. annua* methanolic extract on *P. viburni* nymphs causing mortality and physiological turbulences.

**Keywords:** antioxidant response, *Artemisia annua*, intermediary metabolism, methanolic extract, *Pseudococcus viburni*

## Introduction

Mealybugs are among the most serious pests of agricultural crops found on almost all plant families as sap-sucking insects. Since these pests prefer warm, moist climates they can frequently be found on subtropical trees and greenhouse plants. Favorable weather, being located near citrus orchards and intensive spraying against citrus pests caused a serious outbreak

of mealybugs known as *Pseudococcus viburni* Signoret (Hemiptera: Pseudococcidae) on tea plantations in northern Iran (Mafi 1997). Both nymphs and adults feed intensively on young leaves, shoots and twigs of tea. They also produce honeydew and cover bushes with waxy materials which intervene in proper photosynthesis and decrease tea leaf yield (Mafi 1997).

*Pseudococcus viburni* adults are distinguished by being pinkish-purple to gray, generally lightly dusted with white powdery wax, short, fine lateral wax filaments and one pair of long terminal wax filaments usually more than half as long as the body (Abbassipour and Taghavi 2007). The mealybug population has a sharp density increase in April, followed by a steady decline in density for other times of the season. *Pseudococcus viburni* has four generations per year although it shows seasonal variation with the highest population occurring from June to August (Abbassipour and Taghavi 2007).

As an agricultural product which is directly consumed by humans, no chemical spraying is allowed against *P. viburni* although waxy covers and feeding sites beneath the leaf surface fail to be efficiently controlled by insecticides. *Pseudaphycus flavidulus* (Brethes) (Hymenoptera: Encyrtidae) and *Leptomastix epona* (Walker) (Hymenoptera: Encyrtidae) are the two parasitoids of *P. viburni*, but the biological control of the pest in tea plantations of Iran is based on releasing *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) early in the season (Mafi 1997; Daane *et al.* 2008). Even releasing this ladybird may result in unsuccessful control because of chemical spraying in adjoining citrus orchards, unfavorable weather conditions and the quality of the reared biocontrol agent (Ramzi *et al.* 2018).

Insecticides from nature-based agents like entomopathogens and plants are promising alternatives of chemical compounds to suppress pest populations. There is interest in exploring botanical compounds including extracts, essential oils and individual chemical constituents as crop protectants (Isman 2006). Although these compounds have the least effects on non-target organisms, they have shown toxicity, repellency and have caused several physiological malfunctions in target insects (Isman 2006). Northern Iran is a source of diverse plant species to provide botanical insecticides. One of the most frequent plant species is *Artemisia annua* L. (Asteraceae) which is an annual plant. Its leaves have an intensive aromatic scent with several medicinal properties including anti-malarial, anti-bacterial, anti-inflammatory, plant growth regulatory and cytotoxicity (Bhakuni *et al.* 2001; Shekari *et al.* 2008). Because of the constraints in the proper control of *P. viburni*, providing a plant-based compound might be a potential technique to decrease damage on tea plantations although such a procedure requires bioassay and physiological experiments. In this case, *A. annua* could be a good candidate because of its availability in the region and proved toxicity against insect pests. Hence, the current study aimed to determine the toxicity of *A. annua* methanolic extract against third nymphal instars of *P. viburni* and its potential sublethal effects on intermediary metabolism and antioxidant responses.

## Materials and Methods

### Insect rearing

A stock population of *P. viburni* was reared on squash at  $25 \pm 2^\circ\text{C}$ , 70% relative humidity (RH) and a 16L : 8D photoperiod in the Tea Research Institute of Iran. This stock was kept in a laboratory for at least three generations to avoid any exposure to chemicals.

### Preparation of *Artemisia annua* methanolic extract

*Artemisia annua* were gathered from suburban areas of Lahijan in northern Iran ( $37^\circ 12' 26''$  N  $50^\circ 00' 14''$  E). Initially, the leaves were washed with distilled water and dried in shade at  $25^\circ\text{C}$  prior to being ground into powder. The powdered leaves (30 g) were added to 300 ml of 85% methanol and incubated at  $4^\circ\text{C}$  for 48 h. The solution was stirred for another hour prior to being filtered through filter paper (Whatman No. 4). Then, the solvent (methanol) was removed by vacuum in a rotary evaporator to gain a dark green residue which was dissolved in 10 ml acetone. This stock preparation was used as the starting stock solution and other dilutions were prepared based on the preliminary tests (Moharramipour *et al.* 2003).

### Bioassay

For leaf dipping bioassay,  $3 \times 3$  cm pieces of tea leaves were provided and soaked in different concentrations of *A. annua* extract including 0.2, 0.4, 0.8, 1.6 and 3.2% in addition to control pieces which were soaked in acetone only. After 30 s, the leaf pieces were dried on filter paper (Whatman No. 1) for 60 min and 10 third nymphal instars (24 h old) of *P. viburni* were put on the leaf pieces. Thirty nymphs were used for each concentration including the control in five replicates ( $N = 180$ ). Mortality was recorded after 24 and 48 h and the LC values were estimated by POLO-PC software (LeOra Software 1987).

### Effects of *Artemisia annua* methanolic extract on intermediary metabolism and the antioxidant system

Different pieces of tea leaves of the given dimensions were provided and soaked separately in control and  $\text{LC}_{50}$  solution of *A. annua* extract. After drying, 50 third instar larvae were transferred to control and treatment pieces and kept for 24 and 48 h at  $25 \pm 2^\circ\text{C}$ , 70% RH and a 16L : 8D photoperiod. At the given time intervals, 25 nymphs were randomly selected, transferred to eppendorf tubes and homogenated in 500  $\mu\text{l}$  of distilled water. The samples were centrifuged at 20,000 g

for 20 min at 4°C and the supernatant was used for biochemical experiments.

### Determination of general esterase activity

Assay of the enzyme was carried out using  $\alpha$ - and  $\beta$ -enaphthyl acetates as substrates based on Han *et al.* (1998). Briefly, 20  $\mu$ l of each substrate (5 mM) was added separately to 50  $\mu$ l of fast blue RR salt (1 mM) prior to adding 10  $\mu$ l of enzyme solution. The incubation was prolonged for 5 min and the absorbance was then read at 450 nm.

### Determination of glutathione S-transferase (GST) activity (2.5.1.18)

Briefly, 20  $\mu$ l of CDNB (1-chloro-2,4-dinitrobenzene, 20 mM) and DCNB (1,2-dichloro-4-nitro-benzene, 20 mM) were added separately to 50  $\mu$ l of reduced glutathione solution (20 mM), then 10  $\mu$ l of enzyme solution was added and the absorbance was read at 340 nm after 5 min of incubation (Oppenoorth 1985).

### Assay of alanine (EC 2.6.1.1) and aspartate (EC 2.6.1.1) aminotransferases (ALT, AST)

A biochemical kit manufactured by Biochem Company (Tehran, Iran) was used to assay activities of these enzymes in which pyruvate is combined with 2,4-dinitrophenyl pyruvate and synthesized pyruvate hydrazine (Thomas 1998). Briefly, reagent A (for AST) and reagent B (for ALT) were incubated separately with reagent D. After 5 min, 10  $\mu$ l of the enzyme solution was added and incubation was done for 60 min. At the end, reagent C was added to the solution prior to reading the absorbance at 340 nm.

### Assay of $\gamma$ -glutamyl transferase ( $\gamma$ -GT) (EC 2.3.2.2)

Based on the kit manufactured by ZiestChem Diagnostic Company (Tehran, Iran) and the method of Szasz (1976), 20  $\mu$ l of substrate reagent and L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide was added to 50  $\mu$ l of buffer reagent. Then, 10  $\mu$ l of enzyme solution was added, mixed thoroughly prior to reading the absorbance at 405 nm after 3 min.

### Assay of lactate dehydrogenase (EC 1.1.1.27)

Briefly, 20  $\mu$ l of NAD<sup>+</sup> solution and 20  $\mu$ l of water were added to test and control tubes, separately. Then, 100  $\mu$ l of the buffered substrate and 10  $\mu$ l of the sample were added to the test tubes and incubated for 15 min at 37°C. After that, 100  $\mu$ l of the reagent

(2,4-dinitrophenyl hydrazine) was also added and the reaction mixture was re-incubated for 15 min. Tubes were cooled at room temperature prior to adding 50  $\mu$ l of NaOH (0.4 M). Finally, the absorbance was read at 340 nm after 60 s (King 1965).

### Assay of acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatase (ACP, ALP)

Based on the method of Bessey *et al.* (1946), 10  $\mu$ l of enzyme solution was added to the buffered substrate (Tris-HCl, 20 mM, pH 8 for ALP and pH 5 for ACP), phosphate buffer (0.02 M, pH 7.2) and incubated for 5 min. Afterward, 100  $\mu$ l of NaOH (1 M) was added and the absorbance was read at 405 nm.

### Catalase assay (EC 1.11.1.6)

As described by Wang *et al.* (2001) the reaction mixture consisted of 100  $\mu$ l of sample and 500  $\mu$ l of hydrogen peroxide (1%) which was mixed thoroughly and monitored for 10 min at 28°C prior to reading the absorbance at 240 nm.

### Peroxidase assay (EC 1.11.1)

Based on the method of Addy and Goodman (1972), 100  $\mu$ l of sample was added to 500  $\mu$ l of pyrogallol solution [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 500  $\mu$ l of H<sub>2</sub>O<sub>2</sub> (1%). Then, the absorbance was read at 430 nm every 30 s for 2 min.

### Superoxide dismutase assay (EC 1.15.1.1)

The assay was carried out using the method of McCord and Fridovich (1969) in which xanthine oxidase solution was prepared with 10 mg of bovine albumin and 100  $\mu$ l of xanthine oxidase (5.87 units · ml<sup>-1</sup>) dissolved in 2 ml of phosphate buffer (0.1 M, pH 7). Afterward, 100  $\mu$ l of the solution was added to 500  $\mu$ l of another mixture containing 70  $\mu$ M of nitro blue tetrazolium (NBT) and 125  $\mu$ M of xanthine dissolved in phosphate buffer (PBS). After adding 100  $\mu$ l of the enzyme solution, the incubation was prolonged in darkness for 20 min at 28°C prior to reading the absorbance at 560 nm. The enzymatic activity was reported as  $\Delta A_{560 \text{ nm}} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ protein}$ .

### Ascorbate peroxidase assay (EC 1.11.1.11)

Based on Asada (1984), 100  $\mu$ l of enzyme solution was added to 250  $\mu$ l of reaction solution containing 67 mM potassium phosphate buffer (pH 7) and 2.5 mM of ascorbic acid (250  $\mu$ l). Then, 200  $\mu$ l of H<sub>2</sub>O<sub>2</sub> (30 mM) was added and absorbance was read at 290 nm for 5 min.

### Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)

As described by Balinsky and Bernstein (1963), 100  $\mu$ l of enzyme solution was added to a mixture containing 100  $\mu$ l Tris-HCl buffer (100 mM, pH 8.2), 0.2 mM NADP and 0.1 M of  $MgCl_2$ . At the end, 100  $\mu$ l of glucose-6-phosphate (6 mM) was added and the absorbance was read at 340 nm after 5 min.

### Protein determination

Protein concentrations were determined using bovine serum albumin based on the method described by Lowry *et al.* (1951) (Manufactured by Ziest Chem. Co., Tehran, Iran).

### Statistical analysis

The experiments were designed in a complete randomized design and the data were compared by one-way analysis of variance (ANOVA) using t-test at a probability of less than 5%.

## Results

### Effect of *Artemisia annua* essential oil on mortality and deterrancy of *Pseudococcus viburni*

Table 1 shows the effect of *A. annua* methanolic extract on the third nymphal instars of *P. viburni*. Nymphal exposure to the extract revealed  $LC_{50}$  of 0.287% with confidence limits of 0.171–0.431% after 24 h while these values were found to be 0.194% with confidence limits of 0.073–0.322% after 48 h (Table 1).

### Effect of *A. annua* methanolic extract on the activity of intermediary metabolism

Significant changes were found in the activities of the two detoxifying enzymes, general esterases and glutathione-S-transferases, in the *P. viburni* nymphs treated with *A. annua* extract. By using both  $\alpha$ -naphthyl- and  $\beta$ -naphthyl acetates as substrates, the activities of general esterases in the treated nymphs were significantly higher than the controls except for a time interval of 48 h using  $\alpha$ -naphthyl acetate (Table 2). Activity of glutathione S-transferase in the control nymphs was significantly higher than in *A. annua* treated nymphs at both time intervals once CDNB was used as a reagent (Table 3). By using DCNB, no significant difference was observed after 24 h but the enzymatic activity significantly increased in the treated nymphs compared to the controls (Table 3).

Activities of alanine aminotransferase, aspartate aminotransferase and  $\gamma$ -glutamyl transferase were significantly higher in the control nymphs than in those treated with *A. annua* extract except for time intervals of 24 h in  $\gamma$ -glutamyl transferase and 48 h in alanine aminotransferase (Table 4). Additionally, activities of lactate dehydrogenase, acid- and alkaline phosphatases in the control nymphs were significantly higher than treated ones at all time intervals (Table 5).

### Effect of *A. annua* essential oil on the activities of antioxidant enzymes

$LC_{50}$  concentration of *A. annua* extract led to statistically higher activities of antioxidant enzymes including catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and glucose-6-phosphate dehydrogenase

**Table 1.** Toxicity of *Artemisia annua* essential oil on *Pseudococcus viburni* 3rd nymphal instars

Time of treatment	$LC_{50}$ [%]	Confidence limit (95%)	Slope $\pm$ SE	$\chi^2$	df
24 h	0.287	0.171–0.431	1.215 $\pm$ 0.267	1.223	3
48 h	0.194	0.073–0.322	0.805 $\pm$ 0.254	2.4310	3

**Table 2.** Activity of general esterase (OD  $\cdot$  min<sup>-1</sup>) in the control and *Pseudococcus viburni* 3rd nymphal instars treated with *Artemisia annua* methanolic extract

Treatment	$\alpha$ -Naphthyl acetate		$\beta$ -Naphthyl acetate	
	24 h	48 h	24 h	48 h
Control	0.025 $\pm$ 0.005*	0.007 $\pm$ 0.003	0.057 $\pm$ 0.015*	0.047 $\pm$ 0.010*
$LC_{50}$ concentrations	0.014 $\pm$ 0.005	0.018 $\pm$ 0.004*	0.045 $\pm$ 0.003	0.014 $\pm$ 0.007

\*asterisks show statistical differences in each column (t-test,  $p \leq 0.05$ )

**Table 3.** Activity of glutathione S-transferase ( $\text{OD} \cdot \text{min}^{-1}$ ) in the control and *Pseudococcus viburni* 3rd nymphal instars treated with *Artemisia annua* methanolic extract

Treatment	CDNB		DCNB	
	24 h	48 h	24 h	48 h
Control	0.117 ± 0.015*	0.156 ± 0.011*	0.073 ± 0.011	0.081 ± 0.024
LC <sub>50</sub> concentrations	0.061 ± 0.008	0.039 ± 0.011	0.085 ± 0.003	0.111 ± 0.010*

\*asterisks show statistical differences in each column (t-test,  $p \leq 0.05$ )  
 CDNB – 1-chloro-2,4-dinitrobenzene; DCNB – 1,2-dichloro-4-nitro-benzene

**Table 4.** Activities of transaminases ( $\text{U} \cdot \text{mg}^{-1}$  protein) in the control and *Pseudococcus viburni* 3rd nymphal instars treated with *Artemisia annua* methanolic extract

Treatment	Alanine aminotransferase		Aspartate aminotransferase		$\gamma$ -Glutamyl transferase	
	24 h	48 h	24 h	48 h	24 h	48 h
Control	0.058 ± 0.00*	0.053 ± 0.02	0.049 ± 0.02*	0.044 ± 0.01*	0.064 ± 0.00	0.051 ± 0.01*
LC <sub>50</sub> concentrations	0.047 ± 0.01	0.047 ± 0.02	0.038 ± 0.00	0.037 ± 0.03	0.068 ± 0.00	0.013 ± 0.00

\*asterisks show statistical differences in each column (t-test,  $p \leq 0.05$ )

**Table 5.** Activities of lactate dehydrogenase and phosphatases ( $\text{U} \cdot \text{mg}^{-1}$  protein) in the control and *Pseudococcus viburni* 3rd nymphal instars treated with *Artemisia annua* methanolic extract

Treatment	Lactate dehydrogenase		Acid phosphatase		Alkaline phosphatase	
	24 h	48 h	24 h	48 h	24 h	48 h
Control	0.124 ± 0.01*	0.200 ± 0.02*	0.207 ± 0.02*	0.144 ± 0.01*	0.475 ± 0.05*	0.393 ± 0.06*
LC <sub>50</sub> concentrations	0.045 ± 0.01	0.145 ± 0.00	0.136 ± 0.01	0.070 ± 0.01	0.158 ± 0.10	0.267 ± 0.10

\*asterisks show statistical differences in each column (t-test,  $p \leq 0.05$ )

**Table 6.** Activities of catalase, peroxidase and superoxide dismutase ( $\text{U} \cdot \text{mg}^{-1}$  protein) in the control and *Pseudococcus viburni* 3rd nymphal instars treated with *Artemisia annua* methanolic extract

Treatment	Catalase		Peroxidase		Superoxide dismutase	
	24 h	48 h	24 h	48 h	24 h	48 h
Control	0.113 ± 0.04	0.064 ± 0.01	0.023 ± 0.00	0.041 ± 0.00	0.012 ± 0.00	0.024 ± 0.00
LC <sub>50</sub> concentrations	0.285 ± 0.02*	0.206 ± 0.02*	0.069 ± 0.01*	0.054 ± 0.00*	0.035 ± 0.01*	0.025 ± 0.00

\*asterisks show statistical differences in each column (t-test,  $p \leq 0.05$ )

**Table 7.** Activity of ascorbate peroxidase and glucose-6-phosphate dehydrogenase ( $\text{OD} \cdot \text{min}^{-1}$ ) in the control and *Pseudococcus viburni* 3rd nymphal instars treated with *Artemisia annua* methanolic extract

Treatment	Ascorbate peroxidase		Glucose-6-phosphate dehydrogenase	
	24 h	48 h	24 h	48 h
Control	0.750 ± 0.093	0.797 ± 0.041	0.064 ± 0.015	0.051 ± 0.011
LC <sub>50</sub> concentrations	1.076 ± 0.231*	1.034 ± 0.103*	0.136 ± 0.032	0.058 ± 0.023

\*asterisks show statistical differences in each column (t-test,  $p \leq 0.05$ )

than of the controls although no significant differences were observed in the activities of superoxide dismutase and glucose-6-phosphate dehydrogenase 48 h post-exposure (Tables 6 and 7).

## Discussion

Different concentrations of *A. annua* extract showed significant mortality in the third nymphal instar of

*P. viburni* from 10 to 90% which made it possible to calculate  $LC_{50}$  values at both time intervals. Also, nymphal treatments with the  $LC_{50}$  value of the extract caused significant changes in some enzymatic components of intermediary metabolism and the antioxidant system. Although mealybugs are covered with waxy materials which prevent proper diffusion of insecticides, several studies have shown significant effects of botanical extracts on different species of mealybugs. A 1% concentration of limonene, which is a cyclic terpene of citrus, caused a mortality range of 43 to 98% on the third and fourth nymphal instar of longtail mealybug. The differences in observed mortalities have been attributed to type and percentage of the emulsifiers used (Hollingsworth 2005). Alcohol extracts from several medicinal plants caused mortalities of citrus mealybug, *Planococcus citri* (Risso) (Pseudococcidae: Homoptera) (El-Hefny *et al.* 2011). Also, alcohol extracts of neem, *Pavetta* leaf and garlic bulbs showed different toxicities on papaya mealybug, *Paracoccus marginatus* Williams y Granara de Willink (Hemiptera: Pseudococcidae) (Piragalathan *et al.* 2014). Prishanthini and Vinobaba (2014) and Rizvi *et al.* (2015) reported significant effects of neem, tobacco, calotropis and garlic extracts on the cotton mealybug, *Phanacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) compared to synthetic insecticides. In our previous study, *A. annua* essential oil showed toxicity on the third nymphal instar of tea mealybug after two time intervals by the  $LC_{50}$  values of 0.693 and 0.419% (Ramzi *et al.* 2018). The lower required concentration of *A. annua* extract to gain toxicity against *P. viburni* compared to essential oil may be attributed to the difference in active ingredients in *A. annua* extract versus essential oil which may have synergistic effects on each other. Moreover, the differing results of our study and others were due to the composition of each plant species and the place of their growth (Haghighian *et al.* 2008; Mojarab-Mahboubkar and Sendi 2016).

Intermediary metabolism consists of a series of complex biochemical processes to equilibrate insect hemostasis in the proper order. These processes not only provide nutrient molecules for several tissues but also remove potential harmful chemicals, e.g. insecticides. Overall, the enzymes involved in intermediary metabolism are known as monooxygenases, general esterases, glutathione S-transferases, transaminases, lactate dehydrogenases, phosphatases, etc. General esterases and glutathione S-transferases are the two main detoxifying enzymes which contribute significantly to insect survival. Both enzymes demonstrated significant reactions to environmental extremes like chemicals, temperature and inappropriate food sources such as the presence of plant secondary metabolites (Hemingway and Karunatne 1998; Mardani-Talaei *et al.* 2016). In our study, activities of these enzymes in the

nymphs treated with *A. annua* methanolic extract were lower than those of the controls except for the time interval of 48 h by using  $\alpha$ -naphthyl acetate and DCNB. These findings indicated late induction of these enzymes to detoxifying *A. annua* extract in addition to highlighting the roles of some isozymes in the process. Most studies have demonstrated increased activities of general esterases and glutathione S-transferases after treatment with botanical compounds, although treatment of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) with *A. annua* essential oil have shown adverse results (Mojarab-Mahboubkar *et al.* 2015).

Transaminases are critical enzymes involved in the availability of amino acids for physiological processes of insects. Among them, alanine aminotransferase, aspartate aminotransferase and  $\gamma$ -glutamyl transferase have significant roles in the production of pyruvate and L-glutamate by transferring amino groups of L-alanine to  $\alpha$ -ketoglutarate, converting aspartate and  $\alpha$ -ketoglutarate to oxaloacetate and mobilization of a  $\gamma$ -glutamyl moiety of glutathione to receptor producing glutamate, respectively (Tate and Meister 1985; Klowden 2012). However,  $\gamma$ -glutamyl transferase is involved in synthesizing or degrading glutathione or chemical compounds which enter the body via the  $\gamma$ -glutamyl cycle (Tate and Meister 1985). Our results demonstrated lower activities of all assayed transaminases in the *P. viburni* nymphs treated with *A. annua* extract except for alanine aminotransferase after 24 h and  $\gamma$ -glutamyl transferase after 48 h. These findings may somehow indicate impairment of transaminases following extract exposure or depleting amino acid content from hemolymph or fat bodies.

The third nymphal instars of *P. viburni* treated with *A. annua* methanolic extracts showed lower activities of lactate dehydrogenase, acid- and alkaline phosphatases than control nymphs at all time intervals. Lactate dehydrogenase is an enzyme involved in glycolysis by converting pyruvate to lactate along with the conversion of NADH to  $NAD^+$  (Senthil-Nathan *et al.* 2006). Acid- and alkaline phosphatases are critical to dephosphorylate some biological molecules such as nucleotides, proteins and alkaloids under alkaline and acidic conditions (Zibaei and Bandani 2010). The higher activities of these enzymes in control nymphs indicate proper utilization of large quantities of food, digestion efficiency and positive transportation of nutrients among midgut, hemolymph and fat bodies while the lower activities in the treated nymphs may be attributed to direct toxicity of extract as the feeding inhibitor (Senthil-Nathan *et al.* 2006).

Antioxidants are a vital system to protect organisms from the devastating effects of free radicals on their tissues and physiological functions. The system is comprised of several enzymatic and non-enzymatic components which all together detoxify free radicals

to be inactivated (Felton and Summers 1995; Pardini 1995). Superoxide dismutase catalyzes the superoxide radical ( $O_2^-$ ) into hydrogen peroxide ( $H_2O_2$ ) while catalase and peroxidase scavenge  $H_2O_2$  into water and oxygen (Felton and Summers 1995; Pardini 1995). Moreover, ascorbate peroxidase, which oxidizes ascorbate to decompose hydrogen peroxide (APOX), is an enzyme that decomposes hydrogen peroxide by the concurrent oxidation of ascorbate while decontaminating oxidative agents by oxidation of NADPH (Asada 1984; Felton and Summers 1995). Increased activities of the above mentioned antioxidant enzymes indicate induction of oxidative stress in the *P. vinurni* nymphs following treatment with methanolic extract of *A. annua*. In a similar study, Dhivya *et al.* (2018) reported higher activities of catalase, superoxidase dismutase, glutathione S-transferase and cytochrome P450 in the larvae of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) treated with *Prosopis juliflora* (Sw.) DC seed pod extract. The toxicity may be due to cell death by increasing ionic leak of cell membranes, degeneration of cytoplasmic proteins and membrane organization (Bhakuni *et al.* 2001).

In conclusion, our study demonstrated that *A. annua* methanolic extract has chemical constituents with not only insecticidal properties but also plays intervening roles in intermediary metabolism against third nymphal instars of *P. vinurni*. Moreover, the extract may release free radicals into the body of treated individuals which is responsible for the induction of several antioxidant enzymes. Further studies to identify potential roles of the secondary metabolites in the extract will help identify the active principle which can be developed as a potential insecticide in the control of insect pests. Our results combined with previous ones on the effects of *A. annua* essential oil show significant mortality using low concentrations which highlights the potential of *A. annua* as a botanical insecticide against *P. viburni* nymphs. However, it is recommended to determine its efficiency in the field by considering the fitness of biocontrol agents such as *C. montrouzieri*.

## Acknowledgements

This research was supported by a grant (2-21-21-94103) of the Agricultural Research Education and Extension Organization.

## References

Abbasipour H., Taghavi A. 2007. Description and seasonal abundance of the tea mealybug, *Pseudococcus viburni* (Afinis) (Signoret) (Homoptera: Pseudococcidae) Found on Tea in Iran. *Journal of Entomology* 4: 474–478. DOI: 10.3923/je.2007.474.478

- Addy S.K., Goodman R.N. 1972. Polyphenol oxidase and peroxidase activity in apple leaves inoculated with a virulent or an avirulent strain for *Ervinia amylovora*. *Indian Phytopathology* 25 (4): 575–579.
- Asada K. 1984. Chloroplasts: formation of active oxygen and its scavenging. *Methods in Enzymology* 105: 422–429. DOI: [https://doi.org/10.1016/S0076-6879\(84\)05059-X](https://doi.org/10.1016/S0076-6879(84)05059-X)
- Balinsky D., Bernstein R.E. 1963. The purification and properties of glucose-6-phosphate dehydrogenase from human erythrocytes. *Biochimica Biophysica Acta* 67: 313–315. DOI: [https://doi.org/10.1016/0926-6569\(63\)90239-6](https://doi.org/10.1016/0926-6569(63)90239-6)
- Bessey O.A., Lowry O.H., Brock M.J. 1946. A method for the rapid determination of alkaline phosphates with five cubic millimeters of serum. *Journal of Biological Chemistry* 164: 321–329.
- Bhakuni R.S., Jain D.C., Sharma R.P., Kumar S. 2001. Secondary metabolites of *Artemisia annua* and their biological activity. *Current Science* 80 (1): 35–49.
- Daane K.M., Cooper M.L., Triapitsyn S.V., Andrews Jr J.W., Ripa R. 2008. Parasitoids of obscure mealybug, *Pseudococcus viburni* (Hem.: Pseudococcidae) in California: establishment of *Pseudaphycus flavidulus* (Hym.: Encyrtidae) and discussion of related parasitoid species. *Biocontrol Science and Technology* 18 (1): 43–57. DOI: <https://doi.org/10.1080/09583150701729906>
- Dhivya K., Vengateswari G., Arunthirumeni M., Karthi S., Senthil-Nathan S., Shivakumar M.S. 2018. Bioprospecting of *Prosopis juliflora* (Sw.) DC seed pod extract effect on antioxidant and immune system of *Spodoptera litura* (Lepidoptera: Noctuidae). *Physiological and Molecular Plant Pathology* 101: 45–53. DOI: <https://doi.org/10.1016/j.pmpp.2017.09.003>
- El-Hefny A.S., El-Sahn O.M.N., Yacoub S.S. 2011. Effect of some plant extracts on citrus mealy bug, *Planococcus citri* (Risso). *Egyptian Journal of Agricultural Research* 89 (2): 511–519.
- Felton G.W., Summers C.B. 1995. Antioxidant systems in insects. *Archives of Insect Biochemistry and Physiology* 29 (2): 187–197.
- Haghighian F., Sendi J.J., Aliakbar A.A., Javaherdashti M. 2008. The growth regulatory, deterrence and ovicidal activity of worm wood (*Artemisia annua* L.) on *Tribolium confusum* Duv. and identification of its chemical constituents by GC-MS. *Pestycydy* 1–2: 51–59.
- Han Z., Moores G., Devonshire A., Denholm I. 1998. Association between biochemical marks and insecticide resistance in the cotton aphid, *Aphis gossypii*. *Pesticide Biochemistry and Physiology* 62 (3): 164–171. DOI: <https://doi.org/10.1006/pest.1998.2373>
- Hemingway J., Karunatne S.H.P.P. 1998. Mosquito carboxylesterases: A review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Medical and Veterinary Entomology* 12 (1): 1–12.
- Hollingsworth R.G. 2005. Limonene, a citrus extract, for control of mealybugs and scale insects. *Journal of Economic Entomology* 98 (3): 772–779. DOI: <https://doi.org/10.1603/0022-0493-98.3.772>
- Isman M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* 51: 45–66. DOI: 10.1146/annurev.ento.51.110104.151146
- King J. 1965. The dehydrogenases or oxidoreductases lactate dehydrogenase. p. 83–93. In: “Practical Clinical Enzymology” (King J., ed.). Van Nostrand, London, 363 pp.
- Klowden M.J. 2012. *Physiological Systems in Insects*. 3th ed. Academic Press, New York, 682 pp.
- LeOra Software. 1987. *Polo-PC: a user guide to probit or logit analysis*. LeOra Software, Berkeley, CA.
- Lothar T. 1998. *Clinical Laboratory Diagnostics: Use and Assessment of Clinical Laboratory Results*. TH-Books Verlagsgesellschaft, Frankfurt/Main, Germany, 1727 pp.
- Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. 1951. Pro-

- tein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193 (1): 265–275.
- Mafi S. 1997. Identification of mealybugs in Mazandaran province by considering the dominant species and natural enemies. MSc thesis. Tarbiat Modares University, Tehran, Iran, 112 pp.
- Mardani-Talaei M., Zibae A., Nouri-Ganbalani G., Razmjou J. 2016. Chemical and organic fertilizers affect physiological performance and antioxidant activities in *Myzus persicae* (Hemiptera: Aphididae). *Invertebrate Survival Journal* 13: 122–133.
- McCord J.M., Fridovich I. 1969. Superoxide dismutase: an enzymic function for erythrocyte (hemocytin). *Journal of Biological Chemistry* 244 (22): 6049–6055.
- Moharramipour S., Nazemi R.J., Talebi A.S., Fathipour Y. 2003. The effects of *Nerium oleander*, *Lavandula officinalis* and *Ferula asafoetida* extracts on feeding indices of adult *Tribolium castaneum*. *Journal of Entomological Society of Iran* 23: 69–90. (in Persian)
- Mojarab-Mahboubkar M., Sendi J.J., Aliakbar. 2015. Effect of *Artemisia annua* L. essential oil on toxicity, enzyme activities, and energy reserves of cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Journal of Plant Protection Research* 55 (4): 371–377. DOI: <https://doi.org/10.1515/jppr-2015-0049>
- Mojarab-Mahboubkar M., Sendi J.J. 2016. Chemical composition, insecticidal and physiological effect of methanol extract of sweet wormwood (*Artemisia annua* L.) on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Toxin Reviews* 35 (3–4): 106–115. DOI: <https://doi.org/10.1080/15569543.2016.1203336>
- Oppenoorth F., van der Pas L.J.T., Houx N.W.H. 1979. Glutathione S-transferase and hydrolytic activity in a tetrachlorvinphos-resistant strain of housefly and their influence on resistance. *Pesticide Biochemistry and Physiology* 11 (1–3): 176–188. DOI: [https://doi.org/10.1016/0048-3575\(79\)90057-9](https://doi.org/10.1016/0048-3575(79)90057-9)
- Pardini R.S. 1995. Toxicity of oxygen from naturally occurring redox-active pro-oxidants. *Archives of Insect Biochemistry and Physiology* 29: 101–118. DOI: <https://doi.org/10.1002/arch.940290203>
- Piragalathan A., Pakeerathan K., Thirukkumaran G., Mikunthan G. 2014. Efficacy of different insecticides and bio-rationals against papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) infestation in home gardens. *Middle-East Journal of Scientific Research* 21 (10): 1689–1693. DOI: [10.5829/idosi.mejrs.2014.21.10.82293](https://doi.org/10.5829/idosi.mejrs.2014.21.10.82293)
- Prishanthini M., Vinobaba M. 2014. Efficacy of some selected botanical insecticides against the cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae). *International Journal of Scientific and Research Publications* 4 (3): 1–6.
- Ramzi S., Seraji A., Azadi Gonbad R., Mirhaghpour S.K., Moji Haghighadam Z., Haghighat S. 2018. Toxicity of *Artemisia annua* (Asteraceae) essential oil on the tea mealy bug, *Pseudococcus viburni* Sigornet (Hemiptera: Pseudococcidae). *Archives of Phytopathology and Plant Protection* 50 (19–20): 941–956. DOI: [doi.org/10.1080/03235408.2017.1352223](https://doi.org/10.1080/03235408.2017.1352223)
- Rizvi S.A.H., Ikhtlaq M.N., Jaffar S., Hussain S. 2015. Efficacy of some selected synthetic chemical insecticides and bio-pesticides against cotton mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) under agro ecological conditions of Peshawar, Pakistan. *Journal of Entomology and Zoology Studies* 3 (6): 223–226.
- Senthil-Nathan S., Chung P.G., Murugan K. 2006. Combined effect of biopesticides on the digestive enzymatic profiles of *Cnaphalocrocis medinalis* (Guenée) (the rice leaf folder) (Insecta: Lepidoptera: Pyralidae). *Ecotoxicology and Environmental Safety* 64 (3): 382–389. DOI: <https://doi.org/10.1016/j.ecoenv.2005.04.008>
- Shekari M., Jalali Sendi J., Etenbari K., Zibae A., Shadparvar A. 2008. Effects of *Artemisia annua* L. (Asteraceae) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomelidae). *Pesticide Biochemistry and Physiology* 91 (1): 66–74. DOI: <https://doi.org/10.1016/j.pestbp.2008.01.003>
- Szasz G. 1976. Reaction-rate method for gamma-glutamyltransferase activity in serum. *Clinical Chemistry* 22 (12): 2051–2055.
- Tate A.A., Meister A. 1985. Gamma-glutamyl transpeptidase from kidney. *Methods in Enzymology* 113: 400–419.
- Thomas L. 1998. *Clinical Laboratory Diagnostic: Use and Assessment of Clinical Laboratory Results*. 1st ed. TH-Books Verlagsgesellschaft, Frankfurt/Main, Germany, 1727 pp.
- Wang Y., Oberley L.W., Murhammer D.W. 2001. Evidence of oxidative stress following the viral infection of two Lepidopteran insect cell lines. *Free Radical Biological Medicine* 31: 1448–1455. DOI: [https://doi.org/10.1016/S0891-5849\(01\)00728-6](https://doi.org/10.1016/S0891-5849(01)00728-6)
- Zibae A., Bandani A.R. 2010. A study on the toxicity of a medicinal plant, *Artemisia annua* L. (Asteraceae) extracts to the sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). *Journal of Plant Protection Research* 50 (1): 79–85. DOI: [10.2478/v10045-010-0014-4](https://doi.org/10.2478/v10045-010-0014-4)