

# Biochemical characterization of digestive carbohydrases in the rose sawfly, *Arge rosae* Linnaeus (Hymenoptera: Argidae)

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Abstract: The rose sawfly, Arge rosae Linnaeus, is one of the most destructive pests of rose bushes in the north of Iran. Nowadays, many attempts have been made to reduce pesticide application by looking for new methods of pest control. A non chemical method for controlling insect pests including A. rosae can be achieved by using genetically engineered plants expressing carbohydrase inhibitors. Therefore, in present study we characterized biochemical properties of digestive carbohydrases in the gut of A. rosae for achieving a new method for control of this pest. The specific activity of a-amylase in the digestive system of last larval instars of A. rosae was obtained as 9.46  $\pm$  0.06  $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup> protein. Also, the optimal pH and temperature for α-amylase were found to be at pH 8 and 50 °C. As calculated from Lineweaver-Burk plots, the  $K_m$  and  $V_{max}$ values for α-amylase were 0.82 mg/ml and 7.32 µmol min<sup>-1</sup> mg<sup>-1</sup> protein, respectively, when starch was used as substrate. The effects of ions on amylolytic activity showed that Mg<sup>2+</sup> and Na<sup>+</sup> significantly increased amylase activity, whereas SDS and EDTA decreased the enzyme activity. The highest activities of  $\alpha$ -/ $\beta$ -glucosidase and  $\beta$ -galactosidase were obtained at pH 5.0. By the native PAGE, three, one, one and two bands were clearly detected for  $\alpha$ amylase,  $\alpha$ -/ $\beta$ -glucosidase and  $\beta$ -galactosidase, respectively. No bands were found for a-galactosidase that confirmed the absence or low activity of this carbohydrate in the digestive system of A. rosae. These results could provide the knowledge needed to produce transgenic plants for control of this pest.

Keywords: Arge rosae, α-amylase, α-/β-glucosidases, β-galactosidases

#### Introduction

Alpha-amylases (known as 1, 4- $\alpha$ -D-glucan glucanohydrolase EC 3.2. 1.1) are responsible for starch and glycogen breakdown (Mohamed, 2004). These enzymes have main role in digestion and metabolism of carbohydrates in insects and other organisms.

Alpha-amylase enzymes from different origins i.e. midgut, salivary glands and haemolymph of insects have been characterized (Pelegrini *et al.*, 2006, Dojnov *et al.*, 2008; Asadi *et al.*, 2010; Sharifi *et al.*, 2011; Saberi Riseh and Ghadamyari, 2012).

Hemicelluloses and cellulose, essential energy-producing nutrients, are hydrolyzed by insects' digestive glucosidases to di and oligosaccharides. Also, glucosidases are involved in insect-host plant interactions (Terra and Ferreira, 1994). Alpha-glucosidase (EC 3.2.1.3) can catalyze the releases of  $\alpha$ -D-glucose from

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terminal 1, 4-linked alpha-D-glucose residues. This enzyme acts on several substrates such as sucrose, maltose, maltodextrin and  $pNP-\alpha-D$ glucopyranoside, and it has been reported from digestive system, salivary glands and haemolymph of some insects (Terra et al., 1996; Ghadamyari et al., 2010; Sharifi et al., 2011; Riseh et al., 2012). Beta-glucosidase acts upon  $\beta$ bonds and cleaves β1-4 linkages between two glucoses or cellobiose (Terra et al., 1996). Betaglucosidase in Pieris brassicae (Lepidoptera: Pieridae) is an elicitor of cabbage volatiles that are attractive to Cotesia glomerata L. (Hymenoptera: Braconidae), a gregarious endoparasitoid of P. brassicae. (Mattiaci et al., 1995).

Alpha-D-galactosidases (EC 3.2.1.22) catalyze the hydrolysis of some carbohydrates such as melibiose, raffinose, stachyose, and gluco- or galactomannans successively releasing the  $\alpha$ -linked galactose residues (Meier and Reid, 1982). Beta-D-galactosidase (EC 3.2.1.23) is a hydrolase enzyme that catalyzes the conversion of  $\beta$ -galactosides into monosaccharides. In contrast to  $\alpha$ -amylases and glucosidases, galactosidases are not well understood in insects and study on the properties of these enzymes is necessary not only for comparative studies but also for understanding digestive physiology.

The sawfly. Arge rosae Linnaeus (Hymenoptera: Argidae) is one of the most serious pests of rose plant in Guilan province, Iran. The females lay their eggs in young stems and cause elongated scars on them. The young stems die after oviposition of A. rosae females (Sahragard and Heidari, 2001). The larvae feed on the leaves of rose plants and cause extensive and considerable defoliation. Chemical control has been considered a feasible means for pest control in Iran, including A. rosae, but this method has serious drawbacks such as intoxication of people and animals, side effects of the pesticides on non-target organisms, sublethal effects of the pesticides on target and nontarget organisms, emergence of resistant populations and pesticide residue and their entry into the trophic network (Talebi et al., 2011). Due to planting roses in urban areas, the use of pesticides in these areas holds special risks, as

most pesticides are not very selective, so, alternative methods for pest control that are less hazardous to the environment and human are highly appreciated (Breuer and De Loof, 2000).

Carbohydrases are hydrolytic enzymes present in digestive system, salivary glands and hemolymph of insects that play important roles as they are involved in food digestion, liberation of monosaccharides needed for growth and heme detoxification in blood sucking bugs (Mury et al., 2009). Therefore, any interruption in enzymatic carbohydrate digestion can deprive the A. rosae from utilizing the sources of carbohydrate energy efficiently and inhibition of carbohydrases may lead to an increase in the rose defenses against the rose sawfly. Transgenic plants expressing carbohydrase inhibitors have been considered as safe alternatives against herbivorous pests. because they cause interruption in carbohydrase activity and retard larval growth and development of some insect species. For example, pea and azuki transgenic plants expressing  $\alpha$ -amylase inhibitors showed insecticidal effects on the Bruchus pisorum (L.) and Callosobruchus chinensis (L.) weevils (Ishimoto and Kitamura, 1989; Shade et al., 1994). In our previous research. the identification and biochemical characterizations of different types of A. rosae proteases were studied (Sharifi et al., 2012). The present paper reports on the biochemical properties of  $\alpha$ amylase,  $\alpha$ -/ $\beta$ -glucosidase and  $\beta$ -galactosidase in alimentary canal of A. rosae larvae.

#### **Materials and Methods**

#### Chemicals

Triton X-100, bovine serum albumin, 3, 5-Dinitrosalicylic acid (DNS) and Starch were purchased from Merck (Merck, Darmstadt, P-nitrophenyl-α-D-glucopyranoside Germany). p-nitrophenyl-β-D-glucopyranoside  $(pN\alpha G)$ ,  $(pN\beta G),$ p-nitrophenyl-a-D-galactopyranoside p-nitrophenyl-s-D-galactopyranoside  $(pN\alpha Ga)$ 4-methylumbelliferyl-α-D- $(pN\beta Ga),$ glucopyranoside  $(4-MU\alpha G)$ and 4methylumbelliferyl-a-D-galactopyranoside (4-4-methylumbelliferyl-β-D-MUαGa).

glucopyranoside (4-MU $\beta$ G) and 4methylumbelliferyl- $\beta$ -D-galactopyranoside (4-MU $\beta$ Ga) were obtained from Sigma (Sigma, St Louis, MO, USA). P-nitrophenyl acetate (p-NA) was bought from Fluka (Buchs, Switzerland).

#### Insects

A. rosae larvae were collected from rose plants in Rasht, Guilan province of Iran. The collected individuals were grown and maintained on rose leaves in optimum rearing conditions of  $25 \pm 2$ °C,  $60 \pm 10\%$  RH with a photoperiod of 16 h light and 8 h dark. Same-aged 5<sup>th</sup> instar larvae (24 h after molting) were randomly selected for measuring of carbohydrase activities.

#### Sample preparation and enzyme assays

Last-larval instars were randomly selected for gut extraction. The larvae were immobilized on ice and their digestive systems (without contents) were removed by dissection under a microscope in ice-cold saline buffer. Then, tissues were transferred to a freezer (-20 °C). For measuring enzyme activity, the samples were homogenized in cold double-distilled water using a hand-held glass homogenizer and centrifuged at 15,000 rpm for 10 min at 4 °C.

#### **Enzyme activities**

Alpha-amylase activity was determined in universal buffer (40 mM phosphate-acetic-citric buffer). The supernatant (10 µl) was added to a tube containing 40 µl of the buffer and 50 µl of 1% (w/v) starch and incubated exactly for 30 min. The DNS method according to Bernfeld, (1955) was applied to measure the concentration of reducing sugars obtained from activity of  $\alpha$ amylase. Absorbance of product was measured at 545 nm with a Microplate Reader Model Stat Fax® 3200 (Awareness Technology Inc.). One unit  $\alpha$ -amylase was defined as the amount of the enzyme that liberated one micro mole of maltose from starch per minute.

The activities of  $\alpha$ -/ $\beta$ -glucosidases and  $\alpha$ -/ $\beta$ -galactosidase were measured with pN $\alpha$ G, pN $\beta$ G, pN $\alpha$ Ga and pN $\beta$ Ga as substrates, respectively. Homogenates were incubated with 45 µL of substrate (25 mM) and 115 µL of 40

mM phosphate-acetic-citric buffer for 30 min at 35 °C. After incubation time, 600 µL of NaOH (0.25 M) was added to stop the reaction and then 240 µl of reaction mixture was transferred to microplate wells. P-nitrophenol absorbance was measured at 405 nm using a microplate reader (Stat Fax 3200, Awareness Technology, USA) after 10 min (Ghadamyari et al., 2010). Assays were carried out in triplicate, and for all of them, appropriate blanks without enzyme were run. A standard curve with different concentration of p-nitrophenol was used to express the enzyme activity as µmole. min<sup>-1</sup>mg<sup>-</sup> protein. One unit enzyme is defined as the amount of the enzyme that liberates one micro mole of p-nitrophenol per minute.

### Effect of pH and temperature on enzyme activities

The pH profiles of the  $\alpha$ -amylases,  $\alpha$ -/ $\beta$ -glucosidases and  $\alpha$ -/ $\beta$ -galactosidases were determined at room temperature using universal buffer adjusted to various pHs (pH 2.0 to 11.0) by adding HCl or NaOH (1 N) (Asadi *et al.* 2010). Also, the activities of the enzymes were determined by incubating the reaction mixture at different temperatures ranging from 20 to 60 °C for 30 min (Saberi Riseh *et al.*, 2012). Enzyme activity was measured by the standard assay method mentioned above.

#### **Protein concentration**

Protein concentrations were estimated as described by Bradford (1976), using bovine serum albumin as standard.

### Polyacrylamide gel electrophoresis and zymogram analysis

Non-denaturing polyacrylamide gel electrophoresis (PAGE) (8%) for  $\alpha$ -amylase was carried out as described by Davis (1964) and electrophoresis was performed at constant voltage (100 V) in a refrigerator at 4°C (Asadi *et al.*, 2010). After electrophoresis, the gel was transferred to 2.5% (v/v) Triton X-100 and shaken gently for 30 min at room temperature. Then, the gel was washed twice with deionized water and buffer (25 mM of Tris-HCl pH 7.4).

The washed gel was incubated in substrate solution (1% (w/v) soluble starch) for 1 h. Afterward, the gel was subjected to Lugol solution (I<sub>2</sub> 1.3% and KI 3%) for appearance of the white bands showing  $\alpha$ -amylase activity.

For staining of  $\alpha$ -/ $\beta$ -glucosidases and  $\alpha$ -/ $\beta$ -galactosidase, the samples were mixed with sample buffer and applied onto a polyacrylamide gel (4 and 10 % polyacrylamide for the stacking and resolving gels, respectively). Electrophoresis was performed with 100 V at 4 °C (Sharifi *et al.*, 2011; Sabri Riseh and Ghazanfari 2012). After electrophoresis, the gel was incubated in 3 mM 4-MU $\alpha$ G, 4-MU $\beta$ G, 4-MU $\alpha$ Ga and 4-MU $\beta$ Ga in 0.1 M sodium acetate (pH 5.5) for 15 min at room temperature for appearance of fluorescent bands showing  $\alpha$ -/ $\beta$ -glucosidases and  $\alpha$ -/ $\beta$ -galactosidase activities, respectively. The blue-fluorescent bands were photographed with gel documentation apparatus (Uvitec Cambridge).

#### Kinetic parameters of α-amylases

The Michaelis-Menten constant  $(K_m)$  and maximal velocity  $(V_{max})$  of the  $\alpha$ -amylase were investigated at different concentrations of starch and glycogen over the range of 0.05-2 % (w/v), in 40 mM phosphate, glycine and acetate buffer, pH 8.0. The  $K_m$  and  $V_{max}$  were estimated from the Lineweaver-Burk plots.

### Effect of activators and inhibitors on amylase activities

To investigate the effect of several ions on enzyme activities, assays were carried out in the presence of different concentrations of  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  chloride salts as well as sodium dodecyl sulfate (SDS) and Ethylenediaminetetraacetic acid (EDTA). The enzyme sample was pre-incubated with the compounds for 15 min. After incubation time, the activity was measured by the standard assay method mentioned above. All Experiments were performed in three replicates, and for all of them, appropriate blanks were run.

#### Statistical analysis

The data were compared by one-way analysis of variance (ANOVA) followed by Tukey's test using SAS programs version 8.01(SAS, 1997).

#### **Results and Discussion**

The specific activities of  $\alpha$ -amylase,  $\alpha$ -/ $\beta$ glucosidases and  $\beta$ -galactosidase in 5<sup>th</sup> instar larvae of A. rosae are presented in Table 1. The activity of  $\alpha$ -galactosidase was too low to be detected. Among the tested carbohydrases,  $\alpha$ amylase showed highest specific activity in the digestive system of A. rosae larvae. The activity of  $\alpha$ -amylase in digestive system of A. rosae was higher than that of Allantus viennensis (Hym.: Tenthredinidae) (Jahanjou, 2011). The presence of  $\alpha$ -amylase in mandibular and salivary glands, digestive system and hypo pharyngeal glands of hymenopterans has other been reported (Takenaka et al., 1990; Ohashi et al., 1999; Ricks and Bradling, 2011). Also, the specific activity of  $\alpha$ -amylase in digestive system of A. rosae was higher than that reported for non hymenopteran insects such as Naranga aenescens L. (Lep.: Noctuidae) (Asadi et al., 2010) Glyphodes pyloalis Walker (Lep.: Pyralidae) (Yezdani et al., 2010) and Helicoverpa armigera Hubner (Lep.: Noctuidae) (Kotkar et al., 2009).

**Table 1** The specific activities ( $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup> protein) of digestive carbohydrases in last larval instar of *A. rosae* stage.

Enzyme	activities (μmol min <sup>-1</sup> mg <sup>-1</sup> protein) (Mean ± SE)	Activities (U/ml) (Mean ± SE)
α-glucosidase	$3.4 \pm 0.38$	$1.71 \pm 0.18$
β-glucosidase	$1.8 \pm 0.03$	$0.9 \pm 0.017$
β-galactosidase	$5.2 \pm 0.01$	$2.55\pm0.006$
α-amylase	$9.4 \pm 0.06$	$4.63\pm0.033$

The specific activity of  $\beta$ -galactosidase in the gut of A. rosae was higher than  $\alpha$ - and  $\beta$ glucosidase activities. Alpha and βglucosidase activities in the gut of A. viennensis was reported as  $11.45 \pm 0.23$  and µmol min<sup>-1</sup> mg<sup>-1</sup> protein,  $6.32 \pm 0.25$ respectively. Also, the activity of glucosidases in midgut of A. viennensis was higher than galactosidases (Jahanjou, 2011). So far,  $\beta$ -galactosidase activity is reported at hypopharyngeal of glands some hymenopteran insects such as Scaptotrigona postica, S. mexicana and A. mellifera. It seems that the activities of  $\alpha$ -/ $\beta$ -glucosidases and  $\alpha$ -/ $\beta$ -galactosidase vary depending on

insect species, glycosidic bonds available in diets and host plant foods (Asadi et al., 2012). For example glycolipids available in plant tissues can be hydrolyzed by the  $\beta$ galactosidases in the midgut of lepidopteran insects and then the hydrolyzed glycolipids are used for synthesis of hemolymph and tissue lipid trehalose (Turunen, 1992; Costa and Cruz-Landim, 2005). Also, the same reaction can take place in phytophagous hymenopteran insects such as A. rosae. The highest enzyme activity in the midgut of leafcutting ants, Acromyrmex subterraneus (Hym.: Formicidae) has been detected for  $\alpha$ glucosidase (Erthal et al., 2004), whereas the highest enzyme activity in midgut of A. rosae was obtained for  $\beta$ -galactosidase.

Maximum  $\alpha$ -amylase activity from gut of A. rosae occurs at pH 8.0 (Fig. 1). The optimum activity for a-amylase has been reported at pH 12.0 for midgut lumen of Acherontia atropos (Lep.: Sphingidae), and 10.8 for Lasiocampa quercus (Lep.: Lasiocampidae), 11.3 for Manduca sexta (Lep.: Sphingidae) and 10.8 for Lichnoptera feline (Lep.: Noctuidae) (Dow, 1984). It seems that the optimal pH of  $\alpha$ amylase in hymenoptern insect (Symphyta) is neutral to slightly alkaline whereas,  $\alpha$ -amylases extracted from midgut of lepidopteran larvae are active in alkaline conditions (Asadi et al., 2010; Jahanjou, 2011). The gut pH of A. rosae was alkaline (unpublished data). It seems that the high gut pH in some phytophagous insects such as A. rosae is because of adaptation for feeding on host plants containing tannins (Chapman, 1998), because tannin can bind with proteins in insect's midgut at acidic pH values. Thus, it may decrease the efficiency of food digestion (Dow, 1986).

Maximum activity in the digestive system of *A. rosae* was observed at pH 5.0 for  $\alpha$ -/ $\beta$ -glucosidase and  $\beta$ -galactosidase (Fig. 1). The optimal pH for  $\alpha$ -/ $\beta$ -glucosidase and  $\alpha$ -/ $\beta$ -galactosidase in the digestive system of *A. viennensis* has been reported as pH 6.0 (Jahanjou, 2011). The digestive glucosidases in insects show some differences in optimal pH, for example 4.5-5 for  $\beta$ -glucosidase in *Zygaena* 

*trifolii* Esper (Lep.: Zygaenidae) (Franzl *et al.*, 1989), 4.9-5.6 for  $\alpha$ -glucosidase in the larvae of Apollo butterfly (Nakonieczny *et al.*, 2006) and 8.0 for  $\alpha$ -glucosidase in 3<sup>rd</sup> instar larvae of *Earias vitella* (Lep.: Noctuidae) (Tripathi and Krishna, 1988). This variation in optimal pH between insect species may refer to their phylogenetic relations or may be in response to different diets (Nakonieczny *et al.*, 2006; Asadi *et al.*, 2012). Also, the origin of the  $\alpha$ - and  $\beta$ -glucosidase, i.e. digestive system or salivary glands may justify these differences (Asadi *et al.*, 2012).

al., 2012).



**Figure 1** The effect of pH on the activities of  $\alpha$ amylase,  $\beta$ -galactosidase ( $\beta$ -gala) and  $\alpha$ ,  $\beta$ glucosidases ( $\alpha$ -glu/ $\beta$ -glu) extracted from the digestive system of *Arge rosae*.

The optimum temperature for  $\alpha$ -amylase activity was 50 °C in gut of *A. rosae* (Fig. 2) which is consistent with maximum temperature for  $\alpha$ -amylase activity in the digestive system of *A. viennensis* (Jahanjou, 2011).



**Figure 2** The effect of temperature on the activities of  $\alpha$ -amylase,  $\beta$ -galactosidase ( $\beta$ -gala) and  $\alpha$ ,  $\beta$ -glucosidases ( $\alpha$ -glu/ $\beta$ -glu) extracted from the digestive system of *Arge rosae*.

The A. rosae  $\alpha$ - and  $\beta$ -glucosidase had optimum temperature activity at 30 and 40 °C, respectively. Also, the optimal temperature for  $\beta$ -galactosidase in the digestive system was obtained as 40 °C (Fig. 2). Alpha and  $\beta$ -glucosidase optimum temperature was reported at 45 °C in midgut of G. pyloalis (Ghadamyari et al., 2010); 60 and 50 °C in the digestive system of *Xanthogaleruca* luteola (Coleoptera: Chrysomelidae) (Sharifi et al., 2011). For  $\alpha/\beta$  -galactosidase, optimum temperature was 30 °C for midgut of Brachynema germari Kolenati (Hemiptera: Pentatomidae) (Ramzi and Hosseininaveh, 2010); 40 and 60  $^{\circ}$ C in the digestive system of X. luteola (Sharifi et al., 2011). Most insect  $\alpha$ - and  $\beta$ glucosidases exhibit temperature optima ranging from 20 to 50 °C (Huber and Mathison, 1976; Takenaka and Echigo, 1978).

When midgut homogenates of A. rosae were subjected to native PAGE, three bands were found for  $\alpha$ -amylase (Fig. 3), one band for  $\alpha$ -/ $\beta$ -glucosidases and two bands for  $\beta$ galactosidase (Fig. 4). In the native gel, no bands were observed for  $\alpha$ - galactosidase. Similar to our result, three and one bands have been reported for  $\alpha$ -amylase and  $\alpha$ -/ $\beta$ glucosidases in digestive system of A. viennensis, respectively (Jahanjou, 2011). βglucosidase and  $\beta$ -galactosidase in the digestive system of X. luteola showed three and one isoform, respectively (Sharifi et al., 2011). The results of Riseh et al. (2012) indicated 4, 4, 2, and 1 isoforms of  $\alpha$ - and  $\beta$ glucosidases and  $\alpha$ - and  $\beta$ -galactosidases in the crude digestive system of the last larval instar of Rhynchophorus ferrugineus Olivieri Curculionide), (Col.: respectively. Zymogram pattern of  $\alpha$ - and  $\beta$ -glucosidase activities from the gut of Osphranteria coerulescens Redt. (Col.: Cerambycidae) showed that these activities corresponded to three and four major bands (Aghaali et al., 2012). The results of Asadi et al., (2012) showed that α-glucosidases and ßglucosidases in the alimentary canal of N. aenescense have two isoforms and one isoform, respectively.



**Figure 3** Zymogram of  $\alpha$ - amylase extracted from the digestive system of *Arge rosae*.

Previous researches have reported that insect  $\alpha$ -amylases are activated or inhibited by some ions and chemicals. Some mineral ions and compounds may inhibit the digestive  $\alpha$ -amylases

in midgut of insects and have disadvantageous effect on their food digestion (Hori, 1970; Cohen, 1993; Pavan, 2004). Our results showed that  $Mg^{2+}$ and Na<sup>+</sup> significantly increased amylase activity in digestive system of A. rosae, whereas  $Ca^{2+}$  and K<sup>+</sup> did not show any effect on amylolytic activity (Fig. 5). SDS and EDTA decreased A. rosae αamylase activity. The effect of metal ions and EDTA on the  $\alpha$ -amylase activity in the midgut, salivary glands and haemolymph of N. aenescens showed that  $Mn^{2+}$ ,  $Hg^+$  and  $Hg^{2+}$  ions decreased  $\alpha$ -amylases activity, whereas the  $\alpha$ -amylase activity was enhanced in the presence of Na<sup>+</sup>, K<sup>+</sup>,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Co^{2+}$  and  $Fe^{2+}$  (Asadi *et al.*, 2012). In this study, Ca<sup>2+</sup> showed no effect on the activity of  $\alpha$ -amylases, whereas, the result of Asadi *et al.* (2010) showed the activity of N. aenescens  $\alpha$ amylases was increased by addition of Ca<sup>2+</sup> to the assay mixture. Also, in some insects such as T. molitor, midgut a-amylase was slightly activated by Ca<sup>2+</sup> and Cl<sup>-</sup> (Applebaum, 1961).

α-glu β-glu β-gala



**Figure 4** Zymogram of  $\alpha$ - and  $\beta$ -glucosidases and  $\beta$ -galactosidase extracted from the digestive system of *Arge rosae* (left to right).

As calculated from Lineweaver-Burk plots, the Km values for  $\alpha$ -amylases in digestive system of A. rosae were about  $0.82 \pm 0.051$  and  $5.05 \pm 0.8$  mg/ml (Fig. 6), when starch and glycogen were used as substrates, respectively. Therefore, the  $\alpha$ amylase in digestive system of A. rosae showed 6.3-folds higher affinity to starch than glycogen. The  $K_m$  and  $V_{max}$  for  $\alpha$ amylase in midgut of N. aenescens were reported as 0.07 mg/ml and 0.2 µmol/min, respectively. Also,  $K_m$  values of  $\alpha$ -amylase in midgut and salivary glands of pistachio green stink bug, B. germari were reported as 0.77 and 0.41 mM, respectively (Ramzi and Hosseininaveh, 2010). The result of these researches showed that the  $\alpha$ -amylase  $K_m$  value in midgut of A. rosae was higher than that of N. aenescen.

For carbohydrate metabolism, different forms of carbohydrases such as amylases, galactosidases and glucosidases can be found in insect species, to guarantee their survival and development (Baker, 1983). Therefore, these enzymes are good target candidates for enzyme inhibitors (Franco et al., 2002; Svensson et al., 2003). For example, α-amylase inhibitors are extensively found in many plant seeds and tubers (Franco et al. 2002; Sadasivam et al., 2003) and these molecules play a key role in plant defense toward pests and pathogens (Franco et al., 2000). Plant secondary metabolites such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides and phenolic compounds have insecticidal effects. Also, these compounds mediate in plant defenses against herbivorous pests either by repellence or inhibiting digestive enzymes in the midgut of insects (Hsiao et al., 1985; Asadi et al., 2012) and confer resistance to various plant species against pests. Glycosides are secondary metabolites that can confer resistance to plants against pests. For example, DIMBOA (a glycoside), purified from corn, has detrimental effect on larvae of European corn borer, Ostrinia nubilalis (Hubner) (Klun et al., 1967). compound Also, this retards the development of O. nubilalis and decreases reproductive potential, increases mortality and deters feeding in aphids (Long et al., 1977; Klun et al., 1967). Some Phaseolus varieties contain high concentration of glycosides which confer resistance to a Mexican bean beetle, Epilachna varivestris (Nayar and Frankel, 1963).

Several researches have shown the main role of glucosidases in insect-host plant interactions and plant resistance to pests. Also,  $\alpha$ -amylase inhibitors are extensively reported from seeds of cereals and legumes (Franco et al., 2002; Sadasivam et al., 2003) and so far, pea and azuki transgenic plants expressing  $\alpha$ -amylase inhibitors have been developed for resistance to the B. pisorum and C. chinensis. Since the pesticide applications in urban areas have special risks, so seeking alternative methods, less hazardous to the environment and human, for A. rosae control, is highly appreciated. Therefore, the discovery of novel inhibitors for  $\alpha$ -amylase,  $\alpha$ -/ $\beta$ -glucosidases and  $\beta$ galactosidase available in plants can contribute to managing this pest via pestresistant transgenic plants.

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**Figure 5** Effect of various metal ions and chemicals on relative activity of  $\alpha$ -amylase from digestive system of *Arge rosae* (± SE).



Figure 6 Lineweaver-Burke plot of  $\alpha$ -amylae extracted from digestive system of *Arge rosae* on starch and glycogen as substrates.

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### تعیین خصوصیات بیوشیمیایی کربوهیدرازهای گوارشی در زنبور بر گخوار رز ( Arge rosae ) (Linnaeus (Hymenoptera:Argidae)

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**چکیدہ:** زنبور برگخوار رز، Arge rosae Linnaeus، یکی از مخربترین آفات ہوتەھای رز در شمال ایران میباشد. امروزه با کشف روشهای نوین کنترل آفات، تلاشهای بسیاری در زمینه کاهش کاربرد آفتکشها صورت گرفته است. یکی از روشهای غیرشیمیایی در کنترل آفات از جمله A. rosae استفاده از گیاهان تراریخته حاوی مهارکنندههای کربوهیدراز میباشد. بنابراین در مطالعه حاضر ویژگیهای بیوشیمیایی کربوهیدرازهای گوارشی در لوله گوارش A. rosae جهت دستیابی به روشی جدید در کنترل این آفت تعیین شد. فعاليت ويژه آلفا-آميلاز در لوله گوارشي سن آخر لاروي ۰/۰۶ A. rosae ميكرومول بر دقيقه بر میلی گرم پروتئین بهدست آمد. همچنین pH و دمای بهینه آلفا-آمیلاز بهترتیب ۸ و ۵۰ درجه سلسیوس تعیین شد. هنگامی که از نشاسته به عنوان سوبسترا استفاده شد، مقادیر Km و Vmax آلفا⊣آمیلاز محاسبه شده از نمودار Lineweaver-Burk، بهترتیب ۰/۸۲ میلی گرم بر میلی لیتر و ۷/۳۲ میکرومول بر دقیقه بر میلی گرم پروتئین محاسبه شد. اثر یونها روی فعالیت آمیلولیتیک نشان داد که Mg<sup>2+</sup> Mg<sup>2+</sup> و Na<sup>+</sup> بهطور معنیداری فعالیت ألفا-أميلاز را افزايش داده، درحالي كه SDS و EDTA فعاليت اين أنزيم را كاهش دادند. بيشترين فعاليت ألفا-گلوکوزیداز، بتا–گلوکوزیداز و بتا– گالاکتوزیداز در ۵ pH ۵ بهدست آمد. تعداد باندهای مشاهده شده روی ژل برای آلفا-آمیلاز، آلفا- گلوکوزیداز، بتا- گلوکوزیداز و بتا- گالاکتوزیداز بهترتیب برابر با ۳، ۱، ۱ و ۲ بود. هیچ باندی برای آلفا-گالاکتوزیداز نمایان نشد که عدم حضور یا فعالیت ناچیز این کربوهیدراز را در سیستم گوارشی A. rosae تصدیق می کند. این نتایج می تواند دانش مورد نیاز جهت تولید گیاهان تراریخته برای کنترل این آفت را فراهم کند.

واژگان كليدى: Arge rosae آلفا-آميلاز، آلفا-گلوكوزيداز، بتا-گلوكوزيداز، بتا-گالاكتوزيداز