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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 α-amylase in the digestive system of last larval instars of *A. rosae* was obtained as 9.46 ± 0.06 μmol min^{-1} mg^{-1} 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^{-1} mg^{-1} protein, respectively, when starch was used as substrate. The effects of ions on amylolytic activity showed that Mg^{2+} and Na^+ significantly increased amylase activity, whereas SDS and EDTA decreased the enzyme activity. The highest activities of α-/β-glucosidase and β-galactosidase were obtained at pH 5.0. By the native PAGE, three, one, one and two bands were clearly detected for α-amylase, α-/β-glucosidase and β-galactosidase, respectively. No bands were found for α-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

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Introduction

Alpha-amylases (known as 1, 4-α-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 α-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 *p*NP-α-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 β bonds and cleaves β1-4 linkages between two glucoses or cellobiose (Terra *et al*., 1996). Beta-glucosidase 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 α-linked galactose residues (Meier and Reid, 1982). Beta-D-galactosidase (EC 3.2.1.23) is a hydrolase enzyme that catalyzes the conversion of β-galactosides into monosaccharides. In contrast to α-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, sub-lethal effects of the pesticides on target and non-target 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 α-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 α-amylase, α-/β-glucosidase and β-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, Germany). P-nitrophenyl-α-D-glucopyranoside (pNαG), p-nitrophenyl-β-D-glucopyranoside (pNβG), p-nitrophenyl-α-D-galactopyranoside (pNαGa) p-nitrophenyl-s-D-galactopyranoside (pNβGa), 4-methylumbelliferyl-α-D-glucopyranoside (4-MUαG) and 4-methylumbelliferyl-α-D-galactopyranoside (4-MUαGa), 4-methylumbelliferyl-β-D-glucopyranoside (4-MUβG) and 4-methylumbelliferyl-β-D-galactopyranoside (4-MUβGa) 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 ± 2 °C, 60 ± 10% RH with a photoperiod of 16 h light and 8 h dark. Same-aged 5^{th} 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 α-amylase. Absorbance of product was measured at 545 nm with a Microplate Reader Model Stat Fax® 3200 (Awareness Technology Inc.). One unit α-amylase was defined as the amount of the enzyme that liberated one micro mole of maltose from starch per minute.

The activities of α-/β-glucosidases and α-/β-galactosidase were measured with pNαG, pNβG, pNαGa and pNβGa 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. $min^{-1}mg^{-1}$ 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 α-amylases, α-/β-glucosidases and α-/β-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 ºC 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 α-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).

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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_2 1.3% and KI 3%) for appearance of the white bands showing α-amylase activity.

For staining of α-/β-glucosidases and α-/β-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-MUαG, 4-MUβG, 4-MUαGa and 4-MUβGa in 0.1 M sodium acetate (pH 5.5) for 15 min at room temperature for appearance of fluorescent bands showing α-/β-glucosidases and α-/β-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 α-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 α-amylase, α-/β-glucosidases and β-galactosidase in 5th instar larvae of *A. rosae* are presented in Table 1. The activity of α–galactosidase was too low to be detected. Among the tested carbohydrases, α-amylase showed highest specific activity in the digestive system of *A. rosae* larvae. The activity of α-amylase in digestive system of *A. rosae* was higher than that of *Allantus viennensis* (Hym.: Tenthredinidae) (Jahanjou, 2011). The presence of α-amylase in mandibular and salivary glands, digestive system and hypo pharyngeal glands of other hymenopterans has been reported (Takenaka *et al*., 1990; Ohashi *et al*., 1999; Ricks and Bradling, 2011). Also, the specific activity of α-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 (μmol min^{-1} mg^{-1} protein) of digestive carbohydrases in last larval instar of *A. rosae* stage.

Enzyme	activities (μmol min^{-1} mg^{-1} protein) (Mean ± SE)	Activities (U/ml) (Mean ± SE)
α-glucosidase	3.4 ± 0.38	1.71 ± 0.18
β-glucosidase	1.8 ± 0.03	0.9 ± 0.017
β-galactosidase	5.2 ± 0.01	2.55 ± 0.006
α-amylase	9.4 ± 0.06	4.63 ± 0.033

The specific activity of β-galactosidase in the gut of *A. rosae* was higher than α- and β-glucosidase activities. Alpha and β-glucosidase activities in the gut of *A. viennensis* was reported as 11.45 ± 0.23 and 6.32 ± 0.25 μmol min^{-1} mg^{-1} protein, respectively. Also, the activity of glucosidases in midgut of *A. viennensis* was higher than galactosidases (Jahanjou, 2011). So far, β-galactosidase activity is reported at hypopharyngeal glands of some hymenopteran insects such as *Scaptotrigona postica*, *S. mexicana* and *A. mellifera*. It seems that the activities of α-/β-glucosidases and α-/β-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 β-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 leaf-cutting ants, *Acromyrmex subterraneus* (Hym.: Formicidae) has been detected for α-glucosidase (Erthal *et al*., 2004), whereas the highest enzyme activity in midgut of *A. rosae* was obtained for β-galactosidase.

Maximum α-amylase activity from gut of *A. rosae* occurs at pH 8.0 (Fig. 1). The optimum activity for α-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 α-amylase in hymenoptern insect (Symphyta) is neutral to slightly alkaline whereas, α-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 α-/β-glucosidase and β-galactosidase (Fig. 1). The optimal pH for α-/β-glucosidase and α-/β-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 β-glucosidase in *Zygaena trifolii* Esper (Lep.: Zygaenidae) (Franzl *et al*., 1989), 4.9-5.6 for α-glucosidase in the larvae of Apollo butterfly (Nakonieczny *et al*., 2006) and 8.0 for α-glucosidase in 3rd 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 α- and β-glucosidase, i.e. digestive system or salivary glands may justify these differences (Asadi *et al*., 2012).

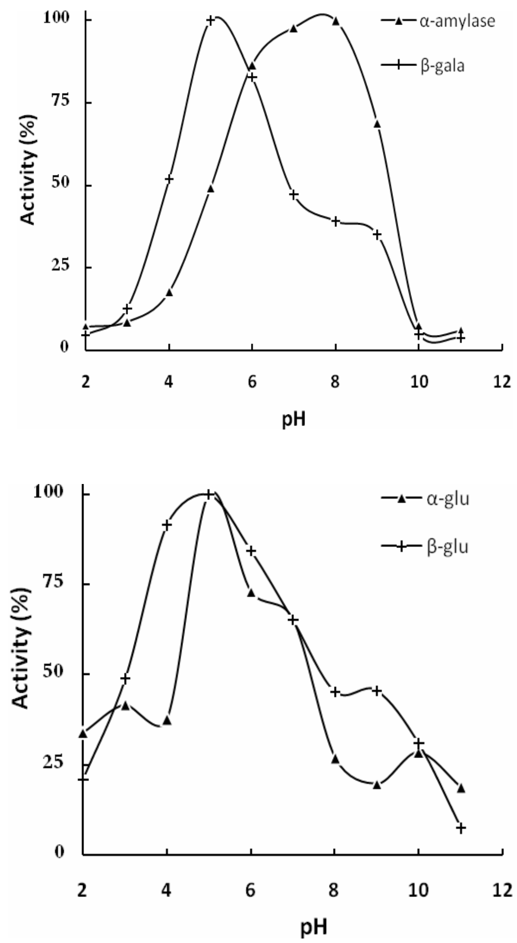

Figure 1 The effect of pH on the activities of α-amylase, β-galactosidase (β-gala) and α, β-glucosidases (α-glu/β-glu) extracted from the digestive system of *Arge rosae*.

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

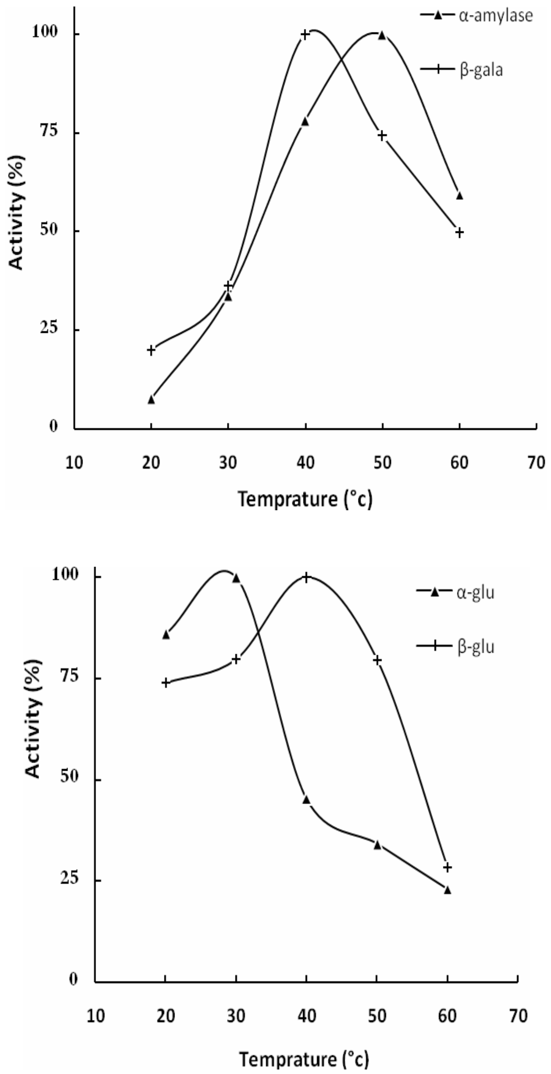

Figure 2 The effect of temperature on the activities of α-amylase, β-galactosidase (β-gala) and α, β-glucosidases (α-glu/β-glu) extracted from the digestive system of *Arge rosae*.

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

When midgut homogenates of *A. rosae* were subjected to native PAGE, three bands were found for α-amylase (Fig. 3), one band for α-/β-glucosidases and two bands for β-galactosidase (Fig. 4). In the native gel, no bands were observed for α- galactosidase. Similar to our result, three and one bands have been reported for α-amylase and α-/β-glucosidases in digestive system of *A. viennensis*, respectively (Jahanjou, 2011). β-glucosidase and β-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 α- and β-glucosidases and α- and β-galactosidases in the crude digestive system of the last larval instar of *Rhynchophorus ferrugineus Olivieri* (Col.: Curculionide), respectively. Zymogram pattern of α- and β-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 β-glucosidases in the alimentary canal of *N. aenescense* have two isoforms and one isoform, respectively.

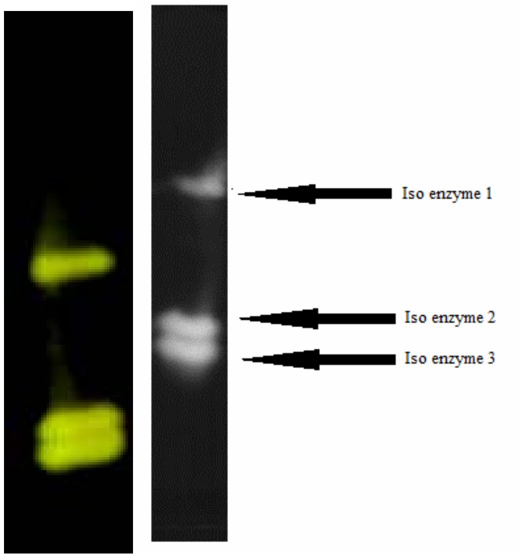

Figure 3 Zymogram of α- amylase extracted from the digestive system of *Arge rosae*.

Previous researches have reported that insect α-amylases are activated or inhibited by some ions and chemicals. Some mineral ions and compounds may inhibit the digestive α-amylases in midgut of insects and have disadvantageous effect on their food digestion (Hori, 1970; Cohen, 1993; Payan, 2004). Our results showed that Mg^{2+} and Na^{+} significantly increased amylase activity in digestive system of *A. rosae*, whereas Ca^{2+} and K^{+} did not show any effect on amylolytic activity (Fig. 5). SDS and EDTA decreased *A. rosae* α-amylase activity. The effect of metal ions and EDTA on the α-amylase activity in the midgut, salivary glands and haemolymph of *N. aenescens* showed that Mn^{2+}, Hg^{+} and Hg^{2+} ions decreased α-amylases activity, whereas the α-amylase activity was enhanced in the presence of Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Co^{2+} and Fe^{2+} (Asadi *et al*., 2012). In this study, Ca^{2+} showed no effect on the activity of α-amylases, whereas, the result of Asadi *et al.* (2010) showed the activity of *N. aenescens* α-amylases was increased by addition of Ca^{2+} to the assay mixture. Also, in some insects such as *T. molitor,* midgut α-amylase was slightly activated by Ca^{2+} and Cl^{-} (Applebaum, 1961).

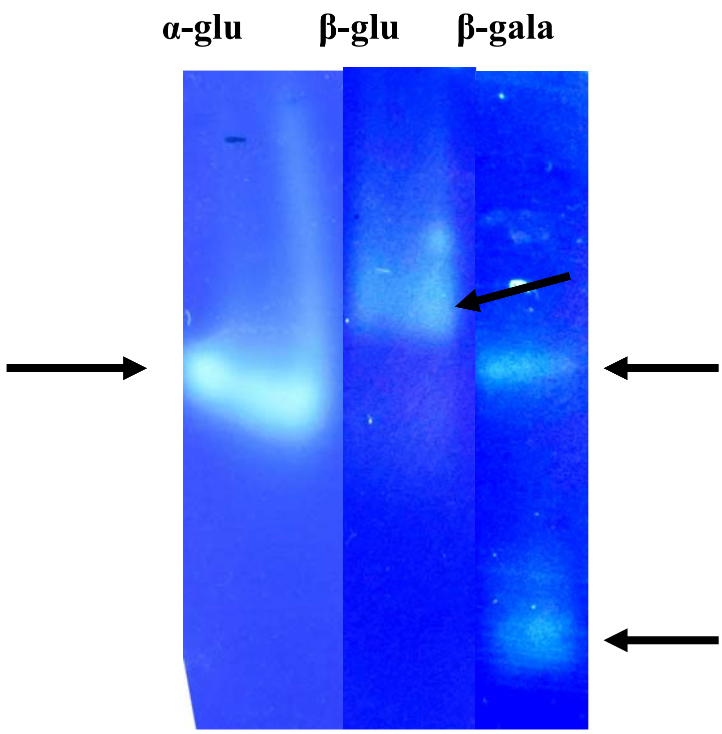

Figure 4 Zymogram of α- and β-glucosidases and β-galactosidase extracted from the digestive system of *Arge rosae* (left to right).

As calculated from Lineweaver-Burk plots, the *Km* values for α-amylases in digestive system of *A. rosae* were about 0.82 ± 0.051 and 5.05 ± 0.8 mg/ml (Fig. 6), when starch and glycogen were used as substrates, respectively. Therefore, the α-amylase in digestive system of *A. rosae* showed 6.3-folds higher affinity to starch than glycogen. The K_m and V_{max} for α-amylase in midgut of *N. aenescens* were reported as 0.07 mg/ml and 0.2 µmol/min, respectively. Also, K_m values of α-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 α-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). Also, this compound 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, α-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 α-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 α-amylase, α-/β-glucosidases and β-galactosidase available in plants can contribute to managing this pest via pest-resistant transgenic plants.

Acknowledgments

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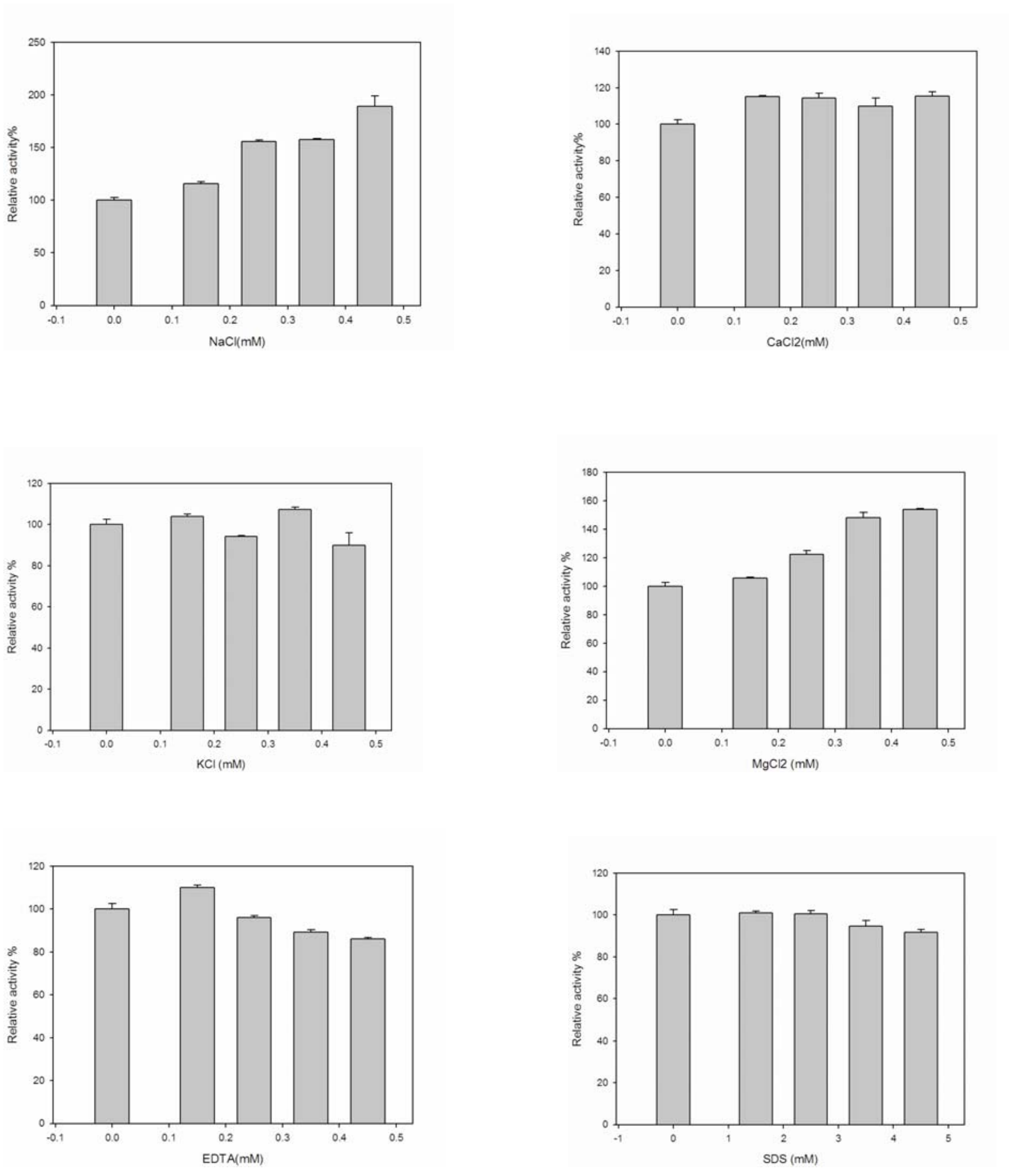

Figure 5 Effect of various metal ions and chemicals on relative activity of α-amylase from digestive system of *Arge rosae* (± SE).

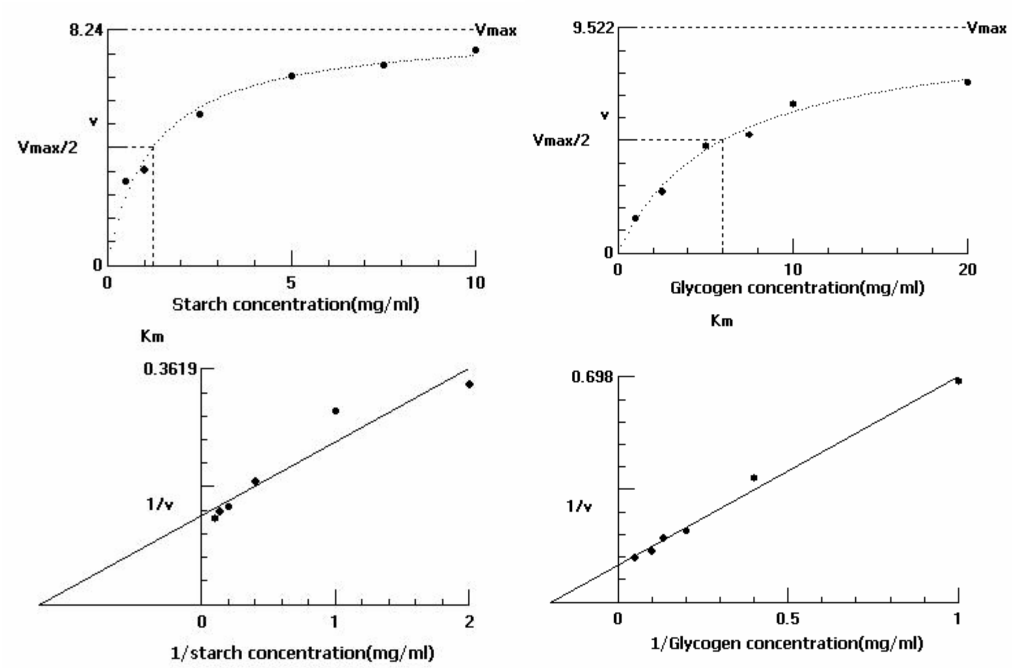

Figure 6 Lineweaver-Burke plot of α-amylae extracted from digestive system of *Arge rosae* on starch and glycogen as substrates.

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تعیین خصوصیات بیوشیمیایی کربوهیدرازهای گوارشی در زنبور برگخوار رز (*Arge rosae* (Linnaeus (Hymenoptera:Argidae)

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چکیده: زنبور برگخوار رز، *Arge rosae* Linnaeus، یکی از مخرب‌ترین آفات بوته‌های 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. فعالیت ویژه آلفا-آمیلاز در لوله گوارشی سن آخر لاروی *A. rosae* ۰/۰۶ ± ۹/۴۶ میکرومول بر دقیقه بر میلی‌گرم پروتئین به‌دست آمد. همچنین pH و دمای بهینه آلفا-آمیلاز به‌ترتیب ۸ و ۵۰ درجه سلسیوس تعیین شد. هنگامی‌که از نشاسته به‌عنوان سوبسترا استفاده شد، مقادیر *Km* و *Vmax* آلفا-آمیلاز محاسبه شده از نمودار Lineweaver-Burk، به‌ترتیب ۰/۸۲ میلی‌گرم بر میلی‌لیتر و ۷/۳۲ میکرومول بر دقیقه بر میلی‌گرم پروتئین محاسبه شد. اثر یون‌ها روی فعالیت آمیلولیتیک نشان داد که Mg^{2+} و Na^{+} به‌طور معنی‌داری فعالیت آلفا-آمیلاز را افزایش داده، درحالی‌که SDS و EDTA فعالیت این آنزیم را کاهش دادند. بیشترین فعالیت آلفا-گلوکوزیداز، بتا-گلوکوزیداز و بتا- گالاکتوزیداز در 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 می‌کند. این نتایج می‌تواند دانش مورد نیاز جهت تولید گیاهان تراریخته برای کنترل این آفت را فراهم کند.

واژگان کلیدی: *Arge rosae*، آلفا-آمیلاز، آلفا-گلوکوزیداز، بتا-گلوکوزیداز، بتا-گالاکتوزیداز