BIOCHEMICAL CHARACTERIZATION OF DIGESTIVE CARBOHYDRASES FROM *XANTHOGALERUCA LUTEOLA* AND INHIBITION OF ITS A-AMYLASE BY INHIBITORS EXTRACTED FROM THE COMMON BEAN

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Abstract - *Xanthogaleruca luteola* Müll. (Col.: Chrysomelidae) is a major urban insect pest on elm trees in Iran. Digestion in the alimentary canal of the elm leaf beetle is facilitated by some carbohydrases which are responsible for the digestion of carbohydrates. The presence of digestive carbohydrases was determined in the digestive system of adult and last larval instar of the elm leaf beetle. The specific activity of α-amylase in the digestive system of adult females and last larval instars were 0.49 ± 0.05 and 0.72 ± 0.07 μmol/min/mg protein, respectively. Also, the amylase activity in the midgut of the last larval instar was 3.125- and 4.16-fold higher than that its activity in the foregut and hindgut, respectively. Results showed that optimum activity for α-amylase was found at pH 5. As calculated from Lineweaver-Burk plots, the K_m values for α-amylase were 0.64 and 1.44 mg/ml, when glycogen and starch were used as substrates, respectively. The effect of pH and temperature on α- and ß-glucosidase and α- and β-galactosidase activities was determined in the digestive system of *X. luteola*. Results showed that the activity of α- and ß-glucosidases in adult females was higher than in larvae, but the ß-galactosidase activity in larvae was more than that of the adult. In adult females the glucosidase activity was higher than the galactosidase activity. The zymogram pattern in the native gel revealed that *X. luteola* α-amylase, β-glucosidase and β-galactosidase in the digestive system had one, three and one isoforms. α-amylase inhibitors, purified from *Phaseolus vulgaris L.* with an ion-exchange DEAE cellulose column showed good inhibitory activity on *X. luteola* gut α-amylase.

Key words: Elm leaf beetle, α- and β-glucosidases, α- and β-galactosidases, α-amylase, α-amylase inhibitor

INTRODUCTION

The α-amylases (α-1,4-glucan-4-glucanohydrolases, EC 3.2.1.1) hydrolyze starch and other polysaccharides to maltose, maltotriose and maltodextrins (Henrissat et al., 2002). These enzymes play a key role in the carbohydrate metabolism of microorganisms, plants and animals and insects (Franco et al., 2002). α-amylases have been found in several insect orders, including Coleoptera, where they are usually reported in the digestive system (Ishimoto and Kitamura, 1989; Silva et al., 1999). Inhibitors of insect

α-amylase have already been shown to be an effective control of insect pests (Shade et al., 1994). Pea and azuki transgenic plants expressing α-amylase inhibitors from common beans were completely resistant to the *Bruchus pisorum* (L.) and *Callosobruchus chinensis* (L.) weevils (Ishimoto and Kitamura, 1989; Shade et al., 1994).

In insects, digestive glucosidases are important for the hydrolysis of di- and oligosaccharides derived from hemicelluloses and cellulose and are involved in insect-plant interactions (Terra and Ferreira,

1994). α-glucosidase (EC 3.2.1.20) is an enzyme that catalyzes the hydrolysis of 1, 4-α-glucosidic linkages, releasing α-glucose. This enzyme strongly hydrolyzes sucrose, maltose, maltodextrin and pNP-α-D-glucopyranoside. It can be found in the alimentary canal, salivary secretions of insects and hypopharyngeal glands of some insects, such as *Apis mellifera* L. (Terra et al., 1996; Baker and Lehner, 1972). So far, α-glucosidases have been isolated and characterized from many insects including *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae), *Sitophilus zeamais* (Coleoptera: Curculionidae), *Apis mellifera* (Hymenoptera: Apidae), *Drosophila melanogaster* (Diptera: Drosophilidae) and *Glyphodes pyloalis* Walker (Lep.: Pyralidae) (Huber and Mathison,1976; Baker, 1991; Silva and Terra, 1997; Tanimura et al., 1979; Ghadamyari et al., 2010). β-glucosidase hydrolyzes β1–4 linkages between two glucoses or glucose-substituted molecules (such as cellobiose) (Terra et al., 1996). In addition to the important digestive role of the enzymes, they can also act as elicitors or triggering agents of plant defense mechanisms when they are present as feeding damage of insect pests (Mattiacci et al., 1995). α-D-galactosidases (EC 3.2.1.22) are exo-acting glycoside hydrolases that cleave α-linked galactose residues from carbohydrates such as melibiose, raffinose, stachyose, and gluco- or galactomannans (Meier, and Reid, 1982). ß-D-galactosidases (EC 3.2.1.23) is a hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides. Our knowledge about the galactosidases of insects is still rudimentary.

The elm leaf beetle, *Xanthogaleruca luteola* Müll. (Col.: Chrysomelidae) is the most serious pest of the elm tree in Iran. Both the adult and larvae feed on the parenchyma of leaves, without consuming the veins, and cause severe damage to trees. If the damage is severe and occurs several years in a row, the trees develop deformed canopies, and suffer vigor loss, physiological disorders and reduced photosynthesis, which predisposes them to the action of other pests, plant disease and stress factors. They become particularly susceptible to scolytid beetles carrying spores of the fungus *Ceratocystis novo ulmi* Brasier, which causes the elm tree disease, a serious threat to survival of these trees (Romanyk and Cadahia, 2002). Defoliation also reduces tree shade in summer and the aesthetical values of elms (Dreistadt et al., 2001). Due to the adverse effect of pesticides on humans, the application of pesticide in an urban area for controlling this pest has some problematic side-effects. The study of insect digestive enzymes is important because the gut is the major interface between the insect and its environment. For an understanding of how digestive enzymes act on their substrates in insects, it is essential to develop methods of insect control. Some work has been done on the carbohydrases in the digestive system of the insect (Ghadamyari et al., 2010; Ramzi and Hosseininaveh, 2010; Kazzazi et al., 2005) but work on the digestive enzyme of *X. luteola* is lacking. The aim of the present study was to determine the biochemical characterization of the carbohydrate hydrolyzing enzyme in the digestive system of the elm leaf beetle in order to gain a better understanding of the digestive physiology of this insect. Plant α-amylase inhibitors show great potential as tools to engineer resistance of crop plants against pests. These inhibitors are proteins found in several plants, and play a key role in natural defenses, especially those that feed on starchy food. These inhibitors are particularly abundant in legumes (Franco et al., 2002) and cereals (Iulek et al., 2000). In this research, we also investigate the inhibitory effects of *Phaseolus vulgaris* L. against *X. luteola* α-amylase.

MATERIALS AND METHODS

Insects

The insects were collected from elm trees leaves in Golestan provinces of Iran and reared on leaves of *Ulmus densa* Litw. Same-aged larvae (24 h after molting) and adult females were randomly selected for the measuring of enzyme activity.

Chemicals

Triton X-100, bovine serum albumin, 3, 5-Dinitrosalicylic acid (DNS), Starch were purchased from Merck (Merck, Darmstadt, Germany).

P-nitrophenyl-α-D-glucopyranoside (pNαG), p-nitrophenyl-ß-D-glucopyranoside (pNßG), p-nitrophenyl-α-D-galactopyranoside (pNαGa) p-nitrophenyl-ß-D-galactopyranoside (pNßGa), 4-methylumbelliferyl-ß-D-glucopyranoside (4-MUG) and 4-methylumbelliferyl-ß-D-galactopyranoside (4-MUGa) were obtained from Sigma (Sigma, St Louis, MO, USA). P-nitrophenyl acetate (p-NA) was bought from Fluka (Buchs, Switzerland) and DEAE Cellulose obtained from Bio-Rad Laboratories Ltd. (UK).

Sample preparation and enzyme assays

Larvae and adults were immobilized on ice and dissected under a stereo microscope in ice-cold saline buffer. Digestive systems were removed and their content was eliminated. The samples were transferred to a freezer (-20 °C). For measuring of enzyme activity, the sample was homogenized in cold double-distilled water using a hand-held glass homogenizer and centrifuged at 10,000 rpm for 10 min at 4°C. After homogenization they were centrifuged at 10,000 rpm for 15 min at 4°C.

Determination of α-amylase activity and its kinetic parameters

α-amylase activity was determined at room temperature in 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 for 30 min. The concentration of reducing sugars obtained from the catalyzed reaction was measured by the dinitrosalicylic acid method according to Bernfeld (1955). Absorbance was measured at 545 nm with a Microplate Reader Model Stat Fax® 3200 (Awareness Technology Inc.). The pH profiles of the α-amylases were determined at room temperature in a mixed buffer containing phosphate, glycine and acetate (40 mM of each) adjusted to various pHs (pH 3 to 12) by adding HCl or NaOH for acidic and basic pH values, respectively (Asadi et al., 2010).

Catalytic activities of the enzymes were investigated at different concentrations of starch and glycogen over the range 0.05-1% (w/v), in 40 mM phosphate, glycine and acetate buffer, pH 5.0. The Michaelis-Menten constant (Km) and maximal velocity ($Vmax$) were estimated from the Lineweaver-Burk plots. The kinetic values are the averages of three experiments and standard errors are less than 10%.

Determination of α- and β-glucosidase and α- and β-galactosidase activities

The activities of α- and β-glucosidases and α- and β-galactosidase were measured with pNαG, pNβG, pNαGa and pNβGa as substrates, respectively. Homogenates were incubated for 30 min at 37°C with 45 μL of substrate (25 mM) and 115 μL of 40 mM phosphate-acetic-citric buffer. The reaction was stopped by addition of 600 μL of NaOH (0.25 M). Optical density was measured at 405 nm using a microplate reader (Stat Fax 3200, Awareness Technology, USA) after 10 min. Controls without enzymes or without substrates were included. A standard curve of absorbance against the amount of p-nitrophenol released was constructed to enable calculation of the amount of p-nitrophenol released during the α- and β-glucosidase and α- and β-galactosidase assays.

Determination of pH optimum and effect of temperature on α- and β-glucosidase and α- and β-galactosidase activities

The activity of α- and β-glucosidases and α- and β-galactosidases was determined at several pH values using 40 mM phosphate-acetic-citric buffer. The effect of temperature on α- and β-glucosidase and α- and β-galactosidase activities were measured using the homogenate adult by incubating the reaction mixture at 20, 30, 40, 50, 60 and 70°C for 30 min, followed by measurement of activity.

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 with 100 V at 4°C. Afterwards, the gel was incubated in 2.5% (v/v) Triton X-100 for 30 min at room temperature with gentle agitation. Then, the gel was rinsed with deionized water and washed in 25 mM of Tris-HCl pH 7.4. The washed gel was incubated in fresh buffer containing 1% (w/v) soluble starch at 30ºC for 60 min. After being washed with distilled water, the gel was subjected to staining with Lugol solution (I2 1.3% and KI 3%) at an ambient temperature until the appearance of clear zones in protein bands with α-amylase activity against a dark blue background.

For zymogram analysis of β-glucosidase and 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. Afterwards, the gel was immersed in 3 mM 4-MUG and 4-MUGa in 0.1 M sodium 10 acetate (pH 5.5) for 10 min at room temperature to develop bands showing ß-glucosidase and ß-galactosidase activities, respectively. The blue-fluorescent bands appear in a few minutes under UV.

Purification of P. vulgaris α-amylase inhibitor from seeds

Seeds were ground to a powder and extracted with 0.15 M NaCl with continuous stirring for 1 h at 4°C. The material was then centrifuged at 6,000 ×g at 4°C for 30 min. The supernatant was heated to 80°C and centrifuged at 6,000 ×g for 15 min. The supernatant was fractionated with ammonium sulfate. Following dialysis, the fraction was applied to an ion-exchange DEAE cellulose column equilibrated with 20 mM Tris-HCl buffer, pH 7.0, with a flow rate of 0.5 ml/min. The column was eluted with a linear NaCl gradient of 0-0.5 M at the flow rate of 0.5 ml/min. The absorbance of the effluent was monitored at 280 nm.

Amylase inhibition assay

10 μl enzyme was pre-incubated with 10 μl inhibitor and 30 μl buffer (pH 5) for 30 min at 37°C; then the same procedure for the amylase assay was performed, and amylase activity was determined by measuring absorbance at 540 nm. Experiments were performed in triplicate.

Statistical analysis

The data were compared by one-way analysis of variance (ANOVA) followed by Tukey's test when significant differences were found at P = 0.05 using SAS program (SAS, 1997).

RESULTS

Alpha-amylase activity and effect of pH on its activity

The activity of α-amylases was assessed in crude extracts. The data revealed that α-amylase is present in the digestive system of larvae and adult females of *X. luteola*. The specific activity of α-amylase in the digestive system of last larval instar was 1.46-fold higher than that of the adult female (Table 1). Also, the amylase activity in the midgut of the last larval instar was 3.125- and 4.16-fold higher than that in the foregut and hindgut, respectively (Fig. 1). Results showed that the optimal pH for α-amylase in the digestive system was 5 (Fig. 2).

Kinetic parameters of α-amylase

α-amylases revealed a Michaelis-Menten type kinetics when hydrolyzing soluble starch and glycogen at

Table 1. The specific activities (nmol/min/mg protein) of digestive carbohydrases in adult and larvae of *X. luteola*

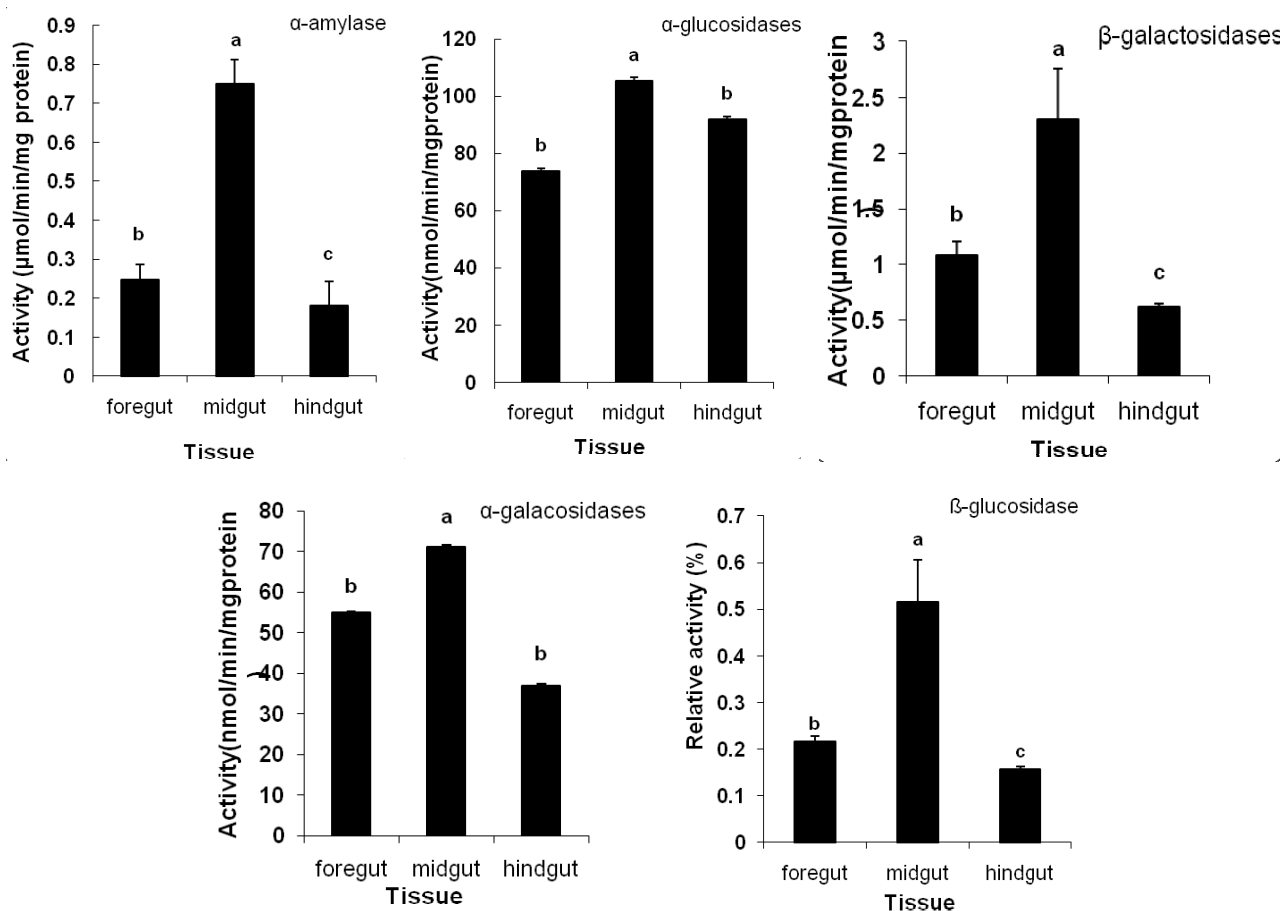| Enzymes | Stage | |
	Last larval instar (Mean±SE)	Adult (Mean±SE)
α-glucosidases	146.09±2.345	451.56±1.03
ß-glucosidases	304.73±0.12	495.93±0.17
α-galactosidases	43.20±0.15	61.45±0.3
β-galactosidases	515.28±0.17	115.10±0.07
α-amylase	72.96± 0.07	49.86± 0.05

Fig. 1. Comparison of the activities of α-amylase, α- and ß-glucosidases and α- and β-galactosidases extracted from different parts of digestive system of *X. luteola*. Different letters indicate that the activity of enzymes in different tissue is significantly different from each other by Tukey's test (p < 0.05).

their optimum pH. As calculated from Lineweaver-Burk plots, the K_m and V_{max} values for soluble starch and glycogen at 37 ºC are presented in Table 2.

α- and β-glucosidase and α- and β-galactosidase activities

The specific activity of α-glucosidase in the digestive system of the adult was 3.08-fold higher than that in the last larval instar, whereas the β-glucosidase activity in the digestive system of the adult female was 1.62-fold higher than its activity in the digestive system of the last larval instar (Table 1). Also, the α-glucosidase activity in the foregut, midgut and hindgut of the last larval instar were 73.9±0.76,

105.2± 1.2 and 91.9± 0.84 nmol/min/mg proteins, respectively. Also, the β-glucosidase activity in midgut was higher than that in foregut and hindgut of last larval instar (Fig. 1).

The specific activity of α-galactosidase in the digestive system of adult female and last larval instar was determined. The obtained results show that the specific activity of α-galactosidase in the digestive system of the adult female was 1.41-fold higher that in the last larval instar, whereas the activity of β-galactosidase in the digestive system of larvae was higher than its activity in the adult female digestive system (Table 1). The α-galactosidase activity in the midgut was higher than in the foregut and hindgut of
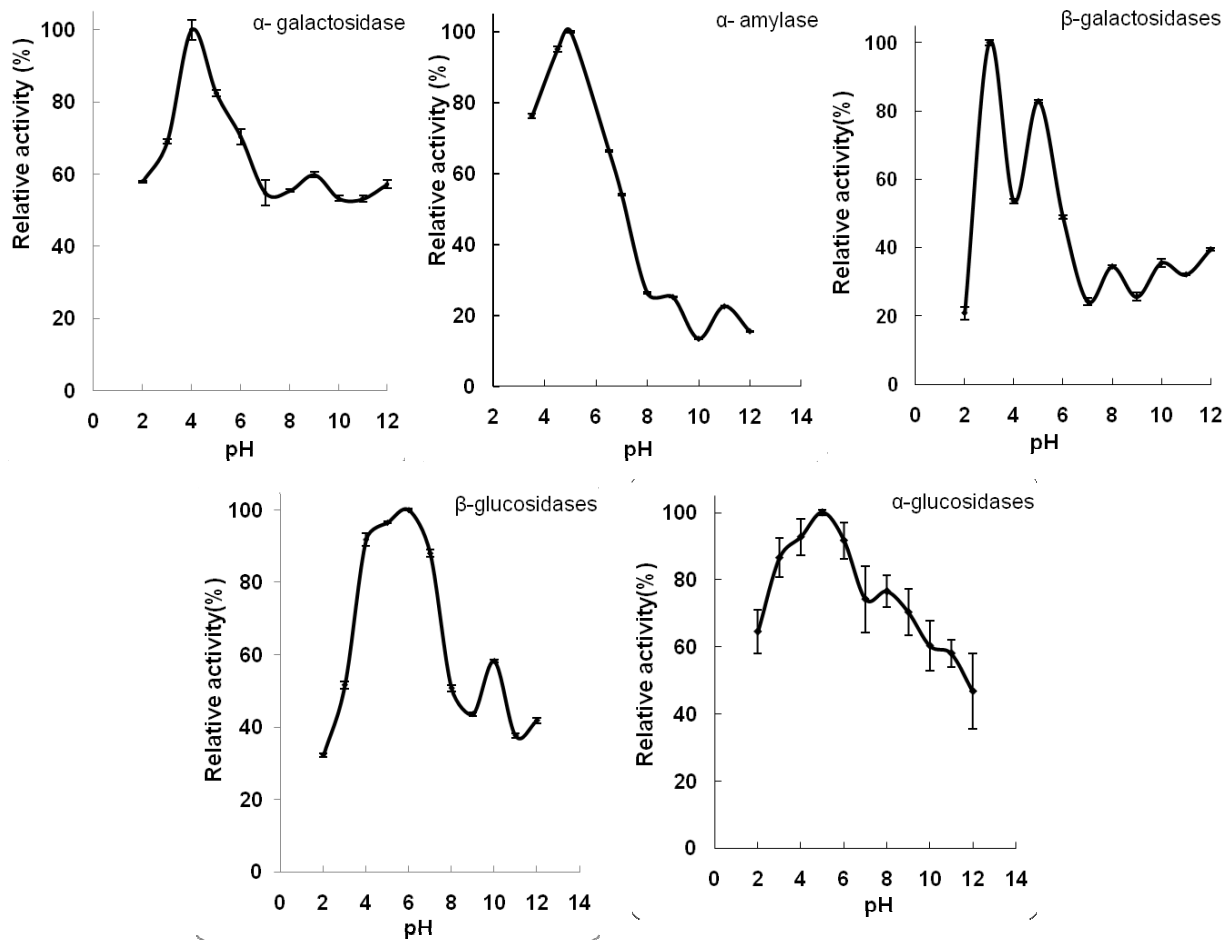
Fig. 2. The effect of pH on the activities of α- and ß-glucosidases, α- and ß-galactosidases and α-amylase extracted from the digestive system of *X. luteola*.

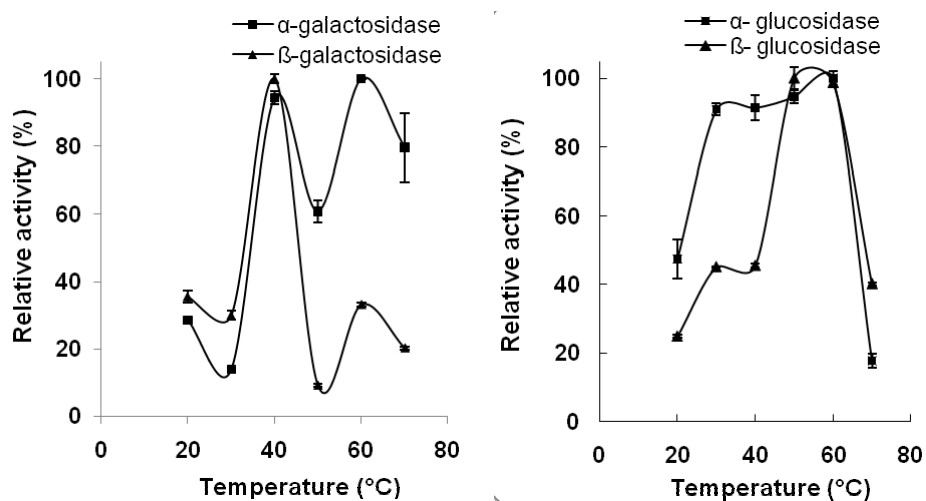**Fig. 3.** The effect of temperature on the activities of α- and ß-glucosidases and α- and ß-galactosidases extracted from the digestive system of *X. luteola*
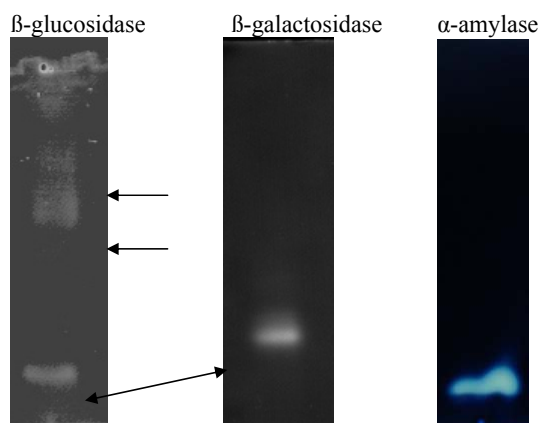
Fig. 4. Zymogram of ß-glucosidase, ß-galactosidase, α-amylase and esterase found in the digestive system of last larval instar of *X. luteola.*

the last larval instar. Also, the β-galactosidase activity in the midgut was 2.12-fold and 3.71-fold higher than its activity in the foregut and hindgut of the last larval instar, respectively (Fig. 1).

Effect of pH and temperature on α- and β-glucosidase and α- and β-galactosidase activities

The effect of pH on the hydrolytic activity towards pNαG, pNßG, pNαGa and pNßGa was tested using 40 mM phosphate-acetic-citric buffer (pH 2–12). Maximum activity in the digestive system was observed at pH 5 and 4 for α-glucosidase and α-galactosidase, respectively, whereas, the optimal pH for ß-glucosidase and ß-galactosidase activity were 6 and 3, respectively (Fig. 2). The *X. luteola* α- and ß-glucosidase has an optimum temperature activity at 60 and 50°C, respectively. Also, the optimal temperature for α-galactosidase in the digestive system was 40 and 60°C (Fig. 3).

Zymogram analysis

The crude extracts of *X. luteola* were analyzed by native PAGE. After amylase activity staining, one major isoform of α-amylase was clearly detected. Also, the zymogram pattern in the native gel revealed that *X. luteola* β-glucosidase and β-galactosidase in the digestive system had three and one isoform, respectively (Fig. 4).
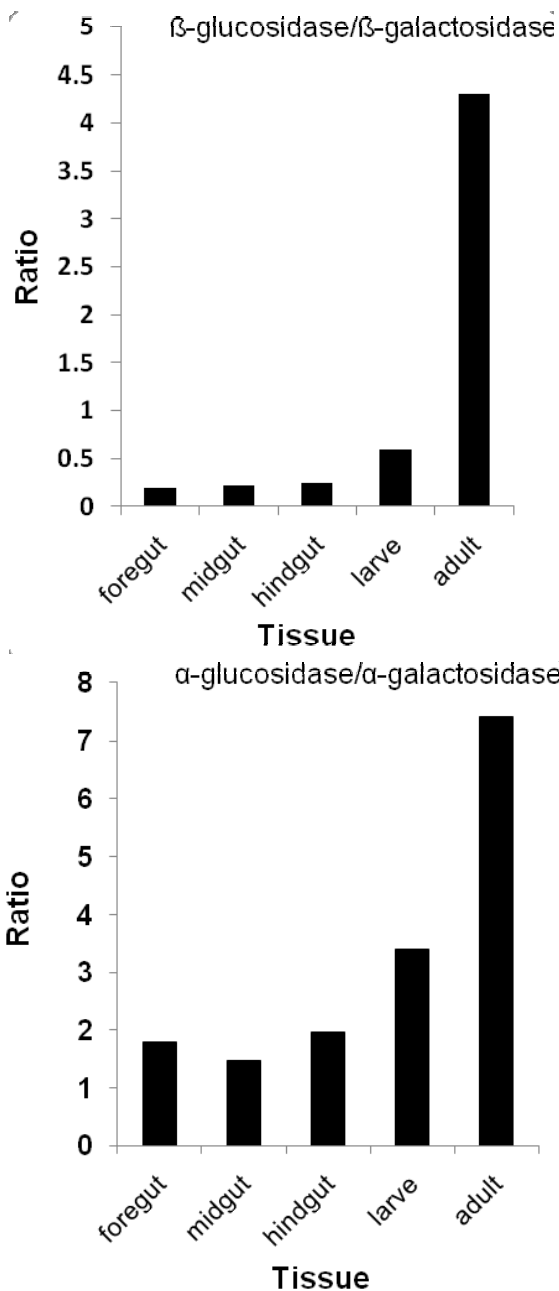

Fig. 5. The ratios of ß-glucosidases/ß-galactosidase and α-glucosidase/α-galactosidase in the digestive system of *X. luteola.*

Effects of P. vulgaris inhibitors on X. luteola amylase activity

The ammonium sulfate fraction was further fractionated on an ion-exchange DEAE cellulose column.
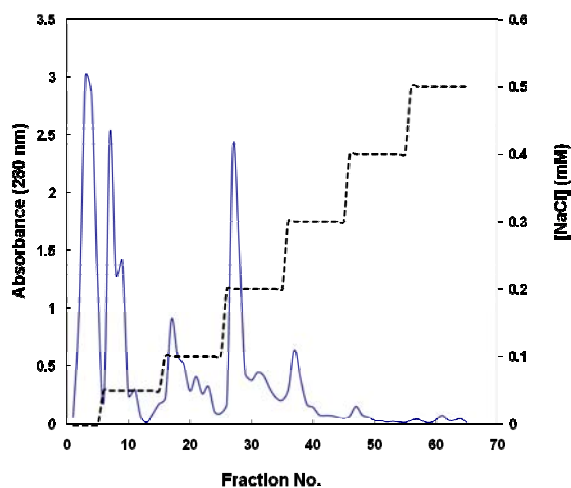
Fig. 6. Retained fraction obtained after ion-exchange DEAE cellulose chromatography, equilibrated with 20 mM Tris-HCl buffer, pH 7.0, with a flow rate of 0.5 ml/min. Dashed line represents a 0-0.5 M NaCl linear gradient.

Table 2. Kinetic parameters of α-amylases from digestive system of *X. luteola* on starch and glycogen

substrate	K_m (mg/ml)	V_{max} (µmol/min/mg protein)	K_m/ V_{max}
Starch	1.34	1.52	0.88
Glycogen	0.64	0.515	1.24

Table 3. Inhibition of α-amylase by inhibitors extracted from *P. vulgaris*

Fraction No.	Mean inhibition (%)
2	0
3	0
4	0
5	0
6	0
7	0
8	0
16	0
17	0
26	35.6
27	66.2
28	72.4
36	31.2
37	36.9
38	30.5

The obtained profile showed three major peaks and three minor peaks (Fig. 6). Assay of peaks (Table 3) revealed that the two peaks of *P. vulgaris* strongly inhibited the *X. luteola* gut α-amylase while the others did not. The peak numbers 27 and 28 had the highest inhibitory effect on α-amylase activity.

DISCUSSION

Our data present evidence that α-amylase is present in the digestive system of the adult and last larval instar of *X. luteola*. The specific activity of α-amylases from the digestive system of larvae was 1.46-fold higher than that of the adult female (Table 1). Our results showed that there is a significant difference in the activity of α-amylases in the foregut, midgut and hindgut of the last larval instar (Fig. 1). The optimum pH activity of *X. luteola* larval amylase was 5 (Fig. 2). α-amylases in the insect are generally most active in a neutral to slightly acidic pH condition (Baker, 1983; Terra et al., 1996). Optimal pH values for amylases in larvae of several coleopterans were 4-5.8 (Baker, 1983). Also, in other non coleopteran insects, the optimum pH were 6.5 in *Lygus hesperus* Knight and *Lygus lineolaris* (Palisot de Beauvois), 6 in *Erinnyis ello* L. (Lepidoptera: Sphingidae) (Terra et al., 1996) and 5 in *Brachynema germari* Kolenati (Hemiptera: Pentatomidae) (Ramzi and Hosseininaveh, 2010).

The activity of α-amylase was also characterized by zymogram analysis after native PAGE which allowed visualization of the enzyme activity *in situ*. The results indicated one α-amylase isoform in the crude digestive system of last larval instar (Fig. 4). α-amylases from the digestive system of *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae) (Kazzazi et al., 2005) and *B. germari* (Ramzi and Hosseininaveh, 2010) showed one isoform. Wisessing et al., (2008) showed that *Callosobruchus maculates* α-amylase had one isoform with a molecular weight of 50 kDa. However, the number of α-amylases identified in different insect species varied from 1 to 8 isoforms, e.g. *Helicoverpa armigera* (Hubner), *Spodoptera litura* (F.), *C. chinensis* and *Corcyra cephalonica* (Stainton) exhibited more than five isoforms where-

as *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) possessed only one isoform (Siva-Kumar et al., 2006). Also, the different forms of α-amylases in insect midgut lumen have been observed in *C. maculatus* and *Zabrotes subfasciatus* (Bohemann) and *Tenebrio molitor* L. (Coleoptera Tenebrionidae) (Campos et al., 1989; Silva et al., 1999). Also, two forms of α-amylase isoforms was reported in the crude midgut, salivary glands and hemolymph of *Naranga aenescens* L. (Lepidoptera Noctuidae) (Asadi et al., 2010).

The kinetic behavior of *X. luteola* α-amylase towards starch and glycogen was significantly different. The affinity of *X. luteola* α-amylase toward glycogen was higher than towards starch. *Km* values calculated for the hemolymph α-amylase of *Mamestra brassicae* L. and silkworm were 0.33 and 0.57 mg/ml, respectively (Tanabe and Kusano, 1984; Matsumura, 1934). Also, Ramzi and Hosseininaveh (2010) showed that the *Km* values of α-amylase in the midgut and salivary glands of *B. germari* were 0.77 and 0.41 mM, respectively.

The present study clearly shows that the larvae and adult female of *X. luteola* possess α- and β-glucosidase and α- and β-galactosidase activities in the digestive system. Comparing the activities against the p-nitrophenyl glycosides of glucose and galactose, the ratios of ß-glucosidase/ß-galactosidase were as 0.59 and 4.3 for larvae and adult females of *X. luteola*, respectively, when activities were determined in whole digestive system (Fig. 5), whereas the ratio of α-glucosidase/α-galactosidase was as 3.38 and 7.3 for larvae and adult female of *X. luteola*, respectively (Fig. 5). These results show that the ß-galactosidase and α-glucosidase activities in the digestive system of larvae are greater than the ß-glucosidase and α-galactosidase activities, whereas the activities of the glucosidases were higher than the galactosidase activities in the adult stage. Feeding is usually intensified at the last larval instar for saving energy as nutrient macromolecules (carbohydrates, protein and lipid). Also, the oviposition usually occurs during high energy demand consequently leading to high

metabolic rates in adults compared with larvae. Therefore, high feeding in larvae and high energy demand in adults may explain these differences in the ratios of α-glucosidase/α-galactosidase and ß-glucosidase/ß-galactosidase in the adult and larvae. The ratios of ß-glucosidase/ß-galactosidase was reported as 88.5 in *Rhynchosciara americana* Wiedemann (Diptera: Sciaridae) (Terra et al., 1979), 105 in *Stomoxys calcitrans* (Deloach and Spotes, 1984), 58 in the midgut tissue of *Rhodnius prolixus* (Terra et al., 1988), and 2.5 in *C. maculates* (Gatehouse et al., 1985). Also, In *Dysdercus peruvianus*, the ß-glucosidase/ß-galactosidase ratio in the midgut tissue is 28.7, but in the whole midgut (epithelium plus luminal contents) the ratio is 3.0, suggesting a major contribution of ß-galactosidase activity by the seed meal present in gut lumen (Silva and Terra, 1997). The ratios of ß-glucosidase/ß-galactosidase found in the adult of *X. luteola* are of the same order as that found in *C. maculates* (Gatehouse et al., 1985) and *D. peruvianus* (Silva and Terra, 1997). It seems the ratios of ß-glucosidase/ß-galactosidase in *X. luteola* and *C. maculates* were lower than *R. americana, S. calcitrans* and *R. prolixus*. Ferreira et al. (1998) reported that high ß-glucosidase activity is found in the foliage feeder, *Abracris flavolineata* De Geer (Orthoptera: Acrididae), in the stored plant product feeder *T. molitor* Linnaeus (Coleoptera: Tenebrionidae), and in the pollen-feeder *Scaptotrigona bipunctata* Lepeletier (Hymenoptera: Apidae). Also, our results showed that the ß-glucosidase activity in adults was higher than that of α- and ß-galactosidase and α-glucosidase activities. In contrast, low ß-glucosidase activity is found among predaceous insects exemplified by *Pheropsophus aequinoctialis* Linnaeus (Coleoptera: Carabidae) and *Pyrearinus termitilluminans* Costa (Coleoptera: Elateridae) (Ferreira et al., 1998). Also, the results of Ferreira et al. (1998) showed that high ß-glucosidase activity could be associated with feeding on plants or plant products; other data disagree with this suggestion. β-glucosidase activity is low in the decaying plant-feeder *R. americana* and in feeders of plant aerial parts, exemplified by *S. frugiperda*, *E. ello* and *Diatraea saccharalis* (Fabricius) (Ferreira et al., 1998).

Our results showed that α-galactosidase activity is relatively low in the digestive system of larvae and adult females. The lowest and highest activity in the digestive system related to α-galactosidase and β-galactosidase, respectively.

The α- and β-glucosidase and α- and β-galactosidase activities was determined at different temperatures ranging from 20 to 80°C. The *X. luteola* α- and ß-glucosidase has an optimum temperature activity at 60 and 50°C, respectively. Also, the optimal temperature for α- and ß-galactosidase in the digestive system was 60 and 40°C, respectively (Fig. 3), which is consistent with the α- and ß-glucosidase activities in *G. pyloalis* (45°C) (Ghadamyari et al., 2010). Ramzi and Hosseininaveh, 2010, showed that α- and ß-galactosidase of *B. germari* has an optimal activity in 30°C and 35°C for midgut and salivary glands, respectively. Biological reactions are catalyzed by proteins – enzymes, and each enzyme has a temperature above which its three dimensional structure is disrupted by heat. Therefore, biological reactions occur faster with increasing temperature up to the point of enzyme denaturation, above which enzyme activity and the rate of the reaction decrease sharply (Agblor et al., 1994; Applebaum).

The zymogram pattern in the native gel revealed that *X. luteola* β-glucosidase and β-galactosidase in the digestive system had three and one isoform, respectively (Fig. 4). In other coleopteran insects, the ß-glucosidase in the digestive system of *Rhynchophorus ferrugineus* (Olivier) and *Osphranteria coerulescens* Redt. has four isoforms. Also, β-galactosidase in the digestive system of *R. ferrugineus* and *O. coerulescens has one and six isoforms, respectively* (unpublished data).

Our results showed that the activities of α-amylase in the midgut of the elm leaf beetle were 3.125- and 4.16-fold higher than that in the foregut and hindgut, respectively (Fig. 1). Also, high activities of α- and ß-glucosidases and α- and β-galactosidase were observed in the midgut, with completion in other parts of digestive system, of the last larval instar. These data suggested that the midgut is the secreting site of carbohdrases in *X. luteola*. In *Odontotermes formosanus* and *Coptotermes formosanus*, the activities of ß-glucosidase in the midgut were higher than those in the foregut and hindgut. This indicates that the midgut of these two termites also has the function of cellulose secretion (Mo et al., 2004). In fact, the midgut of *C. formosanus* could secrete endogenous cellulose (Nakashima et al., 2002).

Amylase, glucosidases and galactosidases play an important role in insect digestion. These enzymes are important in the initial and final phases of food digestion of *X. luteola*. Insect-resistant crops have been one of the major successes of applying plant genetic engineering technology to agriculture. The secondary metabolites in plants can act as protective agents against insects either by repellence or through direct toxicity. Many different types of secondary metabolites, including alkaloids, terpenes, steroids, iridoid glycosides, aliphatic molecules, phenolics (Hsiao, 1985) and others, have been demonstrated to confer resistance to different plant species against insects. Among them, carbohydrase inhibitors seem to play an important role in host plant resistance to insects. Study of the carbohydrates in herbivorous insects is important not only for understanding digestion biochemistry but also for developing insect pest management strategies. Our results showed that α-amylase inhibitors that were purified from *P. vulgaris* by ion-exchange DEAE cellulose chromatography exhibited good inhibitory activity on *X. luteola* gut α-amylase. Peaks 27 and 28 inhibited amylase activity by 66.2 and 72.4%, respectively. Previous research showed that α-amylase inhibitors from *P. vulgaris* seeds are detrimental to the development of the cowpea weevil *C. maculatus* and Azuki bean weevil *C. chinensis* (Ishimoto and Kitamura, 1989; Shade et al.*, 1994)*. It would seem that *P. vulgaris* seeds are promising sources of amylase inhibitor genes for the production of elm trees with a resistance to *X. luteola*. However, additional studies are needed to further investigate this possibility.

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