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# Biochemical characterization of digestive α-amylase, α-glucosidase and β-glucosidase in pistachio green stink bug, *Brachynema germari* Kolenati (Hemiptera: Pentatomidae)

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ABSTRACT

The pistachio green stink bug, *Brachynema germari*, has 3–5 generations per year and causes severe damages to pistachio crops in Iran. Physiological digestive processes, such as digestive carbohydrases, can be used to design new strategies in IPM programs for controlling this pest. The enzyme α-amylase digests starch during the initial stage of digestion. Complete breakdown of carbohydrates takes place in the midgut where α- and β-glucosidic activities are highest. Alpha-amylase and α- and β-glucosidase activities were found in the midgut and salivary glands of pistachio green stink bug adults. Overall enzyme activities were significantly higher in the midgut than in salivary glands. The highest α-amylase and α- and β-glucosidase activities were in section v3, whereas the lowest activities were in section v4. $V_{max}$ was higher and $K_m$ was lower in the midgut than in the salivary glands for these enzymes. In the pistachio green stink bug, the optimal pH was pH 5–6.5 and the optimal temperature was 30 °C to 35 °C for these enzymes. Alpha-amylase activity in the midgut and salivary glands decreased as the concentrations of $MgCl_2$, EDTA and SDS increased. Enzyme activities in both midgut and salivary glands increased in the presence of NaCl, $CaCl_2$, and KCl. NaCl had a negative effect on alpha-amylase extracted from salivary glands.

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## Introduction

The pistachio green stink bug, *Brachynema germari* (Kolenati) (Hemiptera: Pentatomidae) has 3–5 generations per year and causes severe damage to pistachio crops. It feeds on pistachios causing epicarp lesions and the falling of fruits. Chemical and cultural controls are used to decrease its density as part of integrated pest management programs.

Most insects, including *B. germari*, require carbohydrates (amylases and glucosidases) in their diet. The α-amylases are common hydrolytic enzymes that catalyze the hydrolysis of α-D-(1, 4) glucan linkages in glycogen, starch and other related carbohydrates (Strobl et al., 1998; Franco et al., 2000). Alpha-glucosidase catalyzes the hydrolysis of terminal, non-reducing 1,4-linked alpha-D-glucose residues and releases α-D-glucose. This enzyme hydrolyzes several substrates, including sucrose, maltose, maltodextrin and *p*NP-α-D-glucopyranoside, and it is found in insect digestive systems (Terra et al., 1996). Beta-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 (triggering agent of plant defense mechanisms) in plants when they are encountered with the feeding damage of the insect pests (Mattiacci et al., 1995).

Carbohydrate digestion is critical to growth and reproduction in the pistachio green stink bug. Making any interference in enzymatic carbohydrate digestion can deprive the insect from utilizing the sources of energy efficiently. Therefore, knowledge of its digestive enzymes could be crucial in designing new control methods based on inhibition of digestive enzymes. Inhibitors of insect digestive enzymes have been used to control insect pests, especially using resistant varieties bearing the genes encoding the inhibitors or increased amount of the inhibitors (Ghoshal et al., 2001). In addition to their role in digestion, glucosidases have an important role in plant–herbivore co-evolution because of their importance in the breakdown of secondary metabolites of plants (Hemmingi and Lindroth, 2000). It can be important for understanding antibiosis mechanisms of plant defenses against the insects and promoting potential of plant defenses. Therefore, the aim of this study was to characterize α-amylase and α- and β-glucosidases of *B. germari* to better understand the digestive physiology of the insect. This understanding may lead to useful and successful management strategies in insect pest management programs.

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## Material and methods

### *Insects*

Adult *B. germari* were collected from pistachio orchards located in Kerman province (southern Iran) and reared on pistachio nuts in the laboratory at 25 ± 1 °C under a 14/10 (L/D) h photoperiod.

### *Insect dissection and enzyme preparation*

Enzyme samples from adult midguts and salivary glands were prepared using the method of Cohen (1993). Salivary glands and midguts were removed from randomly chosen individuals by dissection in distilled water. Midguts were divided into four distinct divisions (v1, v2, v3 and v4) (Fig. 1). Samples were then placed in a pre-cooled homogenizer and ground in distilled water. The homogenates were centrifuged at 15,000 × *g* for 20 min at 4 °C. The supernatants were stored at −20 °C.

### *Alpha-amylase activity assay*

Alpha-amylase activity was assayed using dinitrosalicylic acid (DNS) (Bernfeld, 1955) with 1% soluble starch as the substrate. Ten microliters of the enzyme extract was incubated for 30 min at 35 °C with 500 µl universal buffer and 40 µl soluble starch. The reaction was stopped with the addition of 100 µl DNS and heating in boiling water for 10 min. DNS is a color reagent which reacts with the reducing groups released from starch by α-amylase action. The boiling water stops the α-amylase activity and catalyzes the reaction between DNS and the reducing groups of sugars. Absorbance was then measured at 540 nm. One unit of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35 °C. A blank without substrate but with α-amylase extract and a control containing no α-amylase extract but with substrate were measured at the same time as the reaction mixtures. All assays were performed in triplicate.

### *Alpha- and β-glucosidase activities*

Alpha- and β-glucosidase activities were assayed by incubating 50 µl of enzyme extract with 75 µl of *p*-nitrophenyl-α-D-glucopyranoside (5 mM), *p*-nitrophenyl-β-D-glucopyranoside (5 mM) and 125 µl of 100 mM universal buffer (pH 5.0) at 37 °C for 10 min. Released *p*-nitrophenol was recorded as the absorbance at 405 nm after the addition of NaOH to the reaction mixture.

### *Effect of pH and temperature on enzyme activity*

The effects of temperature and pH on activity of midgut and salivary gland α-amylase were assayed. Incubation of the reaction mixture was done at a range of temperatures (15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 37 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C and 70 °C) for 30 min. The optimum pH

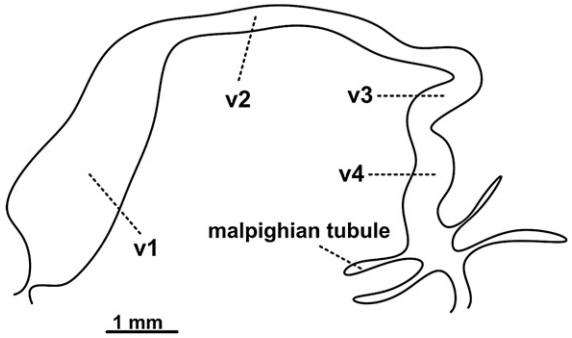


**Fig. 1.** Schematic drawing of the different parts (v1, v2, v3 and v4) of the midgut in the adult *B. germari*.

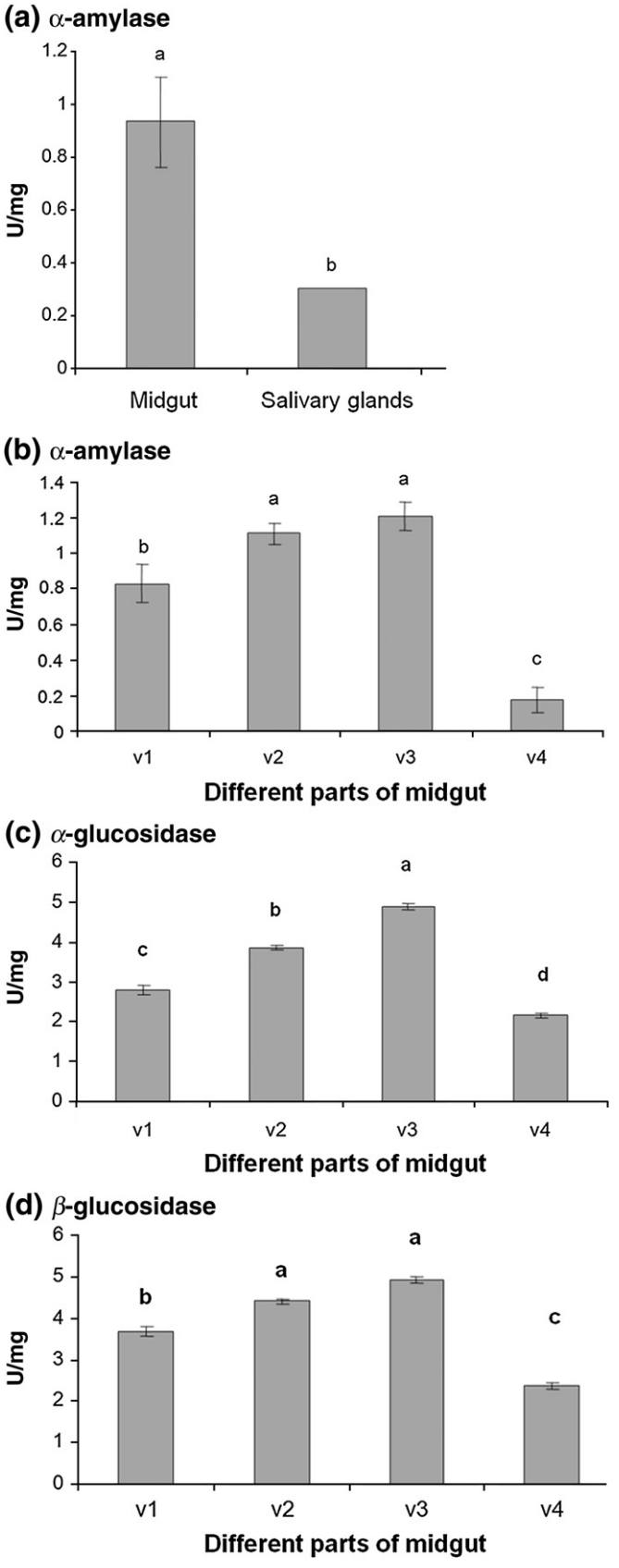


**Fig. 2.** Activity level of α- amylase (a, b) and α- and β-glucosidases (c, d) in the whole midgut (a), salivary glands (a) and different parts of the midgut (v1, v2, v3 and v4) (b, c, d) of adult *B. germari*. Different letters indicate that enzyme activities are significantly different from each other ($p<0.05$).

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