

*Original Contribution***EFFECTS OF OLIVE VARIETIES ON α - AND β -GLUCOSIDASE ACTIVITIES IN THE LARVAE OF *BACTEROCERA OLEAE* GMELIN (DIPTERA: TEPHRITIDAE)****S. Delkash-Roudsari¹, A. Zibae^{1*}, M. R. Abbc-Mozddehi²**¹Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran²Agricultural and Natural Resources Center, Rasht, Iran**ABSTRACT**

The olive fruit fly, *Bacterocera oleae* Gmelin (Diptera: Tephritidae) is a serious pest of olive around the world. In the current research, digestive glucosidases were studied in the alimentary canal of the last larval instar. The specific activity of α - and β -glucosidases were 0.077 and 0.023 U/mg protein in soluble fraction. Results showed that optimal activities of α - and β -glucosidases were at pHs 5-6 and temperatures of 45°C and 25 °C for α - and β -glucosidases, respectively. Ca^{2+} , Cu^{2+} , Fe^{2+} , Na^+ and Zn^{2+} significantly increased activity of α -glucosidase but K^+ and Mg^{2+} had the opposite effects. Meanwhile, Cu^{2+} , K^+ and Na^+ increased β -glucosidase activity and other ions decreased it in the gut of *B. oleae*. Among used specific inhibitors, Phanthroline and DTC significantly decreased activity of α -glucosidases but EGTA and EDTA decreased β -glucosidase activity. Other compounds had no statistical effects on enzymatic activity. Kinetic parameters of α - and β -glucosidases showed a K_m of 4.26 and 0.84 (mM), respectively. Also maximal velocities (V_{max}) were found to be 0.21 and 0.11 U/mg protein. The highest activities of α - and β -glucosidase was found in Frangivento and Fishomi varieties but the enzymatic activities significantly decreased in the larvae fed on Amigdalifolia, Zard and Conservalia varieties. Understanding of digestive enzyme properties, particularly the effect of inhibitors on enzymatic activity and physiology of insects, could be useful to reach safe and useful controls of insect pests before achieving to resistant varieties.

Key words: *Bacterocera oleae*, α -glucosidase, β -glucosidase, different varieties**INTRODUCTION**

The olive fruit fly *Bacterocera oleae* Gmelin (Diptera: Tephritidae) is a serious pest of olive in Africa, Southern Europe, India, western Asia and California (1, 2). The pest has been introduced to north of Iran since 2004 and to date it causes severe damages to olive production (3). Larvae feed on olive fruits and cause loss of the olive crop by tunneling and feasibility of pathogen entrances (1). Management of olive fruit fly rely on insecticides in bait sprays, trapping of adult flies, pheromone and biological control (4).

Polysaccharides like starch in plant cells and glycogen in animal tissues have important roles

in physiological processes so their utilization is critical for insects. Digestion of carbohydrates is initiated by action of amylases prior to glucosidases (5). Insect's glucosidases are recruited to hydrolyze disaccharides and oligosaccharides extracted from hemicelluloses and cellulose (6). α -Glucosidase (EC 3.2.1.20) catalyzes the hydrolysis of 1, 4- α -glucosidic linkages lead to releasing α -glucose. Also it hydrolyzes sucrose, maltose, maltodextrin and pNP- α -Dglucopyranoside (5) The enzyme can be found in alimentary canal, salivary secretions of insects (7) and hypopharyngeal glands of some insects (8, 9). α -Glucosidase has been isolated and characterized in some insects such as *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae) (10), *Sitophilus zeamais* (Coleoptera: Curculionidae) (11), *Apis mellifera* (Hymenoptera: Apidae) (12) and *Chilo suppressalis* Walker (Lepidoptera: Pyralidae)

*Correspondence to: Arash Zibae Ph. D, Assistant Professor of Insect Physiology, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran, 416351314. Phone: +98-0131-6690485, Fax: +96-0131-6690281

(13) and it has shown properties such as molecular masses of 60–80 kDa, pH optima of 5–6.5 and pI values of range 5.0–7.2. The enzyme has been sequenced in dipterans showing the invariant residues: Asp 123, His 128, Asp 206, Arg 221, Glu 271, His 296, and Asp 297 (14). These residues are the same as the active site of α -amylase family, and the three residues Gly 69, Pro 77, and Gly 323 have the structural role for some α -glucosidases (15). β -Glucosidase (*EC 3.2.1.21*; beta-D glucoside glucohydrolase) hydrolyzes β 1, 4- linkages, releasing β -D-glucose (9). Biochemical properties of insect β -glucosidases have been reported as molecular masses of 30–150 kDa, pH optima of 4.5–6.5, and pI values of 3.7–6.8. The enzyme has been isolated from midgut cells of *Rhynchosciara Americana* larvae (16), *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae (17), *Rhynchophorus palmarum* larvae (18) termites *Macrotermes mulleri* (19), *Macrotermes bellicosus* (20), *Macrotermes subhyalinus* (21), honey bees (22) and *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) (13). Currently, β -glucosidase of *Spodoptera frugiperda* Hubner (Lepidoptera: Noctuidae) and *T. molitor* L. (Coleoptera: Tenebrionidae) have been sequenced (17, 23). The enzyme has two glutamic acid residues responsible for catalysis and a structure of (b/a)₈-barrel. pK_as of the nucleophile (Glu 399) and proton donor (Glu 187) in *S. frugiperda* are 4.9 and 7.5, respectively. In addition, it has been found that residue Glu 451 is a key residue to determine the enzymatic preference for glucosides versus galactosides (17, 23).

Because control of *B. oleae* is problematic due to activity of larvae in inner parts of the fruits, it can be a great model to design and develop physiological control approached like using genes encoding toxic molecules and inhibitors. Insect digestive enzymes have various properties and they could be used to design new strategies for insect control. In case, a comprehensive study has been initiated by the authors to complete understanding of digestive enzyme properties in larvae of *B. oleae*. In the first step, digestive α -amylase of larvae were characterized and its inhibition was determined toward proteinaceous inhibitor (24). In this study, we attempted to biochemically characterization of α -glucosidase and β -glucosidase from larvae of *B. oleae* via measuring pH, temperature, effects of ions and inhibitors as well as kinetic parameters. Also, possible changes of the enzymatic

activities were assessed followed by larval feeding on the twelve varieties of olive.

MATERIAL AND METHODS

Insect rearing

For glucosidase characterization, larvae were reared on the olive fruits of Arbequina variety in containers of 20×12 cm. Laboratory conditions were 25±1 °C, 70 % of RH and 16L:8D. Rearing containers were daily checked and cleaned to remove any contaminations. To find effects of host plants, containers with fruits of twelve varieties (*Amigdalifolia*, *Manzallina*, *Arbequina*, *Conservalia*, *Kalamata*, *Balidi*, *Zard*, *Roghani*, *Shengher*, *Mari*, *Fishomi*, *Frangivento* and *Coratina*) were provided and allowed larvae separately to feed on each variety until 3rd larval instar.

Sample preparation and enzyme assays

Third larval instars were separated from fruits, dissected under a stereomicroscope in ice cold saline solution (NaCl, 10 mM), digestive systems were removed and homogenized in distilled water (Equal portion w/v) using a hand-held glass homogenizer. Then, the samples were centrifuged at 13,000 rpm for 20 min at 4°C. Supernatant was separated and kept at -20 °C for subsequent experiments as the enzyme source.

Preparation of membrane-bound fraction

Membrane preparations were exposed from pellets of centrifuged digestive tract (see above) to Triton X-100 for 20 h at 4°C, in a ratio of 10 mg of Triton X-100 per mg of protein. Samples were centrifuged at 13,000 rpm for 20 min so that no sediment was visible at the end of process. Activity of the enzymes remains unchanged at -20°C for periods of at least a month (16, 25).

α - and β -glucosidase assay

Activities of α - and β -glucosidases were measured according to a method described by Ferreira and Terra (16) with *p*-nitrophenyl- α -D-glucopyranoside (pN α G) and *p*-nitrophenyl- β -D-glucopyranoside (pN β G) as substrates, respectively. Samples of soluble and membrane-bound fractions were incubated with 10 μ L of substrate and 40 μ L of universal buffer (pH 7). The absorbance was measured at 405 nm using a microplate reader after 10 min based on the preliminary incubation.

Effect of pH and temperature on α and β -glucosidase activity

The effects of temperature and pH on activities of α and β -glucosidase were examined in the various pH and temperature ranges. Effect of temperature on α and β -glucosidase activities was determined by incubating the reaction mixture at 20-60°C for 30 min followed by measurement of the enzyme activity. The optimum pH for α and β -glucosidase was determined using the universal buffer in pH ranges of 3-12. Other steps were continued as described earlier.

Effect of different cations and inhibitors on α and β -glucosidase activity

Different concentrations of cations (1, 3 and 5 mM) and inhibitors (2, 4, 6, 8 and 10 mM) were assayed to find their effects on α and β -glucosidase activities in *B. oleae* larvae. Used cations were Ca^{2+} , Cu^{2+} , Fe^{2+} , K^+ , Mg^{2+} , Na^+ and Zn^{2+} . Inhibitors were ethylenediamide tetraacetic acid (EDTA), ethylene glycol-bis (β -aminoethylether) N, N, N-, N-tetraacetic acid (EGTA), triethylenetetramine hexaacetic acid (TTHA), diethyldithiocarbamate (DTC), phenanthroline. These compounds were added to the assay mixture, then incubation was continued for 10 min and absorbance was recorded at 405 nm using a microplate reader.

Kinetic parameters (V_{\max} and K_m) of α - and β -glucosidase in gut

Kinetic parameters of α - and β -glucosidases were determined using six different substrate concentrations (0.5, 1, 2, 3, 4 and 5 mM). The Michaelis constant (K_m) and the maximum velocity (V_{\max}) were estimated by Sigmaplot software after reading the absorbance followed by enzymatic assessments.

Table 1. The specific activities (mean \pm SE) of digestive in larvae of *B.oleae*.

	Soluble	Membrane
α -glucosidases (U/mg protein)	0.225 \pm 0.035a	0.042 \pm 0.026b
β -glucosidases (U/mg protein)	0.067 \pm 0.035a	0
Protein(U/mg/dl)	0.994	1.126

*.Statistical differences have been shown by asterisks (t-test, $p \leq 0.05$).

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

The effect of pH on the hydrolytic activity against pNAG, pNBG were assayed using wide range of values provided by universal buffer. The highest activities were observed at pH 5-6 for α -glucosidase and 5 for β -glucosidases

Effect of different varieties on α - and β -glucosidases

α - and β -Glucosidases activities were assayed in the third larval instar reared on each olive variety including Amigdalifolia, Manzallina, Arbequina, Conservalia, Kalamata, Balidi, Zard, Roghani, Shengher, Mari, Fishomi, Frangivento and Coratina. Reaction mixture was 10 μ L of substrate, 40 μ L of universal buffer and 10 microlitre of the samples from each variety, separately.

Protein concentration

Protein concentrations were estimated as described by Lowry *et al.* [26], using bovine serum albumi as the standard.

Statistical analysis

All data were compared by one-way analysis of variance (ANOVA) followed by Tukey's test when significant differences were found at $p \leq 0.05$ using SAS program.

RESULTS

α - and β -glucosidase assay

Results regarding activity localizations of glucosidases revealed the highest activities in soluble rather than membrane-bound fraction (**Table 1**). Also it was found that specific activities of α - and β -glucosidases in the digestive system of *B.oleae* larvae were 0.077 U/mg protein and 0.023 U/mg protein in the last larval instar, respectively (**Table 1**). The results indicated higher activity of glucosidases in soluble fraction by significant activity of α -glucosidase in comparison with β -glucosidase (**Table 1**).

(**Figure 1**). In both cases, enzymatic activity was elevated in acidic range then it sharply decreased (**Figure 1**). Also, the optimal temperature values for α -glucosidase was found at 45°C and 25 °C for β -glucosidases (**Figure 2**). In other values, enzymatic activity was somehow equal (**Figure 2**).

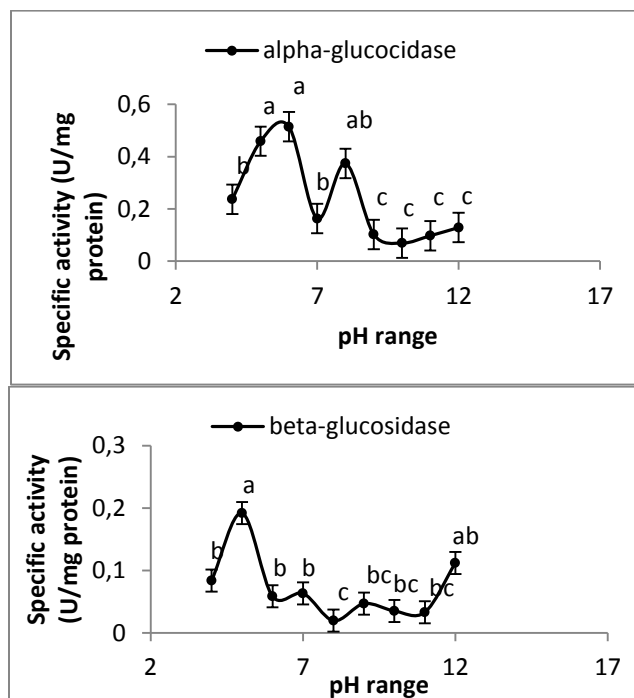


Figure 1. The effect of pH on the activities of α - and β -glucosidases extracted from the digestive system of *B.oleae* larvae. Statistical differences have been shown by various letters (Tukey's test, $p \leq 0.05$).

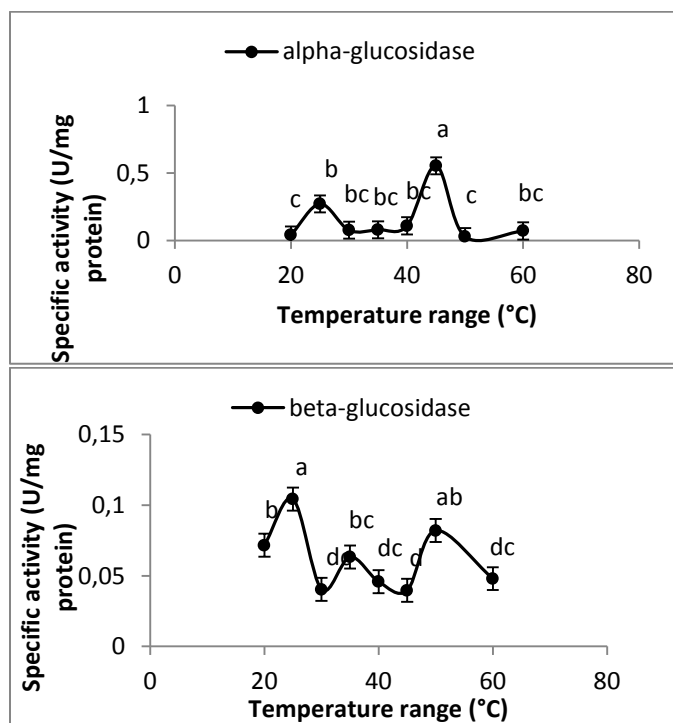


Figure 2. The effect of temperature on the activities of α - and β -glucosidases extracted from the digestive system of *B.oleae* larvae. Statistical differences have been shown by various letters (Tukey's test, $p \leq 0.05$).

Effect of ions and inhibitors on enzyme activity

Activity of α -glucosidase in the gut of *B.oleae* larvae were increased by using concentrations of Ca^{2+} , Cu^{2+} , Fe^{2+} , Na^+ and Zn^{2+} but the enzymatic activity significantly decreased in presence of K^+ and Mg^{2+} (Table 2). In case of β -glucosidase, Cu^{2+} , K^+ and Na^+ increased enzymatic activity and but other ions had adverse effects (Table 3). The effects of several chelating agent were examined

to find their possible effects on glucosidase activities in the larvae of *B.oleae* (Table 4 and 5). Phenanthroline and DTC significantly decrease α -glucosidase activity but other compounds had no effect on the enzymatic activity (Table 4). EGTA and EDTA significantly decreased β -glucosidase activity in the larvae while no effects were observed in case of other compounds.

Table 2. Effect of specific inhibitors on α -glucosidases activity of *B.oleae*.

compound	concentration	specific activity
DTC	C	0.074±0.005 bc
	2	0.159±0.015 b
	4	0.256±0.008 a
	6	0.155±0.004 b
	8	0.032±0.007 c
	10	0.022±0.007 c
Phenanthroline	C	0.134±0.019 a
	2	0.084±0.040 ab
	4	0.025±0.006 b
	6	0.077±0.011 ab
	8	0.019±0.002 b
	10	0.020±0.007 b
EGTA	C	0.073±0.007 c
	2	0.026±0.007 c
	4	0.270±0.019 a
	6	0.087±0.013 c
	8	0.209±0.10 ab
	10	0.114±0.109 bc
EDTA	C	0.029±0.013 ab
	2	0.119±0.006 a
	4	0.038±0.009 ab
	6	0.009±0.002 b
	8	0.089±0.014 ab
	10	0.077±0.011 ab
TTHA	C	0.052±0.009 ab
	2	0.034±0.009 b
	4	0.073±0.020 ab
	6	0.038±0.009 b
	8	0.155±0.009 a
	10	0.085±0.007 ab

Table 3. Effect of specific inhibitors on β -glucosidases activity of *B.oleae*.

compound	concentration	Specific activity
DTC	C	0.006±0.002 a
	2	0.084±0.017 a
	4	0.059±0.006 a
	6	0.066±0.029 a
	8	0.059±0.013 a
	10	0.062±0.008 a
Phenanthroline	C	0.172±0.025 a
	2	0.176±0.030 a
	4	0.232±0.017 a
	6	0.144±0.017 a
	8	0.120±0.013 a
	10	0.112±0.007 a
EGTA	C	0.178±0.030 a
	2	0.030±0.007 b
	4	0.091±0.003 ab
	6	0.032±0.006 b
	8	0.098±0.006 ab
	10	0.091±0.002 ab
EDTA	C	0.135±0.006 a
	2	0.067±0.14 a
	4	0.141±0.004 a
	6	0.124±0.016 a
	8	0.109±0.024 a
	10	0.041±0.011 a
TTHA	C	0.089±0.027 a
	2	0.120±0.006 a
	4	0.105±0.031 a
	6	0.064±0.002 a
	8	0.052±0.012 a
	10	0.086±0.021 a

Table 4. Effect of mono- and divalent actions on α -glucosidases activity of *B.oleae*.

compound	concentration	Specific activity
Ca	C	0.025±0.006 a
	1	0.051±0.005 a
	3	0.096±0.017 a
	5	0.263±0.091 a
	Cu	C
1		0.246±0.017 a
3		0.068±0.010 b
5		0.076±0.004 b
Fe		C
	1	0.089±0.013 a
	3	0.270±0.106 a
	5	0.405±0.138 a
	K	C
1		0.102±0.013 a
3		0.146±0.019 a
5		0.070±0.016 a
Mg		C
	1	0.010±0.002 b
	3	0.040±0.015 b
	5	0.020±0.007 b
	Na	C
1		0.018±0.004 b
3		0.116±0.004 ab
5		0.148±0.016 a
Zn		C
	1	0.121±0.025 b
	3	0.065±0.006 b
	5	0.306±0.014 a

Table 5. Effect of mono- and divalent actions on β -glucosidases activity of *B.oleae*.

compound	concentration	Specific activity
Ca	C	0.182±0.006
	1	0.164±0.006
	3	0.120±0.021
	5	0.098±0.007
	Cu	C
1		0.038±0.009
3		0.098±0.006
5		0.112±0.034
Fe		C
	1	0.054±0.019
	3	0.335±0.069
	5	0.170±0.026
	K	C
1		0.075±0.003
3		0.102±0.007
5		0.100±0.013
Mg		C
	1	0.056±0.008
	3	0.278±0.016
	5	0.035±0.008
	Na	C
1		0.217±0.015
3		0.152±0.014
5		0.093±0.006
Zn		C
	1	0.067±0.017
	3	0.032±0.008
	5	0.077±0.020

Kinetic parameters (V_{max} and K_m) of α - and β -glucosidase in gut

The effect of substrate concentrations on enzymatic activity was calculated and kinetic parameters of α - and β -glucosidases are shown

in Figure 3. V_{max} value of α - and β -glucosidases were found to be 0.11 and 0.21 ($\mu\text{mol}/\text{min}/\text{mg}$ protein) and K_m values were obtained 4.26 and 0.84 (mM), respectively (**Figure 3**).

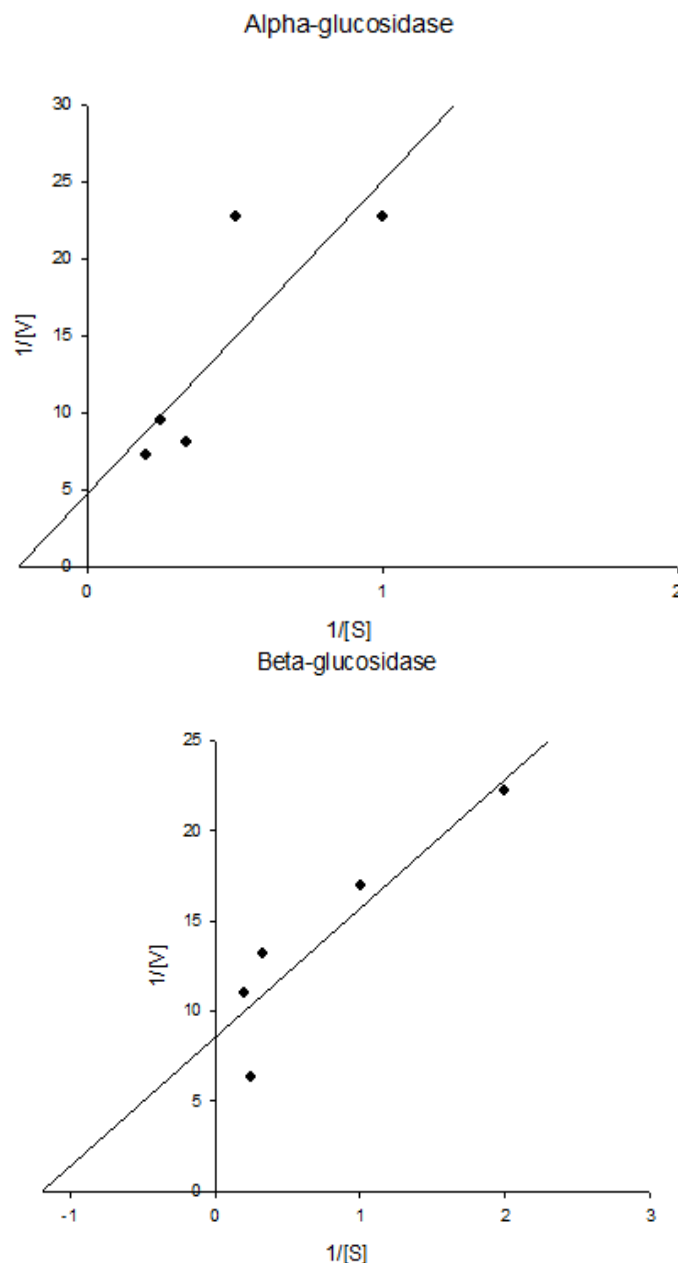


Figure 3. Lineweaver–Burk plots of alpha and beta-glucosidase in gut of *B.oleae* larvae.

Effect of different varieties on α - and β -glucosidases

Activities of α - and β -glucosidase in the larvae fed on Amigdalifolia, Manzallina, Arbequina, Conservalia, Kalamata, Balidi, Zard, Roghani, Shengher, Mari, Fishomi, Frangivento and Coratina varieties of olive have been shown in

Figure 4. The highest activity of α -glucosidase was found in Frangivento and Amigdalifolia varieties but Zard and Conservalia varieties had the significant negative effects on the enzymatic activity (**Figure 4**). The highest activity of β -glucosidase was found in Fishomi variety and the lowest ones have been observed in Zard, Kalamata and Arbequina (**Figure 4**).

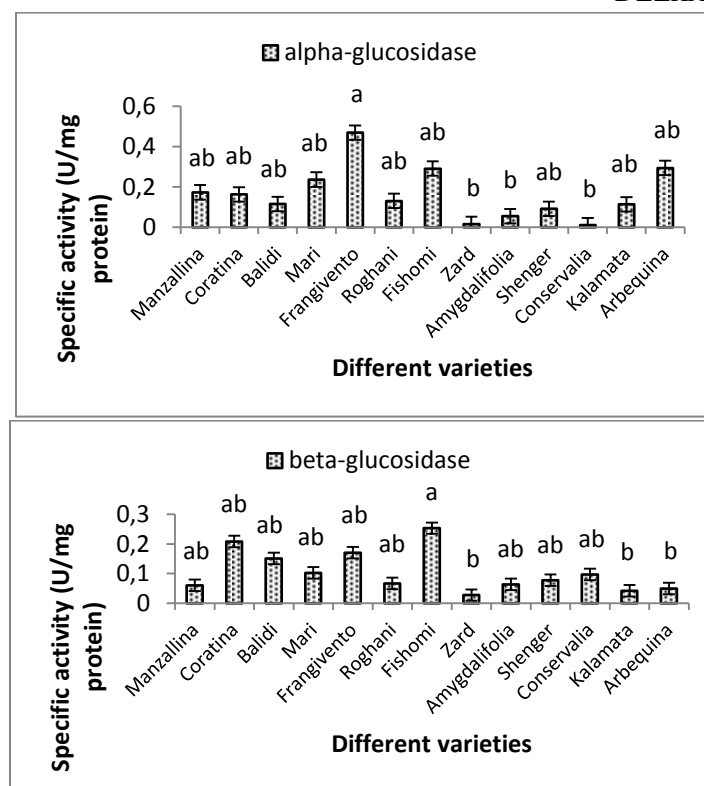


Figure 4. The effect of different varieties on the activities of α - and β - glucosidases extracted from the digestive system of *B.oleae* larvae. Statistical differences have been shown by various letters (Tukey's test, $p \leq 0.05$).

DISCUSSION

Glucosidases are the exo-enzymes which hydrolyze glycosyl bonds and release glucose from oligosaccharides (9). On the hand, glucosidases attack to carbohydrates followed by action of endoamylases like α -amylase. Since, almost all naturally occurring plant glycosides are β -linked o-glycosyl it could be concluded that β -glucosidases are more important than α -glucosidases in the metabolism of plant glycosides. Glycosides are the allelochemicals that deal as the defensive compounds in host plant (27). For example, resistance of corn to the European corn borer *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae) is due to presence of a glycoside known as DIMBOA. Also glycosides have anti-feedant property against phytophagous insects (9). Hemming *et al.* (28) have reported that higher amounts of phenolic glycosides cause lower activity of β -glucosidase in gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). Our data clearly demonstrated that α - and β -glucosidases are present in the digestive system of *B. oleae* larvae. Meanwhile, it was found presence of the enzymes in soluble content of gut rather than membrane-bound samples. It means that these enzymes are free in midgut lumen of larvae and digest

oligosaccharides followed by primary digestion of polysaccharides due to α -amylase activity. These findings are similar to results of Zibae (25) on *Pieris brassicae* L. (Lepidoptera: Pieridae) and Ramzi and Zibae (29) on *Apodiphus amygdali* Germar (Hemiptera: Pentatomidae).

Temperature and pH are the two important factors at biochemical reaction. Our results revealed that α - and β -glucosidases are active in acidic pH conditions correspondances with our previous report on α -amylase of *B. oleae* (24). Jayme *et al.* (30) have shown an optimal pH of 5.5 for α -glucosidase in *Anopheles aquasalis* (Diptera: Culicidae). The optimal pH of β -glucosidase in larvae of the palm weevil, *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) was found at pH 5 (18). Ahsaei *et al.* (31) reported optimal pH value of 5 for β -glucosidase activity in *Lixus incanescens* adults but no activity of α -glucosidase was detected. α -glucosidase in *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) had acidic range of 4 to 6 (32). Differences in optimal pH value could be attributed to phylogenetic relationship of species or reaction to different food sources (33). For example, insects fed on tree foliars have highly alkaline pH in their midgut to overcome toxic materials ingested along with food. Fruits

of different olive varieties have a pH range from 3 to 7 so there might be an adaptability between pH of fruit tissues and optimal pH value of α - and β -glucosidases in *B. oleae* larvae (34). Optimal temperatures for α - and β -glucosidase activities were different for these enzymes which it was found 45°C and 25 °C for α - and β -glucosidases, respectively. The optimal temperature for the enzymatic activity in *R. palmarum* was between 40°C and 50°C (18). The optimal activity for α - and β -glucosidases in rice striped stem borer was found in 45°C (13). In Sunn pest, optimal activity of α -glucosidase was found between 40 to 45°C (Bandani *et al.*, 2010). Increasing temperature causes enzymatic reactions occur faster since enzymes have the temperature above which its structure and activity disrupted (35, 36).

Ions are the important factor in active sites of enzymes and serve in several mechanisms. (1) Ions take and release electrons due to activation of electrophiles and nucleophiles, (2) Ions intervene in the reactions of nucleophiles, (3) they can keep enzyme and substrate in close distance through the coordination bonds causing the higher efficiency of enzymatic activity, (4) Ions put active groups of enzyme and substrate in an effective position to elevate efficiency of enzyme-substrate complex or stability of enzyme (37). In our study, different concentrations of ions had various effects on activity of α - and β -glucosidase activities in larvae of *B. oleae*. Zeng and Cohen (38) reported that Cu^{2+} had the most effect (118%) and Mg^{2+} showed the least effect (96%) on α - and β -glucosidase of *Lygus hesperus* Fabricius (Hemiptera: Miridae). Bandani *et al.* (32) found that Na^+ , K^+ , Mg^{2+} , and Ca^{2+} had positive effects on α -glucosidase activity of *E. integriceps* and urea, SDS, Tween 80, Triton X-100 and EDTA had inhibitory effects on enzymatic activity. Valienamine and Validamine inhibited β -glucosidase in the salivary glands of *Reticulitermes flaviceps* (39). Chemical agents CuCl_2 , ZnCl_2 , FeCl_3 , DTNB and pCMB have an inhibitory effect on β -glucosidase activity of *R. palmarum* (18). NaCl , CaCl_2 and MgCl_2 concentrations had positive effects and KCl , EDTA, SDS but Urea had negative effects on enzyme activity of rice striped stem borer (13). Na^+ , K^+ , Mg^{2+} , and Ca^{2+} have positive effects on the activity of α -glucosidase and α -amylase in other insects (40, 41). Ramzi and Zibae (29) demonstrated that K^+ and Ca^{2+} significantly increased α -glucosidase activity, Na^+ had no effect and Cu^{2+} ,

Fe^{2+} and Mg^{2+} had statistically negative effects in the midgut of *Apodiphus amygdali* Germar (Hemiptera: Pentatomidae). In case of β -glucosidase activity, Ca^{2+} and Fe^{2+} increased the enzymatic activity in the midgut of *A. amygdali*, Na^+ had no effect and other ions significantly decreased it. Terra and Ferreira *et al.* (5) believe that glucosidases are metalloproteins that require calcium for their activity. Additionally, calcium increases stability of the enzymes from variety of sources in both pH and temperature extremes. This could be the reason of glucosidase sensitivity to general chelating agents (5).

Kinetic parameters of α - and β -glucosidase showed that olive fruit fly have K_m values of 4.26 and 0.84 (mM), respectively. These data indicate that β -glucosidase had higher affinity toward the substrate. So, it can be concluded that β -glucosidases are more efficient in carbohydrates digestion of olive fruit fly via affinity to substrates. K_m has an inverse relationship with the substrate concentration required to saturate the active sites of the enzyme. The higher value of K_m indicates the lower affinity of enzyme for substrate. In fact, K_m is the parameter showing stability of the enzyme-substrate complex.

Activities of α - and β -glucosidase were different in the *B. oleae* larvae fed on various olive varieties. These differences could be attributed to molecular properties of the carbohydrases available in the food and contents of glycosides and phenolic components (42). Hemming and Lindroth (28) indicated that performance of Lymantridat moth is affected variation in levels of phenolic glycosides. These compound significantly decreased larval growth, development and reproductive ability of adults. It is believed that midgut glycosidases e.g. α - and β -glucosidases are important in metabolizing phenolic glycosides (28). Overall activity of digestive carbohydrases lead to releasing of glucose and bioactivating of toxic α -glycones or especially reactive orthoquinone methides (28). These process decrease β -glucosidase activity to protect larvae from reactive phenolic glycoside metabolites. Hence, the lower activity of glucosidases in Zard variety could be attributed to presence of higher amount of glycosides in fruits.

CONCLUSIONS

Based on the present study, it can be concluded that glucosidases play an important role in *B. oleae* digestion. The current findings in addition

to our previous report on amylolytic activity might be useful to design a control procedure by recruiting resistant varieties. Delkash-Roudsari *et al.* (24) reported that an amylolytic inhibitor from *Polyganum persicaria* L. caused significant decrease in α -amylase activity of *B. oleae* larvae. Meanwhile, it was found that Zard variety caused the lowest activity of glucosidases in the larvae. So, genetic engineering by considering compounds in *P. persicaria* and Zard variety could be promising to provide resistant varieties of olive against the pest. Finally, understanding of digestive enzyme properties, particularly the effect of inhibitors on enzymatic activity and physiology of insects, could be useful to reach safe and useful controls of insect pests before achieving to resistant varieties.

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