**Biochemical characterisation of α -glucosidase and β -glucosidase
in the alimentary canal of larval *Leptinotarsa decemlineata* SAY, 1824
(Coleoptera: Chrysomelidae)**

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ABSTRACT. Host plant resistance is an environmentally safe method used for reducing a pest population. Basically, when developing resistant cultivars one needs to study the biochemical characteristics of the digestive enzymes in the insect's midgut. In this study, the activities of α - and β -glucosidase were determined from *Leptinotarsa decemlineata* midgut using p-nitrophenyl- α -D-glucopyranoside and p-nitrophenyl- β -D-glucopyranoside as substrates respectively. The results showed that the specific activity of α - and β -glucosidase from 4th instar larvae midguts of *L. decemlineata* were 5.14 and 5.48 Umg⁻¹ protein respectively. The activity of α -glucosidase was optimal at pH 4, whereas the maximum activity of β -glucosidase in the midgut of *L. decemlineata* occurred at pH 4-5.5. Both enzymes were stable at pH 3-8 over an incubation time of 8 hours. The respective activities of α - and β -glucosidase were at their highest at 45 °C and 50 °C, but they were not stable at 50 °C during an incubation time of 8 days. Furthermore, our data showed that MgCl₂, Tris and urea have a moderate but SDS a severe inhibitory effect on enzyme activity. Biochemical characterisation revealed one and three bands of α - and β -glucosidase activities in the midgut of *L. decemlineata* respectively.

KEY WORDS: α -glucosidase, β -glucosidase, alimentary canal, Colorado potato beetle.

INTRODUCTION

One of the most destructive pest of the potato, the Colorado potato beetle (CPB) *Leptinotarsa decemlineata* SAY, 1824 seriously threatens potato production in many parts of the world (HARE 1990, CAPINERA 2001). To date, one effective way of reducing the population of this pest is to apply pesticides. However, excessive use of insecticides has led to the development of resistance in CPB to all currently registered insecticides (GAUTHIER et al. 1981, FORGASH 1985, ARGENTINE et al. 1989, HARE 1990) to such an extent that this pest is one of the top 16 resistant arthropods of the world: its resistance to 48 different active ingredients has been reported (ONSTAD 2007). Therefore, alternative control methods such as resistant or tolerant potato varieties are needed for implementing sustainable management programmes.

Glucosides are important secondary plant metabolites: digestive enzymes in an insect's midgut convert them into toxic aglycones, thereby defending plants against herbivore attacks (WEI et al. 2007). These plant metabolites could be used in genetic engineering techniques to produce plants resistant to herbivores. (MATTIACCI et al. 1995). Studying the biochemical characteristics of the CPB's digestive enzymes is a necessary prerequisite for developing cultivars resistant to CPB (RAZAVI TABATABAEI et al. 2011).

α -Glucosidase (EC 3.2.1.20) and β -glucosidase (EC 3.2.1.21) are enzymes whose main role is played out in the terminal digestion of carbohydrate in an insect's midgut (TERRA & FERREIRA 1994).

α -Glucosidase catalyses the hydrolysis of α -1,4-linked glucose residues and β -glucosidase breaks di- and oligosaccharides by attacking the terminal, non-reducing β -1,4-linked glucose residues (TERRA & FERREIRA 1994). Additionally, β -glucosidase is an enzyme widely present in the majority of animals, plants, fungi, bacteria and other living organisms (Esen 1993). These compounds are involved in plant resistance to herbivorous insect pests. The majority of these compounds are L-glycosides that release cyanide (or other toxic compounds) upon hydrolysis that are toxic to many herbivorous insects (TERRA et al. 1996).

The purification of α - and β -glucosidase and studies of their enzymatic activity under different conditions have been done on many different insect species (SANTOS & TERRA 1985, PRATVIEL et al. 1987, MARANA et al. 1995, SILVA et al. 1999, MARANA et al. 2000, PONTOH & LOW 2002, TOKUDA et al. 2002, AZEVEDO et al. 2003, CARNEIRO et al. 2004, BYEON et al. 2005, SOUZA-NETO et al. 2007, RAMZI & HOSSEININAVEH 2010, RAZAVI TABATABAEI et al. 2011). Considering the importance of carbohydrases and the study of carbohydrate digestion as a target for *L. decemlineata* control, the aim of this study is to provide information about some biochemical properties of α - and β -glucosidase extracted from the midgut of *L. decemlineata* in order to acquire a better understanding of the

digestive physiology of the CPB.

MATERIAL AND METHODS

Sample preparation

Fourth-instar larvae of *L. decemlineata* were collected by sweep-netting around the village of Dehpiaz near the city of Hamedan, Iran, and transferred to the laboratory. They were kept on potato leaves under laboratory controlled conditions (temperature $26\text{ }^{\circ}\text{C} \pm 1$ and photoperiod 14:10 light:dark cycle).

Prior to the experiments it was necessary to provide enzyme samples. Therefore, *L. decemlineata* larvae were immobilised on ice, after which their midguts were removed under a stereomicroscope. After dissection, the midguts were kept in 1 ml doubly-distilled water. Thereafter, they were homogenised in a pre-cooled hand-held glass homogeniser. The homogenates were then centrifuged at 15000 g for 10 min at $4\text{ }^{\circ}\text{C}$ and the supernatant stored at $-20\text{ }^{\circ}\text{C}$ until use (SILVA et al. 1999).

Protein determination and enzyme assays

The protein concentration was determined according to BRADFORD's (1976) method, using ovalbumin as a standard. The activities of α - and β -glucosidase were determined using p-nitrophenyl- α -D-glucopyranoside and p-nitrophenyl- β -D-glucopyranoside as substrate respectively. For the α -glucosidase assay, 10 μl of the gut extract were mixed with 300 μl of 40 mM citrate-phosphate buffer at pH 4. This mixture was then incubated after the addition of 15 mM p-nitrophenyl- α -D-glucopyranoside at $45\text{ }^{\circ}\text{C}$ for 15 min. For the β -glucosidase assay, 10 μl of the gut extract were mixed with 300 μl of 40 mM citrate-phosphate buffer at pH 5. The mixture was then incubated after the addition of 15 mM p-nitrophenyl- β -D-glucopyranoside at $50\text{ }^{\circ}\text{C}$ for 15 min. The reactions were stopped by the addition of 700 μl NaOH (2M); the absorbance was measured at 405 nm after 10 minutes. One unit of enzyme activity (U) is defined as the amount that hydrolyses 1 mmol of substrate per minute.

Effect of pH and temperature on enzyme activities and stabilities

The optimal pH for enzyme activities was measured using 300 μl 40 mM citrate-phosphate buffer with pH set to 2-8 at 0.5 intervals. The enzyme samples were incubated in citrate phosphate buffers at different pHs for 2 and 8 hours. The residual activities of the treated enzyme samples were measured according to the "Enzyme assay" section.

To determine the optimum temperature for the enzyme activities, the reaction mixtures were incubated at different temperatures from 5 to $70\text{ }^{\circ}\text{C}$ at $5\text{ }^{\circ}\text{C}$ intervals for 35 minutes

followed by the “Enzyme assay”. Enzyme stabilities were measured at 5, 26 and 50 °C for 1 to 8 days. Thereafter, the residual activities of the enzymes were determined (PARRY et al. 1996).

Influence of activators and inhibitors on α - and β -glucosidase activities

To test the effects of different ions on the enzyme activities, different concentrations of the chlorides of Na^+ (5, 10, 20 and 40 mmol/l), K^+ (5, 10, 20 and 40 mmol/l), Ca^{2+} (5, 10, 20 and 40 mmol/l) and Mg^{2+} (5, 10, 20 and 40 mmol/l), as well as sodium dodecylsulphate (SDS, 1 mmol/l), Tris (10 and 20 mmol/l) and urea (0.4 and 0.8 mol/l) were added to the assay mixtures, after which the residual enzyme activities were determined following standard assay conditions.

Electrophoresis

Electrophoretic analysis was performed under native-PAGE conditions. The samples were combined with a sample buffer lacking β -mercaptoethanol and SDS (2.1 ml distilled water + 0.5 ml 0.5M Tris-HCl, pH 6.8 + 0.4 ml glycerol + 0.2ml 1% (w/v) bromophenol blue).

Electrophoresis was performed on 10 % (w/v) and 7.5% (w/v) resolving polyacrylamide gels for α - and β -glucosidase respectively (LAEMMLI 1970) at 110 V without boiling the samples. In accordance with native-PAGE, the gel was washed in 40 mM citrate phosphate buffer pH 5 (optimal pH of enzyme activity) at room temperature for 20 min. Then, the gel was incubated in this buffer containing 8 mM fluorogenic substrate 4-methylumbelliferyl- α -D-glucoside for α -glucosidase and 4-methylumbelliferyl- β -D-glucoside for β -glucosidase at 40 °C for 30 minutes. Finally, the α - and β -glucosidase activities were detected by fluorescence under UV illumination and the fluorescent bands were photographed.

Statistical analysis

The significant differences among treatments in a complete randomised design were checked by one-way analysis of variance (Anova) and means were compared using Duncan's multiple range test at the 0.05 significance level.

RESULTS

α - and β -glucosidase activities

α - and β -glucosidase activities were detected in the larval midgut of *L. decemlineata*. The specific activities of α - and β -glucosidase in the midgut were 5.14 and 5.48 Umg^{-1} proteins, respectively.

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

The effect of pH on α - and β -glucosidase activities towards pN α G and pN β G as substrate respectively were measured using 40 mM citrate phosphate buffers (pH 2-8). The maximum activity of α -glucosidase was determined at pH 4, whereas the highest β -glucosidase activity in the midgut of *L. decemlineata* was obtained at pH 4 to pH 5.5. α - and β -Glucosidase activities in the midgut increased steadily from pH 2 to 4 and then decreased with pH increasing from 5 to 8 for α -glucosidase and from 6 to 8 for β -glucosidase (Fig. 1).

Both of these enzymes were stable at pH 3-8 but not at pH 2. Furthermore, α - and β -glucosidase activities increased steadily with temperatures rising from 10 to 45 °C and 50 °C respectively. At temperatures above 45 °C and 50 °C, the enzyme activities gradually decreased (Fig. 2).

The stability of the enzymes at different pH for 2 and 8 hours is shown in Figure 3. The relative activities of α - and β -glucosidase exhibit a similar trend in this figure at different pH for 2 and 8 hours' incubation.

Both enzymes are unstable at 50 °C; they lost about 70% of their maximum activities after 2 days' incubation because of heat inactivation (Fig. 4).

Effect of activators and inhibitors on α - and β -glucosidase activities

The results showed that MgCl₂, Tris and urea have a moderate inhibitory effect, whereas SDS has a severe inhibitory effect on α - and β -glucosidase activities. Different concentrations of CaCl₂ increased midgut α -glucosidase activity whereas the same concentration reduced β -glucosidase activity. NaCl and KCl did not have any effect on α - or β -glucosidase activities (Table 1).

Electropherogram analyses

The results showed single and three distinct α - and β -glucosidase activity bands respectively in the midgut homogenates of 4th instar larvae of *L. decemlineata* obtained using native-PAGE (Fig. 5).

DISCUSSION

Glucosidases are enzymes that are essential for the metabolism and terminal phase of insect carbohydrate digestion. In this study, glucosidase activities were found in the midgut of *L. decemlineata*; this is to be expected in view of the CPB's feeding regime. This finding is coincident with the presence of α - and β -linked oligosaccharides in the CPB's diet.

Fig. 1. Effect of pH on activity of α -glucosidase (a) and β -glucosidase (b) extracted from the midgut of *L. decemlineata*. The different letters indicate that the enzyme activities are significantly different from each other ($p < 0.05$).

Fig. 2. Effect of temperature on the activity of α -glucosidase (a) and β -glucosidase (b) extracted from the midgut of *L. decemlineata*.

Fig. 3. Effect of pH on the stability of α -glucosidase (a) and β -glucosidase (b) extracted from the midgut of *L. decemlineata* after 2 and 8 hours.

Fig. 4. Effect of temperature on the stability of α -glucosidase (a) and β -glucosidase (b) extracted from the midgut of *L. decemlineata* (during 8 days).

Fig. 5. Electropherogram analysis of α -glucosidase (a) and β -glucosidase (b) activity in the midgut of the fourth larval instar of *L. decemlineata*.

Our results demonstrated that α - and β -glucosidase reach their maximum activity at acidic pHs, which is consistent with the hypothesis that there is a correlation between the pH optima of enzymes and the luminal pH of an insect's gut (TERRA & FERREIRA 1994). Furthermore, there are no significant differences between their activities at pHs from 4 to 6, although the curves showed different trends. Likewise, the maximum activities of midgut α - and β -glucosidase were obtained in most insects at acidic pHs (TERRA et al. 1985, TERRA et al. 1988, BAKER 1991, PONTOH & LOW 2002, TOKUDA et al. 2002, BYEON et al. 2005, SOUZA-NETO et al. 2007). In our experiment α - and β -glucosidase exhibited a broad pH stability. Such a broad temperature and pH stability range were previously reported in other insect species (TOKUDA et al. 2002, PONTOH & LOW 2002, BYEON et al. 2005, YAPI et al. 2009).

It was shown that α - and β -glucosidase reach their maximum activity at 45 °C and 50 °C respectively, as the protein structures of the enzymes have their optimal activities within a certain temperature range. Higher temperatures reduce enzyme activity as a result of heat inactivation (PRICE & STEVENS 1989). Additionally, β -glucosidase was not stable above 55–60 °C because it is irreversibly inactivated, although it does display its maximum catalytic activity at 50–55 °C (ESEN 1992).

Table 1. Relative activity (%) of *L. decemlineata* α - and β -glucosidase in the presence of different compounds.

Compounds	Concentration (mmol/L)	Relative activity(%) α -glucosidase	Relative activity(%) β -glucosidase
Control	-	100	100
NaCl	5	94 $\pm$ 0.07	98 $\pm$ 0.11
	10	107 $\pm$ 0.04	92 $\pm$ 0.02
	20	97 $\pm$ 0.05	96 $\pm$ 0.03
	40	98 $\pm$ 0.16	93 $\pm$ 0.11
KCl	5	93 $\pm$ 0.07	90 $\pm$ 0.09
	10	96 $\pm$ 0.07	89 $\pm$ 0.04
	20	90 $\pm$ 0.03	94 $\pm$ 0.02
	40	101 $\pm$ 0.18	95 $\pm$ 0.01
CaCl ₂	5	88 $\pm$ 0.04	76 $\pm$ 0.01
	10	89 $\pm$ 0.14	65 $\pm$ 0.04
	20	95 $\pm$ 0.06	61 $\pm$ 0.05
	40	97 $\pm$ 0.14	61 $\pm$ 0.01
MgCl ₂	5	81 $\pm$ 0.02	73 $\pm$ 0.01
	10	79 $\pm$ 0.14	75 $\pm$ 0.07
	20	70 $\pm$ 0.04	65 $\pm$ 0.04
	40	73 $\pm$ 0.01	49 $\pm$ 0.01
Urea	400	84 $\pm$ 0.16	73 $\pm$ 0.07
	800	79 $\pm$ 0.09	59 $\pm$ 0.11
Tris	10	65 $\pm$ 0.10	82 $\pm$ 0.03
	20	41 $\pm$ 0.09	59 $\pm$ 0.03
SDS	1	7 $\pm$ 0.01	5 $\pm$ 0.01

The present study also implies that divalent cations reduce the activities of α - and β -glucosidase. Many glycohydrolases are inhibited by divalent cations. These cations change the structure of the protein and reduce enzyme activity (BOWERS et al. 2007). However, there are also several glycohydrolases in insects that are activated by cations, such as α -amylase by Ca⁺² (TERRA & FERREIRA 1994) and α -glucosidase by Ca⁺² (GHADAMYARI et al. 2010). In addition, insect α -glucosidase is strongly inhibited by Tris (TERRA & FERREIRA 1994).

The electropherogram analysis of the *L. decemlineata* midgut showed single and three distinct bands for α - and β -glucosidase respectively. In previous studies with different

insect species, α -glucosidase produced at least two (RAZAVI TABATABAEI et al. 2011) or three distinct bands (SILVA et al. 1999). Furthermore, in the midgut of many insect species there are three, four or even five digestive β -glucosidases (AZEVEDO et al. 2003).

Different types of glucosides are phytoanticipins, i.e. plant secondary compounds that occur widely in the plant kingdom (ZAGROBELNY et al. 2004, PANKOKE et al. 2010). Insect β -glucosidases are enzymes that catalyse the hydrolysis of broad range of aryl- and alkyl β -D-glucosides such as toxic glucosides, which have been identified as an elicitor in plant and herbivore interaction (WEI et al. 2007). In many cases, the aglycones usually produced by β -glucosidase activities are more toxic than the glycosides themselves (YU 1989). In this research, as a first step, some biochemical enzymatic properties of α - and β -glucosidase obtained from the midgut of *L. decemlineata* were determined. Therefore, identifying glucosides in different plants and studying their effects on β -glucosidase activity in pests such as the Colorado potato beetle in long term can be useful for producing a genotype resistant to this pest.

REFERENCES

- ARGENTINE J.A., CLARK J.M., FERRO D. N. 1989. Genetics and synergism of resistance to azinphosmethyl and permethrin in the Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* **82**(3): 698–705.
- AZEVEDO T.M., TERRA W.R., FERREIRA C. 2003. Purification and characterization of three β -glycosidases from midgut of the sugar cane borer, *Diathraea saccharalis*. *Insect Biochemistry and Molecular Biology* **33**(1): 81–92.
- BAKER J.E. 1991. Properties of glycosidases from the maize weevil, *Sitophilus zeamais*. *Insect Biochemistry* **21**(6): 615–621.
- BOWERS E.M., RAGLAND L.O., BYERS L.D. 2007. Salt effects on beta-glucosidase: pH-profile narrowing. *Biochimica et Biophysica Acta – Proteins Proteomics* **1774**(12): 1500–1507.
- BRADFORD M. 1976. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* **72**(1-2): 248–254.
- BYEON G.M., LEE K.S., GUI Z.Z., KIM I., KANG P.D., LEE S.M., SOHN H.D., Jin B.R. 2005. A digestive beta glucosidase from the silkworm, *Bombyx mori*: cDNA cloning expression and enzymatic characterization. *Comparative Biochemistry and Physiology* **141**(4): 418–427.
- CAPINERA J.L. 2001. Handbook of vegetable pests. Academic Press, San Diego.
- CARNEIRO N.B., ISEJIMA E.M., SAMUELS R.I., SILVA C.P. 2004. Sucrose hydrolyses from the midgut of the sugarcane stalk borer *Diatraea saccharalis*. *Journal of Insect Physiology* **50**(11): 1093–1101.
- ESEN A. (ed.) 1993. Beta-glucosidases: biochemistry and molecular biology. American Chemical Society, Washington.

- ESEN A. 1992. Purification and partial characterization of maize (*Zea mays* L.) β -glucosidase. *Plant Physiology* **98**(1): 174–182.
- FORGASH A.J. 1985. Insecticide resistance in the Colorado potato beetle. [in:] D.N. FERRO, R.H. VOSS (eds.). *Proceedings of the symposium on the Colorado potato beetle, 17th International Congress of Entomology*. Massachusetts Experiment Station, University of Massachusetts, Amherst, 33–53.
- GAUTHIER N.L., HOFMASTER R.N., SEMEL M. 1981. History of Colorado potato beetle control. [in:] J.H. LASHOMB, R. CASAGRANDE (eds.). *Advances in potato pest management*. Hutchinson Ross Publishing Co., Stroudsburg, 13–33.
- GHADAMYARI M., HOSSEININAVEH V., SHARIFI M. 2010. Partial biochemical characterization of α - and β -Glucosidases of Lesser mulberry pyralid, *Glyphodes pyloalis* WALKER (Lep.: Pyralidae). *Comptes rendus biologiques* **333**(3):197–204.
- HARE J.D. 1990. Ecology and management of the Colorado potato beetle. *Annual Review of Entomology* **35**: 81–100.
- LAEMMLI U.K. 1970. Cleavage of structural proteins during the assembly of the bacteriophage T4. *Nature* **227**(5259): 680–685.
- MARANA S.R., TERRA W.R., FERREIRA C. 1995. Midgut β -d-glucosidases from *Abracris flavolineata* (Orthoptera: Acrididae). Physical properties, substrate specificities and function. *Insect Biochemistry and Molecular Biology* **25**(7): 835–843.
- MARANA S.R., TERRA W.R., FERREIRA C. 2000. Purification and properties of a beta-glucosidase purified from midgut cells of *Spodoptera frugiperda* (Lepidoptera) larvae. *Insect Biochemistry and Molecular Biology* **30**(12): 1139–1146.
- MATTIACCI L.M., DICKE M., POSTHUMUS M. A. 1995. β -Glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America* **92**(6): 2036–2040.
- ONSTAD D. 2007. *Insect resistance management: Biology, Economics and Prediction*. Academic Press, San Diego.
- PANKOKE H., BOWERSAND M.D., DOBLER S. 2010. Influence of iridoid glycoside containing host plants on midgut β -glucosidase activity in a polyphagous caterpillar, *Spilosoma virginica* FABRICIUS (Lep.: Arctiidae). *Journal of Insect Physiology* **56**(12): 1907–1912.
- PARRY M.W. 1996. *A study of the interaction of some fungal metabolites with the insect species Drosophila melanogaster*. Birkbeck College, University of London, London.
- PONTOH J., LOW N.H. 2002. Purification and characterization of β -glucosidase from honeybees (*Apis mellifera*). *Insect Biochemistry and Molecular Biology* **32**(6): 679–690.
- PRATVIEL F., CLERMONT S., PERCHERON F., CHARARAS C. 1987. Studies on glycosidases and glucanases in *Thaumetopoea pityocampa* larvae. II. Purification and some properties of a broad specificity β -D-glucosidase. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **86**(1): 173–178.
- PRICE N.C., STEVENS L. 1989. *Fundamentals of Enzymology*. Oxford University Press, Oxford.
- RAMZI S., HOSSEININAVEH V. 2010. Biochemical characterization of digestive α -amylase, α -glucosidase and β -glucosidase in pistachio green stink bug, *Brachynema germari* KOLENATI (Hemiptera: Pentatomidae). *Journal of Asia-Pacific Entomology* **13**(3): 215–219.

- RAZAVI TABATABAEI P., HOSSEININAVEH V., GOLDANSAZ S.H., TALEBI K. 2011. Biochemical characterization of digestive proteases and carbohydrases of the carob moth, *Ectomyelois ceratoniae* (ZELLER) (Lepidoptera: Pyralidae). *Journal of Asia-Pacific Entomology* **14**(2): 187–194.
- SANTOS C.D., TERRA W.R. 1984. Plasma membrane-associated amylase and trypsin: intracellular distribution of digestive enzymes in the midgut of the cassava hornworm, *Erinnyis ello*. *Insect Biochemistry* **14**(5): 587–595.
- SILVA C.P., TERRA W.R., XAVIER-FILHO J., GROSSI DE SA M.F., LOPES A.R., PONTES E.G. 1999. Digestion in larvae of *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Coleoptera: Bruchidae) with emphasis on α -amylases and oligosaccharidases. *Insect Biochemistry and Molecular Biology* **29**(4): 355–366.
- SOUZA-NETO J.A., MACHADO F.P. LIMA J.B., VALLE D., RIBOLLA P.E.M. 2007. Sugar digestion in mosquitoes: identification and characterization of three midgut Alpha-glucosidases of the Neotropical malaria vector *Anopheles aquasalis* (Diptera: Culicidae). *Comparative Biochemistry and Physiology* **147**(4): 993–1000.
- TERRA W.R., FERREIRA C. 1994. Insect digestive enzymes: properties, compartmentalization and function. *Comparative Biochemistry and Physiology* **109**(1): 1–62.
- TERRA W.R., FERREIRA C., BASTOS F. 1985. Phylogenetic considerations of insect digestion: Disaccharidases and the spatial organization of digestion in the *Tenebrio molitor* larvae. *Insect Biochemistry* **15**(4): 443–449.
- TERRA W.R., FERREIRA C., GARCIA E.S. 1988. Origin, distribution, properties and functions of the major *Rhodnius prolixus* midgut hydrolases. *Insect Biochemistry* **18**(5): 423–434.
- TERRA W.R., FERREIRA C., JORDAO B.P., DILLON R.J. 1996. Digestive enzymes. [in:] M.J. LEHANE, P.F. BILLINGSLEY (eds.). *Biology of the Insect Midgut*. Chapman & Hall, London, 86–114.
- TOKUDA G., SAITO H., WATANABE H. 2002. A digestive β -glucosidase from the salivary glands of the termite, *Neotermes koshunensis* (SHIRAKI): distribution, characterization and isolation of its precursor cDNA by 5'- and 3'-RACE amplifications with degenerate primers. *Insect Biochemistry and Molecular Biology* **32**(12): 1681–1689.
- WEI S.H., SEMEL Y., BRAVDO B.A., CZOSNEK H., SHOSEYOV O. 2007. Expression and subcellular compartmentation of *Aspergillus niger* β -glucosidase in transgenic tobacco result in an increased insecticidal activity on whiteflies (*Bemisia tabaci*). *Plant Science* **172**(6): 1175–1181.
- YAPI D.Y.A., GNARKI D., NIAMKE S.L., KOUAME L.P. 2009. Purification and biochemical characterization of a specific β -glucosidase from the digestive fluid of larvae of the palm weevil, *Rhynchophorus palmarum*. *Journal of Insect Science* **9**: 4.
- YU J. 1989. β -Glucosidase in four phytophagous Lepidoptera. *Insect Biochemistry* **19**(1): 103–108.
- ZAGROBELNY M., BAK S., RASMUSSEN A.V., JORGENSEN B., NAUMANN C.M., MOLLER B.L. 2004. Cyanogenic glucosides and plant-insect interactions. *Phytochemistry* **65**(3): 293–306.

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