J. Crop Prot. 2017, 6 (3): 377-389

Research Article

Biochemical characterization of digestive carbohydrases of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) larvae in response to feeding on six tomato cultivars

Fereshteh Sadeghinasab[1*], Seyed Ali Safavi[1], Mohammad Ghadamyari[2] and Vahid Hosseininaveh[3]

1. Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran.
2. Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.
3. Department of Plant protection, Faculty of Agricultural Sciences and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Abstract: The tomato leaf miner, *Tuta absoluta* (Meyrick) is an imported pest and serious threat to tomato production in farms and greenhouses of Iran. Use of genetically engineered plants expressing carbohydrase inhibitors is one of the non-chemical methods for controlling insect pests, and knowledge about enzymatic properties of carbohydrases will help us to achieve this goal. Therefore, in present study we characterized biochemical properties of digestive carbohydrases in the midgut of last larval instar of *T. absoluta* fed on different tomato cultivars (Kingston, Riogrande, Super Luna, Super Chief, Super strain B and Calj). While the highest amylolytic activity was on Super strain B, the lowest was on Super Chief. The optimal pH and temperature for α-amylase were found to be at pH 9.0 and 45 °C, respectively. As calculated from Lineweaver-Burk plots, the highest K_m and V_{max} values for α-amylase obtained in Super Chief and Super Luna cultivars were 0.565 ± 0.11mM and 2.287 ± 0.4mM/min, respectively. The effects of different compounds on amylolytic activity indicated that $CaCl_2$, $MgCl_2$, NaCl and KCl increased amylase activity, whereas EDTA, $ZnCl_2$ and $BaCl_2$ decreased the enzyme activity in Super Luna cultivar. The highest activity of α-/ß-glucosidases was observed at pH 6.0 and 7.0, respectively, whereas the optimal pH for α/ß-galactosidases was at 5.0. The highest specific activity of α-/ß-glucosidases was determined in Riogrande-fed larvae, whereas the highest α/ß-galactosidases activity was in the larvae fed on Riogrande and Calj cultivars, respectively. By the native- PAGE, two bands were clearly detected for α-amylase. Since the larvae reared on Kingston showed lowest carbohydrase activities, this cultivar could possibly be suggested as the least suitable host for feeding of *T. absoluta*.

Keywords: *Tuta absoluta*, α-amylase, α/ß-glycosidase, α/ß-galactosidase

Introduction

Insects and plants are considered just as a part of the complex interactions in the natural ecosystem (Mello and Silva-Filho, 2002). In these complex interactions, various defense mechanisms are developed by plants to protect them against insect attacks. These defensive mechanisms include defensive protein production (Birkett *et al.*, 2000), secondary metabolites (Baldwin, 2001) and trichome density (Fordyce and Agrawal, 2001). On the other hand, various

Handling Editor: Azam Mikani

* **Corresponding author**, e-mail: fs_nasab@yahoo.com
Received: 29 January 2017, Accepted: 25 August 2017
Published online: 16 September 2017

mechanisms have also been developed by insects in encountering the deleterious effects of the proteinase inhibitors existing in their host plants. These counter mechanisms include enhancement of their digestive enzyme activities or production of less sensitive enzymes to inhibitors by the insects (Paulillo *et al.*, 2000); hydrolyzing inhibitors by different types of digestive proteases (Girard *et al.*, 1998); and overproduction of proteinases insensitive to inhibitors using up regulation to compensate for the inhibited ones (Mello and Silva-Filho, 2002).

The tomato leaf miner, *Tuta absoluta* (Meyrick), is one of the most destructive pests of tomato in different countries including Iran that mainly attacks solanaceous crops. This pest has also been considered as one of the important insect pests of tomato crops since 2010 at different regions of Iran (Baniameri and Cheraghian, 2012). The chemical control as the primary method was used for restricting and control of this pest, unfortunately some populations of *T. absoluta* have developed resistance against the applied insecticides (Roditakis *et al.*, 2013; Silva *et al.*, 2011).

The larvae of many lepidopteran species have become capable of feeding on various host plants to get the essential nutrients for their optimal development and growth. The food sources quality and the secondary metabolites or enzymes inhibitors present in host plants, are the two determinant factors that influence the digestive enzymes of the insects. Poor utilization of nutrients and developmental retardation can occur as the consequence of any interference in digestive enzymes' activity by the enzyme-inhibitors of the host plant (Jongsma and Bolter, 1997).

Carbohydrases have major role in digestive physiology and study on their inhibitors has become an important issue. Great potential has been shown by the plant α-amylase inhibitors as a control tool for making plants resistant to pests. These inhibitors are proteins that can be found in diverse types of plants. Moreover, these proteins are considered as vital elements in natural defenses, particularly against pests feeding on starchy food. Insect α-amylase inhibitors are an effective device for controlling of insect pests (Shade *et al*., 1994). There is a little information about carbohydrases in the insects that feed on various host plants. The digestive enzymes activities were investigated in *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae) that fed on six tomato cultivars (Nemati Kalkhoran *et al*. 2013). The larvae reared on the leaves of Cal JN3 and Korral cultivars showed lowest amylolytic activity and the highest amylolytic activity was determined in the larvae fed on the leaves of SUN 6108 f1.

To achieve a better perception and knowledge about the digestive physiology of tomato leaf minor, the present research has attempted to identify the biochemical properties of the carbohydrate hydrolyzing enzymes in the *T. absoluta* midgut after feeding on six tomato cultivars.

Materials and Methods

Chemicals

Starch, acrylamide, bisacrylamide, ammonium per sulphate p-Nitrophenol and bovine serum albumin were purchased from Merck (Darmstadt, Germany). p-Nitrophenyl-α-D-glucopyranoside (pNαG), p-Nitrophenyl-β-D-glucopyranoside (pNβG), p-Nitrophenyl-α-D-galactopyranoside (pNαGa), and p-Nitrophenyl-β-D-galactopyranoside (pNβGa) were obtained from sigma (St. Louis, USA).

Host plant and insect rearing

Six cultivars of tomato including Kingston, Riogrande, Super Luna, Super Chief, Super strain B and Calj, were used in this study.

The plants were separately planted in plastic pots (19 cm diameter, 14 cm depth) and maintained in insecticide free conditions in a greenhouse at Urmia Agricultural Research Institute, West Azerbaijan, Iran. *T. absoluta* larvae were originally collected

from tomato fields in west Azerbaijan province, Urmia, Isakan (N 37°32'15.1" E 45°14'38.5"). Newly emerged adults were transferred to rearing cages (50 ×50 × 80 cm) containing 2–3 insect free potted tomato plants at a sex ratio of 2 females:3 males and fed with 10% sugar solution. The tomato plants infested with *T. absoluta* eggs were placed in an insect-proof rearing cage to allow larval development. The insects reared separately on each cultivar for three generations and synchronized 4th-instar larvae were used for experiments. All experiments were conducted under laboratory conditions at 26 ± 1ºC, 60-70% RH and 16:8 h light: dark photoperiod.

Sample preparation and enzyme assays

Fourth-instar larvae reared on different tomato cultivars were immobilized on ice and dissected under a stereomicroscope. Midgut was collected and transferred to a freezer (-80 ºC). For measuring the enzyme activity, the samples were homogenized with a hand-held glass grinder on ice, and the homogenates were centrifuged at 12000 × g for 10 min at 4 ºC.

α-amylase activity and its kinetic parameters

The dinitrosalicylic acid (DNS) procedure was used to determine the activity of α-amylase (Bernfeld, 1955). The supernatant (10µl) was added to a tube containing 40 µl of the buffer (50 mM; phosphate, boric-citric acid and glycine) and 50µl of 1% (w/v) starch and incubated for 30 min. The reaction was stopped by the addition of 100 µl DNS. The mixture was heated in boiling water for 10 min. Finally, the absorbance was recorded at 540 nm with a microplate reader model Stat Fax®3200 (Awareness Technology Inc.). Appropriate blanks were included in the experiments as well as. All assays were performed in triplicate.

Catalytic activities (kinetic parameters) of the enzymes were investigated at different concentrations of starch in a range of 0.03-1% (w/v) in 40µl universal buffer, pH 9.0. The Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) were estimated from the Lineweaver- Burk plots. The kinetic values are the averages of three experiments.

Effect of temperature on α-amylase activity

The activity of α-amylase was determined by incubating the reaction mixture at 15, 25, 35, 45, 55 and 65 ºC for 30 min. After the incubation time, remain activity was measured as mentioned above.

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

The activities of α- and β-glucosidases and α- and β-galactosidases were measured with p-Nitrophenyl-α-D-glucopyranoside (pNαG), p-nitrophenyl-β-D-glucopyranoside (pNβG), p-nitrophenyl-α-D-galactopyranoside (pNαGa) and p-nitrophenyl-β-D-galactopyranoside (pNβGa) as substrates, respectively, based on Low *et al.* (1986). Homogenates were incubated for 30 min at 37 ºC with 45µl of substrate (25mM) and 115µl of 50mM phosphate, boric-citric acid and glycine mixed buffer. The reaction was stopped by addition of 600µl of NaOH (0.25M). Optical density was measured at 405nm using microplate reader Stat Fax®3200 (Awareness Technology Inc.). Blanks without enzymes were included.

Effect of pH on enzyme activity

The effect of different pH on the activities of α-amylase, α- and β-glucosidases and α-and β-galactosidases were determined at room temperature in a mixed buffer containing phosphate, boric-citric acid and glycine (50mM of each) adjusted to various pH values (pH 3.0 to 12.0) by adding HCl or NaOH for acidic and basic pH values, respectively.

Protein concentration

Protein concentrations were estimated as originally described by Bradford (1976), using bovine serum albumin as standard.

Effect of activators and inhibitors on α-amylase activity

The effects of various metal ions including Na^+, K^+, Mg^{2+}, Ba^{2+}, Zn^{2+}, Ca^{2+} and ethylene diamine tetraacetic acid (EDTA) (20 mM) on α-amylase activity were determined. After 30 min of incubation of ions and EDTA with enzyme at room temperature, remain activity was measured as mentioned above.

Polyacrylamide gel electrophoresis and zymogram analysis

The visualization of α-amylase activity present in homogenates of larval midgut fed on different tomato cultivars was carried out using non-denaturing polyacrylamide gel electrophoresis (PAGE) based on the procedure of Davis (1960). Electrophoresis was conducted in an 8% (w/v) separating gel and a 5% stacking gel with 100 V at 4 °C. After running, the gel was incubated in 2.5% (v/v) Triton X-100 for 30 min at room temperature with mild agitation. After incubation, the gel was rinsed with deionized water to remove the triton x-100. Gel was then incubated in 1% starch for 120 min, and gel strips were stained with lugol's solution to detect α-amylase activity as white bands in a dark background.

Statistical analysis

The data were compared by one-way analysis of variance (ANOVA) followed by Tukey's test when significant differences were found at $P \leq 0.05$ using SAS software (SAS Institute 1997).

Results

α-amylase activity and effect of pH and temperature on its activity

Amylolytic activity in midgut extracts from larvae fed on the different tomato cultivars are presented in Table 1. Among the tomato cultivars, larvae reared on Super strain B had the highest and those on Super chief showed the lowest levels of amylolytic activity. Results showed that the optimal pH for α-amylase in the digestive system was 9.0 (Fig. 1). The *T. absoluta* α-amylase has an optimum activity at 45 °C (Fig. 2).

Kinetic parameters of α-amylase

Alpha- amylases revealed a Michaelis-Menten type kinetics when hydrolyzing starch at their optimum pH. The K_m and V_{max} values for α-amylase hydrolyzing starch as substrate are presented in Table 2.

Effect of pHs on α- and β-glucosidase and α- and β-galactosidase activities

The effect of pHs on the hydrolytic activity of α- and *β*-glucosidase and α -and *β*-galactosidase towards pNαG, pN*β*G, pNαGa and pN*β*Ga was tested using a mixed buffer containing phosphate, boric-cirtic acid and glycine (50 mM of each) (pH 3.0-12.0). Maximum activity in the midgut was observed at pH 6.0 and 7.0 for α- and *β*-glucosidase, respectively, whereas, the optimal pH for both α-and *β*-galactosidase activity was 5.0 (Fig. 1).

Table 1 The effects of tomato cultivars on specific activity (mM/min/mg protein) of digestive carbohydrases in *Tuta absoluta* larvae.

Enzymes	Specific activity (mM/min/mg protein) (Mean ± SE)					
	Calj	Riogrande	Kingston	Super Luna	Super strain B	Super Chief
α-amylase	4.14 ± 0.38ab	5.05 ± 0.17a	5.11 ± 0.29a	4.18 ± 0.47ab	5.29 ± 0.07a	3.19 ± 0.16b
α-glucosidases	14.24 ± 1.9ab	19.42 ± 0.62a	7.16 ± 0.71c	10.58 ± 0.83bc	9.60 ± 0.38c	7.41 ± 0.50c
β-glucosidases	3.77 ± 0.33b	12.21 ± 0.34a	3.09 ± 0.09b	3.15 ± 0.14b	3.84 ± 0.26b	3.58 ± 0.05b
α-galactosidase	0.99 ± 0.04b	1.82 ± 0.17a	0.75 ± 0.02bc	0.44 ± 0.01cd	0.67 ± 0.03bc	0.27 ± 0.04d
β-galactosidase	1.65 ± 0.11a	1.54 ± 0.26a	0.85 ± 0.08b	1.62 ± 0.05a	1.01 ± 0.07ab	1.11 ± 0.14ab

Different letters show significant differences among values using Tukey's test at $p \leq 0.05$.

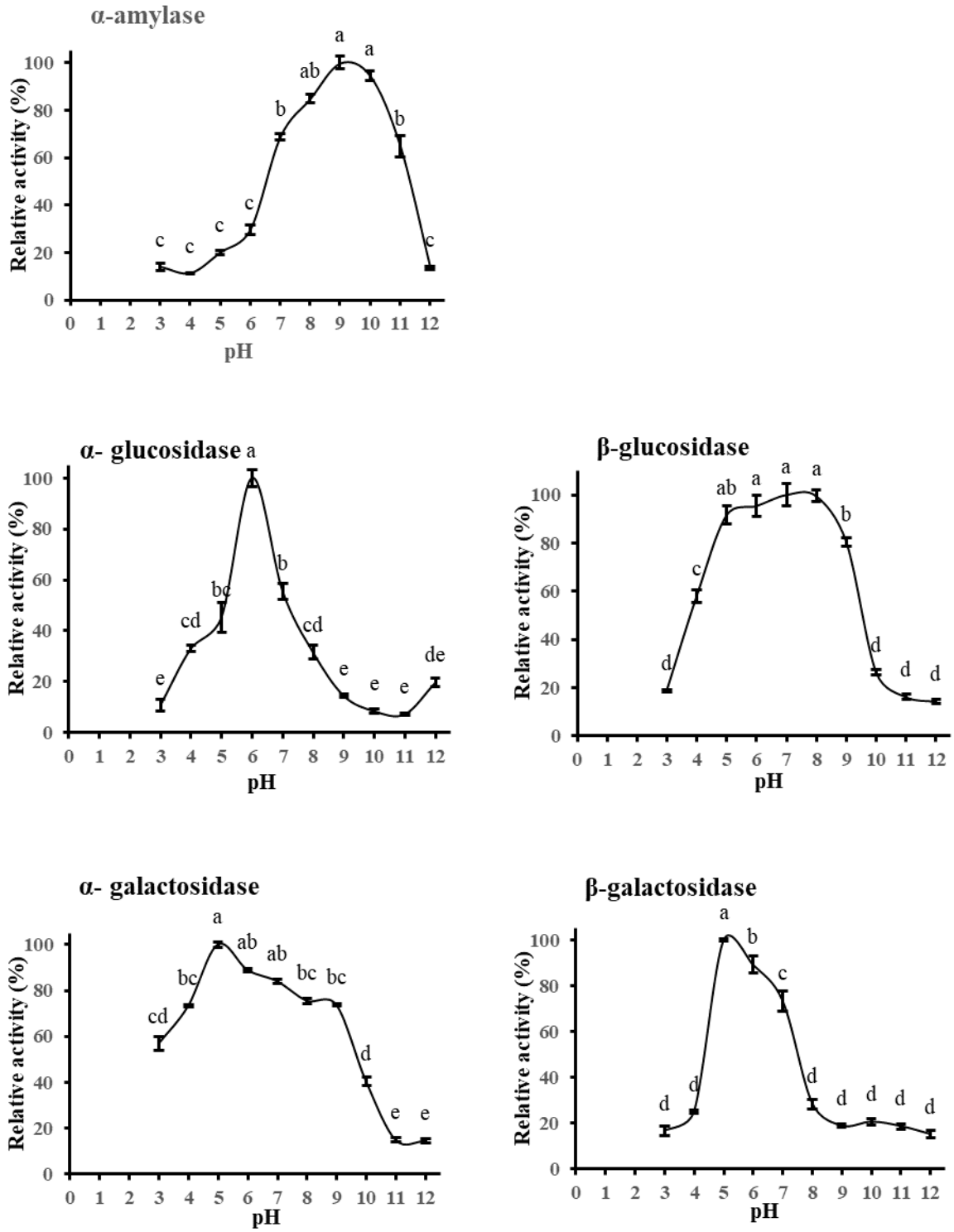

Figure 1 The effect of pHs on the activities of digestive carbohydrases extracted from the midgut of *Tuta absoluta* larvae.

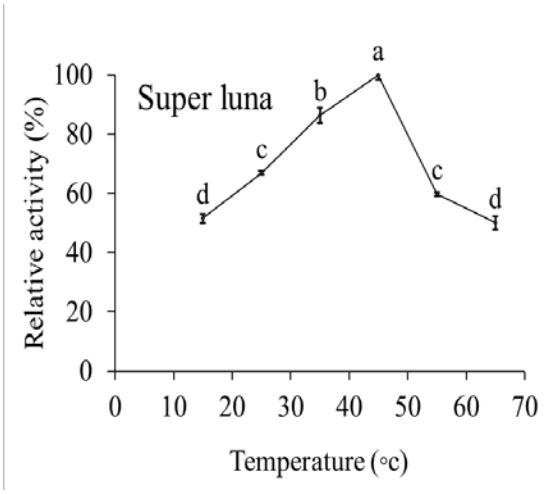

Figure 2 The effect of temperature on the activity of α-amylase extracted from the midgut of *Tuta absoluta* larvae.

Effect of tomato cultivars on activities of α- and β-glucosidase and α- and β-galactosidase

Alpha and β-glucosidase activity of *T. absoluta* reared on six different tomato cultivars are shown in Table 1. The highest level of α-and β-glucosidase activity was present in Riogrande -fed larvae. The lowest α- and β -glucosidase activity was detected in midgut extracts larvae fed on Kingston (Table 1).

The specific activities of α -and *β*-galactosidase in midgut of fourth larval instar were determined. The obtained results showed that the highest α- and β- galactosidase activity was in Riogande and Calj -fed larvae, while, the lowest activity was in midgut extract from larvae fed with Super chief and Kingston, respectively (Table 1).

Effect of activators and inhibitors on α-amylase activity

$CaCl_2$, $MgCl_2$, NaCl and KCl enhanced α-amylase activity, whereas activity of α-amylase was inhibited by ethylene diamine tetra acetic acid (EDTA), $ZnCl_2$ and $BaCl_2$ (Table 3).

Zymogram analysis of α-amylase

The crude extracts of *T. absoluta* were analyzed by native PAGE. After α-amylase activity staining, two major isoforms of α-amylase were clearly detected (Fig. 3).

Table 3 shows effects of different compounds (20 mM) on digestive amylase activity in *T. absoluta* larvae fed on Super Luna cultivar.

Table 3 Effects of different compounds (20 mM) on digestive amylase activity in *Tuta absoluta* larvae fed on Super Luna cultivar.

Compounds	Relative activity (%)
Control (no addition)	100c
Na^+	134.684 ± 8.23b
K^+	127.342 ± 0.81b
Mg^{2+}	191.646 ± 1.84a
Ba^{2+}	76.962 ± 2.60d
Zn^{2+}	48.860 ± 1.69e
Ca^{2+}	192.152 ± 3.42a
EDTA	65.569 ± 1.19de

Different letters show significant differences among values using Tukey's test at $p \leq 0.05$.

Table 2 Effect of tomato cultivars on kinetic parameters of α-amylases extracted from midgut of *Tuta absoluta* larvae.

Cultivars	K_m (mM)	V_{max} (mM/min)	K_m/V_{max}
Calj	0.282 ± 0.06abc	1.786 ± 0.1ab	0.158
Riogande	0.045 ± 0.00c	0.971 ± 0.01c	0.046
Kingston	0.207 ± 0.03bc	1.258 ± 0.1bc	0.165
Super luna	0.179 ± 0.08bc	2.287 ± 0.4a	0.078
Super strain B	0.385 ± 0.06ab	1.240 ± 0.1bc	0.310
Super chief	0.565 ± 0.11a	2.156 ± 0.2ab	0.262

Different letters show significant differences among values using Tukey's test at $p \leq 0.05$.

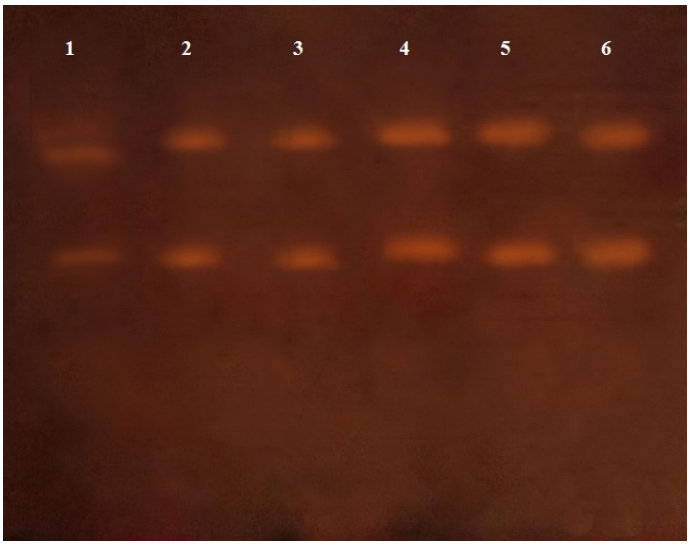

Figure 3 Zymogram of α-amylase extracted from the midgut of *Tuta absoluta* larvae reared on different cultivars (1 Calj, 2 Riogrande, 3 Kingston, 4 Super Luna, 5 Super Chief and 6 Super strain B.).

Discussion

The activities of digestive enzymes are directly influenced by some important factors such as temperature, density of consumed diet, pH (Sivakumar *et al*., 2006) and secondary metabolites in the diet. These enzymes are necessary for providing nutrition and energy to the growing larva of insects. The optimal pH for α-amylase was obtained at alkaline pH in the present research. Regarding the lepidopteran insects, the alkaline pH range has been determined to be thesuitable for the activity of α-amylase. In order to feed on tannin-rich plant materials, lepidopteran insects have adapted to alkaline pH in their midguts (Chapman 1998). In this condition, the digestion efficiency was increased as a result of less binding of tannin with proteins (Dow, 1986). The optimum pH was demonstrated in most of the studies, on lepidopteran α-amylase activity, to be at 8-12, reported e.g. pH 9.0 for α-amylase activity of *Pieris brassicae* (Linn), *Chilo suppressalis* (Walker), *Tecia solanivora* (Povolny) and *H. armigera* (Zibaee *et al*. 2008; Zibaee, 2012; Valencia Jime´nez *et al*. 2008; Ozgur *et al*. 2009). Esmaeily and Bandani (2015) reported the optimum pH of 8.0 for α-amylase activity of *T. absoluta*. Therefore, the consumed chemical compounds and the kind of insect species are two main factors on the optimum pH for the α-amylase activity (Hori, 1971; Zeng and Cohen, 2000; Swart *et al*., 2006).

While, the pH 6.0 and 5.0 were observed to be the optimum activity for α-glucosidase and α-galactosidase in midgut of *T. absoluta*, the β-glucosidase and β-galactosidase activity were shown to have the maximum activity at pH 7.0 and 5.0, respectively. According to Kaur *et al*. (2014), the phylogenetic relation or response of the insect to different diets may suggest the dissimilarities in the insect's maximum pH of α- and β-glucosidases and α- and β-galactosidases.

We also evaluated the α-amylase activity of *T. absoluta* fourth instar larvae fed on different cultivars. Our results showed that the food quality or the chemical and inhibitor compounds existing in food maybe essential factors in the activity of carbohydrases in midgut of insects as previously proved by Slansky (1982) and Mendiola-Olaya *et al*. (2000). The results revealed that the highest and lowest amylolytic activity were detected in larvae reared on Super strain B and Super chief, respectively, compared to other cultivars. This

result suggests that, the responsible factor for any significant differences in the larvae's amylase activities can be due to the dissimilarities in carbohydrate contents in different host plant cultivars. In a study, the influence of various tomato cultivars on the digestive enzymes activities of *H. armigera* larvae was investigated (Nemati Kalkhoran *et al.* 2013). The results of their study demonstrated that the highest amylolytic activity (0.062 ± 0.00004 mU mg^{-1}) was shown by the larvae reared on the leaves of SUN 6108 f1, while, the larvae fed on the leaves of Cal JN3 as well as Korral had the lowest activities of 0.027 ± 0.00004 mU mg^{-1} and 0.027 ± 0.0001 mU mg^{-1}, respectively. Insect species and the physiological diversities in the tomato cultivars are possibly among the reasons for such disagreements.

Measuring activity of α-/β-glucosidases and α-/β-galactosidases in last larval instar of *T. absoluta* fed on various tomato cultivars showed significant differences in their specific activities. Availability of compounds containing β-glycosidic bonds in insect diet can influence the glucosidase and galactosidase activities (Gholamzadeh Chitgar *et al.* 2014). The previous studies on phytophagous lepidopterans showed the presence of α- and β-glucosidase in the salivary glands and in the midgut (Ghadamyari *et al.*, 2010; Franzl *et al*., 1989; Marana *et al*., 2000). Comparing the tested carbohydrases, the highest activity was shown by α–glucosidase. The obtained findings agree with the results of Riseh *et al*. (2012). They reported that the activity of α-glucosidase was higher than that of other galactosidases and glucosidases in the *Rhynchophorus ferrugineus* Olivieri larvae's digestive system and female adult. Also, similar results were obtained for the *Choreutis nemorana* (Hübner) last larvae instar (Gholamzadeh Chitgar *et al.* 2014). Contrary to these findings, high activity of β-glucosidase was found by Ferreira *et al* (1998) in a number of insects belonging to diverse orders such as *Tenebrio molitor* (L.) (Col.: Tenebrionidae), *Abracris flavolineata* (De Geer) (Orth.: Acrididae) and *Scaptotrigona bipunctata* (Lepeletier). (Hym.: Apidae). Additionally, according to Aghaali *et al.* (2012), comparing to other glucosidases and galactosidases, β-galactosidase activity was higher in the digestive system of *Osphranteria coerulescens* Redt. larvae than that of other carbohydrases.

The reaction rate of enzymatic-catalysis will increase with temperature due to the increase in kinetic energy as well as the interaction frequency of molecules. Kaur *et al*. (2014) found that α-amylase is active over a wide range of temperatures from 30-60 °C. Accordingly, the optimum temperature activity for α-amylase of *T. absoluta* was obtained at 45 °C.

The substrate's affinity of enzyme is determined by K_m. High amount of K_m value would results in less affinity between substrate and enzyme. In addition, as demonstrated by Kaur *et al*. (2014), when an enzyme is completely saturated with substrate, the number of converted substrate molecules into product by an enzyme in a unit time is revealed by V_{max}. In order to find the kinetic parameters of α-amylase extracted from *T. absoluta* larvae in the presence of starch substrate, Linweaver-Burk analysis was applied. Regarding α-amylase in Super Chief and Super Luna cultivars, the findings showed 0.565 ± 0.11 mM and 2.287 ± 0.4 mM/min as the highest K_m and V_{max} values, respectively.

The K_m values for the α-amylase of *Bombyx mori* (L.), *P. brassicae* and *Mamestra brassicae* (L.), were obtained as 0.57, 1.37 and 0.33 mg/ml, respectively (Tanabe and Kusano, 1984; Sharifloo *et al*., 2016; Matsumura, 1934). According to the report by Sharifi *et al.* (2011), using starch as substrate, the K_m and V_{max} values of 1.34 mg/ml and 1.52 µmol/min/mg protein were obtained for α-amylase of *Xanthogaleruca luteola* (Müller), respectively. In addition, in another study by Ramzi and Hosseininaveh (2010), the K_m and V_{max} values of 0.77 mM and 0.064 µmol min^{-1}were observed for the α-amylase in *Brachynema germari* Kolenati midgut, respectively.

Insect α-amylases are metallo-enzymes which need metal ions for maintaining integrity, structural stability and activity (Terra and

Ferreira, 2012; Kaur *et al*. 2014). Mg^{2+}, Ba^{2+}, Fe^{2+}, Mo^{+}, K^{+}, Mg^{+}, Cu^{+}, Hg^{+}, Zn^{2+}, nitrite, urea, phytic acid, tannic acid, EDTA, SDS, tris, triton X-100, triethylene tetramine hexa acetic acid (TTHA), ethylene glycol tetra acetic acid (EGTA) inhibited α-amylase activity of various insects (Kaur *et al.* 2014). Two factors were shown to be the possible cause of the inhibition of amylase by some metal ions. First, this could be due to their binding to either catalytic residue. The other possibility is suggested to be the substitution of Ca^{2+} from the substrate binding site of α-amylase. In the present research $ZnCl_2$, EDTA and $BaCl_2$ decreased amylase activity in *T. absoluta* larvae significantly. The presence of $CaCl_2$, $MgCl_2$, NaCl and KCl were shown to increase the activities of the enzyme. These findings are in accordance with the results of previous studies (Zibaee *et al.*, 2008; Asadi *et al.*, 2010; Yezdani *et al*., 2010).

The Zymogram analysis showed two bands of α-amylase activity in midgut of *T. absoluta*. Considering other insects such as *Spodoptera littoralis* (Fabricius) and *C .nemorana*, the presence of a mixture of diverse α-amylase iso-enzymes has been stated (Bigham *et al*., 2013; Darvishzadeh *et al*., 2014). Thus, it can be argued that the presence of this diversity in α-amylase iso-enzymes could be associated with the significance of this enzyme in the food digestion of the insect.

Conclusion

According to the results of the present research, the larvae fed on Kingston cultivar showed lowest carbohydrase activity. This could be because of changes in secondary chemicals or digestive system inhibitors existing in this cultivar, which rendered it as unsuitable host for *T.absoluta* larvae. It could beassumed that Kingston cultivar contains a range of inhibitors that will make it a good choice to develop from it *T. absoluta*-resistant transgenic plants. Of course, other biological data such as growth, development, survival and fecundity data are necessary to confirm resistance of this cultivar. Due to the diversity in the activity of carbohydrate hydrolyzing enzymes in *T. absoluta* larvae, depending on their diet, and complex interactions between plant and insect digestive system, the inhibition of the insect digestive system should be considered in designing strategies for sustainable crop protection.

References

Aghaali, N., Ghadamyari, M. and Ajamhasani, M. 2012. Biochemical characterization of glucosidases and galactosidases from rosaceae branch borer, *Osphranteria coerulescens* Redt. (Col.: Cerambycidae). Romanian Journal of Biochemistry, 49 (2): 125-137.

Asadi, A., Ghadamyari, M., Sajedi, H. R., Jalali, J. and Tabari, M. 2010. Biochemical characterization of midgut, salivary glands and haemolymph α-amylases of *Naranga aenescens*. Bulletin of Insectology, 63 (2): 175-181.

Baldwin, I. T. 2001. An ecologically motivated analysis of plant-herbivore interactions in native tobacco. Plant Physiology, 127: 1449-1458.

Baniameri V. and Cheraghian A. 2012. The first report and control strategies of *Tuta absoluta* in Iran. Bulletin OEPP/EPPO Bulletin, 42 (2): 322-324.

Bernfeld, P. 1955. Amylase, α and β. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in Enzymology. Academic Press, New York, pp.149-158.

Bigham, M., Hosseininaveh V. and Darvishzadeh A. 2013. Activity of digestive proteinases and carbohydrases in the alimentary tract of the fig leaf roller, *Choreutis nemorana* Hübner (Lepidoptera: Choreutidae). Archives Phytopathology and Plant Protection, 46: 2035-2042.

Birkett, M. A., Campbell, C. A. M., Chamberlain, K., Guerrieri, E., Hick, A. J., Martin, J. L., Matthes, M., Napier, J. A., Pettersson, J., Pickett, J. A., Poppy, G. M., Pow, E. M., Pye, B. J., Smart, L. E.,

Wadhams, G. H., Wadhams, L. J. and Woodcock, C. M. 2000. New roles for *cis*-jasmone as an insect semiochemical and in plant defense. Proceedings of the National Academy of Sciences of the United States of America, 97: 9329-9334.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

Chapman, R. F. 1998. The Insects: Structure and Function. Cambridge University Press, Cambridge, 788 pages.

Darvishzadeh, A., Hosseininaveh, V. and Salimian Rizi, S. 2014. Enzymatic activity of α-amylase in alimentary tract *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae): Characterization and Compartmentalization. Arthropods, 3 (3): 138-146.

Davis, B. J. 1960. Disc electrophoresis II. Method and application to human serum protein. Annals of the New York Academy of Sciences, 121: 404-427.

Dow, J. A. T. 1986. Insect midgut function. Advances in Insect Physiology, 19: 187-328.

Esmaeily, M. and Bandani, A. R. 2015. Interaction between larval *α*-amylase of the tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) and proteinaceous extracts from plant seeds. Journal of Plant Protection Research, 55 (3): 278-286.

Ferreira, C., Torres, B. B. and Terra, W. R 1998. Substrate specificities of midgut β-glycosidases from insects of different orders. Comparative Biochemistry and Physiology, 119B: 219-225.

Franzl, S., Ackermann, I. and Nahrstedt, A. 1989. Purification and characterization of a β-glucosidase (linamarase) from the haemolymph of *Zygaena trifolii* Esper 1783 (Insecta, Lepidoptera). Experientia, 45: 712-718.

Fordyce, J. A. and Agrawal, A. A. 2001. The role of plant trichomes and caterpillar group size on growth and defense of the pipe vine swallowtail *Battus philenor*. Journal of Animal Ecology, 70: 997-1005.

Ghadamyari, M., Hosseininaveh, V. and Sharifi, M. 2010. Partial biochemical characterization of α- and β-glucosidases of lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lep.: Pyralidae). Comptes Rendus Biologies, 333: 197-204.

Gholamzadeh Chitgar, M., Ghadamyari, M., Sharifi, M. and Hassan Sajedi, R. 2014. Partial characterization digestive carbohydrases midgut Figure tree skeletonizer moth *Choreutis nemorana* (Lep.: Choreutidae). Trakia Journal of Sciences, 12 (1): 27-37.

Girard, C., Le Métayer, M., Bonadé-Bottino, M., Pham-Delègue, M. H. and Jouanin, L. 1998. High level of resistance to proteinase inhibitors may be conferred by proteolytic cleavage in beetle larvae. Insect Biochemistry and Molecular Biology, 28: 229-237.

Hori, K. 1971. Studies on the feeding habits of *Lygus disponsi* Linnavuori (Hemiptera: Miridae) and the injury to its host plants. I. Histological observations of the injury. Applied Entomology and Zoology, 6: 84-90.

Jongsma, M. A. and Bolter, C. 1997. The adaptation of insect to plant protease inhibitors. Journal of Insect Physiology, 43: 885-896.

Kaur R., Kaur N. and Gupta A. K. 2014. Structural features, substrate specificity, kinetic properties of insect α-amylase and specificity of plant α-amylase inhibitors. Biochemistry and Physiology, 116: 83-93.

Low, N. H., Vong, K. V. and Spornes, P. 1986. A new enzyme, β-glucosidase in honey. Journal of Apicultural Research, 25: 178-181.

Marana, S. R., Terra, W. R. and Ferreira, C. 2000. Purification and properties of a ß-glycosidase purified from midgut cells of *Spodoptera frugiperda* (Lepidoptera) larvae. Insect Biochemistry and Molecular Biology, 30: 1139-1146.

Matsumura, S. 1934. Genetical and physiological studies on amylase activity in the digestive juice and haemolymph of the

silkworm, *Bombyx mori*. L. Bulletin Nagano-Ken Sericulture Experiment Station, 28: 1-24.

Mello, M. O. and Silva-Filho, M. C. 2002. Plant-insect interactions: An evolutionary arms race between two distinct defense mechanisms. Brazilian Journal of Plant Physiology, 14: 71-81.

Mendiola-Olaya, E., Valencia-Jiménez, A., Valdes-Rodriguez, S., Delano-Frier, J. and Blanco-Labra, A. 2000. Digestive amylase from the larger grain borer, *Prostephanus truncatus* Horn. Comparative Biochemistry and Physiology, 126B: 425.433.

Nemati Kalkhoran, M., Naseri, B., Rahimi Namin, F. and Kouhi, D. 2013. Life table parameters and digestive enzymes activity of *Helicoverpa armigera* (Lep.: Noctuidae) on different tomato cultivars. Journal of Entomological Society of Iran, 33 (2): 45-58.

Ozgur, E., Yucel, M. and Oktem, H. A. 2009. Identification and characterization of hydrolytic enzymes from the midgut of the cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Turkish Journal of Agriculture and Forestry 33: 285-294.

Paulillo, L. C. M. S., Lopes, A. R., Cristofoletti, P. T., Parra, J. R. P., Terra, W. R. and Silva-Filho, M. C. 2000. Changes in midgut endopeptidase activity of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) are responsible for adaptation to soybean proteinase inhibitors. Journal of Economic Entomology, 93: 892-896.

Ramzi, S. and 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: 215-219.

Riseh, N. S., Ghadamyari, M. and Motamediniya, B. 2012. Biochemical characterization of α & β-glucosidases and α- & β-galactosidases from red palm weevil, *Rhynchophorus ferrugineus* Olivieri (Col.: Curculionide). Plant Protection Science, 48: 85-93.

Roditakis E., Skarmoutsou C. and Staurakaki M. 2013. Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. Pest Management Science, 69: 834-840.

SAS Institute. 1997. SAS/STAT user's guide for personal computers. Cary, NC: SAS Institute.

Shade, R. E., Schroeder, H. E., Pueyo, J. J., Tabe, L. M., Murdock, L. L., Higgins, T. J. V., and Chrispeels, M. J. 1994. Transgenic pea seeds expressing the α-amylase inhibitor of the common bean are resistant to bruchid beetles. Bio/Technology 12: 793-796.

Sharifi, M., Ghadamyari, M., Moghadam, M. M. and Saiidi, F. 2011. Biochemical characterization of digestive carbohydrases from *Xanthogaleruca luteola* and inhibition of its α-amylase by inhibitors extracted from the common bean. Archives of Biological Science, 63: 705-716.

Sharifloo, A. Zibaee, A., Jalal Sendi, J. and Talebi Jahromi, Kh. 2016. Characterization of a Digestive α-Amylase in the Midgut of *Pieris brassicae* L. (Lepidoptera: Pieridae) , Frontiers in Physiology, 7: 96.

Silva G. A., Picanço M. C. and Bacci L., Crespo A. L. B., Rosado J. F. 2011. Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. Pest Management Science, 67: 913-920.

Sivakumar, S., Mohan, M., Franco, O. L. and Thayumanavan, B. 2006. Inhibition of insect pest α-amylases by little and finger millet inhibitors. Biochemistry and Physiology, 85: 155-160.

Slansky, F. 1982. Insect nutrition: an adaptationists perspective. The Florida Entomologist, 65: 45-71.

Swart, C. C., Deaton, L. E. and Felgenhauer, B. E. 2006. The salivary gland and salivary enzyme of the giant water bugs (Heteroptera; Belostomatidae). Comparative Biochemistry and Physiology, 145: 114-122.

Tanabe, S. and Kusano, T. 1984. Changes of the haemolymph amylase activity during development and its properties in the

cabbage armyworm, *Mamestra brassicae* L. Kontyu, 542 (4): 472-481.

Terra, W. R. and Ferreira, C. 2012. Biochemistry and molecular biology of digestion, In: Gilbert, L. I. (Ed.), Insect Molecular Biology and Biochemistry, Elsevier, New York, pp: 365-418.

Valencia-Jiménez A., Arboleda V. J. W., López-Ávila A. L. and Grosside-Sá M. F. 2008. Digestive α-amylases from *Tecia solanivora* larvae (Lepidoptera: Gelechiidae): response to pH, temperature and plant amylase inhibitors. Bulletin of Entomological Research, 98 (06): 575-579.

Yezdani, E., Sendi, J. J., Zibaee, A. and Ghadamyari, M. 2010. Enzymatic properties of α-amylase in the midgut and the salivary glands of mulberry moth, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). Comptes Rendus Biologies, 333: 17-22.

Zeng, F. and Cohen, A. C. 2000. Partial characterization of α-amylase in the salivary glands of *Lygus hesperus* and *L. Lineolaris*. Comparative Biochemistry and Physiology, 126B: 9-16.

Zibaee, A., Bandani, A. R., Kafil, M. and Ramzi, S. 2008. Characterization of α-amylase in the midgut and the salivary glands of rice striped stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). Journal of Asia-Pacific Entomology, 11 (4): 201-205.

Zibaee A. 2012. Digestive enzymes of large cabbage white butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) from developmental and site of activity perspectives. Italian Journal of Zoology, 79: 13-26.

تعیین خصوصیات بیوشیمیایی کربوهیدرازهای گوارشی در لارو شب‌پره مینوز گوجه‌فرنگی *Tuta absoluta* (Lep.: Gelechidae) در پاسخ به تغذیه از شش رقم گوجه‌فرنگی

فرشته صادقی نسب[1*]، سیّدعلی صفوی[1]، محمّد قدمیاری[2] و وحید حسینی نوه[3]

۱- گروه گیاه‌پزشکی، دانشکده علوم کشاورزی، دانشگاه ارومیه، ایران.
۲- گروه گیاه‌پزشکی، دانشکده علوم کشاورزی، دانشگاه گیلان، ایران.
۳- گروه گیاه‌پزشکی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران.

* پست الکترونیکی نویسنده مسئول مکاتبه: fs_nasab@yahoo.com
دریافت: ۱۰ بهمن ۱۳۹۵؛ پذیرش: ۳ شهریور ۱۳۹۶

چکیده: شب‌پره مینوز گوجه‌فرنگی، *Tuta absoluta* (Meyrick) تهدیدی جدی برای تولید محصول گوجه‌فرنگی در مزارع و گلخانه‌های ایران محسوب می‌شود. یکی از روش‌های غیرشیمیایی کنترل آفات استفاده از گیاهان تراریخته حاوی مهارکننده‌های کربوهیدرازها می‌باشد، بنابراین مطالعه خصوصیات آنزیمی کربوهیدرازها برای دستیابی به این هدف به ما کمک خواهد کرد. در پژوهش حاضر ویژگی‌های بیوشیمیایی کربوهیدرازهای گوارشی استخراج شده از روده میانی لارو سن آخر شب‌پره مینوز پرورش یافته روی ارقام مختلف گوجه‌فرنگی (Kingston, Riogrande, Super Luna, Super Chief, Super strain B, Calj) تعیین شد. بیش‌ترین فعالیت ویژه آلفا-آمیلاز در لاروهای تغذیه کرده از رقم Super strain B و کم‌ترین آن در رقم Super Chief مشاهده شد. اسیدیته و دمای بهینه برای فعالیت آلفا-آمیلاز به‌ترتیب ۹ و ۴۵ درجه سلسیوس تعیین گردید. بیش‌ترین مقادیر K_m و V_{max} در لاروهای تغذیه کرده از ارقام Super Chief (۰/۱۱ ± ۵۶۵. ۰میلی‌مولار) و Super Luna (۰/۴ ± ۲/۲۸۷ میلی‌مولار بر دقیقه) به‌دست آمد. اثر ترکیبات مختلف روی فعالیت آمیلولیتیک نشان داد که ترکیبات $CaCl_2$, $MgCl_2$, NaCl و KCl به‌طور معنی‌داری فعالیت آلفا-آمیلاز را افزایش دادند، در حالی‌که EDTA، ZnCl2 وBaCl2 موجب کاهش فعالیت این آنزیم در رقم سوپر لونا شدند. بیش‌ترین فعالیت آلفا و بتا-گلوکوزیداز به‌ترتیب در اسیدیته ۶ و ۷ به‌دست آمد، در حالی‌که مقدار اسیدیته بهینه برای فعالیت آلفا و بتا-گالاکتوزیداز برابر ۵ بود. بیش‌ترین فعالیت ویژه آلفا و بتا-گلوکوزیداز در لاروهای تغذیه کرده از رقم Riogrande مشاهده شد، درحالی‌که بیش‌ترین فعالیت ویژه آلفا و بتا-گالاکتوزیداز به‌ترتیب در لاروهای تغذیه کرده از ارقام Riogrande و Calj به‌دست آمد. تعداد باندهای مشاهده شده روی ژل برای آلفا-آمیلاز برابر ۲ بود. از آن‌جایی‌که لاروهای تغذیه کرده از رقم Kingston کم‌ترین فعالیت کربوهیدرازی را نشان دادند احتمالاً این رقم میزبان نامناسبی برای شب‌پره مینوز گوجه‌فرنگی می‌باشد.

واژگان کلیدی: *Tuta absoluta* ، آلفا-آمیلاز، آلفا و بتا-گلوکوزیداز، آلفا و بتا-گالاکتوزیداز