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Research Article

Targeting *Plutella xylostella* digestive enzymes by applying resistant Brassicaceae host cultivars

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Abstract: The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most destructive insect pests, feeding exclusively on wild and cultivated cruciferous species. The attacked plants produce considerable amount of glucosinolates in response to insects' feeding. Herein, we studied digestive activities of *P. xylostella* on four different genotypes of family Brassicaceae including two canola cultivars (SLM_{046} and RGS_{003}) and two cabbage cultivars (Green-Cornet and Glob-Master). The highest proteolytic and amylolytic activities of *P. xylostella* were observed on Green-Cornet and the lowest occurred on RGS_{003} and Glob-Master, respectively. The highest activity of α-glucosidase and β-glucosidases were observed on Green-Cornet and SLM_{046} and the lowest was observed on Glob-Master and RGS_{003}. The zymogram analysis revealed different isozymes of protease, trypsin-like and α-amylase in the midgut extract of *P. xylostella*. Activity of the above mentioned isozymes was inhibited in larvae feeding on RGS_{003} and Glob Master as resistant host cultivars. Also, larvae feeding on the resistant genotypes showed more glucosidase activities, indicating possibility of high glycosinolate existence in the resistant genotypes. By these results we can state that host plant property can affect insect digestive physiology through inhibiting digestive enzyme activities. These findings provide insights into the direct effects of host plants on insect physiology which are conducive to change in insect fitness.

Keywords: Brassicaceae, digestive enzymes, plant resistance, *Plutella xylostella*

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a specialist herbivore on Brassicaceae (Talekar and Shelton, 1993), causing serious damage to various oilseed (canola) and food crops (Sarauer *et al.*, 2003). Excessive application of chemical pesticides has led to increased insecticide-resistance in *P. xylostella* (Chougule *et al.*, 2008). Furthermore, *P. xylostella* was the first crop pest which showed resistance to *Bacillus thuringiensis* toxin in field condition, indicating high potential of *P. xylostella* to show resistance to other different

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groups of insecticides. It means that more attention should be devoted to find efficient non-chemical approaches to control *P. xylostella* and managing the resistance to this pest. Disruption of insect midgut physiology and digestive biochemistry is a benign approach which can be used instead of chemicals to delay the resistance development in *P. xylostella* (Sarauer *et al.*, 2003; Kotkar *et al.*, 2009). Alternate trophic levels can be in positive interaction and regarding any change in the lower trophic levels may affect the upper trophic ones (Soufbaf *et al.*, 2012, Kianpour *et al.*, 2014), study of insect digestive enzymes feeding on genetically different host plants can help to unveil resistant host plants and secondary metabolites involved in the resistance to be used in pest control strategies (Behmer and Grebenok, 1998; Winter and Bergelson, 2001; Mehrabadi *et al.*, 2012; Marchioro and Foerster, 2013).

The lepidopteran larval midgut embraces a series of protease enzymes including trypsins, chymotrypsins, elastases, cathepsin-B-like proteases, aminopeptidases and carboxypeptidases, all of which play role in protein digestion. Based on recent studies, 95% of total digestive activity in lepidopteran species was done by serine proteases (Bown *et al.*, 1997; Naseri *et al.*, 2010). Other nutrients like polysaccharides and starch & related carbohydrates are catalyzed by amylase and α-amylase, respectively (Franco *et al.*, 2000; Kotkar *et al.*, 2009). Myrosinase is the main enzyme in *P. xylostella* which enables it to feed on resistant Brassicaceous plants through catalyzing glucosinolate (Li *et al.*, 2000).

Although various researchers have investigated different aspects of digestive enzymes in *P. xylostella*, nevertheless the effect of resistant and susceptible host plants on digestive activity of *P. xylostella* has remained approximately unknown (Kotkar *et al.*, 2009; Naseri *et al.*, 2010; Garcia- Carreno *et al.*, 2012). Herein, we aimed to study digestive physiology of *P. xylostella* on various Brassicaceae host plants with emphasis on protease, amylase, glucosidase and galactosidase activity.

Materials and Methods

Insects and plants

Seeds of two cultivars of canola *Brassica napus* L. including SLM_{046}, RGS_{003} and two cultivars of cabbage *Brassica oleracea* var. *capitata* including Glob-Master and Green-Cornet were prepared from Seed and Plant Improvement Institute, Karaj, Iran. Several seeds of *Brassica* plants were sown in a standard potting mix in plastic pots without any fertilizer and pesticides. When they reached 5- to 8- leaf stage (about 5weeks after planting), the plants were transferred to separate pots. The cabbage and canola genotypes were nine and six weeks old, respectively, when they were used in experiments. To colonize *P. xylostella*, its larvae and pupae were collected from cabbage fields of Tehran University and maintained in a growth chamber set at 25 ± 1 ˚C, 65 ± 5% and a photoperiod of 16:8 (L: D) hours. The fourth instars of the F_2 generation were used for all trials.

Sample preparation

The last-larval instars (< 8 h old) were cold-immobilized and dissected under a stereoscopic microscope and their midguts were removed. The isolated midguts were cleaned and transferred into a 2 ml microtubes containing 1.5 ml distilled water and then homogenized using a hand-held glass grinder on ice. The homogenates were centrifuged at 1600 × g for 10 min at 4 ºC and the supernatant was removed and stored at -20 ºC. All experiments were replicated three to five times with appropriate blanks.

Total proteolytic activity

Total proteolytic activity was measured using azocasein as substrate, according to Cohen (1993). A volume of 20 µl of 1.5% Azocasein solution was added to each microtube containing 30 µl of universal buffer (acidity 3 to 12). Trial solution was incubated at 37 ºC for 60 min and then 25 µl of 30% trichloroacetic acid (TCA) was added to the

reaction mixture. In the next step, the reaction mixture was cooled down at 4 ºC for 60 min and centrifuged at 16000 × g for 10 min. An equal volume of 2 M NaOH was added to the supernatant and the absorbance was determined at 450 nm.

The activity of trypsin, chymotrypsin and elastase- like enzymes was measured using BApNA, SAAPFpNA and SAAApNA specific substrate, respectively (Vinokurov *et al.,* 2005). Five microliters of 1 mM substrate were added to 10 µl enzyme extract and 85 µl of universal buffer (acidity 3 to 12) and the absorbance was measured at 405 nm for 40 min at 2 min intervals. Rate of enzymatic hydrolysis of the substrate was evaluated by an increase in its absorbance due to release of 4-nitroaniline. The absorbance is routinely measured at 400 nm or higher (Erlanger *et al.,* 1961).

α-amylase assay

α- amylase activity was evaluated using dinitrosalicylic acid (DNS) method and 1% soluble starch as substrate, according to Bernfeld (1955). Twenty microliter of enzyme extract with 80 µl of 40 mM universal buffer (acidity 3 to 12) and 20 µl substrate were incubated for 30 min at 50 ºC. The reaction was terminated by adding 50 µl DNS and heating in the boiled water for 10 min. The absorbance was measured at 540 nm. One unit of amylase activity was defined as amount of enzyme required to produce 1 mg maltose per 30 min at 37 ºC under conditions of the assay (Kotkat *et al.,* 2009).

Glucosidase and galactosidase assay

Glucosidase and galactosidase activities were measured using specific substrates. Accordingly, pNPαGlu (p-nitrophenyl-α-$_D$-glucopyranside), pNPβGlu (p-nitrophenyl-β-$_D$-glucopyranside), pNPαGala (p-nitrophenyl-α-$_D$- galactosidase) and pNPβGala (p-nitrophenyl-β-$_D$- galactosidase) were used to determine α-glucosidase, β- glucosidase, α-galactosidase and β- galactosidase activities, respectively. To do so, 15 µl of the enzyme extract with 85 µl of 40 mM universal buffer (acidity 3 to 12) and 10 µl 5 mM substrate were incubated at 35 ºC for 30 min and the absorbance was determined at 405 nm after adding 2 M NaOH to terminate the reaction (Li *et al.,* 2000). For p-nitrophenol concentration, molar extinction coefficient was calculated at 405 nm (Hardie, 1993)

Electrophoretic detection of proteolytic activity was performed using a native (Non-denaturing SDS- PAGE) 12.5% and 4% resolving and stacking polyacrylamide gels, respectively (Laemmli, 1970), followed by protocol of Garcia-Carreno *et al.* (1993). The gel was incubated in 2% casein in 50 mM universal buffer for 30 min at 4 °C, and then the gel immersed to the same solution for 90 min at 25 °C. The gel was stained using 0.1% cimmassie brilliant blue R-250 in methanol-acetic acid- water (50: 10: 40). Destaining was done in methanol- acetic acid- water (50: 10: 40) and the bands indicating protease activity appeared as clear zones on a dark blue background.

Specific proteolytic activity was determined using p-nitroanilide substrates BrpNA, SAAPFpNA and SAAApNA, and a native (PAGE) 10% and 4% resolving gel and stacking polyacrylamide gels, respectively. After electrophoresis, the stacking gel was cut and resolving gel was incubated for 15 min in 100 mM universal buffer (pH 10 and 11) for determining the trypsin- cymotrypsin- and elastase- like enzyme activities, respectively. Then, the buffer was removed and the gel was covered with a nitrocellulose membrane that had been presoaked for 40 min in the substrate solution (BApNA, 1 mg ml^{-1}) and slightly air dried. The gel and membrane both were incubated in a moist chamber at 37 °C until the faint yellow bands become visible on the membrane. Then, the gel was removed and the membrane was incubated for 5 min in three solutions serially including 0.1% sodium nitrite in 1 M HCl, 0.5% ammonium sulfanate in 1 M HCl and 0.05% N- (1-naphthyl)-ethylenediamine in 47.5% ethanol. Pink bands on the white background indicating the specific proteolytic activities.

The α-amylase activity was evaluated using a native (PAGE) 10% and 4% resolving gel and stacking polyacrylamide gels, respectively. The sample buffer contained 12 mM $CaCl_2$ in 1% starch in 0.1 M phosphate- acetate- borate buffer with acidity 8.0 after 1 h incubation. The Gel then was washed and incubated by a solution of 10 mM I_2 and 14 mM KI to stop the reaction. Areas of α-amylase activity were visualized as light strips on a dark blue background.

Detection of α-glucosidase activity in gel was done using the fluorescent substrate 4-methylumbelliferyl- α- D- glucopyranoside. After electrophoresis, the gel was incubated with 3 mM substrate in 40 mM phosphate-acetate- borate buffer (pH 6.0) for 30 min. The bands of α-glucosidase activities were shown as fluorescent band under UV.

Protein assay

Protein concentrations were measured with bovine serum albumin as a standard (2, 1.5, 1, 0.5, 0.125 and 0.063 mg mL^{-1}) (Lowry *et al.*, 1951).

Data Analysis

The Kolmogorov- Smirnov test was applied to test for a normal distribution. The data were analyzed by one- way analysis of variance (ANOVA) (SPSS, 14.0). Mean comparison was done using Duncan Multiple Range test ($p < 0.05$).

Result

Protease activity

The effect of pH on general protease activity of midgut extract is shown in Fig. 1. As shown, the lowest and highest proteolytic activities were observed at pH 5.0-6.0 (acidic conditions) and pH 9.0, respectively. Figure 2 shows that the highest protease activity was observed in larvae feeding on Green-Cornet and SLM_{046} genotypes. The specific proteolytic activity was also measured using specific substrates (Fig. 3). The high level of specific proteolytic activity was observed over a broad alkaline pH range (pH 9.0-11.0). Like general protease activity pattern, the highest activity of specific protease enzymes was observed on Green-Cornet (Fig. 4A-C). The highest activity of Trypsin- like and Chymotrypsin & Elastase like enzymes occurred at pH 10 and pH 11, respectively.

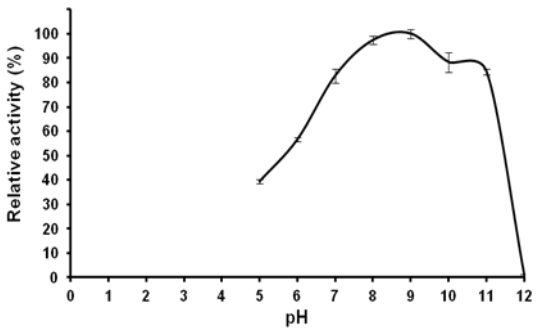

Figure 1 General proteolytic activity in the larval midgut extracts of *Plutella xylostella* at different pHs. Enzymes was assayed in different buffer systems of pH 5.0-12.0 using azocasein as substrate through spectrophotometric method. Bars represent standard errors (± SE).

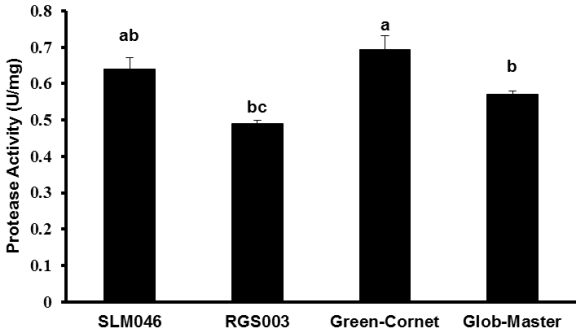

Figure 2 General proteolytic activity of midgut extracts of *Plutella xylostella* in the *Plutella xylostella* larvae reared on the four Brassicaceae cultivars using azocasein as substrate, pH 9.0. Bars represent standard errors (± SE).

α-amylase activity

The effects of different pHs on α-amylase activity showed two different activity peaks in which the first (small peak) was at pH 4.0 and the second (big peak) occurred at pH 8.0 (Fig. 5). The highest and lowest amylolytic activities were seen in larvae feeding on Green-Cornet and Glob Master (the cabbage cultivars) (Fig. 6).

Glucosidase and galactosidase activity

The maximum activity of α-galactosidase was seen at pH 6.0. So, in alkaline pH, α-galactosidase activity decreased and its minimum activity was observed at pH 8.0 (Fig. 7A). Similarly, the β-galactosidase activity had a negative correlation with increased pH and its highest activity was observed at pH 4.0 (Fig. 7B). The α and β-glucosidases had the same activity trend including increased activity by increasing acidity to pH 6.0-7.0. Then the activity decreased by increasing acidity (Fig. 7C-D). The highest α-galactosidase activity was observed on Green-Cornet (Fig. 8A). The larvae fed on SLM_{046} and Glob Master showed the highest β- galactosidase activity ($F= 0.14$, d. f. = 19.0, $p > 0.05$) (Fig. 8B). Different Brassicaceae cultivars showed no significant difference in α- glucosidase activity (Fig. 8C). The high activity of β- glucosidase was observed on SLM_{046} and Green-Cornet cultivars (Fig. 8D).

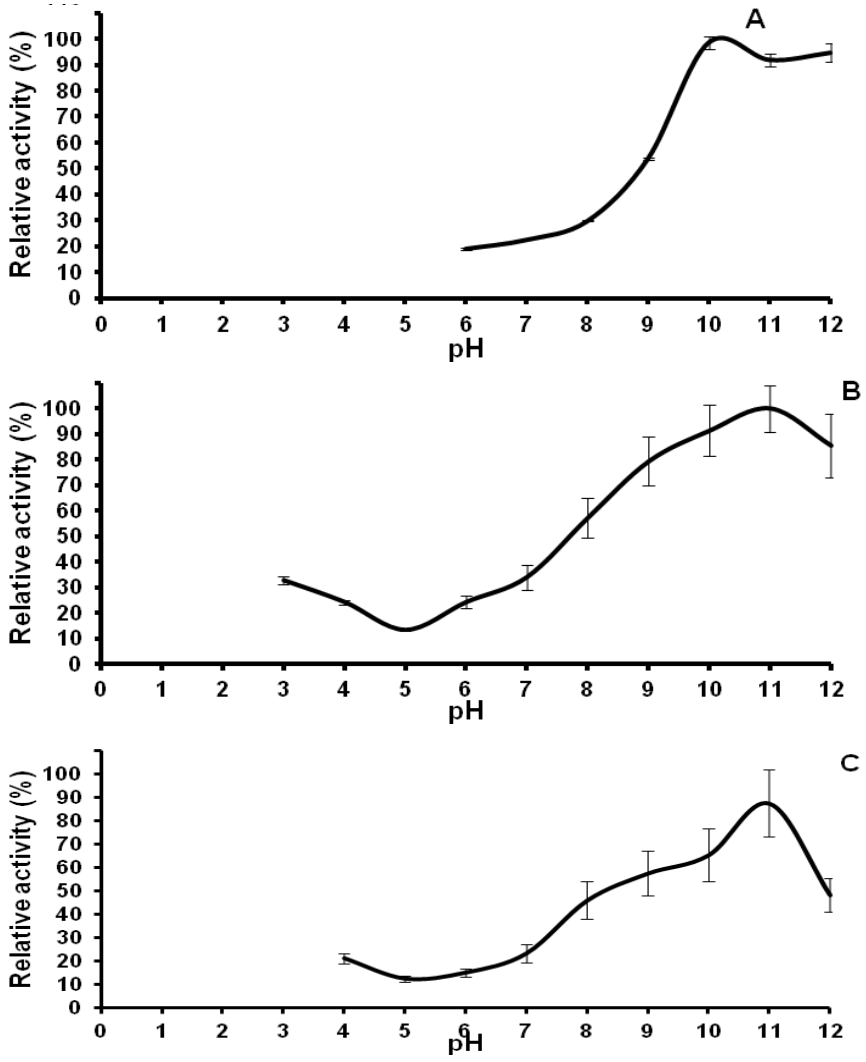

Figure 3 Trypsin- (A), Cymotrypsin- (B) and Elastase- like (C) activities in the larval midgut extracts of *Plutella xylostella* at different pH levels on the serine peptidase substrate. BApNA (A), SAAPFpNA (B) and SAAApNA (C), were used as substrate at different pHs. The high level of specific proteolytic activity was observed over a broad alkaline pH range (pH 9.0-11.0). Bars represent standard errors (± SE).

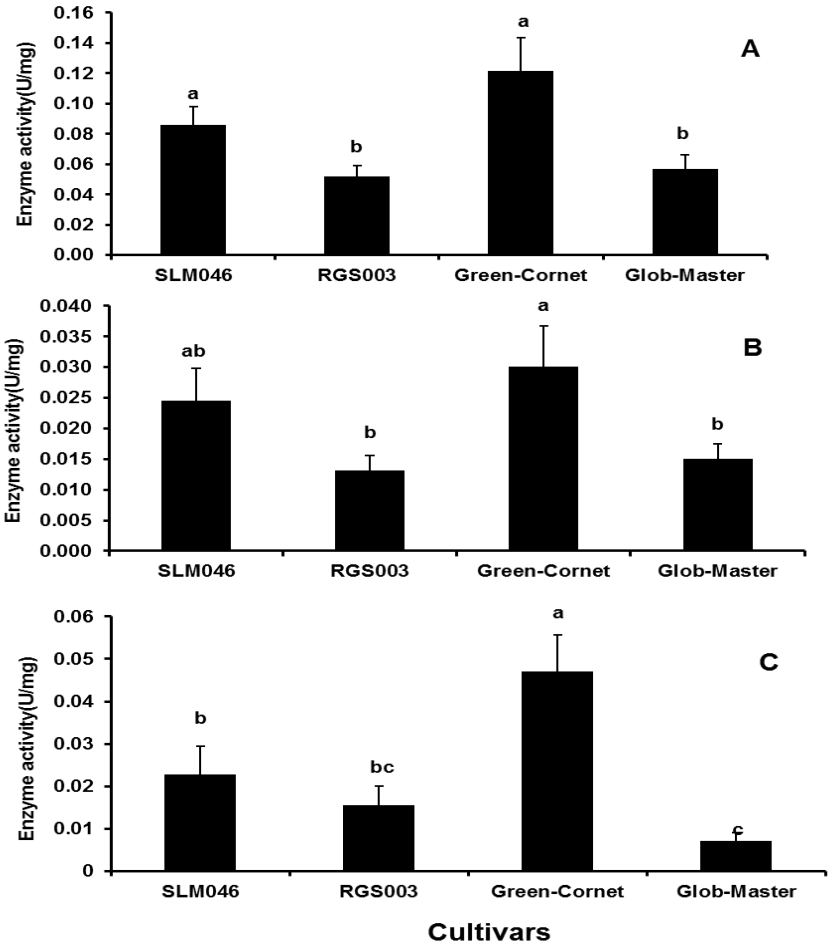

Figure 4 Trypsin- (A), Cymotrypsin- (B) and Elastase- like (C) activities in midgut extracts of the *Plutella xylostella* larvae reared on the four Brassicaceae cultivars, using BApNA (pH 10), SAAPFpNA (pH 11) and SAAApNA (pH 11) as substrate, respectively. Bars represent standard errors (± SE).

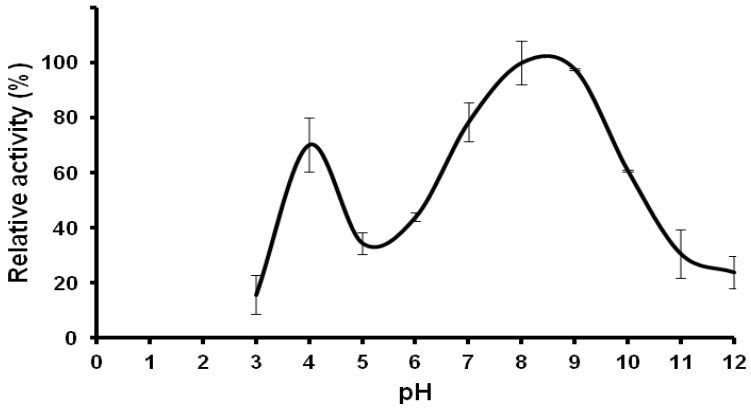

Figure 5 α amylolytic activity in the larval midgut extracts of *Plutella xylostella* measured by spectrophotometric assay in different buffer systems (pH 3.0-12.0) using 1% starch and dinitrosalicylic acid (DNS) method as substrate. Bars represent standard errors (± SE).

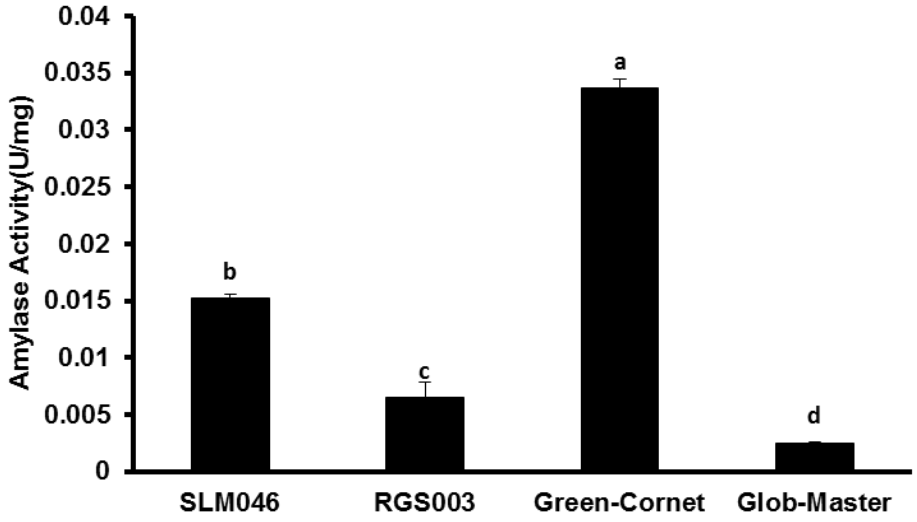

Figure 6 α amylolytic activity of midgut extracts in the *Plutella xylostella* larvae reared on Brassicaceae cultivars using 1% starch as substrate at pH 8.0. Bars represent standard errors (± SE).

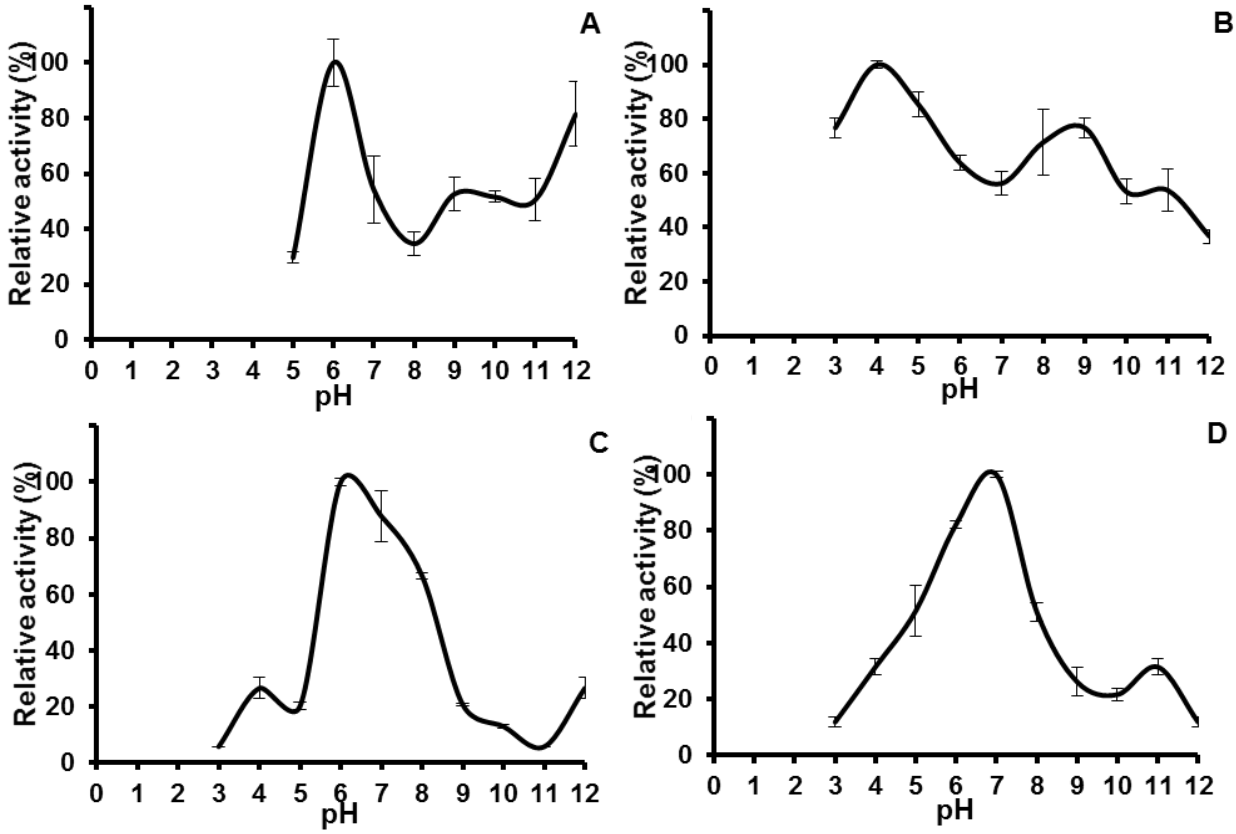

Figure 7 α-galactosidase (A), β-galactosidase (B), α-glucosidase (C) and β-glucosidase (D) activities of larval midgut extracts from *Plutella xylostella* was assayed trough spectrophotometric assay in different pH levels using pNPαGala, pNPβGala pNPαGlu and pNPβGlu as substrates. Bars represent standard errors (± SE).

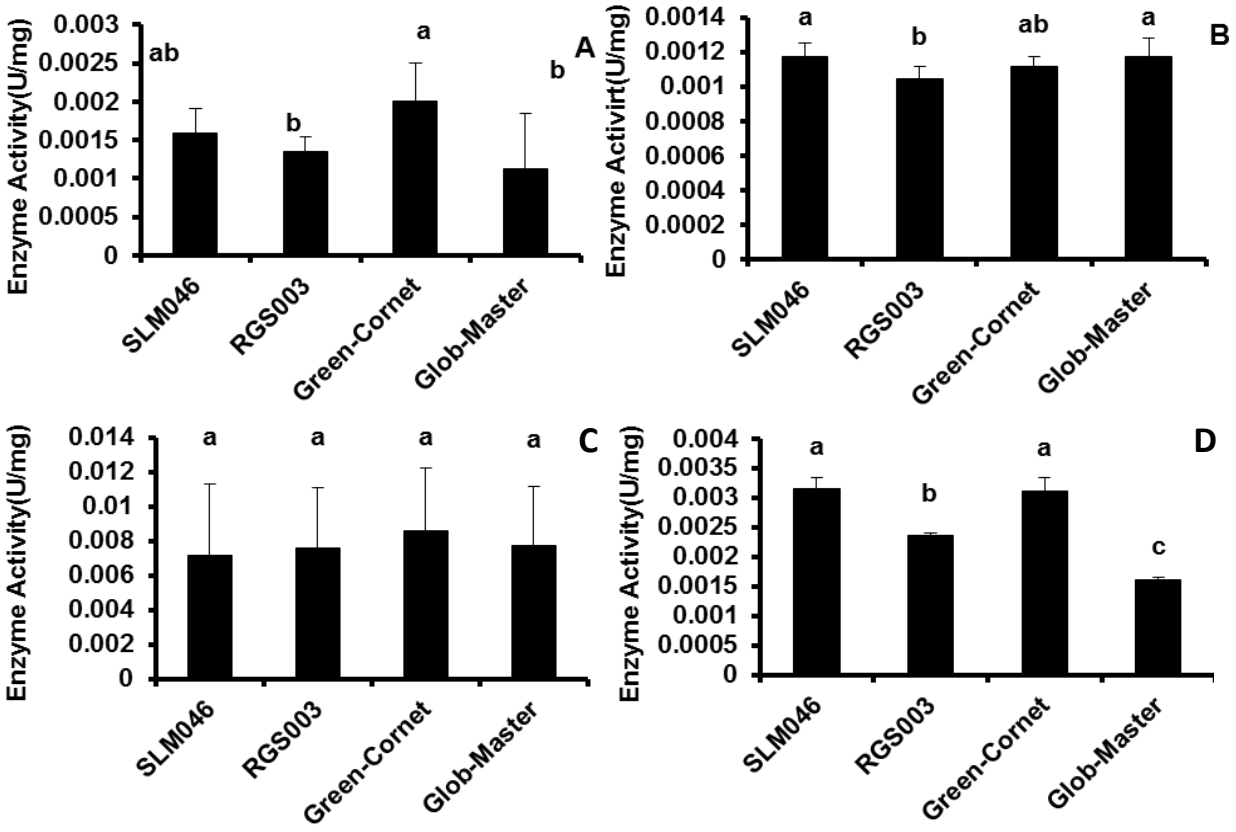

Figure 8 α-galactosidase(A), β-galactosidase (B), α-glucosidase (C) and β-glucosidase (D) activities of larval midgut extracts from *Plutella xylostella* larvae reared on Brassicaceae cultivars using pNPαGala (pH 6), pNPβGala (pH 4), pNPαGlu (pH 6) and pNPβGlu (pH 7) as substrates. Bars represent standard errors (± SE).

Zymogram analysis

Different isoforms of protease were detected in the fourth instar larvae feeding on different Brassicaceae cultivars (Fig. 9). The zymogram analysis confirmed at least five different isoforms of protease in the *P. xylostella* gut extracts (P_1-P_5) which the P_1, P_2 and P_3 were found to be more effective than P_4 and P_5. Also larvae feeding on SLM_{046} and Green-Cornet had higher protease activity than those feeding on the other cultivars. The isoforms pattern in larvae feeding on RGS_{003} and Glob-Master were faint.

Expression of Trypsin-like enzymes spectra was measured using the electrophoresis technique (Fig. 10), unveiled at least four different bands of trypsin-like enzyme activity (T1-T4) in the midgut extract of larvae feeding on different Brassicaceae cultivars. Like protease isoform, the high activity of trypsin-like enzyme was detected in the midgut extract of larvae feeding on SLM_{046} and Green Cornet cultivars. Expression T1 isoform was highly inhibited in RGS_{003} and Glob Master.

Further characterizations of α-amylase activity in *P. xylostella* larvae under native-PAGE and using starch as substrate are shown in Fig. 11. Zymogram analysis also revealed an amylase isozyme in the fourth instar larvae *P. xylostella* (Fig. 11). As seen in the protease zymogram analysis, pattern of α amylase activity was weak in larvae feeding on the RGS_{003} and Glob Master compared with SLM_{046}- and Green-Cornet genotypes.

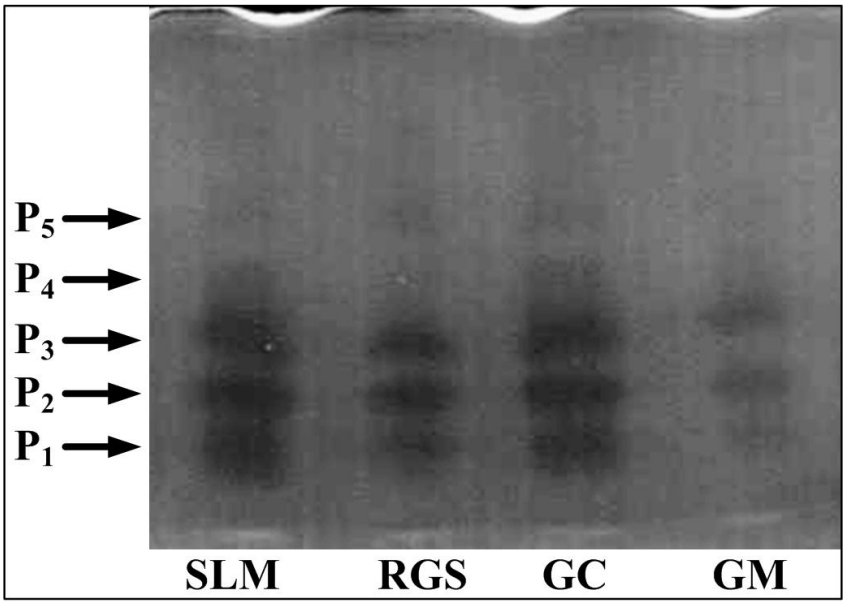

Figure 9 Zymogram analyses of the effect of some Brassicaceous host plants on proteolytic activity of fourth larval midgut extract of *Plutella xylostella* in non-reducing SDS-PAGE. Protease activity bands are indicated by arrows (P_1-P_5). Abbreviation: SLM, SLM_{046}; RGS, RGS_{003}; GC, Green-Cornet; GM, Glob-Master.

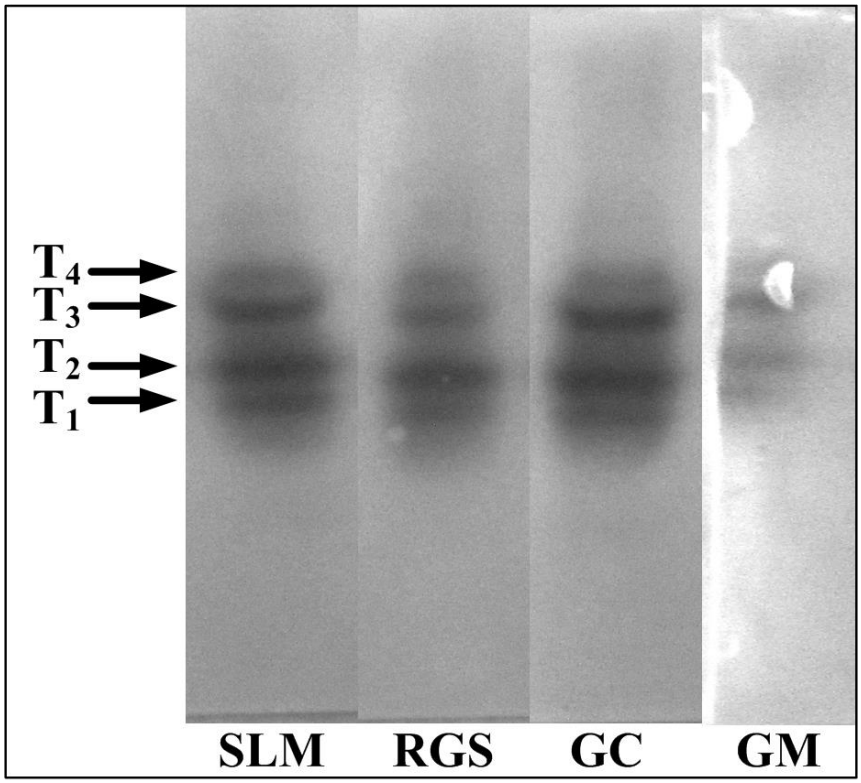

Figure 10 Zymogram analyses of tryptic activity of larval midgut extracts from *Plutella xylostella* using an overlay technique on nitrocellulose membrane. Tryptic activity bands are indicated by arrows (P_1-P_4). Abbreviation: SLM, SLM_{046}; RGS, RGS_{003}; GC, Green-Cornet; GM, Glob-Master.

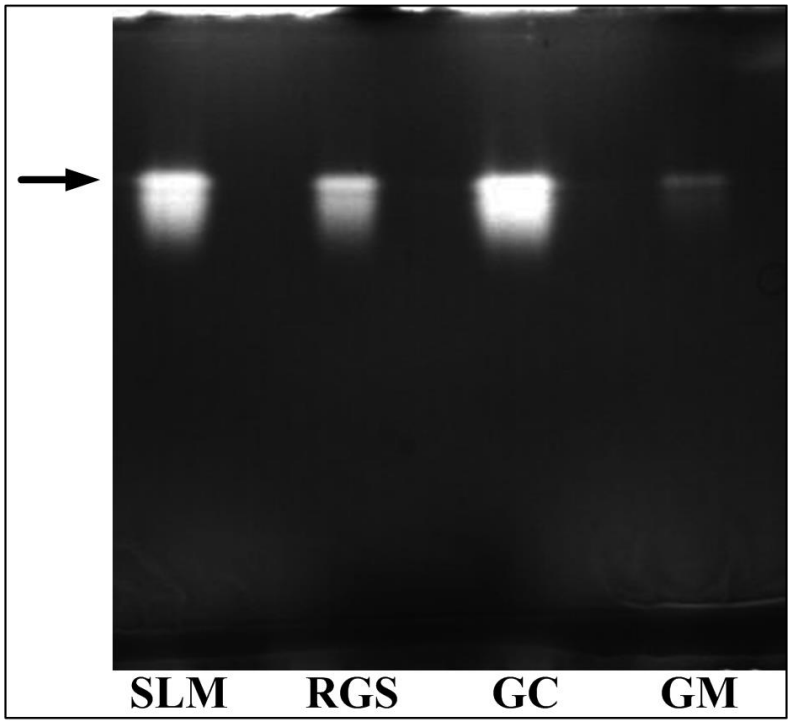

Figure 11 Zymogram analysis of α-amylase activity in the midgut of the last larval instar of *Plutella xylostella* using native-PAGE. α-amylase activity band are indicated by arrow. Abbreviation: SLM, SLM_{046}; RGS, RGS_{003}; GC, Green-Cornet; GM, Glob-Master.

Discussion

Diet density, temperature and pH are amongst the important factors which may affect digestive enzymes activity through affecting midgut protein content (Sivakumar *et al.*, 2006). *P. xylostella* has a series of complex and diverse proteolytic digestive enzymes which enables it to feed on different Brassicaceae cultivars. Here, we showed that different Brassicaceae cultivars affected the digestive physiology of *P. xylostella* which is in line with findings of Yang *et al.* (2009) who showed digestive enzymes of *P. xylostella* were affected by feeding on genetically different host plants (Yang *et al.*, 2009). We found the highest level of general proteolytic activity on SLM_{046} and Green-Cornet which was in accordance with findings of Soufbaf *et al.* (2010) who showed high susceptibility of these two cultivars to *P. xylostella*. Furthermore, Kianpour *et al.* (2014) showed that larvae *P. xylostella* on SLM046 and Green-Cornet were weighty rate even in short feeding time, indicating high suitability of these cultivars and confirms results of the present study.

Zymogram studies revealed five different types of protease isozymes (e.g. P1-P5) in larvae feeding on Brassicaceae cultivars. The P_1, P_2 and P_3 were found to be more prevalent than P_4 and P_5. The highest levels of proteolytic activity was observed in larvae feeding on susceptible cultivars, SLM_{046} and Green-Cornet. Cultivars RGS_{003} and Glob-Master affected P_5 and decreased its activity compared with the other cultivars which can be owing to the high levels of plant protease inhibitors (PPIs) existing in RGS_{003} and Glob-Master or low protein content of these genotypes. Decreased protease activity in insects due to high levels of PPIs in host plants has been shown by Naseri *et al.* (2010) who showed higher protease activity in *Helicorverpa armigera* (Hubner) (Lepidoptera: Noctuidae) feeding on susceptible cultivars

and artificial-fed diet than when they feed on susceptible ones.

We showed that trypsin serine protease is main specific protease in larvae *P. xylostella* which is in accordance with other studies (Naseri *et al.,* 2010; Tabatabaei *et al.,* 2011). Although chymotrypsin and elastase were also found in *P. xylostella* their activity was not great enough to be detected by zymogram. Using zymogram analysis, at least four major trypsin isoforms were detected in the midgut extracts of *P. xylostella* larvae. The larvae feeding on SLM_{046} and Green-Cornet had more tryptic activity than those feeding on RGS_{003} and Globe-Master. Existence of various isoforms for trypsin enzyme has been reported for other lepidopteran insects (Tabatabaei *et al.,* 2011).

Evolutionarily, herbivorous insects attempt to possess a diverse array of digestive enzymes and improve their adaptability by expressing different types of an enzyme to overcome plant protease inhibitors (PIs) and ensure digestion (Mehrabadi *et al.,* 2012). In contrast, host plants try to hinder insect herbivory by producing efficient inhibitor compounds (i.e., enzyme inhibitors) (Mehrabadi *et al.,* 2011). Accordingly, the low protease activity of *P. xylostella* larvae on RGS_{003} and Globe-Master cultivars, may be related to the high levels of PIs in these two cultivars which make them unsuitable to be fed by *P. xylostella* larvae. Low food utilization indices of *P. xylostella* on RGS_{003} and Globe-Master (Kianpour *et al.,* 2014) corroborates the unsuitability of these two cultivars.

We found that the optimal acidity of α-amylase activity in *P. xylostella* larvae occurred at alkaline condition which is in line with finding of other researchers (Abraham *et al.,* 1992; Markwick *et al.,* 1996; Valencia-Jimenez *et al.,* 2008). Like trypsin enzyme, the higher activity of α-amylase was observed on SLM_{046} and Green-Cornet, indicating lack of α-amylase inhibitors in both cultivars. Furthermore, we found one additional α-amylase isozyme by feeding on SLM_{046} and Green-Cornet which was lacking in larvae feeding on RGS_{003} and Glob-Master which may confirm the PIs-mediated resistance in RGS_{003} and Glob-Master.

Herbivorous insects feeding on Brassicaceae host plants, showed different reactions in responding to changed Glycosinolate level (Eigenbrode *et al.,* 1996) in which the most variation was observed in expression of glucosidase enzymes, especially β-glucosidase. By studying rate of glucosides hydrolase and biochemical properties of these enzymes in *P. xylostella* and their response to *P. xylostella* feeding on different plant hosts, we found that the high activity of α- and β-galactosidase occurred on susceptible cultivars which is in line with other reports (Terra and Ferriera, 1994; Azevedo *et al.,* 2003). The optimal acidity for α-glucosidase and β-glucosidase activities were at pH 6.0 and 7.0, respectively, showing the glucosidase group is more efficient at slightly acidic to neutral pH such as that seen in some lepidopteran insects (Frandsen and Svensson, 1998; Tabatabaei *et al.,* 2011).

By these results we can conclude that susceptibility in Brassicaceae cultivars is related to low level of produced glucosinolate as a main defensive element in these cultivars and ability of *P. xylostella* in producing different enzymes which enables them to hydrolyze secondary metabolites in low concentration. Considering the efficiency of conversion of digested food into larval biomass depends on activity of the digestive enzymes (Lazarevic *et al.,* 2003; Kotkar *et al.,* 2009), therefore, it can be argued that inhibition of digestive enzymes in *P. xylostella*, can be one of the important developed defensive mechanisms in resistant Brassicaceae which makes them unsuitable hosts. Activity of *P. xylostella* digestive enzymes (proteases, amylase and glycoside-hydrolysing enzymes) was inhibited substantially by feeding on resistant Brassicaceae cultivars. This mechanism most probably occurs by expressing enzyme inhibitors which needs to be further investigated.

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مهار آنزیم‌های گوارشی شب‌پره پشت‌الماسی *Plutella xylostella* با استفاده از ارقام مقاوم خانواده چلیپائیان

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چکیده: شب‌پره پشت‌الماسی *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) یکی از مخرب‌ترین آفات خانواده چلیپائیان هست که به‌شکل کاملاً تخصص‌یافته روی گونه‌های وحشی و زراعی این خانواده تغذیه می‌کند. گیاهان مورد حمله در واکنش به تغذیه این آفت، مقادیر زیادی گلوکوزینولات تولید می‌کنند. در پژوهش حاضر فعالیت آنزیم‌های گوارشی *P. xylostella* روی چهار ژنوتیپ مختلف از خانواده چلیپائیان بررسی شد. ژنوتیپ‌های مورد مطالعه شامل دو ژنوتیپ کلزا (SLM_{046} و RGS_{003}) و دو رقم کلم (Green-Cornet و Glob-Master) بود. نتایج نشان داد که بیش‌ترین فعالیت آنزیم‌های پروتئولایتیک و آمیلولایتیک شب‌پره پشت‌الماسی روی رقم Green-Cornet و کم‌ترین آن روی RGS_{003} و Glob-Master بود. بیش‌ترین فعالیت آنزیم‌های آلفاگلوکوزیداز و بتاگلوکوزیداز روی Green-Cornet و SLM_{046} و کم‌ترین آن روی Glob-Master و RGS_{003} بود. نتایج به‌دست آمده از مطالعه زایموگرام نشان داد که ایزوزایم‌های مختلفی از پروتئازها، شبه تریپسین‌ها و آلفاآمیلاز در ترکیب استخراج شده از معده میانی شب‌پره پشت‌الماسی وجود دارد که فعالیت این آیزوزایم‌ها در لاروهایی که روی ارقام مقاوم Glob-Master و RGS_{003} تغذیه کردند، متوقف شد. هم‌چنین لاروهایی که روی ژنوتیپ‌های مقاوم تغذیه کردند فعالیت گلوکوزیدازی بیش‌تری داشتند که نشان می‌دهد احتمالاً مقادیر زیادی گلوکوزینولات در ژنوتیپ‌های مقاوم مورد مطالعه وجود دارد. این نتایج نشان می‌دهد که توانایی ذاتی گیاهان مقاوم می‌تواند فیزیولوژی گوارشی شب‌پره پشت‌الماسی را از طریق مهار فعالیت آنزیم‌های گوارشی آن تحت تأثیر قرار دهد. نتایج این پژوهش حاوی نکات ارزشمندی درخصوص اثرات مستقیم گیاهان میزبان روی فیزیولوژی شب‌پره پشت‌الماسی است.

واژگان کلیدی: *Brassicaceae*، آنزیم‌های گوارشی، مقاومت گیاهی، *Plutella xylostella*