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Gut Proteolytic Profile of Larval *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in Response to Feeding on Different Fabaceous Host Plants

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ABSTRACT

The impact of three different fabaceous host plants including cowpea (*Vigna unguiculata*), chickpea (*Cicer arietinum*), and mung bean (*Vigna radiata*) seeds was investigated using biochemical approaches on possible changes of gut proteolytic activity of the cowpea weevil, *Callosobruchus maculatus* at 30±1˚C and 70±5% RH and a photoperiod of 8:16 (L:D). Results revealed that pH of 4-5 and 9 was optimal for the activity of larval gut proteases using azocasein and hemoglobin as general substrates. Different serine (BApNA, SAAPFpNA, PMSF, TLCK, and TPCK) and cysteine (Z-Arg-Arg- pNA, Z-Phe-Arg-pNA and DTT) specific substrates inhibitors and activator were used as a further proof of the proteolytic profile in the gut of *C. maculates*. Although combinations of serine and cysteine proteases were observed, the cysteine proteases had the highest rate on the studied hosts. The protease activity, especially cystein protease, was the highest on cowpea, which was supported by hemoglobin (0.156±0.045 U mg^{-1}), Z-Phe-Arg-pNA (2.85 U mg^{-1}) substrates and DTT (90.00±0.10%) as an activator. Due to the importance and frequency of cysteine proteinases and their effects on biological and physiological process, it would be better to design pest management programs based on cysteine plant proteinase inhibitors as transgenic plants.

Keywords: Chickpea**,** Cowpea, Cysteine proteinases, Digestive enzymes, Mung bean.

INTRODUCTION

Legume grains such as common bean (*Phaseolus vulgaris* Linneaus), cowpea (*Vigna unguiculata* Linneaus), chick pea (*Cicer arietinum* Linneaus), lentil (*Lens culinaris* Linneaus) and mung bean (*Vigna radiate* Linneaus) have become important sources of dietary proteins and carbohydrates, but yields are often diminished by infestation of seed weevils. Among the most important pests of stored grains of legumes, the bruchid cowpea weevil, i.e. *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) has been widespread in most tropical and sub-tropic countries, namely, Africa, South America, USA, Europe, and Asia (Ouedraogo *et al*., 1996; Silva *et al*., 1999; Pereira *et al*., 2006; Kazemi *et al*., 2009). It causes serious damage to grains so that over 30 percent of peas are destroyed by this weevil annually in Iran. As legumes provide the cheapest and richest source of plant protein, the control of this pest is essential (Taheri, 1995).

Control of *C. maculatus* currently relies upon application of chemical insecticides (Murad *et al*., 2008), which has negative effects on environment and human health. One approach to control herbivorous insects is to enhance the resistance of important crops by plant proteinase inhibitors which

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are the major plant defense mechanisms against herbivorous insects (Lawrence and Koundal, 2002). Several proteinaceous inhibitors are present in plant seeds and vegetative organs and act on the digestive enzymes (e.g. amylases and proteinases) (Biggs and McGregor, 1996; Lawrence and Koundal, 2002). Making any interference and inactivation in enzymatic protein digestion by inhibitors could lead to blocking of gut enzymes, resulting in poor nutrient utilization, retarded growth and development, depriving the reproduction efficiency and death because of starvation (Jongsma and Bolter, 1997; Gatehouse and Gatehouse, 1999). These enzymes and correlated genes can be used in the development of efficient and harmless biotechnological tools such as transgenic resistant plants for agricultural insect pest control without negative environmental and human health effects (Pereira *et al*., 2006). Therefore, knowledge of insect digestive enzymes could be crucial in designing new control methods based on inhibition of digestive enzymes (Azzouz *et al*., 2005).

The search for possible targets in bruchid physiology that could be used in control strategies made an important advance in the late 1970's, when Gatehouse and his co-workers published results of their study suggesting that trypsin inhibitors were involved in resistance of a cultivar of *V. unguiculata* to the cowpea weevil (Gatehouse *et al*., 1979). It became evident from these studies that bruchid larvae utilize cysteine and aspartic proteinases to digest dietary proteins, which are not affected by serine proteinase inhibitors from legume seeds (Gatehouse *et al*., 1985; Kitch and Murdock, 1986; Wieman and Nielsen, 1988; Campos *et al*., 1989; Lemos *et al*., 1990; Silva and Xavier-Filho, 1991).

Considering the importance of protein digestion as a target for bruchid control, it is clear that the role of digestive proteins in larvae of bruchids deserves attention and clarification. There are documentations regarding the serin protease of gut activity in this case (Gatehouse *et al*., 1979; Silva and Xavier-Filho, 1991). Due to the importance of cystein protease activity on digestive system of *C. maculatus*, there is little information regarding the cystein protease activity. Characterization of cystein proteinases in response to the different host plants and inactivation of enzymatic protein digestion by inhibitors could lead to blocking of gut enzymes. The major purpose of this study was to determine the effect of different host plants on digestive proteinase activity of *C. maculatus*.

MATERIALS AND METHODS

Rearing of Insects

Stock culture of *C. maculatus* was prepared from stored pest lab in the Department of Agricultural Entomology (established in 2014) at Urmia University. The cowpea weevil was reared on the seeds of three different fabaceous plants such as cowpea (*Vigna unguiculata*), chickpea (*Cicer arietinum*), and mung bean (*Vigna radiata*) seeds. Stock cultures (9-cm diameter and 12-cm height), were reared in plastic jars containing seeds of the mentioned host plants for two generations before being used in the experiments. The insects were kept at 30±1˚C and 70±5% RH and photoperiod of 16:8 (D:L).

Preparation of Samples from Insects and Seeds

The fourth instar larvae were cold immobilized and dissected to remove the whole gut in 250 mM of NaCl (Silva *et al*., 1999). Only actively feeding larvae with their gut tracts filled with food were chosen for dissection. After the removal of the whole gut, the adhering unwanted tissues were removed, then, the gut tissue was homogenized and transferred to 1.5 mL microfuges and was centrifuged at 13,000 (g) for 20 minutes at 4°C. The clear supernatant was transferred to a pre-chilled

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microtube. The samples were stored at -20°C until further use. The seeds of fabaceous were ground by electrical grinder, and the resultant flour was kept at 4°C until further use.

Protein Quantification

Protein concentration of gut larvae was determined by the method of Bradford (1976) using Bovine Serum Albumin (BSA; Bio-Rad, biorad.com) as the standard (0.1, 0.3, 0.5, 0.7, 0.9, 1.0 and 1.2 mg mL^{-1}). Protein content of fabaceous flours were quantified using BSA as standard according to Bradford (1976) with minor modifications. In brief, a quantity (200 mg) of each host plant flour was homogenized in distilled water (10 mL), centrifuged at 13,000×g for 10 minutes, and 100 µL of the homogenate was added to 3 mL of Bradford reagent. The samples were incubated in darkness at 37°C, and absorbance was read at 595 nm (Bouayad *et al.*, 2008).

General Proteolytic Activity

General proteinase activity of larval guts was determined using azocasein (Sigma-Aldrich, A2765) (pH 2–12) and hemoglobin (pH 2–12) as substrates at broad pH range. The universal buffer system (50 mM sodium acetate–phosphate–borate) was used to determine the optimal pH of proteolytic activity (Elpidina *et al.*, 2001). For azocaseinolytic activity, the reaction mixture consisted of 30 µL of 2% azocasein solution in 50 mM universal buffer of specified pH and 15 µL enzymes. The reaction mixture was incubated at 37ºC for 60 minutes. Proteolysis was stopped by addition of 30 µL of 30% TriChloroAcetic acid (TCA). Appropriate blanks in which TCA was added first to the substrate were prepared for each assay. Precipitation was achieved by cooling at 4ºC for 60 minutes and the reaction mixture was centrifuged at 16,000×g for 10 minutes. An equal volume of NaOH 1N was added to the supernatant and the absorbance was recorded at 440 nm using a microplate reader (BioTek ELX808).

The assay with a hemoglobin substrate was performed according to Cohen (1993) with slight modifications. Hemoglobin solution (2%), 30 µL, was added to 90 µL of the desired pH universal buffer. Reactions were started with the addition of 15 µL enzyme extract and incubation at 37ºC for 120 minutes. For reaction termination, 30 µL of 30% TCA was added to the reaction mixture. Precipitation was achieved by cooling at 4ºC for 60 minutes and the reaction mixture was centrifuged at 16,000×g for 10 min. Appropriate blanks were used as described before. The peptides liberated from hemoglobin were estimated using Folin-Ciocalteu reagent at 650 nm (Folin and Ciocalteu, 1927).

Specific Protease Activity

Trypsin- and chymotrypsin-like activities (as two sub-classes of serine proteases) were assayed using final concentrations of 1 mM BApNA (Nα-Benzoyl-L-Arginine4-NitroAnilide hydrochloride; Sigma-Aldrich, B3279) and 1 mM SAAPFpNA (N-Succinyl-Alanine-Alanine-Proline-phenylalanine-p-NitroAnilide; Sigma-Adrich, S7388) as substrates, respectively. A reaction mixture consisted of 10 µL enzyme, 85 µL of universal buffer at broad pH range (8-12), and 5 µL of the above mentioned substrates. The reaction mixture was incubated at 37ºC for 10 minutes before adding 30 µL 30% acetic acid to terminate the reaction. The absorbance of the resulting mixture was measured spectrophotometrically at 405 nm by p-nitroaniline release.

Two specific substrates benzyloxycarbonyl-Arg-Arg-*p*NA (Sigma-Aldrich, C8536) and benzyloxycarbonyl-Phe-Arg-*p*NA (Sigma-Aldrich, B2133) were used for cysteine proteinase assays. Hydrolysis of the substrates was determined by measuring the absorbance by p-

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nitroaniline released after 10 min incubation at 405 nm using microplate reader (BioTek ELX808).

Inhibition and Activation Assay

The effect of different inhibitors and activator was determined on proteolytic activities of the gut enzyme extract. The inhibitors and their concentrations were: general protease inhibitor, 1 mM PMSF (PhenylMethylSulfonyl Fluoride; Sigma-Aldrich, P7626); inhibitor, 0.1 mM TLCK (Nα-p-Tosyl-L-Lysine Chloromethyl Ketone; Sigma-Aldrich, T5012); chymotrypsininhibitor and 0.1 mM TPCK (N-Tosyl-L-Phenylalanine Chloromethyl Ketone; Sigma-Aldrich, T7254) and protease activators, 5 mM DTT (DiThioThreitol; Sigma-Aldrich, D0632). To determine the effect of these compounds on enzymatic activities, the enzymes were pre incubated with the appropriate inhibitors and activator at room temperature for 15 minutes, then, substrates were added and the assays were carried out as described in the enzyme assay section.

Statistics and Data Analysis

Data were analyzed by one-way Analysis Of Variance (ANOVA) followed by comparison of the means with the Tukey's test at α= 0.01 using SPSS (ver. 20) statistical software .

RESULTS

Protein Quantification of Larval GutGut Extracts and Fabaceous Seed Flours

Statistical tests indicated significant differences in the content of protein among flour of various host plants tested (F= 1.219; df= 2,6; P< 0.05). The highest content of protein was obtained in cowpea (0.871±0.021 mg mL^{-1}) followed by chickpea (0.866±0.014 mg mL^{-1}) and mung bean (0.777±0.052 mg mL^{-1}). The protein concentration of gut extracts of the larvae reared on different fabaceous host plants was significantly different (F= 1.568; df= 2,6; P< 0.05). The protein content in gut of the larvae fed on cowpea, chickpea and mung bean were 1.43, 1.10, and 1.50 mg mL^{-1}, respectively (Table 1).

Protease Activity

The effect of pH on proteolytic activity of the gut extract from *C. maculatus* larvae is presented in Figure 1. Proteolytic activity with azocasein as a protein substrate showed that the substrate was hydrolyzed over a broad range of acidic and alkaline pH values with a peak at 4-5 and 9 (Figure 1-a). Similarly, the same pattern was observed using hemoglobin substrate at acidic (pH 4) and alkaline (pH 8) (Figure 1-b) on the three mentioned host plants. Serine and cysteine proteolytic activity data (P< 0.05) by general substrates from *C. maculatus* fourth instar larvae reared on

Table 1. Mean (±SE) protein contents (mg ml^{-1}) of flours and gut extract from fourth instar gut of *Callosobruchus maculatus*, feeding on different fabaceous host plants.[a]

Hosts	Protein content of flours (mg mL^{-1})	Protein content of gut larvae (mg mL^{-1})
Cowpea	0.871±0.021[a]	1.43 ± 0. 011[a]
Chickpea	0.866±0.014[b]	1.10 ± 0.023[b]
Mung bean	0.777±0.052[c]	1.50 ± 0.047[a]

[a] The means followed by different letters in the same columns are significantly different (Tukey, P< 0.05).

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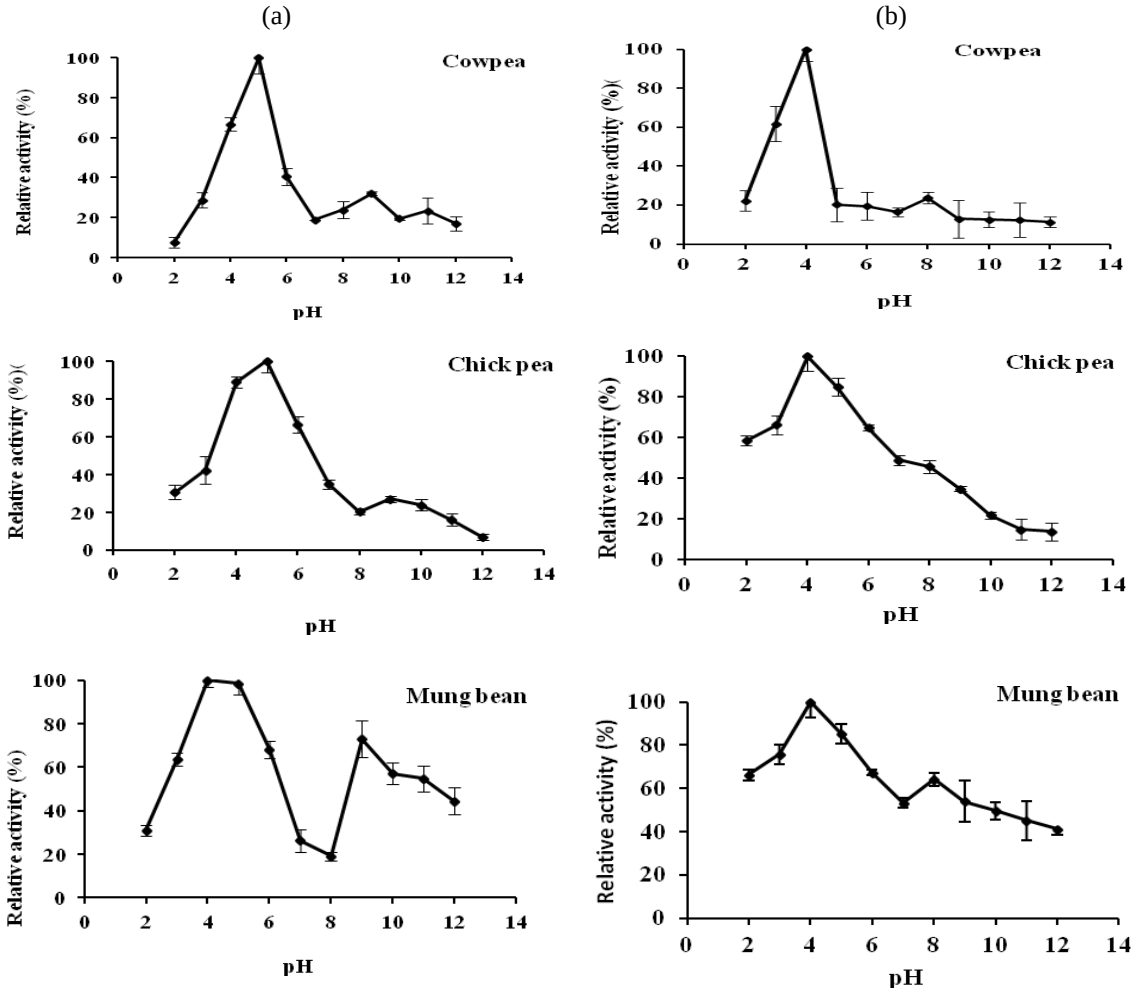

Figure 1. The effect of pH on the proteolytic activity of larval gut extracts from *Callosobruchus maculatus* on the substrates azocasein (a) and hemoglobin (b).

various fabaceous are shown in Table 2. The lowest serine proteolytic activity was in larvae reared on chickpea using azocasein (0.002±0.0001 U mg^{-1}) and hemoglobin (0.032± 0.001 U mg^{-1}), whereas the highest activity was on cowpea and mung bean using both substrates (Table 2). In the case of cysteine proteinase activity, although the cysteine activity was not affected using hemoglobin substrate, it was higher than serine proteinase activity by both substrates (Table 2).

Specific Proteolytic Activity

The results obtained when assaying larval digestive extracts (susceptible hosts: cowpea) with specific substrates showed the presence of both trypsin- (100.00±10.20%) and chymotrypsin-like (4.00±0.001%) activities (Figure 2-a). The substrates BApNA and SAAPFpNA were hydrolyzed at alkaline pH with maximum activity at pH 8-10, respectively. The gut extract showed hydrolytic activity against the substrates (Z-Arg-Arg- pNA) and (Z-Phe-Arg-pNA), indicating the presence of cysteine protease which hydrolyzed at acidic pH, with maximum activity at pH 3-4, respectively (Figure 2-b) on cowpea.

There was a significant difference among different host plants, regarding specific activity value of trypsin (F= 2.439; df= 2,6; P< 0.05), Cathepsin L (F= 1.003; df= 2,6, P< 0.05) and Cathepsin B (F= 0.465; df=

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Table 2. Mean (±SE) serine (U mg^{-1}) and cysteine proteolytic (U mg^{-1}) activities of the gut extracts from thefourth instar larvae of *Callosobruchus maculatus* on different fabaceous at optimal pH suing general substrates.[a]

Hosts	Serine activity (U mg^{-1})		Cysteine activity (U mg^{-1})	
	Azocasein	Hemoglobin	Azocasein	Hemoglobin
Cow pea	0.003±0.0002[a]	0.037±0.002[a]	0.009±0.001[a]	0.156±0.045[a]
Chick pea	0.002±0.0001[b]	0.032±0.001[b]	0.0116±0.002[a]	0.121±0.032[a]
Mung bean	0.0035±0.001 [a]	0.077±0.014[a]	0.00244±0.000[b]	0.120±0.022[a]

[a] The means followed by different letters in the same columns are significantly different (Tukey, P< 0.05).

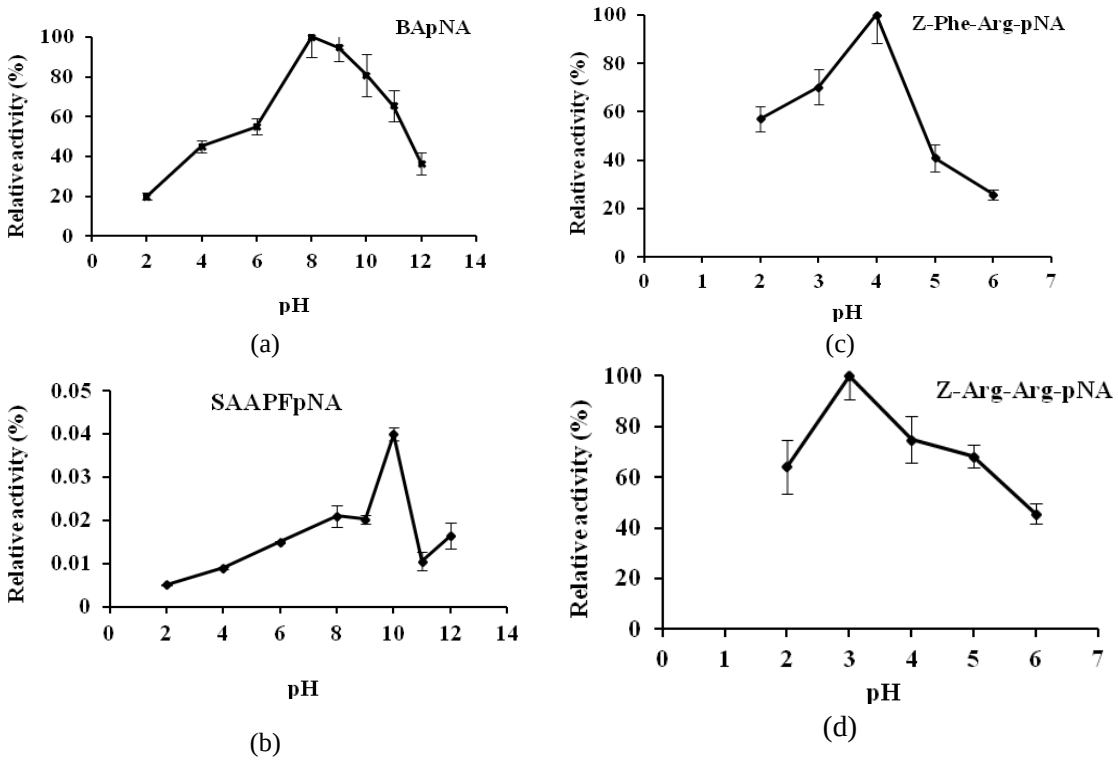

Figure 2. Tryptic (a); chymotryptic (b); cathepsin L (c), and cathepsin B (d) activity of larval gut extract of *Callosobruchus maculatus* fed on susceptible host (cowpea).

2,6; P< 0.05) activity. Trypsin activity of the larvae that fed on chickpea was significantly higher than the other host plants. A gut trypsin proteinase specific activity value was estimated at 0.452, 0.623, and 0.188 (U mg^{-1}) substrate hydrolyzed on cowpea, chickpea, and mung bean, respectively (Figure 3).

The specific activity values of cathepsin L determined for Z-Phe-Arg-pNA were 2.855, 1.721, and 1.517 (U mg^{-1}) substrate hydrolyzed on cowpea, chickpea, and mung bean, respectively. The specific activity values of cathepsin B determined for Z-Arg-Arg-pNA were 1.60, 2.252, and 1.936 (U mg^{-1}) substrate hydrolyzed on cowpea, chickpea and mung bean, respectively, which was the highest rate by the larvae that fed on chickpea (Figure 3).

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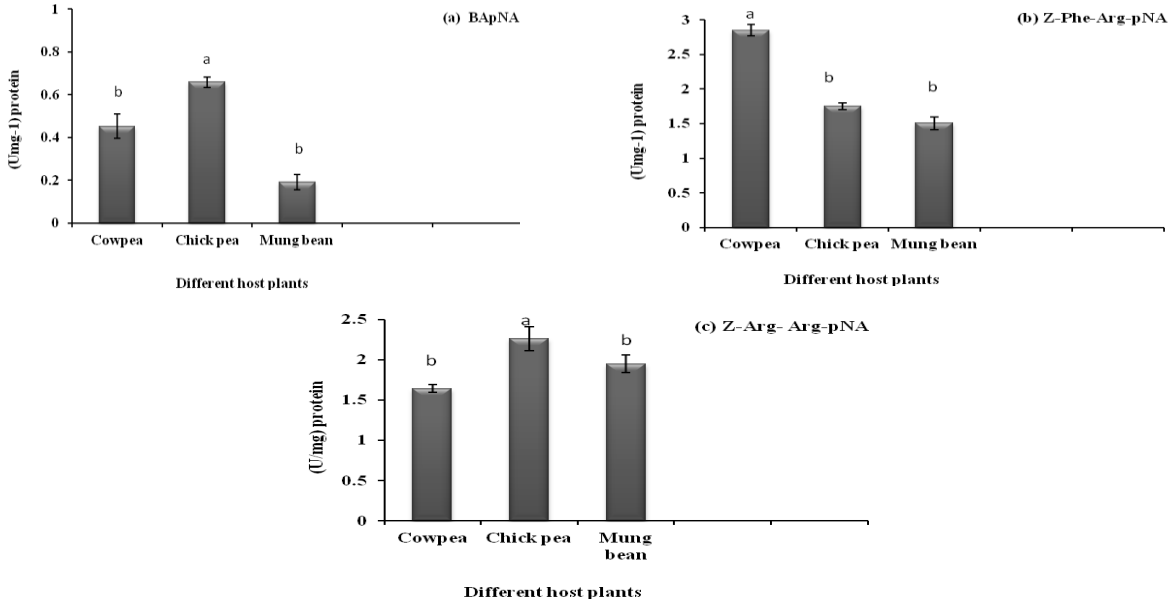

Figure 3. Tryptic (BApNA; a), Cathepsin L (Z-Phe-Arg-pNA; b), and Cathepsin B (Z-Arg-Arg-pNA; c) activity of larval gut extract fed on different host plants at desired pH. (Unit activity= Micromoles substrate hydrolyzed per minute).

Effects of Inhibitors and Activator on Protease Activity

The total proteolytic activity of larval gut extract was further characterized using general and specific serine proteinase inhibitors and cysteine activator (Table 3). It was found that DTT compound affecting Cysteine proteases significantly changed proteolytic activity, confirms the presence of cysteine proteases in the gut extract of *C.maculatus* (Table 3). Therefore, the highest cysteine activation was observed by DTT on cowpea. PMSF, as a serine protease inhibitor, significantly decreased proteolytic activity along with trypsin-like (TLCK) and chymotrypsin-like (TPCK) (Table 3). However, considerable inhibition of larval azocaseinolytic activity by PMSF, TLCK, and TPCK was observed for trypsin-like proteinases compared with chymotryosin-like proteinases. The highest inhibition by PMSF and TLCK inhibitors were observed in gut of larvae fed on cowpea as (13.63±0.01%) and (11.93±1.15%), respectively (Table 3).

DISCUSSION

Host plants have a critical role in the fitness of herbivorous insects because their

Table 3. The effects of some protease inhibitors and activator, on the general proteolytic activity from gut extract of *Callosobruchus maculatus* on different fabaceous at optimal pH using general substrates.[a]

Hosts	Inhibition (%)			Activator
	PMSF	TLCK	TPCK	DTT (%)
Cow pea	13.63±0.01[a]	11.93±1.15[a]	3.70±0.005[b]	90.00± 0. 10[a]
Chick pea	13.50±0.06[a]	11.65±0.03[a]	4.20±0.023[b]	12.10± 3.20[b]
Mung bean	5.28±0.001 [b]	4.70±0.01[b]	6.10±0.32[a]	12.00± 2.20[b]

[a] The means followed by different letters in the same columns are significantly different (Tukey, P< 0.05)

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components could directly affect the digestive processes of insect pests (Chown and Nicolson, 2004). Changes of proteolytic activity of different host plants and their transgenic varieties have been reported in different insects (Chen, 2008). Results of the current study demonstrated the presence of major proteases in the gut of *C. maculatus*, and their changes after feeding on different host plants.

According to the obtained results, the flour of mentioned fabaceous hosts considerably influenced the digestive enzymatic activity of fourth instars of *C. maculatus*. It is generally accepted that the insect larvae regulate the release of digestive enzymes in response to the level of metabolites ingestion (Lwalaba *et al*., 2010). Since variations in protein content of the hosts may lead to differences in the proteolytic activity of *C. maculatus*, the highest protein content of gut extract and proteolytic activity were detected in the larvae reared on cowpea, which is attributed to the high protein content of this host.

The azocasein and hemoglobin are routinely used as conventional substrates for detecting general proteolytic activity in many cases (Cohen, 1993; Elpidina *et al*., 2001). Our data show that the proteinases in gut extracts from *C. maculatus* larvae are capable of hydrolyzing both substrates. Gut pH is a major factor affecting the regulation of enzymatic reactions in digestion (Terra and Ferreira, 1994). Experiments to determine the optimal pH for general proteolytic activity in the gut of *C. maculatus* demonstrated two peaks for the gut extract at acidic and alkaline pH using azocasein and hemoglobin (2%), suggesting that there were cysteine and serine proteinase activities in the gut extract of larvae. According to the obtained results, the most conventional proteinase activity happens at acidic pH (2-5) compared with alkaline pH (8-9), and it seems that the cysteine proteinases were the major hydrolyzing enzyme in the gut extract of *C. maculatus* compared with serine proteinase. This was due to the fact that in many Coleoptera, the main endopeptidases have cysteine or aspartic acid at their active centers (Murdock *et al*., 1987), which are primarily responsible for protein digestion in *C. maculatus* (Lawrence and Koundal, 2002). Investigations of other coleopteran species also show that the cysteine proteases are the prevailing proteolytic enzymes in their gut according to optimal pH of their activity, specific substrates and inhibition assays (Kitch and Murdock, 1986; Lemos *et al*., 1990; Murdock *et al*., 1987; Campos *et al*., 1989).

The ability to hydrolyze specific synthetic substrates, the elucidation of the pH at which maximal hydrolysis occurs, and their sensitivity to protease inhibitors confirmed the presence of trypsin-like and chymotrypsin-like activities in gut extracts of insects (Hosseininaveh *et al*., 2007). The specific substrates including trypsin, chymothrypsin, cathepsin L and cathepsin B were used to prove the presence of protease activity which is involved in digestion. In the case of serine proteases, trypsin-like, and chymotrypsin-like proteases were active in gut extract, but trypsin-like proteinase had significant activity in the gut compared with the chymotrypsin-like proteases, which is supported by specific serine proteinase inhibitors on cowpea (Table 3). Larval proteinases with alkaline pH optima (8 and 10) hydrolyzed BApNA and SAAPFpNA, respectively. Alkaline pH values are optimal for trypsin and chymotrypsin-like proteases activities. The presence of serine proteases has been previously reported in different species of beetles, such as *Lasioderma serricorne* Fabricius (Oppert *et al.*, 2002), *Tribolium castaneum* Herbst (Oppert *et al.*, 2003), *Tenebrio molitor* L. (Vinokurov *et al.*, 2006), *Trogoderma granarium* Everts (Dermestidae: Coleoptera) (Hosseininaveh *et al.*, 2007) and *Plagiodera versicolora* Laicharting (Coleoptera: Chrysomelidae) (Zibaee and Hajizadeh, 2013), *Oryctes elegans* Prell (Coleoptera: Scarabaeidae) (Saberi Riseh *et al*., 2014).

Further studies with specific cysteine proteinase substrates (Z-Phe-Arg-pNA and Z-Arg-Arg- pNA) with optimal pH (4 and 3, respectively), found in the gut. Although the cathepsin B had higher activities in the gut extract of larvae fed on chickpea, the larvae fed on cowpea had the highest cathepsin L

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activity in their gut. Combinations of serine and cysteine proteases have been reported in many coleopteran beetles (Oppert *et al*., 2002, 2003; Vinokurov *et al*., 2006; Hosseininaveh *et al*., 2007; Zibaee and Hajizadeh, 2013).

It has been well understood that insect reared on different host plants or transgenic plants containing heterologous anti-feedants like plant protease inhibitors had different digestive enzymatic responses (Saikia *et al*., 2011). Plant species have many allelochemicals that may affect the digestive physiology of insects. Polyphagous insects, which are associated with a wide range of host plants, are more likely to encounter a wide range of biosynthetically unrelated allelochemicals (toxins) in their host (Zibaee and Hajizadeh, 2013). In fact, lower activities of specific proteases in the gut of insects may indicate that seed proteins are not efficiently utilized due to the presence of allelochemicals and plant inhibitors. Plant inhibitors of proteinaceous enzymes are part of the reserve storage proteins of seeds and have been also considered to be a part of the constitutive and inducible array of defense mechanisms against attack by insects (Blanco-Labra *et al*., 1996).

The effect of four leguminous species (cowpea, green gram, chickpea, and lentil) on biological and demographic parameters of *C. maculatus* has been studied by Kazemi *et al*. (2009). Based on their findings, the larvae reared on cowpea had the shortest developmental time and the highest population parameters (e.g. net reproductive rate and intrinsic rates of increase), suggesting the suitability of cowpea compared with the other tested leguminose species. The enzymatic current study can be interpreted in line with their findings, as compared with the cystein protease, it is due to the their inhibition by trypsin inhibitors (Lemos *et al*., 1990; Silva and Xavier-Filho, 1991), resulting in hyperproduction of cystein protease. There is some evidence regarding the importance and frequency of these enzymes, which are responsible for life process (Murdock *et al*., 1987; Lawrence and Koundal, 2002).

CONCLUSIONS

The results of this study showed that *C. maculatus* had different types of specific proteases in the gut extracts of larvae, suggesting the adaptation of this insect to different host plants. Changes of proteolytic activity due to feeding on different host plants indicate a correlation between digestive proteolytic activity and nutrient composition of the various diets, and reflect the adaptive nature of the polyphagous pest (Zibaee and Hajizadeh, 2013).

This study indicates that the fabaceous hosts affect the type and frequency of digestive enzymes in *C. maculates* larvae. Although, both serine and cysteine proteinases activity were observed in gut extract, the cysteine proteinases were the common proteinases in gut of *C. maculatus* larvae and the cathepsin L was the main endoproteinases in the larval digestive system which fed on cowpea. For pest management programs, it is recommended to use transgenic plants with cysteine protease through biotechnological approaches, because of their importance and frequency in digestive system process.

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پروفایل پروتئینی روده لارو سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) در واکنش به تغذیه از میزبان‌های مختلف حبوبات

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چکیده

تاثیر سه میزبان مختلف حبوبات شامل لوبیای چشم بلبلی(*Vigna unguiculata*)، نخود (*Cicer arietinum*) و ماش (*Vigna radiata*)روی تغییرات احتمالی فعالیت پروتئولیتیکی سوسک چهارنقطه‌ای حبوبات *C. maculates* با استفاده از روش‌های بیوشیمیایی در شرایط دمایی ۱± ۳۰ درجه سانتی‌گراد، رطوبت نسبی ۵± ۷۰ و دور ه روشنایی ۸: ۱۶ روشنایی به تاریکی مورد مطالعه قرار گرفت. نتایج نشان داد که pH بهینه برای فعالیت پروتئازی روده لاروها، با استفاده از سوبستراهای عمومی آزوکازئین و هموگلوبین ۴-۵ و ۹ بود. از سوبستراها، مهارکننده‌ها و فعال کننده تخصصی سرینی (BApNA, SAAPFpNA, PMSF, TLCK,TPCK) و سیستئینی (Z-Arg-Arg-pNA, Z-Phe-Arg-pNA, DTT) جهت پروفایل پروتئینی نیز استفاده گردید. اگرچه، ترکیبی از پروتئازهای سرینی و سیتئینی مشاهده شد، پروتئازهای سیستئینی بیشترین میزان فعالیت را در هر سه میزبان نشان دادند. نتایج نشان داد که لاروهای تغذیه شده با لوبیای چشم‌بلبلی، بیشترین فعالیت سیستئین پروتئازی با سوبسترای عمومی (۰/۰۴۵±۰/۱۵۶ واحد فعالیت آنزیمی/میلی گرم)، کاتپسین L (۲/۸۵ واحد فعالیت آنزیمی/میلی گرم) و فعال کننده DTT (۰/۱۰ ± ۹۰/۰۰ درصد) داشتند. به دلیل اهمیت و فراوانی پروتئازهای سیستئینی و اثرات آنها روی فرآیندهای فیزیولوژیکی و زیستی، بهتر است برنامه‌های مدیریتی بر اساس مهارکننده های پروتئینازی سیستئینی در قالب گیاهان تراریخته طراحی شود.

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