J. Crop Prot. 2019, 8 (4): 389-402

Research Article

Effect of different barley cultivars on nutritional physiology of *Tribolium castaneum* (Coleoptera: Tenebrionidae)

Foroogh Rahimi Namin, Gadir Nouri-Ganbalani, Bahram Naseri* and Jabraeil Razmjou

Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran.

Abstract: Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan and serious pest of cereal grains and their products in storage. In this research, nutritional indices and digestive enzymatic activity of *T. castaneum* fourth instar and adults were studied on ten barley cultivars (Fajr30, Behrokh, Sahra, Makuyi, Neek, Lout, Bahman, Nosrat, Abidar, and Sahand) at 30 ± 1 °C, relative humidity of 75 ± 5% and darkness conditions. The results showed that fourth instars and adults reared on cultivars Makuyi and Fajr30 had the lowest weight gain, efficiency of conversion of ingested food (ECI), relative growth rate and growth rate (GR) values. However, *T. castaneum* fed on cultivar Lout showed the highest weight gain, ECI and GR. The highest amylolytic activity of larvae was on cultivar Bahman, whereas the lowest activity was on cultivars Makuyi and Neek. Amylolytic activity of adults was the highest on cultivar Makuyi, and the lowest on cultivar Lout. Moreover, proteolytic activity of fourth instar was the highest when larvae were fed on cultivar Sahra and the lowest when they were fed on cultivars Behrokh and Makuyi. The highest proteolytic activity of adults was on cultivar Abidar, and the lowest on cultivar Bahman. The results of this study indicated that cultivars Fajr30 and Makuyi were less nutritive and cultivar Lout was more nutritive to *T. castaneum*. Therefore, more attention should be paid to manage the pest on cultivar Lout as a susceptible cultivar.

Keywords: Nutritional index, digestive enzyme, barley, red flour beetle

Introduction

Barley, *Hordeum vulgare* L., is one of the main cereals used for feed and malt production in Iran and worldwide (Khodabandeh, 2003; Schulte *et al.,* 2009; Kordan andGabrys, 2013; Houshyar, 2017). Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is known as a widespread and serious pest of cereals and their products in storage (Campbell and Abrogast, 2004; Shafique *et al.,* 2006, Javed *et al.,* 2016; Naseri *et al.,* 2017). Both adult and larvae are able to exploit different stored products, and cause economic damages by contaminating food stuffs and decreasing their nutritional value. Extensive grain losses could occur by *T. castaneum* when susceptible cultivars are stored for a long time under favorable conditions (Sarwar, 2013, 2015).

Synthetic insecticides are often effective to control stored product pests such as *T.*

Handling Editor: Saeid Moharramipour

* **Corresponding author**, e-mail: bnaseri@uma.ac.ir
Received: 12 February 2019, Accepted: 13 July 2019
Published online: 21 August 2019

castaneum. Since these insecticides may have dangerous side effects to humans, non-target organisms and environment (Iram *et al.,* 2013; Shweta and Prakash, 2013), it is crucial to develop control approaches that are safe and eco-friendly. To accomplish this goal, understanding the nutritional physiology of *T. castaneum* on various host cultivars may be practical to select host cultivars resistant to this pest (Fabres, 2014; Naseri *et al.,* 2017).

The effect of different seed inhibitors on digestive amylolytic activity of *T. castaneum* larvae was evaluated by Khan *et al.* (2012), who reported that the inhibitors extracted from wheat, maize and kidney bean showed higher inhibitory effects than the others. Kheradpir (2014) studied the food preference by *T. castaneum* among different flour types and found the susceptibility of wheat flour to this insect. Also, Sagheer *et al.* (2014) investigated the effect of wheat, corn and barley flours on nutritional indices of *T. castaneum* and noted that barley flour was unsuitable diet for this species. Feeding efficiency and digestive enzymatic activity of *T. castaneum* on different food commodities were evaluated by Naseri *et al.* (2017), who stated that soybean flour was the most unsuitable food for its development.

Despite the economic losses caused by *T. castaneum* larvae and adults on stored barley seeds (Javed *et al.,* 2016), no published articles are available regarding the nutritional physiology of this important pest on various barley cultivars. Therefore, the aim of this research was to evaluate the nutritional indices and activity of two main digestive enzymes (amylase and protease) of *T. castaneum* when fed on various barley cultivars. The results from this study will be helpful to improve better pest management strategies on stored barley cultivars.

Materials and Methods

Chemicals

Substrates, dinitrosalicylic acid (DNS) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, USA). Bovine serum albumin (BSA) was purchased from Roche Co. (Germany). Electrophoresis chamber was obtained from Bio-Rad Laboratories (Richmond, CA), and materials used to prepare the Electrophoresis gel were purchased from Merck Co. (Germany).

Barley sources

Tested eight barley cultivars (Fajr30, Behrokh, Sahra, Makuyi, Neek, Lout, Bahman, and Nosrat) were provided by Agricultural and Natural Resources Research Center of Isfahan, Iran. Seeds of two other barley cultivars (Abidar and Sahand) were obtained from Agricultural and Natural Resources Research Center (Ardabil, Iran). The selected cultivars are the most commonly cultivated in barley-growing regions of Iran. Before beginning of the experiments, all grains were ground to fine flour. Moisture level of tested cultivars ranged from 2.83% in cultivars Behrokh, Sahra and Makuyi to 8.76% in cultivar Sahand.

Insect rearing and experimental conditions

Adults of *T. castaneum* were collected from the laboratory colony from Department of Plant Protection, University of Mohaghegh Ardabili, (Ardabil, Iran), and maintained for two generations on a flour-baking yeast mixture (10% yeast) as a food substrate. After rearing on various barley cultivars for two generations, 20 pairs of male and female adults (1-5 days old) were each transferred into plastic containers (18 × 12 × 6 cm) containing various tested cultivars, and allowed to mate and lay eggs for 7 days. Then, the adults were removed by sieving, and the eggs laid were kept for offspring emergence. One-day old larvae were used to start the experiments. All experimental insects were reared at 30 ± 1 °C, relative humidity of 75 ± 5%, and darkness conditions (Naseri *et al.,* 2017).

Nutritional indices

A gravimetric method described by Waldbauer (1968), based on dry weight, was used to determine the nutritional indices of *T. castaneum* larvae and adults on tested cultivars. Seven groups of 10 fourth instars or adults were weighed and transferred to Petri dishes (diameter 6 cm, depth 1 cm) containing barley flour (≈ 200 mg) of each cultivar. Each larvae and adults were weighed again after 3 and 6 days to achieve the mean changes in the body weight. To obtain the percentage dry weight of food and insects (larvae and adults), 5 specimens of each were weighed, and dried in an oven (at 60 °C for 48 h), then re-weighed. Nutritional indices of *T. castaneum* were calculated via the formulae described by Waldbauer (1968) and Farrar *et al.* (1989) as follows:

Efficiency of conversion of ingested food (ECI) = P/E; relative consumption rate (RCR) = $E/A \times T$; relative growth rate (RGR) = $P/A \times T$; and growth rate (GR) = P/T; where A = mean dry weight of larvae or adults over unit time, E = dry weight of food consumed, P = dry weight gain of larvae or adults, and T = duration of feeding period (days).

Pupal mass and growth indices

To determine pupal weight of *T. castaneum*, seven groups of 10 pupae were weighed 48 hours after pupation. The larval growth index (LGI), standardized insect-growth index (SII) and fitness index (FI) of *T. castaneum* were determined on various barley cultivars using formula described by Pretorius (1976) and Itoyama *et al.,* 1999:

$$LGI = l_x/L$$
$$SII = P_w/L$$
$$FI = (P \times P_w)/(L + P_d)$$

where, lx = survival rate of larvae, L = larval period, P_w = pupal weight, P = percentage of pupation and P_d = pupal period.

Preparation of digestive enzymes

Tribolium castaneum fourth instars fed on each barley cultivar were ice-immobilized and dissected in distilled water under stereomicroscope (Stemi SV6 ZEISS, Germany). The guts (100 larvae for each cultivar) were then cleaned of adhering unwanted tissues and homogenized into 300 µl of distilled water. The whole body of 100 adults fed on various barley cultivars were collected into 300 µl of distilled water and homogenized with a handheld glass grinder on ice. The homogenates were then centrifuged at 12,000 *g* at 4 °C for 15 min. The obtained supernatants were collected in new micro tubes and stored in aliquots (at -20 °C) until further use (Borzoui and Bandani, 2013; Naseri *et al.,* 2017).

Activity assessment of enzymes

Digestive amylolytic activities of *T. castaneum* fourth instar and adults fed on various barley cultivars were estimated according to DNS method using 1% starch as substrate (Bernfeld, 1955). Enzyme extract (20 µl) was mixed with 50 mM acetate buffer (500 µl) at pH 5.0. Then, the mixture was incubated, after addition of 1% starch solution (40 µl), at 37 °C for 30 min. The reaction was stopped by adding 100 µl of DNS and heating in boiling water for 10 min., and the absorbance was read at 540 nm after cooling on ice. All assays were carried out in three replicates with blanks containing no enzyme extract.

General proteolysis of *T. castaneum* fourth instar and adults was assayed using 1.5% (w/v) solution of azocasein substrate in 50 mM acetate buffer (pH 5.0). Enzyme extract (20 µl) was added to 80 µl substrate and incubated at 37 °C for 50 min. Proteolysis was stopped by addition of 100 µl of 30% TCA. For each assay, appropriate blanks in which TCA was added firstly to the substrate were prepared. Precipitation was achieved by cooling at 4 °C for 30 min and centrifugation at 15,000 *g* for 10 min. The supernatant (100 µl) was added to 100 µl of 2 M NaOH and the absorbance was recorded at 440 nm. Assays were carried out in three replicates with blanks containing no enzyme extract (Elpidina *et al.,* 2001).

General protein concentrations in the crude enzyme extract of fourth instar and adults were determined using BSA as a standard according to the method of Bradford (1976).

Activity assessment of enzymes in gel

Native polyacrylamide gel electrophoresis (PAGE) was used to fractionate α-amylase on 10% polyacrylamide gel (Laemmli, 1970). Enzyme extracts from fourth instar and adults fed on various barley cultivars were loaded on zymogram gel, and electrophoresed at a voltage of 120 V for 2 h. After electrophoresis, the gel was rinsed twice with distilled water and left in a solution of 2.5% (v/v) Triton X-100 for 15 min. Then the gel was dipped into 1% starch substrate prepared in 50 mM acetate buffer (pH 5.0) containing 2 mM $CaCl_2$ and 10 mM NaCl and incubated at 37 °C for 1.5 h. Finally, to stop the reaction and stain the un-reacted starch background, the gel was treated with a solution of 1.3% I_2 and 3% KI. Zones of amylolytic activity appeared as light bands against dark background. General proteolysis in the electrophoretic gel was determined using 1% gelatin as substrate (Laemmli, 1970; Saadati *et al.,* 2011). Enzyme extracts from fourth instar and adults fed on various barley cultivars were loaded on zymogram gel and electrophoresed at a voltage of 80 V for 4 h. After electrophoresis, the gel was rinsed twice with distilled water and put in a solution of 2.5% (v/v) Triton X-100 for 30 min. Subsequently, the gel was dipped into 50 mM acetate buffer (pH 5.0) and incubated at 37 °C overnight. Finally, the gel was stained in 40% methanol, 7% glacial acetic acid and 0.05% Coomassie Brilliant Blue R and destained until proteolytic activity appeared as light bands against dark background.

Statistical analysis

Normality of the data was checked using the Kolmogorov-Smirnov test before analysis and all of data were normal. The result of each experiment was analyzed by one-way analysis of variance (ANOVA) using statistical software Minitab 16.0. Statistical differences among the means were assessed at 5% level by Tukey's test. The Pearson correlation coefficient was used to evaluate the correlations between the nutritional indices of larvae and adults with their enzymatic activity when fed on various barley cultivars using Minitab 16.0.

Results

Nutritional indices of fourth instar and adult

Nutritional indices of *T. castaneum* fourth instar were significantly different on tested barley cultivars (Table 1). The lowest weight gain ($F = 294.15$; $df = 9, 60$; $P < 0.01$), ECI ($F = 161.54$; $df = 9, 60$; $P < 0.01$), RGR ($F = 184.98$; $df = 9, 60$; $P < 0.01$) and GR ($F = 298.39$; $df = 9, 60$; $P < 0.01$) values were on cultivars Makuyi and Fajr30. The highest food consumed ($F = 219.88$; $df = 9, 60$; $P < 0.01$) and RCR ($F = 165.24$; $df = 9, 60$; $P < 0.01$) values were on cultivar Sahand and the lowest values were on cultivar Nosrat.

The data in Table 2 shows nutritional indices of adults reared on various barley cultivars. The lowest weight gain ($F = 62.86$; $df = 9, 60$; $P < 0.01$), ECI ($F = 161.54$; $df = 9, 60$; $P < 0.01$), RGR ($F = 42.87$; $df = 9, 60$; $P < 0.01$) and GR ($F = 62.86$; $df = 9, 60$; $P < 0.01$) values were on cultivars Fajr30 and Makuyi. Moreover, food consumed by adults was the lowest on cultivar Neek. Also, the adults fed on cultivars Neek and Lout showed the lowest RCR value.

Pupal mass and growth indices

The pupal mass and growth indices of *T. castaneum* are indicated in Table 3. The heaviest pupal weight ($F = 306.68$; $df = 9, 60$; $P < 0.01$) was on cultivar Lout, and the lightest one was on cultivar Makuyi. Among various barley cultivars, the LGI varied from 1.65 on cultivar Fajr30 to 4.25 on cultivar Sahra. The highest SII index ($F = 1559.52$; $df = 9, 60$; $P < 0.01$) was on cultivar Lout, whereas the lowest value was on cultivars Fajr30 and Makuyi. Moreover, the highest value of FI ($F = 2326.80$; $df = 9, 60$; $P < 0.01$) was on cultivar Lout, and the lowest was on cultivars Fajr30 and Makuyi (Table 3).

Table 1 Mean (± SE) nutritional indices of *Tribolium castaneum* fourth instar on various barley cultivars.

Barley cultivar	Weight gain (mg)	Food consumed (mg)	ECI[1] (%)	RCR[2] (mg/mg/day)	RGR[3] (mg/mg/day)	GR[4] (mg/day)
Abidar	0.534 ± 0.015d	4.55 ± 0.09b	11.72 ± 0.28e	0.401 ± 0.012b	0.047 ± 0.002b	0.076 ± 0.002b
Fajr 30	0.141 ± 0.008e	3.36 ± 0.24c	4.35 ± 0.41f	0.274 ± 0.019d	0.011 ± 0.001e	0.020 ± 0.001e
Behrokh	0.375 ± 0.006c	2.10 ± 0.03ef	17.90 ± 0.39cd	0.167 ± 0.003ef	0.030 ± 0.001c	0.053 ± 0.002c
Sahra	0.344 ± 0.017cd	1.74 ± 0.10fg	19.92 ± 0.59c	0.133 ± 0.001fg	0.026 ± 0.001cd	0.049 ± 0.002cd
Makuyi	0.168 ± 0.006e	2.97 ± 0.11cd	5.77 ± 0.43f	0.271 ± 0.010d	0.015 ± 0.001e	0.024 ± 0.001e
Neek	0.785 ± 0.025a	4.50 ± 0.12b	17.53 ± 0.70cd	0.334 ± 0.008c	0.058 ± 0.001a	0.112 ± 0.003a
Lout	0.842 ± 0.011a	2.60 ± 0.10de	32.69 ± 1.22a	0.188 ± 0.009e	0.061 ± 0.001a	0.120 ± 0.001a
Bahman	0.292 ± 0.015d	1.77 ± 0.07fg	16.54 ± 0.54d	0.140 ± 0.005efg	0.023 ± 0.001d	0.041 ± 0.002d
Sahand	0.792 ± 0.016a	6.85 ± 0.07a	11.57 ± 0.30e	0.513 ± 0.008a	0.059 ± 0.001a	0.113 ± 0.001a
Nosrat	0.318 ± 0.019cd	1.29 ± 0.06g	24.72 ± 1.08b	0.108 ± 0.006g	0.026 ± 0.001cd	0.045 ± 0.001cd

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Tukey's test ($P < 0.01$).
[1] Efficiency of conversion of ingested food, [2]Relative consumption rate, [3] Relative growth rate, [4]Growth rate

Table 2 Mean (± SE) nutritional indices of *Tribolium castaneum* adult on various barley cultivars.

Barley cultivar	Weight gain (mg)	Food consumed (mg)	ECI[1] (%)	RCR[2] (mg/mg/day)	RGR[3] (mg/mg/day)	GR[4] (mg/day)
Abidar	0.118 ± 0.005b	1.64 ± 0.06b	7.22 ± 0.17d	0.123 ± 0.005b	0.0089 ± 0.0003cd	0.017 ± 0.001b
Fajr 30	0.080 ± 0.003c	2.12 ± 0.08a	3.77 ± 0.11f	0.179 ± 0.007a	0.0067 ± 0.0002f	0.011 ± 0.000c
Behrokh	0.115 ± 0.004b	2.27 ± 0.06a	5.11 ± 0.21e	0.169 ± 0.005a	0.0086 ± 0.0003de	0.017 ± 0.001b
Sahra	0.168 ± 0.005a	1.57 ± 0.07bc	10.78 ± 0.30b	0.108 ± 0.004bc	0.0115 ± 0.0003b	0.024 ± 0.001a
Makuyi	0.081 ± 0.002c	2.14 ± 0.06a	3.82 ± 0.19f	0.187 ± 0.006a	0.0071 ± 0.0002ef	0.012 ± 0.000c
Neek	0.111 ± 0.003b	1.20 ± 0.03d	9.32 ± 0.33c	0.080 ± 0.003d	0.0074 ± 0.0002def	0.016 ± 0.000b
Lout	0.184 ± 0.004a	1.32 ± 0.04cd	13.89 ± 0.24a	0.077 ± 0.002d	0.0106 ± 0.0002bc	0.026 ± 0.001a
Bahman	0.110 ± 0.003b	1.54 ± 0.03bc	7.07 ± 0.28d	0.117 ± 0.001b	0.0083 ± 0.0003def	0.016 ± 0.001b
Sahand	0.117 ± 0.004b	1.48 ± 0.03bcd	7.87 ± 0.19d	0.093 ± 0.002cd	0.0074 ± 0.0002def	0.017 ± 0.001b
Nosrat	0.181 ± 0.008a	1.58 ± 0.08bc	11.47 ± 0.20b	0.119 ± 0.006b	0.0136 ± 0.0006a	0.025 ± 0.001a

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Tukey's test ($P < 0.01$).
[1] Efficiency of conversion of ingested food, [2]Relative consumption rate, [3] Relative growth rate, [4]Growth rate.

Table 3 Pupal weight (mg), larval growth index (LGI), standardized insect-growth index (SII), and fitness index (FI) of *Tribolium castaneum* on various barley cultivars.

Barley cultivar	Index (mean ± SE)			
	Pupal weight (mg)	GI	SII (mg/day)	FI (mg/day)
Abidar	1.872 ± 0.008de	3.990	0.093 ± 0.000de	1.366 ± 0.006bc
Fajr 30	1.725 ± 0.010f	1.646	0.047 ± 0.000f	0.430 ± 0.002f
Behrokh	1.914 ± 0.012d	4.231	0.096 ± 0.001cd	1.332 ± 0.008c
Sahra	2.020 ± 0.012c	4.250	0.104 ± 0.001b	1.354 ± 0.008bc
Makuyi	1.622 ± 0.008g	1.753	0.044 ± 0.000f	0.441 ± 0.002f
Neek	2.248 ± 0.033b	3.323	0.098 ± 0.001c	1.160 ± 0.017e
Lout	2.401 ± 0.011a	4.033	0.127 ± 0.001a	1.443 ± 0.006a
Bahman	1.898 ± 0.005de	4.168	0.094 ± 0.000.de	1.369 ± 0.004bc
Sahand	2.250 ± 0.012b	3.612	0.104 ± 0.001b	1.399 ± 0.008ab
Nosrat	1.820 ± 0.007e	3.877	0.092 ± 0.000e	1.221 ± 0.005d

Mean values in a column followed by different lowercase letters are significantly different on the basis of ANOVA with Tukey's test ($P < 0.01$).

Activity assessment of enzymes

Amylolytic activities of *T. castaneum* fourth instar and adults reared on various barley cultivars are shown in Fig. 1. The highest amylolytic activity of fourth instar (F = 47026.65; df = 9, 20; $P < 0.01$) was in larvae reared on cultivar Bahman, whereas the lowest activity was observed in larvae fed on cultivars Makuyi and Neek (Fig. 1A). The highest amylolytic activity of adults was on cultivar Makuyi (F = 12302.93; df = 9, 20; $P < 0.01$), and the lowest activity was on cultivar Lout (Fig. 1B).

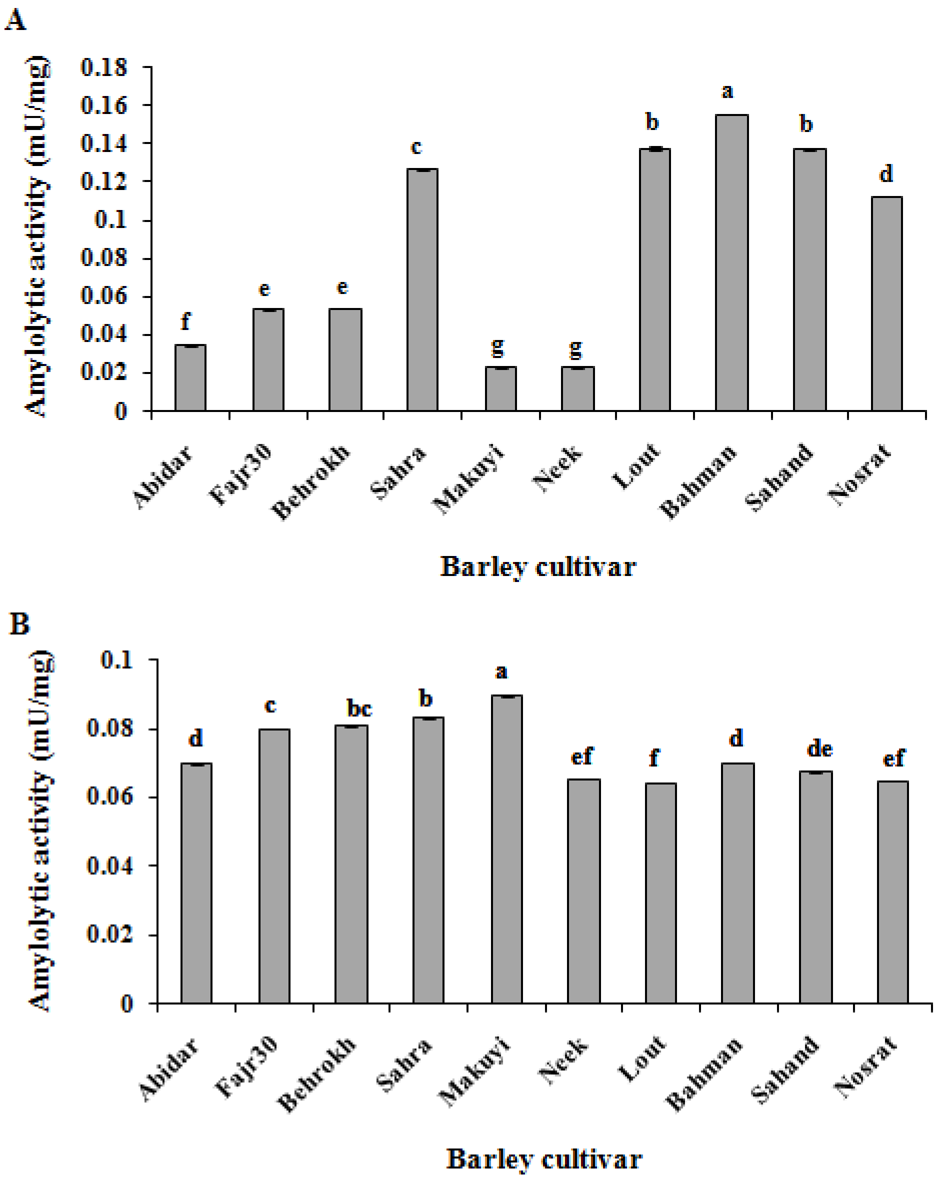

Figure 1 Mean (± SE) amylolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) ($n = 3$) fed on various barley cultivars. The means followed by different letters are significantly different (Tukey's test, $P < 0.01$).

Figure 2 indicates general proteolytic activity of *T. castaneum* fourth instar and adults reared on various barley cultivars. The fourth instar (F = 2480.49; df = 9, 20; $P < 0.01$) reared on cultivar Sahra showed the highest level of proteolytic activity, whereas the lowest activity was in larvae reared on cultivars Behrokh and Makuyi (Fig. 2A). Our data showed that the highest proteolytic activity of adults (F= 2395.03; df = 9, 20; $P < 0.01$) was on cultivar Abidar, whereas the lowest activity was on cultivar Bahman (Fig. 2B).

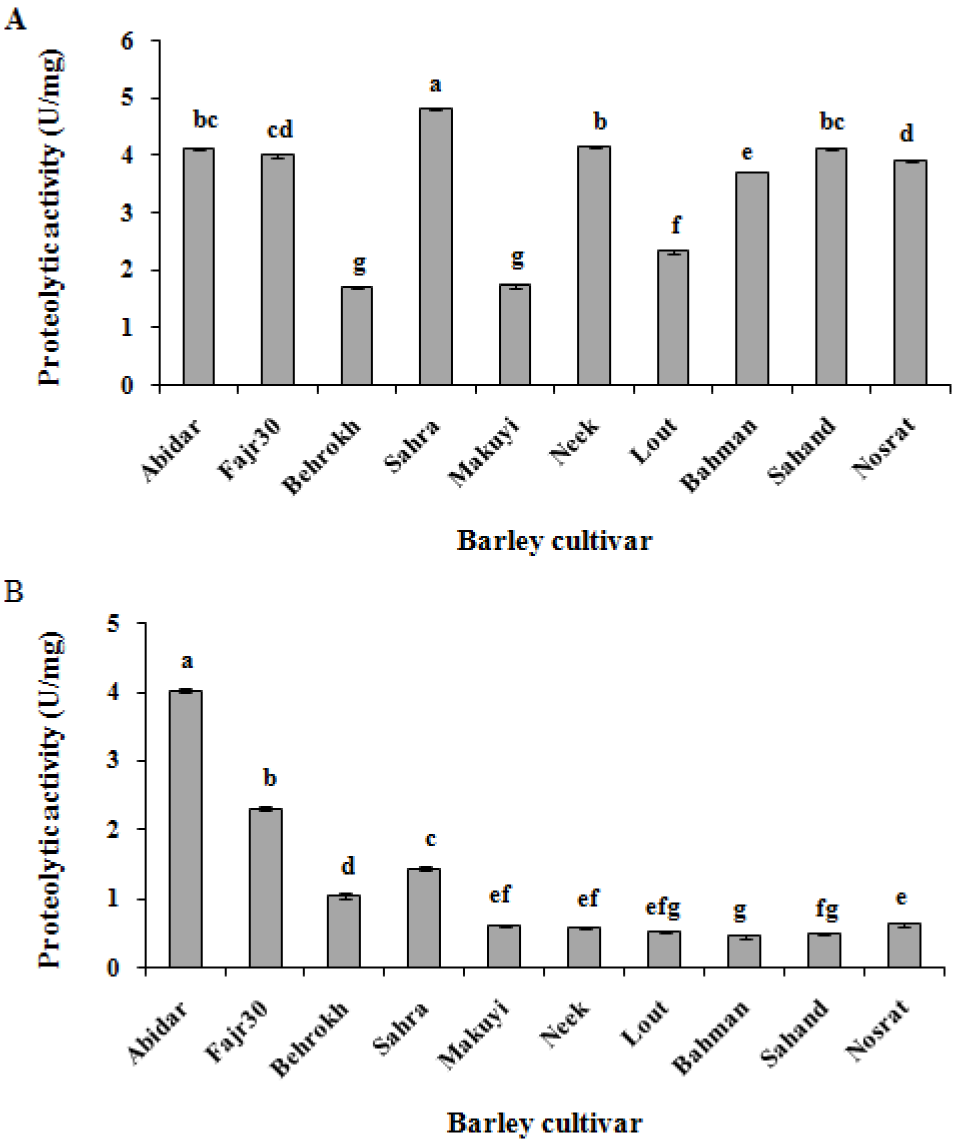

Figure 2 Mean (± SE) general proteolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) ($n = 3$) fed on various barley cultivars. The means followed by different letters are significantly different (Tukey's test, $P < 0.01$).

Activity assessment of enzymes in gel

Results from polyacrylamide gel test nearly agreed with those from the quantitative assays. Fourth instar reared on various barley cultivars showed two strong bands of amylolytic activity (isoenzymes 2 and 3). According to the amylolytic activity pattern, larvae fed on cultivars Sahra, Neek and Lout exhibited a weak intensity of band (isoenzyme 1) as compared to the others (Fig. 3A). By contrast, the adults fed on barley cultivars showed two bands on cultivars Sahra and Makuyi, and one band on the other cultivars which nearly showed the same activity pattern (Fig. 3B).

According to the zymogram of general proteolytic activity, larvae fed on cultivar Makuyi had only one band of proteolytic activity (isoenzyme3). However, larvae that were fed on the other cultivars showed three bands (isoenzymes 1-3) (Fig. 4A). The general proteolytic activity of adults fed on various barley cultivars, except for cultivars Abidar and Fajr30, showed one proteolytic band (isoenzyme 3). However, three bands of proteolytic activities were detected on cultivars Abidar and Fajr30 (Fig. 4B).

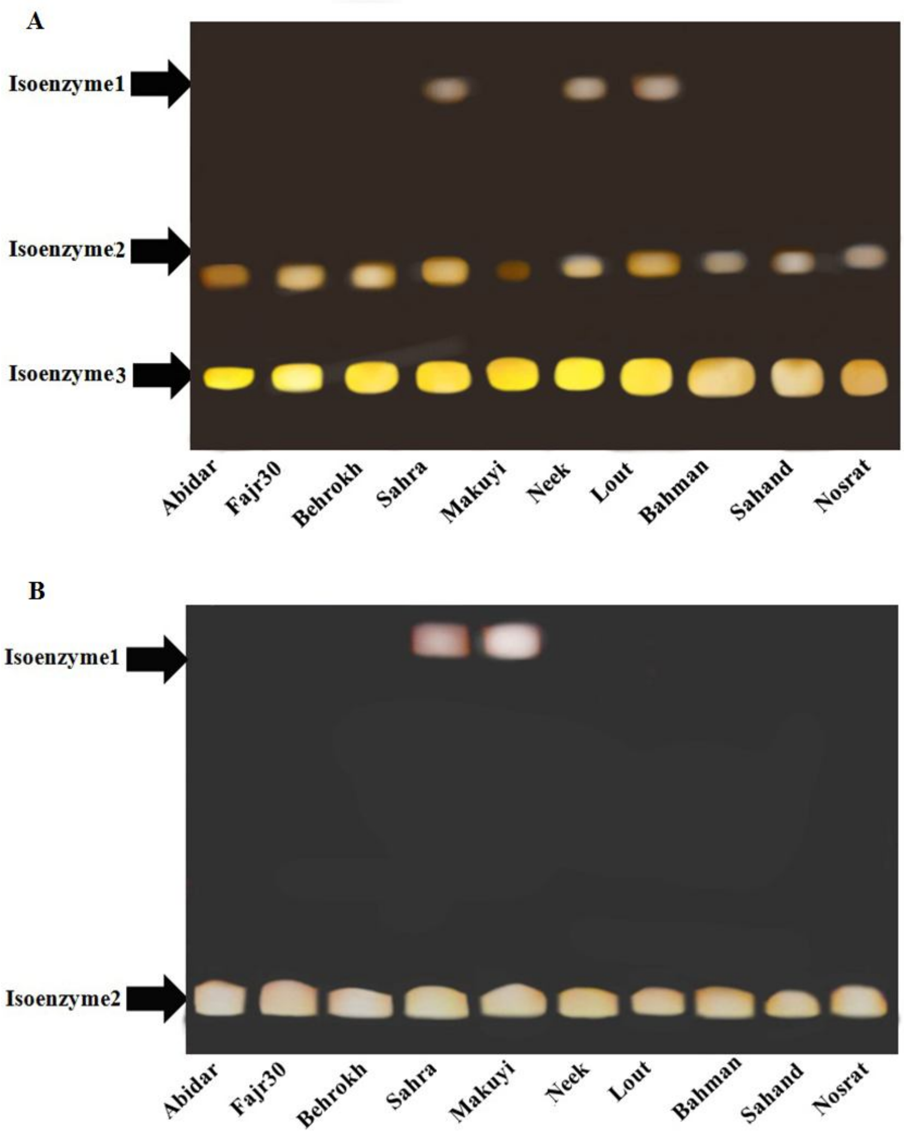

Figure 3 Mean (± SE) amylolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) in gel visualization of isozymes.

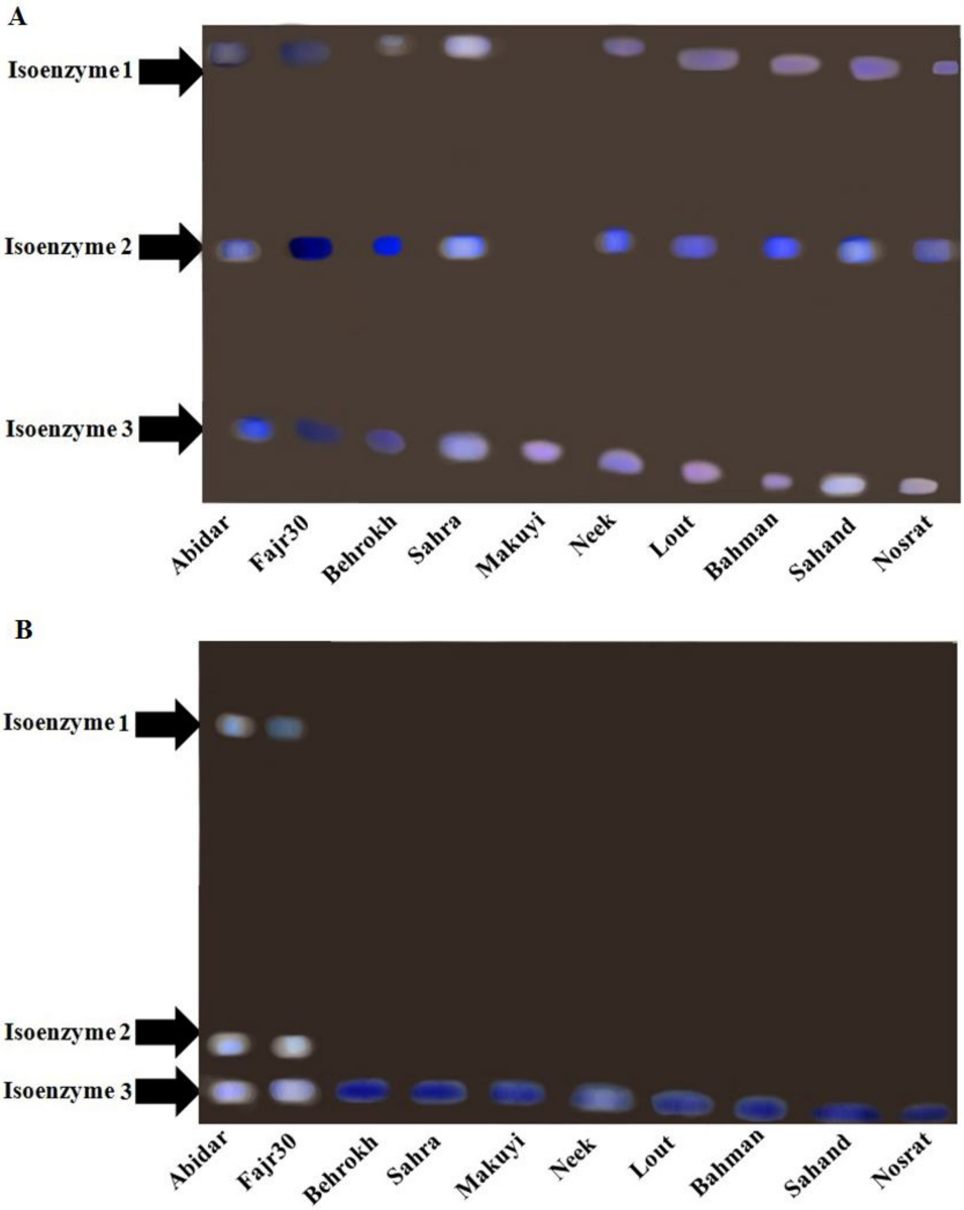

Figure 4 Mean (± SE) general proteolytic activity of *Tribolium castaneum* fourth instar (A) and adult (B) in gel visualization of isozymes.

Correlation analysis

Table 4 demonstrates the correlation analysis coefficients of the nutritional indices of *T. castaneum* larvae and adults with their enzymatic activity when fed on various barley cultivars. No correlations were observed between all nutritional indices of larvae with their amylolytic and proteolytic activities. However, correlation coefficients in adults showed that the food consumed (r = 0.787) and RCR (r = 0.794) values exhibited a positive correlation with amylolytic activity. There was a negative correlation between ECI values with the amylolytic activity of adults. Moreover, no significant correlations were observed between all nutritional indices of adults with their proteolytic activity.

Table 4 Correlation coefficients (*r*) of nutritional indices of *Tribolium castaneum* larvae and adults with their enzymatic activity when fed on various barley cultivars.

Parameters	Larva				Adult			
	Amylolytic activity		Proteolytic activity		Amylolytic activity		Proteolytic activity	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Weight gain	0.191	0.597	0.102	0.780	-0.412	0.236	-0.223	0.536
Food consumed	-0.197	0.584	0.236	0.511	0.787	0.007	0.221	0.540
ECI[1]	0.532	0.114	-0.074	0.840	-0.646	0.044	-0.271	0.449
RCR[2]	-0.294	0.410	0.204	0.571	0.794	0.006	0.240	0.503
RGR[3]	0.142	0.696	0.109	0.765	-0.294	0.409	-0.095	0.794
GR[4]	0.189	0.602	0.102	0.778	-0.453	0.189	-0.215	0.551

[1] Efficiency of conversion of ingested food, [2] Relative consumption rate, [3] Relative growth rate, [4] Growth rate.

Discussion

Since feeding requirements of the insects can change throughout their development (Barton Browne, 1995), we considered the effect of various barley cultivars not only on the nutritional physiology of *T. castaneum* larva, but also on the adult stage. In agreement with the other works (Naseri and Borzoui, 2016; Naseri *et al.,* 2017), the results of this study showed that the nutritional physiology of *T. castaneum* fourth instar and adult was significantly affected by feeding on various barley cultivars.

The insects prefer host plants that are more suitable for their growth and reproduction (Futuyma and Moreno, 1988; Jaenike, 1990; Via, 1990). Evaluation of nutritional indices can be a criterion to understand an insect's response to its host plants (Lazarevic and Peric-Mataruga, 2003). Among barley cultivars tested in this study, the lowest weight gain of larvae fed on cultivars Fajr30 and Makuyi demonstrated their low-nutritive values for this pest. The poor ability of larvae to convert food consumed to body matter was an important reason for their lower weight gain and ECI values on cultivars Fajr30and Makuyi than the others. Moreover, the lowest ECI values on these cultivars could be correlated with the kernel hardness and/or lower moisture levels of them. The lowest survival rate of *T. castaneum* larvae was observed when they were fed on cultivars Fajr30 and Makuyi (Rahimi Namin *et al.,* 2018), which is associated with lower quality of these two cultivars.

The highest ECI and RGR values in fourth instar fed on cultivar Lout suggested the high ability of larvae to incorporate the ingested food into growth. The ECI value of the fourth instar on cultivar Lout, in this study, was nearly similar to that reported by Naseri *et al.* (2017) for *T. castaneum* fourth instar on barley (unknown cultivar) (35.33%).

The lowest ECI, RGR and GR values in the adults fed on cultivars Fajr30and Makuyi showed that these cultivars have lower amount of some nutrients, which affected their growth and increased consumption related with changes in efficiencies (Slansky and Feeny, 1977; Rahimi Namin *et al.,* 2014). In this research, the RGR value of adults on cultivars Fajr30 and Makuyi was nearly 3.5-folds lower than Sagheer *et al.* (2014) reported for *T. castaneum* adults on barley (0.024 mg/mg/day), indicating that the barley cultivar was more suitable than those used in our research. High ECI value in the adults fed on cultivar Lout revealed that they had a high efficiency to convert eaten food to body matter on this cultivar, which can be correlated with their low food consumption (Abisgold and Simpson, 1987; Rahimi Namin *et al.,* 2014; Golizadeh and Abedi, 2017).

In the present research, larvae reared on cultivar Fajr30 had the lightest pupal weight as compared to the others, suggesting that cultivar Fajr30 was unsuitable cultivar for *T. castaneum* larvae. Also, lower value of LGI in the larvae fed on cultivars Fajr30 and Makuyi can be correlated with a lower survival rate of larvae and longer larval period on these cultivars. Moreover, the lowest SII value on cultivars Fajr30 and Makuyi may be related to lower pupal weight or longer larval period on these cultivars. Obtained results showed that FI was the lowest on cultivars Fajr30 and Makuyi, which can be attributed with a lower percentage of pupation or lower pupal weight on these cultivars (Table 3).

This work demonstrated that various barley cultivars significantly affected the amylolytic and proteolytic activities of *T. castaneum* fourth instar and adult. Although low amylolytic activity of the fourth instar was on cultivar Makuyi; the highest amylolytic activity of the adults was on this cultivar. According to Batista-Pereira *et al.* (2002), the amount of food consumption by an insect depends on the food digestibility. The RCR value and amount of food consumed by larvae on cultivar Makuyi (Table 4) were the main factors, which had a positive correlation with the digestive amylolytic activity (Sivakumar *et al.,* 2006; Naseri and Borzoui, 2016).

The low proteolytic activity level in larvae fed on cultivar Makuyi (Fig. 2A), might be due to the high kernel hardness (Rahimi Namin *et al.,* 2018) or response of the insect to the ingested protease inhibitors (PIs). Also, high proteolytic activity of adults on cultivar Fajr30 (Fig. 2B) might be correlated with the high protein content (Rahimi Namin *et al.,* 2018) or presence of PIs in this cultivar. The role of PIs on level of enzymatic activity in *T. castaneum* is unknown, and should be investigated in future works.

The larval dietary conditions are vital for the insects whose larval food sources vary from those of adults and the effects of poor larval nutrition cannot be compensated by adult feeding (O'Brien *et al.,* 2004; Plesnar-Bielak *et al.,* 2017). According to Plesnar-Bielak *et al.* (2017), even if *T. castaneum* larvae and adults live on the same food, larval diet is a main factor affecting adult life history. In the current research, higher ECI and enzymatic activity for *T. castaneum* fourth instar as compared to the adults indicated a better feeding performance of larvae than adult stage. Consequently, controlling of the larvae should be targeted to improve effective management of *T. castaneum* on barley cultivars.

In conclusion, the observations of the present research revealed that cultivars Fajr30 and Makuyi were less nutritive for feeding and growth of *T. castaneum* owing to lower nutritional performance (especially ECI) and larval growth indices. Furthermore, results from digestive enzymes tests showed that amylolytic and proteolytic activities of larvae and adults, in most cases, were relatively lowest when they were reared on cultivar Makuyi. Therefore, it is concluded that Makuyi was an unsuitable barley cultivar and can be suggested to be grown and stored in areas where the damage of *T. castaneum* is greater. In the future investigations, responses of *T. castaneum* to the PIs from barley and other cereals should be evaluated for selection of proper PIs, which can be utilized in transgenic expression for the insect pest resistance.

Acknowledgments

This work was financially supported by the University of Mohaghegh Ardabili, (Ardabil, Iran), which is greatly appreciated.

References

Abisgold, J. D. and Simpson, S. J. 1987. The physiology of compensation by locusts for changes in dietary protein. Journal of Experimental Biology, 129: 329-346.

Barton Browne, L. 1995. Ontogenetic changes in feeding behavior. In: Chapman, R. F. and Boer, G. de. (Eds.), Regulatory Mechanisms in Insect Feeding. Chapman and Hall, pp: 307-342.

Batista-Pereira, G. L., Petacci, F., Fernandes, B. J., Correa, A. G., Vieira, P. C., Fatima da Silva, M. and Malaspina, O. 2002. Biological activity of astilbin from *Dimorphandra mollis* against *Anticarsia gemmatalis* and *Spodoptera frugiperda*. Pest Management Science, 58: 503-507.

Bernfeld, P. 1955. Amylases, α and β. Methods in Enzymology, 1: 149-154.

Borzoui, E. and Bandani, A. R. 2013.Wheat and triticale proteinaceous seed extracts inhibit gut α-amylase and protease of the carob moth, *Ectomyelois ceratoniae*. Molecular Entomology, 4 (3): 13-21.

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

Campbell, J. F. and Arbogast, R. T. 2004. Stored-product insects in a four mill: population dynamics and response to fumigation treatments. Entomologia Experimentalis et Applicata, 112: 217-225.

Elpidina, E. N., Vinokurov, K. S., Gromenko, V. A., Rudenshaya, Y. A., Dunaevsky, Y. E. and Zhuzhikov, D. P. 2001. Compartmentalization of proteinases and amylases in *Nauphoeta cinerea* midgut. Archives of Insect Biochemistry and Physiology, 48: 206-216.

Fabres, A., Ma da Silva, J. C., Fernandes, K. V. S., Xavier-Filho, J., Rezende, G. L. and Oliveira, A. E. A. 2014. Comparative performance of the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) on different plant diets. Journal of Pest Science, 87 (3): 495-506.

Farrar, R. R., Barbour, J. D. and Kennedy, G. G. 1989. Quantifying food consumption and growth in insects. Annals of the Entomological Society of America, 82: 593-598.

Futuyma, D. J. and Moreno, G. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics, 19: 207-233.

Golizadeh, A. and Abedi, Z. 2017. Feeding performance and life table parameters of Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) on various barley cultivars. Bulletin of Entomological Research, 107 (5): 689-698.

Houshyar, E. 2017. Environmental impacts of irrigated and rain-fed barley production in Iran using life cycle assessment (LCA). Spanish Journal of Agricultural Research, 15 (2): 1-13.

Iram, N., Arshad, M. and Akhter, N. 2013. Evaluation of botanical and synthetic insecticide for the control of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Bio Assay, 8 (3): 1-10.

Itoyama, K., Kawahira, Y., Murata, M. and Tojo, S. 1999. Fluctuations of some characteristics in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) reared under different diets. Applied Entomology and Zoology, 34: 315-321.

Jaenike, J.1990. Host specialization in phytophagous insects. Annual Review of Ecology and Systematics, 21: 243-273.

Javed, M., Zeeshan Majeed, M., Khaliq, A., Arshad, M., Ahmad, M. H. and Sufyan, M. 2016. Quantitative losses in some advanced genotypes of barley incurred by *Tribolium castaneum* L. (Herbst.). International Journal of Agronomy and Agricultural Research (IJAAR), 8 (2): 45-50.

Khan, N. D., Khan, Z. H. and Kamble, L. H. 2012. Inhibitory potential of sorghum α-amylase inhibitor on the digestive enzyme of red flour beetle, *Tribolium castaneum*. Trends in Life Sciences, 1 (4): 20-25.

Kheradpir, N. 2014. Food preference of *Tribolium castaneum* among four flour types. European Journal of Experimental Biology, 4 (1): 436-439.

Khodabandeh, N. 2003. Cereals. 7^{th}ed. Tehran, Tehran University Press.

Kordan, B. and Gabrys, B. 2013. Effect of barley and buckwheat grain processing on the development and feeding of the confused flour beetle. Journal of Plant Protection Research, 53 (1): 96-101.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Lazarevic, J. and Peric-Mataruga, V. 2003. Nutritive stress effects on growth and digestive physiology of *Lymantria dispar* larvae. Yugoslav Medical Biochemistry, 22: 53-59.

Naseri, B. and Borzoui, E. 2016. Life cycle and digestive physiology of *Trogoderma granarium* (Coleoptera: Dermestidae) on various wheat cultivars. Annals of the Entomological Society of America, 109 (6): 831-838.

Naseri, B., Borzoui, E., Majd, Sh. and Mansouri, S. M. 2017. Influence of different food commodities on life history, feeding efficiency, and digestive enzymatic activity of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Economic Entomology, 110 (5): 2263-2268.

O'Brien, D. M., Boggs, C. L. and Fogel, M. L. 2004. Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. Oikos, 105: 279-291.

Plesnar-Bielak, A., Woch, K. R., Małszycki, M. A., Alkhawlany, A. T. H., Hołysz, A., Assis Correia, J. F., Turk, N., Ugrin, M., Kramarz, P. and Prokop, Z. M. 2017. Larval and adult nutrition effects on reproductive traits in the red flour beetle. Journal of Zoology, 302: 79-87.

Pretorius, L. M. 1976. Laboratory studies on the developmental reproductive performance of *Helicoverpa armigera* on various food plants. Journal of the Entomological Society of Southern Africa, 39: 337-334.

Rahimi Namin, F., Naseri, B. and Razmjou, J. 2014. Nutritional performance and activity of some digestive enzymes of the cotton bollworm, *Helicoverpa armigera*, in response to seven tested bean cultivars. Journal of Insect Science, 14 (93): 1-18.

Rahimi Namin, F., Naseri, B., Nouri Ghanbalani, Gh. and Razmjou, J. 2018. Demographic studies of *Tribolium castaneum* (Coleoptera: Tenebrionidae) on various barley cultivars. Journal of Stored Products Research, 79: 60-65.

Saadati, F., Bandani, A. R. and Moslemi, A. 2011. Effect of plant seeds protein extract on the sunn pest, *Eurygaster integriceps* Puton, growth and development and its gut serine protease activity. African Journal of Biotechnology, 10: 11502-11510.

Sagheer, M., ul-Hasan, M., Bilal, M., ul-Hassan, M. N., Ahmad Khan, F. Z., Haidri, S. R. and Farhan, M. 2014. Nutritional indices of *Tribolium castaneum* (Herbst) and its response to plant extracts in relation to three types of flours. International Journal of Agronomy and Agricultural Research (IJAAR), 4 (5): 51-56.

Sarwar, M. 2013. Development and boosting of integrated insect pests' management in stored grains. Research and Reviews: Journal of Agriculture and Allied Sciences, 2 (4): 16-20.

Sarwar, M. 2015. Categorization of some advanced local wheat lines against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). International Journal of Life Science and Engineering, 1 (3): 108-113.

Schulte, D., Close, T. J., Graner, A., Langridge, P., Matsumoto, T., Muehlbauer, G. and Stein, N. 2009. The international barley sequencing consortium-at the threshold of efficient access to the barley genome. Plant Physiology, 149 (1): 142-147.

Shafique, M., Ahmad, M. and Chaudry, A. 2006. Feeding preference and development of *Tribolium castaneum* (Herbst.) in wheat products. Pakistan Journal of Zoology, 38 (1): 27-31.

Shweta, S. and Prakash, S. 2013. Development of resistance in *Tribolium castaneum*, Herbst (Coleoptera: Tenebrionidae) towards deltamethrin in laboratory. International Journal of Scientific and Research Publications, 3 (8): 2250-3153.

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

Slansky, F. Jr. and Feeny, P. 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecological Monographs, 47: 209-228.

Via, S. 1990. Ecological genetics and host adaption in herbivorous insects: the experimental study of evolution in natural and agricultural systems. Annual Review of Entomology, 35: 421-446.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Advances in Insect Physiology, 5: 229-288.

تأثیر ارقام مختلف جو روی فیزیولوژی تغذیه‌ای *Tribolium castaneum* (Coleoptera: Tenebrionidae)

فروغ رحیمی نمین، قدیر نوری قنبلانی، بهرام ناصری* و جبرائیل رزمجو

گروه گیاه‌پزشکی، دانشکده کشاورزی و منابع طبیعی، دانشگاه محقق اردبیلی، اردبیل، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: bnaseri@uma.ac.ir

دریافت: ۲۳ بهمن ۱۳۹۷؛ پذیرش: ۲۲ تیر ۱۳۹۸

چکیده: شپشه‌ی قرمز آرد، *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)، آفت جدی و همه‌جازی دانه‌های غلات و فرآورده‌های آنها در انبار می‌باشد. در این پژوهش، شاخص‌های تغذیه‌ای و فعالیت آنزیم‌های گوارشی لاروهای سن چهارم و حشرات کامل *T. castaneum* روی ده رقم جو (فجر۳۰، بهرخ، صحرا، ماکویی، نیک، لوت، بهمن، نصرت، آبیدر و سهند) در دمای ۱ ± ۳۰ درجه سلسیوس، رطوبت نسبی ۵ ± ۷۵ درصد و شرایط تاریکی مطالعه شدند. نتایج نشان داد که لاروهای سن چهارم و حشرات کامل پرورش یافته روی ارقام ماکویی و فجر۳۰ کم‌ترین مقادیر افزایش وزن، کارایی تبدیل غذای خورده شده (ECI)، نرخ رشد نسبی و نرخ رشدی (GR) را داشتند. با این حال، حشرات تغذیه شده با رقم لوت بیش‌ترین افزایش وزن، ECI و GR را داشتند. بیش‌ترین فعالیت آمیلولیتیک لاروها روی رقم بهمن بود، درحالی‌که کم‌ترین فعالیت این آنزیم روی ارقام ماکویی و نیک بود. فعالیت آمیلولیتیک حشرات کامل روی رقم ماکویی بیش‌ترین و روی رقم لوت کم‌ترین مقدار به‌دست آمد. هم‌چنین، فعالیت پروتئولیتیک لاروها روی رقم صحرا بیش‌ترین و روی رقم بهرخ و ماکویی کم‌ترین بود. بیش‌ترین فعالیت پروتئولیتیک حشرات کامل روی رقم آبیدر و کم‌ترین فعالیت این آنزیم روی رقم بهمن بود. نتایج این مطالعه نشان داد که ارقام فجر۳۰ و ماکویی از ارزش غذایی پایین‌تر و رقم لوت از ارزش غذایی بالاتری برای تغذیه *T. castaneum* برخوردار بودند. بنابراین باید توجه بیش‌تری به مدیریت آفت روی رقم لوت به‌عنوان رقم حساس داشت.

واژگان کلیدی: شاخص تغذیه‌ای، آنزیم گوارشی، جو، شپشه قرمز آرد