Journal of Stored Products Research 87 (2020) 101591

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Nutritional responses and digestive enzymatic profile of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) on 10 commercial rice cultivars

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A R T I C L E I N F O

Article history:
Received 27 December 2019
Received in revised form
14 February 2020
Accepted 29 February 2020
Available online xxx

Keywords:
Nutritional index
Grain resistance
Rice cultivar
Digestive enzyme
Khapra beetle

A B S T R A C T

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is an important polyphagous pest of stored grains in the world. Nutritional performance and digestive amylase and protease activity of fifth instar larvae were studied on different commercial rice cultivars (Sadri, Tarom, Neda, Fajr, Nemat, Shafagh, Kadous, Gohar, Gilane, and Khazar) at 33 ± 1 °C, relative humidity of 65 ± 5%, and a photoperiod of 14:10 (L:D) h. Larvae fed with cultivar Gilane consumed more food and increased body weight higher than those fed with other cultivars. However, the lowest values of nutritional indices were found in larvae fed with cultivar Khazar. The lightest pupal weight was on cultivars Fajr, Nemat and Sadri. The larval growth index differed from 1.99 to 2.67 on cultivars Khazar and Tarom, respectively. Among various tested cultivars, the highest standardized insect-growth index was on cultivar Gilane, whereas the lowest index was on cultivars Fajr, Nemat and Sadri. Furthermore, the fitness index showed the highest value on cultivars Gilane and Neda, and the lowest on cultivar Sadri. The amylolytic activity was the highest in larvae fed with cultivar Gilane, and the lowest in larvae fed with cultivars Fajr and Khazar. The cultivar Gilane-fed larvae exhibited the highest proteolytic activity as compared with other cultivars. The cluster analysis showed that Nemat, Neda, Fajr and Khazar were the least suitable (partially resistant) cultivars to *T. granarium*, which could be further investigated to identify proteins that contribute to khapra beetle resistance.

1. Introduction

The rice (*Oryza sativa* L.) provides a great deal of human food for more than 50% of the world's population (Ashtiani Araghi et al., 2010). In Asian countries including Iran, it is a strategic cereal crop that supplies about 80% of daily food calories for the people (Bao and Bergman, 2004).

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is one of the polyphagous and key pests of stored cereal grains such as wheat, barley, maize, rice and their products in Asia, Africa, and some areas of southern Europe (Lowe et al., 2000; Ahmedani et al., 2009; Athanassiou et al., 2016, 2019). It is an invasive species with a high potential to establish in different geographical areas of the world (Burges, 2008). It is well-known that the germination potential, nutritive value and weight of grains infested by the khapra beetle larvae can be decreased; resulting reduced the marketability of them (Jood and Kapoor, 1993; Jood et al., 1996; Burges, 2008). Moreover, the infestation of stored grains and their products by setae and body parts of *T. granarium* larvae can lead to gastrointestinal and allergic irritations (Jood et al., 1996). When the susceptible rice cultivars are stored for a long time, especially in traditional storage systems, *T. granarium* could cause severe economic loss to them. Therefore, identifying the cultivars that are resistant or less nutrient to *T. granarium* is necessary to minimize grain losses by this insect.

Using storage insecticides has been the first control option of post-harvest insect pests like *T. granarium* (Throne et al., 2000; Finkelman et al., 2006). However, repeated and improper use of insecticides can lead to the insect's resistance and pollution of stored products and the environment (Hagstrum and Subramanyam, 1996; Mohandass et al., 2007). It is, therefore, critical to develop benign alternative control approaches that will result in better protection of stored products against this pest (Lawrence and Koundal, 2002; Majd-Marani et al., 2017). To achieve

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https://doi.org/10.1016/j.jspr.2020.101591

this purpose, knowledge of the feeding and growth performances of stored product pests on different host cultivars could be a prerequisite to identify secondary chemicals of host grains that are unfavorable to *T. granarium* (Borzoui and Naseri, 2016; Borzoui et al., 2017; Naseri et al., 2017).

For insects with a non-feeding adult stage (e.g. *T. granarium*), the amount and quality of food eaten by larvae strongly affect the storage of resources in pupal and adult stages (Awmack and Leather, 2002). The ability of insects to feed and develop on various host cultivars is associated with nutrient levels of consumed food and some physical and biochemical features of host plants (Joern and Behmer, 1997; Karasov et al., 2011). The nutritional quality of host cultivars can alter the growth, reproductive rate, and feeding behavior of herbivorous insects (Tsai and Wang, 2001). Study of the resistance indices of various host cultivars to insect pests can improve our knowledge about their suitability for development and population increase of the insects (Golizadeh and Abedi, 2016, 2017). In recent years, evaluation of nutritional performance and digestive physiology of the khapra beetle larvae fed with different grain cultivars is suggested as one of the major tools of an integrated pest management program (Naseri and Borzoui, 2016; Majd-Marani et al., 2018).

There are several published papers regarding the resistance of various stored grains such as wheat, barley, maize, and rice to the khapra beetle by measuring its biology, nutritional indices and digestive physiology. For example, Sayed et al. (2006) investigated the resistance of wheat cultivars against *T. granarium*, and reported cultivar Mehran-89 as the least suitable host for feeding of the larvae. Borzoui et al. (2015) studied the life history and nutritional performance of *T. granarium* fed with different diets and found that walnut was an unsuitable diet for this pest. Studying the effect of different barley cultivars on the nutritional physiology of the khapra beetle, Seifi et al. (2015) reported that cultivar Bahman was the most unfavorable diet for feeding of this beetle. Evaluating the effect of wheat cultivars on feeding performance of *T. granarium*, Golizadeh and Abedi (2016) declared that cultivar Kuhdasht was not suitable host for the pest. According to Majd-Marani et al. (2018), nutritional performance and activity of some digestive enzymes of *T. granarium* larvae were affected when they were fed with various maize hybrids.

In a recent study, Barzin et al. (2019) investigated the feeding indices and digestive profile of *T. granarium* on six rice cultivars, and reported that Gilane and Khazar were susceptible and resistant cultivars, respectively. Due to the economic importance of *T. granarium* on a wider range of stored rice cultivars, we decided to compare the resistance status of other commercial rice cultivars apart from those tested by Barzin et al. (2019). The selected cultivars are among the most widely grown rice cultivars in Iran. Therefore, this study aimed to evaluate the influence of ten commercial rice cultivars on feeding performance, growth indices and digestive enzymatic activity (protease and amylase) of the khapra beetle. The results of this study will be helpful in the identification of resistant rice cultivar(s) for the development of transgenic rice grains.

2. Materials and methods

2.1. Sources of rice and insect

De-husked and white rice (*O. sativa*) cultivars including Sadri, Tarom, Neda, Fajr, Nemat, Shafagh, Kadous, Gohar, Gilane, and Khazar were obtained from the Agricultural and Natural Resources Research Center of Guilan (Rasht, Iran), and powdered using an electric grinder. Two cultivars of Gilane and Khazar were selected because they were reported as susceptible and resistant cultivars against *T. granarium*, respectively (Barzin et al., 2019). All selected cultivars were free of infestation and insecticides.

The primary population of *T. granarium* was obtained from colonies maintained at the Department of Plant Protection, University of Mohaghegh Ardabili, (Ardabil, Iran). The insects were reared on ground wheat grains (as an ideal host) and kept under laboratory conditions (33 ± 1 °C, relative humidity of 65 ± 5%, and a photoperiod of 14:10 (L:D) h), according to the method described by Karnavar (1967).

2.2. Nutritional performance

To determine the nutritional performance of *T. granarium* larvae on tested rice cultivars, a gravimetric method, based on dry weight, was used (Waldbauer, 1968). Three groups of 10 fifth instar larvae were weighed and placed to Petri dishes (diameter 6 cm, depth 1 cm) including ground rice grains from each cultivar. Each larval group was weighed again after 7 days to achieve the changes in the body mass. To determine the percentage of dry weight, 20 larvae were weighed, and dried in an oven (at 60 °C for 48 h), then reweighed. Nutritional indices of *T. granarium* were measured using the formulae described by Waldbauer, 1968:

Efficiency of conversion of ingested food (ECI) = P/E; relative consumption rate (RCR) = $E/W_0 \times T$; and relative growth rate (RGR) = ECI × RCR; where, W_0 = initial dry weight of larvae, E = dry weight of food eaten, P = dry weight gain of larvae, T = feeding time (day).

2.3. Pupal mass and growth indices

To determine the pupal mass of *T. granarium*, seven groups of 10 pupae were weighed on the day of emergence. The larval growth index (LGI), standardized insect-growth index (SII) and fitness index (FI) of *T. granarium* were calculated on various rice cultivars (Itoyama et al., 1999): $LGI = l_x/L$; $SII = P_w/L$; $FI = (P \times P_w)/(L + P_d)$; where, l_x = survival rate of larvae, L = larval period, P_w = pupal mass, P = percentage of pupation and P_d = pupal period.

2.4. Enzymes preparation

The fifth instar larvae of *T. granarium* fed with each rice cultivar were ice-immobilized and dissected on ice under stereomicroscope (Stemi SV6 ZEISS, Germany). One-hundred midguts were homogenized into 300 μl of distilled water using a handheld glass grinder. The homogenates were then centrifuged at 15,000 *g* at 4 °C for 10 min. The obtained supernatants were stored in aliquots (at −20 °C) until further use (Hosseininaveh et al., 2007; Borzoui and Bandani, 2013).

2.5. Activity of digestive enzymes

Digestive amylolytic activity of *T. granarium* fifth instar larvae fed with various rice cultivars were estimated based on Bernfeld's method with minor modification (Bernfeld, 1955). The enzyme extract (20 μl) was mixed with Tris-Hcl (130 μl) at pH 8.0. The enzymatic activity was started by adding 60 μl of 1% soluble starch (Sigma chemical Co., St Louis, USA, 99% purity) to this mixture, and incubated at 37 °C for 30 min. The reaction was terminated by adding 100 μl of 3,5-dinitrosalicylic acid and heating in boiling water for 10 min. Then, the absorbance was read at 540 nm after cooling on ice. All assays were conducted in three replicates with blanks containing no enzyme extract.

General proteolytic activity of *T. granarium* fifth instar larvae was assayed using 1.5% soluble azocasein substrate in 50 mM glycine-NaOH buffer (pH 10) (Borzoui et al., 2015). The enzymatic activity was started by mixing 50 μl of enzyme extract with 50 μl of the substrate and incubated at 37 °C for 50 min. Azocasein digestion was stopped by the addition of 100 μl of 30% trichloroacetic acid (TCA). For each assay, proper blanks in which TCA was added firstly to the substrate were prepared. Precipitation was caused by cooling at 4 °C for 30 min and centrifugation at 15,000 *g* for 10 min. The supernatant (100 μl) was mixed with 2 M NaOH (100 μl) and the absorbance was read at 440 nm. Assays were carried out in three replicates with blanks containing no enzyme extract (Elpidina et al., 2001).

General protein concentration in the crude enzyme extract of fifth instar was determined by Bradford's procedure using bovine serum albumin (BSA; Roche Co., Germany) as a standard (Bradford, 1976).

2.6. Grain properties

Protein content in grains of rice cultivars tested in this study was quantified using BSA as a standard (Bradford, 1976). Moreover, starch content in grains of tested cultivars was evaluated using starch as a standard (Bernfeld, 1955). Briefly, 200 mg of the ground grains of each rice cultivar was homogenized in 35 ml of distilled water and heated to boiling. The amount of 100 μl of each sample was mixed with 2.5 ml of iodine reagent (0.02% I2 and 0.2% KI), and the absorbance was read at 580 nm. The grain hardness was determined by standard particle size index (%) method (AACC 55e30, 2000). To determine moisture content of tested cultivars, 2 g of each ground cultivar was weighed and dried in an oven (100 °C) for 3 h, then re-weighed.

2.7. Statistical and cluster analyses

All the data obtained from each trial were examined for normality using Kolmogorov–Smirnov test (PROC GLM; SAS Institute, 2009), and analyzed by one-way analysis of variance (ANOVA). As the data were normally distributed, no data transformation was done. Statistical differences among the means were evaluated by the Tukey test at the significance level of 0.05. The Pearson correlation coefficient was used to analyze the correlations of grain properties of tested cultivars with nutritional performance and enzymatic activity of *T. granarium* using SPSS 16.0. A dendrogram of various rice cultivars according to nutritional performance, growth indices and activity of tested digestive enzymes of *T. granarium* fed with these cultivars was drawn by Ward's method using SPSS 16.0.

3. Results

3.1. Nutritional performance

The mean nutritional indices of *T. granarium* fifth instar larvae were significantly different on tested rice cultivars (Table 1). The lowest food consumption ($F = 193.67$; $df = 9, 20$; $P < 0.01$), weight gain ($F = 111.63$; $df = 9, 20$; $P < 0.01$), ECI ($F = 14.00$; $df = 9, 20$; $P < 0.01$), RCR ($F = 14.00$; $df = 9, 20$; $P < 0.01$) and RGR ($F = 17.71$; $df = 9, 20$; $P \leq 0.01$) values were on cultivar Khazar. The food consumption and weight gain of larvae was the highest when they were fed with cultivar Gilane. Larvae reared on cultivar Sadri showed the highest RCR and RGR indices; however, cultivar Tarom-fed larvae had the highest ECI value.

3.2. Pupal mass and growth indices

The survival rate of larvae, pupal mass and growth indices of *T. granarium* are shown in Table 2. The survival rate of larvae ranged from 96% on cultivars Gilane and Tarom to 83% on cultivar Khazar. The pupal mass ($F = 4.74$; $df = 9, 20$; $P < 0.01$) was the heaviest on cultivars Gilane, Neda and Kadous, and the lightest on cultivars Fajr, Nemat and Sadri. The LGI value differed from 1.99 on cultivar Khazar to 2.67 on cultivar Tarom (($F = 36.69$; $df = 9, 20$; $P < 0.01$). Among various tested cultivars, the highest SII ($F = 7.31$; $df = 9, 20$; $P < 0.01$) was on cultivar Gilane, whereas the lowest value was on cultivars Fajr, Nemat and Sadri. Furthermore, the FI value ($F = 5.16$; $df = 9, 20$; $P < 0.01$) was the highest on cultivars Gilane and Neda, and the lowest on cultivar Sadri.

3.3. Activity of digestive enzymes

Table 3 shows amylolytic and proteolytic activities of *T. granarium* fifth instar larvae on various rice cultivars. The highest amylolytic activity was in larvae fed with cultivar Gilane, whereas the lowest activity was in larvae fed with cultivars Fajr and Khazar ($F = 17.80$; $df = 9, 20$; $P < 0.01$). The fifth instar larvae reared on cultivar Gilane showed the highest proteolytic activity ($F = 6.14$; $df = 9, 20$; $P < 0.01$).

3.4. Grain properties

Data of some biochemical and physical properties in grains of tested rice cultivars are indicated in Table 4. Starch content was the highest in cultivar Gilane, and the lowest in other examined cultivars ($F = 5.22$; $df = 9, 20$; $P < 0.01$). Among rice cultivars, the highest protein content was measured in cultivars Gilane and Tarom, whereas the lowest content was in cultivars Khazar and Fajr

Table 1
Mean (±SE) nutritional indices of fifth instar *Trogoderma granarium* fed with different rice cultivars.

Cultivar	E (mg)	P (mg)	ECI (%)	RCR (mg/mg/day)	RGR (mg/mg/day)
Gilane	2.54 ± 0.02a	0.83 ± 0.02a	32.95 ± 0.97abc	0.075 ± 0.0005b	0.025 ± 0.001abc
Nemat	1.84 ± 0.02d	0.52 ± 0.02c	28.14 ± 0.82c	0.063 ± 0.0008bc	0.017 ± 0.0006cde
Shafagh	1.53 ± 0.05e	0.45 ± 0.03cd	29.79 ± 1.55bc	0.051 ± 0.004cd	0.015 ± 0.001de
Khazar	1.06 ± 0.03g	0.22 ± 0.01f	20.76 ± 1.56d	0.033 ± 0.0004d	0.007 ± 0.0008f
Sadri	2.39 ± 0.07 ab	0.75 ± 0.02a	31.47 ± 0.19bc	0.098 ± 0.01a	0.031 ± 0.005a
Neda	1.31 ± 0.02f	0.40 ± 0.08de	30.71 ± 0.58bc	0.037 ± 0.0004d	0.011 ± 0.0004ef
Tarom	2.11 ± 0.04c	0.80 ± 0.02a	38.22 ± 0.48a	0.073 ± 0.01b	0.028 ± 0.006 ab
Kadous	2.33 ± 0.08b	0.65 ± 0.01b	28.10 ± 0.42c	0.069 ± 0.001bc	0.019 ± 0.0001cde
Gohar	1.88 ± 0.02d	0.65 ± 0.03b	34.95 ± 1.76 ab	0.059 ± 0.007bc	0.021 ± 0.003bcd
Fajr	1.24 ± 0.06f	0.36 ± 0.01e	29.33 ± 2.24bc	0.050 ± 0.006cd	0.014 ± 0.001de

The means within a column followed by the same letter are not statistically different (Tukey, $P < 0.01$). E = Food consumption, P = Weight gain, ECI = Efficiency of conversion of ingested food, RCR = Relative consumption rate, RGR = Relative growth rate.

Table 2
Survival rate of larvae, and mean (±SE) pupal mass, larval growth index (LGI), standardized-insect growth index (SII), and fitness index (FI) of *Trogoderma granarium* fed with different rice cultivars.

Cultivar	Survival rate (%)	Pupal mass (mg)	LGI	SII (mg/day)	FI (mg/day)
Gilane	96	4.79 ± 0.06a	2.69 ± 0.02a	0.134 ± 0.002a	0.211 ± 0.003a
Nemat	87	4.14 ± 0.07b	2.17 ± 0.01def	0.103 ± 0.002c	0.175 ± 0.005bc
Shafagh	94	4.23 ± 0.12 ab	2.54 ± 0.03 ab	0.115 ± 0.005 ab	0.182 ± 0.009abc
Khazar	83	4.50 ± 0.10 ab	1.99 ± 0.01f	0.108 ± 0.005abc	0.200 ± 0.007 ab
Sadri	89	3.52 ± 0.28b	2.22 ± 0.03de	0.088 ± 0.008c	0.147 ± 0.013c
Neda	85	4.98 ± 0.03a	2.13 ± 0.01def	0.124 ± 0.0009 ab	0.213 ± 0.002a
Tarom	96	4.20 ± 0.40 ab	2.67 ± 0.07a	0.117 ± 0.008 ab	0.184 ± 0.013abc
Kadous	92	4.78 ± 0.06a	2.43 ± 0.06bc	0.126 ± 0.004 ab	0.200 ± 0.007 ab
Gohar	90	4.52 ± 0.32 ab	2.33 ± 0.05cd	0.116 ± 0.005 ab	0.189 ± 0.008abc
Fajr	84	3.58 ± 0.35b	2.07 ± 0.01ef	0.088 ± 0.008c	0.155 ± 0.016bc

The means within a column followed by the same letter are not statistically different (Tukey, P < 0.01).

Table 3
Mean (±SE) amylolytic and proteolytic activities of midgut extracts from fifth instar larvae of *Trogoderma granarium* fed with different rice cultivars.

Cultivar	Amylolytic activity (mU/mg)	Proteolytic activity (U/mg)
Gilane	0.649 ± 0.013a	0.166 ± 0.036a
Nemat	0.549 ± 0.006 ab	0.061 ± 0.004b
Shafagh	0.539 ± 0.019b	0.059 ± 0.002b
Khazar	0.352 ± 0.005c	0.039 ± 0.001b
Sadri	0.599 ± 0.006 ab	0.084 ± 0.022b
Neda	0.528 ± 0.015b	0.054 ± 0.016b
Tarom	0.606 ± 0.051 ab	0.036 ± 0.001b
Kadous	0.598 ± 0.006 ab	0.063 ± 0.003b
Gohar	0.593 ± 0.001 ab	0.062 ± 0.010b
Fajr	0.421 ± 0.032c	0.050 ± 0.004b

The means within a column followed by the same letter are not statistically different (Tukey, P < 0.01).

(F = 8.40; df = 9, 20; P < 0.01).

Hardness index of tested rice cultivars (F = 3.20; df = 9, 20; P = 0.014) was the highest in Gilane and Tarom (softer grain cultivars), and lowest in cultivar Khazar (harder grain cultivar). Moisture content among the grain of ten rice cultivars was not significantly different (F = 1.82; df = 9, 20; P = 0.126).

3.5. Correlation analysis

Table 5 indicates the correlation analysis of the nutritional performance and enzymatic activity of *T. granarium* with protein content, starch content, hardness index and moisture content of tested rice cultivars. Positive correlations were observed among examined indices with protein content, except for amylase activity. Moreover, food consumption, weight gain and protease activity showed positive correlations with starch content. Nutritional indices and enzymatic activity of this pest had positive correlations with hardness index. However, no significant correlations were seen among RCR, ECI and enzymatic activities with moisture content of tested grains.

3.6. Cluster analysis

A dendrogram of ten rice cultivars according to the nutritional performance and enzymatic activity of *T. granarium* is shown in Fig. 1. Different cultivars were grouped in two main clusters labeled A and B. The cluster A includes Kadous, Gohar, Shafagh, Gilane, Tarom and Sadri as susceptible cultivars; and cluster B consists of Nemat, Neda, Fajr and Khazar as resistant ones.

4. Discussion

The results of this study indicated that *T. granarium* developed successfully on grains of all 10 rice cultivars and that tested cultivars could significantly influence its nutritional responses and digestive physiology. These findings are in agreement with those reported in previous studies (Myers and Hagstrum, 2012; Barzin et al., 2019).

In our study, the lowest food consumed and weight gain of *T. granarium* larvae on cultivar Khazar resulted in a decreased survival rate, LGI and digestive amylolytic activity. Physicochemical analysis of tested rice cultivars indicates that the grain of cultivar Khazar is harder and the amounts of protein and starch are lower than other cultivars. So, the nutritional quality of cultivar Khazar for feeding of larvae was lower than other tested cultivars. In contrast, larvae fed with Gilane rice grains consumed the most food (2.54 mg) and had the greatest body weight compared to all other cultivars. These larvae increased their growth indices and digestive enzymes activity higher than those fed with other cultivars. Higher nutritive value of cultivar Gilane grains in terms of starch and protein contents, and softer grains of this cultivar could explain its suitability for feeding and growth of *T. granarium*. Positive

Table 4
Mean (±SE) some biochemical and physical properties in grains of tested rice cultivars.

Cultivar	Starch content (mg/g)	Protein content (mg/g)	Hardness index (%)	Moisture content (%)
Gilane	0.108 ± 0.01a	2.52 ± 0.02a	89.72 ± 2.09a	11.84 ± 3.01a
Nemat	0.053 ± 0.002b	1.87 ± 0.02d	82.54 ± 0.91 ab	4.72 ± 0.59a
Shafagh	0.074 ± 0.0006b	2.20 ± 0.03b	85.73 ± 1.28a	6.62 ± 0.80a
Khazar	0.052 ± 0.002b	1.57 ± 0.01e	75.64 ± 2.26b	4.26 ± 0.70a
Sadri	0.060 ± 0.01b	1.91 ± 0.02cd	84.00 ± 0.95 ab	4.79 ± 0.54a
Neda	0.053 ± 0.002b	1.87 ± 0.03d	82.41 ± 3.65 ab	4.79 ± 0.83a
Tarom	0.077 ± 0.004b	2.50 ± 0.04a	86.47 ± 2.15a	9.95 ± 3.96a
Kadous	0.063 ± 0.002b	2.01 ± 0.01cd	84.26 ± 2.72 ab	6.44 ± 1.44a
Gohar	0.061 ± 0.007b	2.04 ± 0.07c	84.03 ± 1.00 ab	6.50 ± 1.64a
Fajr	0.53 ± 0.001b	1.64 ± 0.03e	81.81 ± 1.50 ab	4.56 ± 1.83a

The means within a column followed by the same letter are not statistically different (Tukey, P < 0.01).

Table 5
Correlation coefficients (r) of nutritional indices and enzymatic activity of *Trogoderma granarium* fed with different rice cultivars with protein content, starch content, hardness index and moisture content of tested rice cultivars.

Parameter	Protein content		Starch content		Particle size index		Moisture content	
	r	p	r	p	r	p	r	p
E	0.662	0.000	0.514	0.004	0.571	0.001	0.430	0.018
P	0.794	0.000	0.534	0.002	0.602	0.000	0.486	0.006
RGR	0.616	0.000	0.347	0.061	0.436	0.016	0.408	0.025
RCR	0.461	0.010	0.287	0.124	0.383	0.037	0.328	0.077
ECI	0.716	0.000	0.323	0.082	0.483	0.007	0.357	0.052
PA	0.372	0.043	0.440	0.015	0.363	0.049	0.271	0.147
AA	0.42	0.20	0.349	0.059	0.396	0.030	0.235	0.212

E = Food consumption, P = Weight gain, RGR = Relative growth rate, RCR = Relative consumption rate, ECI = Efficiency of conversion of ingested food, PA= Proteolytic activity, AA = Amylolytic activity.

correlations between food consumption and larval weight gain with examined grain properties highlight the role of these properties in the ability of larvae to feed and complete their development on tested rice cultivars. However, Barzin et al. (2019) reported the role of grain hardness in rice cultivars as a major factor responsible for the amount of food consumption and weight gain of *T. granarium* larvae. Differences in the most rice cultivars used in our study with those examined by Barzin et al. (2019) would explain this disagreement. The range of food consumption and weight gain of larvae in this study is higher than that reported for *T. granarium* fed on wheat (Golizadeh and Abedi, 2016; Naseri and Borzoui, 2016), rice (Barzin et al., 2019) and maize (Majd-Marani et al., 2018) cultivars. So, it is possible that rice cultivars tested in this study are more nutrient than the wheat, rice and maize studied by above-mentioned workers for *T. granarium* larvae.

Among nutritional indices tested here, the ECI is the most important statistic showing potential of larvae to convert eaten food to body matter. The highest ECI value of larvae was seen on cultivar Tarom, suggesting that it was more favorable than other examined cultivars for feeding of larvae. Moreover, cultivar Khazar-fed larvae showed the lowest ECI value, indicating the poor capability of larvae to convert ingested food to body tissue. It is reported that lower ECI value of *T. granarium* on cultivar Khazar could be attributed to lower weight gain of larvae on this host (Naseri and Borzoui, 2016; Barzin et al., 2019). Furthermore, the lowest hardness index and protein content in grains of cultivar Khazar could be a reason for lower nutritional performance, especially ECI value, of larvae fed with this cultivar. The range of ECI values of *T. granarium* in this study is higher than that reported for the khapra beetle larvae fed with wheat (Naseri and Borzoui, 2016; Golizadeh and Abedi, 2016), barley (Seifi et al., 2015; Golizadeh and Abedi, 2017), maize (Majd-Marani et al., 2018) and rice (Borzoui et al., 2015; Barzin et al., 2019) cultivars. Therefore, it can be noted that the nutritional quality of rice cultivars examined in this study is higher than cereal cultivars tested by above-mentioned authors for development of larvae.

The results of this study showed significant differences in the pupal mass of *T. granarium* among different rice cultivars. The pupal mass was the highest on cultivars Neda, Gilane and Kadous, suggesting that the resource accumulation (such as protein and carbohydrate) during the larval stage was the highest when *T. granarium* was fed with these cultivars. Since the pupal mass is an indicator of the fecundity and longevity of adult insects (Khosravi et al., 2010), heavier pupal mass of *T. granarium* on cultivars Neda, Gilane and Kadous will probably lead to the formation of adults with a high reproductive potential. The mean pupal mass of *T. granarium* on various tested cultivars, in our study, is approximately similar to that reported for this pest on wheat (Naseri and Borzoui, 2016) and rice (Barzin et al., 2019) cultivars. However, Barzin et al. (2019) expressed that due to the high mortality of larvae fed with an unsuitable rice cultivar, the pupal mass of *T. granarium* was not significantly different among various six rice cultivars.

The insects' growth indices are known as one of the critical fitness indicators that show host cultivar resistance to them (Barzin et al., 2019). These indices can be determined by survival rate, duration of larval and pupal stages, and pupal mass. Due to high mortality of larvae feeding on cultivar Khazar, *T. granarium* exhibited the lowest LGI value when fed on it. The range of this index in our study is higher than that reported for *T. granarium* on barley cultivars (Seifi et al., 2015) and maize hybrids (Majd-Marani et al., 2018). However, it is somewhat close to that found on rice cultivars (Barzin et al., 2019). So, it can be suggested that rice is more nutrient than barley and maize for growth of this insect. Lower SII value of *T. granarium* on cultivars Sadri, Nemat and Fajr is mainly attributed to lighter pupal mass and longer development time of larvae on these cultivars than the others. In contrast, cultivar Gilane-fed larvae showed heavier pupae and shorter development time of larvae than those fed with the other cultivars. The highest SII value in our study is nearly similar to that noted for *T. granarium* on wheat cultivar MV17 (Seifi et al., 2015).

Food macronutrients, especially the protein and carbohydrate contents, can affect food suitability to post-harvest insects such as *T. granarium* by altering digestive physiology of them (Bernardi et al., 2012; Naseri and Borzoui, 2016). Larvae of *T. granarium* use

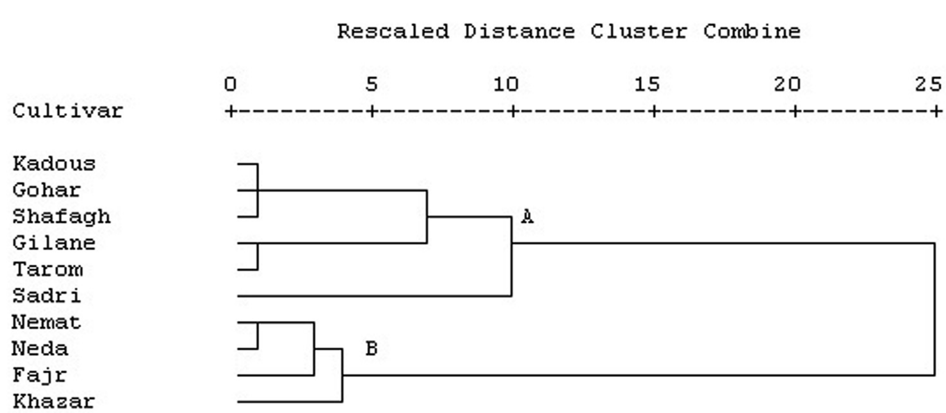

Fig. 1. Dendrogram of different rice cultivars according to nutritional performance, growth indices and digestive proteolytic and amylolytic activities of *T. granarium* fed with these cultivars using Ward's method.

different mechanisms to detect the macronutrient content of different diets and adjust the levels of digestive enzymes for successful development and reproduction (Naseri and Borzoui, 2016). Our findings revealed that larvae fed with cultivar Gilane showed the highest level of enzymatic activity, whereas those fed with cultivars Khazar and Fajr had the lowest activity. Differences in the hardness index, and concentration of protein and starch between grains of cultivar Gilane and other tested cultivars could explain high levels of enzymatic activity on this cultivar. The amount of protein and starch in grains of cultivar Gilane was higher than other cultivars. It seems that *T. granarium* larvae prefer to feed on well balanced food (cultivar Gilane) to the others, which is similar to Borzoui et al. (2015) findings, who stated the highest amylolytic activity of the khapra beetle larvae on rye (with high starch content). According to the correlation analysis, a positive relationship was observed between proteolytic activity of larvae with hardness index, protein content and starch content of tested grains. It is reported that the amount of food consumption by an insect has a direct effect on the midgut enzymatic activity (Sivakumar et al., 2006). So, the highest amount of food eaten by larvae on cultivar Gilane is another reason for higher level of enzymatic activity. Moreover, low levels of enzymatic activity in larvae fed with cultivars Khazar and Fajr could be attributed to low amount of protein and starch and hard grain of these two cultivars. In line with our results, Majd-Marani et al. (2018) and Barzin et al. (2019) emphasized the effect of grain hardness on digestive protease and amylase activity of *T. granarium* larvae. In a study, Mardani-Talaee et al. (2017) found that amylolytic and proteolytic activities of *T. granarium* were significantly decreased when larvae were reared on barley cultivars with harder grain.

The range of amylolytic activity on various rice cultivars, in this study, is higher than that found on barley cultivars (Seifi et al., 2015) and on maize hybrids (Majd-Marani et al., 2018). However, it was lower than that obtained for *T. granarium* fed with rice (Mohammadzadeh and Izadi, 2018). Our data on the activity of digestive amylase is close to that expressed by Barzin et al. (2019) on rice cultivars. Furthermore, the proteolytic activity of *T. granarium* on cultivars tested in the present research is lower than that found for *T. granarium* fed with barley cultivars (Seifi et al., 2015) and rice (Mohammadzadeh and Izadi, 2018). This variation could be attributed to different host species used by Seifi et al. (2015) and different larval instar (last instar) tested by Mohammadzadeh and Izadi (2018).

Cluster analysis was used to group rice cultivars by constructing a dendrogram based on the nutritional responses, growth indices and enzymatic activity of *T. granarium*. Having some chemical and physiological similarities of rice cultivars might be a reason for grouping of them in the same cluster. However, no considerable differences were observed between these two groups in respect of physicochemical features tested in our study. Therefore, further experiment is required to identification of nutritional and anti-nutritional (e.g. digestive enzymes inhibitors) substances of tested rice cultivars that can influence the feeding and growth of *T. granarium*. By comparing associated parameters, it would be concluded that cultivars grouped in cluster A (Kadous, Gohar, Shafagh, Gilane, Tarom and Sadri) were susceptible, and cultivars classified in cluster B (Nemat, Neda, Fajr and Khazar) were partially resistant for the growth and development of this pest. The resistant rice cultivars, especially Khazar and Fajr, can be introduced to incorporate into the genetically engineered crops to reduce grain damage by this pest. However, susceptible cultivars grouped in cluster A will require more attention during their storage to prevent economic loss caused by *T. granarium*.

Declaration of competing interest

We have no conflict of interest.

CRediT authorship contribution statement

Bahram Naseri: Conceptualization, Methodology, Writing - review & editing, Supervision. **Pezhman Aeinehchi:** Conceptualization, Formal analysis, Writing - review & editing. **Abbas Rahimi Ashjerdi:** Methodology, Investigation.

Acknowledgment

We acknowledge the University of Mohaghegh Ardabili, Ardabil, Iran, for collaboration by support for the experiment.

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