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LIFE TABLE PARAMETERS AND DIGESTIVE ENZYMATIC ACTIVITY OF PLODIA INTERPUNCTELLA (HÜBNER) (PYRALIDAE) ON ARTIFICIAL DIET CONTAINING BRAN OF VARIOUS WHEAT CULTIVARS

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ABSTRACT. The eggs and larvae of the Indian meal moth, *Plodia interpunctella* (Hübner), are widely used for mass rearing of parasitoids and predators. Life table parameters and digestive enzymatic activity (protease and α-amylase) of *P. interpunctella* were studied on bran of different wheat cultivars ('Backcross Roshan', 'Khooshe Pishgam', 'Khoshki line 9', 'Arg', 'Alvand', 'Pishtaz', 'WS-89-2', 'Sepahan' and 'Bam') when incorporated into artificial diets under laboratory conditions (25 ± 1°C, 65 ± 5% R.H., and a 16:8 h light-dark photoperiod). The net reproductive rate (R_p) of *P. interpunctella* was highest on 'Backcross Roshan' (78.05±4.80 female/female/generation) and lowest on 'Pishtaz' (21.82±0.96 female/female/generation). The intrinsic rate of increase (r_m) ranged from 0.0520±0.0006 to 0.0836±0.0008 (day¹), which was lowest on 'Pishtaz' and highest on 'Backcross Roshan'. The highest and lowest levels of proteolytic activity were recorded in the fifth instar larvae fed with 'Backcross Roshan' (1.19 ± 0.16 U mg¹) and 'Bam' (0.24 ± 0.08 U mg¹). Also, the highest level of amylolytic activity was recorded on 'Alvand', 'Backcross Roshan', 'Arg' and 'Khoshki line 9' (0.85 ± 0.02, 0.83 ± 0.09, 0.78 ± 0.05, 0.77 ± 0.04 mU mg¹, respectively) while the lowest activity was on 'Sepahan' and 'Bam' (0.36 ± 0.06 and 0.43 ± 0.06 mU mg¹, respectively). The results show that, among the different wheat cultivars tested, 'Backcross Roshan' was the most suitable cultivar for rearing *P. interpunctella*, as an alternative host, in order to optimize the mass production of natural enemies.

Additional key words: Indian meal moth, population growth, digestive physiology, wheat bran

The Indian meal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), is a major cosmopolitan pest of granaries, food processing plants, warehouses, retail stores, and households. Plodia interpunctella larvae are able to feed on a wide range of dried vegetable and animal materials including grain, cereal products, oilseeds, dried fruits, dried vegetables, nuts, animal feed (Cox and Bell, 1991), walnut, almond, pistachio and dates in Iran (Sepasgozarian 1979) and other parts of the world (Azelmat et al. 2005). In addition, the eggs and larvae of P. interpunctella have been used as alternative hosts in the mass rearing of several natural enemies, such as Orius albidipennis (Reuter) (Hemiptera: Anthocoridae) (Ghadamyari et al. 2001), Venturia canescens (Gravenhorst) (Hymenoptera: Ichneumonidae) (Spanoudis and Andreadis 2012), Habrobracon hebetor (Say) (Hymenoptera: Braconidae) (Akinkurolere et al. 2009, Ghimire and Phillips 2010) and Trichogramma brassicae (Bezdenko) (Hymen optera: Trichogrammatidae) (Iranipour et al. 2009), under laboratory conditions.

Biological aspects such as the development and survivorship of the pre-imaginal stages, as well as physiological indices of insect pests, can be affected by temperature, moisture, photoperiod, host commodity and the quality of food eaten (Johnson et al. 1997, Na and Ryoo 2000, Musa and Ren 2005, Bouayad et al. 2008). The developmental time and adult progeny production of the Indian meal moth are significantly influenced by the type of diet consumed during the larval stage (Cline and Highland 1985). Life table

parameters can provide a comprehensive description of the development, survivorship, and fecundity of a population, and is an appropriate tool to study the dynamics of animal populations, especially arthropods (Maia et al. 2000), and may be useful in constructing population models (Carey 1993) and understanding interactions with other insect pests and natural enemies (Omer et al. 1996). Southwood (1966) stated that the life table parameters, particularly the intrinsic rate of increase (r_{m}) , are the most useful parameters to compare and estimate the population growth potential of different species under specific climatic and food conditions (Ricklefs and Miller 2000). Consequently, this research examined the life table parameters in order to compare the population growth of P. interpunctella on artificial diet containing bran of various wheat cultivars. Several researchers have previously studied the biology of P. interpunctella on various diets. For example, Arbogast (2007) evaluated the development of immature stages of P. interpunctella under different temperature, humidity and dietary conditions, and found that moisture content had a significant effect on the developmental period of this insect. Bouayad et al. (2008) examined the effect of the four commodities (wheat flour, dates, sorghum and barely) on the post-embryonic development of this insect pest, and reported that the shortest development time was on wheat flour and dates. Also, population growth parameters of the Indian meal moth on three date cultivars were studied by Pourbehi et al. (2013), who observed the highest value of intrinsic rate of increase on cultivar Zahedi.

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However, since insects derive most of their nourishments from proteins and soluble carbohydrates, proteases and amylases, which are the key insect-gut digestive enzymes, are essential for their survival (Ishaaya et al. 1971). Bouayad et al. (2008) examined the effect of four commodities (wheat flour, dates, sorghum and barely) on the α -amylase activity of P. interpunctella, and reported that the lowest amylolytic activity was found in the larvae reared on wheat flour and dates. Farshbaf Pour Abad et al. (2010) examined some properties of α -amylase in the Indian meal moth larvae fed pistachio, and detected the highest amylolytic activity in the fifth instar larvae. Recently, Borzouei (2012) considered the effect of different diets on proteolytic and amylolytic activities of *P. interpunctella*, and stated that the highest enzymatic activity was in the larvae fed on artificial diet containing wheat bran, yeast, honey and glycerol. Also, Nasirian et al. (2014) examined the feeding performance of P. interpunctella on different artificial diets and detected the highest efficiency of conversion of ingested food (ECI) on bran of the wheat cultivar 'Backcross Roshan'.

To date, no published information exists concerning life table and digestive enzymatic activity (amylases and proteases) of *P. interpunctella* on artificial diet containing bran of various wheat cultivars. Therefore, this research was performed to elucidate the life table parameters and activity of two main digestive enzymes of *P. interpunctella* in response to feeding on artificial diet prepared by bran of nine wheat cultivars. The findings of this research will be useful in selecting the most suitable wheat cultivar for optimal feeding, rapid development, maximum survival and high fecundity of *P. interpunctella* in order to optimize the mass rearing of some natural enemies under laboratory conditions.

MATERIALS AND METHODS

Artificial diet. Different wheat cultivars including 'Backcross Roshan', 'Khooshe Pishgam', 'Khoshki line 9', 'Arg', 'Alvand', 'Pishtaz', 'WS-89-2', 'Sepahan' and 'Bam' were acquired from the Agricultural and Natural Resources Research Center of Isfahan, Iran, and used to prepare artificial diets. To prepare the dry part of the artificial diet, 800 gr of bran of each wheat cultivars was mixed with 160 gr of brewer's yeast. For preparing the aqueous part, 200 ml of honey and 200 ml of glycerol were dissolved and mixed together. Thereafter, the dry and liquid components were mixed, and held at room temperature for one week in closed containers (Silhacek and Miller 1972).

Insect colony. Eggs of the Indian meal moth were obtained from a laboratory colony maintained on an artificial diet (Silhacek and Miller 1972) from Tarbiat

Modares University (Tehran, Iran). Test insects were maintained on their respective test diets under laboratory conditions (25 \pm 1°C, 65 \pm 5% R.H., and a 16:8 h light-dark photoperiod) for two generations prior to the start of the life table analysis.

Life table parameters. Age-specific survival rate (l_x) and fecundity (m_x) on different artificial diets were calculated according to Carey (1993) as well as the intrinsic rate of increase (r_m) , net reproductive rate (R_0) , finite rate of increase (λ) , mean generation time (T) and doubling time (DT) (Birch 1948, Southwood and Henderson 2000) were also estimated on different artificial diets.

Extraction of digestive (gut) enzymes. The fifth instar larvae of P. interpunctella fed with artificial diets prepared by bran of various wheat cultivars for 24 h were chilled and quickly dissected under a stereomicroscope. The guts were gathered into a known volume of distilled water and were homogenized using a handheld glass grinder on ice. The homogenates were centrifuged at $16000 \times g$ for 10 min at 4 °C. The resulting supernatants were then collected into new micro tubes and stored at -20°C until further use.

Protein determination. Protein concentration in the gut of the fifth instar larvae of *P. interpunctella* was quantified by the method of Bradford (1976) using bovine serum albumin (BSA) (Roche Co., Munich, Germany) as standard.

Proteolytic activity. General proteolytic activity in the midgut of *P. interpunctella* fifth instar larvae was assayed using azocasein (Sigma chemical Co., St Louis, USA) as a substrate at the optimal pH. The method of Elpidina et al. (2001) was used with slight modifications in determining the optimal pH of proteolytic activity in the gut. To evaluate the proteolytic activity, the reaction mixture containing 80 µL of 1.5% azocasein solution in 50 mM universal buffer (pH 12) and 50 µL of crude enzyme was incubated at 37 °C for 50 min. The reaction was ended by adding 100 µL of 30% trichloroacetic acid (TCA). Precipitation was achieved by cooling at 4° C for 30 min, and the reaction mixture was centrifuged at $16000 \times g$ for 10 min. The supernatant (100 μ L) was added to 100 µL of 2 M NaOH and the absorbance was read at 440 nm. Appropriate blanks that TCA had been added before the substrate was prepared for each examine. One unit of protease activity was determined as an increase in optical density mg-1 protein of the tissue min-1 due to azocasein proteolysis. All experiments were done in triplicates with three different supernatants.

Amylolytic activity. Amylolytic activity in the midgut of *P. interpunctella* fifth instar larvae was assayed using 1% soluble starch (Sigma chemical Co., St Louis, USA)

as substrate at the optimal pH, according to the method of Bernfeld (1955). A quantity of 20 μL of the enzyme extract was incubated with 500 μL of universal buffer (pH 10) and 40 μL of soluble starch for 30 min at 37 °C. The reaction was stopped by adding 100 μL of DNSA (Sigma chemical Co., St Louis, USA) and heated in boiling water for 10 min. The absorbance was read at 540 nm after cooling on ice. Unit activity was characterized as the amount of enzyme required to produce 1 mg of maltose (Sigma chemical Co., St Louis, USA) in 30 min at 37 °C under the given assay conditions. All experiments were carried out in triplicates with three different supernatants.

Data analysis. Life table parameters and digestive enzymatic activity of *P. interpunctella* reared on different artificial diets were analyzed by one-way ANOVA, followed by the comparison of means with LSD test at $\alpha = 0.05$, using statistical software Minitab 16.0. All data were tested for normality before analysis. Differences in each life table parameter on different artificial diets were tested for significance by estimating variances using the jackknife procedure (Meyer et al. 1986, Maia et al. 2000).

RESULTS

Survival and fecundity. Age-specific survival rate (l_x) and fecundity (m_x) of P. interpunctella on different artificial diets are shown in Figure 1. The survival rate of individuals to adulthood from the initial cohort stage was estimated to be 0.88, 0.81, 0.82, 0.77, 0.81, 0.44, 0.73, 0.81 and 0.62 on 'Back cross Roshan', 'Khoshki line 9', Khooshe Pishgam, Arg', Alvand', 'Pishtaz', 'WS-89-2', 'Sepahan' and 'Bam, respectively. Our results showed

that the death of the last female on mentioned wheat cultivars artificial diets occurred in the age of 57, 57, 62, 58, 62, 63, 58, 60 and 63 days, respectively (Fig. 1).

First oviposition on the examined cultivars occurred in the age of 48, 50, 54, 52, 55, 57, 52, 53, and 58 days, 'Back cross Roshan', 'Khoshki line 9', Khooshe Pishgam, Arg', Alvand', 'Pishtaz', 'WS-89-2', 'Sepahan' and 'Bam, respectively. The highest daily fecundity (m_x) of P interpunctella adult emerged from the larvae reared on these cultivars was 16.4, 16.3, 17.87, 13.4, 18.16, 11.8, 18.0, 13.9 and 16.2 females/female/day, respectively that occurred in the ages of 52, 52, 57, 55, 60, 60, 54, 56 and 60 days, 'Back cross Roshan', 'Khoshki line 9', 'Khooshe Pishgam', 'Arg', Alvand', 'Pishtaz', 'WS-89-2', 'Sepahan' and 'Bam', respectively (Fig. 1).

Life table parameters. The net reproductive rate (R_0) of P. interpunctella was the highest (78.05 ± 4.80) female/female/generation) on 'Backcross Roshan' (F = 13.17; df = 8, 36; P < 0.01). However, no significant differences were observed for the R_0 value of P. interpunctella fed with 'Backcross Roshan', 'Alvand' and 'Khooshe Pishgam' (Table 1). However, the intrinsic rate of increase (rm) ranged from 0.0520 ± 0.0006 to 0.0836± 0.0008 (day-1), which was lowest on 'Pishtaz' and highest on 'Backcross Roshan' (F = 69.67; df = 8, 36; P<0.01). Furthermore, the finite rate of increase (λ) value of this insect showed significant differences based on rearing diet (F = 68.86; df = 8, 36; P<0.01), being lowest on 'Pishtaz' (1.053 ±0.001 day-1) and highest on 'Backcross Roshan' (1.087 ± 0.001 day-1). Among the different artificial diets, the mean generation time (T)was longest on 'Bam' (60.09±0.11 days) and 'Pishtaz' (59.36 ± 0.34 days) and shortest on 'Backcross Roshan'

Table 1. Mean (\pm SE) life table parameters of *Plodia interpunctella* on artificial diet containing bran of various wheat cultivars under laboratory conditions

	Parameter (mean ± S				SE)	
Wheat cultivars	$R_{_{0}} \\$ (female/female/generation)	$r_{_m} \ ({ m day}^{_1})$	λ (day ⁻¹)	T (day)	DT (day)	
'Backcross Roshan'	78.05±4.80a	0.0836±0.0008a	1.087±0.001a	52.12±0.40e	8.28±0.08f	
'Khooshe Pishgam'	64.97±6.98ab	$0.0739 \pm 0.0016 bcd$	$1.076 \pm 0.002 bcd$	$56.57 \pm 0.39 c$	$9.37{\pm}0.20{\rm cde}$	
'Khoshki line 9'	58.31±5.92be	$0.0772 \pm 0.0014 b$	1.080 ± 0.002 b	$52.75 \pm 0.47e$	$8.97 \pm 0.17e$	
'Arg'	49.02±3.92be	$0.0715 \pm 0.0013 \mathrm{cd}$	$1.074 \pm 0.001 cd$	54.51±0.16d	$9.69 \pm 0.18 ed$	
'Alvand'	$64.49 \pm 5.90 ab$	$0.0719 \pm 0.0010 \mathrm{cd}$	1.074 ± 0.001 cd	$57.99 \pm 0.71 \mathrm{b}$	$9.63 \pm 0.13 cd$	
'Pishtaz'	21.82±0.96d	$0.0520 \pm 0.0006 \mathrm{f}$	$1.053 \pm 0.001 f$	59.36±0.34a	13.33±0.16a	
'WS-89-2'	60.57±3.69b	$0.0756 \pm 0.0007 be$	$1.078 \pm 0.001 bc$	54.28±0.35d	$9.16 \pm 0.09 de$	
'Sepahan'	$50.26 \pm 1.91 bc$	$0.0705 \pm 0.0007 d$	$1.071 \pm 0.001 \mathrm{d}$	$55.59 \pm 0.32 cd$	9.83±0.10c	
'Bam'	41.80±1.83e	$0.0621 \pm 0.0007e$	$1.064 \pm 0.002e$	60.09±0.11a	11.15±0.14b	

The means followed by different letters in the same column are significantly different (LSD, P<0.01)

 R_0 = net reproductive rate, rm = intrinsic rate of increase, λ = finite rate of increase, T = mean generation time, DT = doubling time

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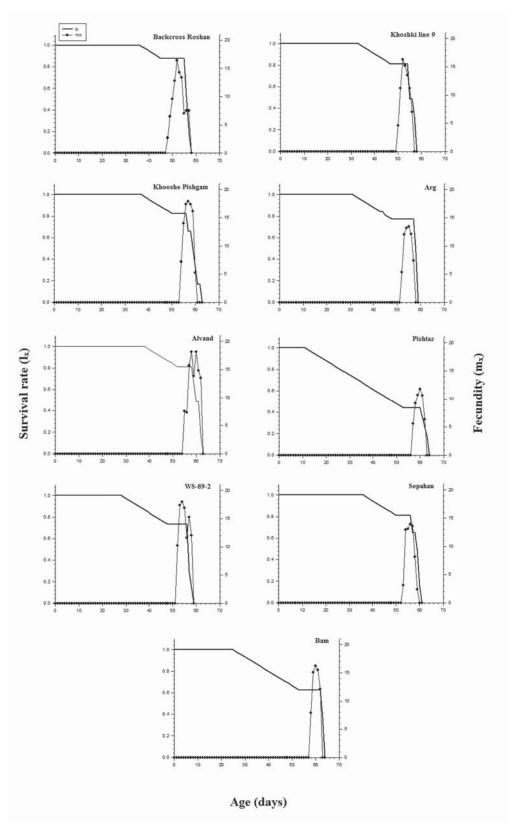


Fig. 1. Age-specific survival rate (l_x) and fecundity (m_x) of *Plodia interpunctella* fed on artificial diet containing bran of various wheat cultivars.

	Index (mean ± SE)		
Wheat cultivars	Proteolytic activity (U mg ⁻¹)	Amylolytic activity (mU mg-1)	
Backeross Roshan'	1.19 ± 0.16a°	$0.83 \pm 0.09a$	
Khooshe Pishgam'	$0.53 \pm 0.11 bcd$	$0.68 \pm 0.02ab$	
Khoshki line 9'	0.83 ± 0.28 abc	$0.77 \pm 0.04a$	
'Arg'	0.90 ± 0.03 ab	$0.78 \pm 0.05a$	
Alvand'	0.80 ± 0.30 abc	$0.85 \pm 0.02a$	
'Pishtaz'	0.41 ± 0.04 bcd	$0.69 \pm 0.15 ab$	
'WS-89-2'	0.33 ± 0.10 cd	$0.54 \pm 0.05 \mathrm{bc}$	
'Sepahan'	0.35 ± 0.03 cd	$0.36 \pm 0.06c$	
'Bam'	$0.24 \pm 0.08d$	$0.43 \pm 0.06c$	

Table 2. Mean (± SE) proteolytic (U mg¹) and amylolytic (mU mg¹) activities of *Plodia interpunctella* fifth instar larvae fed on artificial diet containing bran of various wheat cultivars under laboratory conditions

The means followed by different letters in the same column are significantly different (LSD, P < 0.01; $P < 0.05^{\circ}$)

and 'Khoshki line 9' (52.12 ± 0.40 and 52.75 ± 0.47 days, respectively) (F = 49.30; df = 8, 36; P<0.01). Moreover, the doubling time (DT) value of P. interpunctella on 'Pishtaz' was longer (13.33 ± 0.16 days) than the other cultivars (F = 98.67; df = 8, 36; P<0.01).

Proteolytic activity. The highest value of proteolytic activity was found in larvae reared on 'Backcross Roshan' (1.19 \pm 0.16 U mg⁻¹), and the lowest value was on 'Bam' (0.24 \pm 0.08 U mg⁻¹). (F = 12.50; df = 8, 18; P<0.05)(Table 2).

Amylolytic activity. The highest amylolytic activity was found in the larvae fed with 'Alvand', 'Backcross Roshan', 'Arg' and 'Khoshki line 9' $(0.85 \pm 0.02, 0.83 \pm 0.09, 0.78 \pm 0.05$ and 0.77 ± 0.04 mU mg⁻¹, respectively) and the lowest activity was in the larvae fed with 'Sepahan' and 'Bam' $(0.36 \pm 0.06$ and 0.43 ± 0.06 mU mg⁻¹, respectively) (F = 18.90; df = 8, 18; P < 0.01)(Table 2).

DISCUSSION

Plodia interpunctella larvae are known for their ability to develop on a large variety of food hosts, and their development is largely influenced by the quality of food (LeCato 1976, Bouayad et al. 2008). In this study, it was shown that artificial diets containing bran of various wheat cultivars had significant effect not only on the life table parameters of P. interpunctella, but also on the two main digestive enzymes of this insect.

The higher rm value of *P. interpunctella* fed with an artificial diet containing bran of the wheat cultivar 'Backcross Roshan' was due to the greater fecundity, lower mortality and shorter development time of the immature stages on this cultivar. Nasirian et al (2014) showed that among different artificial diets, the efficiency of conversion of ingested food (ECI) value of

the fifth instar larvae of *P. interpunctella* was highest on cultivar 'Backcross Roshan', demonstrating that the fifth instar larvae fed with this cultivar, were more efficient at converting ingested food to body biomass. However, a lower rm value on cultivar 'Pishtaz' was mainly as a result of the lower fecundity and survivorship as well as longer development time of the immature stages of *P.* interpunctella. The r_m values of P. interpunctella in this study are different from the values reported by Pourbehi et al. (2008). Some probable reasons for these variations are due to physiological differences depending on the type of host and genetic differences in geographic populations of the insect. Although, a high value of r_m shows the suitability of a host to insect feeding, a low value shows that the host species is unsuitable to the insect. Since the cultivar 'Backcross Roshan' was a suitable host, P. interpunctella had the greatest chance of population increase. The net reproductive rate (R_o) indicates adult female production of *P. interpunctella* by a female during its lifetime. The highest net reproductive rate was on 'Backcross Roshan', which was 1.5 fold higher than that reported by Pourbehi et al. (2013), and this may be attributed to the difference in host-diet used for rearing P. interpunctella. However, the highest finite rate of increase (λ) and the shortest doubling time (DT) of P. interpunctella were on cultivar 'Backcross Roshan', and this is similar to that reported by Pourbehi et al. (2013) on date cultivar Zahedi (a suitable cultivar). The conformity between the results of the present study and the afore-mentioned study may be attributed to the polyphagous nature of the Indian meal moth. Moreover, the lowest net reproductive rate and longest doubling time of *P. interpunctella* was on cultivar 'Pishtaz', indicating that this cultivar is unsuitable for the mass rearing of this pest.

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In the present research, the effects of various artificial diets on physiological responses were evaluated at the level of activity of two key digestive enzymes (i.e. protease and α -amylase) in *P. interpunctella* fifth instar larvae. In lepidopteran insects, differences in digestive enzymatic activity can influence feeding performance, especially ECI, in the ultimate instars (Slansky and Scriber 1985). Nasirian et al. (2014) showed that the highest value of ECI in P. interpunctella fifth instar larvae was on artificial diet containing bran of the wheat cultivar 'Backcross Roshan', demonstrating a positive correlation between feeding performance and digestive enzymatic activity. The gut enzymatic activity depends on the chemical composition of food sources and enzyme-inhibitors (Mendiola-Olaya et al. 2000). Borzouei (2012) reported that the highest proteolytic and amylolytic activity of P. interpunctella was on artificial diet (containing wheat bran, yeast, honey and glycerol) and the lowest activity was on raisin. Due to the fact that the lepidopteran larvae need a diet with high protein content, Borzouei (2012) found a positive correlation between the amounts of dietary protein and the level of proteolytic activity. The highest value of proteolytic activity was found in the fifth instar larvae fed with 'Backcross Roshan', which is probably related to the high protein content of the diet. The level of proteolytic activity on 'Backcross Roshan' was 5.5-fold higher than that detected for artificial diet-fed larvae of armigera Helicoverpa (Hübner) (Lepidoptera: Noctuidae) (Sarate et al. 2012), and was almost 2.5-fold lower than that reported by Mansouri et al. (2013) for (Zeller) (Lepidoptera: Phthorimaea operculella Gelechiidae) on potato germplasm Savalan (a suitable host). Farshbaf Pour Abad et al. (2010) reported that the amylolytic activity in the fifth instar larvae of P. interpunctella was higher than that observed in the fifth instar larvae of this research. The results of the present study indicate that the highest amylolytic activity of the fifth instar larvae on 'Alvand' is approximately 2.5 fold higher than that reported by Jafarlu et al. (2012), for the female fifth instar larvae of Anagasta kuehnieklla (Zeller) (Lepidoptera: Pyralidae) on wheat flour. The possible reasons for the afore-mentioned inconsistency could be attributed to differences in artificial diets, variations in experimental conditions and examined insect species. Silva et al. (2001) stated that the α amylase gene is regulated according to the starch or glucose contents in the dietary substrate. In another study, Bouayad et al. (2008) showed that this regulation also existed in *P. interpunctella* larvae and the levels of α-amylase activity depended on both glucose repression and starch induction. Also, Borzouei (2012) showed that amylolytic activity decreased with increase in the

amounts of dietary carbohydrates. As for the relationships between digestive enzymatic activity and protein/starch contents of various wheat cultivars, it seems that there is an insect mechanism that accurately assesses food contents and regulates the levels of these vital digestive enzymes (Kotkar et al. 2009). Typically, the variations in protease and amylase activities in *P. interpunctella* larvae fed with various artificial diets may be attributed to the differences in either the protein and starch contents of the diet or the response of the insect to dietary enzymes-inhibitors. To validate the findings of this study, additional studies should be considered in the future.

Since *P. interpunctella* is a suitable alternative host for the rearing of some predators and parasitoids (Ferkovich and Shapiro 2004, Ghimire and Phillips 2010, Spanoudis and Andreadis 2012), optimizing the mass rearing of this insect on artificial diets would be economically useful. By combining the results of the current study regarding life table parameters and the digestive physiology of the Indian meal moth on artificial diet based on bran of various wheat cultivars, it was found that the cultivar 'Backcross Roshan' is the most suitable host for preparing the artificial diet for P. interpunctella rearing. For a better confirmation and more application of the results of this study, supplementary researches need to focus on the study of the specific digestive enzymes properties of the Indian meal moth fed with various artificial diets containing bran of different wheat cultivars.

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