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Evaluation of artificial diets based on different legume seeds on the nutritional physiology and digestive function of *Helicoverpa armigera* (Hübner)

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Abstract

Helicoverpa armigera (Hübner) is considered a serious agricultural pest worldwide. We explored the effects of artificial diets containing ten legumes, including broad beans (Shadan, Feyz, Saraziri, Barekat, and Mahta cultivars), white kidney beans (Dehghan cultivar), red kidney beans (Goli cultivar), common beans (Khomein cultivar), cowpeas (Mashhad and Arabi cultivars) on the feeding responses of *H. armigera* by quantifying specific primary and secondary metabolites in the studied legumes and determining larval nutritional indices and digestive enzyme activities. The results showed that the highest efficiency of conversion of digested food (ECD) and relative growth rate values (RGR) of whole larval instars were obtained in the Dehghan and Goli cultivars. However, the lowest values of ECD and RGR were observed in the larvae fed on the Khomein and Mahta cultivars. The highest proteolytic and amylolytic activities of larvae were found on the Dehghan and Mashhad cultivars. The highest and lowest values of standardized insect-growth index and index of plant quality were observed in larvae feeding on the Dehghan and Khomein cultivars, respectively. Additionally, significant variations in phytochemical metabolites were recorded among the studied legume cultivars. Significant negative or positive correlations were also found between feeding characteristics and enzymatic activities of *H. armigera* with the biochemical composition of the studied legumes. The cluster analysis results revealed that artificial diets containing Mahta and Khomein cultivars were unsuitable for *H. armigera*, and can be used as candidates for integrated pest management programs or for screening insect inhibitors to produce genetically modified pest-resistant plants.

Introduction

Cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a primary and important pest of cotton, bean, chickpea, corn, tobacco, tomato, pigeonpea, and other high-value agricultural crops in Iran and other countries worldwide (Fitt, 1989; Liu *et al.*, 2004; Talekar *et al.*, 2006; Hemati *et al.*, 2012*a*; Fallahnejad-Mojarrad *et al.*, 2017; Atashi *et al.*, 2021). The use of synthetic chemical insecticides for the management of *H. armigera* has resulted in the development of insecticide resistance in the pest (Ismail, 2020). On the other hand, the use of pesticides in agriculture may impose a serious negative impact on the environment (Radwan *et al.*, 2019; Hemmati *et al.*, 2022). Due to the emergence of resistant insects and environmental risks of conventional insecticides, it is necessary to employ alternative pest management approaches that are more cost-effective, and sustainable (Korrat *et al.*, 2012).

Recent study efforts are focused on the use of resistant host plants, which can reduce the need for insecticides and improve the profitability, efficacy, and environmental acceptability of crop production (Kennedy *et al.*, 1987; Toews *et al.*, 2000; Hemmati *et al.*, 2021*a*). Moreover, plant resistance can be an economically beneficial, effective approach in integrated pest management and easy to use (Ofuya and Credlandt, 1995; La Rossa *et al.*, 2013; Shishehbor and Hemmati, 2022).

Feeding indices help evaluate the performance of pests in response to nutrition on various plant cultivars (Lazarevic and Peric-Mataruga, 2003; Hemati *et al.*, 2012*b*). In addition to nutritional indices studies, understanding the insects' digestive physiology is also important when developing environmentally-safe strategies of control, such as using transgenic plants and digestive enzyme inhibitors (Jongsma and Bolter, 1997; Babamir-Satehi *et al.*, 2022). Evaluating the digestive function of insects on various host plants is a suitable tool for determining the relationship between host plants and herbivores that could improve the integrated pest management (IPM) strategies (Kotkar *et al.*, 2009; Hemati *et al.*, 2012*a*; Zamani Fard *et al.*, 2022). Furthermore, the standardized insect-growth index (SII) and the index of plant quality (IPQ) are used to evaluate the host plant quality for insect pests (Shishehbor and Hemmati, 2022).

Based on available studies, the level of infection with *H. armigera* varies considerably from cultivar to cultivar on host plants. Legumes are one of the most important plant sources of high levels of carbohydrates, calcium, iron, and especially protein and the second largest source of human food after cereals that widely cultivated as one of the most nutritious foods throughout world (Kotkar *et al*., 2009; Sanon *et al*., 2010). Although different researchers have studied the effects of host plants on feeding efficiency and digestive enzyme activity of *H. armigera*, there is no information about the effect of various broad bean cultivars on physiological characteristics and growth indices of this pest. Previous studies have been conducted on the effects of various cultivars of soybean on nutritional indices of cotton bollworm (Naseri *et al*., 2010*a*; Soleimannejad *et al*., 2010). Previously, Hemati *et al.* (2012*a*, 2021*b*) investigated the digestive proteolytic and amylolytic activities, feeding responses, and growth indices of this pest on various legumes including common bean, chickpea, tomato, white and red kidney bean, potato, and cowpea and indicated that tomato was a relatively unsuitable host plant for nutrition of larvae cotton bollworm.

Baghery *et al.* (2013) demonstrated the growth rate and nutritional performance of *H. armigera* on seeds of five host plants, including chickpea, cowpea, navy bean, corn, and soybean. They found that the cowpea and corn were more and less nutritious than the other hosts, respectively. Furthermore, Kouhi *et al.* (2014) studied nutritional indices of *H. armigera* on different tomato cultivars, and their results showed that the Rio grande UG cultivar is an unsuitable host for the cotton bollworm. Rahimi Namin *et al.* (2014) evaluated larval nutritional indices and activities of digestive enzymes in response to bean cultivars. They reported that red kidney bean Sayyad was the unsuitable host for feeding *H. armigera*. Furthermore, Naseri and Arghand (2015) determined the effect of an artificial diet containing seeds of five corn hybrids on the nutritional performance of this pest and screened the higher feeding performance of the larvae on SC260 and SC704 and lower on SC500 and SC700 hybrids. Fallahnejad-Mojarrad *et al.* (2017), and Fathipour *et al.* (2018) expressed that various cultivar plants significantly affected on growth and nutrition of *H. armigera*.

No published data were available in the correlation between tested parameters of cotton bollworm with phytochemical metabolites of legumes based on artificial diets. Therefore, the objectives of the present study were as follows: (A) feeding responses and digestive enzyme activities of *H. armigera* on an artificial diet based on different legume cultivars; (B) the investigation of some phytochemical metabolites in the tested legume seeds; and (C) analysis of the possible relationship between feeding properties and digestive function of *H. armigera* with biochemical compounds of various legume seeds. The outcomes of this study help identify plant inhibitors produced by legume cultivars that can be cloned into different host plants to confer protection against *H. armigera*.

Materials and methods

Legume seed source

Seeds of different legumes, including broad bean (*Vicia faba* L.; Shadan, Feyz, Saraziri, Barekat, Mahta cultivars), common bean (*Phaseolus vulgaris* L.; Khomein cultivar), white and red kidney bean (*P. vulgaris*; Goli and Dehghan cultivars), and cowpea (*Vigna sinensis* L.; Arabi and Mashhad cultivars) were obtained from the Agricultural Research Center of Safiabad, Dezful, Iran. Legumes were powdered separately and were placed in paper bags, kept in refrigerators at 4°C for larvae feeding. These legume seeds are commonly grown in Iran and used as food.

Cotton bollworm rearing

H. armigera larvae were collected from the tomato field in the Behbahan region (Khuzestan province) in the southwest of Iran in October 2020. The larvae of cotton bollworm were reared on an artificial diet based on the seed powder of each legume under controlled conditions: 25 ± 1°C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D) hours. The artificial diet contained: powdered legume seed (125 g), wheat germ (15 g), yeast (17.5 g), sorbic acid (0.55 g), ascorbic acid (1.75 g), sunflower oil (2.5 ml), agar (7 g), methyl-p-hydroxy benzoate (1.1 g), formaldehyde 37% (1.25 g), and distilled water (325 ml) (Shorey and Hale, 1965). To prevent cannibalism, third instar larvae of cotton bollworm were transferred to individual Petri dishes and kept until pupation (Twine, 1971). The adults of cotton bollworm were fed a honey solution (10%). Before starting the experiments, larvae were reared on an artificial diet based on each legume seed powder for two generations. Then the third-generation colony was used for the feeding indices and digestive enzymes experiments.

Nutritional performances of cotton bollworm

After mating, females of *H. armigera* that laid eggs were removed, and eggs were kept in Petri dishes with 8 cm diameter and 1 cm depth (40 eggs per cultivar). The first and second instar larvae of *H. armigera* were reared together in plastic dishes of 25 cm in height and 15 cm in width until the emergence of the third instar larvae. Then, the third instar larvae were separately reared in Petri dishes to prevent any cannibalistic behavior. From the third to fifth instar stage, the larval weight was measured before and after feeding on powdered artificial diets containing various legume until pre-pupation (25 replicates for each cultivar). The diets were replaced with new ones every 24 h. After daily feeding, the larval weight, the amount of the remaining leaves, and the feces produced by larvae were documented. To determine the percentage of dry weight of *H. armigera* food and larvae, 20 samples were weighed for each legume, dried in an oven at 60°C for 48 h and then weighed again. Nutritional performances of *H. armigera* larvae were calculated by Waldbauer (1968) formulae:

$$\text{Consumption index (CI)} = [(E/A)];$$
$$\text{Approximate digestibility (AD)} = [(E-F)/E];$$
$$\text{Efficiency of conversion of ingested food (ECI)} = [(P/E) \times 100];$$
$$\text{Efficiency of conversion of digestion food (ECD)} = [(P/E-F) \times 100];$$
$$\text{Relative consumption rate (RCR)} = [(E/W_0 \times T)] \text{ and}$$
$$\text{Relative growth rate (RGR)} = [P/W_0 \times T].$$

where A = average of larval dry weight over time (mg), E = dry weight of the food consumed (mg), F = dry weight of feces produced, P = dry weight gain of larvae (mg), T = the feeding duration (day), and W_0 = primary weight of larvae (mg).

Moreover, the pupal weight of *H. armigera* was measured after 24 h of pupation on each cultivar. The SII was determined by Itoyama *et al.* (1999) formula:

$$\text{SII} = [\text{Pupal weight (Pw)}/\text{Larval period(T)}].$$

Furthermore, IPQ was determined using Koricheva and Haukioja (1992) formula:

$$\mathrm{IPQ} = [\text{Pupal weight (Pw)/Frass dry weight (Fw)}].$$

Digestive enzyme assay

The fifth instar larvae of *H. armigera* reared on each legume seed were decomposed in pre-cooled distilled water under a stereomicroscope. The midguts of 20 larvae of *H. armigera* were homogenized on ice and prepared as described by Hosseininaveh *et al.* (2007). Homogenates were centrifuged at 15,000***g*** at 4°C for 10 min. The supernatants were collected and frozen (−20°C) for enzymatic assays. All assays were done in three replicates (per each legume cultivar) with a blank lacking midgut extract.

Amylase activity of cotton bollworm

Amylase activity of cotton bollworm larvae fed with different legumes was evaluated by the dinitrosalicylic acid (DNSA) method, with 1% starch as a substrate in the universal buffer system (10 mM succinate-glycine-2, morpholinoethan sulfunic acid) at pH 9. The mixtures containing midgut extracts and 1% starch were incubated at 37°C for 30 min. The enzymatic reaction was stopped by adding 50 µl of DNSA reagent and heating in boiling water for 15 min. The adsorption of mixture was read at 540 nm after cooling on ice (Bernfeld, 1955). The amount of maltose released during the α-amylase assays were estimated using the standard curve generated by using known amounts of maltose (2, 1, 0.5, 0.25, and 0.125 mg ml^{-1}).

Protease activity of cotton bollworm

Proteolytic activity was examined using azocasein (1.5%) as substrate in the universal buffer system (50 mM sodium phosphate-borate) at pH 12, and reading the absorbance at 440 nm. The reaction mixture containing 50 µl of the midgut extract and 80 µl of the substrate in 50 mM universal buffer was incubated at 37°C for 50 min. Proteolysis was stopped by adding 100 µl of 30% trichloroacetic acid, followed by cooling at 4°C for 30 min and centrifugation at 14,000 ×***g*** for 10 min. An equal volume of 2 M NaOH was added to the supernatant, and the absorbance was measured at 440 nm (Elpidina *et al.*, 2001). Furthermore, protein concentrations were estimated using the Bradford (1976) protein assay. The standard curve was constructed using known amounts of bovine serum albumin (BSA) (2, 1.5, 1, 0.5, 0.25, 0.125, and 0.063 mg ml^{-1}).

Biochemical properties of legume seeds

Biochemical characteristics of seeds of different legume cultivars, including protein, starch, total phenolic, and flavonoid amounts, were investigated to detect the relationship between biochemical compositions with feeding responses and enzyme activities of *H. armigera*. Biochemical assays were performed in three replicates for each cultivar, and distilled water was used as control. The powdered legumes were used to measure all phytochemicals of the tested cultivars.

The Bradford procedure was utilized to evaluate the protein content of the seeds. Briefly, 200 mg of the powdered seeds of each cultivar was homogenized in 10 ml of distilled water, and then 100 µl of the homogenate was mixed to 3 ml of Bradford reagent. The absorption was measured at 595 nm using BSA as a standard (Bradford, 1976).

The Bernfeld method (1955) was utilized to estimate the starch content of legumes. The powdered legume seeds (0.2 g) were homogenized in 35 ml distilled water and heated to boiling. Then, 100 µl of each sample was mixed with 2.5 ml of iodine reagent (0.2% KI and 0.02% I_2). The absorbance was recorded at 580 nm.

Furthermore, to measure the total phenol content, the Folin–Ciocalteau reagent was used (Sonald and Laima, 2001). Briefly, the powdered seeds were homogenized in methanol. After centrifugation, the supernatants were transferred to 1.5 ml Folin–Ciocalteau reagent 10% and sodium carbonate solution 7% was added to the mixture and incubated for 30 min at dark conditions. Standards and samples were measured with a spectrophotometer (S2100SUV, UNICO, USA) at 725 nm wavelength. Gallic acid was expressed quantified as the standard.

The method described by Jia *et al.* (1999) was used to determine the flavonoids content in the seeds. Briefly, 2 g of powdered seeds was homogenized in acidified ethanol (1 acid acetic:100 ethanol) and centrifuged at 13,000 ×***g*** for 12 min. The homogenates were filtered with Whatman paper, and heated at 80°C in boiling water for 12 min. After cooling the filtrates, the absorbance was read at 430 nm, and the flavonoid content of seeds was calculated using a standard curve made by known amounts of catechin.

Data analysis

Normality of data was checked using the Kolmogorov–Smirnov test by the statistical software SPSS v. 22.0. Furthermore, the result of each cultivar experiment was analyzed by one-way analysis of variance. Statistical differences of means were assessed with the Tukey test at 5% significance level. Dendrogram of various legumes was performed based on all parameters tested for *H. armigera* by Ward's method. Pearson's correlation analysis was investigated to analyze the relationship between nutritional responses and enzyme activity of *H. armigera* with biochemical traits of legume cultivars (SPSS Inc., 2007).

Results

Feeding responses of cotton bollworm

Nutritional performance of third larval instar of *H. armigera* reared on the different legume cultivars is indicated in table 1. The highest value of consumption index (CI) was observed on Mahta (3.74) and Mashhad (3.65) cultivars. Furthermore, the lowest value of CI was on Shadan cultivar (2.43) ($F = 7.42$; df = 9, 240; $P < 0.01$). The highest approximate digestibility (AD) value of the third instar larvae was on Feyz cultivar (63.35%) and the lowest was on Arabi cultivar (43.11%) ($F = 9.07$; df = 9, 240; $P < 0.01$). The larvae reared on Dehghan cultivar had the highest efficiency of conversion of ingested food (ECI, 33.01%) ($F = 60.92$; df = 9, 240; $P < 0.01$), efficiency of conversion of digested food (ECD, 74.67%) ($F = 35.03$; df = 9, 240; $P < 0.01$), and relative growth rate (RGR, 0.347) ($F = 100.14$; df = 9, 240; $P < 0.01$) values. However, the lowest values of ECI (10.30%), ECD (20.45%), and RGR (0.083) indices were found on Khomein cultivar compared with the other hosts. Among the

Table 1. Nutritional indices of the third instar larvae of *Helicoverpa armigera* fed on artificial diets based on the different legume seeds

Legume cultivars	CI	AD (%)	ECI (%)	ECD (%)	RCR (mg mg^{-1} day^{-1})	RGR (mg mg^{-1} day^{-1})
Broad bean (Barekat)	2.80 ± 0.16 cd	55.47 ± 2.41 ab	17.48 ± 0.85 cd	34.41 ± 3.05 cd	0.785 ± 0.046 ef	0.131 ± 0.006 d
Broad bean (Mahta)	3.74 ± 0.18 a	58.07 ± 1.17 ab	11.85 ± 0.37 ef	20.77 ± 0.92 de	1.083 ± 0.048 bc	0.125 ± 0.004 d
Broad bean (Saraziri)	3.18 ± 0.21 abc	51.08 ± 3.22 bcde	16.18 ± 1.11 d	35.01 ± 3.62 c	0.916 ± 0.059 cde	0.135 ± 0.003 d
Broad bean (Feyz)	3.48 ± 0.23 abc	63.35 ± 1.99 a	14.79 ± 0.65 e	24.37 ± 1.58 cde	0.997 ± 0.065 cd	0.139 ± 0.005 d
Broad bean (Shadan)	2.43 ± 0.10 d	54.58 ± 2.80 abc	18.33 ± 0.10 cd	37.90 ± 3.98 c	0.690 ± 0.028 f	0.123 ± 0.006 d
White kidney bean (Dehghan)	3.16 ± 0.09 abc	44.97 ± 1.40 de	33.01 ± 0.94 a	74.67 ± 2.63 a	1.066 ± 0.036 bc	0.347 ± 0.012 a
Common bean (Khomein)	3.38 ± 0.12 abc	50.74 ± 1.39 bcde	10.30 ± 0.34 f	20.45 ± 0.64 e	0.810 ± 0.029 def	0.083 ± 0.004 e
Red kidney bean (Goli)	2.90 ± 0.13 bcd	45.91 ± 2.06 cde	26.01 ± 1.32 b	60.54 ± 4.52 b	1.020 ± 0.041 bc	0.259 ± 0.014 b
Cowpea (Arabi)	3.57 ± 0.13 ab	43.11 ± 2.24 e	20.27 ± 0.89 c	52.31 ± 4.42 b	1.299 ± 0.0406 a	0.261 ± 0.0130 b
Cowpea (Mashhad)	3.65 ± 0.12 a	54.48 ± 1.53 abcd	16.70 ± 0.55 cd	31.56 ± 1.70 cde	1.215 ± 0.039 ab	0.202 ± 0.008 c

CI, consumption index; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate.
The means followed by different letters in the same column are significantly different (Tukey, $P < 0.01$).

various host cultivars, the highest value of relative consumption rate (RCR) was observed on Arabi cultivar (1.299), and the lowest value was obtained on broad bean Shadan cultivar (0.690) ($F = 17.95$; df = 9, 240; $P < 0.01$) (table 1).

The nutritional indices of the fourth instar larvae of the cotton bollworm were significantly different on the various legume seeds tested. The larvae fed with Khomein cultivar (3.26) had the highest CI value, and the lowest value was observed when the larvae were fed with both Shadan (2.24) and Barekat (2.26) cultivars ($F = 7.14$; df = 9, 240; $P < 0.01$). The highest values of ECI index were recorded on both Goli (20.68%) and Dehghan (16.62%) cultivars, however, the lowest one was observed on Mahta (8.16%) and Khomein (8.40%) cultivars ($F = 31.96$; df = 9, 240; $P < 0.01$). The maximum values of ECD were found on both Dehghan (72.46%) and Goli (71.20%) cultivars and the minimum value was obtained on the Khomein cultivar (22.52%) ($F = 40.01$; df = 9, 240; $P < 0.01$). The cotton bollworm larvae reared on Dehghan (0.266) and Goli (0.252) cultivars had the highest values of RGR index ($F = 81.688$; df = 9, 240; $P < 0.01$). Among the various legume species, Dehghan cultivar had the highest value (1.41) of RCR ($F = 24.28$; df = 9, 240; $P < 0.01$) (table 2).

The highest CI of the fifth instar larvae was observed on the Khomein cultivar (1.70), whereas the lowest CI index (1.00) was found on the Barekat cultivar ($F = 6.457$; df = 9, 240; $P < 0.01$). The highest AD index was found in the cotton bollworm fed on the Feyz cultivar (38.28%), and the lowest one was observed on the Arabi cultivar (24.68%) ($F = 3.649$; df = 9, 240; $P < 0.01$). The larvae fed with the Dehghan cultivar showed the highest ECI (21.56%) and ECD (69.97%) values, while the lowest ECI index was achieved on Mahta cultivar (5.78%) and the minimum values of ECD were found on the Mahta (23.14%) and Khomein (24.88%) cultivars (ECI, $F = 35.22$; df = 9, 240; $P < 0.01$; ECD, $F = 21.36$; df = 9, 236; $P < 0.01$). The fifth instar

Table 2. Nutritional indices of the fourth instar larvae of *Helicoverpa armigera* fed on artificial diets based on the different legume seeds

Legume cultivars	CI	AD (%)	ECI (%)	ECD (%)	RCR (mg mg^{-1} day^{-1})	RGR (mg mg^{-1} day^{-1})
Broad bean (Barekat)	2.26 ± 0.13c	41.60 ± 2.88 a	11.45 ± 0.70 cd	31.14 ± 2.79 de	0.821 ± 0.048 d	0.091 ± 0.007 d
Broad bean (Mahta)	2.74 ± 0.11 b	32.66 ± 2.02 ab	8.16 ± 0.34d	28.01 ± 1.84 de	0.997 ± 0.038 cd	0.081 ± 0.005 d
Broad bean (Saraziri)	2.57 ± 0.10 bc	29.97 ± 2.53 b	12.48 ± 0.91bc	47.71 ± 3.96 bc	0.915 ± 0.033 d	0.112 ± 0.007 cd
Broad bean (Feyz)	2.50 ± 0.08bc	41.01 ± 1.69 a	11.83 ± 0.37 c	29.94 ± 1.41de	0.910 ± 0.028 d	0.107 ± 0.004 d
Broad bean (Shadan)	2.24 ± 0.07 c	41.85 ± 2.78 a	12.44 ± 0.46 bc	33.54 ± 2.61de	0.799 ± 0.026 d	0.099 ± 0.003 d
White kidney bean (Dehghan)	2.62 ± 0.13 bc	28.02 ± 1.70 b	19.62 ± 0.93 a	72.46 ± 2.61a	1.406 ± 0.057 a	0.266 ± 0.008 a
Common bean (Khomein)	3.26 ± 0.12 a	40.79 ± 2.37 a	8.40 ± 0.30 d	22.52 ± 1.65e	0.984 ± 0.033 cd	0.083 ± 0.004 d
Red kidney bean (Goli)	2.39 ± 0.06 bc	33.83 ± 2.14 ab	20.68 ± 1.09 a	71.198 ± 3.67a	1.229 ± 0.032 ab	0.252 ± 0.013 a
Cowpea (Arabi)	2.46 ± 0.14 bc	29.72 ± 2.08 b	15.45 ± 0.92 b	55.543 ± 3.37 b	1.170 ± 0.063 bc	0.170 ± 0.007 b
Cowpea (Mashhad)	2.50 ± 0.10 bc	32.68 ± 2.81 ab	11.14 ± 0.87 cd	38.702 ± 3.26 cd	1.368 ± 0.064 ab	0.144 ± 0.010 bc

CI, consumption index; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate.
The means followed by different letters in the same column are significantly different (Tukey, $P < 0.01$).

Table 3. Nutritional indices of the fifth instar larvae of *Helicoverpa armigera* fed on artificial diets based on the different legume seeds

Legume cultivars	CI	AD (%)	ECI (%)	ECD (%)	RCR (mg mg^{-1} day^{-1})	RGR (mg mg^{-1} day^{-1})
Broad bean (Barekat)	1.00 ± 0.7d	30.357 ± 2.23 abc	10.09 ± 0.99 cd	43.30 ± 5.17 cd	0.445 ± 0.029 d	0.042 ± 0.003 de
Broad bean (Mahta)	1.24 ± 0.09 bcd	30.57 ± 2.72 abc	5.78 ± 0.48 e	23.14 ± 2.48 e	0.622 ± 0.047 cd	0.037 ± 0.004 e
Broad bean (Saraziri)	1.39 ± 0.08 abc	25.46 ± 1.65 bc	10.71 ± 0.63 cd	46.62 ± 3.96 c	0.669 ± 0.037 bc	0.072 ± 0.005 bcd
Broad bean (Feyz)	1.25 ± 0.08 bcd	38.28 ± 2.31 a	8.11 ± 0.81 cde	24.01 ± 2.66 e	0.606 ± 0.036 cd	0.048 ± 0.005 cde
Broad bean (Shadan)	1.31 ± 0.05 bcd	33.45 ± 3.19 abc	9.34 ± 0.58 cde	35.18 ± 4.06 cde	0.654 ± 0.026bc	0.061 ± 0.005 cde
White kidney bean (Dehghan)	1.11 ± 0.05 cd	31.60 ± 1.87 abc	21.56 ± 1.04 a	69.97 ± 2.18 a	0.740 ± 0.036bc	0.155 ± 0.007 a
Common bean (Khomein)	1.697 ± 0.10 a	27.66 ± 1.54 bc	7.15 ± 0.62 de	24.88 ± 1.78 e	0.586 ± 0.032 cd	0.041 ± 0.004 de
Red kidney bean (Goli)	1.477 ± 0.09 ab	28.44 ± 1.90 abc	16.77 ± 0.85 b	64.17 ± 4.26 ab	0.961 ± 0.051 a	0.165 ± 0.013 a
Cowpea (Arabi)	1.25 ± 0.07 bcd	24.68 ± 1.58 c	11.73 ± 1.05 c	50.86 ± 4.87 bc	0.822 ± 0.045 ab	0.101 ± 0.011 b
Cowpea (Mashhad)	1.49 ± 0.08 ab	35.71 ± 2.98 ab	7.97 ± 0.81 de	27.18 ± 3.63 de	0.956 ± 0.050 a	0.077 ± 0.009 bc

CI, consumption index; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate.
The means followed by different letters in the same column are significantly different (Tukey, $P < 0.01$).

larvae of *H. armigera* reared on Dehghan (0.165) and Goli (0.155) cultivars obtained the highest values of RGR. In contrast, the lowest RGR value was recorded when the larvae were fed with Mahta cultivar (0.037) ($F = 39.71$; df = 9, 240; $P < 0.01$). The maximum value of RCR index was recorded on the Goli (0.961) and Mashhad (0.956) cultivars and the lowest value was recorded on the Barekat cultivar (0.445) ($F = 17.35$; df = 9, 240; $P < 0.01$) (table 3).

There were significant differences among all nutritional response parameters of the total larvae (third to fifth instars) of cotton bollworm on various legume seeds tested. The larvae fed on the Khomein (2.46) and Barekat (1.72) cultivars had the highest and lowest CI index ($F = 9.29$; df = 9, 240; $P < 0.01$). The highest value of AD index was obtained on the all Feyz (45.50%), Barekat (42.83%) and Shadan (42.42%) cultivars, while the lowest one was documented on Arabi cultivar (31.01%) ($F = 7.441$; df = 9, 240; $P < 0.01$). The highest ECI index was achieved by rearing larvae on Dehghan cultivar (22.34%) and the lowest value was found on both Khomein (8.30%) and Mahta (8.48%) cultivars ($F = 121.43$; df = 9, 240; $P < 0.01$). Total instar larvae fed on Dehghan (70.71%) and Goli (62.18%) cultivars found the highest ECD value, while the lowest ECD index was documented on both Mahta (22.14%) and Khomein (22.53%) cultivars ($F = 71.01$; df = 9, 240; $P < 0.01$). The larvae of cotton bollworm fed with Dehghan (0.201 mg mg^{-1} day^{-1}) and Goli (0.192) cultivars showed the highest values of RGR index. Furthermore, the lowest RGR value was achieved when the larvae were fed with Khomein cultivar (0.059) ($F = 139.59$; df = 9, 240; $P < 0.01$). The maximum value of RCR index was obtained on the Mashhad (1.02), Goli (0.95), and Arabi (0.93) cultivars and the minimum value was recorded on Barekat cultivar (0.602) ($F = 31.28$; df = 9, 240; $P < 0.01$) (table 4).

The results in fig. 1 show that the highest whole larval instars weights (75.01) ($F = 27.92$; df = 9, 240; $P < 0.01$) and feces produced values (97.90 mg) ($F = 7.320$; df = 9, 240; $P < 0.01$) were achieved on Dehghan cultivar, and the lowest larval weights were achieved in larvae fed on Khomein (52.16 mg) and Mahta (52.38 mg) cultivars (fig. 1a, c). The highest value of food consumed was detected on the Mashhad cultivar (153.21 mg), and the lowest was also recorded on the Mahta cultivar (113.91 mg)

Table 4. Nutritional indices of whole instar larvae of *Helicoverpa armigera* fed on artificial diets based on the different legume seeds

Legume cultivars	CI	AD (%)	ECI (%)	ECD (%)	RCR (mg mg^{-1} day^{-1})	RGR (mg mg^{-1} day^{-1})
Broad bean (Barekat)	1.72 ± 0.07 d	42.83 ± 2.19a	12.14 ± 0.34 d	30.20 ± 1.86 cde	0.602 ± 0.023 e	0.072 ± 0.003 def
Broad bean (Mahta)	2.18 ± 0.08 ab	39.74 ± 1.57ab	8.48 ± 0.22 e	22.14 ± 1.00 e	0.798 ± 0.026 bc	0.067 ± 0.002ef
Broad bean (Saraziri)	2.07 ± 0.08 bc	33.92 ± 1.52bc	12.36 ± 0.46 d	38.63 ± 2.07 c	0.744 ± 0.026 cd	0.091 ± 0.004d
Broad bean (Feyz)	2.01 ± 0.08 bcd	45.50 ± 1.50a	11.22 ± 0.37d	25.46 ± 1.28 de	0.727 ± 0.026 cd	0.081 ± 0.003de
Broad bean (Shadan)	1.82 ± 0.04 cd	42.42 ± 2.48a	12.32 ± 0.30 d	31.31 ± 1.79 cde	0.655 ± 0.016 de	0.081 ± 0.003de
White kidney bean (Dehghan)	1.93 ± 0.06 bcd	32.54 ± 1.53bc	22.34 ± 0.60 a	70.71 ± 2.18 a	0.910 ± 0.027 ab	0.201 ± 0.005 a
Common bean (Khomein)	2.46 ± 0.08 a	38.57 ± 1.50abc	8.30 ± 0.20 e	22.53 ± 0.81 e	0.712 ± 0.023 cde	0.059 ± 0.002 f
Red kidney bean (Goli)	2.01 ± 0.06 bcd	34.26 ± 1.72bc	20.16 ± 0.62 b	62.18 ± 3.26 a	0.953 ± 0.024 a	0.192 ± 0.008 a
Cowpea (Arabi)	1.99 ± 0.07 bcd	31.01 ± 1.52 c	15.33 ± 0.36 c	51.817 ± 2.39 b	0.933 ± 0.029 a	0.143 ± 0.005 b
Cowpea (Mashhad)	2.18 ± 0.06 ab	38.57 ± 2.02 abc	11.35 ± 0.47 d	31.78 ± 2.36 cd	1.021 ± 0.028 a	0.115 ± 0.004 c

CI, consumption index; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate.
The means followed by different letters in the same column are significantly different (Tukey, $P < 0.01$).

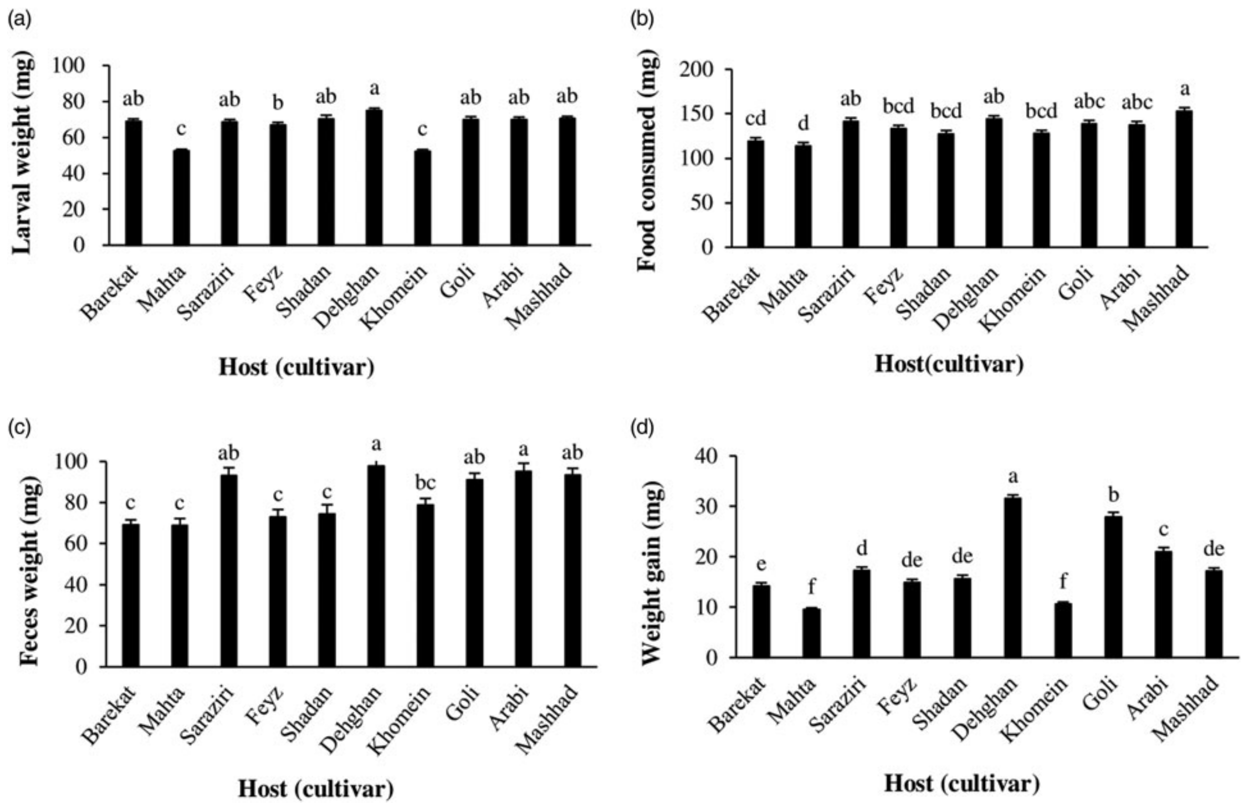

Figure 1. (a) Mean larval weight, (b) food consumed, (c) feces produced, and (d) larval gain weight of *Helicoverpa armigera* fed on artificial diets based on the different legume seeds.

($F = 7.320$; df = 9, 240; $P < 0.01$) (fig. 1b). The larvae of cotton bollworm fed on the Dehghan cultivar (31.58 mg) observed the maximum value of larval gain weight ($F = 109.66$; df = 9, 240; $P < 0.01$) (fig. 2d).

IPQ and SII indexes of cotton bollworm

There were significant differences in the SII and IPQ values of *H. armigera* fed on different host plant cultivars. The highest SII ($F = 75.44$; df = 9, 240; $P < 0.01$) and IPQ ($F = 25.66$; df = 9, 240; $P < 0.01$) indexes were obtained when the larvae were reared on the Dehghan cultivar (SII: 3.014; IPQ: 0.676), whereas the larvae reared on the Khomein cultivar had the lowest values (SII: 1.50; IPQ: 0.44) (fig. 2).

Enzyme activity of cotton bollworm

The activities of digestive proteases and amylases in fifth instar larval reared on artificial diets based on the different legume

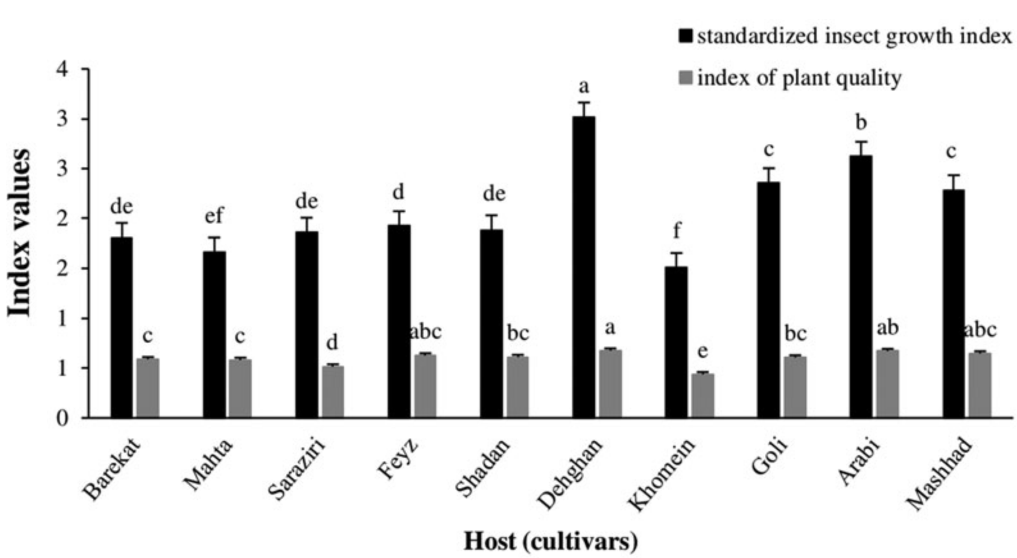

Figure 2. Index of plant quality (IPQ) and standardized insect-growth index (SII) of *Helicoverpa armigera* fed on artificial diets based on the different legume seeds.

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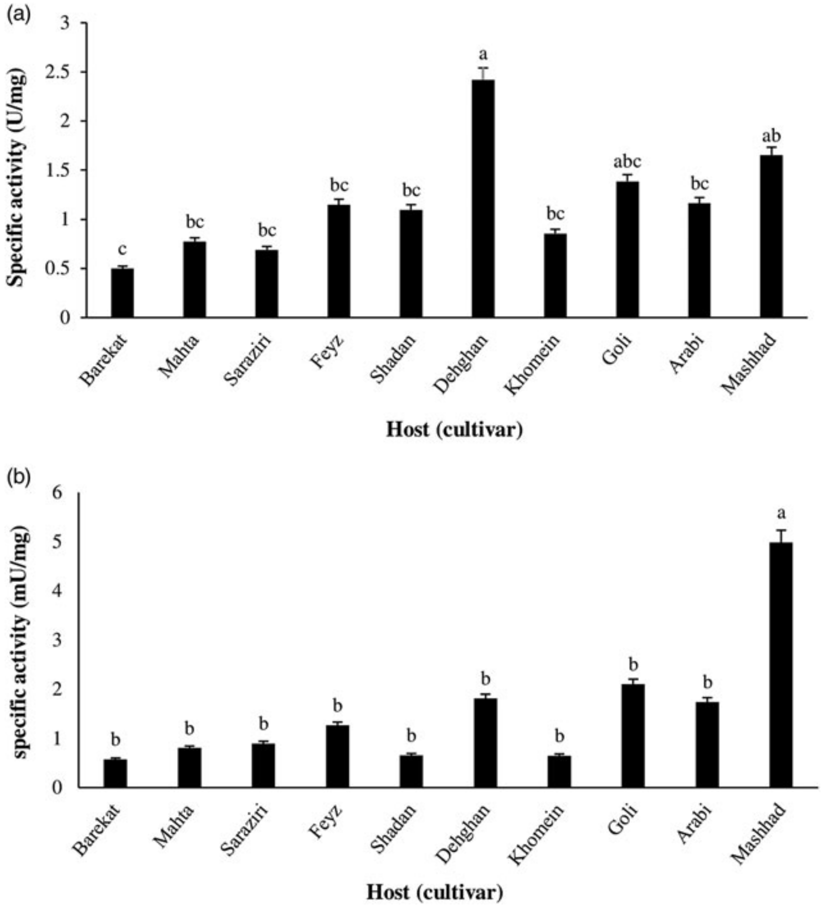

Figure 3. General proteolytic (a) and amylolytic (b) activity of midgut extracts from *Helicoverpa armigera* fed on artificial diets based on the different legume seeds.

seeds are presented in fig. 3. The maximum and minimum proteolytic activity of the fifth instars were obtained in the larvae reared on Dehghan (1.65 U mg^{-1}) and Barekat (0.498 U mg^{-1}) cultivars ($F = 6.86$; df = 9, 20; $P < 0.01$) (fig. 3a). Moreover, the larvae fed on Mashhad cultivar showed the highest levels of amylolytic activity (4.98 mU mg^{-1}), in contrast, no significant difference in amylolytic activity of fifth instar larvae value was recorded among larvae feeding on the other cultivars ($F = 10.54$; df = 9, 20; $P < 0.01$) (fig. 3b).

Cluster analysis

The dendrogram-based feeding indices and growth parameters with enzymatic activity of *H. armigera* on different legume cultivars are presented in fig. 4. Two clusters A and B are apparent in the dendrogram. Sub-cluster A_1 includes Feyz, Shadan, and Barekat cultivars, and sub-cluster A_2 includes Mahta and Khomein cultivars. Sub-cluster B_1 consisted of Saraziri and Mashhad cultivars and Dehghan, Goli, and Arabi cultivars were in sub-cluster B_2 (fig. 4).

Biochemical characteristics of various legume seeds

The primary and secondary metabolites of the tested legume cultivars are given in table 5. There were significant differences in the phytochemical metabolites of various seed cultivars. The highest protein content was measured in the Dehghan cultivar (0.342 mg ml^{-1}) ($F = 31.23$; df = 9, 20; $P < 0.01$). In contrast, the lowest amount of starch seeds was achieved in the Mahta cultivar (1.81 mg ml^{-1}) ($F = 6.58$; df = 9, 20; $P < 0.01$). The highest total phenolic content was detected in the Mahta (11.98 mg ml^{-1}), Feyz (12.46 mg ml^{-1}), and Shadan (11.97 mg ml^{-1}) cultivars, and the lowest content was observed in the Dehghan cultivar (9.21 mg ml^{-1}) ($F = 10.79$; df = 9, 20; $P < 0.01$). Furthermore, the highest amount of flavonoids was obtained for the Saraziri (111.80 mg ml^{-1}), Feyz (111.93 mg ml^{-1}), and Shadan (112.20 mg ml^{-1}) cultivars, whereas the lowest amount was measured for Arabi (93.60 mg ml^{-1}) and Mashhad (94.13 mg ml^{-1}) cultivars ($F = 60.39$; df = 9, 20; $P < 0.01$).

Correlation analysis

The analysis of correlation coefficients of nutritional performances and growth characteristics of *H. armigera* with their enzymatic activity when fed on different legume is given in table 6. Significant correlations were detected between feeding and growth of cotton bollworm and the amylolytic and proteolytic enzymes activity on various legume cultivars. The food consumed, larval gain, feces produced, RCR, and RGR parameters of *H. armigera* showed significant negative correlations with amylolytic and proteolytic enzymes activity ($P < 0.01$). In contrast, the digestive enzymes of larvae positively correlated with the tested growth characteristics of *H. armigera*, including SII and IPQ indexes ($P < 0.01$). There was no significant correlation among larval weight, AD, ECI, and ECD parameters of cotton bollworm with the amylolytic enzyme activity of larvae. Likewise, the CI and AD variables of *H. armigera* were not significantly correlated with amylolytic activity ($P > 0.05$). Furthermore, a significant negative

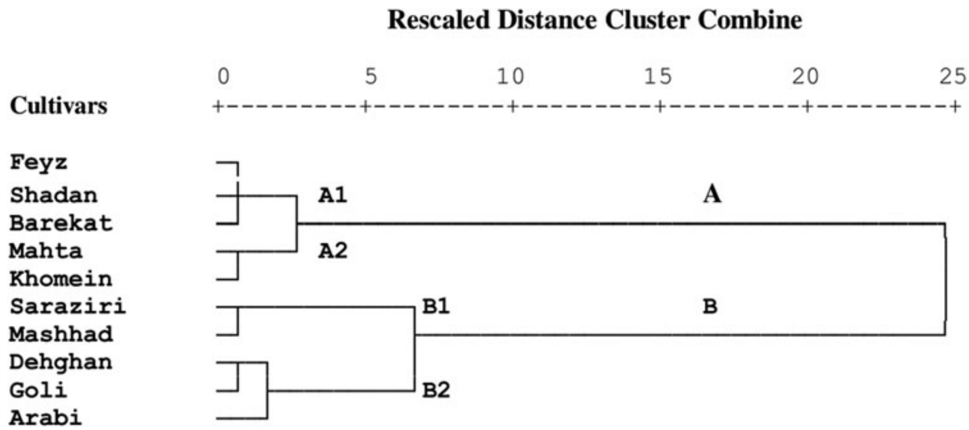

Figure 4. Dendrogram of different cultivars based on nutritional and growth indices and enzymatic activity of *Helicoverpa armigera* fed on artificial diets based on the different legume seeds (Ward's method).

Table 5. Biochemical characteristics (mean ± SE) (mg ml^{-1}) of tested different legume seeds

Mung bean cultivar	Protein content	Starch content	Total phenolic content	Flavonoids content
Broad bean (Barekat)	0.183 ± 0.007 c	1.97 ± 0.033 abc	11.34 ± 0.23 ab	106.80 ± 1.71 abc
Broad bean (Mahta)	0.159 ± 0.005 c	1.81 ± 0.063 c	11.98 ± 0.03 a	109.07 ± 0.58 ab
Broad bean (Saraziri)	0.174 ± 0.001 c	1.96 ± 0.036 abc	10.93 ± 0.18 ab	111.80 ± 0.92 a
Broad bean (Feyz)	0.181 ± 0.015 c	2.25 ± 0.089 ab	12.46 ± 0.73 a	111.93 ± 0.59 a
Broad bean (Shadan)	0.179 ± 0.007 c	1.89 ± 0.069 bc	11.97 ± 0.07 a	112.20 ± 1.06 a
White kidney bean (Dehghan)	0.342 ± 0.014 a	2.29 ± 0.101 a	9.21 ± 0.10 c	99.00 ± 0.61 cd
Common bean (Khomein)	0.279 ± 0.013 b	2.13 ± 0.045 abc	10.25 ± 0.08 bc	100.93 ± 1.45 bcd
Red kidney bean (Goli)	0.272 ± 0.017 b	2.32 ± 0.128 a	11.18 ± 0.46 ab	102.67 ± 3.07 bc
Cowpea (Arabi)	0.312 ± 0.012 ab	2.18 ± 0.027 abc	9.89 ± 0.30 bc	93.60 ± 2.31 d
Cowpea (Mashhad)	0.304 ± 0.020 ab	2.31 ± 0.077 a	9.96 ± 0.33 bc	94.13 ± 2.09 d

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

correlation was observed between larval weight, ECI, ECD, and proteolytic enzyme activity of *H. armigera* ($P < 0.01$) (table 6).

In addition, the analysis of correlation coefficients between nutritional indices and enzyme activity of *H. armigera* with biochemical traits of the tested cultivars is illustrated in table 7. SII index, amylolytic, and proteolytic activities of *H. armigera* showed a significant high positive correlation with protein content. In contrast, the food consumed, larval gain, and ECI, ECD, RCR, and RGR indices of larvae negatively correlated with protein content of legume cultivars ($P < 0.01$). There were significant negative or positive correlations between all nutritional and physiological traits of *H. armigera* with the starch amounts. The food consumed, larval gain, ECD, and RGR variables of larvae were significantly positively correlated with total phenolic content of the legume seeds. Furthermore, significant negative correlation was detected between total phenolic content of various legumes with SII parameter and amylolytic and proteolytic enzymes activity of larvae ($P < 0.01$). SII and IPQ variables and enzymes activity of *H. armigera* showed negative correlations with flavonoids content; conversely, food consumed, larval gain, ECI, ECD, RCR, and RGR indices of larvae were positively correlated with this biochemical trait of the studied legumes (table 7).

Discussion

The growth rate of *H. armigera* can be affected by quantity and quality of food consumed. Our study showed that feeding properties and proteolytic and amylolytic activities of *H. armigera* were significantly affected by artificial diets based on the different legume seeds. These results are consistent with the previous studies related to *H. armigera* fed on various plants (Soleimannejad *et al.*, 2010; Hemati *et al.*, 2012*b*; Baghery *et al.*, 2013; Rahimi Namin *et al.*, 2014; Naseri and Arghand, 2015; Fallahnejad-Mojarrad *et al.*, 2017).

The quality of food may affect the life table, feeding performance, and nutritional physiology of insects, and these traits can indicate the degree of plant resistance. Furthermore, the importance of nutrient quality or phytochemical metabolites and physical characteristics of host plants, in insect–plant interactions, is demonstrated (Kennedy *et al.*, 1987; Gvozdenac *et al.*, 2018; Shishehbor and Hemmati, 2022).

To the best of our knowledge, no findings exist on the nutritional physiology studies of this pest on various broad bean cultivars. The present research confirmed the findings of previous studies by others that low host quality, such as the presence of

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Table 6. Correlation coefficients (*r*) of nutritional characteristics of *Helicoverpa armigera* with their digestive enzymes fed on various legume cultivars

Parameter	Amylolytic activity	Proteolytic activity
Larval weight	−0.032 (0.867)	−0.495 (0.005)
Food consumed	−0.516 (0.004)	−0.562 (0.001)
Larval gain	−0.359 (0.050)	−0.488 (0.006)
SII	0.435 (0.016)	0.747 (0.000)
IPQ	0.423 (0.020)	0.539 (0.002)
ECI	−0.269 (0.151)	−0.432 (0.017)
ECD	−0.327 (0.078)	−0.395 (0.031)
RCR	−0.622 (0.000)	−0.382 (0.037)
RGR	−0.444 (0.014)	−0.474 (0.008)

SII, standardized insect-growth index; IPQ, index of plant quality; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate. The number in parenthesis is *P* value. Correlations were evaluated based on Pearson's correlation test ($P < 0.01$).

inhibitors, reduces feed and growth of *H. armigera* (Naseri *et al*., 2010*b*; Hemati *et al*., 2012*b*; Kouhi *et al*., 2014; Fathipour *et al*., 2018). The study results showed that *H. armigera* fed on the artificial diets based on the various legume cultivars could develop and feed at the different rates. Among different tested legumes, the highest CI index value of fourth, fifth, and whole instar larvae of cotton bollworm was on Khomein cultivar. Furthermore, the rate of food intake relative to average larval weight was highest during the feeding period, while the larvae fed with this cultivar.

The findings indicated that the fifth instar larvae reared on the Feyz cultivar had the highest AD value. It seems that the high AD index could not compensate for the low ECD index, which consequently resulted in growth retardation. Another reason may be the increased instar duration, in which large amounts of ingested food must be allotted to maintain metabolism (Scriber and Slansky, 1981).

The highest ECI value for whole instar larvae on the artificial diets containing Dehghan cultivar indicated that the larvae were able to convert digested food to body mass and the weights of larvae improved on this cultivar. The ECI index measures an insect's ability to incorporate ingested food into growth (Lazarevic and Peric-Mataruga, 2003). Variation in ECD value can be related to differences in the physiological and biochemical characteristics of the ingested food (Nathan *et al*., 2005). The lowest ECD value in the larvae of *H. armigera* was expressed on the Khomein and Mahta cultivars, suggesting the higher metabolic cost affecting catabolism, and, consequently, excretion (Scriber and Slansky, 1981).

Furthermore, the feeding duration is an effective factor in the RCR and RGR values (Hemati *et al*., 2012*b*). Among various legume cultivars, the lowest RCR value of the fifth and whole larval instars of cotton bollworm was obtained in the Barekat cultivar. Probably, the Barekat cultivar has low nutritive content for larvae, and consequently, a longer immature period was needed to complete growth. Conversely, the highest RGR value of whole instar larvae of *H. armigera* on artificial diets containing Goli and Dehghan seeds indicates that the rate of increase in larval weight was the highest on these legumes. It is reported that the nutritional responses of an insect are positively correlated to body biomass (Nathan *et al*., 2005). The lowest RGR value of whole instar larvae of cotton bollworm on Khomein cultivar may be due to decreased food consumption when the ingested food must be allocated to maintenance metabolism. The difference in the RGR index on various legume cultivars might be attributed to differences in primary and secondary plant metabolites among the legumes.

It has been shown that variations in allelochemical and biochemical traits and macronutrients among host plants could affect pest's performance (Tsai and Wang, 2001; Chen *et al*., 2009). The findings revealed that various legume species affect the nutritional indices of *H. armigera*, such as Dehghan and Goli cultivars as the suitable and Khomein and Mahta cultivars as the unsuitable host for feeding and developing of this pest.

In the present research, the lowest SII value in the larvae fed on Khomein cultivar may be related to a longer larval period or lower pupal weight on this cultivar. Additionally, the lowest value of IPQ on the Khomein cultivar can be correlated with a lower pupal weight and higher frass dry weight on this cultivar. The high values of feces production and food consumption on the Khomein cultivar might be related to the higher contents of secondary metabolites in this cultivar compared to the other cultivars (Shishehbor and Hemmati, 2022).

Previous studies have shown that insects, especially polyphagous pests, rapidly modify their digestive enzyme profiles in response to ingested plant protease inhibitors by down and up adjustment of gut proteases (Harsulkar *et al*., 1999; Hemmati *et al*., 2021*a*). In this study, protease and amylase as main digestive enzymes in midgut of *H. armigera* were considered. Digestive proteases play two major functions in insect physiology including inactivating protein toxins consumed as a consequence of feeding and breaking down proteins into amino acids essential for growth and development (Nation, 2002). The number of proteins in legume seeds may affect the major insect digestive enzymes such as proteases (Biggs and McGregor, 1996). Inactivation of digestive enzymes by inhibitors leads to blocking gut proteases and other digestive enzymes such as amylases, leading to poor nutrient utilization, growth retardation, and death because of starvation (Hemmati *et al*., 2021*b*).

The enzymatic activity of *H. armigera* was affected with food eaten, that reported by other researchers (Hemati *et al*., 2012*a*; Rahimi Namin *et al*., 2014; Fallahnejad-Mojarrad *et al*., 2017). The low gut amylolytic activity of cotton bollworm in all cultivars (except Mashhad legume) may be related to the presence of amylase inhibitors in these legume seeds. Moreover, the low gut proteolytic activity level in larvae fed on artificial diet containing Barekat cultivar might be correlated to the ingested protease inhibitors or the low protein content in this legume. The role of amylase and protease inhibitors on the level of enzymatic activity in *H. armigera* is known and should be demonstrated in future research.

In this study, cluster analysis showed that concerning growth and nutritional indices and enzymatic activity of *H. armigera*, various legume cultivars could be divided into four distinct classes. The grouping in each class might be due to a high level of physiological similarity of various legume cultivars. Cluster A_1 included the Feyz, Shadan, and Barekat, and cluster B_1 consisted of Saraziri and Mashhad cultivars as intermediate groups. Cluster A_2 included Mahta and Khomein cultivars as relatively unsuitable group, which could be due to the lowest values of IPQ and the highest values of secondary metabolites in these cultivars. Cluster B_2 consisted of Dehghan, Goli, and Arabi cultivars as relatively suitable group.

The present study suggests the existence of interactions between feeding parameters and activity of digestive enzymes of

Table 7. Correlation coefficients (*r*) of nutritional and physiological characteristics of *Helicoverpa armigera* with biochemical traits of various legume cultivars

Parameter	Protein	Starch	Total phenolic	Flavonoids
Larval weight	−0.236 (0.210)	−0.486 (0.006)	0.112 (0.556)	0.047 (0.807)
Food consumed	−0.530 (0.003)	−0.451 (0.012)	0.463 (0.010)	0.421 (0.021)
Larval gain	−0.575 (0.001)	−0.643 (0.000)	0.370 (0.044)	0.513 (0.004)
SII	0.722 (0.000)	0.551 (0.002)	−0.589 (0.001)	−0.569 (0.001)
IPQ	0.322 (0.083)	0.330 (0.075)	−0.140 (0.460)	−0.315 (0.090)
ECI	−0.550 (0.002)	−0.600 (0.000)	0.338 (0.068)	0.499 (0.005)
ECD	−0.623 (0.000)	−0.623 (0.000)	0.428 (0.018)	0.602 (0.000)
RCR	−0.600 (0.000)	−0.652 (0.000)	0.320 (0.084)	0.557 (0.001)
RGR	−0.657 (0.000)	−0.702 (0.000)	0.390 (0.033)	0.611 (0.000)
Amylolytic activity	0.525 (0.003)	0.475 (0.008)	−0.393 (0.032)	−0.505 (0.004)
Proteolytic activity	0.653 (0.000)	0.598 (0.000)	−0.525 (0.003)	−0.410 (0.024)

SII, standardized insect-growth index; IPQ, index of plant quality; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate. The number in parenthesis is *P* value.
Correlations were evaluated based on Pearson's correlation test ($P < 0.01$).

H. armigera with the biochemical metabolites of legume cultivars. Significant negative or positive correlations were found in correlation analysis between growth characteristics and digestive enzymes activity of cotton bollworm with primary and secondary metabolites of legume seeds. Correlation analysis showed that the growth and nutrition of *H. armigera* fed on various legume cultivars were influenced by the digestive enzyme activity of this pest. The rate of feeding in reared insects was the lowest in Mahta and Khomein cultivars, probably due to low nutritional level, biochemical properties, and high concentration of protein inhibitors, indicating that larvae feeding on these cultivars were less effective in converting ingested and digested food to biomass.

Correlation analysis revealed that amylolytic and proteolytic activities of *H. armigera* were significantly negatively correlated with the many nutritional and growth characteristics of *H. armigera* on different legume seeds. Furthermore, a positive correlation was observed between high amylolytic and proteolytic activities in the midgut with the high SII index of *H. armigera* larvae fed on Goli and Dehghan cultivars, suggesting that the quality and nutritional value of these legumes are suitable for the feeding of larvae. Variations in the protein contents of host plants might lead to differences in the proteolytic activity of *H. armigera* feeding on these plants.

In conclusion, the present results revealed significant differences in the nutritional performance and activities of major digestive enzymes of *H. armigera* larvae reared on diets containing different legume seeds. By combining the findings of the present research about the feeding responses and proteolytic and amylolytic activities of larvae fed on different legumes, it is highly probable that the Mahta and Khomein cultivars contain plant inhibitors that mediate antibiosis to insects reflected by the poor performance of *H. armigera* on these cultivars. Further research is required to identify the plant compound(s) responsible for delaying growth and development of *H. armigera*. Identification of these inhibitory compounds in host plants provides an interesting opportunity to generate transgenic pest-resistant plants as a sustainable pest management strategy. Moreover, a wider range of plants can be tested for selecting cultivars that are more resistant to *H. armigera*. A comprehensive biochemical and molecular analysis of midgut proteases and carbohydrases may shed light on the adaptive responses of larvae to feeding on different cultivars.

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Conflict of interest. None.

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