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COMPARATIVE REPRODUCTIVE PERFORMANCE AND DIGESTIVE ENZYMATIC ACTIVITY OF *HELICOVERPA ARMIGERA* (NOCTUIDAE) ON SEVEN BEAN CULTIVARS

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ABSTRACT. The cotton bollworm, *Helicoverpa armigera* (Hübner), is one of the most important pests of many host crops in Iran and worldwide. The effect of different cultivars of bean (*Phaseolus vulgaris* L.) including white kidney bean (cultivars Pak, Daneshkadeh and Shokufa), common bean (cultivar Talash) and red kidney bean (cultivars Akhtar, Sayyad and Naz) was studied on the reproductive parameters of *H. armigera* under laboratory conditions (25±1 °C, 65 ± 5% RH, and photoperiod of 16: 8 (L: D) h) and on the activity of some digestive enzymes of the larvae under field conditions. The highest rates of gross fecundity and gross fertility were on red kidney bean Naz, and the lowest values of these parameters were on white kidney bean Pak and red kidney bean Akhtar, respectively. The highest rates of net fecundity and net fertility were observed on common bean Talash, whereas the lowest values of these parameters were seen on red kidney bean Akhtar. The lowest proteolytic activity of fourth and fifth instar larvae of *H. armigera* was respectively on the leaf of red kidney bean Akhtar and red kidney bean Naz. Among the pods of different bean cultivars, proteolytic activity of fourth and fifth instar larvae was the lowest when they were fed on the green pod of red kidney bean Akhtar. The lowest amylolytic activity of fourth and fifth instar larvae of *H. armigera* was on the leaf of red kidney bean Naz. The fourth and fifth instar larvae reared on the green pod of common bean Talash showed the lowest amylolytic activity. The results indicated that red kidney bean Akhtar was an unsuitable host for *H. armigera* feeding. The findings of this study could be used in designing new strategies to control *H. armigera*.

Additional key words: *Helicoverpa armigera*, reproductive performance, digestive enzyme, bean

Among the agricultural plants, beans (*Phaseolus vulgaris* L.) are important legumes because they have a high percentage of protein, have the capability to fix nitrogen and are rich in mineral nutrients as compared to other agricultural crops (Daliry et al. 2010). The gram pod borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the polyphagous and voracious pests of agricultural crops—especially beans, in Iran (Farid, 1986) and many countries of the world (Zalucki et al. 1994, Singh and Mullick 1997, Reddy et al. 2004). The larvae of *H. armigera* attack most plant structures (stems, leaves, flower, heads, and fruits) and create serious economic losses in cultivated crops including cotton, bean, corn, tobacco, and tomato (Liu et al. 2004). Polyphagy, high mobility, high reproductive potential as well as facultative diapause are important traits that make *H. armigera* as an important pest of different host crops (Raheja 1996). Since the application of chemical insecticides has increased the risk of environmental contamination and development of insecticide resistance in *H. armigera* populations (McCaffery 1998, Naseri et al. 2009), several studies have been done to identify control measures that are environmentally-safe and economically acceptable.

For polyphagous insects, the availability of different host plants plays a central role in population outbreaks (Singh and Parithar 1988). Different nutritional values of host plants can influence growth, development and population dynamics of *H. armigera* (van Steenis and El-khawass, 1995; Du et al. 2004). Some proteins in seeds and vegetative organs of host plants may affect the key gut digestive enzymes of insects including amylases and proteinases (Biggs and McGregor 1996). Because the abundance and activity of α-amylases (α-1, 4-glucan-4-glucanohydrolases, EC 3.2.1.1) of the insects are dependent upon food sources, many lepidopteran insects living on a polysaccharide rich diet require digestive α-amylase for starch digestion (Valencia-Jiménez et al. 2008). The ability to synthesize particular digestive enzymes in herbivorous insects can result in successful development and reproductive potential (Ishaaya et al. 1971). Plants with protein inhibitor mechanisms decrease not only insect fecundity and fertility but also are effective on digestive enzymatic activity. Inhibition of an insect's digestive enzyme activity by enzyme-inhibitors of a host plant can result in poor nutrient utilization and developmental retardation (Jongsma and Bolter 1997, Gatehouse and Gatehouse 1999).

Host plant resistance among crop plants is a major part of an integrated pest management program (IPM). Developing cultivars that are resistant to *H. armigera* would supply an effective complementary approach in IPM to reduce the extent of losses caused by this pest (Jallow et al. 2004). Plants with an antibiosis mechanism may decrease directly fecundity of insect pests (Sarfraz et al. 2006). Knowledge about some properties of digestive enzymes is critical to physiologically-based control methods (Fathipour and Naseri 2011). Thus, studying the insects' digestive system is an important tool to find a new control techniques in IPM programs (Lawerence and Koundal 2002). So, we have focused on

two key digestive enzymes (protease and amylase) of *H. armigera* and fecundity in response to feeding on different bean cultivars.

Because the bean cultivars used in this study have different nutritional values for *H. armigera* larvae (Namin et al. 2014), it was hypothesized that the adult females emerging from larvae fed on some bean cultivars will have higher reproductive potential than those reared on any other cultivar. Furthermore, because there were significant differences in protein and starch contents among tested bean cultivars (Namin et al. 2014), we hypothesized that *H. armigera* larvae feeding on bean cultivars poor in protein and starch contents would show lower levels of digestive enzymatic activity that can affect reproductive performance of this insect.

Hitherto, several studies have been done on the effect of different host plants on reproductive performance (Liu et al. 2004, Fathipour and Naseri, 2011, Naseri et al. 2011, Hemati et al. 2013) and digestive enzymes activity of *H. armigera* (Kotkar et al. 2009, Hemati et al. 2012, Namin et al. 2014, Nemati Kalkhoran et al. 2014). However, no published articles are available regarding the effect of different bean cultivars on reproductive performance, as well as on the digestive enzymatic activity (amylases and proteases) of this species under field conditions. Because the insects are ectothermic animals, developmental and physiological processes of them can be affected by environment temperature (Johnson et al. 1992, Na and Ryoo 2000). Our objective here was to compare the effect of different bean cultivars on reproductive parameters (under laboratory conditions) and some digestive enzymatic activity (under field conditions) in *H. armigera* reared on bean cultivars to develop pest management programs for beans in Iran. We hope that the results of this research along with the findings of previous research on life table parameters (Naseri et al. 2014) and nutritional indices (Namin et al. 2014) of *H. armigera* on different bean cultivars would provide useful information to develop a comprehensive pest management program of the pest on beans.

MATERIALS AND METHODS

Plant. Different bean (*P. vulgaris*) cultivars including white kidney bean (WKB) (cultivars Pak, Daneshkadeh and Shokufa), common bean (CB) (cultivar Talash) and red kidney bean (RKB) (cultivars Akhtar, Sayyad and Naz) were acquired from the Seed and Plant Improvement Institute (Khomein, Iran) and were cultivated in the research farm of the University of Mohaghegh Ardabili (Ardabil, Iran) in May 2012. Selected bean cultivars in this research are commercially cultivated legumes in Iran (Namin et al. 2014). The protein content of these cultivars ranged from 0.857 mg mL^{-1} in WKB cultivar Daneshkadeh to 0.737 mg mL^{-1} in CB cultivar Talash. Also, the starch content differed from 2.244 mg mL^{-1} in RKB cultivar Akhtar to 0.438 mg mL^{-1} in WKB cultivar Pak (Namin et al. 2014).

The experiments were started when bean cultivars reached reproductive stage. For this research, the young leaves and green terminal pods (all of the equal size) of bean cultivars were transferred to a growth chamber at 25 ± 1 °C, 65 ± 5% RH, with a photoperiod of 16: 8h (L: D). The leaves of different bean cultivars were used for feeding of first and second *H. armigera* larval instars and the green pods were used for feeding of the third to fifth *H. armigera* larval instars (Green et al. 2002, Naseri et al. 2009, Namin et al. 2014).

Laboratory rearing of *H. armigera*. *H. armigera* eggs used in this research were obtained from a laboratory colony maintained on an artificial diet, as described by Shorey and Hale (1965), from Tarbiat Modares University (Tehran, Iran) and transferred to a growth chamber at 25 ± 1°C, 65 ± 5% RH and a photoperiod of 16:8 h(L:D). Before starting the experiments, *H. armigera* was reared for two generations on different bean cultivars, and data were collected from third generation.

Reproductive parameters. To study the reproductive parameters of *H. armigera* on different bean cultivars, fifty eggs (within 12 h) were collected from adults that were reared on each cultivar and then used for each experiment. After hatching, first instar larvae were transferred individually into plastic Petri dishes (diameter 8 cm, depth 2 cm), containing the detached fresh leaves of each tested cultivar. To ventilate, a hole (diameter 1 cm) covered by a mesh net was made in the Petri dishes. A piece of water-soaked cotton was wrapped around the petioles of detached leaves and green pods to prevent desiccation. The leaves and green pods were changed daily until prepupation. The larvae in each Petri dish were checked daily for the mortality or ecdysis. Prepupae and pupae were kept inside small plastic tubes (diameter 2 cm, depth 5 cm). Duration of pre-pupal and pupal stages and their mortality were also recorded daily.

After emergence of adult moths, a pair of female and male moths were transferred into oviposition containers (diameter 11.5 cm, depth 9.5 cm), which was closed at the top with a fine mesh net for aeration. The internal walls of oviposition containers were covered with the same mesh net as an oviposition substrate. A small cotton wick soaked in 10% honey solution was inserted in each oviposition container for the adult's feeding.

The oviposition containers were checked daily for adult's mortality and number of deposited eggs. The reproductive parameters of *H. armigera* were calculated as follows (Carey 1993):

Gross fecundity rate= $\sum_{x=\alpha}^{\beta} M_x$

Gross fertility rate= $\sum_{x=\alpha}^{\beta} h_x M_x$

Gross hatch rate= $\frac{\sum_{x=\alpha}^{\beta} h_x M_x}{\sum_{x=\alpha}^{\beta} M_x}$

Net fecundity rate= $\sum_{x=\alpha}^{\beta} L_x M_x$

Net fertility rate= $\sum_{x=\alpha}^{\beta} L_x h_x M_x$

where, L_x is the days lived in interval x and x+1, M_x is the average number of offsprings produced by females at age x, and h_x is the hatching rate; a is the age of female at the first oviposition and $^{\beta}$ is the age of female at the last oviposition.

Fitness index. Fitness index (*FI*) of *H. armigera* was calculated on seven tested bean cultivars using the following formula (Itoyama et al. 1999):

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

where, *P*= percentage of pupation, *Pw*= pupal weight (gr), *L*= larval period (day) and *Pd*= pupal period (day).

Digestive enzymes activity

Chemicals. Azocasein, Bradford reagent, dinitrosalicylic acid (DNS), maltose and starch were obtained from Sigma chemical Co., St Louis, USA, (Sigma, www.sigmaaladrich.com). Bovine serum albumin (BSA) was purchased from Roche Co., Germany, (Roche, www.roche.com).

Preparation of digestive enzymes. Fifty neonate larvae were reared on the leaves and green pods of each bean cultivar until fourth and fifth instars according to the method mentioned at "Laboratory Rearing" section. For assessing the digestive amylolytic and proteolytic activities, the fourth and fifth instar larvae of *H. armigera* were transferred to the research field of the University of Mohaghegh Ardabili (Ardabil, Iran) in August 2012, and they were reared on the leaves and green pods of each related bean cultivar for 24 h. These larvae were collected from the field after 24 h feeding, immediately anesthetized on ice and dissected under a stereoscopic microscope. The midguts were washed in pre-cooled distilled water, cleaned by removal of unwanted tissues; they were then collected into a known volume of distilled water. The homogenates were centrifuged at 16000 × g for 10 min at 4 °C and the resulting supernatants were collected in new micro tubes and stored at -20°C in aliquots for further use (Hosseininaveh et al. 2007).

Proteolytic activity assay. General proteolytic activity in the midgut of fourth and fifth instar *H. armigera* larvae fed on the leaves and green pods of the tested bean cultivars (for 24 h feeding) was assayed using azocasein as a substrate at the optimal pH (Elpidina et al. 2001). To evaluate the azocaseinolytic 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. Proteolysis was finished by the addition of 100 µL 30% trichloroacetic acid (TCA), and continued with cooling at 4 °C for 30 min and centrifugation at 16000 × g for 10 min. An equal quantity of 2 M NaOH was added to the supernatant, and the absorbance was read at 440 nm (using ELIZA-Reader, Anthos 2020, England). Appropriate blanks, to which TCA had been added prior to the substrate, were prepared for each treatment. Unit activity was represented as an increase in optical density per mg protein of the tissue per min due to azocasein proteolysis. All experiments were carried out in triplicate.

Amylolytic activity assay. Digestive amylolytic activity in crude homogenates of midgut extracts from *H. armigera* fourth and fifth instar larvae fed on bean cultivars was determined using the dinitrosalicylic acid (DNS) method, with 1% soluble starch as a substrate at the optimal pH (Bernfeld 1955). A quantity of 50 µL of the midgut enzyme extracts was incubated with 250 µL of universal buffer (pH 9) and 20 µL of soluble starch for 30 min at 37 °C. The reaction was stopped by the addition of 100 µL DNS and heating the tubes in boiling water bath for 10 min. The absorbance was read at 540 nm using spectrophotometer (JENWAY 6705 UV/Vis, USA) after cooling on ice. One unit activity was characterized as the amount of enzyme required to produce 1 mg of maltose in 30 min at 37 °C under the given assay conditions. All experiments were carried out in triplicate.

Protein quantification. Protein concentrations were assayed by the method of Bradford (1976) using BSA as a standard.

Statistical analysis. Reproductive parameters and digestive enzymes activity of *H. armigera* on seven bean cultivars were analyzed with one-way ANOVA using the statistical software Minitab 16 (Minitab, State College, PA, USA). Statistical differences among the means were evaluated using the Tukey test at $\alpha = 0.05$. All data were tested for normality before analysis. A dendrogram of different bean cultivars based on the reproductive parameters and digestive enzymatic activity in fourth and fifth instar larvae of *H. armigera* reared on these cultivars was constructed after cluster analysis by Ward's method (Ward 1963) using SPSS 16.0 statistical software (SPSS, Chicago, IL, USA).

RESULTS

Reproductive parameters. The results of the effect of different bean cultivars on reproductive parameters of *H. armigera* are given in Table 1. According to the results, the highest rate of gross fecundity ($F = 2.48$, df = 6, 66, $P<0.01$) was on RKB cultivar Naz, whereas the lowest value of this parameter was on WKB cultivar Pak. Among different bean cultivars, the gross fertility rate ($F = 1197.60$, df = 6, 66, $P<0.01$) was the highest on RKB cultivar Naz. The gross hatch rate of *H. armigera* on CB cultivar Talash was higher than other tested cultivars. The highest and lowest rates of the net fecundity ($F = 7.47$, df = 6, 71, $P<0.01$) and the net fertility ($F = 7.32$, df = 6, 71, $P<0.01$) were on CB cultivar Talash and RKB cultivar Akhtar, respectively.

Fitness index. Different bean cultivars as larval food had a significant effect ($F = 26.87$; df = 6, 154; $P<0.01$) on fitness index of *H. armigera*, which was the highest on CB cultivar Talash, and lowest on RKB cultivar Akhtar (Fig. 1).

Proteolytic activity. The general proteolytic activity in midgut extracts from *H. armigera* fourth and fifth instar larvae fed on the leaves and green pods of different bean cultivars under field conditions is indicated in Tables 2 and 3. The highest proteolytic activity of the fourth instar *H. armigera* was in the larvae fed on leaves ($F = 5.50$; df = 6, 7; $P<0.05$) of WKB cultivar Pak and green pods ($F = 29.19$; df = 6, 7; $P<0.05$) of RKB cultivar Naz and WKB cultivar Daneshkadeh. Also, the lowest proteolytic activity of the fourth instar was observed when larvae were fed on the leaves of RKB cultivar Akhtar, and green pods of WKB cultivars shokufa and Pak, RKB cultivars Akhtar and Sayyad, and CB cultivar Talash. Proteolytic activity of the fifth instar *H. armigera* was the highest when larvae were fed on the leaves ($F = 5.05$; df = 6, 7; $P<0.05$) and green pods ($F = 6.53$; df = 6, 7; $P<0.05$) of WKB cultivar Pak.

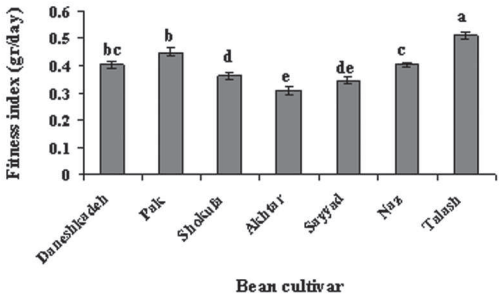

FIG. 1. Mean (±SE) fitness index of *Helicoverpa armigera* fed on different bean cultivars under laboratory conditions. The means followed by different letters are significantly different (Tukey, $P<0.01$)

Amylolytic activity. Tables 2 and 3 indicates amylolytic activity in midgut extracts from *H. armigera* fourth and fifth instar larvae reared on the leaves and green pods of different bean cultivars under field conditions. Regarding the results of this study, amylolytic activity of fourth instar larvae of *H. armigera* was influenced by feeding on the leaves ($F = 3.69$; df = 6, 7; $P<0.05$) and green pods ($F = 25.83$; df = 6, 7; $P<0.05$) of different bean cultivars. The fourth instar larvae fed on the leaves of WKB cultivar Shokufa showed the highest levels of amylolytic activity, whereas the lowest activity was in the larvae reared on the leaves of RKB cultivar Naz. Although the highest amylolytic activity was observed in the fourth instar larvae fed on green pods of WKB cultivar Daneshkadeh, the lowest activity was in the larvae fed on green pods of CB cultivar Talash. The highest amylolytic activity of *H. armigera* fifth instar fed on the leaves ($F = 18.94$; df = 6, 7; $P<0.05$) of different bean cultivars was on WKB cultivar Pak , which was not significantly different from WKB cultivars Shokufa and Daneshkadeh, and CB cultivar Talash. However, the lowest activity was in the larvae reared on RKB cultivar Naz. The fifth instar larvae of *H. armigera* fed on the green pods ($F = 16.18$; df = 6, 7; $P<0.05$) of RKB cultivars Sayyad, Naz and Akhtar showed the highest levels of amylolytic activity. However, amylase activity was the lowest in midgut extracts from fifth instar larvae fed on the green pods of WKB cultivar Pak and CB cultivar Talash.

Cluster analysis. A dendrogram according to the reproductive parameters, proteolytic and amylolytic activities in fourth and fifth instar larvae of *H. armigera* reared on seven bean cultivars is shown in Figure 2. The dendrogram shows two clusters labelled A (including subclusters A1 and A2) and B. Different cultivars of bean were grouped within each cluster according to the comparison of reproductive parameters and digestive enzymatic activity of the pest on these cultivars. Cluster A included subclusters A1 (WKB cultivars Pak, Shokufa, and Daneshkadeh, CB cultivar Talash, and RKB cultivar

TABLE 1. Mean (±SE) reproductive parameters of *Helicoverpa armigera* fed on different bean cultivars under laboratory conditions

Host (cultivar)	Parameter				
	Gross fecundity rate (eggs female-1)	Gross fertility rate (eggs female-1)	Gross hatch rate (%)	Net fecundity rate (eggs female-1)	Net fertility rate (eggs female-1)
white kidney bean (Daneshkadeh)	954.5±179ab	372.3±69.8b	39	353.9±91.9ab	138.0±35.8b
white kidney bean (Pak)	697.5±191b	308.1±83.6b	44	392.8±94.9ab	172.8±41.8ab
white kidney bean (Shokufa)	719.3±117b	309.3±50.3b	43	440.0±62.1ab	189.2±26.7ab
red kidney bean (Akhtar)	894.2±254ab	214.6±60.9b	24	173.5±111.5b	41.6±26.5b
red kidney bean (Sayyad)	1208.4±88ab	338.3±24.8b	28	480.9±76.2ab	134.7±21.3b
red kidney bean (Naz)	1505.0±204a	878.1±53.5a	52	297.3±112.5ab	154.6±58.4ab
common bean (Talash)	744.8±153ab	394.7±80.9b	53	609.5±95.8a	323.0±50.8a

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

Sayyad) and A2 (RKB cultivar Akhtar), and cluster B included RKB cultivar Naz.

DISCUSSION

The results of this study show that various bean cultivars have significant effects on the reproductive performance and digestive physiology of *H. armigera*. The females of *H. armigera* reared on RKB cultivar Akhtar had lower rates of gross fertility, net fecundity and net fertility than the other tested cultivars. Naseri et al. (2014) have previously demonstrated that *H. armigera* reared on RKB cultivar Akhtar showed the lowest values of r_m and R_0, suggesting that this cultivar was less suitable to this pest compared with the others.

Among different bean cultivars, we observed the highest gross fecundity rate of *H. armigera* on RKB cultivar Naz probably due to high nutrient values. This rate approximately was 2-fold lower than that reported by Hemati et al. (2013) for *H. armigera* on chickpea Arman (2947.8 eggs female^{-1}). This variation indicates that RKB cultivar Naz has lower nutrient values than chickpea Arman. The gross fertility rate of *H. armigera* ranged from 878.1 to 214.6 eggs female^{-1} on RKB cultivars Naz and Akhtar, respectively. According to the results of Naseri et al. (2011), the lowest gross fertility rate of *H. armigera* on different soybean cultivars was on Gorgan3 (149 eggs female^{-1}), which was in disagreement with our results, suggesting that the quantity and/or the quality of nutrients in bean are more suitable than those in soybean as a food for *H. armigera* larvae. Among various bean cultivars tested in the current study, the net fecundity and net fertility rates of *H. armigera* on RKB cultivar Akhtar were lower than those reported by Hemati et al. (2013) for *H. armigera* on common bean Khomein (780.4 eggs and 694.5 8 eggs female^{-1}, respectively). Some possible reasons for such disagreement may be due to the genetic differences as a result of laboratory rearing or variations in geographic populations of the pest.

The lowest value of fitness index of *H. armigera* on RKB cultivar Akhtar showed nearly 2-fold lower than that reported for fitness index of this pest on corn hybrid SC700 (0.69 gr/day) (Arghand et al. 2014). Some probable reasons for these variations are due to the physiological differences depending on the type of the host plant and genetic differences in geographic populations of the pest. In this study, we examined the effects of different bean cultivars on physiological responses at the level of activity of two key digestive enzymes (protease and α-amylase) in *H. armigera* fourth and fifth instar larvae as well. The activity of digestive enzymes, including proteases and α-amylases, depends on the nature of food or ingested chemical compounds and enzyme-inhibitors (Mendiola-Olayaet

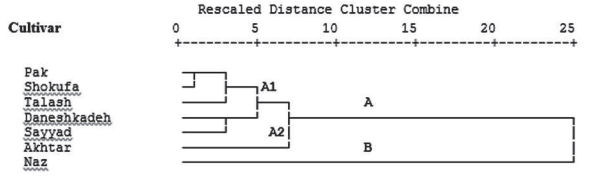

FIG. 2. Dendrogram of different bean cultivars based on reproductive parameters and digestive enzymatic activity in fourth and fifth instar larvae of *Helicoverpa armigera* reared on these cultivars (Ward's method).

TABLE 2. Mean (±SE) proteolytic and amylolytic activity of midgut extracts from *Helicoverpa armigera* fourth instar larvae fed on leaves and pods of different bean cultivars under filed conditions

	Proteolytic activity of fourth instar (U/mg)		Amylolytic activity of fourth instar (mU/mg)	
Host (cultivar)	Reared on leaves	Reared on pods	Reared on leaves	Reared on pods
white kidney bean (Daneshkadeh)	0.244±0.021 ab	3.987±0.253 a	0.466±0.050 b	1.521±0.068 a
white kidney bean (Pak)	0.585±0.137 a	2.090±0.103 b	0.651±0.184 ab	1.114±0.044 ab
white kidney bean (Shokufa)	0.498±0.005 ab	1.653±0.184b	0.868±0.032 a	0.735±0.093 bc
red kidney bean (Akhtar)	0.104±0.005b	1.612±0.033 b	0.495±0.092 ab	1.050±0.076 b
red kidney bean (Sayyad)	0.171±0.135 ab	1.613±0161 b	0.593±0.141 ab	0.555±0.039 c
red kidney bean (Naz)	0.458±0.063 ab	3.641±0.308a	0.205±0.029 b	0.714±0.132 bc
common bean (Talash)	0.271±0.017 ab	2.417±0.084 b	0.630±0.109 ab	0.326±0.054 c

The means followed by different letters in the same column are significantly different ($P<0.05$, Tukey).

et al. 2000). Protein as the primary component of the insect diets is digested into amino acids by proteases. Also, the complex polysaccharides are broke down into simple sugars by amylases. The synthesis of particular enzymes in herbivorous insects ensure appropriate quality and quantity of the reproductive success (Ishaaya et al. 1971); therefore, any interfering in normal food digestion and absorption by enzyme-inhibitors of a host plant can result in population growth retardation, especially intrinsic rate of increase (Jongsma and Bolter 1997, Gatehouse and Gatehouse 1999). Accordingly, study of life table parameters, digestive enzyme activity and reproductive performance of *H. armigera* can play an important role to recognize anti-feeding compounds and using them for designing comprehensive pest management strategies against this pest.

Among seven bean cultivars, the highest proteolytic activity was observed in fourth and fifth instar larvae of *H. armigera* on the leaves of WKB cultivar Pak. However, lower proteolytic activity was in fourth instar larvae of *H. armigera* on the leaves of RKB cultivar Akhtar. Moreover, the fifth instar larvae reared on the green pods of WKB cultivar Pak had the highest proteolytic activity, whereas a low proteolytic activity of this instar was on the green pods of RKB cultivar Akhtar. The results of life table parameters of *H. armigera* reared on different bean cultivars indicated that the highest intrinsic rate of increase was on WKB cultivar Pak and CB cultivar Talash, and the lowest one was on RKB cultivar Akhtar (Naseri et al. 2014).

By combining the results from Naseri et al. (2014) and the findings of the current research it could be suggested that RKB cultivar Akhtar is an unsuitable cultivar against *H. armigera*. The resistance of this cultivar may be due to the absence of primary nutrients necessary for the development of *H. armigera* larvae or the presence of some secondary biochemicals (Naseri et al. 2014). However, according to Namin et al. (2004), various bean cultivars had significant effects on the nutritional performance of *H. armigera*. They suggested that higher protease and amylase activities in *H. armigera* larvae fed on some bean cultivars might be due to the differences in protein and starch contents of the diet. Therefore, since *H. armigera* larvae had the complexity of the digestive enzymes (Brito et al. 2001, Kotkar et al. 2009), their different ability to access these macronutrients via digestive enzymes or nutritional imbalances between protein and carbohydrate in the tested cultivars could explain the differences in the digestive enzymatic activity of this pest on various bean cultivars. Nutrient balance, especially P/C ratio, has been reported as an important factor for growth and development of many insect pests (Lee et al. 2002, Bede et al. 2007).

The results of this study showed that the highest proteolytic activity was in fifth instar larvae fed on leaves of WKB cultivar Pak (3.974 U/mg) and the lowest activity was on RKB cultivar Naz (0.666 U/mg). Nemati Kalkhoran et al. (2014) reported that the highest proteolytic activity of *H. armigera* larvae reared on the leaves of different tomato cultivars, under field conditions, was on Hed riogrande (3.235 U/mg) and the lowest was on Korral (0.940 U/mg), which are different from our results in this study. Some possible reasons for

TABLE 3.Mean (±SE) proteolytic and amylolytic activity of midgut extracts from *Helicoverpa armigera* fifth instar larvae fed on leaves and pods of different bean cultivars under filed conditions

	Proteolytic activity of fifth instar (U/mg)		Amylolytic activity of fifth instar (mU/mg)	
Host (cultivar)	Reared on leaves	Reared on pods	Reared on leaves	Reared on pods
white kidney bean (Daneshkadeh)	0.812±0.329 b	2.569±0.032b	1.150±0.089 a	0.906±0.029 ab
white kidney bean (Pak)	3.974±1.30 a	3.947±0.379a	1.377±0.185 a	0.328±0.209 b
white kidney bean (Shokufa)	1.077±0.195 ab	2.585±0.068b	1.056±0.046 a	0.971±0.004 ab
red kidney bean (Akhtar)	1.281±0.014 ab	2.260±0.267b	0.835±0.066 ab	1.616±0.205 a
red kidney bean (Sayyad)	0.823±0.214 b	3.463±0.325ab	0.342±0.160 bc	1.622±0.115 a
red kidney bean (Naz)	0.666±0.347 b	2.548±0.232b	0.238±0.009 c	1.155±0.105 a
common bean (Talash)	2.450±0.163 ab	2.990±0.082ab	1.342±0.058 a	0.219±0.150 b

The means followed by different letters in the same column are significantly different ($P<0.05$, Tukey).

the discrepancy might be due to either physiological differences of host plants or variations in the examined larval instars of *H. armigera*. The proteolytic activity of fifth instar larvae of *H. armigera* fed on the green pods of cultivar WKB cultivar Pak showed approximately 2.5 fold lower activity than those fed bean Dehghan (Hemati et al. 2012). The proteolytic activity of fifth instar larvae of *H. armigera* fed on the green pods of RKB cultivar Akhtar showed approximately 2 fold higher activity than those fed on WKB cultivar Shokufa (Namin et al. 2014). Some possible reasons for disagreement might be due to the differences in rearing condition or variations in the geographic populations of *H. armigera*.

In the current study, the fourth and fifth instar larvae fed on the leaves of RKB cultivar Naz showed lower amylolytic activity than the others. Namin et al. (2014) reported that the fourth instar larvae of *H. armigera* fed on RKB cultivar Naz showed the highest values of ECI (efficiency of conversion of ingested food) and ECD (efficiency of conversion of digested food). It has been reported that the efficiency of conversion of digested food into larval biomass depends on the activity of digestive enzymes (Lazarevic et al. 2004). As a result, it can be expressed that despite a low digestive amylolytic activity of *H. armigera* on RKB cultivar Naz, the larvae fed on this cultivar had more performance to convert the ingested and digested food to body biomatter.

The current research shows that the fourth and fifth instar larvae of *H. armigera* fed on green pods of CB cultivar Talash had lower proteolytic and amylolytic activity than those fed on the other tested cultivars. Namin et al. (2014) reported that although proteolytic activity of the last instar of *H. armigera* was the lowest on CB cultivar Talash, the highest survival and larval growth index were observed on this cultivar. Furthermore, the highest intrinsic rate of increase (r_m) of *H. armigera* was reported on CB cultivar Talash (Naseri et al. 2014). Therefore, *H. armigera* larvae fed on CB cultivar Talash required less energy to produce digestive enzymes, and stored more energy for growth and reproduction. It could be suggested that CB cultivar Talash, among tested cultivars, was a suitable host for *H. armigera*.

The results of the cluster analysis indicated that grouping within each cluster might be due to a high physiological similarity among different bean cultivars, whereas the separate clusters might indicate significant variability in physiological characteristics between clusters. The comparative reproductive parameters and proteolytic and amylolytic activities in fourth and fifth instar larvae of *H. armigera* on seven bean cultivars revealed that subcluster A2 was the least suitable cultivar, and cluster B was the most suitable cultivar for *H. armigera*. However, cultivars classified in subcluster A1 had an intermediate status.

It is noticeable that the host plant cultivars are different in suitability for herbivorous insects; thus, they can affect life history traits of the insects such as development, survival and reproductive rates (Tsai and Wang 2001, Kim and Lee 2002). The greater total reproduction and shorter developmental time of insects on a host plant indicate greater suitability of that plant (van Lenteren and Noldus 1990). The quality and

quantity of nourishment ingested by an insect can lead to diverse negative effects including reduced intrinsic rate of increase and reproductive parameters, which we found the majority of these effects in *H. armigera* reared on RKB cultivar Akhtar. Also, the lowest value of proteolytic activity was observed on RKB cultivar Akhtar that may be due to the lack of nutritional components. However, our study about reproductive parameters was conducted under laboratory conditions, preventing the evaluation of other ecological factors that can play an important role in the reproductive performance of the moths. So, for a better understanding of the *H. armigera* – bean interaction to control of this pest, more attention should be allocated to study the demographic and reproductive parameters of this pest on different bean cultivars under field conditions.

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