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Life table parameters and digestive enzymes activity of *Helicoverpa armigera* (Lep.: Noctuidae) on different tomato cultivars

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Abstract

The tomato fruit borer, *Helicoverpa armigera* (Hübner), is a destructive insect pest of many host crops in Iran and worldwide. The effect of different tomato cultivars (SUN 6108 f1, Rio grande UG, Korral, CH falat, Hed rio grande and Cal JN3) was studied on life table parameters of *H. armigera* under laboratory (25 ± 1 °C, 65 ± 5% RH and a photoperiod of 16: 8 (L: D) hours), and on the activity of some digestive enzymes of its sixth larval instars under field conditions. The larval period was longest on Hed rio grande (35.500 ± 1.340 days) and shortest on Korral (24.290 ± 0.688 days). The intrinsic rate of increase (r_m) ranged from 0.094 ± 0.003 to 0.159 ± 0.002 (day^{-1}), which was lowest on Rio grande UG and highest on Korral. The larvae reared on the leaves of SUN 6108 f1 showed the highest amylolytic activity (0.062 ± 0.00004 mU mg^{-1}), whereas the lowest activity was in the larvae fed on the leaves of Cal JN3 (0.027 ± 0.00004 mU mg^{-1}) and Korral (0.027 ± 0.0001 mU mg^{-1}). The amylolytic activity of larvae fed on the fruits of tomato cultivars was highest on Cal JN3 (0.047 ± 0.0001 mU mg^{-1}). Also, the highest general proteolytic activity of *H. armigera* was in the larvae reared on the leaves of Hed rio grande (3.235 ± 0.004 U mg^{-1}) and fruits of Rio grande UG (2.757 ± 0.135 U mg^{-1}). It could be concluded that Rio grande UG is unsuitable host for the growth and development of *H. armigera*.
Key words: tomato fruit borer, life table, enzyme activity, host plant

چکیده

پارامترهای جدول زندگی و فعالیت آنزیم‌های گوارشی *Helicoverpa armigera* (Lep.: Noctuidae) روی ارقام مختلف گوجه‌فرنگی

مریم نعمتی کلخوران، بهرام ناصری، فروغ رحیمی نمین و داوود کوهی

کرم میوه‌خوار گوجه‌فرنگی، *Helicoverpa armigera* (Hübner)، یکی از آفات جدی انواع محصولات زراعی در ایران و جهان است. تأثیر ارقام مختلف گوجه‌فرنگی (SUN 6108 f1، Rio grande UG، Korral، CH falat، Hed rio grande و Cal JN3) روی پارامترهای جدول زندگی *H. armigera* تحت شرایط آزمایشگاهی (دمای ۱ ± ۲۵ درجه‌ی سلسیوس، رطوبت نسبی ۵ ± ۶۵ درصد و دوره‌ی نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی) و فعالیت برخی از آنزیم‌های گوارشی لاروهای سن ششم این آفت تحت شرایط مزرعه‌ای مورد مطالعه قرار گرفت. طولانی‌ترین دوره‌ی لاروی روی Hed rio grande (۱/۳۴۰ ± ۳۵/۵۰۰ روز) و کوتاه‌ترین آن روی Korral (۰/۶۸۸ ± ۲۴/۲۹۰ روز) بود. نرخ ذاتی افزایش جمعیت (r_m) از ۰/۰۰۳ ± ۰/۰۹۴ تا ۰/۰۰۲ ± ۰/۱۵۹ بر روز در نوسان بود که کم‌ترین آن روی Rio grande UG و بیش‌ترین آن روی Korral بود. لاروهای پرورش‌یافته روی برگ‌های SUN 6108 f1 بیش‌ترین فعالیت آمیلولیتیک را نشان دادند (۰/۰۰۰۰۴ ± ۰/۰۶۲ mU mg^{-1})، درحالی‌که کم‌ترین فعالیت در لاروهای تغذیه‌شده روی برگ‌های Cal JN3 (۰/۰۰۰۰۴ ± ۰/۰۲۷ mU mg^{-1}) و Korral (۰/۰۰۰۱ ± ۰/۰۲۷ mU mg^{-1}) بود. بیش‌ترین فعالیت آمیلولیتیک لاروهای تغذیه‌شده با میوه‌های ارقام گوجه‌فرنگی مربوط به Cal JN3 (۰/۰۰۰۱ ± ۰/۰۴۷ mU mg^{-1}) بود. همچنین، بیش‌ترین فعالیت پروتئولیتیک کل *H. armigera* در لاروهای پرورش‌یافته روی برگ‌های Hed rio grande (۰/۰۰۴ ± ۳/۲۳۵ U mg^{-1}) و میوه‌های Rio grande UG (۰/۱۳۵ ± ۲/۷۵۷ U mg^{-1}) بود. می‌توان چنین نتیجه‌گیری کرد که رقم Rio grande UG نامناسب‌ترین میزبان برای رشد و نمو *H. armigera* می‌باشد.

واژگان کلیدی: کرم میوه‌خوار گوجه‌فرنگی، جدول زندگی، فعالیت آنزیمی، میزبان گیاهی

Introduction

Herbivorous lepidopteran larvae, as one of the most important crop pests, can eat on various parts of host plants and cause substantial economic losses to crop products, as well as influence their quality (Valencia-Jiménez *et al.*, 2008). The tomato fruit borer, *Helicoverpa armigera* (Hübner), is considered as one of the most destructive and cosmopolitan insect pests of many host crops in Iran and other parts of the world. Immatures of *H. armigera* can complete their development on various host plants including tomato, cotton, soybean, bean, chickpea and many others (Farid, 1986; Zalucki *et al.*, 1986; Jallow *et al.*, 2004; Yu *et al.*, 2008). Several greenhouse and field evidences have demonstrated that tomato is a preferred host plant for *H. armigera* (Jallow *et al.*, 2001; Jallow & Matsumura, 2001), and the avoidable loss of 35% is caused by this insect in tomato (Latif *et al.*, 1997). The availability of *H. armigera* to different host plants, high mobility, broad geographical diversity, facultative

diapause, high fecundity potential and trend to develop resistance to synthetic insecticides are the several main factors that supply increasingly to its pest status (Fitt, 1989; McCaffery, 1998).

It is known that the physiological processes and development of insects are affected by various biotic and abiotic factors such as the quality and quantity of food (Johnson *et al.*, 1992; Na & Ryoo, 2000; Musa & Ren, 2005). The larvae of many lepidopteran species can feed on various host plants in order to derive essential nutrients for their optimal development. Protein as the primary component of the insect diets is digested into amino acids by proteases. Also, amylases break down the complex polysaccharides into simple sugars. The synthesis of particular enzymes in herbivorous insects ensure appropriate quality and quantity of the reproductive success (Ishaaya *et al.*, 1971), therefore, any interference in the activity of digestive enzymes by enzyme-inhibitors of host plant can result in poor nutrient utilization and developmental retardation (Jongsma & Bolter, 1997; Gatehouse & Gatehouse, 1999). It is noticeable that various host plants can influence life history traits of the insects such as development, survival and fecundity (Tsai & Wang, 2001; Kim & Lee, 2002). Furthermore, the study of host plant resistance can be considered as an important tool for identifying the antidigestive or antifeedant compounds and their supplementary use in the pest management strategies (Lewis *et al.*, 1997). The life table parameters, particularly the intrinsic rate of increase (r_m), are the most important parameters that can be used to estimate the population growth of insect species under specified conditions (Andrewartha & Birch, 1954; Ricklefs & Miller, 2000). Host plants demonstrating higher values of r_m are more susceptible than those with lower values of r_m. Consequently, the life table parameters were used, in the current research, to compare the potential of population growth of *H. armigera* on different tomato cultivars.

Several studies have hitherto been carried out on the effect of different host plants on biological parameters (Singh & Rembold, 1992; Singh & Mullick, 1997; Kulkarni *et al.*, 2004; Naseri *et al.*, 2009; Soleimannejad *et al.*, 2010; Bagheri *et al.*, 2011) and on digestive enzymes activity of *H. armigera* (Kotkar *et al.*, 2009; Naseri *et al.*, 2010; Fallahnejad-Mojarrad *et al.*, 2011; Hemati *et al.*, 2011). However, no published information exists on the life table and digestive enzymes (amylases and proteases) activity of this species on different tomato cultivars. Consequently, the objective of this study was to investigate the effect of different tomato cultivars on life table parameters of *H. armigera* under laboratory conditions, as well as the effect of leaves and fruits of the examined cultivars on its digestive enzymes activity under field conditions to evaluate susceptibility or resistance of tomato cultivars to this pest. Our findings may provide useful information for designing comprehensive pest management strategies against *H. armigera*.

Materials and methods

Tomato sources

Seeds of the six tomato, *Lycopersicon esculentum* Mill, cultivars including SUN 6108 f1, Rio grande UG, Korral, CH falat, Hed rio grande and Cal JN3 were obtained from Plant and Seed Improvement Research Institute (Karaj, Iran) and were planted in the research field of University of Mohaghegh Ardabili (Ardabil, Iran) in May 2011. The research was initiated when tomato cultivars reached to the reproductive stage. For this study, the young leaves and green equal-size of terminal fruits of different tomato cultivars were transferred to a growth chamber at 25 ± 1 °C, 65 ± 5% RH and a photoperiod of 16: 8 (L: D) hours. The experiments were conducted during the morning to afternoon on mid-July to mid-September 2011.

Insect rearing

The eggs of *H. armigera* were achieved from a laboratory colony maintained on a cowpea-based artificial diet, as described by Teakle (1991), from Tarbiat Modares University (Tehran, Iran). The insects tested on different tomato cultivars had already been reared for two generations on the same cultivars. All

experimental insects were maintained inside a growth chamber at 25 ± 1 °C, 65 ± 5% RH and a photoperiod of 16: 8 (L: D) hours. Tomato cultivars leaves were used for feeding of first and second larval instars and the green fruits were used for feeding of the third to sixth larval instars (Green *et al.*, 2002; Naseri *et al.*, 2009).

Development time

Fifty eggs of *H. armigera* (within 12 hours) were taken from the adult moths, which had already been reared for two generations on each tomato cultivar under laboratory conditions. After hatching, neonate larvae were transferred with a fine camel hair brush individually into plastic Petri dishes (8 cm in diameter by 2 cm in height) with a hole covered by a fine mesh net for ventilation, containing the fresh leaves of each tomato cultivar. The petioles of detached leaves were inserted in water-soaked cotton to retain freshness. The leaves and fruits of each tomato cultivar were replaced with new ones every day, and observations were recorded daily for the mortality/survival of larvae in the same instar or moulting to next instar up to pupation and emergence of adult. Sixth instar larvae were kept in plastic containers (3 cm in diameter by 5 cm in height) for pre-pupation and pupation.

After emergence of adult moths, a pair of female and male moths was transferred to oviposition container (11.5 cm in diameter by 9.5 cm in height), 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. The number of deposited eggs was counted daily. To supply a source of carbohydrate for adult feeding, a small cotton wick soaked in 10% honey solution was inserted into the oviposition containers.

Larval (sixth and whole larval instars), pre-pupal, pupal and immature periods and their mortality were recorded on different tomato cultivars. Also, every pupa was weighed 24 hours after pupation. In this research, the larval growth index (*LGI*), standardized insect-growth index (*SII*) and fitness index (*FI*) of *H. armigera* were calculated on different tomato cultivars using following formulae (Pretorius, 1976; Itoyama *et al.*, 1999): $LGI = l_x / L$; $SII = P_w / L$; $FI = (P \times P_w) / (L + P_d)$; where l_x = survival rate of larvae; L = larval period; P = percentage of pupation; P_d = pupal period; and P_w = pupal weight.

Life table parameters

Age-specific survival rate (l_x) and fecundity (m_x) of *H. armigera* on different tomato cultivars were calculated according to Carey (2001). Life table parameters including 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 & Henderson, 2000) for *H. armigera* on different tomato cultivars were estimated.

Digestive enzyme activity - Chemicals

All enzyme substrates, Bradford reagent and the dinitrosalicylic acid (DNS) were acquired from Sigma Chemical Co., St Louis, USA. Bovine serum albumin (BSA) was purchased from Roche Co., Germany.

Preparation of digestive enzymes

About 50 neonate larvae were reared on leaves and fruits of each examined cultivar until the sixth instar under laboratory conditions. Sixth instar larvae of *H. armigera* were transferred to tomato field and reared on leaves and fruits of each related tomato cultivar for 24 hours. Sixth instar larvae were collected from field after 24 hours and immediately anesthetized on ice and dissected under a stereoscopic microscope in the laboratory. The midguts adhering of unwanted tissues were 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, stored at -20 °C in aliquots for further use (Hosseininaveh *et al.*, 2007).

Amylolytic activity assay

Amylolytic activity in crude homogenates of midgut extracts from sixth instar larvae of *H. armigera*

was assayed by the dinitrosalicylic acid (DNS) method (Bernfeld, 1955), with 1% soluble starch as substrate at the optimal pH. The universal buffer system (10 mM succinate-glycine-2, morpholinoethan sulfonic acid) was used to assess the optimal pH of amylolytic activity over a pH range of 2-12. A quantity of 20 µL of the enzyme extract (0.841 mg mL^{-1}) was incubated with 500 µL of universal buffer and 40 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 a boiling water bath for 10 min. The absorbance was read at 540 nm (using spectrophotometer, JENWAY 6705 UV/Vis, USA) after cooling on ice. Unit activity was characterized as the amount of enzyme required to release 1 mg of maltose in 30 min at 37 °C under the given assay conditions. All experiments were carried out in triplicates.

Proteolytic activity assay

General proteolytic activity present in the midgut of *H. armigera* sixth instar larvae fed on the leaves and fruits of different tomato cultivars were determined using azocasein substrate over a pH range of 7-12. The universal buffer system (50 mM sodium phosphate-borate) was used to assay the optimal pH of proteolytic activity in the midgut (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 7 to 12) and 50 µL of crude enzyme was incubated at 37 °C for 50 min. The reaction was stopped by the addition of 100 µL of 30% trichloroacetic acid (TCA) and continued by cooling at 4 °C for 30 min and centrifugation at 16000 × g for 10 min. A quantity of 100 µL of the supernatant was added to 100 µL of 2 M NaOH 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. One unit of protease activity was defined as an increase in optical density mg^{-1} protein of the tissue min^{-1} due to azocasein proteolysis (Elpidina *et al.*, 2001). All experiments were carried out in triplicates.

Protein quantification of larvae

General protein concentrations in the midgut of sixth instar larvae of *H. armigera* fed on leaves and fruits of tomato cultivars were determined using bovine serum albumin (BSA) as a standard according to the method of Bradford (1976).

All data were analyzed by one-way ANOVA followed by comparison of the means with LSD test at $\alpha = 0.05$ using statistical software Minitab 16.0. All data were tested for normality before analysis.

Results

Survival and fecundity

Age-specific survival rate (l_x) and fecundity (m_x) of *H. armigera* on different tomato cultivars are shown in fig. 1. The survival rate of individuals developed to adults from the initial cohort stage was estimated 0.64, 0.18, 0.84, 0.24, 0.22 and 0.70 on SUN 6108 f1, Rio grande UG, Korral, CH falat, Hed rio grande and Cal JN3, respectively. The results of the current study showed that the death of the last female on the mentioned tomato cultivars occurred in the age of 58, 59, 62, 56, 61 and 55 days, respectively (fig. 1). The oviposition beginning of the first female on the examined cultivars (the same order mentioned above) occurred in the age of 37, 49, 41, 47, 50 and 38 days, respectively. The highest daily fecundity (m_x) of *H. armigera* adults emerged from the larvae reared on these cultivars was 317, 172, 330, 108, 185 and 57 females/female/day, and occurred in the age of 48, 51, 47, 53, 51 and 38 days, respectively.

Development time

The results of the effect of different tomato cultivars on larval, pre-pupal and pupal periods, as well as the development time of immature stages of *H. armigera* are shown in table 1. Although the incubation period of *H. armigera* was not significantly different on various tomato cultivars, the larval period ($F = 8.43$; df = 5, 80; $P < 0.01$) was longest on Hed rio grande (35.500 ± 1.340 days) and shortest on Korral (24.290 ± 0.688 days).

In this research, the sixth instar larval period of *H. armigera* (F = 2.47; df = 5, 87; $P < 0.05$) was longest on Rio grande UG (9.200 ± 0.975 days) and shortest on Korral (6.684 ± 0.626 days) (table 1). The results showed that pre-pupal and pupal period was not significantly different on six tomato cultivars. Duration of immature stages (F = 5.85; df = 5, 39; $P < 0.01$) was longest on Hed rio grande (51.670 ± 2.330 days) and shortest on Korral (43.250 ± 0.834 days) (table 1).

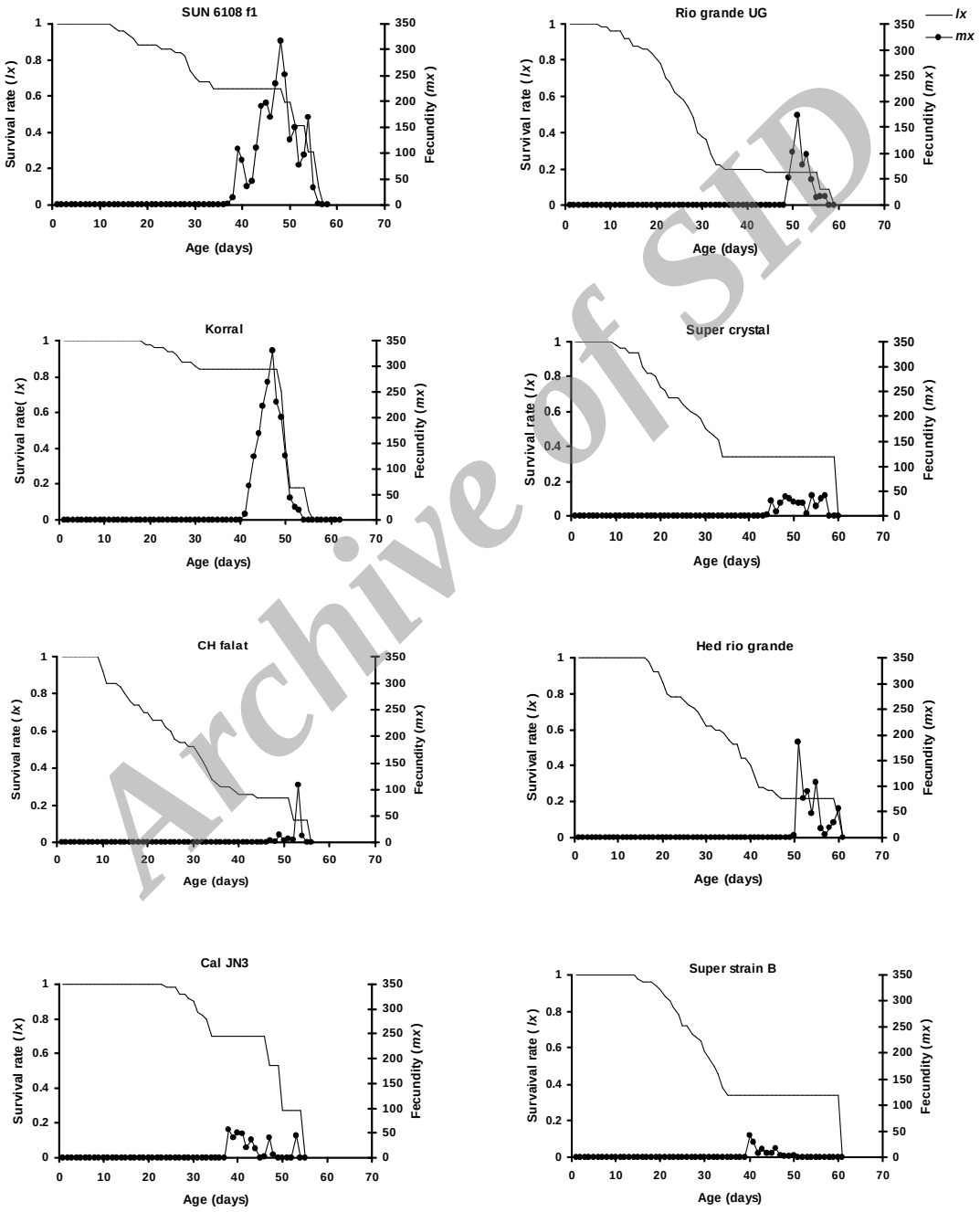

Fig. 1. Age-specific survival rate (*lx*) and fecundity (*mx*) of *Helicoverpa armigera* fed on different tomato cultivars under laboratory conditions.

Table 1. The mean (± SE) duration of immature stages (days) of *Helicoverpa armigera* reared on different tomato cultivars under laboratory conditions.

Cultivar	Incubation	Sixth instar larval period	Whole larval period	Pre-pupal period	Pupal period	Immature stages
SUN 6108 f1	3.00a	7.227 ± 0.456bc*	25.842 ± 0.876e	3.222 ± 0.236a	12.667 ± 0.310a	44.080 ± 1.120b
Rio grande UG	3.00a	9.200 ± 0.975a	29.200 ± 1.680d	3.250 ± 0.250a	13.600± 0.510a	48.750 ± 0.854a
Korral	3.00a	6.684 ± 0.626c	24.290 ± 0.688f	3.318 ± 0.232a	13.333 ± 0.222a	43.250 ± 0.834b
CH falat	3.00a	8.333 ± 0.620abc	30.000 ± 1.440c	3.714 ± 0.286a	12.750 ± 0.479a	48.000 ± 1.960a
Hed rio grande	3.00a	9.067 ± 0.643a	35.500 ± 1.340a	3.500 ± 0.327a	12.333 ± 0.333a	51.670 ± 2.330a
Cal JN3	3.00a	8.600 ± 0.815ab	32.200 ± 1.470b	3.273 ± 0.273a	13.429 ± 0.369a	50.170 ± 2.070a

The means followed by different letters in the same column are significantly different ($P < 0.01$ and $P < 0.05^*$, LSD).

Growth indices

Growth indices of *H. armigera* on different tomato cultivars are shown in fig. 2. The results showed that the highest and lowest values of the larval growth index were respectively on Korral and CH falat. The standardized insect-growth index of *H. armigera* showed significant difference (F = 5.88; df = 5, 57; $P < 0.01$) among tomato cultivars, being highest on Korral and lowest on Super strain B (fig. 2). Also, the results showed that different tomato cultivars as larval food had a significant effect (F = 70.68; df = 5, 34; $P < 0.01$) on the fitness index of this pest, which was highest on Korral and lowest on Super crystal and Super strain B (fig. 2).

Life table parameters

The net reproductive rate (R_0) of *H. armigera* on Korral (374.600 ± 27.400 female/female/generation) was longer than the other tomato cultivars (F = 18.96; df = 5, 26; $P < 0.01$). The intrinsic rate of increase (r_m) ranged from 0.094 ± 0.003 to 0.159 ± 0.002 (day^{-1}), which was lowest on Rio grande UG and highest on Korral (F = 35.49; df = 5, 26; $P < 0.01$). Furthermore, the finite rate of increase (λ) value of this pest showed significant differences (F = 34.60; df = 5, 26; $P < 0.01$), being lowest on Rio grande UG (1.098 ± 0.003 day^{-1}) and highest on Korral (1.173 ± 0.002 day^{-1}) (table 2). Among different tomato cultivars, the mean generation time (T) was longest on Hed rio grande (45.036 ± 0.441 days) and shortest on Cal JN3 (33.567 ± 0.445 days) (F = 83.49; df = 5, 26; $P < 0.01$). Moreover, the doubling time (DT) value of *H. armigera* on Rio grande UG was longer (7.392 ± 0.216 days) than the other cultivars (F = 46.55; df = 5, 26; $P < 0.01$).

Amylolytic activity of *H. armigera*

Amylolytic activity in midgut extracts from *H. armigera* sixth instar larvae reared on the leaves (F = 69973.57; df = 5, 12; $P < 0.01$) and fruits (F = 14778.57; df = 5, 12; $P < 0.01$) of various tomato cultivars under field conditions is indicated in fig. 3. The sixth instar larvae reared on the leaves of SUN 6108 f1 (0.062 ± 0.00004 mU mg^{-1}) showed the highest levels of amylolytic activity, whereas the lowest activity was in the larvae reared on the leaves of Cal JN3 (0.027 ± 0.00004 mU mg^{-1}) and Korral (0.027 ± 0.0001 mU mg^{-1}). The results demonstrated that the highest amylolytic activity of *H. armigera* sixth instar larvae fed on the fruits of different tomato cultivars was on Cal JN3 (0.047 ± 0.0001 mU mg^{-1}), whereas the lowest activity was in the larvae reared on SUN 6108 f1 (0.009 ± 0.0002 mU mg^{-1}) and CH falat (0.009 ± 0.0001 mU mg^{-1}).

Proteolytic activity of *H. armigera*

The general proteolytic activity data in midgut extracts from *H. armigera* sixth instar larvae reared on the leaves (F = 96.68; df = 5, 6; $P < 0.01$) and fruits (F = 108.37; df = 5, 12; $P < 0.01$) of different tomato cultivars under field conditions is shown in fig. 3. Proteolytic activity of *H. armigera* was the highest in the larvae reared on the leaves of Hed rio grande (3.235 ± 0.004 U mg^{-1}) and lowest in the larvae fed on Korral (0.940 ± 0.005 U mg^{-1}). The highest proteolytic activity of sixth instar larvae reared on the fruits of

different tomato cultivars was on Rio grande UG (2.757 ± 0.135 U mg^{-1}) and the lowest was on Korral (0.945 ± 0.004 U mg^{-1}).

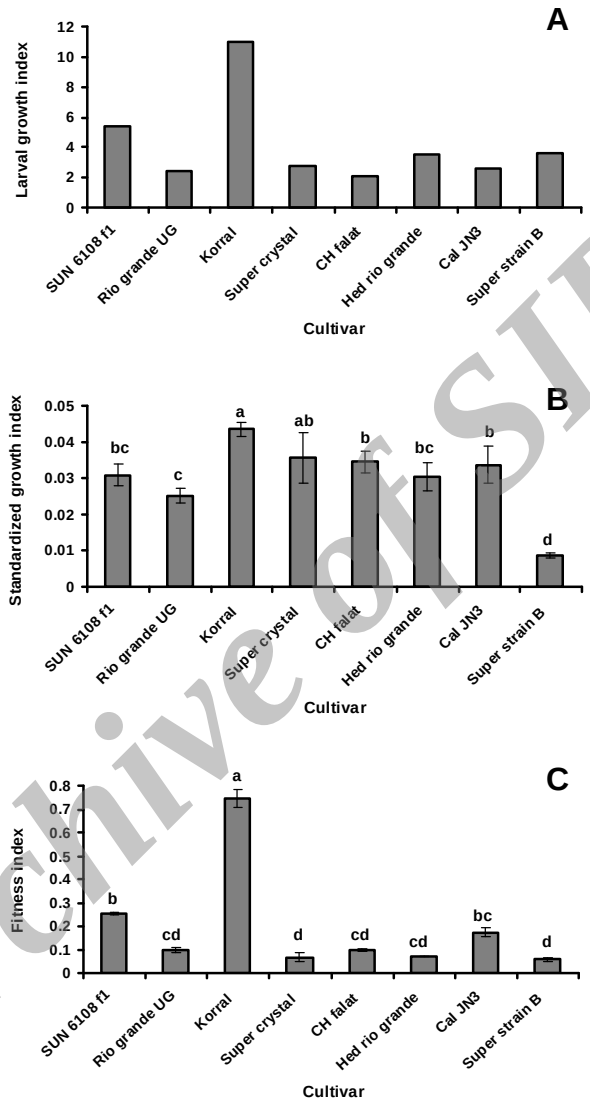

Fig. 2. The larval growth (A), standardized insect-growth (B) and fitness (C) indices of *Helicoverpa armigera* on different tomato cultivars under laboratory conditions.

Table 2. Life table parameters of *Helicoverpa armigera* reared on different tomato cultivars under laboratory conditions.

Cultivar	Parameter (mean ± SE)				
	R_0	r_m (day^{-1})	λ (day^{-1})	T (day)	DT (day)
SUN 6108 f1	169.100 ± 31.100b	0.144 ± 0.005b	1.155 ± 0.006b	35.715 ± 0.311e	4.803 ± 0.167b
Rio grande UG	52.680 ± 5.080c	0.094 ± 0.003c	1.098 ± 0.003c	42.331 ± 0.286b	7.392 ± 0.216a
Korral	374.600 ± 27.400a	0.159 ± 0.002a	1.173 ± 0.002a	37.234 ±0.533d	4.353 ± 0.057b
CH falat	54.410 ± 5.140c	0.099 ± 0.003c	1.105 ± 0.003c	40.235 ± 0.246c	6.966 ± 0.187a
Hed rio grande	77.700 ± 17.800c	0.097 ± 0.004c	1.102 ± 0.005c	45.036 ± 0.441a	7.114 ± 0.317a
Cal JN3	117.200 ± 37.100bc	0.144 ± 0.008b	1.154 ± 0.009b	33.567 ± 0.445f	4.811 ± 0.278b

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

Discussion

In the current research, the survival rate of *H. armigera* had various trend on the six tomato cultivars. The survival rate of individuals developed to adults from the initial cohort varied from 0.18 on Rio grande UG to 0.84 on Korral. According to the reports by Fathipour & Naseri (2011) and Arghand (2011), this value respectively ranged from 0.72 to 0.96 on soybean varieties, and 0.36 to 0.56 on corn hybrids. This variation can be due to differences of host plant species or different plant parts consumed by the larvae that may have very diverse primary and secondary chemicals.

The incubation period of *H. armigera* showed no significant difference among tomato cultivars tested, indicating that this parameter was not affected by the host plant cultivar. Our results for the incubation period of *H. armigera* on different tomato cultivars (3.00 days) are in agreement with those reported by several authors (Jallow & Matsumura, 2001; Borah & Dutta, 2002; Naseri *et al*., 2009; Arghand, 2011).

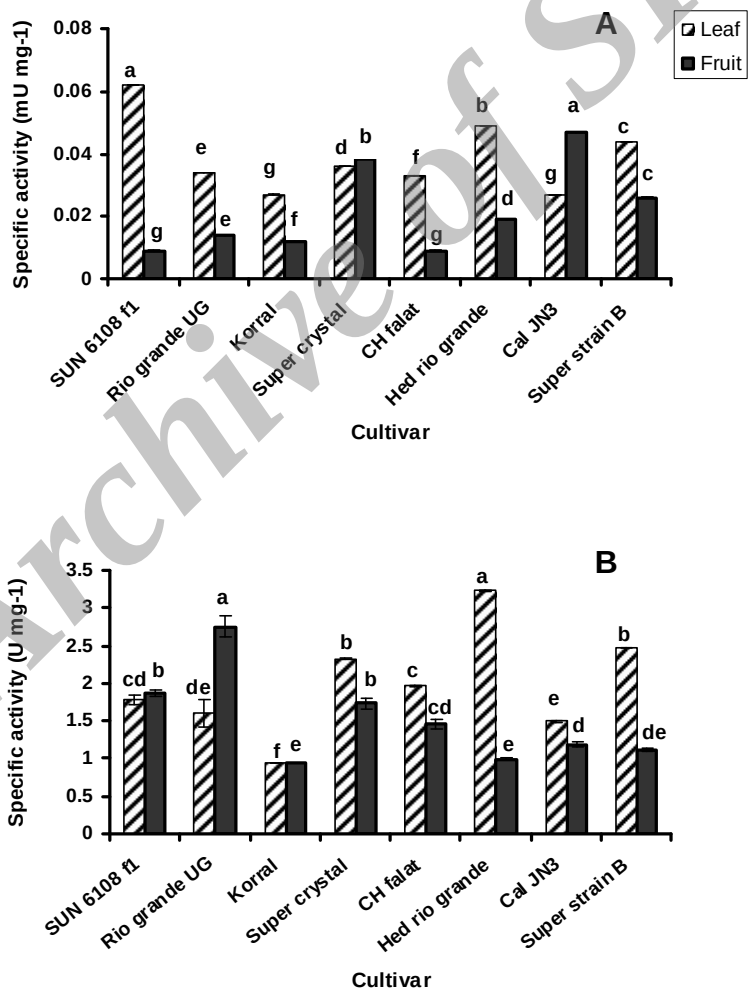

Fig. 3. Amylolytic (A) and general proteolytic (B) activity of midgut extracts from *Helicoverpa armigera* sixth instar larvae reared on leaves and fruits of different tomato cultivars under field conditions using soluble starch 1% (pH 10) and azocasein 1.5% (pH 12) as substrate, respectively. Bars represent means of three independent estimations associated with standard error. The means followed by different letters are significantly different (LSD, $P < 0.01$).

In this study, there were six larval instars of *H. armigera* on all examined tomato cultivars. This situation has been previously reported by several authors (Jones *et al.*, 1981; Goyal & Rathore, 1988; Borah & Dutta, 2002; Arghand, 2011). However, Lokar *et al.* (1993), Saour & Causse (1996) and Fathipour & Naseri (2011) reported that larval stages of *H. armigera* are completed in five instars. These variations might be due to either the differences in geographic population of *H. armigera*, or variations in the nutritional quality and quantity of the host plant species (Poitout & Cayrol, 1969; Nadgauda & Pitre, 1983; Bernays & Chapman, 1994).

There was significant difference in the development of larval stages reared on each tomato cultivar. The results showed that the mean larval period of *H. armigera* was 39.505 ± 1.249 days on six tomato cultivars. According to Fathipour & Naseri (2011) and Arghand (2011), the larval period of *H. armigera* was 20.652 ± 0.865 days on soybean cultivars, and 21.800 ± 0.956 days on corn hybrids, respectively, indicating that soybean and corn are more suitable host plants for *H. armigera* larvae than tomato. It is noticeable that the type of host plant, genetic variations and different geographic populations of the insect can affect the duration of larval stage in *H. armigera* (Fathipour & Naseri, 2011).

In the current research, the development time of immature stages showed a significant difference among the tomato cultivars, with values ranging from 43.250 ± 0.834 days on Korral to 51.670 ± 2.330 days on Hed rio grande UG (table 1). Liu *et al.* (2004) reported that the development time of *H. armigera* was 32.91 ± 0.55, 33.91 ± 0.61 and 35.07 ± 0.27 days on tobacco, hot pepper and tomato, respectively. Results present here confirm that the tomato cultivar tested by Liu *et al.* (2004) was more suitable host plant for the growth and development of *H. armigera* than the cultivars of tomato tested in our study. According to Fathipour & Naseri (2011) and Arghand (2011), the development time of *H. armigera* was 35.185 ± 0.960 days on soybean cultivars and 39.184 ± 0.792 days on corn hybrids, respectively. It suggests that the tomato cultivars examined in the current study may be more unsuitable host plants for *H. armigera* than soybean and corn. In comparison with other studies (Coaker, 1959; Cowgill & Lateef, 1995; Fathipour & Naseri, 2011), there was no significant effect of larval food on the pre-pupal and pupal period of *H. armigera*.

In this study, the highest and lowest values of larval growth index of *H. armigera* were on Korral (11.013) and CH falat (2.133), respectively. However, Fathipour & Naseri (2011) and Arghand (2011) reported that the lowest larval growth index was 2.68 on soybean (cultivar L17), and 1.54 on corn (hybrid DC370), respectively.

The r_m value of *H. armigera* estimated in the current research ranged from 0.094 ± 0.003 to 0.159 ± 0.002 day^{-1}, which was minimum on Rio grande UG and maximum on Korral. The higher r_m value of *H. armigera* on Korral was due to the greater fecundity, lower mortality and shorter development time of the pest fed on this cultivar. However, lower r_m value on Rio grande UG was mainly a result of the lower fecundity and survivorship, as well as the longer development time of *H. armigera* on this cultivar. The intrinsic rate of increase for *H. armigera* was estimated 0.155 on soybean (Fathipour & Naseri, 2011) and 0.142 on pearl millet (Patal & Koshyia, 1997). Some probable reasons for these variations are due to physiological differences depending on the type of the host plant and genetic differences in geographic populations of the pest. A high value of r_m shows the susceptibility of a host plant to insect feeding, while a low value demonstrates that the host plant species is resistant to the pest. So, since some tomato cultivars including Korral and SUN 6108 f1 were susceptible hosts, *H. armigera* had the greatest chance to increase its population on these cultivars. However, Rio grande UG was more unsuitable host plant, suggesting its partial resistance to *H. armigera* compared to the other cultivars. The finite rate of increase was 1.173 ± 0.002 day^{-1} on Korral, which is nearly similar to that reported by Fathipour & Naseri (2011) on soybean (cultivars

Williams, DPX and M4). In the current research, the lowest net reproductive rate (R_0) and longest doubling time (*DT*) of *H. armigera* was on Rio grande UG. The doubling time varied from 4.353 ± 0.057 days on Korral to 7.392 ± 0.216 days on Rio grande UG. Fathipour & Naseri (2011) noted that the shortest doubling time of *H. armigera* was 3.750 days on soybean, cultivar M9.

It is known that the variations in climatic conditions, especially temperature and relative humidity, can affect the feeding performance and digestive enzymes activity of insects. Furthermore, study of ecophysiological aspects of insect pests under field conditions gives us a better understanding and rational insight for planning and developing strategies to control of the insect pests. Therefore, to gain more practical information in the current study, the activity of the two key digestive enzymes (α-amylase and protease) of *H. armigera* sixth instars larvae was also evaluated under field conditions. Digestive enzymes activity of insects depends on either the quality of food sources or consumed chemical compounds and enzyme-inhibitors (Slansky, 1982; Mendiola-Olaya *et al.*, 2000). It was previously reported that the insects adapt to plant enzyme-inhibitors by different ways such as producing inhibitor-insensitive, inhibitor-resistant and inhibitor declining enzymes in their midgut (Broadway, 1997; Girard *et al.*, 1998). Because the polyphagous insects are demonstrated to be more adaptive to different types of inhibitors, this provides an idea of the complexity of the digestive enzymes secreted by *H. armigera* in response to the different host plants cultivars (Brito *et al.*, 2001; Kotkar *et al.*, 2009). The highest amylolytic activity of *H. armigera* sixth instar larvae fed on leaves of SUN 6108 f1 and fruits of Cal JN3, respectively showed 5.5 and 7 fold lower than those reported for amylolytic activity of *H. armigera* on white kidney bean Dehghan (Hemati *et al.*, 2011). The lower amylolytic activity of this pest on tomato may be attributed to the lower starch contents of tomato in comparison with white kidney bean. It seems that there could be some enzyme-inhibitors in the leaves and fruits of the above-mentioned tomato cultivars, which should be investigated in future studies. Moreover, the lowest amylolytic activity of *H. armigera* sixth instar larvae fed on the fruits of SUN 6108 f1 showed nearly 10 fold lower than those reported for amylolytic activity of fifth instar larvae of *H. armigera* on tomato Meshkin (Hemati *et al.*, 2011), while it was nearly similar to those reported by Kotkar *et al.* (2009) for amylolytic activity of *H. armigera* on tomato. Some possible reasons for such disagreement might be because of either physiological differences of tomato cultivars or variation in examined larval instar of *H. armigera*.

Although the proteolytic activity of *H. armigera* larvae fed on leaves of Hed rio grande showed approximately 2.5 fold lower activity than those fed on white kidney bean Dehghan (Hemati *et al.*, 2011), it was similar to the proteolytic activity of *H. armigera* larvae fed on tomato Meshkin (Hemati *et al.*, 2011), indicating that the protein content in tomato is lower than that in bean (Kotkar *et al.*, 2009). The lowest proteolytic activity of *H. armigera* sixth instar larvae fed on fruits of Korral showed nearly six fold higher than those reported by Kotkar *et al.* (2009) for proteolytic activity of *H. armigera* larvae on tomato. Possible reasons for this discrepancy might be because of either variation in examined larval instars or physiological differences of tomato cultivars. Also, the value of proteolytic activity of *H. armigera* on fruits of Rio grande UG was similar to that reported by Naseri *et al.* (2010) on cowpea-based artificial diet. Within different tomato cultivars, a higher general proteolytic activity in the sixth instar larvae fed on leaves of Hed rio grande, may be attributed to the variations in either protein content or to the response of the insect to eaten enzymes-inhibitors of the diet (Broadway & Duffy, 1986).

In the current research, lowest levels of the amylolytic and proteolytic activity of *H. armigera*, as well as the shortest development time of sixth instar larvae, and highest r_m and R_0 values were observed in *H. armigera* reared on Korral, indicating that this

cultivar was a suitable host plant for development and population increase of *H. armigera*. The highest proteolytic activity of *H. armigera*, as well as the longest sixth instar larval period and lowest r_m and R_0 values were on Rio grande UG, suggesting that this cultivar was a partially resistant host against *H. armigera*.

For a better understanding of the insect-plant interaction to control *H. armigera* on different tomato cultivars, additional study will be required to determine demographic parameters of the pest under semi-field and field conditions. Also, the identification and extraction of secondary biochemicals of resistant tomato cultivars will greatly help to design useful strategies for the management of this pest.

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