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Research article

Adaptation of *Habrobracon hebetor* (Hymenoptera: Braconidae) to Rearing on *Ephestia kuehniella* (Lepidoptera: Pyralidae) and *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

Food characteristics strongly regulate digestive enzymatic activity of insects through direct influences on their midgut mechanisms. Insect performance is better on diets that contain nutrients in proportions that fit its digestive enzymes. Little is known about the influences of rearing history on parasitism success of *Habrobracon hebetor* Say. This research focused on the effect of nutrient regulation on survival, development, and parasitism of *H. hebetor*. Life history and digestive enzyme activity of fourth-stage larvae of *H. hebetor* were studied when reared on *Ephestia kuehniella* Zeller. This parasitoid was then introduced to *Helicoverpa armigera* (Hübner), and above-mentioned parameters were also studied in the first and fourth generations after transfer. In term of parasitism success, *H. hebetor* preferred *E. kuehniella* over *He. armigera*. When the first and fourth generations of *He. armigera*-reared *H. hebetor* were compared, the rearing history affected the life history and enzymatic activity of the parasitoid. A better performance of *H. hebetor* was achieved after it was reared on *He. armigera* for the four generations. Because, digestive α-amylase and general protease of the parasitoid were matched with the new host, it used reserve energy for a better performance. Thus, a better performance of *H. hebetor* could be obtained when the parasitoid was reared on its original host for at least four generations.

Key words: *Habrobracon hebetor*, biocontrol, rearing history, digestive physiology

Parasitoids play a key role in integrated pest management programs due to their capability to keep pest populations under economic thresholds (Belda and Riudavets 2013). *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is a polyphagous and gregarious ectoparasitoid that can parasitize the larvae of some Lepidoptera species, such as *Ephestia kuehniella* (Zeller) (Pyralidae), *Ectomyelois ceratoniae* (Zeller) (Pyralidae), *Plodia interpunctella* (Hübner) (Pyralidae), and *Helicoverpa armigera* (Hübner) (Noctuidae) (Benson 1974, Kovalenkov 1984, Nay and Perring 2005, Altuntas et al. 2010). It is suggested as one of the best natural enemies of lepidopteran pests (Chen et al. 2013), which can be used in their management (Brower et al. 1996).

Several factors in a parasitoid's host, including physiological conditions and species of host, as well as the type of host diet, can affect the development of the parasitoid (Mironidis and Savopoulou-Soultani 2009). When host diet is suitable for the parasitoids' larvae, they will reach the adult stage earlier and will be larger (Dmitriew and Rowe 2011). The morphophysiological traits of adult parasitoids depend on the host quality that their larvae are able to achieve (Harvey et al. 2004, Jervis et al. 2008). Also, in a multitrophic level, the quality of diets can flow up to upper trophic levels, affecting the biological traits (Karimzadeh et al. 2013) and digestive physiology (Karasov et al. 2011) of natural enemies. As a result, the dietary source that a parasitoid fed on is determinant in its foraging behavior and population dynamics (King 1987, Cicero et al. 2011). Therefore, it is required to find the information regarding the physiologically mediated parasitoid–host relationships to have successful production of biocontrol agents (Saadat et al. 2014b). Due to the wide host range, several studies have focused on the effect of quantity and quality of host on ecology and life history of *H. hebetor* (Yu et al. 2003, Magro et al. 2006, Ghimire and Phillips 2010, Isitan et al. 2011, Saadat et al. 2014a). However, in spite of the importance of *H. hebetor* adaptation to rearing on laboratory hosts in a successful biological control program, no information is available regarding the effects of such adaptation on life-history parameters of *H. hebetor*. Only, a paper was published

concerning the developmental time of *H. hebetor* reared on different lepidopteran host species and its relationship with digestive enzymes (Saadat et al. 2014b). Therefore, this study is the first attempt about the adaptation of *H. hebetor* to rearing on *E. kuehniella* and *He. armigera* and its effect on life history and digestive physiology of this parasitoid under laboratory conditions. As *E. kuehniella* larvae, which feed on stored-products rich in carbohydrate, provide physiological conditions that are more suitable for *H. hebetor* than *He. armigera* larvae (Saadat et al. 2014b), it is hypothesized that *E. kuehniella* was superior to *He. armigera* for mass rearing of this parasitoid.

In this research, the biology and digestive enzyme activity of the fourth-stage larvae of *H. hebetor* were studied when they were fed on *E. kuehniella*. After that, this parasitoid was introduced to *He. armigera* and its biology and digestive enzymes activity were studied in the first and fourth generations. Consequently, the success of this parasitoid after changing of host was also studied.

Materials and Methods

Insect Host Rearing

Mediterranean flour moth, *E. kuehniella*, was obtained from infested wheat flour in the laboratory, and the cotton bollworm, *He. armigera* was originally collected from infested tomato fields near Karaj, Alborz Province, Iran. Mediterranean flour moth was reared on a wheat flour, rolled oats, and yeast (6:3:1, wt/wt/wt) at 29 ± 1°C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h. Larvae of *He. armigera* were reared on tomato leaves (first and second instars) and fruits (third to fifth instars) at 25 ± 1°C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h in a growth chamber (Saadat et al. 2014b).

Parasitoid Rearing

A laboratory stock colony of *H. hebetor* was established from individuals collected from tomato fields near Karaj, Alborz Province, Iran. The parasitoids were reared on fifth-stage larvae of *E. kuehniella* for three generations. After establishing proper population on *E. kuehniella*, adult parasitoids were transferred to *He. armigera* and reared at 25 ± 1°C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h in a growth chamber for three generations for possess to population reared on each host.

Developmental Time

To study the developmental time of the parasitoid, the 50 parasitoids (48- to 72-h-old parasitoid) that reared on *E. kuehniella* for three and six generations, and those reared on *He. armigera* for three generations were collected. After that, 200 fifth-stage larvae of each moth were introduced to parasitoids to parasitize the host for 24 h. Then, from each species, 50 parasitized larvae were placed in individual Petri dishes (6 cm in diameter, 1 cm in depth) and left in a growth chamber at 25 ± 1°C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h. Petri dishes were visited daily, and the duration of immature stages and adult longevity were recorded for *H. hebetor* reared on *E. kuehniella* in fourth and seventh generations, and those reared on *He. armigera* in once and fourth generations.

Survival, Realized Fecundity, and Egg Fertility

To determine the number of eggs laid by female *H. hebetor* reared on *E. kuehniella* in the fourth and seventh generations and those reared on *He. armigera* in the first and fourth generations, we released one pair of newly emerged *H. hebetor* into a glass tube cage (4.5 cm in diameter, 9.0 cm in height) with 20 *E. kuehniella* and *He. armigera* fifth-stage larvae, separately. They were allowed 24 h for parasitization, after which they were transferred to a new Petri dish. The Petri dishes were checked daily, and all newly laid eggs were counted and recorded for *H. hebetor* reared on *E. kuehniella* in the fourth and seventh generations and those reared on *He. armigera* in the first and fourth generations. All eggs collected in this study were maintained for at least 10 d to determine the percentage of hatch (fertility).

Enzyme Assay

Enzymes Preparation

The enzyme preparation was done according to the methods of Borzoui and Bandani (2014), and the supernatants were stored at −20°C as an enzyme source for subsequent analysis.

Amylolytic and Proteolytic Activity Assay

The dinitrosalicylic acid method (Bernfeld 1955) was used to assay the digestive amylolytic activity of *H. hebetor* larvae fed on the two host species. All experiments were replicated three times.

The general proteolytic activity was assayed according to Elpidina et al. (2001) and Gatehouse et al. (1999).

In-Gel α-Amylase and Protease Assay

The amylolytic activity in the gel was detected using the method of Kazzazi et al. (2005), and the proteolytic activity in the gel was detected using the methods described by Laemmli (1970) and Saadati et al. (2011).

Data Analysis

Before the analysis, all data were examined for normality using the Kolmogorove–Smirnov test. Life history and enzymatic activity of *H. hebetor* reared on two hosts and different generations were analyzed based on a factorial design using two-way analysis of variance (ANOVA; PROC GLM; SAS 2003). Statistical differences among the means were compared using Tukey's test at $\alpha = 0.05$.

Results

Developmental Time

Braconid wasp, *H. hebetor*, indicated a significant difference in developmental time of immature stages (from egg to adult stage) on two hosts and different generations ($F = 24.48$; df = 3, 126; $P < 0.01$). *H. hebetor* had the longest developmental time of immature stages on *He. armigera* in the first generation after transfer (Table 1). The incubation period was longest in the parasitoids reared for four generations on *He. armigera* (Table 1), which was significantly different from other treatments ($F = 3.59$; df = 3, 171; $P < 0.01$). Also, the larvae developed more slowly on *He. armigera* in the first generation after transfer ($F = 18.16$; df = 3, 154; $P < 0.01$). Similarity, pupal period ($F = 13.35$; df = 3, 126; $P < 0.01$) was longest in the larvae reared on *He. armigera* in the first generation after transfer. By contrast, prepupal period was not significantly different on various treatments ($F = 0.62$; df = 3, 143; $P = 0.6$). The two hosts showed a significant effect on the longevity of male ($F = 10.22$; df = 3, 56; $P < 0.01$) and female ($F = 48.14$; df = 3, 66; $P < 0.01$) of *H. hebetor*. The larvae reared for one generation on *He. armigera* had the shortest male longevity. The records for the longest longevity of female *H. hebetor* were for the larvae reared on *E. kuehniella* for four and seven generations. By contrast, the

Table 1. Mean (±SE) duration (days) of immature stages and longevity of *H. hebetor* reared on two lepidopteran host species in different generations

	Generation	Incubation period	Larval period	Prepupal period	Pupal period	Immature period	Male longevity	Female longevity
H. hebetor reared on *E. kuehniella*	Fourth generation	2.31 ± 0.07 b	5.09 ± 0.11 b	0.90 ± 0.01 a	6.42 ± 0.10 b	14.71 ± 0.17 c	7.38 ± 0.13 a	25.84 ± 0.31 a
	Seventh generation	2.43 ± 0.08ab	5.00 ± 0.09 b	0.87 ± 0.01 a	6.51 ± 0.09 b	14.76 ± 0.15 c	7.21 ± 0.12 a	25.48 ± 0.31 a
H. hebetor reared on *He. armigera*	First generation	2.38 ± 0.09ab	6.21 ± 0.09 a	0.88 ± 0.01 a	7.63 ± 0.11 a	17.05 ± 0.18 a	5.60 ± 0.14 b	14.85 ± 0.23 c
	Fourth generation	2.73 ± 0.09 a	5.38 ± 0.10 b	0.90 ± 0.01 a	6.87 ± 0.13 b	15.99 ± 0.15 b	6.55 ± 0.13 a	22.69 ± 0.38 b

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

Table 2. Mean (±SE) percentage survival, realized fecundity (eggs laid per female), fertility (eggs hatched per female), and offspring sex ratio (female/male + female) of *H. hebetor* on two lepidopteran host species in different generations

	Generation	Survival (%)	Realized fecundity	Egg fertility (%)	Sex ratio
H. hebetor reared on *E. kuehniella*	Fourth generation (23)	80.00 ± 2.43 a	186.60 ± 4.31 a	80.04 ± 0.93 a	65.21 ± 1.57 a
	Seventh generation (25)	83.33 ± 1.49 a	191.40 ± 3.19 a	78.89 ± 0.83 a	64.28 ± 1.52 a
H. hebetor reared on *He. armigera*	First generation (8)	48.88 ± 2.22 c	61.62 ± 3.43 c	39.00 ± 1.94 c	28.59 ± 1.93 c
	Fourth generation (12)	62.22 ± 2.81 b	127.50 ± 3.41 b	57.36 ± 1.87 b	40.25 ± 1.46 b

The means followed by different letters in the same column are significantly different (Tukey, $P < 0.05$). Numbers in parentheses show the number of *H. hebetor* adults paired for recording realized fecundity at each treatment

records for the shortest longevity of female were on *He. armigera* in the first generation after transfer (Table 1).

Survival, Realized Fecundity, Egg Fertility, and Sex Ratio

The highest survival rate of the immature stages of *H. hebetor* was on *E. kuehniella* (fourth and seventh generations; $F = 49.31$; df = 3, 20; $P < 0.01$), and lowest survival rate was on *He. armigera* (first generation; Table 2). The highest realized fecundity ($F = 156.71$; df = 3, 64; $P < 0.01$) and egg fertility ($F = 191.81$; df = 3, 64; $P < 0.01$) of *H. hebetor* were recorded for females came from larvae reared on *E. kuehniella* (fourth and seventh generations). By contrast, the lowest realized fecundity and egg fertility were for females came from larvae fed on *He. armigera* (first generation; Table 2). The results indicated a significant difference in offspring sex ratio (female/total) of *H. hebetor* reared on two treatments ($F = 123.01$; df = 3, 20; $P < 0.01$). The sex ratio of parasitoid was the highest on *E. kuehniella* (fourth and seventh generations) and the lowest on *He. armigera* (first generation; Table 2).

Enzymes Assay

The α-amylase activity measured for fourth-stage larvae reared on two treatments showed significant differences. The lowest α-amylase activity was recorded for the larvae reared on *He. armigera* (fourth generation). The larvae reared on *E. kuehniella* (fourth and seventh generations) had the highest α-amylase activity (Fig. 1).

The lowest general protease activity was found in the larvae reared on *He. armigera* (first generation). The larvae reared on *E. kuehniella* (fourth and seventh generations) showed the highest general protease activity (Fig. 2).

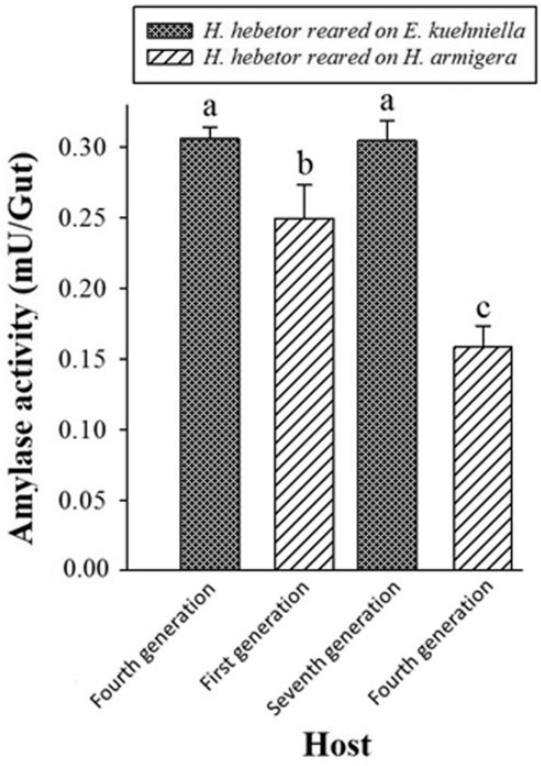

Fig. 1. Mean (±SE) α-amylase activity of midgut extracts from *H. hebetor* larvae reared on two lepidopteran host species in different generations. The means followed by different letters are significantly different (Tukey, $P < 0.05$).

In-Gel Activity Assay

The results showed that the fourth-stage larvae reared on *E. kuehniella* (fourth generation) had three α-amylase (A2, A3, and A4) and two general protease (P1 and P2) bands in the gel assays (Figs. 3 and 4). Four α-amylase (A1, A2, A3, and A4) and two general protease (P2 and P3) bands were observed in the fourth-stage larvae reared on *He. armigera* in the first generation after transfer (Figs. 3 and 4). Also, the fourth-stage larvae reared on *He. armigera* for four generations had three α-amylase (A1, A3, and A4) and two general protease bands (P2 and P3; Figs. 5 and 6).

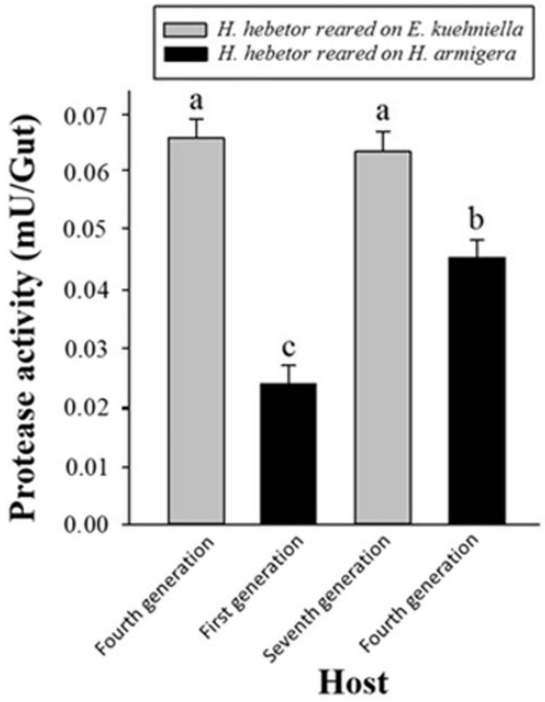

Fig. 2. Mean (±SE) general protease activity of midgut extracts from *H. hebetor* larvae reared on two lepidopteran host species in different generations. The means followed by different letters are significantly different (Tukey, $P < 0.05$).

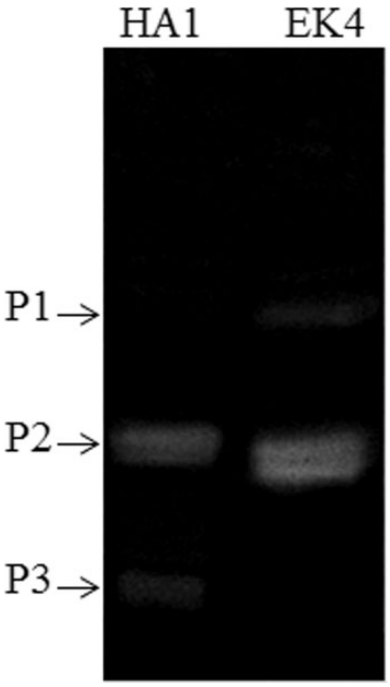

Fig. 4. General protease zymogram of midgut extract from *H. hebetor* larvae reared on two lepidopteran host species in different generations using gelatin as substrate. Numbers are as follows: EK4, *H. hebetor* reared on *E. kuehniella* for four generations; HA1, *H. hebetor* reared on *He. armigera* for one generation.

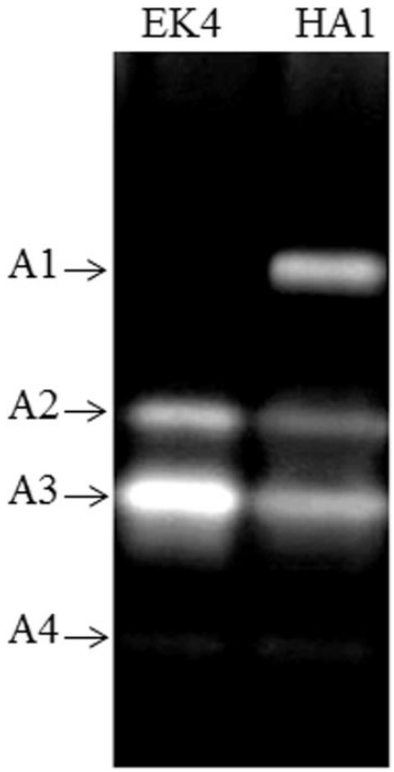

Fig. 3. Amylolytic zymogram of midgut extract from *H. hebetor* larvae reared on two lepidopteran host species in different generations using 1% starch as substrate. Numbers are as follows: EK4, *H. hebetor* reared on *E. kuehniella* for four generations; HA1, *H. hebetor* reared on *He. armigera* for one generation.

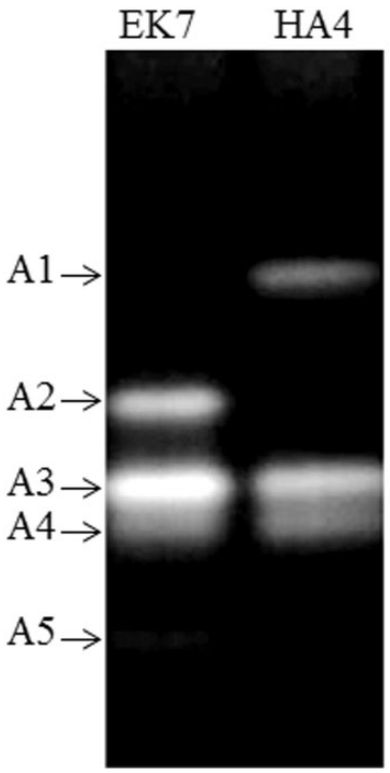

Fig. 5. Amylolytic zymogram of midgut extract from *H. hebetor* larvae reared on two lepidopteran host species in different generations using 1% starch as substrate. Numbers are as follows: EK7, *H. hebetor* reared on *E. kuehniella* for seven generations; HA4, *H. hebetor* reared on *He. armigera* for four generations.

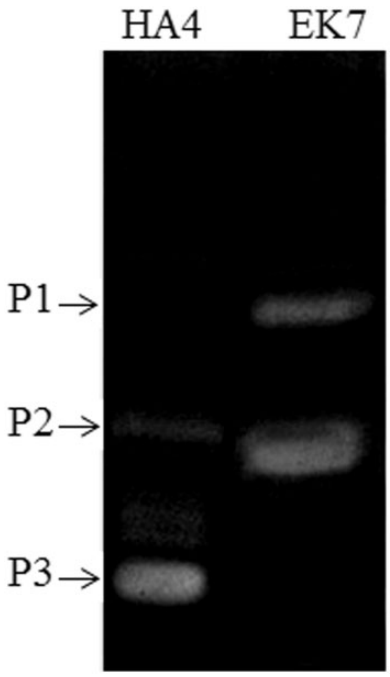

Fig. 6. General protease zymogram of midgut extract from *H. hebetor* larvae reared on two lepidopteran host species in different generations using gelatin as substrate. Numbers are as follows: EK7, *H. hebetor* reared on *E. kuehniella* for seven generations; HA4, *H. hebetor* reared on *He. armigera* for four generations.

Discussion

Using of parasitoids, such as *H. hebetor*, could be an important strategy to control lepidopteran pests (Chen et al. 2011). The success of *H. hebetor* as a biological control agent is limited by temperature (Carrillo et al. 2005, Chen et al. 2011), relative humidity and photoperiod (Farghaly and Ragab 1987, Forouzan et al. 2008), maternal effects (Saadat et al. 2014b), as well as searching behavior of parasitoids (King 1987, Takasu and Lewis 1995). In this study, the biological characteristics and digestive enzymatic profile of *H. hebetor* were studied in response to feeding on two hosts and different generations after transfer.

The results of this study showed that *E. kuehniella* was superior to *He. armigera* for rearing of *H. hebetor*. There were significant increases in fecundity and fertility, significant decreases in larval duration, and generally lower mortality rate for *E. kuehniella*-fed *H. hebetor* relative to *He. armigera*-fed *H. hebetor*. This indicates that *He. armigera* is not containing the ingredients necessary for *H. hebetor* development and thus reduced the fitness of the parasitoid. Wäckers (2001) expressed that conversion in parasitoid–host dynamics can be attributed to the attendance of unsuitable diet. Also, Bouayad et al. (2008) reported that the developmental traits of insects are mostly affected by the quality and the quantity of diet. Ghimire and Phillips (2014) investigated the effect of different lepidopteran hosts, such as *E. kuehniella* and *He. armigera*, on the biological parameters of *H. hebetor*. According to their results, *E. kuehniella* was a more suitable host when measured daily fecundity on each host, egg-to-adult survivorship, and progeny sex ratio. Our results indicated that the α-amylase and general protease activities of larvae fed on *E. kuehniella* (fourth and seventh generations) were more than larvae fed on *He. armigera* (first and fourth generations). Similarly, Saadat et al. (2014b) reported that the activity of α-amylase and protease of *H. hebetor* fourth-stage larvae reared on *E. kuehniella* is higher than larvae reared on *He. armigera*. In insects, type and amount of carbohydrate and protein consumed is an important factor with direct effect on type of isoforms and activity of digestive enzymes responsible for providing energy to larval stage (Sivakumar et al. 2006). This regulation may befall at different levels, including transcriptional and/or covalent modification of the digestive enzymes (Lehane et al. 1995). In the larvae reared on *E. kuehniella* for four generations, type and number of α-amylase and general protease bands was similar to those reared for seven generations. Also, all of treatments used in the current survey indicated the presence of one main common α-amylase isoform (A3). Unique α-amylase isoforms were identified in *He. armigera*-fed *H. hebetor* (A1 and A4). This study revealed complex, diverse, and flexible nature of α-amylase and general protease enzymes of *H. hebetor* for carbohydrate and protein digestion during larval development and upon feeding on various hosts. Higher α-amylase and general protease activities in *E. kuehniella*-fed larvae may be due to nutritionally balanced combination of host. Only a few researches have characterized the α-amylase and general protease activity in insect's parasitoids and their relation with host (Ghimire and Phillips 2014, Saadat et al. 2014a). Alvarez-Alfageme et al. (2012) expressed that α-amylase activity in wasps extract was lower in larvae than in females and this difference might be due to the ability of female wasps to feed on floral and extra-floral nectar that is carbohydrate-rich sources.

In the present research, immature period was significantly increased, male and female longevity were significantly decreased when parasitoid was reared on *He. armigera* in the first generation as compared with the fourth generation after transfer. It has been well established that adaptation to food has a considerable effect on life cycle, fecundity, and fertility. Alvarez-Alfageme et al. (2007) expressed the negative effects of inhibitors reduced by the capability of natural enemies to adapt their digestive metabolism to the unsuitable host. The larvae reared on *He. armigera* in the first generation after transfer had a higher α-amylase activity and a lower general protease activity than larvae reared on *He. armigera* for four generations. Also, type and number of α-amylase bands were differed in the larvae reared on *He. armigera* in the first generation as compared with the fourth generation after transfer. On the other hands, it was discovered a difference in expression level and type of general proteases. For dietary components, there are relationships between their levels in diet and the presence or number of gut enzymes (Silva et al. 2001, Karasov et al. 2011). But, it seems that there is not a good match to secrete α-amylase and protease enzymes with amount of protein and carbohydrate of the host in *He. armigera*-fed *H. hebetor* larvae in the first generation after transfer, and non-occurrence to host in the first generation clearly affects life history and parasitism success of *H. hebetor*.

Conclusion

In conclusion, an extremely important result in the present study is the fact that change of host could affect the final outcome of the parasitoid. When *H. hebetor* reared for several generations on *E. kuehniella* and then transferred to *He. armigera*, it required more than one generation timing for enzymatic adaptation until improved growth features on the new host. This is very important subject in mass rearing and release of parasitoids against pests in field and

storage conditions. Therefore, due to *H. hebetor* enzymatic adaptation to *He. armigera* under laboratory conditions, the parasitoid should be reared on *He. armigera* for three generations before being used in biological control of this pest.

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