Article

Effect of continuous rearing generations on some biological parameters of *Habrobracon hebetor* (Hymenoptera: Braconidae) under insectarium conditions

Ghadir Momenian, **Mohammad Hasan Sarayloo**, **Ali Afshari**

Department of Plant Protection, Faculty of Plant Productions, Gorgan Agricultural Sciences and Natural Resources University, Gorgan, Iran

E-mail: gmomenian@gmail.com

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Abstract

Habrabracon hebetor (Say) (Hymenoptera: Braconidae) is one of the most important biocontrol agents of insect pests in conducting IPM programs. In the present study, the effect of successive mass rearing by several generations on some biological parameters of *H. hebetor* was studied under laboratory conditions (28±2^{o}C, R.H. 65±5 % & L:D (16:8)). The results of the analysis of variance showed that continuous rearing in different generations had a significant effect on all parameters ($p<0.05$). Eighth generation (G8) had the longest pre-adult stages longevity and the percentage of sex ratio with averages of 12.14 days and 66.48 (female / female+male), respectively. The longest oviposition period and highest female longevity with the averages of 18.88 and 21.88 days, respectively, was observed in the ninth generation (G9). The highest percentage of larval and pupal mortality was observed in the G1 with averages of 1.20 and 10.83%, respectively. The results also showed that the highest number of eggs laid (fecundity), pupal hatching, daily parasitism, and paralysis of larvae with averages of 14.24 eggs per female, 99.56%, 3.98 larvae, and 59.8 larvae, respectively, belonged to the fifth generation (G5). Finally, the results showed that mass rearing in continuous generations affected the biological parameters of *H. hebetor* and the fifth generation (G5) had the highest quality compared to other generations.

Keywords *Habrabracon hebetor*; Continuous generations; longevity; mortality; sex ratio.

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1 Introduction

Effects of pesticide misuse such as increasing pressure to select pesticide-resistant populations, loss of beneficial insects, the prevalence of secondary pests, contamination of plant and livestock foods, as well as Pollution of water resources and the environment to pesticide residues has highlighted the need to use a biocontrol strategy (Kamkar and Damghani, 2013; Badran et al., 2020). Biological control or pest control

using natural enemies is one of the most important methods proposed for sustainable agricultural development (Ahmad and Jam, 2015). Hymenopteran parasitoids serve as biological control agents for agricultural and forestry pests and as an alternative and environmentally friendly approach to overcoming the challenges of using synthetic insecticides in harvesting and post-harvesting systems (Godfray, 1994; Ueno and Ueno, 2007; Mbata and Warsi, 2019; Wang et al., 2019). Among these, after the family Ichneumonidae, the family Braconidae has been identified as the second-largest family in the order Hymenoptera with about 17,000 species whose members attack the larval stages of Lepidoptera, Coleoptera, and Diptera (Centrella et al., 2010; Belda and Riudavets, 2013; Badran et al., 2020).

The *Habrabracon hebetor* Say (Hymenoptera: Braconidae) is a ubiquitous insect that has been reported as a larval ecto- and cumulative parasitoid of a large number of Lepidoptera (Darwish et al., 2003; Gündüz and Gülel, 2005). This parasitoid also has significant efficiency in controlling storage pests and is one of the important ectoparasitoids of storage pests, especially the family Pyralidae (Brower et al., 1996; Ba et al., 2014; Baoua et al., 2018; Mbata and Warsi, 2019). Stored product pests cause severe economic losses due to the infestation of commodities in stored grain ecosystems, including silos, bakeries, food processing industries, etc. (Brower and Press, 1990; Cline and Press, 1990; Dubey et al., 2008; El- Aziz, 2011; Mbata and Warsi, 2019). The parasitoid *H. hebetor* mainly attacks the larvae of Lepidopteran stored product pests including *Plodia interpunctella* Hübner, *Ephestia kuehniella* Zeller, *Ephestia cautella* Walker, *Anagasta kuehniella* Zeller, *Galleria mellonella* Linnaeus and *Amyelois transitella* Walker (Schöller, 2014; Solá Cassi, 2017; Mbata and Warsi, 2019). Some strains of *H. hebetor* also attack field pests such as *Helichoverpa armigera* Hübner, *Maruca testulalis* Geyer, *Spodoptera litura* Fabricius and *Earias vittella* Fabricius (Dabhi et al., 2012; Mbata and Warsi, 2019).

Mass rearing systems of biocontrol agents (even under suitable conditions) can cause the declining quality of the controlling agent and, consequently, reduce its controlling power during release. Because even if the biological agent is selected correctly, but the necessary conditions for its mass rearing are not provided, its controlling power will be affected. Therefore, today, evaluating the ability of biological agents to control pests (quality control) in the success of bio-control programs has become a very important issue (Van Lenteren, 2003; Bueno et al., 2017). However, it should be noted that the quality control of each biological control agent is unique and should be evaluated with different indices. On the other hand, it is often impossible to evaluate all of these indices on a large scale. Therefore, today, some important characteristics, including biological (sex ratio, longevity, and the number of eggs laid or fecundity) and behavioral (mobility and flight) characteristics are considered as indices (Ardeh and Ghazavi, 2010). Numerous studies have been performed on the quality control of parasitoids, especially *H. hebetor*.

Takahashi, quoted by Benson (1974), evaluated the interaction between *H. hebetor* and *Ephestia cautella* in 20 consecutive generations in a laboratory and observed adult population fluctuations of *H. hebetor* in generations 6, 11, and 15 (G6, 11 and 15). Benson (1974) in the study of population dynamics of *H. hebetor* and *E. cautella* in 11 consecutive generations observed fluctuation in the number of adult wasps produced in different generations. In the above study, the lowest number of adult insects, parasitism rate, and sex ratio were seen in the 6th generation (G6) and the highest number of adult insects was seen in the 9th generation (G9). Salmanova (1991) also reported a decrease in the quality of *Trichogramma* wasps due to the continuous rearing in the laboratory conditions. Duton and Bigler (1995) reported that the ability to fly of *Trichogramma brassicae* decreases with increasing number of reared generations. Rojas et al. (1995) in their study during 8 consecutive reared generations of *Bracon thurberiphage* on *Heliothis virescens* reported reduced fertility. Thomson and Hoffman (2002) in their study of the effect of 6 consecutive generations of *Trichogramma carverae* on its fecundity showed that fecundity is reduced by increasing the number of reared generations but

the parasitoid efficiency in the field is not reduced. Gondez and Goglel (2005), by examining the effect of *H. hebetor* age on fecundity and sex ratio, showed that the fecundity of *H. hebetor* did not change in the first five days of life, but then decreased significantly. The sex ratio of offspring on both hosts (*G. mellonella* and *A. kuehniella*) was in favor of males and the production of females was higher on *G. mellonella* larvae than that on *A. kuehniella* larvae. Pratissoli et al. (2004) reported a decrease in the viability of *Trichogramma pretiosum* with increasing number of rearing generations. Badran et al. (2020) reported that the second generation of *H. hebetor* had the longest pre-adult development time (12.4 days) and the highest fecundity (1304 eggs/female) among 20 generations reared on *E. kuehniella*. Ghaemmaghami et al. (2021a, b) stated that the female adult longevity of *T. brassicae* varied significantly between successive generations (45 generations). They also reported that the highest values of gross reproduction rate (GRR), net reproduction rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ) and mean generation time (T) were found in generations 5 and 10 (G5 and G10). Considering the importance of *H. hebetor* as one of the important agents in biocontrol strategy, this study was conducted to evaluate the effect of long-term mass rearing on the quality of *H. hebetor*.

2 Materials and Methods

2.1 Rearing of larvae of the Mediterranean flour moth (*E. kuehniella*)

To achieve a sufficient and homogeneous larval colony in terms of rearing and nutritional conditions, the larvae were reared in the same laboratory conditions (25±2°C, 70±5% RH, and a photoperiod of 16:8 (L:D) h). Plastic basins with 14 cm in high and 48 cm in diameter were used for rearing, which was filled with food mixture and moth eggs to a height of 3 cm. After disinfecting the nutrient composition with Aluminium phosphide (phostoxin) tablets, the eggs were poured evenly on the nutrient composition into the basins, which included wheat flour (75%) and wheat bran (25%) (Yazdanian, 2001). The number of eggs consumed per kg of nutrient composition was 0.18 to 0.2 g of eggs. To provide the moisture needed to hatch the eggs and prevent them from drying out, the openings of the basins were covered with a thin black cotton cloth, and water was sprayed with a spray.

2.2 Collecting of adult *H. hebetor*

In the spring, adult *H. hebetor* collected from rapeseed fields, which were in the flowering stage, by using funnel-shaped traps consisting of a slotted funnel and two layers of ruching. 40-50 4^{th} and 5^{th} larvae were placed between two layers of ruching and the set was fixed in the opening of the funnel. The traps were placed on a wooden base or rapeseed plants after preparation. 50 female wasps were collected. To eliminate environmental effects, reduce test errors, and standardize test materials, adult wasps were reared for one generation in laboratory conditions and used as the primary colony in the main experiment.

2.3 Mass rearing of *H. hebetor*

last age larvae of *A. kueniella* were used to rear *H. hebetor* wasps. To separate the larvae from the intertwined nutrient composition, basins were placed on a gentle heat source. Then a black cloth was placed on the basins so that the cloth was in contact with the nutrient composition inside the basins. As the temperature inside the plastic basins gradually increased, the flour moth larvae began to rise out of the basins and came out of the diet. On a sheet of A4 paper, 5 of the 5^{th} instar larvae of flour moth were placed. Each wasp was released into a clear glass with 10 cm in height and 5 cm in diameter and covered with cloth and netting. The glasses were placed upside down on the papers containing the larvae. After 24 hours, the glasses were removed and the parasitized larvae were kept in the experimental cabin under the desired conditions until adult parasitoids emerged.

2.4 Experiment

After observing the wasps of each generation in the rearing cabin, 25 pairs (male and female) of them were randomly selected from the population inside the cabin and placed in a test tube with a length of 10 cm and aperture diameter of 1 cm for 24 hours to mate. The test tubes were blocked with cotton soaked in 5% honey water. After 24 hours, the wasps are removed from the test tubes and a pair of them are released into a disposable glass with 10 cm in height and 5 cm in diameter and covered their opening with a cloth and net. 10 larvae of Mediterranean flour moths were supplied to wasps indirectly on a paper daily, and cotton pieces soaked in 5% honey water were hung from the top of the glass to feed the wasps. Every day after counting the wasp eggs, the sheets containing the eggs were cut and transferred to plastic Petri dishes and other measurements were recorded inside them.

2.5 Parameters studied

2.5.1 Pre-adult stages longevity

The number of days from egg stage to emergence of adult insects was counted in each replication and each generation and the average longevity of pre-adult stages in each generation was obtained.

2.5.2 Pre-adult stages mortality

Offspring mortality at each developmental stage (eggs, larvae, pupae) was calculated and the results were expressed as a percentage.

2.5.3 Oviposition period

The duration of this period was calculated by counting the number of days between the first and last oviposition of females.

2.5.4 Female and male longevity

Female and male longevity was determined by counting the number of days between emergence to death time of adult wasps. Dead insects were removed from the containers and then their sex was determined.

2.5.5 Number of eggs laid (=Fecundity)

The number of eggs on the larvae under each glass was examined daily at a specific time (every morning) and the number of eggs laid in each replicate was counted and recorded separately using a stereomicroscope.

2.5.6 Percentage of egg hatching

After 2 days of egg hatching, the larvae were counted and this number was considered as hatched eggs.

2.5.7 Sex ratio of offspring

After emerging of adult wasps developed from offspring, the number of female and male adults in each replication was counted. Finally, the total number of female adults produced was divided by the total number of adults produced by each individual, and the results were calculated as a percentage.

2.5.8 Adult emergence

The total number of adult insects in each generation was counted in each replication and divided by the total number of pupae and the results were expressed as a percentage.

2.5.9 Parasitism

The number of parasitized larvae in each replicate and each generation was counted every day and the total number of parasitized larvae was divided over the lifespan of the wasp and the results were considered as the average of daily parasitism per female.

2.5.10 Paralysis rate of host larvae

The number of paralyzed larvae per day was counted in each generation and the total number of paralyzed larvae was divided over the lifespan of the wasp and was considered as the average of daily paralysis per female.

2.6 Statistical analysis

The present study was conducted as a completely randomized design. Analysis of variance of data for this experiment was performed by SAS statistical software (version 9.4). Also, SPSS 16 software was used to normalize the data and to draw graphs. Comparisons of means of data were performed using Duncan's multiple range test at one and five percent probability levels. Data were converted to square root to normalize them.

3 Results

3.1 Pre-adult stages longevity

The results of the analysis of variance showed that successive rearing in different generations had a significant effect on the mean longevity of pre-adult stages at the level of one percent probability (Table 1). The highest longevity with averages of 12.14 and 11.82 days belonged to the eighth and tenth generations (G8 and G10), respectively, which did not differ significantly together but had significant differences with other generations (Table 2). The lowest longevity of pre-adult stages was seen in the first, third, and fourth generations (G1, G3, and G4), which were significantly different from other generations but were not significantly different together. Also, no significant difference was observed between the longevity of pre-adult stages in the second and fifth generations (G2 and G5) together and the sixth, seventh and ninth generations (G6, G7, and G9) together.

3.2 Pre-adult stages mortality

The results of the analysis of variance showed that the effect of different generations on mortality of eggs, larvae, and pupae of H. hebetor was significant at the level of 1% probability (Table 1). The highest mean percentage of egg mortality was seen in the tenth generation (G10) with an average of 38.68%, which was not significantly different from that in the seventh generation (G7) at the level of one percent probability but was significantly different from the percentage of egg mortality in the other generations (Table 2). The lowest percentage of egg mortality was observed in the second generation (G2) with an average of 9.13%, which was significantly different from the percentage of mortality in the sixth, seventh, eighth, ninth, and tenth generations (G6, G7, G8, G9, and G10), but no significant difference was observed between this generation and other generations (Table 2). Also, the highest percentage of larval mortality was in the first and tenth generations (G1 and G10) with averages of 1.20 and 1.27%, which were not significantly different from each other and the fifth generation (G5), but a significant difference was observed between the first and tenth generations with other generations (Table 2). The lowest percentage of larval mortality belonged to the fourth and eighth generations (G4 and G8) with the same average of 0.82%, which did not differ significantly together but had a significant difference with the percentage of larval mortality obtained in the first, second, fifth, ninth and tenth generations (Table 2). The highest percentage of pupal mortality occurred in the first generation (G1) with an average of 10.83%, which was not significantly different from the percentage of mortality obtained in the second, third, and tenth generations (G2, G3, and G10), but was significantly different from that in other generations. The lowest percentage of pupal mortality belonged to the fifth, seventh, and sixth generations (G5, G7, and G6) with averages of 0.88, 1.35, and 1.52%, respectively, which were not significantly different together, but differed significantly with mortality obtained in other generations at a 1% probability level (Table 2).

3.3 Oviposition period

Based on the results of the analysis of variance, the effect of different generations on the oviposition period of adult females was significant at the level of 5% probability (Table 1). Based on the results of the mean comparison, the longest oviposition period was recorded in the ninth generation (G9) with an average of 18.88 days, which was significantly different from the mentioned period in the first, second, third, sixth, and tenth generations, but was not significantly different from mean obtained in the other generations (Table 2). The lowest duration of this period was observed in the sixth generation (G6) with an average of 10.41 days, which

was significantly different from the average obtained in the fourth, fifth, seventh, and ninth generations, but was not significantly different from the oviposition period in other treatments (Table 2).

Table 1 Results of variance analysis of the effect of long-term mass rearing (different generations) on the pre-adult stages longevity and pre-adult stages mortality, the oviposition period, and the female and male longevity of *H. hebetor*.

Parameters	Df	Mean square	F
Pre-adult stages longevity	9	42.52	84.59**
Pre-adult stages mortality			
Egg	9	23.58	15.11**
Larva	9	0.73	17.95**
Pupa	9	13.11	20.53**
Oviposition period	9	2.70	1.71*
Female longevity	9	2.09	1.54*
Male longevity	9	1.34	1.75*

* and ** indicate the significant differences between parameters obtained in different generations reared at the levels of 5% and 1% probability, respectively.

3.4 Female and male longevity

The results of the analysis of variance showed that the effect of rearing generation on the female and male longevity was significant at the level of 5% probability (Table 1). The mean female longevity in the ninth generation (G9) with an average of 21.88 days was higher than that in other treatments, which was significantly different from the mean obtained in the first, second, and sixth generations at the level of 5% probability. The lowest female longevity was related to the sixth generation (G6) with an average of 13.86 days, which was significantly different from the average longevity recorded in the ninth and tenth generations (G9 and G10) but was not significantly different from averages obtained in the other generations (Table 2). The highest and lowest male longevity with averages of 17.60 and 11.16 days belonged to the tenth and first generations (G10 and G1), respectively, which had significant differences together (Table 2). The male longevity obtained in the first generation (G1) showed a significant difference with values recorded in the eighth, ninth, and tenth generations at the level of 5% probability and the longevity obtained in the tenth generation (G10) had a significant difference with those in the first, second, third and fourth generations at the level of 5% probability.

Table 2 Comparison of mean the pre-adult stages longevity and pre-adult stages mortality, the oviposition period and the female and male longevity of *H. hebetor* in different generation reared.

Parameters	Generation reared (G)									
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Pre-adult stages longevity (day)	9.86e	10.50d	9.54e	9.78e	10.62d	11.26c	11.40c	12.14a	11.60bc	11.82ab
Pre-adult stages mortality (%)										
Egg	16.42de	9.13e	16.24de	17.89de	13.12e	24.16bcd	32.76ab	23.39cd	28.52bc	38.68a
Larva	1.20a	1.00bc	0.85cd	0.82d	1.13ab	0.87cd	0.84cd	0.82d	1.03b	1.27a
Pupa	10.83a	8.94abc	9.74ab	6.72bc	0.88d	1.52d	1.35d	6.81bc	5.90c	8.12abc
Oviposition period (day)	12.92bc	12.56bc	13.20bc	16.29ab	15.92ab	10.41c	16.36ab	14.72abc	18.88a	12.32bc
female longevity (day)	15.08bc	15.60bc	16.44abc	17.00abc	18.48abc	13.86c	19.44abc	19.88abc	21.88a	20.48ab
male longevity (day)	11.16c	13.24bc	13.52bc	13.60bc	14.36abc	13.84abc	14.76abc	15.60ab	16.68ab	17.60a

* Different letters in each row indicate a statistically significant difference ($p < 0.05$).

3.5 Number of eggs laid (=Fecundity)

The results of variance analysis showed that the effect of generation on fecundity and egg production of females *H. hebetor* was significant at the level of 1% probability (Table 3). The highest number of eggs laid was obtained in the fifth generation (G5) with a daily average of 14.24 eggs per female, which was significantly different from the values obtained in the eighth and tenth generations (G8 and G9). The lowest average number of eggs laid belonged to the eighth generation (G8) with an average of 8.43 eggs per female, which was significantly different from fecundity calculated in the third, fourth, fifth, and sixth generations, but was not significantly different from that in the other generations (Table 4).

3.6 Percentage of egg hatching

Based on the results obtained from the analysis of variance, the effect of successive generations on the percentage of egg hatching was significant at the level of 1% probability (Table 3). Based on the mean comparison results, the highest mean percentage of egg hatching was related to the second generation (G2) with an average of 89.29%, which was significantly different from means obtained in the sixth, eighth, ninth, and tenth generations, but significant difference was not observed between the percentage of egg hatching in the second generation and other generations (Table 4). The lowest mean percentage of egg hatching was

related to the tenth and ninth generations (G10 and G9) with averages of 59.96 and 70.24%, respectively, which were significantly different from each other and values obtained in other generations (Table 4).

3.7 Sex ratio of offspring

Based on the analysis of variance related to the sex ratio of offspring, it was found that the average percentage of sex ratio of offspring in different generations had a significant difference at the level of 1% probability (Table 3). The highest percentage of sex ratio was related to the eighth generation (G8) with an average of 66.48 (female/female+male) which was significantly different from the sex ratio calculated in the second and ninth generations (48.86 and 44.57, respectively) at the level of 1% probability. The lowest percentage of sex ratio was related to the ninth generation (G9) with an average of 44.57% (female/female+male), which was not significantly different from the values obtained in the second and fifth generations, but there was no significant difference between the percentage of sex ratio in the ninth generation and other generations (Table 4).

Table 3 Results of variance analysis of the effect of long-term mass rearing (different generations) on the fecundity, percentage of egg hatching, the sex ratio, and the adult emergence of *H. hebetor*.

Parameters	Df	Mean square	F
Fecundity (the number of eggs laid	9	3.09	3.30**
Percentage of egg hatching	9	1856.38	12.42**
Sex ratio of offsprings	9	1158.99	4.41**
Adult emergence	9	330.77	15.63**

** indicates the significant differences between parameters obtained in different generations reared at the level of 1% probability.

Table 4 Comparison of mean the fecundity, percentage of egg hatching, the sex ratio, and the adult emergence of *H. hebetor* in different generations reared.

Parameters	Generation reared (G)									
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Fecundity (egg/female)	12.66ab	12.19ab	13.00a	12.79a	14.24a	13.22a	10.38ab	8.43b	12.14ab	8.44b
Percentage of egg hatching (%)	85.62a	89.29a	82.66abc	81.88abc	86.26a	75.16cd	84.82ab	77.54bcd	70.24d	59.96e
Sex ratio of offsprings (female/female+male)	63.20a	48.86bc	60.46ab	66.01a	57.07abc	62.29ab	58.62ab	66.48a	44.57c	57.82ab
Adult emergence (%)	89.28d	91.06bcd	90.26cd	92.56bcd	99.56a	98.50a	98.65a	93.19bc	94.60b	91.88bcd

* Different letters in each row indicate a statistically significant difference ($p < 0.05$)

3.8 Adult emergence

Based on the results obtained from the analysis of variance, the effect of successive generations on the percentage of adult emergence of *H. hebetor* was significant at the level of 1% probability (Table 3). The highest mean adult emergence was related to the fifth, sixth, and seventh generations with averages of 99.56, 98.50, and 98.65%, respectively, which were not significantly different from each other but were significantly different from values recorded in other generations (Table 4). The lowest mean adult emergence was obtained in the first generation with an average of 89.28%, which had a significant difference with the percentage of adult emergence in the eighth and ninth generations, but a significant difference was not observed between the first generation and other generations (Table 4).

3.9 Parasitism

The results obtained from the analysis of variance showed that the effect of generations reared on the daily mean parasitism was significant at the level of 5% probability (Table 5). The highest daily mean parasitism was related to the fifth generation (G5) with an average of 3.98 (larvae/day), which was significantly different from the level of parasitism in the sixth, eighth and tenth generations, but was not significantly different from parasitism rates in other generations (Table 6). The lowest daily mean parasitism belonged to the tenth generation (G10) with an average of 2.12 (larvae/day), which had a significant difference from the mean parasitism in the first, third, fourth, fifth, and ninth generations (Table 6).

Table 5 Results of variance analysis of the effect of long-term mass rearing (different generations) on the parasitism and the paralysis rate of host larvae by *H. hebetor*.

Parameters	Df	Mean square	F
Parasitism	9	8.02	4.87*
Paralysis rate of host larvae	9	18.35	8.79**

* and ** indicate the significant differences between parameters obtained in different generations reared at the levels of 5% and 1% probability, respectively.

Table 6 Comparison of mean the parasitism and the paralysis rate of host larvae by *H. hebetor* in different generations reared.

Parameters	Generation reared (G)									
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Parasitism (larvae/day)	3.25ab	3.11abcd	3.20abc	3.35ab	3.98a	2.61bcd	3.00abcd	2.31cd	3.36ab	2.12d
Paralysis rate of host larvae (larvae/day)	8.19ab	7.75ab	8.26ab	8.59a	8.72a	7.90ab	8.23ab	7.09b	8.42a	5.84c

* Different letters in each row indicate a statistically significant difference ($p<0.05$)

3.10 Paralysis rate of host larvae

Based on the results of the analysis of variance, the effect of successive generations on the daily paralysis rate of Mediterranean flour moth larvae was significant at the level of 1% probability (Table 5). The results of the mean comparison showed that the highest mean daily paralysis of larvae was related to the fourth, fifth, and ninth generations with the averages of 8.59, 8.59, and 8.42 (larvae/day), respectively, which had a significant difference with the means obtained in the eighth and fourth generations, but did not have significant differences with each other and other generations (Table 6). The lowest mean daily paralysis of host larvae was observed in the tenth generation (G10) with an average of 5.84 (larvae/day), which was significantly different from the averages obtained in other generations (Table 6).

4 Discussion

Parasitic wasps represent an alternative and environmentally friendly approach in postharvest systems for the management of pest populations because parasitoids are environmentally safe and do not negatively impact humans or beneficial organisms (Mbata and Warsi, 2019). *H. hebetor* is an important ectoparasitoid that has already been demonstrated to have biocontrol potentials and can regulate a wide range of stored product moth pests including *P. interpunctella* (Warsi et al., 2018) and *E. kuehniella* (Ghimire and Phillips, 2014). Lack of information on the quality and performance of natural enemies reared continuously for many generations is one of the most important issues which should be considered when establishing a biological control program, as successive mass rearing of natural enemies on factitious hosts in laboratories or insectaries may decrease the performance of the reared insects under field conditions (Bertin et al., 2017). Determining the performance of natural enemies under sequential mass rearing can provide insights into the most efficient generation of these beneficial insects and to what generation we can use natural enemies reared under these conditions without any loss in their performance (Badran et al., 2020). In the present study, we evaluated the quality of *H. hebetor* reared on *E. kuehniella* over 10 sequential generations using some biological parameters as our measurement of parasitoid quality. We found that these parameters of *H. hebetor* varied significantly among generations. The results of other researches also showed fluctuations in the values of biological parameters among generations reared (Badran et al., 2020; Ghaemmaghami et al., 2021a).

According to the results of the present study, an increased trend was observed during the pre-adult stages longevity with increasing number of generations, although, between the first and fourth generations, the longest period of this period was related to the second generation (G2). Badran et al. (2020) reported the longest pre-adult period of *H. hebetor* after 20 generations reared on *E. kuehniella* in the second generation (G2) with an average of 12.15 days. In the study of these researchers, the duration of the pre-adult period increased from the fourth to the fifteenth generation (G4 to G15). In the present study, although the longest duration of this period was observed in the eighth generation (G8) with an average of 12.14 days, the results of the two studies were similar in the duration of this period. Also, in both studies, an increasing trend was observed during the period with increasing the number of rearing generations. The results of the present study are inconsistent with some other studies. For example, Borzoui et al. (2016) showed a decrease during the larval stage of *H. hebetor* reared on *E. kuehniella*. Also, Magro and Parra (2004) reported a shorter larval stage of *H. hebetor* on *E. kuehniella* with increasing number of generations.

According to the results, mortality at the egg stage increased with increasing number of generations. At this development stage, the second and fifth generations had the lowest mortality with averages of 9.13 and 13.12%, respectively. In the larval stage, the mortality rate decreased from the first to the eighth generation (except for the fifth generation) with increasing the number of generations, but the mortality rate increased in the ninth and tenth generations. In the pupal stage, the mortality rate was high in the early generations (G1 to

G4), but this rate decreased with the increase in the number of generations to the seventh generation (G7), but it increased after the seventh generation (G7). Badran et al. (2020) showed that the survival rate of larvae and pupae increases with increasing the number of generations to the fifteenth generation (G15), in other words, the mortality rate decreases with increasing number of generations. This indicates that in insect rearing, the mortality rate in the early generations is high due to the imposition of artificial conditions, but this rate decreases after a few generations and adaptation to laboratory conditions.

Based on the results of the present study, the highest and lowest mean duration of the oviposition period were observed in the ninth and sixth generations (G9 and G6), respectively. Duration of the oviposition period increased when the number of generations increased to the fourth generation, but it decreased from the fourth to the sixth generation. The duration of this period fluctuated from the sixth generation to the next, but the oviposition period in the tenth generation was shorter than that in the first generation. Badran et al. (2020) reported that the oviposition period of *H. hebetor* gradually decreased with increasing generations, which this trend was observed in some generations in the present study. The researchers observed the longest oviposition period in the second and fourth generations and the shortest period in the 15th and 20th generations. A decrease in the duration of the oviposition period with increasing number of generations was also reported in other studies. For example, Ghaemmaghami et al. (2021a) stated that the oviposition period of *T. brassicae* on *S. cerealella* increased from 4.65 days in the fifth generation to 2.55 days in the 45th generation. Lü et al. (2017) also showed that the biological parameters of *Trichogramma dendrolimi* decreased after 20 generations.

The results showed that the highest and lowest mean female longevity was observed in the ninth and sixth generations, respectively, and for male longevity in the tenth and first generations, respectively. The study of this parameter in the 10 generations studied shows that the female and male longevity increased with increasing number of generations. The results of other researchers are different from the results of the present study. Badran et al. (2020) showed that female longevity decreased from the second generation (G2) to the twentieth generation (G20). Host size, age, and species can affect parasitoid longevity (Milonas, 2005; Charles and Paine, 2016). Badran et al. (2020) stated that the quality of *Ephestia* larvae may decrease with successive rearing, which may reduce the longevity of *H. hebetor*. The researchers also reported that the initial egg density of the parasitoid could affect the longevity of the female. An increase in the number of eggs laid by the female during rearing may decrease female longevity (Milonas, 2005; Charles and Paine 2016). These results may have been inconsistent due to the differences in the populations studied, the number of rearing generations, and the experimental conditions.

The results showed that the highest and lowest number of eggs laid occurred in the fifth and eighth generations, respectively. The findings of the present study showed that the reproduction rate of this parasitoid increased from the first to the fifth generation and then decreased. This result is consistent with the results of Attaran (1996) on *H. hebetor*, Benson (1974) on *H. hebetor*, Takahashi quoted by Benson (1974) on *H. hebetor*, Gündüz and Gülel (2005) and Jooyandeh (2008) on *H. hebetor*, Rojas et al. (1995) on *Bracon thurberiphage*, Thomson and Hoffman (2002) on *Trichogramma carverae*. However, it was different from the results of Badran et al. (2020) on *H. hebetor* and Ghaemmaghami et al. (2021a, b) on *T. brassicae*. Badran et al. (2020) reported that the highest and lowest fecundity occurred in the second and twentieth generations, respectively, and the reproduction rate decreased with increasing number of generations. The fecundity obtained in the present study (8.43-24.24 eggs/female) was less than the values obtained in the study of Badran et al. (2020) with an average of 49.98 -136.07 eggs/female. Ghaemmaghami et al. (2021b) reported that the highest and lowest fecundity of *T. brassicae* were found in G5 (47.41 eggs/female) and G45 (20.25 eggs/female), respectively. Amir-Maafi and Chi (2006) and Farag et al. (2015) reported the average of fecundity 78.3 eggs/female and 6.9 eggs/female. Nasab et al. (2014) estimated the mean fecundity of this

parasitoid on *E. kuehniella* between 14.94-24.46 egg/female, while the fecundity on hosts reared on wheat, soybeans, and wheat + soybean decreased over three generations, but this parameter increased on hosts reared on barley and rice over three generations. The discrepancy between the present study and the mentioned research could be due to *Wolbachia* infection, the difference in the duration of the oviposition period, using feral populations of *H. hebetor* with different genetic patterns instead of laboratory or insectary lines, using mature pyralid larvae as hosts in the experiment or supplying adult wasps with honey as a carbohydrate source (Ghimire and Phillips, 2010, 2014; Bagheri et al., 2019). Also, the decrease of host genetic diversity of *E. kuehniella* due to inbreeding over successive rearing on a standard diet may reduce host quality and thus lead to a reduction in *H. hebetor* fecundity over generations (Bertin et al., 2017).

Based on the results of the mean comparison, the highest mean percentage of egg hatching was observed in the second generation (G2) and the lowest mean percentage of egg hatching was recorded in the tenth and ninth generations (G10 and G9). Also, the percentage of egg hatching over 10 generations had a decreasing trend despite observing fluctuations. Badran et al. (2020) reported that the gross reproduction rate (GRR) and net reproduction rate (NRR) of *H. hebetor*, which represent the number of offspring produced per person excluding mortality and normal mortality, respectively, increased by increasing the number of generations to the tenth generation, which was different from the results of the present study. On the other hand, Ghaemmaghami et al. (2021a) stated that the values of the above two parameters for *T. brassicae* decreased with increasing number of generations, which is consistent with the results of the present study.

Sex ratio is an economically important trait in parasitoids that affects the financial profitability of mass rearing (Badran et al., 2020; Ghaemmaghami et al., 2021a). The highest and lowest sex ratio was related to the eighth generation (G8) and the ninth generation (G9), respectively. The results showed that the sex ratio decreased in the ninth and tenth generations compared to the first generation (G1). Badran et al. (2020) reported that the sex ratio (female/female+male) in *H. hebetor* under successive rearing showed an increasing trend from the second generation (17%) to the tenth generation (60%) and then this ratio gradually decreased after the tenth generation. They also reported some fluctuations in the sex ratio of *H. hebetor* under successive rearing. Parasitoids reared for many successive generations usually show some fluctuations in sex ratio (Wylie, 1979). In the present study, fluctuations in sex ratio were observed during ten generations. Mahdi Nasab et al. (2014) reported the increased trend in the sex ratio of *H. hebetor* on *E. kuehniella* reared on wheat and barley over three generations and decreased trend on rice. The researchers also confirmed some fluctuations in sex ratio over three rearing generations. Ghaemmaghami et al. (2021a) also found some fluctuations in the sex ratio of *T. beassicae* during long-term mass rearing on *S. cerealella*. The above studies as well as the findings of Pratissoli et al. (2004) and Lü et al. (2015) who showed fluctuations in the sex ratio of parasitoids experiencing successive rearing is in the agreement with the results of the present study. The association of some bacterial endosymbionts, such as *Wolbachia*, with *H. hebetor* could be the reason for more female production in *H. hebetor*. The important role of *Wolbachia* in shifting the sex ratio towards more female production in parasitoids has been confirmed by various researchers (Rousset et al., 1992; Pintureau et al., 2000; Tagami et al., 2001; Karimi et al., 2012; Weinert et al., 2015; Badran et al., 2020; Ghaemmaghami et al., 2021a).

The results of the present study showed that the highest mean adult emergence was related to the fifth, sixth, and seventh generations, and the lowest mean was observed in the first generation. According to the results, the percentage of adult emergence increased up to the fifth generation (G5). The highest percentage of adult emergence was seen in the fifth generation, but then gradually decreased. This result was similar to the results of Prezotti et al. (2004) on *T. pretiosum*, Nordlund et al. (1997) on *T. minutum*. Taghikhani et al. (2019) recorded the highest and lowest adult emergence of *T. brassicae* for the fifth and second generations,

respectively in the laboratory. They stated that the highest rate of adult emergence was recorded for the first three generations in the insectarium, while the last five generations showed a significant decrease in the rate of adult emergence. The researchers reported that the emergence rate of insectary-reared wasps was lower than the rate obtained in the laboratory, which may highlight the unsuitability of the rearing method, the factitious host, and/or the climatic conditions. According to Soares et al. (2012), the emergence rate of parasitoid wasps, especially *Trichogramma*, may depend on the size and quality of the host egg, the number of parasitoids that develop per egg, and temperature.

According to the results, the highest and lowest daily mean parasitism belonged to the fifth and tenth generations, respectively. The results of the parasitism showed that the rate of parasitism increased to the fifth generation and then decreased with increasing generation. This result was similar to the results of Benson (1974) on *H. hebetor* and Prezotti et al. (2004) on *T. pretiosum* but was different from the results of Nordlund et al. (1997) on *T. minutum*. Ghaemmaghami et al. (2021a) reported that the rate of *T. brassicae* parasitism decreased with increasing number of rearing generations. Taghikhani et al. (2019) stated that there was a significant difference in the rate of parasitism among the generations of *T. brassicae*. The maximum rate of parasitism was observed in the third generation (G3), followed by a decreasing trend until the eighth generation (G8). The results of the mean comparison showed that the highest mean daily paralysis of larvae was recorded for the fourth and fifth generation (G4 and G5), while the lowest was observed in the tenth generation (G10). According to the results, the paralysis rate of larvae increased from the first to the fifth generation, followed by fluctuations, so that it reached its lowest value in the tenth generation (G10).

Our results showed that a lack of information about the consequences of long-term mass rearing of *H. hebetor* may lead to the failure of biological control programs. We also showed that mass rearing in continuous generations had an effect on the biological parameters of *H. hebetor* and the fifth generation (G5) had the highest quality compared to other generations. Due to reduced female fecundity, after these generations, the colony needs to be rejuvenated by adding natural individuals.

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