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Biological parameters of *Bracon hebetor* (Hym.: Braconidae) parasitizing *Ephestia kuehniella* (Lep.: Pyralidae): effect of host diet

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Abstract: The effect of host diet on developmental time, fecundity and life-table parameters of *Bracon hebetor* (Say) (Hymenoptera: Braconidae) against the Mediterranean flour moth *Ephestia kuehniella* (Zeller) was studied in the laboratory condition at 26 ± 1 °C, 65 ± 5% relative humidity and a photoperiod of 16L:8D h. The diets used were rice, barley, corn and wheat flours. The developmental time (egg to adult) of the first and second generations ranged from 9.00 to 14.00 days on barley flour and wheat flour, respectively. The survival of immature generations 1 and 2 ranged from 27 to 63%. The sex ratio of wasp progeny (females/total) ranged from 36.72 to 57.83% on wheat flour and rice flour, respectively. In the first and second generations, the fecundity of *B. hebetor* reared on barley and rice flours was greatest. Life table parameters varied significantly with host diets. In generation 2, *B. hebetor*-parasitized larvae reared on rice flour showed the highest net reproduction rate (R_0 = 106.13) and the highest intrinsic rate of increase (r_m = 0.269) of parasitoids. Based on the life-table analyses, rice flour was found to be the best diet for rearing the parasitoid. The results of this study can be used to improve mass-rearing programs of *B. hebetor*.

Keywords: *Bracon hebetor*, host diet, developmental time, fecundity, life table parameters

Introduction

Biological control has come to have a significant role in integrated pest management (IPM) because of its advantages over chemical methods, pest resistance to conventional pesticides and compatibility with other IPM methods (DeBach and Rosen, 1991; Dent, 2000). Non chemical control methods have gained importance in IPM, as a policy aiming to minimize the application of residual chemical insecticides such as methyl bromide (Fields and White, 2002) and greater restrictions on the use of dichlorvos (Scholler, 2010). The control of stored product pests requires the control of large numbers of pests hidden in large amounts of product or structurally complex building (Scholler, 2010). So, biological control is especially vital with regard to stored product facilities (Scholler *et al*., 1997; Scholler and Flinn, 2000).

Bracon hebetor (Say) (Hymenoptera: Braconidae) is a gregarious, idiobiont, cosmopolitan ectoparasitoid that attacks lepidopteran larvae, mainly moths in the family Pyralidae, including the Indian meal moth *Plodia interpunctella* (Hubner), Mediterranean flour moth *Ephestia kuehniella* (Zeller) and dried fruit moth *Vitula edmansae* (Packard) (Brower *et al.,* 1996; Hagstrum and Smith, 1997). It also attacks a number of non-pyralid

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lepidopteran species that occur in both grain storage and field habitats in Asian countries (Gerling, 1971; Cock, 1985; Nikam and Pawar, 1993; Amir-Maafi and Chi, 2006).

The mass rearing of parasitoids, as biological control agents of insect pests, has improved substantially in recent years (Anderson and Leppla, 1992). The production of beneficial insects, especially parasitoids, is based mainly on the use of natural and artificial diets. Natural diets are used to produce hosts that in turn are employed for mass rearing of parasitoids. The quantity and quality of the food sources provided to the host have significant effects on both development and physiological activities of parasitoids (Gulel, 1988). By and large, a host species that allows a parasitoid species to develop to maturity is considered nutritionally suitable for that parasitoid species (Gulel, 1988). It has been documented that quantitative and qualitative changes of natural nutrients during the larval developmental period of parasitoid species affect the developmental time, adult size, longevity, fecundity and sex ratio in the progeny (Hagley and Barber, 1992; Tillman and Cate, 1993).

In this study, we compared the developmental time, longevity, fecundity (total number of eggs per female), sex ratio and life-table parameters of *B. hebetor* when its host species *E. kuehniella* was given four different natural food types. The aim was to find the most suitable conditions for rearing and maintaining *B. hebetor* for mass production and biological control applications.

Materials and Methods

Diets

Rearing diets consisted of rice *Oryza sativa* L., barley *Hordeum vulgare* L., corn *Zea mays* L. and wheat *Triticum aestivum* L. flours. To prepare the flours, the respective grains were washed in distilled water and dried at 40°C for 1 day, before being powdered and softened to eliminate any debris. New cultures were initiated by transferring eggs of *E. kuehniella* from the stock culture to each of four plastic containers (45 × 15 cm).

Insect rearing

A culture of *E. kuehniella* originated from a flour mill in Gorgan, Iran, was reared at 26 ± 1 °C, 65 ± 5% RH and a photoperiod of 16L: 8D h. The colony was maintained in cylindrical plastic containers (45 × 15 cm) each containing 150-200 eggs of the moth and 200-250 g of wheat flour, which provided the larvae with excess food throughout their development (Attaran, 1996).

A laboratory stock colony of *B. hebetor* was established from individuals collected from tomato fields near Ahvaz, Khuzestan Province, Iran. The parasitoids were cultured on full-grown fifth instar larvae of *E. kuehniella* reared on wheat diet in Petri dishes (diameter 9 cm and height 1 cm). Air circulation was achieved through a hole (diameter 4 cm) in the lid. The Petri dishes were kept at laboratory conditions as previously mentioned.

Host diet suitability experiments

For generation 1, approximately 30 full-grown 5^{th} instar *E. kuehniella* were placed in a petri dish (diameter 9 cm) with five pairs of parasitoids. After parasitization, for 2-3 h the 30 larvae were collected and placed individually into a Petri dish. Only one wasp egg was left on each host. Each of the 30 petri dishes were considered as a replication and these experiments were repeated 4-6 and 11-14 times for different diets in the first and second generations, respectively. The development of the wasp larvae was checked daily. The developmental time, survival, and sex ratio of the progeny were recorded upon emergence.

To study longevity, fecundity, and life-table parameters on different host diets, the method of Amir-maafi and Chi (2006) was adapted. Two fifth-instar host larvae reared on each diet were introduced into a Petri dish and left undisturbed to settle for 6-12 h. One pair of newly emerged parasitoids (age < 24 h) obtained in the above experiment was introduced and allowed to oviposit for 24 h. The wasp had access to honey smeared on the inside of the dish. If a male was found dead, it was replaced by another of similar age. Each pair was transferred daily into new

Petri dish. Larvae in the previous dish were transferred to Petri dishes containing excess food medium at 26 °C, to evaluate survivorship and sex ratio of the second generation. The procedure for these experiments was similar to those described for the first generation. The developmental time, the number of survivors and sex ratio of these progeny were recorded when they reached adulthood. The procedure was carried out until the female parent was dead. Each longevity and fecundity experiment was replicated 15-23 times for different diets. Petri dishes were kept at above-mentioned conditions. The same procedure was followed to evaluate the longevity and fecundity of the subsequent generation of *B. hebetor* on different diets.

Data analysis

The development times, egg-to-adult survivorship, sex ratio, adult longevity, and fecundity were analyzed by one-way analysis of variance (PROC GLM, SAS Institute, 2005). The egg-to-adult survivorship and progeny sex ratio data were arcsine transformed to meet assumptions of normality and heterogeneity of variance (Ghimire and Philips, 2010). The means were separated using the least significant difference (LSD) test (α = 0.05).

Demographic parameters were analyzed based on female age-specific life tables. The Jackknife procedure was used to estimate pseudovalues of life table parameters (Maia *et al*., 2000). This method was first applied to life-table analysis by Meyer *et al.,* (1986), and it has been used widely to estimate population growth rates of animals.

Results

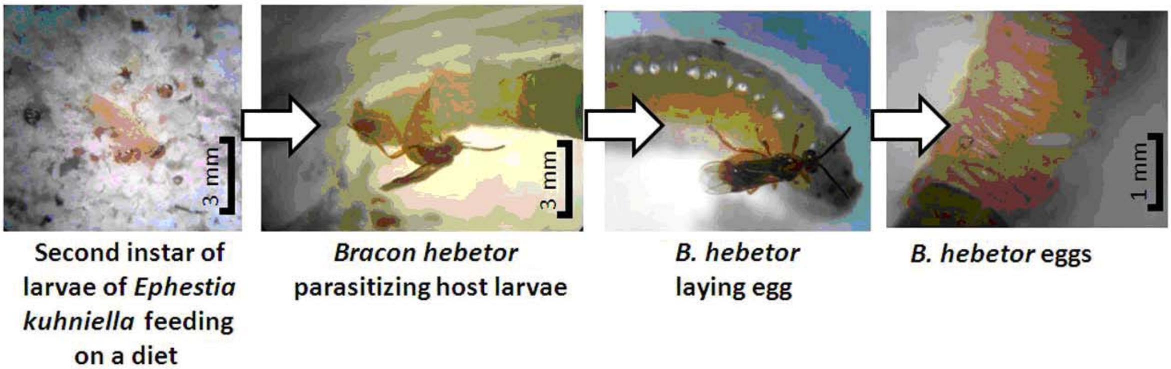
The obtained results showed that *B. hebetor* was able to complete its development during two successive generations on *E. kuehniella* that were reared on different diets. For generations 1 and 2, the egg-to-adult development times for *B. hebetor* varied significantly with host diet (Table 1). Development time was shortened with barley flour in the second female generation. Pre-adult duration of both female and male of the first generation on rice flour diet was significantly higher than those of the other treatments. In both generations, the percentage of females was not significantly affected by the host diet.

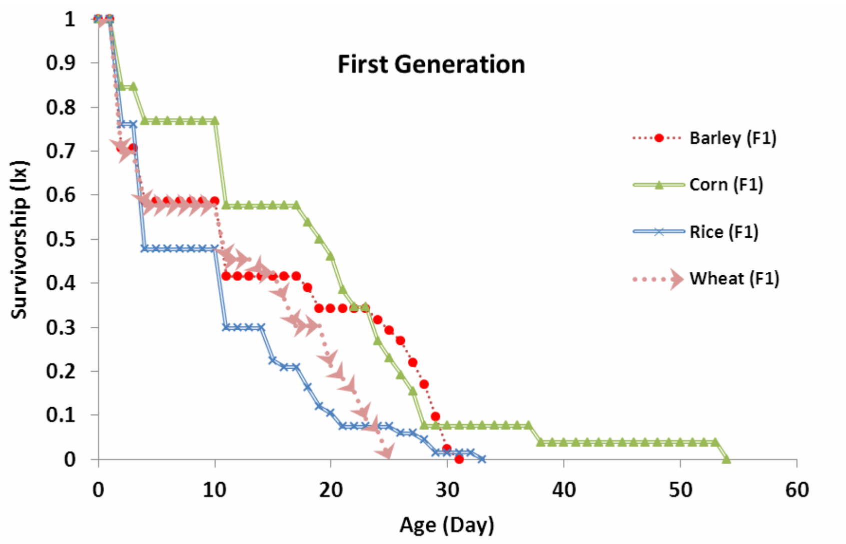
Survival of the first generation on the corn flour diet was significantly higher than that of the other treatments. In the second generation, rice flour diet followed by corn flour were significantly higher than the other treatments (Fig. 1). Also, *B. hebetor* exhibited adult survivorship that was not significantly different from that of other treatments (Table 2).

Table 1 Egg-to-adult developmental time (days) and sex ratio (mean ± SE) of *B. hebetor* parasitizing *E. kuehniella* reared on different diets.

Treatments	Pre-adult duration of female	Pre-adult duration of male	Sex ratio (female%)
Generation 1			
Rice flour	12.096 ± 0.20a[a] (31)[b]	12.06 ± 0.15a (29)	57.83 ± 0.12a (6)
Barley flour	11.12 ± 0.20b (25)	11.23 ± 0.17b (38)	47.33 ± 0.12a (6)
Corn flour	10.968 ± 0.11b (32)	10.72 ± 0.12c (37)	55.00 ± 0.07a (4)
Wheat flour	11.363 ± 0.08b (44)	11.20 ± 0.13b (40)	50.00 ± 0.07a (5)
F	10.85	12.19	0.21
P	< 0.0001	< 0.0001	0.8910
Generation 2			
Rice flour	11.215 ± 0.11b (88)	13.18 ± 0.134a (94)	49.78 ± 0.07a (14)
Barley flour	9.67 ± 0.21c (81)	9.00 ± 0.23b (67)	54.12±0.06a (13)
Corn flour	11.01 ± 0.10b (100)	8.2 ± 0.10b (91)	54.30 ± 0.07a (13)
Wheat flour	11.839 ± 0.7a (168)	14.00 ± 0.04a (310)	36.72 ± 0.03a (11)
F	58.52	78.80	1.75
P	< 0.0001	< 0.0001	0.05

Values are expressed as means ± standard error.
a. Values in the same column followed by different letters are significantly different (p < 0.05).
b. Number of individuals tested.





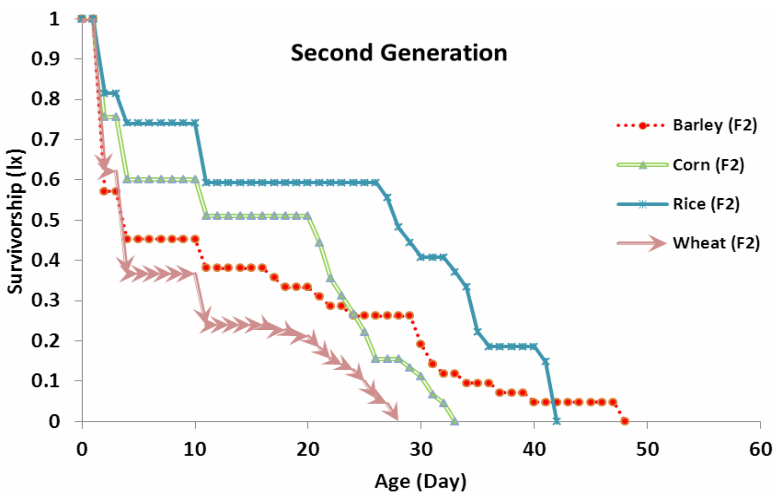


Figure 1 Age-specific survivorship (l_x) of the first and second generations of *Bracon hebetor* parasitizing *E. kuehniella* reared on different diets.

Table 2 Percent immature survival (mean ± SE) of *B. hebetor* parasitizing *E. kuehniella* reared on different diets.

Treatments	Egg	Larvae	Pupate	Total
Generation 1				
Rice flour	73.91 ± 3.50ab[a] (214)[b]	54.90 ± 9.13b (158)	60.07 ± 6.03b (90)	27.31 ± 5.51b (59)
Barley flour	63.59 ± 5.34b (212)	78.05 ± 11.42ab (135)	67.08 ± 9.51ab (102)	36.02 ± 8.22b (77)
Corn flour	80.89 ± 1.61a (152)	90.96 ± 1.56a (123)	86.94 ± 5.51a (112)	63.69 ± 3.59a (96)
Wheat flour	62.44 ± 3.05b (245)	77.69 ± 5.80ab (153)	77.00 ± 5.52ab (121)	38.36 ± 5.65b (94)
F	3.51	2.19	1.80	3.64
P	0.035	0.023	0.017	0.0315
Generation 2				
Rice flour	73.40 ± 4.05a (371)	82.88 ± 3.98a (277)	81.92 ± 2.84a (240)	50.66 ± 4.61a (194)
Barley flour	60.80 ± 2.98bc (360)	62.62 ± 3.55b (215)	91.37 ± 2.88a (142)	37.49 ± 2.81a (130)
Corn flour	68.28 ± 3.44ab (475)	69.74 ± 6.03ab (341)	76.24 ± 8.82a (254)	41.20 ± 5.78a (226)
Wheat flour	57.49 ± 2.76c (1281)	76.03 ± 5.13ab (732)	83.65 ± 3.63a (562)	36.84 ± 3.99a (478)
F	4.28	2.59	1.29	1.96
P	0.0094	0.063	0.228	0.133

Values are expressed as means ± standard error.
a. Values in the same column followed by different letters are significantly different ($p < 0.05$).
b. Number of individuals tested.

In generations 1 and 2, the longevity of *B. hebetor* females varied significantly with host diets (Table 3). In generation 2, the type of host diet had a notable effect on the longevity of females. Accordingly, the longest longevity for females (24 days) was recorded on larvae reared on rice flour.

Host diet had a significant effect on the fecundity of *B. hebetor*, as detailed in Table 3. In generation 1, the greatest total fecundity (193.94 eggs/female) was recorded from the hosts reared on barley flour (Table 3). In generation 2, the greatest mean number of eggs, in decreasing numerical order, was observed on rice (324 eggs/female), barley (310 eggs/female), wheat (166 eggs/female) and corn flours (150 eggs/female).

Life table parameters varied significantly with host diets, data of which is detailed in Table 4. In generation 1, *B. hebetor*-parasitized larvae reared on barley flour showed the highest net reproduction rate (R_0 = 49.248) and the highest intrinsic rate of increase (r_m = 0.276) of parasitoids.

Discussion

The results of our study show that the type of host diet can have a significant effect on a wide range of characteristics related to the performance of *B. hebetor*, including development, survival and fecundity. Those variables have important implications for parasitoid population dynamics. Our results from rearing *B. hebetor* for two subsequent generations on specific diets indicate that rice flour was the most suitable of the four treatments. These findings are in agreement with earlier work by Radhika and Chitra (1997) and Singh *et al.,* (2006), who reported that a host diet of *Corcyra cephalonica* (Stainton) had a significant effect on the development and reproduction of *B. hebetor*.

Although *B. hebetor* was able to complete development on all four of the diets tested, not all of the diets were equally suitable. The development time for *B. hebetor* on the different diets ranged from 9 to 12 days for both generations. These data are in line with previous results, of 10.83 days (Amir-Maafi and Chi, 2006) (based on two-sex life table), 12.85 days (Eliopoulos and Stathas, 2008) and 10.2 days (Ghimire and Philips, 2010), for the same host species.

Only a few studies have reported an r_m value for *B. hebetor* (Amir-Maafi and Chi, 2006; Eliopoulos and Stathas, 2008; Nikam and Pawar, 1993; Singh *et al*., 2006). Nikam and Pawar (1993) obtained an r_m value for *B. hebetor* of 0.215 on *C. cephalonica* reared on sorghum flour; Amir-Maafi and Chi (2006) (based on two-sex life table) obtained an r_m value of 0.137 on *E. kuehniella* reared on wheat flour. Singh *et al.,* (2006) obtained r_m values of 0.210, 0.185, 0.151 and 0.127 on *C. cephalonica* reared on wheat, maize, sorghum and rice flour, respectively. Eliopoulos and Stathas (2008) obtained r_m values of 0.121, 0.163, 0.191 and 0.185 for host densities of 1, 5, 15 and 30, respectively. Our estimated r_m for *B. hebetor* of 0.291 reared on rice flour for the second generation is well above the published estimates of r_m for this braconid wasp on different host diets.

Table 3 Longevity and fecundity (mean ± SE) of *B. hebetor* parasitizing *E. kuehniella* reared on different diets.

Treatments	Pre-oviposition period	Oviposion period	Post-oviposion period	Longevity of female	Longevity of male	Mean total number of egg/female	Daily number of egg
Generation 1							
Rice flour	0.45 ± 0.13a[a] (20)[b]	6.45 ± 1.14b (20)	1.95 ± 0.060b (20)	8.850 ± 1.23b (20)	8.95 ± 0.82a (20)	66.05 ± 13.99c (20)	6.526 ± 0.596a (20)
Barley flour	0.24 ± 0.106ab (17)	11.18 ± 0.796a (17)	4.00 ± 0.085a (17)	15.41 ± 0.95a (17)	10.18 ± 1.103a (17)	193.94±17.83a (17)	9.241 ± 1.73a (17)
Corn flour	0.13 ± 0.09b (15)	13.00 ± 2.46a (15)	2.20 ± 0.088b (15)	15.33 ± 2.42a (15)	7.20 ± 0.93a (15)	150.50±25.53ab (15)	6.888 ± 0.603a (15)
Wheat flour	0.00 ± 0.0b (15)	7.00 ± 0.78b (15)	2.266 ± 0.05b (15)	9.267 ± 0.93b (15)	7.00 ± 0.93a (15)	106.13±13.43bc (15)	8.256 ± 1.71a (15)
F	3.40	5.13	6.01	6.32	2.43	7.84	1.33
P	0.0231	0.0031	0.0012	0.0008	0.0736	<0.0001	0.269
Generation 2							
Rice flour	0.0625 ± 0.05a (16)	20.688 ± 1.31a (16)	3.312 ± 0.60a (16)	24.063 ± 1.43a (16)	13.18 ± 0.89a (16)	324.50 ± 33.33a (16)	11.36 ± 1.26ab (16)
Barley flour	0.125 ± 0.088a (16)	17.063 ± 2.43a (16)	2.687 ± 0.62a (16)	19.875 ± 2.36b (16)	9.00 ± 1.31b (16)	310.75 ± 45.64a (16)	13.075 ± 5.44a (16)
Corn flour	0.0870 ± 0.085a (23)	12.261 ± 1.063b (23)	2.391 ± 0.246a (23)	14.739 ± 0.88c (23)	8.20 ± 1.07b (23)	150.26 ± 18.13b (23)	8.91 ± 0.62b (23)
Wheat flour	0.0588 ± 0.062a (17)	10.412 ± 0.606b (17)	2.647 ± 0.35a (17)	13.118 ± 0.83c (17)	14.00 ± 1.01a (17)	166.71 ± 13.47b (17)	10.058 ± 0.65ab (17)
F	0.14	10.00	0.76	12.02	7.34	10.64	2.67
P	0.937	< 0.0001	0.522	< 0.0001	0.0003	< 0.0001	0.05

Values are expressed as means ± standard error.
a. Values in the same column followed by different letters are significantly different (p < 0.05).
b. Number of individuals tested.

Table 4 Life table parameters (mean ± SE) of *B. hebetor* parasitizing *E. kuehniella* reared on different diets.

Treatments	R_0	λ	r_m	T	DT
Generation 1					
Rice flour	14.00 ± 2.968c[a]	1.209 ± 0.100c	0.190 ± 0.008c	13.690 ± 0.879ab	3.559 ± 0.140a
Barley flour	49.248 ± 4.732a	1.322 ± 0.009a	0.276 ± 0.007a	13.798 ± 0.266ab	2.447 ± 0.057b
Corn flour	47.270 ± 7.630a	1.272 ± 0.028b	0.240 ± 0.021b	15.630 ± 1.500a	2.730 ± 0.236b
Wheat flour	29.078 ± 3.686b	1.312 ± 0.009ab	0.270 ± 0.007ab	12.288 ± 0.286b	2.512 ± 0.059b
F	12.80	12.31	12.64	2.21	14.83
P	< 0.0001	< 0.0001	< 0.0001	0.0952	< 0.0001
Generation 2					
Rice flour	106.133 ± 10.902a	1.311 ± 0.010a	0.269 ± 0.0086a	16.970 ± 0.789ab	2.515 ± 0.074b
Barley flour	71.623 ± 10.864b	1.272 ± 0.016b	0.239 ± 0.012b	17.462 ± 1.223a	2.823 ± 0.141a
Corn flour	42.876 ± 5.1740c	1.280 ± 0.0074b	0.246 ± 0.0056ab	14.957 ± 0.524bc	2.747 ± 0.61ab
Wheat flour	24.895 ± 2.0131c	1.260 ± 0.0049b	0.230 ± 0.0038b	13.707 ± 0.302c	2.888 ± 0.057a
F	20.24	4.18	3.94	5.25	3.26
P	< 0.0001	0.009	0.0118	0.0026	0.0268

Values are expressed as means ± standard error.
a. Values in the same column followed by different letters are significantly different (p < 0.05).

Overall, these results show that flour diets can be used for the mass rearing of *B. hebetor* using *E. kuehniella* as the host. However, we observed differences in the biology of the parasitoid, showing a higher longevity and a faster developmental time, when hosts were reared on rice flour, indicating that this diet is the most suitable for the mass rearing of *B. hebetor*. Further studies are necessary to analyse the chemical composition of diets and their influence on the development and reproduction of both host and parasitoid species.

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پارامترهای زیستی *Bracon hebetor* (Hym.: Braconidae) پارازیتوئید *Ephestia kuehniella* (Lep.: Pyralidae): تأثیر رژیم غذایی میزبان

هاجر فعال‌محمدعلی و پرویز شیشه‌بر

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چکیده: تأثیر رژیم‌های مختلف غذایی میزبان *Ephestia kuehniella* (Zeller) روی دوره پیش از بلوغ، پارامترهای تولیدمثلی و جدول زندگی *Bracon hebetor* (Say) در شرایط آزمایشگاهی (۱ ± ۲۶ درجه سلسیوس، رطوبت نسبی ۵ ± ۶۵٪ و دوره نوری ۱۶: تاریکی ۸ ساعت) ارزیابی شد. از رژیم‌های غذایی آرد برنج، آرد جو، آرد ذرت و آرد گندم استفاده گردید. طول دوره رشدی از مرحله تخم تا حشره کامل در نسل اول و دوم از ۹/۶۷ تا ۱۲/۰۹ روز بود و درصد بقا مراحل نابالغ در نسل اول و دوم از ۲۷ تا ۶۳ درصد متغیر بود. صرف‌نظر از نوع رژیم غذایی، نسبت جنسی (ماده/کل) بین ۵۷/۸۳-۴۷/۳۳ درصد مشاهده شد. بیشترین مقدار تولیدمثل زنبور *B. hebetor* در نسل اول و دوم روی میزبان با رژیم غذایی جو و برنج مشاهده گردید. پارامترهای جدول زندگی براساس نوع رژیم غذایی میزبان اختلاف معنی‌داری با یکدیگر داشتند. در نسل دوم، زنبورهای *B. hebetor* پرورش یافته روی لاروهای تغذیه کرده روی آرد برنج بیشترین نرخ خالص تولید مثل (R_0=۱۰۶/۱۳) و بیشترین نرخ افزایش رشد جمعیت (r_m = ۰/۲۶۹) را نشان دادند. تجزیه و تحلیل جدول زندگی براساس رژیم غذایی نشان داد که رژیم غذایی برنج بهترین رژیم غذایی برای پرورش زنبور پارازیتوئید بود. نتایج این تحقیق در بهبود پرورش انبوه زنبور پارازیتوئید *B. hebetor* برای کنترل پروانه‌های آفت قابل استفاده است.

واژگان کلیدی: *Bracon hebetor*، رژیم غذایی میزبان، دوره رشدی، تولیدمثل، پارامترهای جدول زندگی

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