



Influence of host diet on the biological characteristics of *Bracon hebetor* Say (Hymenoptera: Braconidae)

Konukçu besinin *Bracon hebetor* Say (Hymenoptera: Braconidae)'un bazı biyolojik özelliklerine etkisi

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ABSTRACT

The quantity and quality of host insect diets is strongly related with the development of parasitoid offspring. To evaluate the prediction that effects of host artificial diets on the ecto-larval parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae); *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae were reared with different host diets. We used corn flour + bran (control), oat flour + bran, rye flour + bran, carob flour + bran, einkorn flour + bran, and potato flour + bran (1:1) as host diet. The number of eggs laid by the parasitoid *B. hebetor* was lowest (3.5 ± 0.41) on carob flour+ bran. The rate of developing larvae was highest on corn flour+bran (70.74%) and einkorn flour+bran (65.69%). Potato flour + bran (66.59 ± 1.34 h) and einkorn flour + bran (61.87 ± 1.26 h) are the best host diet for the parasitoid larval development time. The most suitable diet for pupal development time is potato flour + bran (212.02 ± 4.08 h) and rye flour + bran (220.85 ± 3.09 h). Considering the total development time, similarly potato flour + bran (330.21 ± 4.86 h) and rye flour + bran (341.62 ± 4.63 h) are suitable diets. Female parasitoids showed longer longevity than male parasitoids for all host nutrients, except in the case of carob flour + bran. The sex ratios of the parasitoids were affected for each host diet. The highest fecundity was obtained in mixture of potato flour + bran with 242 individuals. The results of this study can contribute to the improvement of the mass rearing of *B. hebetor* for the biological control of different pests.

Key Words: Host diet, *Ephestia kuehniella*, *Bracon hebetor*, Biology

ÖZ

Konukçu besininin kalite ve kantitesi parazitoit nesillerinin gelişimini etkilemektedir. Konukçu besininin ekto-larval parazitoit *Bracon hebetor* Say (Hymenoptera: Braconidae) üzerindeki etkisini belirlemek için konukçu *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvaları farklı besinlerde yetiştirilmiştir. Çalışmada kullanılan besinler mısır unu + kepek (kontrol), yulaf unu + kepek, çavdar unu + kepek, keçiboynuzu unu+ kepek, siyez unu + kepek ve patates unu + kepek (1:1)'dir. *B. hebetor*'un en az yumurta bıraktığı besin (3.5 ± 0.41) keçiboynuzu unu+ kepek karışımıdır. *B. hebetor*'un gelişen larva oranının en yüksek olduğu besin mısır unu + kepek (%70.74) ve siyez unu + kepek (%65.69) karışımlarıdır. Larval gelişim için en uygun konukçu besinleri ise patates unu + kepek (66.59 ± 1.34) ve siyez unu + kepek (61.87 ± 1.26 sa) karışımlarıdır. Pupa gelişme süresi için en uygun besinler patates unu + kepek (212.02 ± 4.08 sa) ve çavdar unu + kepek (220.85 ± 3.09 sa) olarak bulunmuştur. Parazitoitin toplam gelişme süresi dikkate alındığında benzer olarak patates unu + kepek (330.21 ± 4.86 sa) ve çavdar unu + kepek (341.62 ± 4.63 sa) uygun besinler olarak bulunmuştur. Keçiboynuzu unu + kepek besin karışımı hariç dişi parazitoitlerin yaşam süresi erkek parazitoitlerin yaşam süresinden daha uzun bulunmuştur. Parazitoitin cinsiyet oranında konukçu besinlerinden etkilendiği saptanmıştır. En yüksek doğurganlık ise 242 birey ile patates unu + kepek karışımında bulunmuştur. Bu çalışma sonuçlarının önemli zararlıların parazitoiti olan *B. hebetor*'un kitle üretimine katkı sağlayacağı kanısındayız.

Anahtar Kelimeler: Konukçu besini, *Ephestia kuehniella*, *Bracon hebetor*, Biyoloji

Introduction

Carbohydrates, proteins, and lipids are the primary nutrient compounds in all insects and play an essential role in energy storage and utilization (Clements, 1992; Candy et al., 1997; Arrese and Soulages, 2010). Parasitoid population dynamics are affected by variability in the quantity and quality of host nutrients (Godfray, 1993; Thompson, 1999; Arakawa et al., 2004; Jervis et al., 2008). The rearing strategy of parasitoids on hosts fed different nutrients could critically impact the behaviours and biological characteristics (developmental time, adult size, longevity, fecundity and sex ratio) of parasitoid progeny (Rivero and Casas, 1998; Harvey et al., 2004; Jervis et al., 2008).

The relation between the diet of the host insect and parasitoid fitness is defined in ecology as a complex multitrophic relationship (Slansky Jr, 1986; Lill et al., 2002). The quality of the host's diet is the main factor affecting the fitness of the host insect in the rearing of parasitoids. On the other hand, the fitness of the host insect is defined as the highest level of growth and development for the host (Price, 1997; Speight et al., 1999, Schowalter, 2000; Avmack and Leather, 2002). Additionally, the efficiency of a parasitoid significantly depends on its reproductive capacity. The success of oogenesis in parasitoids is associated with the nutrient biochemistry of the female parasitoid during the early stage of development (Hagen et al., 1984; Wheeler, 1996; Papaj, 2000). Especially for idiobiont parasitoids, the food source that the host represents is the primary factor determining the reproductive capacity of the parasitoid. The importance of the host diet is once again highlighted here.

The hosts of the larval idiobiont ectoparasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae) belong to the orders Lepidoptera, Coleoptera and Hymenoptera. *B. hebetor* is reared and sold commercially for the management of several lepidopteran species (Gerling, 1971; Cock, 1985; Nikam and Pawar, 1993; Prozell and Schöller, 2003; Amir-Maafi and Chi, 2006; Ghimire et al.,

2010). *Ephestia kuehniella* (Lepidoptera: Pyralidae) is used as an effective host for current and daily mass rearing. In this study, the host *E. kuehniella* was reared on different diet combinations (potato flour + bran, oat flour + bran, rye + bran, einkorn flour + bran, corn flour + bran) to enhance the fitness of *B. hebetor*.

Materials and Methods

Rearing of *Ephestia kuehniella*

Ephestia kuehniella, which was used as a host for the experiments, was reared in a climate-controlled room at $25 \pm 1^\circ\text{C}$ and 60-70% relative humidity under 16:8 light:dark conditions. A mixture of corn flour:bran (1:1) was used as a diet for the rearing of *E. kuehniella*. To prevent possible contamination during the rearing process, the mixture was sterilized for 3 hours at 60°C . Other materials were disinfected with 1% sodium hypochlorite before use in rearing. First, a 200-gram diet mixture was weighed and added to disinfected plastic rearing containers ($19 \times 24 \times 7$ cm). On average, 1000 host eggs were added to the 200-gram diet mixture. After the completion of development, the adults were collected and transferred to a box. This process was repeated every day (Tunca and Demiray, 2021).

Rearing of *Bracon hebetor*

The parasitoid *Bracon hebetor* was reared in the climate-controlled room at a temperature of $25 \pm 1^\circ\text{C}$ and 60-70% relative humidity under 16:8 light:dark conditions. *E. kuehniella* larvae were used in the rearing of *B. hebetor*. A total of 40-45 mature larvae of *E. kuehniella* were transferred with soft forceps, and 4 pairs of parasitoids were released into the petri dish. In addition, 1-2 drops of diluted honey were rubbed on paper strips and placed in the petri dish as food for the parasitoids. After a 24-hour period, the parasitoid pairs were removed from the petri dish. Adult parasitoids that had completed their development were used both in the experiments and for the continuity of parasitoid rearing (Tunca and Demiray, 2021).

Experiments

Different nutrient mixtures were used in this study: corn flour + bran (control), oat flour + bran, rye flour + bran, carob flour + bran, einkorn flour + bran, and potato flour + bran (1:1). These mixtures were prepared by weighing 200 g of the nutrient mixtures and transferring them to plastic boxes (19 × 24 × 7 cm). Approximately 1000 *E. kuehniella* eggs were transferred to these mixtures. Then, the eggs in the plastic boxes were allowed to undergo development in the dark at 25 ± 1°C and 60-70% relative humidity under 16:8 light:dark conditions until the fifth larval period. The mature larvae of *E. kuehniella* were removed from the rearing boxes and placed into 3 cm petri dishes, after which a pair of mated female-male parasitoids, which were fed for three days, were also placed into the same petri dishes. The parasitoid pair was removed from the petri dish after 24 hours of parasitization time. The eggs laid by the female parasitoids on the host larvae reared in the different diets were examined with a stereomicroscope (Leica EZ4). The parasitoid eggs, larvae, pupae and adults were observed under the stereomicroscope to follow the biological development of the parasitoid at the same time every day. For each host nutrient, the number of eggs deposited by the parasitoid, the *B. hebetor* larval ratio, larval development time, pupal development time, total development time, sex ratio, and female and male development times were determined separately. In addition, the F1 fecundity and longevity of *B. hebetor* were investigated.

Data analysis

All data were analysed with one-way ANOVA followed by Tukey's multiple comparison test. All analyses were carried out considering the 5% significance level. Statistical analyses were performed using Minitab version 17. The percentage data were normalized using an arcsine transformation ($p_0 = \arcsine\sqrt{p}$) (Zar, 1999).

Results

The mean numbers of eggs laid by the parasitoid *B. hebetor* in *E. kuehniella* larvae that developed in the different diets are shown in Table 1. There were significant differences in the numbers of eggs laid by *B. hebetor* depending on the host nutrient (F5, 232=12.41, P<0.05); the lowest number of eggs was left by parasitoids raised on *E. kuehniella* larvae that developed in the carob flour + bran mixture (3.5 eggs), while the highest number of eggs was left by parasitoids raised on *E. kuehniella* larvae that developed in the oat flour + bran mixture (10.2 eggs).

The parasitoid larval rates are presented in Table 1 and show significant differences among means (F5, 223=2.77, P=0.019). According to the results, the maximum developing larvae rate was found in parasitoids raised on *E. kuehniella* fed with corn flour + bran (70.74%). This nutrient mixture was followed by einkorn flour + bran (65.69%), rye flour + bran (62.69%), potato flour + bran (54.70%), oat flour + bran (51.27%) and carob flour + bran (45.73%).

The effects of the host nutrients used in the experiments on the larval, pupal and total developmental times of the parasitoid *B. hebetor* are given in Table 1. The larval development times of the parasitoid significantly differed depending on the diet used to rear the host (F5, 1035=31.83, P<0.05). According to the results, the longest larval development time was found in parasitoids raised on *E. kuehniella* fed carob flour + bran (97.14 h) and oat flour + bran (82 h). The shortest larval development time was found in parasitoids raised on *E. kuehniella* fed with einkorn flour + bran (61.87 h), potato flour + bran (66.59 h) and corn flour + bran (70.52 h-control).

Table 1 The effect of tritrophic relationship on the mean number of eggs laid by the parasitoid *Bracon hebetor* in the host, the developing larvae rate, the larval development time, the pupal development time, the total development time, and the female and male development time

Properties	Potato flour+ bran	Oat flour+ bran	Rye flour+ bran	Carob flour+ bran	Einkorn flour+bran	Corn flour+bran (Control)
Average number of eggs deposit on host *	7.78±0.59 AB	10.28±0.56 A	9.58±0.87 AB	3.5±0.41 C	8.6±0.49 AB	6.92±0.34 B
The rate of developing larvae (%)	54.70 BC	51.27 C	62.69 AB	45.73 D	65.69 A	70.74 A
Larval development time (hour)*	66.59±1.34 DE n= 173	82.00± 1.33 B n=252	72.89± 1.85 C n=189	97.14± 5.61 A n= 21	61.87± 1.26 E n=211	70.52± 1.24 CD n=195
Pupal development time (hour)*	212.02± 4.08 D n=163	258.81 ± 1.22 B n=245	220.85 ± 3.09 D n=188	289.14±5.10 A n=21	249.42±1.78 B n=209	235.73±1.70 C n=191
Total development time (hour)*	330.21±4.86 D n=163	396.64±2.08 B n=245	341.62±4.63 D n=188	442.29±7.51 A n=21	366.78±2.45 C n=209	360.25± 2.49 C n=191
Female development time (hour)**	334.89±8.13 EF n=65	412.85±3.22 A n=84	359.66±7.70 CDE n=71	448±16 AB n=3	376.85±4.08 BC n=84	369.03±4.67 BCD n=85
Male development time (hour)	327.18±6.03 F n=98	388.17±2.44 B n=161	330.66±5.57 F n=117	441.33±8.49 A n=18	360.57±2.97 CD n=125	354.56±2.52 DE n=106

*:Each line is evaluated within itself, and the difference between different capital letters is statistically significant.-

**:Two lines are evaluated within themselves, and the difference between different capital letters is statistically significant.-

Table 2. The effect of tritrophic relationship on female and male longevity of parasitoid *Bracon hebetor*

Properties	Potato flour+ bran	Oat flour+ bran	Rye flour+ bran	Carob flour+ bran	Einkorn flour+bran	Corn flour+bran (Control)
Female longevity	513.6 ±34.1 A n=25	434.4 ± 25.4 B n=40	441.2 ± 35.5 B n=26	240 ± 99.9 D n=3	466.6 ± 29.2 B n=43	516 ± 52 A n=16
Male longevity	237.1± 28 D n=25	229.2± 16.7 D n=40	248.3 ± 21.3 D n=26	336 ± 24 C n=3	342.1 ± 32.2 C n= 43	210 ± 43.2 E n=16

*: The difference between different capital letters is statistically significant.-

Similarly, the pupal development time of the parasitoid significantly differed depending on the diet used to rear the host ($F_5, 1011=63.90, P<0.05$). The longest pupal development time of the parasitoid was found on the hosts reared in carob flour + bran (289.14 h), and the shortest development time was found on the hosts reared in potato flour + bran (212.02 h) and rye flour + bran (220.85 h). When the total development time of *B. hebetor* was examined, the differences among host nutrients were statistically significant ($F_5, 1011=63.49, P<0.05$). The longest total development period was found on the hosts reared in carob flour + bran (442.29 hr), and the shortest total development period was found on the hosts reared in potato flour + bran (330.21 hr) and rye flour + bran (341.62 hr).

The effects of the host diets used in the experiments on the development times of male and female parasitoids are shown in Table 1. GLM analysis showed that the interaction between host nutrients and sex was not significant ($F_5, 1005=1.26, P=0.281$). However, one-way analysis of variance revealed a significant difference ($F_{11}, 1005=34.06, P<0.05$). The longevity of male and female parasitoids differed among the host nutrients ($F_{11}, 294=11.79, P<0.05$). Female parasitoids lived longer than male parasitoids for all host nutrients, except in the case of carob flour + bran (Table 2). The sex ratios of the parasitoids

were also determined for each host nutrient. The sex ratios of the parasitoids were (1.50:1), (1.91:1), (1.64:1), (6:1), (1.48:1), and (1.24:1) (male: female) for potato flour + bran, oat flour + bran, rye flour + bran, carob flour + bran, einkorn flour + bran and corn flour + bran, respectively.

In addition, the real fecundity of *B. hebetor* that developed on host larvae reared on the different diets was calculated. In total, 242 individuals were found on the host insects reared in potato flour + bran, 175.1 individuals were found on the host insects reared in oat flour + bran, 195.4 individuals were found on the host insects reared in rye flour + bran, 69 individuals were found on the host insects reared in carob flour + bran, 182.7 individuals were found on the host insects reared in einkorn flour + bran and 229.6 individuals were found on the host insects reared in corn flour + bran flour. In addition, real fecundity graphics were created to show the numbers of individuals that a female parasitoid produced daily on each host reared in the different nutrients (Figures 1 and 2). According to Figure 2, it is clear that the parasitoid *B. hebetor* showed consistent fecundity within the tritrophic relationship. However, it was observed that the fertility period was much shorter among those parasitoids that developed on hosts reared in carob flour + bran compared to others that developed on hosts reared in the other nutrients.

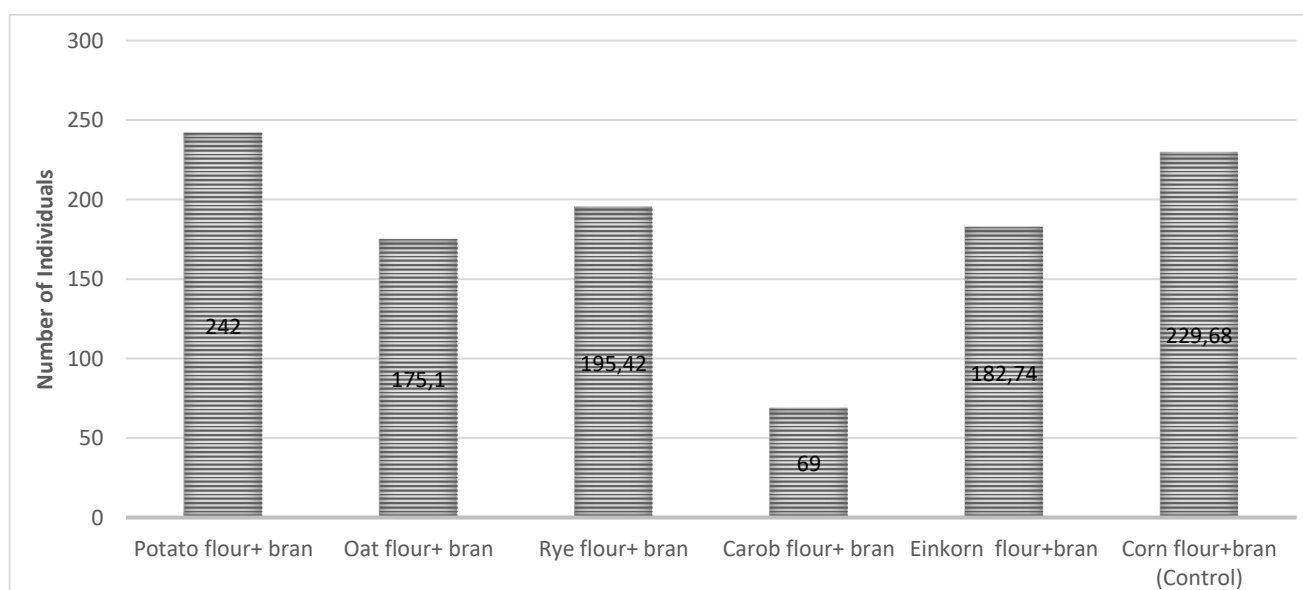


Figure 1 Real fecundity of *Bracon hebetor*, developed on host reared in each nutrient within the scope of tritrophic relationship.



Figure 2 Within the scope of tritrophic relationship, the daily number of individuals produced by the *Bracon hebetor* for each host reared on different nutrients.

Discussion

The different host diets used to rear *E. kuehniella* significantly affected the biological properties of the gregarious ectolarval parasitoid *B. hebetor*, including its development, survival and fecundity. There are many studies showing an important relationship between the host insect nutrient content (artificial food or host plant) and host preference/efficiency of the parasitoid in nature or under laboratory conditions (Campadelli and Barlotti, 1986; Fox et al., 1990; Harvey et al., 1995; Mallampalli et al., 1996; Teder and Tammaru, 2002; Coley et al., 2006; Mauricio et al., 2007; Faal-Mohammad-Ali and Shishehbor, 2013; Amadou et al., 2019).

It has been determined that the average number of eggs laid on the host by the parasitoid varies according to the nutrients used to rear the host larvae, and it is thought that the rates of *B. hebetor* larvae developing on *E. kuehniella* larvae reared in the different foods are directly related to the parasitoid. Here, corn flour + bran, einkorn flour + bran, rye flour + bran and potato flour +

bran were found to be important. When this result is combined with the larval development time, it is observed that the shortest development times are on the hosts reared in the mixtures of einkorn flour + bran, potato flour + bran and corn flour + bran. The first noteworthy observation is that these three foods have high carbohydrate ratios. The low carbohydrate and high protein contents in oat flour reduce the diet suitability for *Ephestia* larvae. Low or even zero development was observed in *Ephestia* larvae in our previous diet experiments when the protein ratio was high. Researchers think that proteins and some polysaccharides can become toxic or undesirable products during digestion. A similar situation was confirmed by researchers using rearing diets for insects with high protein content, such as soy flour and wheat germ (Fukushima, 1991; Cohen, 2004). It was expected that the high sugar content (sucrose, fructose and glucose) and fibre ratio in carob flour may cause digestive difficulties in larvae. Gilmour (1961) reported that some sugars, such as melibiose and galactosides, can be digested by insects, while some cannot. It has been stated that

sugars such as raffinose, stachyose, and α -galactosides can only be digested by insects with the enzyme α -galactosidase. On the other hand, the important reason for the slow development of the host in carob flour is the high fibre content and high levels of cellulose, hemicellulose, lignin and insoluble polyphenols in the fibre content (Goulas et al., 2016). Most insects cannot digest cellulose and use it for metabolic activities (Gilmour, 1961; House 1974). The possibility of encountering a similar situation in *Ephestia* larvae has been thought to be very high. Therefore, our study revealed that nutritional compatibility changes the growth rate of *Ephestia* larvae and that these changes affect the development of *B. hebetor*. This result is also supported by other studies (Barbosa, 1988; Van Emden, 1995; Teder and Tammaru, 2002; Mauricio et al., 2007; Faal-Mohammad-Ali and Shishehbor, 2013; Amadou et al., 2019). Additionally, nutrients that positively affect the larval development time of *B. hebetor* also appear to be advantageous in terms of the pupal development time and total development time.

In this study, there were no differences in the development times of male and female parasitoids developing on the hosts reared in the different nutrient mixtures, with the exception of the oat flour + bran and rye flour + bran mixtures. As explained above, the content of oat flour is not suitable for host larvae. No difference was observed between rice flour and corn flour in female development time. However, a shorter development time was obtained in the male development time in rice flour than in corn flour.. Rye flour has lower carbohydrate and fat contents than the other foods, and therefore rye flour was found to be a suitable food item. This situation might also cause a difference in the developmental times of male and female parasitoids. Corn flour is more economical than rice flour. A similar difference was expected in the carob flour + bran mixture, but a difference was not found between the development times of male and female parasitoids. The small difference is considered to be incidental due to the small number of samples. According to a study by Faal-Mohammad &

Shishehbor (2013), there was no difference in the development times of *B. hebetor* male and female parasitoids in the F1 generation that developed on host *E. kuehniella* reared in rice, barley, corn, and wheat flour.

The longest female parasitoid survival times were found on the hosts reared in the mixture of corn flour+ bran (516 hours) and potato flour + bran (513 hours). The longest male life expectancies of parasitoids were 342 and 336 hours on the hosts reared in the mixtures of einkorn flour + bran and carob flour + bran, respectively. A long life span of female parasitoids is advantageous for biological control applications in which parasitoids are effectively used. Therefore, the potato flour + bran mixture and corn meal + bran mixture provide ideally important nutrients for hosts of female *B. hebetor* with long longevity at the level of the tritrophic relationship. A similar study conducted by Eslampour and Aramideh (2016) showed that *Habrobracon hebetor* had the longest female and male life spans of 552 and 360 hours, respectively, on hosts reared in a mixture of whole wheat flour and 20% glycerol. It has been determined that the sex ratio of parasitoids developing on hosts reared with different diets was found to be in favour of males. However, the sex ratio appears to be most advantageous in the mixtures of potato flour + bran (1.50:1), einkorn flour + bran (1.48:1) and corn flour + bran (1.24:1). Eslampour and Aramideh (2016) found that the percentages of female *H. hebetor* developing on hosts reared in mixtures of whole wheat flour, whole wheat flour + 20% wheat germ, whole wheat flour + 20% glycerol, and whole wheat flour + 10% wheat germ + 10% glycerol were 59%, 55%, and 59%, respectively. Faal-Mohammad-Ali and Shishehbor (2013) found that the *B. hebetor* female percentages were 57%, 47%, 55% and 50% in rice, barley, corn and wheat flour, respectively. According to the results of all three studies, the host nutrient might affect the sex ratio of the parasitoid.

According to the experimental results, the highest fecundities of the parasitoids were found

on the hosts reared in a mixture of potato flour + bran (with 242 individuals) and corn flour + bran (with 229.68 individuals). The lowest fecundity of the parasitoids was determined to be 69 individuals on the hosts reared in a mixture of carob flour + bran. Eslampour and Aramideh (2016) found the fecundity of *B. hebetor* to be 102.8 individuals on hosts fed whole wheat flour, 136 individuals in whole wheat flour + 20% wheat rusheymi, 182 individuals in whole wheat flour + 20% glycerol, and 181 individuals in whole wheat flour + 10% wheat rusheymi + 10% glycerol. Another study provided a similar result; the fertility of *B. hebetor* was calculated based on the eggs laid by parasitoids. According to the results of the study, 66 eggs of parasitoids were found on hosts fed rice flour, 193.9 eggs were found on hosts fed with barley flour, 150.5 eggs were found on hosts fed with corn flour, and 106 eggs were found on hosts fed with wheat flour (Faal - Mohammad-Ali and Shishehbor, 2013). In our study, we observed that some parasitoids did not survive from the egg stage to the adult parasitoid stage. Therefore, the correct approach for calculating fertility for *B. hebetor* is based on the number of individuals. Figure 2 shows the numbers of individuals produced by parasitoids on hosts fed the different diets. However, the fecundity of parasitoids on a daily basis during their lifetimes was found to be similar.

Our study found that the diet of the host *E. kuehniella* affects the biological properties of the parasitoid *B. hebetor*. In this study, it was determined that the diets that contributed positively to the biological properties of the parasitoid were potato flour + bran, corn flour + bran and einkorn flour + bran. *B. hebetor* is an important larval parasitoid of many pests that can be found in warehouses and open fields. In addition, this parasitoid is a well-known biological control agent that has been reared en masse and used effectively for different biological control applications. We believe that this study will contribute to the mass production of *B. hebetor*.

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