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Adult longevity, fertility and sex ratio of *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) parasitizing *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae): effect of host artificial diets

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Abstract
Habrobracon hebetor is a larval gregarious ecto-parasitoid of several species of Lepidoptera in the family Pyralidae. Host diet strongly influences the reproductive success of the parasitoid. In this study, we assessed the reproductive performance of the parasitoid, *H. hebetor* in a series of laboratory experiments using four different host diets (wheat flour, wheat flour mixed with 20٪ wheat germ, wheat flour mixed with 20٪ glycerol and wheat flour mixed with 10٪ wheat germ, 10٪ glycerol) for rearing *E. kuehniella*. The result shows that total fertility was highest on whole wheat flour mixed with glycerol and whole wheat flour mixed with glycerol and germ (181.2 ± 10.7 and 182.4 ± 14.7, respectively). Longevity of *H. hebetor* females and males were significantly higher on whole wheat flour mixed with glycerol (23.4 ± 1.3 and 15.6 ± 1.2,). The progeny sex ratio was not significantly affected by the host diet.

Keywords: Biological control; stored product pest; Glycerol; Wheat germ; Reproduction

1. Introduction
The Mediterranean flour moth *Ephestia kuehniella* (Zeller) is a serious pest of stored grain products, particularly flour [1]. Its eggs and larvae are widely used to rear parasitoids and predators for biological control and research into behavior, biochemistry and molecular biology [2, 3]. Interest has been focused in recent years on the development of non-chemical strategies such as cultural, physical, biological, varietal and genetic control measures in place of conventional pesticides for the management of stored-product insects [4, 5, 6]. Using parasitoids and predators as natural enemies, is one of the important strategies in integrated pest management for field and especially stored-product protection. *Habrobracon hebetor* (Say) is considered a potential biological control agent of stored-product moths and has had some use in commercial pest control [7, 8, 9]. It attacks a variety of important lepidopterous pests of stored product, mainly moths in the family Pyralidae, and pests of field crops [10, 11]. It also attacks a number of non-pyralid lepidopteran species that occur in both grain storage and field habitats in Asian countries [12, 13]. Variability in host nutrient quantity and quality is likely to have significant effects on a number of parasitoid fitness components [14, 15]. 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 used 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 [16]. The behaviors, physical characteristics, and also physiology of many adult hymenopteran parasitoids are believed to be affected by the resources their larvae are able to obtain [15, 17, 18]. And synovigenic parasitoid like *H. hebetor* may acquire some nutrients necessary for reproduction during larval feeding [15; 18]. *H. hebetor* has been produced and sold commercially for management of stored-product moths [5, 19], so it is absolutely essential to investigate different aspects of rearing *H. hebetor* to optimize its efficiency. The present study evaluated the effect of glycerol and wheat germ as the components of artificial diet on adult longevity, fertility and progeny sex ratio of *H. hebetor* parasitizing *E. kuehniella*.

2. Materials and methods
2.1. Parasitoid origin and rearing
Biological studies of *H. hebetor* were conducted at the laboratory of plant protection department of Urmia University in October 2014. *H. hebetor* used in this study originated

from an insectarium maintained by the Plant Protection Bureau of Urmia and were associated with an infestation of the Mediterranean flour moth, *E. kuehniella*. The parasitoid was then cultured on full-grown larvae of *E. kuehniella* reared on wheat diet in the laboratory at 29±1 °C, 60±5% relative humidity (r. h), and a photoperiod of 16:8 (L: D) h.

2.2. Artificial diet Experiments

To evaluate the effect of Mediterranean flour moth larval artificial diets on parasitoid longevity and reproductive investment, larvae of *E. kuehniella* were reared in different diets. For preparing the artificial diets, dry and liquid components were mixed, completely blended, and kept at room temperature for one week in closed containers [20]. The treatments were: (1) larvae reared on a mixture of whole wheat flour and 3% yeast, (2) larvae reared on whole wheat flour, 3% yeast and 20% glycerol (w/w), (3) larvae reared on whole wheat flour, 3% yeast and 20% wheat germ, and (4) larvae reared on whole wheat flour, 3% yeast, 10% glycerol and 10% wheat germ (w/w). All the cultures were maintained in the similar condition as described above. All the materials used in the experiments, were obtained from the local market in Urmia, Iran.

Experiments were carried out in the laboratory using plastic petri dishes (90 by 15 mm) with larvae of each host diet. The last-instar, wandering stage larvae were used in this experiment because *H. hebetor* females preferred to attack wandering larvae at a rate 10-fold more than they attack young larvae [21]. 40 pairs of (age < 48 h) males and females from the colony reared on wheat diet were allowed to mate for one day in 500-ml plastic vials containing 50 last-instar *E. kuehniella,* and then five pairs of *H. hebetor* were introduced into a petri dish with thirty last-instar host larvae reared on each diet and allowed to oviposit for 24 h. There are adequate resources in a single larva to support the development of four *H. hebetor* [22]. The number of eggs on a larva was counted, and up to three eggs was left on each larva, and the remaining eggs were removed to ensure that there were sufficient nutritional resources available for development. The emergence of parasitoids was monitored daily after one week. Recently emerged adult *H. hebetor* (age < 24 h) were separated into pairs, placed in petri dishes and provided five last-instar larvae of *E. kuehniella* daily until the female died. There were 5 replicates for each host diet and all replicates of all the host diets were run at the same time. Each pair was transferred daily into new petri dish. 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. Petri dishes with parasitized larvae were kept in the laboratory under the same conditions as described above. The longevity of males and females, fertility and sex ratio of the F1 generation were evaluated.

2.3. Data analysis

All data were arcsine transformed to meet the assumptions of normality and heterogeneity of variance [5] and were compared by means of one way ANOVAs, followed by Tukey's multiple comparison test, and mean separation tests at (α=0.05). Statistical analyses were performed using SPSS version 22.

3. Results

3.1. Parasitoid fertility

The total number of parasitoid progeny produced from *E. kuehniella* larvae differed significantly in response to various host diet with 95% confidence ($F_{3,19}$= 10.59, P= 0.001; Table 1). The highest mean number *H. hebetor* adult was produced from whole wheat flour mixed with glycerol (182.4 ± 14.7) and whole wheat flour mixed with wheat germ and glycerol (181.2 ± 10.7) followed by whole wheat flour mixed with wheat germ (136.4 ± 14.2) and the lowest mean number of parasitoid progeny was produced from whole wheat flour (102.8 ± 4.6; Table 1) (Fig. 1).

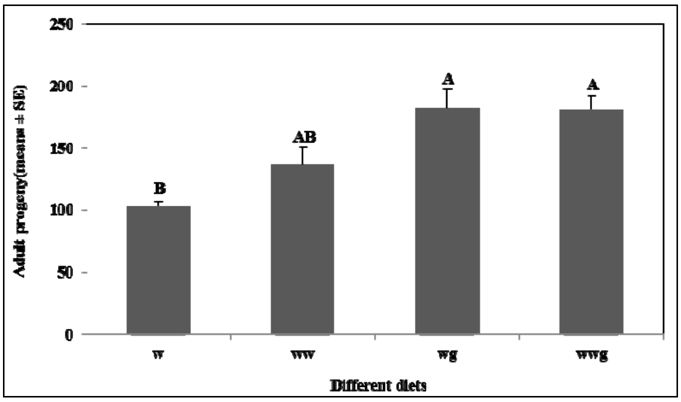

Fig 1. Effect of different artificial host diets on the total adult progeny of parasitoid *H. hebetor*. Bars associated with different letters are significantly different at α = 0.05 using HSD-tukey procedures. (w– whole wheat flour; ww– wheat flour+20% wheat germ; wg– wheat flour+20% glycerol; wwg– wheat flour+10% wheat germ+ 10% glycerol).

3.2. Adult longevity

Mean longevity of male *H. hebetor* was (15.6 ± 1.2) days on the whole wheat flour mixed with glycerol. This was significantly longer than that obtained with the other diets (7–10 days) with 95% confidence ($F_{3,19}$=21.99, P= 0.001; Table 1). There was no significant variation in the longevity of *H. hebetor* males among the other diets. The lowest Mean longevity of male was about (7.8 ± 0.3) days on the whole wheat flour. Mean longevity of female *H. hebetor* was significantly higher on the whole wheat flour mixed with glycerol (23.4 ± 1.3) ($F_{3,19}$=23.42, P= 0.001; Table 1), followed by whole wheat flour mixed with germ and glycerol (16.4 ± 1.2), whole wheat flour mixed with germ (14.4 ± 0.9) and lowest on whole wheat flour (11.4 ± 0.5) (Fig. 2).

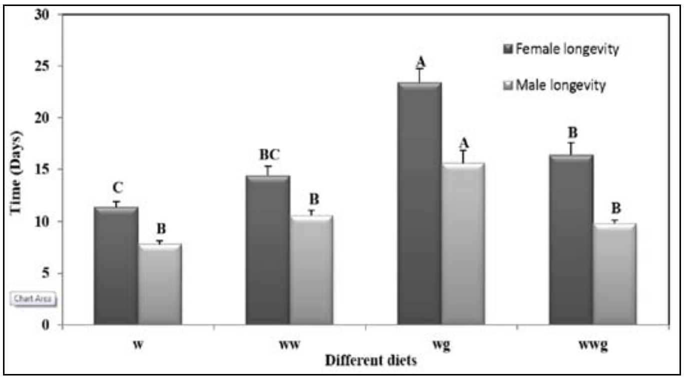

Fig 2. Effect of different artificial host diets on adult longevity of parasitoid *H.hebetor*. Bars associated with different letters are significantly different at α = 0.05 using HSD-tukey procedures (comparing each sex differ from each other).

Table 1. Reproductive statistics (mean ± SE) of *H. hebetor* parasitizing *E. kuehniella* reared on four different host diets.

Host diets	Total adult fertility	Longevity of females (d)	Longevity of males (d)	Progeny sex ratio (female %)
Whole wheat flour	102.8±4.6^{b} (89.9–115.6)	11.4±0.5^{c} (9.98–12.8)	7.8±0.3^{b} (6.76–8.83)	44.7±2.2^{a} (38.4–50.9)
Whole wheat flour+20% wheat germ	136.4±14.2^{ab} (96.9–175.8)	14.4±0.9^{bc} (11.8–16.9)	10.6±0.5^{b} (9.18–12)	59.9±7.1^{a} (40–79.9)
Whole wheat flour+20% glycerol	182.4±14.7^{a} (141.3–223.4)	23.4±1.3^{a} (19.6–27.1)	15.6±1.2^{a} (12.24–18.95)	55±3.9^{a} (44.1–65.9)
Whole wheat flour+10% wheat germ+10% glycerol	181.2±10.7^{a} (151.2–211.1)	16.4±1.2^{b} (13–19.7)	9.8±0.3^{b} (8.76–10.83)	59.8±2.6^{a} (52.5–67.1)
F	10.59	23.4	21.9	2.6
DF	3, 19	3, 19	3, 19	3, 19
P	< 0.001	< 0.001	< 0.001	0.088
N	5	5	5	5

Means in a column for a given value followed by the same letters are not significantly different at α =0.05 using Tukey procedures. Range of data (minimum to maximum) is given in the parenthesis.

3.3. Progeny sex ratio

The sex ratio (proportion of the female progeny) of emerging adults was not significantly affected by the host diet ($F_{3, 19}$= 2.60, P= 0.088; Table 1). The experiments conducted here produced progeny that were female-biased (Fig. 3), which is ideal for mass rearing in support of biological control.

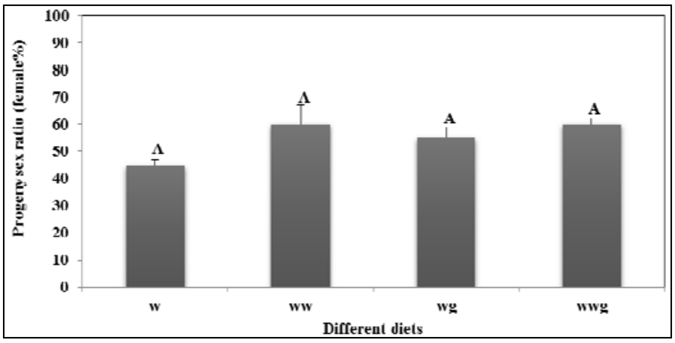

Fig 3. Effect of different artificial host diets on the progeny sex ratio (female %) of parasitoid *H. hebetor*. Bars associated with same letter are not significantly different at α = 0.05 using HSD-tukey procedures.

4. Discussion

The findings of the current study demonstrated that artificial diet components can have a significant effect on a parasitoid's reproductive parameters, such as longevity, progeny production, and sex ratio. Mean lifetime fertility was generally higher on the whole wheat flour mixed with glycerol and whole wheat flour mixed with wheat germ and glycerol (182.4 and 181.2 adults per female, respectively) as compared whole wheat flour (102.8 adults per female). The mean number of adult progeny produced on whole wheat flour is higher than the 66.3 eggs/female on *E. kuehniella* [13] (Fig. 1). The low fecundity observed in their study was possibly a consequence of supplying only two fifth instars per parasitoid for oviposition. *H. hebetor* females may avoid laying more eggs than could complete development on a host, as proposed by Yu *et al* [23]. The mean number of adult progeny produced by *H. hebetor* females on four different diets reported here is less than 568.2 adults/female reported by Ghimire and Phillips [24]. This difference could be explained by rearing *E. kuehniella* on the same diet as used for rearing *P. interpunctella* (a standardize diet of corn meal, chick laying mash, chick starter mash, and glycerol) by those authors.

The lowest mean longevity of females was about 11.4 days on the whole wheat flour that is similar to the values reported by Faal-Mohammad-Ali and Shishehbor [25], when the host diet was wheat flour (Fig. 2). This result is less than 17.2 days reported by Amir-maafi and Chi [13]. This difference might be due to the variation in the strain of *H. hebetor*, because those authors collected the parasitoids associated with *Heliothis* spp. in tomato fields. In our study the longest mean longevity of females was about 23.4 days on the whole wheat flour mixed with glycerol. These female parasitoids lived just as long as those reared on the rice flour in generation 2 (24 days), but lived longer than those reared on barley flour and corn flour (15.41, 14.33 days, respectively) by Faal-Mohammad-Ali and Shishehbor [25]. The results from this study are considerably less than 60.3 days reported by Ghimire and Phillips [24]. The possible explanation for this variation could be difference in host diet as mentioned above. The lowest mean longevity of male *H. hebetor* was 7.8 days on the whole wheat flour. This is similar to the values reported by Magro and Parra [26] and Faal-Mohammad-Ali and Shishehbor [25]. The longest mean longevity of male *H. hebetor* was 15.6 days on the whole wheat flour mixed with glycerol.

Reproductive fitness of *H. hebetor* could be accelerated by the addition of glycerol to wheat flour. We also found that the host larval growth rate on wheat flour mixed with glycerol was greater than other diets (data not shown). Silhacek and Murphy [27] point out that the glycerol requirement was to provide the host larvae with a digestible energy source that entered the glycolytic pathway.

We conclude that glycerol as a component of artificial diet is more effective than wheat germ to maximize *H. hebetor* reproductive parameters of fertility, adult longevity and progeny sex ratio that allow for the better levels of parasitoid progeny and survival, which can be useful to improve mass production of wasps for purposes of biological control of stored-product or field moth pests.

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