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Effect of Different Diet Concentrations on Longevity and Fecundity of Parasitic Wasp *Bracon hebetor* (Say.) (Hymenoptera: Braconidae)

Sumra Ashraf[1], Zain ul Abdin[1,*], Saqi Kosar Abbas[2], Rao Sohail Ahmad Khan[3], Muhammad Tahir[1], Sehrish Rasool[1], Maryam Anwar[1] and Fiaz Hussain[1]

[1]*Department of Entomology, University of Agriculture, Faisalabad, 38040, Pakistan*
[2]*Bahauddin Zakariya University, Bahadur Sub-Campus, Layyah, Pakistan*
[3]*Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, 38040, Pakistan*

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Authors' Contribution
ZA and RSAK designed the project and supervised the work. SA performed the experimental work. FH helped in execution of experimental work. SA and SKA wrote the article. MT helped in rearing of insects. SR helped in collection and management of data. MA helped in handling of experimental insects.

Key words
Bracon hebetor,* Fecundity, Longevity, Honey syrup, Date syrup, Sugar syrup, Parasitic wasp, Parasitoid, *Galleria mellonella.

ABSTRACT

To enhance their life period and reproductive potential, the adults of parasitoids mostly depend on supplemental food sources, such as sugars and other carbohydrates. These food resources are commonly obtained from animal secretions or plant exudates which include honeydew, fruit juices and both floral and extra-floral nectar. A direct behavioral assay was conducted to investigate the dietary preference and effects of different diets on the fecundity, sex ratio and longevity of *B. hebetor.* Three different diets [Honey syrup, sugar syrup, date syrup and a control (water)] were used with different solution percentage (25, 50, 75 and 90%). It was observed that honey fed wasp pair produces significantly more number of eggs, adults and also lived significantly longer than other treatments. Highest fecundity and longevity of parasitoid was recorded in case of parasitoids feeding on 50% honey solution followed by 25% sugar and date syrups solutions respectively. It has provided useful information for the mass rearing of this potential biocontrol agent which may be used in the successful implementation of biocontrol program for the suppression of pyralid pest insects damaging different agricultural crops of economic importance.

INTRODUCTION

Like other insects, parasitoids also require continuous supply of balanced food to perform their activities. Food consumption, especially from sugar rich sources, enhance the reproductive potential and insect parasitoid longevity (Lee and Heimpel, 2005, 2008; Lavandero *et al*., 2006; Winkler *et al*., 2009). In the context of biological control program, sugar feeding plays a vital role in the performance of parasitoids (Gurr *et al*., 2004). For parasitoids various sugars have different nutritional qualities and strongly increased the longevity while the others have small effect or even may be toxic to parasitoids (Wäckers, 2001; Wäckers *et al*., 2006; Kehrli and Bacher, 2008). Along with enhancing the longevity and fecundity (Heimpel *et al*., 1997a, b; Wäckers, 2001; Costamagna and Landis, 2004; Winkler *et al*., 2006), carbohydrate consumption also affects other aspects of reproductive success (Azzouz *et al*., 2004) in insect parasitoids.

Honey is used as best food source and standard for comparison with other food sources. Malati and Hatami (2005) investigated that feeding on honey significantly enhanced the life span of adults of *Trichogramma brassicae* Bezdenko. Eliopoulos (2007) determined that adult life span and number of adult emergence in *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) was strongly dependent on honey feeding.

Onagbola *et al*. (2007) studied the life span, reproductive potential and sex ratio of progeny of an ectoparasitoid *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae) which were affected by feeding on different diets. Wu *et al*. (2008) showed that *Meteorus pulchricornis* parasitoids when provided with 30% sugar solution lived significantly longer and produced more progeny compared to those fed on other concentration of sugars in the absence of host. Wyckhuys *et al*. (2008) checked that *Binodoxys communis* had more longevity when provided with honey and sucrose as compared to those provided with honeydew of *Aphis glycines*. Harvey *et al*. (2012) reported that reproductive potential and longevity in two hyperparasitoids, *Lysibia nana* and *Gelis agilis*, showed significant variations when they were provided with different dietary sugars and honey.

Bracon hebetor (Say.) (Hymenoptera: Braconidae), is

* Corresponding author: zainunibas@gmail.com
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a cosmopolitan larval ectoparasitoid that utilizes several pests belonging to the family Pyralidae (Lepidoptera) as host (Fagundes *et al*., 2005; Shojaei *et al*., 2006; Yasodha and Natarajan, 2006; Desai *et al*., 2007; Kyoung *et al*., 2008; Mohapatra *et al*., 2008; Dweck *et al*., 2010). The *B. hebetor* females prefer to attack and oviposit on last instar (fifth) larvae, although younger instars are also parasitized. The broad host range, high reproductive rate and short generation time makes *B. hebetor* is an excellent natural biological control agent of many pyralid pests, including *Galleria mellonella* (Lepidoptera: Pyralidae) while no work has been done on the diet preference effects on the reproductive potential and fecundity of this biocontrol agent (Gunduz and Gulel, 2005).

The project was planned to check that if fecundity and longevity of *B. hebetor* is affected when fed on honey and different sugars and to determine at which diet maximum female emergence is obtained. It is hypothesized that fecundity and longevity of *B. hebetor* will be affected due to dietary sugars and honey which provide maximum longevity and fecundity compared to other sugars.

MATERIALS AND METHODS

Rearing of host Galleria mellonella

The larvae, pupae and adults of the host, greater wax moth, *G. mellonella* were collected from the infested bee hives located at the campus of University of Agriculture, Faisalabad, Pakistan. Pairs of adult male and female of *G. mellonella* were placed in the jars (having fresh wax) for mating. After 24 h mating period, female starts egg laying as egg firing by extruding its ovipositor and flattering wings. Eggs were placed in incubator for hatching under controlled conditions. The insect culture containing eggs, larvae, pupae and adults was maintained at 27ºC±1 temperatures and 65±5% relative humidity (RH) by following a slightly modified approach as described by Anam *et al*. (2015).

Rearing of Bracon hebetor

The ectophagous larval parasitic wasp *B. hebetor* was reared in the laboratory on the late stage (5th instar) larvae of greater wax moth *G. mellonella* by following a slightly modified approach as described by Manzoor *et al*. (2011) and Anam *et al*. (2015). The adults of the parasitoid, *B. hebetor* were collected directly from the berseem crop, *Trifolim alexandrium* L., located at the campus of the University of Agriculture, Faisalabad, Pakistan. The collected parasitoids were identified on the basis of morphological characters by making comparison with the available literature. The parasitoid cultures was also maintained in glass jars, placed at 27ºC ±1, 65±5% relative humidity (RH) and 18 h light /6 h dark photoperiod.

Experimental procedure

Different concentrations (25%, 50%, 75% and 90%) of pure honey and date syrup were prepared with distilled water in 25ml glass vial. Similarly fine powder of sugar was dissolved in water to make its 25%, 50%, 75% and 90% concentrations in different pre-labeled glass vials of 25 ml. Honey syrup, date syrup and sugar syrup were used as treatments and different concentrations (25%, 50%, 75% and 90%) were used as different levels of these treatments.

Three groups of vials were prepared G1, G2, and G3 for three different diets like honey, sugar and date syrup, respectively. Further, five vials for each group were also prepared and vials for each group were provided with one larva of *G. mellonella*, a couple of freshly emerged parasitoid males and females. The cotton plugs soaked in four different concentrations (25%, 50%, 75% and 90%) of all the three diets (honey syrup, date syrup and table sugar syrup) were wrapped on the mouth of each vial of each group. The 5th vial was provided with water soaked cotton plug which acted as control. After egg laying, parasitoids were removed from the vials and shifted in other three groups. This practice was repeated again and again till the death of female to check its longevity. The number of eggs laid on each host were recorded after 24 h intervals and then parasitized hosts were incubated individually till adult emergence. The number of eggs hatched, emerged larvae feed on parasitized host larvae and pupate outside the body. The number of adults emerged and their sex ratio were also recorded on daily basis from each vial.

Determination of sex ratio

Sex ratio of *B. hebetor* and the percentage of female emergence in response to different diets was calculated by using the following formula:

$$\%\ \text{of males emerged} = \frac{\text{Total No. of males emerged}}{\text{Total No. of adults emerged}} \times 100$$

Data analysis

The data was statistically analyzed by Analytical software (2003), Statistix 8.1 by using the factorial design and the means were separated by Tukey HSD test at $P<0.05$.

RESULTS

Number of egg laying and adult emergence of Bracon hebetor *due to different diets*

Data given in the Table I show the mean values of fecundity and adult emergence of *B. hebetor* in response to different diets. Results show that there is a statistically significant difference in fecundity and adult emergence of

B. hebetor fed on different concentrations of honey, sugar and date syrup (Tukey's HSD test, $P< 0.05$). Parasitoid female fed on 50% honey solution laid significantly more number of eggs (ANOVA, df=3, F=128.22, 129±4, P<0.05) and produced more progeny (107.4±4.91, P<0.05) than those fed on other concentrations of different diets. When sugar and date syrups were provided to parasitoids as food, maximum fecundity (96.4±2.50 and 77±1.94, respectively) and maximum adult emergence (ANOVA, df=3, F=104.09, P<0.05, 85.5±2.85 and 66.4±2.204, respectively) were recorded at 25% concentration but it was still smaller than those which fed on 50% honey solution. Whereas in control treatment average fecundity of 27.1±0.70 (P<0.05) and adult emergence 15.5±0.70 (P<0.05) was recorded.

Sex ratio on different diets

Figure 1 shows the mean percentage of male and female sex ratio emerged by feeding on different diets. The results showed that maximum female emergence (48.4±3.36%, p<0.05) was observed by feeding on 50% honey solution followed by 43±1.74, 43.5±1.51 and 37.5±0.71% after sugar, date syrup and control treatment, respectively. Minimum female percentage (41.1±0.51%, p<0.05) was recorded in date syrup on 90% solution. Male mean percentage was recorded maximum (59.7±1.83%) on 90% honey solution while minimum (51.5±6.02%, p<0.05) was observed on 50% honey solution.

Table I.- Mean effect of different diets on fecundity and adult emergence of *Bracon hebetor.*

Treat-ments	Concentrations							
	25%		50%		75%		90%	
	Fecundity	Adults emerged	Fecundity	Adult	Fecundity	adult	Fecundity	Adult
Honey	63.4±1.805d	50.4±2.94de	129±4.00 a	107.4±4.91a	98.4±2.42 b	81.6±4.06 b	47.8±1.93ef	35.8±2.009f
Sugar	96.4±2.50 b	85.5±2.85 b	65.6±3.18cd	55.4±2.56cd	48.4±3.23ef	39.6±2.85ef	45.0±2.00 f	35±1.41 f
Date syrup	77.0±1.94 c	66.4±2.20 c	58±3.20 de	50.6±2.73de	44.0±1.87 f	34.4±2.85 f	42.4±1.74 f	32.6±1.63 f
Control	27.0±0.70 g	15±0.70 g	26.4±1.69 g	16±0.70 g	27.0±0.70 g	15±0.70 g	28.0±0.70 g	16±0.70 g

Means followed by different letter(s) within each column (denoted by lower-case letters) are significantly significant by Tukey's HSD test at P <0.05.

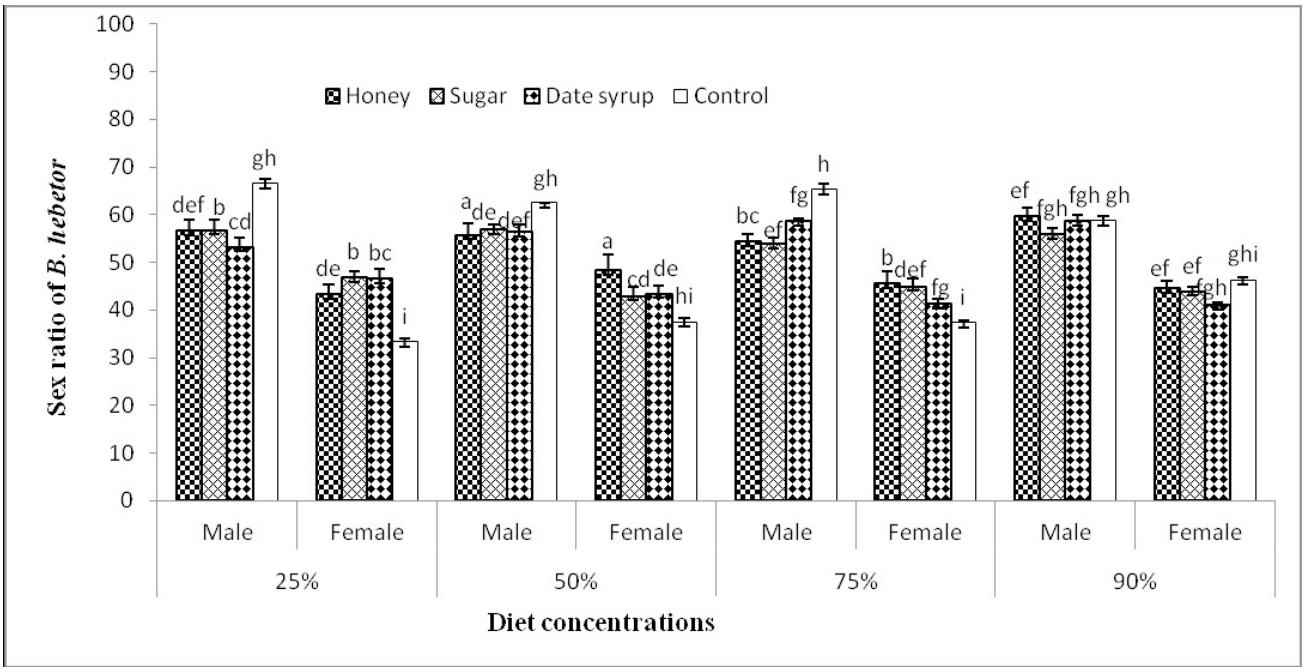

Fig. 1. Effect of different diets on sex ratio of the parasitoid, *Bracon hebetor.*

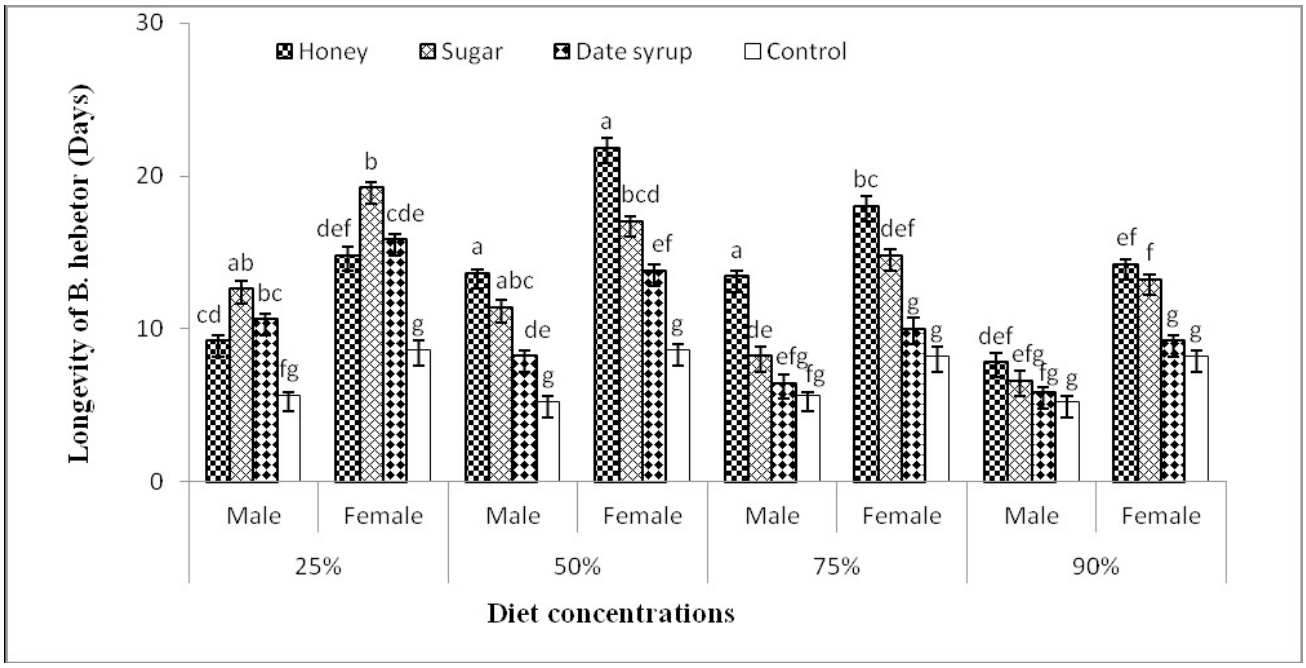

Fig. 2. Effect of different diets on longevity of male and female of the parasitoid, *Bracon hebetor.*

Longevity of Bracon hebetor *on different diets*

Figure 2 indicates the mean value of longevity of male and female of *B. hebetor* in response to different diets. Maximum female and male life span (21.8±0.66 and 13.6±0.24 days, respectively) was recorded when parasitoids fed on 50% honey solution than those fed on other concentrations of honey and of other diets. In case of sugar feeding parasitoids, female showed longer life span of 19.2±0.37 days and male had longer life span of 12.6±0.50 days at 25% concentration. Date syrup feeding female and male parasitoids showed maximum of 15.8±0.37 and 10.6±0.4 days longevity respectively at 25% concentration. More dilutions in the case of all treatments caused decline in the longevity of parasitoids.

DISCUSSION

Several studies have shown that the survival, longevity, sex ratio and fecundity of parasitoids largely depend upon diet. Many authors have reported that supply of an artificial source of food is very important and beneficial for the use of parasitic wasps in agricultural systems (Heimpel *et al*., 1997a).

Our results show that different solutions of honey, sugar and date syrup have significant effect on longevity, fecundity and female emergence of *B. hebetor*. Parasitoids fed on 50% honey solution lived relatively longer and deposited more number of eggs than those fed on all other solutions. This indicated that 50% honey was a better diet for *B. hebetor,* its longevity and fecundity decreased with increase or decrease in honey concentration. This was probably due to the sugar and water content of the honey (Barbehenn *et al*., 1999). Lower concentration of honey contains more water than carbohydrates while higher concentration of honey contains more energy than water, *i.e.* too much energy or lack of water which will shorten the parasitoid longevity. Similar results were reported by Leatemia *et al*. (1995) in which the longevity of females *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) fed on 20% honey was 26.5±5.4 days, on 40% honey it was 28.5±5.4 days, on 60% honey it was 25.4±6.1 days and on 80% honey it was at 20.7±6.1 days. Temerak (1983) showed that best diet provided to parasitoids to elongate their life span was honey. After honey, maximum egg laying, female emergence and longevity of *B. hebetor* was recorded after feeding on sugar followed by date syrup.

It was interesting to note that honey, sugar and date syrup solution caused the parasitoids to produce more eggs and also allowed them to live longer. Similar results were reported for *Meteorus pulchricornis* (Wesmael) in which the sugar concentration maximized lifespan as well as lifetime progeny production (Wu *et al*., 2008). In addition, Karimi and Hatami (2010) reported that fecundity of honey fed adults increased approximately four folds compared to unfed females. Adults fed on

honey showed highest percent emergence over unfed females. Paraiso *et al*. (2011) recommended provision of honey to *Trichogramma* females in mass rearing programs to establish a sustainable population of *T. fuentesi* in the laboratory. It was also reported that mean time to 50% survival of unfed parasitoids *T. platneri*, was 2.0±0.1 days, whereas honey-fed parasitoids lived significantly longer (2.6±0.1days) (Mansfield and Mills, 2002). Our findings also showed that honey fed parasitoids lived longer than those provided with water only. Figure 1 showed that male emergence in all the treatments were more as compared to females. Kuhlmann and Mills (1999) also reported that females of *Trichogramma* species with longer longevity are known to produce more males, probably, due to sperm depletion. But sex ratio remained the same (more number of males than females) in all treatments which are in contrast to Onagbola *et al*. (2007) who reported that feeding on sugar-rich foods alter the sex ratio of progeny.

The composition and concentration of sugars may affect the longevity and number of offspring of parasitoids (Fuchsberg *et al*., 2007; Wu *et al*., 2008), but the effects may differ among parasitoid species (Lee *et al*., 2004; Chen and Fadamiro, 2006; Wyckhuys *et al*., 2008). Our findings also showed that as the concentrations of honey, sugar and date syrup change, longevity and number of adults emerged also change (Table I).

The present results are mostly in accordance with the above reported findings as mentioned by Harvey *et al*. (2012) that wasps fed on glucose, as well as on a honey imitating solution that contained approximately the same sugars in similar relative concentrations found in honey, produced only half of the progeny compared to wasps fed on honey. Shaw (1997) stated that honey is an efficient diet for parasitic wasps as it is for bees. It contains proteins and vitamins as well as sugar and seems to be adequate for egg maturation for most, possibly all species that nourish and mature their eggs successively through their adult life.

CONCLUSION

It may be concluded that 50% honey solution was found to be a better food than other concentrations of honey or other diets (sugar, date syrup and control) evidenced that it relates to parasitoid longevity and fecundity increment. A useful information has been generated which may be exploited in mass rearing programmes of the wasp *B. hebetor* for successful implementation of biocontrol strategies for the suppression of pest insects of Pyralidae

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Statement of conflict of interest

Authors have declared no conflict of interest.

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