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Original Article

Refinement of Rearing Technique of a Potent Larval Parasitiod *Bracon hebetor* (Say), (*Braconidae: Hymenoptera*)

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Abstract: *Bracon hebetor* is a larval parasitoid of various lepidopterous insect pests. It was reared in laboratory on large scale on a host, waxmoth, *Galleria mellonella*, at different temperature and humidity percentages. *Galleria mellonella* was reared on an artificial diet at 30 ± 1°C and 75 ± 5 % R.H. It was observed in the experiment for storage of pupae that the number of surviving pupae decreased as the storage periods increased. However, the percentage of emergence reduced from 90 to 86% on 15 °C for the 1st and 2nd weeks, respectively. The study on reproductive and post-reproductive life (PRL) of the female prarasitoid revealed that the longevity ranged from 17 to 32 days with an average of 23.17 ± 3.80 days. It is assumable that the observed increase in PRL, with host density, was due to the females, obtaining more energy by taking more frequent blood-meals from greater number of hosts.

Keywords: Refinement, mass rearing, larval parasioitd, *Bracon hebetor* (Say)

INTRODUCTION

Bracon hebetor, is an ectoparasitoid of the larvae of many pyralid pest-species, that attack stored grains [1]. The parasitoid is considered to have a potential for the biological control, of many other lepidopterous pests, of various crops, because it is highly aggressive [2]. It occurs, naturally, throughout the world. There is a growing evidence that *B. hebetor* can also be an important bio-control agent of *Helicoverpa armigera* [3]. It is used in Turkmenistan, on cotton and to a lesser extent, in Uzbekistan, where they rely more on *Trichogramma pintoi* [4]. It attacks the larval stage of stored grain pyralid moths, such as, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) [5]. It has been widely used in studies of host parasitoid interactions, because of its high reproductive rate, short generation time and considerable range of host species [6].

The parasitoid, *B. hebetor* has a great potential as a bio-control agent of the stored product moth [7]. The biology of *B. hebetor*, has been intensively studied because of it's suitability as a model organism: which is easy to rear in the laboratory and has also a great potential for being used as a biological control agent of many lepidopterous pests. *Bracon hebetor*, was used to control the *Ecomyelios ceratoniae*, on dates, in warehouses. It has also been found, that it was very active, in the field, to control certain lepidopterous pests [8]. *Bracon hebetor* (Say), while attacking the larvae of *Plodia interpunctella* (Hübner), also showed similar ovipositional-behavior, related to the host-size, depositing more eggs on larger hosts, than on smaller ones. Presumably, the parasitoid is adjusting clutch-size to the nutritional value of the host, thereby avoiding larval competition, among its progeny [9].

The storage of the parasitoid in the form pupae is very important for its timely releases. Similarly the reproductive and post-reproductive life span of the female is very important for the laboratory rearing of the parasitoid. This information is very helpful in the mass rearing of the insects, especially the bio-control agents. The present research experiments were conducted to study the storage of parasitoid pupae at different temperatures for different time periods in order to determine the suitable temperature for storage. The experiments also included the study of reproductive and post-reproductive life span of the adult females.

MATERIAL AND METHODS

The research study was conducted in a laboratory at the Department of Agri. Entomology, University of Agriculture, Faisalabad. The experiments were laid down in

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completely randomized design.

Collection of Insects

(i) *Bracon hebetor* (Say.) (Larval parasitoid)

The adults, were collected, mostly, from the fields of berseem, in the months of January and February. However, the parasitized larvae of the armyworm and american bollworm, were brought to the laboratory, and placed in the vials, for the emergence of parasitoid-adults. The adults, thus, collected were used for the mass-rearing experiments.

(ii) *Galleria mellonella* (Host)

The adults of *Galleria mellonella*, were collected from the apiaries. The infested hives, in the apiaries, were examined for the collection of adults as well as for the larvae. The insects, thus, collected were brought to the laboratory and used for mass-rearing.

Rearing of Insects

(i) *Bracon hebetor* (Say.) (Larval parasitoid)

The adults of *B. hebetor*, were reared on a large scale, by using the larvae of the greater wax moth, *Galleria mellonella* (L.), as a host, at 25 ± 1 °C, 65 ± 5% R.H and 16:8 LDHS. A number of 2 to 3 larvae, of the 3rd or 4th instars, of *G. mellonella*, were placed in the glass-vial, with a fertilized female of the parasitoid, *B. hebetor* (Say.). The female, was provided with a cotton swab, soaked in 20 % honey-solution, for feeding the parasitoid. After 24 hours, the females were shifted to the other vials, with a lot of the newly emerged larvae of the host, *G. mellonella* and honey-soaked, cotton-swab. This process was continued up to the end of female parasitoid's life. The parasitized larvae of the host, *G. mellonella*, were incubated, under the optimum conditions of 25 ± 1 °C of temperature and 65 ± 10 % of the relative humidity.

(ii) *Galleria mellonella* (L.), (host)

The adults were released in plastic jars (dia, 5 cm and depth 30 cm) for mating and egg collection and provided with the artificial diet/folded card sheets, for feeding as well as for egg-laying. The larvae of *G. mellonella*, collected from the field as well as those hatched from the eggs, laid by the adults, on folded sheets, were reared on a semi-natural diet, comprising: 600 g of crushed-wheat grains, 150 g of the Honeybee-wax, 150 g of the yeast, and 100 ml of the plain/commercial glycerin. These jars, were incubated in the growth chamber, at an optimum condition of 30 ± 1 °C, 75 ± 5 % R.H and 24 hours of DLHS [10]. The larvae, thus, reared were used for the mass-rearing of *B. hebetor*.

RESULTS AND DISCUSSION

Storage of *Bracon hebetor*, Late-age Pupae, at Various Temperatures and for Different Periods

To evaluate the parasitic activity of the parasitoid, *Bracon hebetor*, under low temperature, 3 to 4 day old pupae, were isolated from the laboratory culture, and distributed in the glass jars (dia, 3cm and depth 10 cm), sterilized with dry temperature, by using an electric oven run at 100°C, for 4 hours. Each jar had 50 pupae, which were stored, at 5, 10, 15 and 20°C for 1, 2, 3 and 4 weeks, respectively, using four replicates for each treatment.

The results, showed that the number of adults (females and males), emerged from the stored pupae, decreased as the stored periods increased, within the same temperature, and this also reflected upon the emergence percentage which ranged from 94 to 68% on 5°C for the periods of 14 to 4 week, respectively, 94 to 72% on 10°C and 90 to 86% on 15°C for the first and second weeks, respectively. The percentage of adult emergence reached 96% for the first week, on 20°C. Those results were supported by the studies of Farghaly and Ragab [11] as well as by those of Shawkit *et al*. [12], who emphasized that the percentage of the adults-emerged, from the stored pupae of this parasitoid, at the low temperature decreased as the storage period increased, within the same temperature. Data about the sex-ratio, revealed that the percentage of the males emerged, was always more than that of the females. These results were supported by Al-Ramahi and Ali [13], which indicated that the sex ratio of *B. hebetor* was 1:1.3 (F:M) as well as by Ahmed *et al.* [14], who pointed out that the sex ratio was 1:3.98 (F, M). This, was explained, by them, to be due to the differences in temperature and host-density; while, it was thought by Benson [15] to be due to the death factor, as the Male eggs, will hatch before the female eggs and due to this, the males larvae will have a better survival due to the better availability of food, around.

The results of the study, in this case, however, differ from those of Reinert and King [16], who reported that the sex ratio of *B.*

hebefor was 2:1 (female, male). Data about the adult longevity, showed that the longevity of females and males decreased as the stored periods, of the pupae increased, at the same temperature, and within all the temperatures. These results were supported by the studies of Farghaly and Ragab [11] and Shawkit *et al*. [12], who stored the pupae of *B. hebetor*, for 10, 15 and 30 days, respectively. These results also showed, that the number of the larvae, which developed from the eggs laid by the females, decreased as the storage-periods and the temperature of the pupae, increased. These results were supported by the studies of Farghaly and Ragab [11], who reported that, the number of the larvae were reduced to 43.03 larvae after 4 week, at 5°C from 66.3 at 10°C, and to 52.61 larva, at 15°C, after the same period. Finally, Table 1 showed that there existed a negative relationship between the number of the parasitized larvae by the females, which developed from the stored pupae, at various temperatures and for different storage periods. The results, in this case, were supported by those of Shawkit *et al*. [12], who reported that, the females which developed from the stored-pupae at 15°C, for 2 weeks, can parasitize 48.16 larvae. These conclusions were further supported by Farghaly and Ragab [11], who indicated that the number of parasitized larvae per-female - developed from stored pupae, at 5°C, for 4 weeks - were 20.03, 22.01 and 47.86 larvae per-female-developed from pupae, stored at 10 and 15°C, for 4 weeks, respectively.

Table 1. The adult emergence, etc. of *B. hebetor* (Say), from its late pupal-stage, at different storage periods and temperatures.

Temp. (ºC)	Storage Period (weeks)	No. of storage pupa	No. of adults emerged			Sex ratio		Longevity of adults (days)		No. of larvae developed/ female	No. of larvae parasitized/ female
			F	M	%	F	M	F	M		
5	1	50	19	28	94	40.42	59.57	17-28 23.94a*±2.71	12-18 13.36b±2.01	39-109 76.94a±7.82	25-60 43.05d±5.24
	2	50	20	25	90	42.22	55.77	12-17 14.31c±2.09	6-8 6.76d±1.05	32-102 68.57c±9.86	25-52 36.07ef±6.23
	3	50	17	21	76	44.73	55.26	6-9 7.29d±2.11	4-7 5.0e±1.00	35-107 64.58d±8.75	20-39 27.05g±3.99
	4	50	16	18	68	47.05	52.94	3-6 6.81d±0.01	2-4 2.88f±1.01	23-77 47.25g±5.25	12-25 19.06h±1.09
10	1	50	18	29	94	38.29	61.70	16-27 23.22a±5.25	13-19 15.86a±2.06	33-103 72.5b±9.99	31-103 62.66b±8.15
	2	50	19	24	86	44.18	55.81	13-20 16.05±4.09	13-20 14.29ab±2.01	32-107 69.73c±9.45	22-52 32.94f±2.13
	3	50	16	23	78	41.02	58.97	7-12 9.06±2.13	6-8 6.88d±1.09	35-93 59.93e±8.76	17-35 27.06g±2.19
	4	50	14	22	72	38.88	61.11	6-8 5.00b±3.19	6-88 4.86e±.76	32-74 53.07f±9.82	13-27 21.09h±1.99
15	1	50	19	28	90	39.13	60.86	19-25 21.05ab±7.1	9-20 13.32b±1.07	37-92 65.38d±4.79	32-102 66.77ab±9.85
	2	50	17	26	86	44.18	55.81	14-20 14.57c±2.09	6-16 10.70c±0.81	38-83 54.63f±5.62	37-73 48.68cd±7.52
20	1	50	21	27	93	43.75	56.25	17-27 21.14ab±2.0	13-18 14.40ab±3.15	32-83 61.80e±9.12	43-92 70.71a±7.73

*Means in a column not sharing a common letter are statistically different (P<0.05)

Reproductive and post-reproductive life of the *Bracon hebetor* (Say)

Reproductive and post-reproductive life of the female of the parasitoid, *Bracon hebetor* (Say) was also studied. 30 repeats of female, were tested in this experiment, to evaluate some of the life-span parameters. In this study, the reproductive and post-reproductive life of the parasitoid females, when they completed their egg-layings and started parasitizing the larvae of the host, upon the end moment of her life, were evaluated.

The results showed that the longevity of females ranged from 17 to 32 days with an average of 23.17 ± 3.80 days (Table 2). This was supported by the studies of Farghaly and Ragab [11], who found that the longevity of the female of *B. hebetor* was extended upto 30 days, when they were fed on 20% honey; solution and the number of the eggs laid, per female, ranged from 43 to 160, during the reproductive-life (R.L.). They also found that the number of parasitized larvae per-female, was 75.09 ± 1.93. The current study showed that the duration of egg laying per-female, ranged from 13 to 25 days, with an average of 17.96 ± 3.76 days. These results were supported by the studies of Clark [17], who reported that the females continued laying eggs on the host, during its RL, which was extended upto 27.8 ± 1.3 days; while, they differed from the findings of Hagstrum and Smittle [18], who reported that the female of *B. hebetor* did parasitize the host-larvae, during RL but did not oviposit. These results, also showed that the number of parasitized larvae per-female was 26.3 ± 2.87 and that the post-reproductive life PRL extended upto 5.13 ± 5.70 days. These results, were supported by the studies of Jervis *et al*. [19], who reported that the females can live only for few days, after the completion of egg-laying and that they continued paralyzing the host-larvae. He further explained, that, during PRL, females presumably maintain themselves by host-feeding and that had no quantitative data, on the frequency of host-feeding by females, during PRL. Thus, it is reasonable to assume that the observed increase in PRL, with host density, was due to the females, obtaining more energy by taking more frequent blood-meals from the greater number of hosts. He also mentioned that the PRL in *B. hebetor,* can occurre even at the lowest level of host-availability [20]

Table 2. The reproductive and post-reproductive life of *B. hebetor* (Say), at different biological parameters, under laboratory condition (29±1 °C, 65±5% R.H., 16-18 (L.D).

Biological parameter	Reproductive life/ female (RL)		Post-reproductive life/ female (PRL)	
	Range	Mean ± SD	Range	Mean ± SD
Longevity of female per day	17-32	23.17±3.80	17-32	23.17±3.80
No. of eggs laying/female	37-153	81.63±28.29	0-0	0.00±0.00
No. of larvae parasitized /female	25-109	73.37±3.38	12-52	26.3±2.87
Eggs laying duration per day	13-25	17.96±3.76	-	-
Post-reproductive duration	-	-	2-7	5.13±5.70

Influence of Two Type of Diets on the Fecundity of *B. hebetor*

To improve the colony of the parasitoid, *B. hebetor* (Say), for the mass rearing, two types of diets of 20% honey and 10% sugar, were tested, in order to evaluate their impact on the fecundity of the females, and to choose the best out of them, for the mass rearing of the parasitoid, under laboratory conditions, maintained at 29 ± 1°C and 65 ± 5% RH, with 16.8 LD hours.

The impact on some biological parameters of *B. hebetor*, feeding on two types of diets (Honey solution 20% and Sugar solution 20%), at 29 ± 1°C and 25 ± 1°C, is reported in Table 3. The results were revealed that the level of all the biological parameters, which were studied in this experiment, increased by feeding the adults (females) on 20% honey solution, at 29 ± 1°C, or, in other words, there was a positive correlation between the use of 20% honey solution at 29 ± 1°C and change in the studied biological parameters. These results were supported by the studies of Radhika and Chitra [8], who reported that using the honey solution for feeding the females of *B. hebetor*, caused, an increase not only in the number of eggs laid but also in the larvae, as well as the pupae and adults numbers developed *vis*-a-*vis* increasing the longevity of the adults over two generations. Clark [17] also indicated that the longevity of the adults was extended upto 27.8 ± 1.3 days,

Table 3. biological parameters of the parasitoid, *Bracon hebetor* (Say) (females), fed on the honey and sugar solutions, at various incubation conditions.

	Type of diet with incubation condition	No. of eggs/ Female	Eggs incubation (Day)	No. of larvae developed/ female	Larval duration (Day)	No. of pupae developed/ female	Pupal duration (Day)	No. of adults developed/ female F	No. of adults developed/ female M	Sex ratio F	Sex ratio M	Longevity of adults F	Longevity of adults M	No. of larvae parasitized/ female
Honey solution 20% 29±1ºC	Range	37-162	2-3	32-157	2-4	28-142	3-5	11-64	17-78	1:	1.4	19-30	13-20	27-100
	Average	84.10a	2.1a	78.3a	30.06b	69.57a	4.10b	29.03a	42.00a	40.54b	59.46a	25.30a	17.40a	73.63a
Honey solution 20% 25±1ºC	Range	45-104	2-3	39-97	3-5	33-87	5-7	14-36	18-51	1:	1.32	19-28	10-20	27-102
	Average	75.2a	2.13a	68.2a	4.1a	59.23b	6.00a	25.30a	33.93b	42.61b	57.39a	25.37a	13.3b	69.63a
Sugar solution 20% 29±1ºC	Range	25-85	2-3	20-78	2-4	18-72	3-5	6-43	11-43	1:	1.41	14.22	7.12	33.97
	Average	56.96b	2.03a	48.73b	3.1b	41.66c	3..93b	17.30b	24.36 c	41.38b	58.63a	16.27b	9.16c	62.80a
Sugar solution 20% 25±1ºC	Range	27-87	2-3	17-72	3-5	16-72	5-7	11-35	9-45	1:	1.39	12-19	5-12	26-82
	Average	53.76b	2. 23a	47.8b	3.96a	38.1c	5.90a	18.00b	21.1c	45.85a	85.14b	13.90c	7.3 d	40.80b

*Mean in a column not sharing a common letter are statistically different (P<0.05)

when the insects were fed on honey solution. Benson [15] reported that the longevity of the adults of *B. hebetor,* was also, in the same way, extended for 3 weeks. The results were also supported by the studies of Ode *et al.* [5] who indicated that the adults of *B. hebetor*, lived longer, when fed on honey solution and the females had a higher daily and life time fecundities alongwith the males, which copulated with more females; while, Ide and Lanfranco [21] showed that the adults of larval parasitoid, *Orgilus obscurator* Ness (Hymenoptera: Braconidae), when fed on honey-solution, increased the longevity of the adults upto 23 and 33 days, for the males and females, respectively. This ultimately increased all the biological parameters of the parasitoid. Finally, the current study differed with the results, reported by Doten [22], who mentioned that the life span of *B.* hebetor was extended for few months, when it was fed on sugar-solution. In the light of above experiments, it can be concluded that the storage of the parasitoid in the form pupae is very important for its timely releases. The parasitoid pupae can be stored at 5°C for a period of almost 4 weeks. Similarly the reproductive and post-reproductive life span of the female especially is very important for the laboratory rearing of the parasitoid. The most suitable temperature found for the rearing of the parasitoid ranges from 25-30°C.

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