Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

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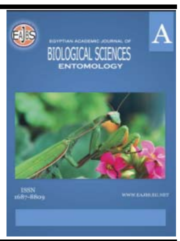
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol.8 (3)pp.69-78 (2015)*

Egypt. Acad. J. Biolog. Sci., 8(3): 69-78 (2015)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
www.eajbs.eg.net



Developmental and Reproductive Biology of the Ecto-larval Parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) on Sesame Capsule Borer, *Antigastra catalaunalis* (Duponchel) (lepidoptera-pyralidae)

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ARTICLE INFO

Article History
Received:1/11/2015
Accepted: 7/12/2015

Keywords:
Bracon hebetor
Antigastra catalaunalis
Preimaginal development
Reproductive biology

ABSTRACT

The parasitoid, *Bracon hebetor* Say (Hymenoptera: Braconidae) is a gregarious larval ectoparasitoid of several lepidopteran species. The reproductive biology of *B. hebetor* was studied on the larvae of its host, the sesame capsule borer *Antigastra catalaunalis* (Duponchel) (Lepidoptera: Pyralidae) under laboratory condition. The obtained results revealed that the total preimaginal period of *B. hebetor* on *A. catalaunalis* was significantly affected by ambient temperature being shortest at 35°C (6.9 days) and longest (12.5 days) at 25ºC. Mating status had a significant effect on the ovipositional periods, fecundity and longevity being 154.9 and 105.8 eggs /female in mated and virgin females, respectively. Oviposition pattern for mated females showed a gradual increase then declined as females aged with only one maximum peak 12 days post emergence with greatest mean daily fecundity of 21.4 eggs/female/day. The supplemental food had a significant effect on adults of *B. hebetor*. Females of *B. hebetor* fed with pure honey together with the host larvae of *A. catalaunalis* parasitized a higher number of host (23.3 larvae) and laid a higher number of eggs per female (154.9 eggs). The numbers of paralyzed and parasitized hosts, number of daily laid eggs/host instar, longevity and percentages of emerged wasps were significantly affected by host instars. *B. hebetor* paralyzed all larval instars of *A. catalaunalis* but parasitized only third, fourth and fifth instar larvae. The fifth larval instar of *A. catalaunalis* was the most suitable instar for *B. hebetor* larval development than earlier instars as indicated by the highest total lifetime fecundity/female of 154.9 eggs and the highest percentage of emerged wasps of 93.5%.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an old and important oil seed crop being cultivated in Egypt and tropics, subtropical regions of the world (Seegeler, 1983; Iwo *et al.*, 2002; Mahmoud, 2013). Sesame is considered to have both nutritional and medicinal values. It is clear that the increase in sesame production during last decade was mainly due to the increase in its growing area, especially in newly reclaimed sandy soils (Mahmoud, 2013).

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol.8 (3)pp.69-78 (2015)*

Sesame is attacked by different insect pests during its different growing stages, but sesame leaf webber, and capsule borer worm, (*Antigastra catalaunalis* Dup.) is considered one of the key pests of sesame in Egypt. It is very devastating pest of sesame crop and damages the crop from seedling to flower and capsule stages by its devastating larval stage, which, web the top leaves, bores into the pods and shoots, and causes significant yield loss, (Suliman *et al*., 2004; Narayanan and Nadarajan, 2005; Ahirwar *et al*., 2010).

To control this pest, farmer use chemical insecticides, which could be toxic to natural enemies, thus disturbing the biological balance in the field and contaminating the environment. Sesame capsule borer has several natural enemies (Kumar and Goel, 1994; Muralibaskaran *et al*., 1990). Thus, the use of natural enemies for sesame capsule borer management is a very important strategy. There are various groups of biocontrol agents; the braconid parasitoids are the second most important family of parasitoid wasps in biological control and have been introduced in successful IPM programs (Greathead, 1986).

The braconid wasp genus *Bracon* is a synovegenic, ecto-parasitoid that attacks larvae of several species of Lepidoptera, mainly pyralid moths. *Bracon hebetor* Say (Hymenoptera: Braconidae) is a highly polyphagous gregarious ecto-parasitoid of several species of lepidopteran larvae (Magro and Parra, 2001; Jhansi and Babu, 2002; Fagundes *et al*., 2005; Kyoung *et al*., 2008). It attacks a variety of important lepidopterous stored product and of field crop pests (Athanassiou and Eliopoulos, 2003; Gupta and Sharma, 2004). *B.hebetor* was found to be very effective against *A. catalaunalis*, (Jakhmola, 1983). *B. hebetor* females first paralyze their host larvae by stinging and then laying variable numbers of eggs singly on or near the surface of paralyzed hosts (Antolin *et al*., 1995). The paralyzed host larvae are then used as food sources for developing wasp and also for the adult females. A good understanding of host-parasitoid association is crucial to the success of biological control programs. A host's value to the reproductive fitness of a parasitoid mainly depends on the number and quality of her progeny producing from that host. Thus, physiological suitability of the host is necessary for the successful development of parasitoid progeny (Wiedenmann and Smith, 1997)

The main objective of this study is to evaluate the developmental and reproductive biology of the ecto-larval parasitoid, *Bracon hebetor* as a biological control agent against the sesame capsule borer, *Antigastra catalaunalis* under laboratory conditions. This study will broaden the knowledge on this species and provide practical information for studies concerning the use of *B. hebetor* in programs of integrated management in sesame plant against *A. catalaunalis*.

MATERIAL AND METHODS

Rearing of *A. catalaunalis*

Samples of sesame branches infested with *A. catalaunalis* larvae were collected from sesame fields at the Experimental Farm, Ismailia Agricultural Research Station, Egypt during 2012 season. The collected larvae were kept in glass jars (20x10 cm) and fed on sesame leaves until pupation under laboratory conditions of 25±2ºC and 60±5%R.H. Pupae were sexed and isolated in tubes (2x10 cm.) until adult emergence. Newly emerged moths were kept in glass jars (20x10cm). Sesame Branches bearing fresh sesame leaves were introduced daily for moths. Deposited eggs were reared and the newly hatched larvae were transferred to another jars and provided daily with fresh sesame leaves until pupation and emergence of adults (Ahirwar *et al*., 2010). Larvae of 5th instar were collected and offered for the parasitoid *B. hebetor* as hosts.

Rearing of *Bracon hebetor*

A laboratory stock colony of *B. hebetor* was established from individuals of the host larvae *A. catalaunalis*, collected from sesame fields at the Experimental Farm, Ismailia Agricultural Research Station, Egypt. Parasitized larvae of *A. catalaunalis* were kept under laboratory condition of 25±2ºC and 70±5%.RH. until adult emergence. Fertilized females were maintained in glass tubes (7x2 cm.) stoppered with a piece of cotton with few droplets of honey as nutrition, *A. catalaunalis* fifth instar larvae were used as hosts. Parasitized hosts were removed daily and placed in Petri dishes that kept until pupation and emergence of adult parasitoids. Healthy larvae were introduced daily to the parasitic adult until the death of all females (Hajar and Parviz, 2013).

Biological studies of *B. hebetor*:

Effect of temperature on preimaginal development of *B. hebetor* on *A. catalaunalis*

The effect of different temperature regimes (25, 30 and 35ºC) on the biology of *B. hebetor* was studied under laboratory conditions of 60± 5 % R.H. Twenty five full-grown larvae of *A. catalaunalis* were placed in a Petri dish (diameter 9 cm.) with five pairs *B. hebetor*. Small droplets of honey were put on the inner wall to serve as food. After parasitization, larvae were collected and placed individually into Petri dishes. Only one wasp egg was left on each host as replicate. Observation took place every day under binocular microscope to determine the developmental periods for different stages of *B. hebetor* until adult emergence**.**

Effect of mating on ovipositional periods, fecundity and longevity

The ovipositional periods, number of paralyzed and parasitized larvae, longevity and the number of eggs laid per mated and virgin female of *B. hebetor* were studied during its life span at 30ºC and 60 ±5% R.H. Pupal stages were placed individually in Petri dishes until they reached adulthood. Upon adult eclosion, males and females were kept individually isolated for the unmated cohorts. To obtain mated *B. hebetor*, individual males and females were placed together for mating for six hours then males were removed so that the longevity of individual mated predators could be determined as was done for the unmated ones. Twenty mated and virgin females (within 24 hours) were placed, each, in a Petri dish (9 cm in diameter) with droplets of honey to serve as food. Each female was confined with five fifth instar larvae of *A. catalaunali*s for 24 hours. The number of paralysed larvae was calculated. Paralysed larvae included parasitized and unparasitized larvae. The parasitized host larvae were collected and replaced with another set of unparasitized larvae daily until the death of female parasitoids. The removed parasitized host larvae were investigated under binocular and the deposited eggs were counted. Longevity of *B. hebetor* adults was determined for mated and unmated females and males.

Effect of different supplemental diets on adult stage

Longevity and oviposition capacity of *B. hebetor* females under different supplemental diets in presence of the host larvae *A. catalaunali*s at 30ºC, 60± 5% were evaluated using 4 treatments. These food treatments were pure honey, honey solution 50%, water and starvation. Each treatment arranged; twenty mated female wasps at the first day after emergence, separated into Petri dishes each contained five host larvae of 5th instar *A. catalaunali*s. Unparasitized host larvae were replaced every day until females of the parasitoid died. Longevity and oviposition capacity of *B. hebetor* (paralyzed larvae, parasitized larvae and number of eggs laid per female) was recorded.

Effect of host instar on *B. hebeto*r

Every instar from the first to fifth larval host of *A. catalaunali*s was arranged to contact with *B. hebetor* mated females (within 24 hours) during its life span to assess suitability of the larval age of *A. catalaunali*s to parasitism. Ten mated females were introduced singly into Petri dishes containing five individuals of the same age for host larval instars. Healthy host larvae were replaced every day throughout their life time. Number of host paralysed and parasitized, number of wasp emerged, longevity of females, percent of wasp emerged from every instars were recorded.

Statistical analysis

All experiments were subjected to analysis of variance (ANOVA), using Co Stat6311 Windows Computer Program. The data on preimaginal development, ovipositional periods, fecundity and longevity of mated and virgin females, Longevity of *B. hebetor* adults under different supplemental diets and suitability of larval age *A. catalaunalis* to the parasitoid were analyzed by one-way ANOVA's, and compared using Duncan's Multiple Range Tests (DMRT), at 0.05 level of significance.

RESULTS

Effect of temperature on preimaginal development of *B. hebetor* on *A. catalaunalis*

Data in Table (1) indicated that the developmental time of *B. hebetor* decreased significantly as temperature increased. Moreover, *B. hebetor* developed successfully when reared under the three tested temperature; being shortest (6.9 days) at 35ºC and longest (12.5 days) at 25ºC. Obviously, significant differences were found in developmental time for all stages of *B. hebetor* among the tested temperature regimes. The total preimaginal period was also significantly affected by temperature.

Table1: Effect of temperature on preimaginal development of *B.hebetor* reared on *A.catalaunalis*

Tem.	Egg period/day	Total Larval period/day	Prepupal period/day	Pupal period/day	Total dvelopmental period (Egg-Adult)
25 ºC	1.7±0.02[a] (1.5-1.8)	4.0± 0.04[a] (3.9-4.5)	0.9 ± 0.01[a] (0.8-1.0)	5.8±0.1[a] (5.0-7.0)	12.5 ± 0.1[a] (11.2-14.1)
30ºC	1.1±.0.02[b] (1.0-1.2)	3.0± 0.03[b] (3.0-3.5)	0.7±0.01[b] (0.6-0.8)	4.4 ±0.1[b] (4 .0-5.0)	9.3 ± 0.08[b] (8.6 - 9.9)
35ºC	0.7 ±0.03[c] (0.7-0.9)	2.3 ±0.02[c] (2.1-2.5)	0.5±0.01[c] (0.5-0.6)	3.3±0.01[c] (2.0-4.0)	6.9 ± 0.03[c] (5.3 - 7.8)
F value	359.6	632.0	205.0	82.675	361.2

Means in the same column followed by different letters are significantly different ($p < 0.05$) according to Duncan's Multiple Range Tests (DMRT)

Effect of mating on ovipositional periods, fecundity and longevity

Data presented in Table (2) indicate that the mean duration of pre- ovipositional period of *B. hebetor* on *A. catalaunali*s varied from (0.7 day) in mated females to (0.9 days) in virgin females. The mean ovipositional periods were 18.6 and 16.3 days in mated and virgin females, respectively. The mean duration of the post ovipositional periods were (4.0 day) in mated and (5.0 day) in virgin females. The mean total number of paralyzed larvae by the female was 34.2 and 29.6 larvae in mated and virgin females, respectively. Whereas the mean total number of parasitized larvae was 23.3 larvae in mated females and 20.6 larvae in virgin females. The mean total number of eggs laid per female during its life span was 154.9 eggs in mated females and 105.8 eggs in virgin females. Mated females lived longer (23.3 days) than virgin (21.7 days). Longevity of males was longer in unmated (8.4 days) than in mated individuals (6.8 days).

Table 2: Ovipositional periods, number of paralyzed, parasitized host larvae and fecundity of mated and virgin females of *B. hebetor* reared on *A. catalaunalis* at 30ºC, 60 ± 5% R.H.

Treatment	Ovipositional Periods/ days			Paralyzed host larvae	Parasitized host larvae	Total eggs /female	Daily eggs /female	Longevity/day	
	Pre	Ovi	Post					Female	Male
Mated	0.7±0.01[b] (0.6-0.8)	18.6± 0.4[a] (15.4-5.3)	4.0±0.1[b] (3.0-5.0)	34.25±07[a] (30-40)	23.3± 0.7[a] (20-30)	154.9±3.9[a] (124-200)	8.165±0.1[a] (6.6-9.4)	23.3± 0.4[a] (20-30)	6.8± 0.1[b] (6.0-9.0)
Virgin	0.9±0.02[a] (0.7-1.0)	16.3± 0.4[b] (14.0-0.1)	5.0±0.1[a] (4.0-6.0)	29.6± 0.7[b] (25-35)	20.6± 0.8[b] (15-30)	105.8±2.8[b] (80-125)	6.5±0.1[b] (5.2-8.5)	21.7±0.4[b] (18-25)	8.4±1.8[a] (7.0-0.0)

Means in the same column followed by different letters are significantly different (P< 0.05).

Oviposition pattern of *B. hebetor* parasitizing larvae *A. Catalaunalis*

Oviposition was not constant over time. Oviposition patterns for mated females showed a gradual increase then declined as female aged. The oviposition rhythm observed (*n*=20 females) reached its highest level at the day 12 of age, with maximum oviposition between (8 and 6) days of females age. The greatest mean daily fecundity, averaged over female lifetime (21.4 eggs/female/day) (Fig.1).

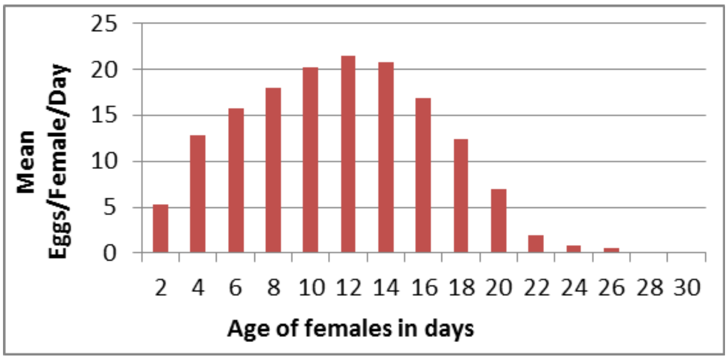

Fig. 1: Oviposition rhythm of *B. hebetor* parasitizing larvae of *A. catalunalis*

Effect of different supplemental diets on adult stage

As shown in Table (3); significant differences in female longevity were observed among the different supplemental diets. The adults of *B. hebeto*r that were fed with pure honey together with *A. catalaunalis* host larvae, lived longer than those fed 50% honey solution, water and starvation with the same host larvae.

The shortest lifetime for females was 9.5 days for those starved with the host larvae, whereas the longest was 23.3days recorded for those fed pure honey with the host larvae. Adults that were fed with pure honey, parasitized a higher number of hosts and laid a higher number of eggs (23.3 larvae and 154.9 eggs) than those fed 50% honey solution (23.0 larvae and 130.2 eggs). The respective values for those fed on water were (7.0 larvae and 44.6 eggs) or starved individuals with only host larvae (6.8 larvae and 40.9 eggs). Statistically the effect of supplemental nutrition on wasp longevity was significant, ($P < 0.05$).

Table 3: Longevity of *B.hebetor* adults under different supplemental diets in presence of the host larvae of A. *catalaunalis* at 30 ºC, 60 ± 5% R.H.

Diet	Longevity	Oviposition Capacity		
		No. of paralyzed larvae	No. of parasitized larvae	Total no. of eggs laid /Female
Starved	9.5 ±0.3 (7 -12)[d]	9.4 ± 0.1(8 -10)[c]	6.8±0.1 (4 – 8)[b]	40.9±1.9 (30-61)[c]
Water	11.7 ±0.4(10-16)[c]	13.7±0.6 (8 -12)[b]	7.0±0.1(6.0-8.0)[b]	44.6±2.1(32-65)[c]
Honey solution	21.0 ±0.3(18-23)[b]	33.5±0.7(30-38)[a]	23.0±0.5(20-26)[a]	130.2±4.4(90-170)[b]
Pure honey	23.3 ±0.4(20-30)[a]	34.2±0.7(30-40)[a]	23.3±0.7(20-30)[a]	154.9±3.9(131-200)[a]
F value	255.6	401.9	386.1	304.2

Mean values followed by the same letter within a column do not differ significantly at P < 0.05 by DMRT.

Effect of host instar on *B. hebeto*r

Paralysis of hosts by *B.hebetor* females was significantly affected by host instars. Data presented in (Table 4) indicated that the 1st and 2nd larval instars of *A. catalaunalis* were not suitable for development of *B. hebetor* immature stages. Mated females of *B. hebetor* could paralyze the first and second larval instars but refrained to parasitize them. The mean paralysed 1st and 2nd larval instars of *A.catalaunalis* was 6.7 and 10.2 larvae and the longevity of mated females of *B. hebetor* on those instars was 8.3 and 10.0days, respectively. Although mated females of *B. hebetor* could paralyze and parasitize the third larval instar at respective values15.8 and 3.8 larvae, but this was accompanied by low fecundity (25.1 eggs) and lower emergence rate of emerged wasp 29.4%. The fourth and fifth larval instars of *A. catalaunalis* appeared to be suitable for *B. hebetor* larval development than earlier ones. On fourth larval instar, the mean paralyzed, parasitized, total no. of eggs/female and percent of emerged wasps were 24.6 larvae, 14.8 larvae, 110.9 eggs, and 78.5%, respectively. Data further revealed that the fifth instar was the most suitable larval instar for *B. hebetor* larval development as indicated by highest total number of laid eggs per female (154.9 eggs) and the highest percentage of emerged wasps (93.5%).

Table 4: The suitability larval age of *A. catalaunalis* to the parasitoid *B. hebetor* at 30 ºC, 60 ±5% R.H.

Host larval instar	No. of paralysed host	No. of parasitized host	Total no. of eggs/female	Total no. of wasp emerged	Emerged wasp /host	Longevity of female	Emerged wasp (%)
1st instar	6.7±0.6[e]	0.0±0.0[d]	0.0±0.0[d]	0.0±0.0[d]	0.0±0.0[c]	8.3±0.3[d]	0.0±0.0[d]
2nd instar	10.2±0.8[d]	0.0±0.0[d]	0.0±0.0[d]	0.0±0.0[d]	0.0±0.0[c]	10.0±0.3[d]	0.0±0.0[d]
3rd instar	15.8±1.1[c]	3.8±1.0[c]	25.1±7.1[c]	11.8±3.5[c]	2.0±0.6[b]	12.7±1.4[c]	29.4±9.0[c]
4th instar	24.6±1.2[b]	14.8±0.9[b]	110.9±3.3[b]	87.8±3.6[b]	6.2±0.3[a]	18.1±0.8[b]	78.5±1.2[b]
5th instar	34.2±0.9[a]	23.3±1.0[a]	154.9±6.9[a]	144.9± 6.8[a]	6.3± 0.4[a]	23.3±0.9[a]	93.5±0.6[a]
F value	119.6	164.2	224.2	291.7	70.9	43.3	112.8

Mean values followed by the same letter within a column do not differ significantly at P < 0.05 by DMRT.

DISCUSSION

Temperature is a critical abiotic factor influencing the dynamics of insects and their natural enemies. Knowledge on adaptations of the natural enemies to climatic conditions plays an essential role in pest management (Obrycki and Kcring, 1998; Thanavendan and Jeyarani, 2010). In the present study, the egg-to-adult developmental periods for *B. hebetor* varied significantly with the temperature. The preimaginal development of *B.hebetor* was temperature dependent, with development being significantly faster at 35°C than at the lower tested temperatures. Similar findings were reported for *B. brevicornis* on different host larvae (Thanavendan and Jeyarani, 2010) and *B. kirkpatricki* on *Spodoptera exiguae* (Engroff and Watson, 1975) that the life cycle is very short at 35°C, while at 20°C it was prolonged. This conclusion goes with that observed in the present study, where the life cycle of *B. hebetor* was greatly influenced by the change in temperature regimes.

Mating status had significant effects on the ovipositional periods, fecundity and longevity. Mating status affected the ovipositional periods of *B. hebetor* and pre and post oviposition periods were shorter in mated than in virgin females while the oviposition period was longer in mated than in virgin ones. *B. hebetor* unfertilized eggs develop as haploid males, but fertilized eggs are diploid and can develop into males or females (Alisha *et al*., 1999). Also mating of *B. hebetor* had significant effect on paralyzed and parasitized host larvae of *A. catalaunalis*. *B. hebetor* females first paralyze their host larva by stinging and then laying variable numbers of eggs singly on or near the surface of paralyzed hosts (Antolin *et al*., 1995). The paralyzed host larvae are used as food sources for developing immature wasps and also for the adult females, this could be because *B. hebetor* females continually produce eggs throughout their lifetime (synovigenic) and reproductive females are engage in host-feeding, which is essential for the maturation of additional eggs (Benson, 1973; Rosenheim and Rosen, 1992; Redolfi and Campos,2010).

Oviposition was not constant over time. Oviposition patterns for mated females increased gradually then declined as females aged. The daily oviposition rhythm proved uniform in its rise and fall, with only one maximum peak at 12 days of age without pronounced fluctuations, in comparison with other ectoparasitoid species (Redolfi *et al*., 1987). Newly emerged females contain very few eggs and need three to four days of maturation and host-feeding to attain their maximum daily eggs production (Petters and Grosch, 1977). The results indicate that in programs of biological control, depending on the food resources within the agricultural ecosystem, females *B. hebetor* should be released at 2 to 8 days of age.

Longevity and fecundity of female wasps are two decisive factors for the effectiveness of parasitoid species as biological control agents. Accessibility and suitability of nutrient sources determine parasitoid survival and reproduction. The reproductive success is influenced by supplemental feeding (Thompson, 1999). In nature, there are different types of food that wasps can find and feed for their life and existence such as aphid’s honey dew, honey/nectar from flowers, rain-water or dew-water. Data analysis showed that the effect of supplemental nutrition on *B. hebetor* wasp longevity was statistically significant. Similar result for oviposition capacity of the ectoparasitoid female *Elasmus* sp. fed with pure honey parasitized a higher number with subsequent a higher number of eggs as compared to those fed 50% honey solution and pure water **(**Phan Thanh *et al*.,2011).

Host age /instar is an important factor that effects parasitoid fitness (Vinson 1976) in this study the reproductive biology of *B. hebetor* different when reared on different larval instars of *A. catalaunalis*. Data further indicated that the 1st and 2nd larval instars of *A. catalaunalis* were not suitable for development of *B. hebetor*. Mated females of *B .hebetor* could paralyze the first and second larval instars but refrained to parasitize the same ages due to lack of enough food sources, for the developing off spring. The fifth larval instar *A. catalaunalis* was the most suitable instar for *B. hebetor* larval development as indicated by the highest total eggs laid per female and the highest percentage of emerged wasps. Hajar Faal- and Parviz (2013) mentioned that *B. hebetor* was reared on full grown fifth instar larvae of *E. kuehniella* to study longevity, fecundity, and life-table parameters on different host diets. Also, Benson (1973) mentioned that, *B.hebetor* females preferred to attack and oviposit on last instar (fifth) larvae although; younger instars were also stung and used by parasitoid females.

CONCLUSION

This study presents experimental evidence that the gregarious ectoparasitoid *Bracon hebetor* developed in a rather short time. The daily oviposition rhythm proved that in programs of biological control, depending on the food resources within the agricultural ecosystem, females should be released at 2 to 8 days of age. The fifth larval instar of *A. catalaunalis* was the most suitable instar for *B. hebetor* larval development than earlier instars as indicated by the highest total eggs laid/ female and the highest percentage of emerged wasps. Pure honey could be used as a supplemental food for mass rearing of *B. hebetor* for obtaining higher number of progeny. *B. hebetor* is promising natural enemy in biocontrol stragy because of its behaviour, fecundity and its high percentage of emergence. This study will broaden the knowledge on this species and provide practical information for studies concerning the use of *B. hebetor* in programs of integrated management in sesame plant against *A. catalaunalis*.

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