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Biometrics of *Bracon hebetor* on larvae of *Helicoverpa armigera* reared on different hosts

Shah Nivedita, Ganguli Jayalaxmi, Bhowmick AK and Chunni Kumari

Abstract
Bracon hebetor (Say) is a cosmopolitan idiobiont braconid that parasitizes several lepidopteran larvae including *Helicoverpa armigera*. Parasitoids have always shown their preference towards a particular host species and in this aspect, the diet of the host also plays an important role. Likewise, the development and growth-related parameters of parasitoids developing on hosts fed on different diets also vary. The present studies were taken up during 2019, at the Biological control laboratory, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh. to study the morphometric variations of different immature and adult stages of *B. hebetor* reared on the larva of *Helicoverpa* armigera fed on various host plants.

Keywords: *Bracon hebetor*, Parasitoids, *Helicoverpa armigera*, morphometric variations

Introduction
Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is a severe insect pest of agricultural importance having worldwide occurrence, including India (Chakravarty *et al*., 2019) [2]. It also exhibits an exceptionally wide host range and has been reported to be feeding upon 96 different crop species in India, including most of the commonly grown pulses (Srivastava and Joshi 2011) [9]. The problem of this pest is magnified due to its direct attack on fruiting structures, voracious feeding habits, and availability of different host plants in desired crop phenology. Applying insecticides with different modes of action and from different classes has not been able to control *H. armigera* (Downes *et al*., 2017) [3], frequently resulting in pest resistance and deletion of many biocontrol agents. As a result, interest in biological control has increased, which favors conservation and the sustainable use of biological resources. Among the various biocontrol agents, hymenopteran parasitoids constitute nearly 78% of the estimated number of reported parasitoid species and due to this great abundance, they have served as models of selection for nearly all modern research on insect parasitoids (Hawkins *et al*., 1994) [5]. *Bracon hebetor* (Say) is a cosmopolitan idiobiont braconid that parasitizes *Helicoverpa armigera*. The morphological characteristics of immature stages play an important role in the recognition, taxonomy, and classification of parasitoid wasps (Zhao *et al*., 2014) [10]. For the understanding of host-parasitoid relations, the recognition of immature stages at different stages of development is relevant.

Material and Methods
Studies on the morphology of *B. hebetor* were carried out on *H. armigera* reared on nine different hosts *viz*. chickpea, pigeon pea, marigold, tomato, cabbage, maize, soybean, artificial diet, and *Corcyra cephalonica* (Standard check) at the Biological Control Laboratory, Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the year 2019-2020. During the study period the average temperature and relative humidity were maintained at 28 ± 0.5 ºC, 65 ± 5% RH, 16:8 h L:D photoperiod. The culture of *H. armigera* was multiplied and maintained on different hosts under laboratory conditions. A pair of newly emerged *B. hebetor* was introduced into a small plastic container (100 ml) covered with a piece of white muslin cloth over which one 4th instar larvae reared on the respective host were placed. After placing the larva it was covered with another piece of white muslin cloth of the same size and secured tightly with a rubber band. A cotton swab dipped in honey was placed inside the container on one side as a food source for the adults. Length and width of the various immature stages were recorded with the help of a digital stereo-trinocular microscope by taking the largest length and width in dorsal view, whereas the

adult length and wingspan were recorded by placing them in slides and spreading their wings for accurate measurement. Five replicates were used for each host species. Morphological parameters of *B. hebetor* from egg to adult were studied under laboratory conditions.

Statistical Analysis

The parasitoid *B. hebetor* developing on the 4th instar larvae of *H. armigera* reared on different host plants were studied by using mean and standard deviation by applying Completely Randomised Design.

Results and Discussions

There was stage overlap during the egg to adult development of *B. hebetor*. The amplitude of each immature stage was small, especially in the first instars.

1. **Adults:** The fully developed adults emerged through the pupal case by breaking the cocoon with its jaw. The females were bigger and had prominent ovipositor (Fig: 1), while the males were smaller in size. Also, they could be identified by the number of flagellomeres in their antennae. The antennae of both males and females were filiform. Females possessed 13 flagellomeres of equal size, whereas males had 20 equal-sized flagellomeres. Similar findings have been reported by (Pezzini *et al.*, 2017 and Dweck and Gadallah 2008) [7, 4]. Adults were orangish in colour with prominent dark black eyes. The sizes of *B. hebetor* male and female differed significantly. Data presented in table 1 revealed that maximum length and wingspan in adult male was observed in *C. cephalonica* and on the larvae reared on marigold and chickpea (2.726 and 3.488 mm, 2.685 and 3.359, 2.678 and 3.427 mm) respectively, whereas minimum was observed on the larvae reared on cabbage (2.540 and 3.195 mm). Similarly, maximum length and a wingspan of an adult female were observed in *C. cephalonica* and larvae reared on chickpea and marigold (4.445 and 0.386, 4.427 and 0.382, 4.410 and 0.378 mm) respectively, but the length of the ovipositor of females differed non-significantly (Fig: 5). It varied from 0.386 to 0.371 mm on the larvae of *H. armigera* feeding on different hosts.
2. **Egg:** Adult females of *B. hebetor* laid eggs in a group of 2-4 as the female ovary contains 4 ovarioles only (Ahmed 2012) [6]. Most of the eggs were laid on the ventral surface of the larvae and when the surface was full, deposition of eggs started on the dorsal side. These findings match with the findings of (Ahmed 2012) [6] who also reported that the eggs of *B. hebetor* were mostly laid in groups. The eggs were very small, opaque white, with a smooth surface, typically hymenopteriform, elongated, more or less elliptical, approximately four times longer than wider (Fig: 2). The present findings corroborate the findings of (Pezzini *et al.*, 2017 and kumari 2020) [7, 1] who recorded the same morphological characters of eggs. The data presented in table 2 revealed that the size of eggs varied significantly when the same larvae (*H. armigera*) fed on different hosts were provided to *B. hebetor* females. The longest length and width were observed on the larvae reared on the factious laboratory host *C. cephalonica* (0.537 and 0.130 mm) followed by the larvae reared on chickpea, tomato, and marigold (0.517 and 0.126, 0.511 and 0.125, 0.506 and 0.124mm) respectively whereas shortest length and width were observed on soybean, maize, artificial diet, pigeon pea and cabbage (0.504 and 0.124, 0.502 and 0.121, 0.497 and 0.120, 0.493 and 0.118, 0.484 and 0.116mm) respectively.
3. **Larvae/grubs:** In general, the four larval instars were similar to each other, differing mainly in the size and shape of segments. Larvae presented a gradual loss of transparency, becoming opaquer at each successive instar with the enlargement of the intestine (Fig: 3) Perusal of the data in table 2, revealed that differences in the 1st instar larvae were significant. 1st instar larvae which developed on *C. cephalonica* recorded maximum length and width (0.425 and 0.107 mm) followed by larvae reared on chickpea and marigold (0.419 and 0.101, 0.410 and 0.100 mm) whereas minimum length and width were recorded on pigeon pea and cabbage (0.401 and 0.095, 0.398 and 0.095 mm). A similar trend was observed for the 2nd, 3rd, and 4th instar larvae. In 2nd instar larval length differed non-significantly but larval width differed significantly and it was maximum for *C. cephalonica* and larvae reared on chickpea and marigold (0.941 and 0.370, 0.914 and 0.365, 0.913 and 0.361 mm) respectively. In the 3rd instar, larval length differed significantly but larval width was found to be nonsignificant. It was maximum for the laboratory host *C. cephalonica* and larvae reared on chickpea and marigold (2.146 and 0.801, 2.130 and 0.789, 2.069 and 0.771 mm) respectively and minimum for larvae reared on cabbage (1.917 and 0.688 mm). In the 4th instar again larval length and width differed significantly and it was maximum for *C. cephalonica* (2.778 and 0.980 mm) and minimum for larvae reared on cabbage (2.632 and 0.967 mm). Present findings are in accordance with the findings of (Pezzini *et al.*, 2017) [7]. They also reported the larval length and width of 1st, 2nd, 3rd and 4th instars as (0.44 ± 0.073 and 0.10 ± 0.019,0.89 ± 0.142 and 0.36 ± 0.022, 1.87 ± 0.283 and 0.60 ± 0.086, 2.67 ± 0.139 and 0.90 ± 0.079 mm).The preference and variation in the length and width of the larval instars might be due to the differences in of nutrient content of the host.
4. **Pre-pupa:** At the end of the fourth instar, the pre-pupal transformation occurred with the fully formed, oval-shaped and, lighter white-coloured cocoon and absence of movement. (Fig: 3(E)). Perusal of the data in table 2 revealed that differences in pre-pupal length and width were found to be significant and it was maximum for *C. cephalonica* and larvae reared on chickpea (3.008 and 0.997, 2.998 and 0.994 mm) respectively and minimum for larvae reared on cabbage (2.663 and 0.938 mm) (Fig:4).

Pupa: Pupa was somehow larger as compared to pre-pupa with an off white coloured cocoon that gradually became brownish and finally completely black at the end of the pupal phase. (Fig: 3(F)). On average, it took five days to turn into a pupa. The adult braked the cocoon with its jaws and emerged, leaving the cocoon. Data presented in table 2 revealed that pre-pupal length and width differed significantly and it was maximum for *C. cephalonica* (3.335 and 1.291mm) and minimum for larvae reared on cabbage (3.160 and 1.214 mm). The present findings are concurrent with the findings of (Ghirtlahre 2014) [8] who also observed the pupal length and width (3.55 and 1.51, 3.57 and 1.45 mm) of *B. hebetor* on the larvae of *H. armigera* when reared on chickpea and tomato respectively.

Fig 1: Female *B. hebetor* parasitising *H. armigera* larva

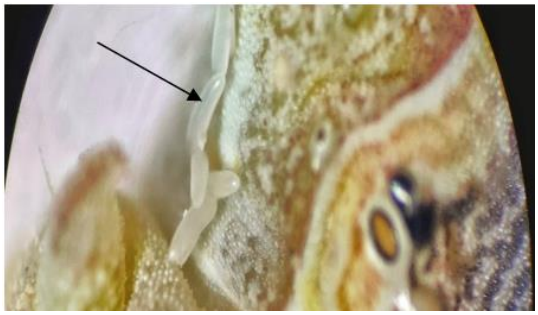

Fig 2: Eggs of *B. hebetor* laid on the external surface of *H. armigera* larva

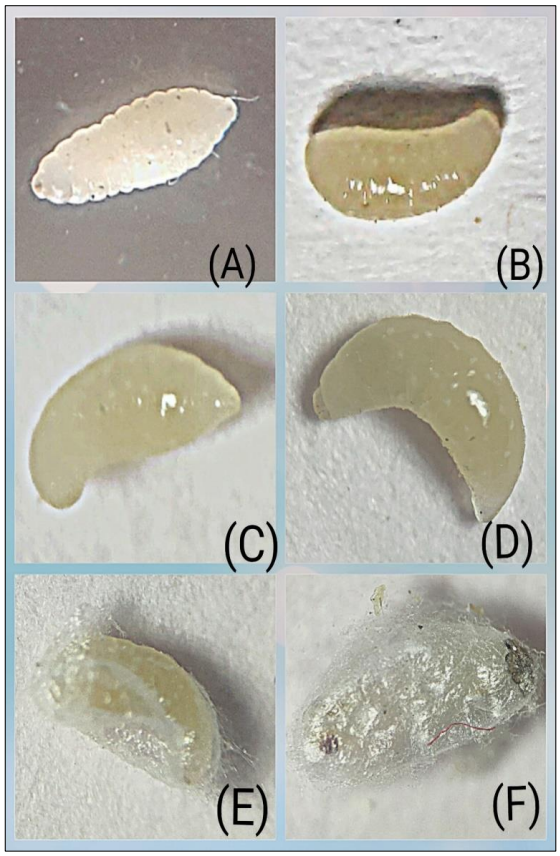

Fig 3: Various immature stages of development of *B. hebetor* (A) 1st instar (B) 2nd instar (C) 3rd instar (D) 4th instar (E) Pre-pupa (F) Pupa

Fig 4: Morphometrics of prepupa of *B. hebetor* in trinocular microscope

Fig 5: Morphometrics of the ovipositor of female *B. hebetor* in trinocular microscope

Table 1: Effect on the size of adults of *Bracon hebetor* reared on larvae of *Helicoverpa armigera* fed on different host plants

***H. armigera* reared on different hosts**	**Mean size of adult *B. hebetor* (mm)**				
	Male		**Female**		
	Length	**Wingspan**	**Length**	**Wingspan**	**Ovipositor length**
Chickpea	2.678	3.427	3.499	4.427	0.382
Pigeon pea	2.563	3.204	3.303	4.170	0.374
Marigold	2.685	3.359	3.489	4.410	0.378
Tomato	2.666	3.355	3.439	4.329	0.381
Cabbage	2.540	3.195	3.261	4.157	0.371
Maize	2.641	3.279	3.360	4.237	0.375
Soybean	2.646	3.343	3.382	4.283	0.377
Artificial diet	2.612	3.268	3.314	4.170	0.374
C. cephalonica	2.726	3.488	3.511	4.445	0.386
Mean ± SD	2.640 ±0.059	3.324± 0.098	3.395 ± 0.093	4.292± 0.116	0.378 ±0.005
SEm±	0.038	0.086	0.058	0.073	0.004
CD at 5%	0.109	NS	0.167	0.209	NS

NS- Nonsignificant

Table 2: Effect on body length and width of immature stages of *Bracon hebetor* reared on larvae of *Helicoverpa armigera* fed *on* different hosts

H. armigera reared on different hosts	Mean length and width of different stages of *B. Hebetor* (mm)													
	Egg		1st instar		2nd instar		3rd instar		4th instar		Pre pupa		Pupa	
	Length	width	Length	width	Length	width	Length	width	Length	width	Length	width	Length	width
Chickpea	0.517	0.126	0.419	0.101	0.914	0.365	2.130	0.789	2.759	0.974	2.998	0.994	3.277	1.273
Pigeon pea	0.493	0.118	0.401	0.095	0.891	0.345	1.952	0.701	2.700	0.967	2.801	0.939	3.190	1.218
Marigold	0.506	0.124	0.410	0.100	0.913	0.361	2.069	0.771	2.751	0.971	2.995	0.993	3.268	1.264
Tomato	0.511	0.125	0.413	0.100	0.904	0.360	2.048	0.735	2.744	0.969	2.994	0.992	3.274	1.251
Cabbage	0.484	0.116	0.398	0.095	0.889	0.345	1.917	0.688	2.632	0.967	2.663	0.938	3.160	1.214
Maize	0.502	0.121	0.408	0.098	0.895	0.351	1.992	0.709	2.720	0.968	2.986	0.990	3.209	1.236
Soybean	0.504	0.124	0.406	0.098	0.900	0.355	1.997	0.743	2.724	0.972	2.992	0.993	3.232	1.240
Artificial diet	0.497	0.120	0.403	0.097	0.900	0.353	1.967	0.704	2.723	0.969	2.967	0.979	3.201	1.235
C. cephalonica	0.537	0.130	0.425	0.107	0.941	0.370	2.146	0.801	2.778	0.980	3.008	0.997	3.335	1.291
Mean ± SD	0.506± 0.015	0.123± 0.004	0.409 ±0.009	0.099± 0.004	0.905 ±0.016	0.356± 0.009	2.024± 0.079	0.738± 0.041	2.726 ± 0.042	0.971± 0.004	2.934± 0.120	0.979± 0.024	3.238± 0.054	1.247± 0.025
S.Em±	0.01	0.003	0.005	0.002	0.015	0.004	0.052	0.031	0.028	0.003	0.06	0.015	0.034	0.016
CD at 5%	0.029	0.007	0.014	0.004	NS	0.012	0.15	NS	0.082	0.008	0.173	0.044	0.098	0.047

Conclusion

Thus, among the nine hosts tested, *C. cephalonica* was found to be suitable for the rearing of *B. hebetor* which was evident by the significant differences shown between the size of the eggs, maximum length, and width of the larval, pupal, and adult size. However, *H. armigera* larvae reared on chickpea and marigold were found as the next suitable host for rearing *B. hebetor*, in case, the laboratory host *C. cephalonica* was not available, followed by the larvae reared on tomato, soybean, maize, and artificial diet, whereas pigeon pea and cabbage were found to be least suitable for the rearing of the ectoparasitoid, *B. hebetor*, having a detrimental effect on the biometrics of *B. hebetor ie*. minimum size of the immatures and adults.

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