

Effect of Adult Nutrition at Different Concentrations on Reproduction and Longevity of *Bracon brevicornis*, Larval Parasitoid of Coconut Black-Headed Caterpillar

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Abstract: Coconut black-headed caterpillar (*Opisina arenosella*) or BHC is one of the main coconut palm defoliators in many Asian countries, including Malaysia, where it was discovered in 2017. Since then, an effort has been made to manage the pest by the use of larval parasitoids, started with a study on the effects of sucrose diet on reproduction, longevity and parasitism of a naturally occurring parasitoid known as *Bracon brevicornis* (Hymenoptera: Braconidae). The aim of this study was to determine the optimal sucrose concentration for improving its rearing technique. Two experiments were conducted, i.e., a) reproductive performance study; and b) longevity study. In each study, different sucrose concentrations were tested on mated females in five (5) replications. Feeding on 20% sucrose concentration resulted in significantly ($P < 0.05$) highest fecundity (82.4 ± 0.9), hatchability (100%), pupation, fertility (82.7%) and longest reproductive period (12.8 ± 0.3 days) of *B. brevicornis* among treatments. Meanwhile, 40% sucrose was able to prolong its adults' lifespan (20.6 days). Adults fed on 20% sucrose had the highest parasitism (33.4 ± 0.6 host larvae), despite the fact that 30% sucrose had no statistically significant difference. Thus, this study suggested that 20% sucrose could be suitable to increase fertility and parasitism by *B. brevicornis*. However, additional research on its effect on behaviours is necessary to acquire a better knowledge of its efficiency in the field.

Keywords: *Bracon brevicornis*, *Corcyra cephalonica*, Sucrose Diet, Reproductive Performance, Parasitism, Larval Ectoparasitoid

1. Introduction

Coconut black-headed caterpillar (*Opisina arenosella*) or BHC is one of the main defoliators of coconut in many Asian countries and was reported to be found in Malaysia in 2017 [1]. Previous studies documented the attack of BHC on numerous types of crops including oil palm, jackfruit, cashew, date palms, ornamental palms [2, 3] and others. According to Mohan et al., [4], upon outbreak, the pest larvae could cause up to 45.5% of crop loss on infested palm in the following year.

Bracon brevicornis (Hymenoptera: Braconidae) is an idiobiont ectoparasitoid that parasitised several number of insect pests which include *Eublemma amabilis*, *Tuta absoluta*, *Pectinophora gossypiella*, *Maruca testulalis* Geyer, *Helicoverpa armigera* (Hubner) Hardwick, *Spodoptera litura* Fab, *Earias vittella*, *Ephestia kuehniella*, *Galleria mellonella*, *Sesamia cretica*, *Spodoptera littoralis*, *Pectinophora gossypiella*, *Plodia interpunctella*, *S. litura*, *S. frugiperda* [5-10] and others. This parasitoid has the potential to be used as a biological control agent for many pests due to its ability to paralyse its host through stinging and envenomation. During the stinging, *B. brevicornis* females would puncture

the host's body with their ovipositor (tube-like organ that helps in egg deposition) in order to obtain its body fluid while simultaneously injecting venom that causes paralysis. Temerak mentioned that *B. brevicornis* sometimes paralyze host larvae permanently without depositing eggs on them [11], which could be advantageous in pest management.

Parasitoid nutrition is important in evaluating its reproductive performance and life span. Several studies mentioned that diluted honey [10] or sugary water [12] in general, without specifying certain concentrations, could be used when rearing *B. brevicornis* on *S. frugiperda* or *E. kuehniella*. However, Wei et al. reported that honey-feeding and host hemolymph-feeding parasitoid shows similar effect on reproduction and longevity [13]. Meanwhile, optimum diet concentration would enhance parasitoid performance in various aspects [14]. There are studies conducted to investigate the effect of supplementary nutrition on enhancement of reproduction and longevity of *B. cephi* and *B. lissogaster* [15], as well as *Habrobracon hebetor*, (Hymenoptera: Braconidae) [16], and found that sucrose with suitable concentrations could improve the reproductive performance and lifespan of the parasitoids. Thus, the purpose of this study was to investigate the effect of providing supplementary nutrition of different sucrose concentrations on the reproduction, longevity and parasitism of *B. brevicornis* as an effort to improve mass rearing of the parasitoid on *Corcyra cephalonica* (Lepidoptera: Pyralidae) as host and its use as a biocontrol agent.

2. Materials and Methods

2.1. Source of Host

C. cephalonica was used as parasitoid host in this experiment. The Lepidopteran eggs were obtained from rearing culture at the Centre for Agriculture and Bioscience International (CABI) and reared in laboratory at MARDI Bagan Datuk using mixture of fine rice husk and rice bran (1:1) as rearing media. Plastic containers with lids (35 x 27 x 7cm) were used as rearing containers. The middle part of the lids was cut out and lined with muslin cloth for aeration. The *C. cephalonica* eggs were scattered onto the rearing media and allowed to hatch into larvae. When the larvae reached pupal and later adult stage, the adults were transferred into other plastic container (26 x 16cm) to allow mating and eggs laying. The eggs were collected and scattered into new container for hatching. The hatched larvae were allowed to develop into late larval stage before being used in the experiment.

2.2. Source of Parasitoid

B. brevicornis was obtained from sampling of BHC in coconut planting area. Parasitised BHC larvae showing symptoms of crinkled body with presence of *B. brevicornis* pupae nearby the dead larvae. The parasitoid pupae were collected and placed individually into plastic vial (4 x 8cm) before covered with muslin cloth. Diluted honey was offered as source of food. Upon adult emergence, male and female of

B. brevicornis were identified and prepared to be used in the experiment.

2.3. Experimental Layout

2.3.1. Reproductive Performance

From pre-liminary trials, three (3) sucrose concentrations were prepared (20%, 30%, 40%) by diluting the sucrose into distilled water. Sucrose was chosen for being the most common carbohydrate found in floral nectar [17, 18]. Meanwhile, distilled water was used as control. The prepared diets were swabbed onto the inner wall of plastic vial. Newly emerged males and females were starved for four (4) hours before carefully transferred into plastic vials for mating and the vials were later covered with muslin cloth. Ten (10) *C. cephalonica* larvae were placed onto the muslin cloth before placing another layer of muslin cloth and secured with rubber band. The larvae were replaced daily until parasitoids died. Number of dead *C. cephalonica* larvae, *B. brevicornis* eggs, larvae, pupae, newly emerged adults (Figure 1), longevity and reproductive period, were observed and recorded. Experiment was conducted following Complete Randomized Design (CRD) with five (5) replications. Data on percentage of hatchability (1) and fertility (2) were calculated as below:

$$(\text{Number of eggs hatched} / \text{Total number of eggs laid}) \times 100 \quad (1)$$

$$(\text{Number of adults emerged} / \text{Total number of eggs laid}) \times 100 \quad (2)$$

2.3.2. Longevity

Longevity of *B. brevicornis* adults were also observed by offering them with the same diets as above without presence of hosts. Parasitoids were placed in the same size plastic vial individually and covered with muslin cloth. The diets were swabbed onto the inner wall of the plastic vial and newly emerged *B. brevicornis* adults were allowed to feed them. Their longevities were observed and recorded from the day they emerged until they died. This experiment was also done following Complete Randomized Design (CRD) with five (5) replications. All experiments were conducted under 30±3°C temperature, 80±5% relative humidity and 12 h:12 h light and dark illumination.

2.4. Statistical Analysis

Data on reproductive performance, longevity and parasitism were subjected to Analysis of Variance (ANOVA) after testing for normality using Kolmogorov-Smirnov Test. Multiple mean comparisons were done using Fisher's Least Significant Different (LSD) at 95% confidence level.

3. Results

3.1. Reproductive Performance

Results of this study show that *B. brevicornis* significantly produced the highest number of eggs, larvae, pupae and adults ($P < 0.05$) in response to feeding with 20% sucrose followed by 30% sucrose, 40% sucrose and distilled water (Table 1). Meanwhile, results in Table 2 revealed that *B. brevicornis* fed

with 20% sucrose recorded the highest fecundity and significantly different to those with 40% sucrose as well as distilled water ($P=0.01$). Nevertheless, hatchability and fertility were relatively the highest in 20% sucrose even though not statistically different with 30% and 40% sucrose. On the other hand, reproductive period of *B. brevicornis* shows longest when fed with 20% sucrose (12.8 ± 0.3 days) as compared to 30%, 40% sucrose and distilled water with 10.0 ± 0.4 , 8.6 ± 0.8 and 4.2 ± 0.3 days respectively (Table 3).

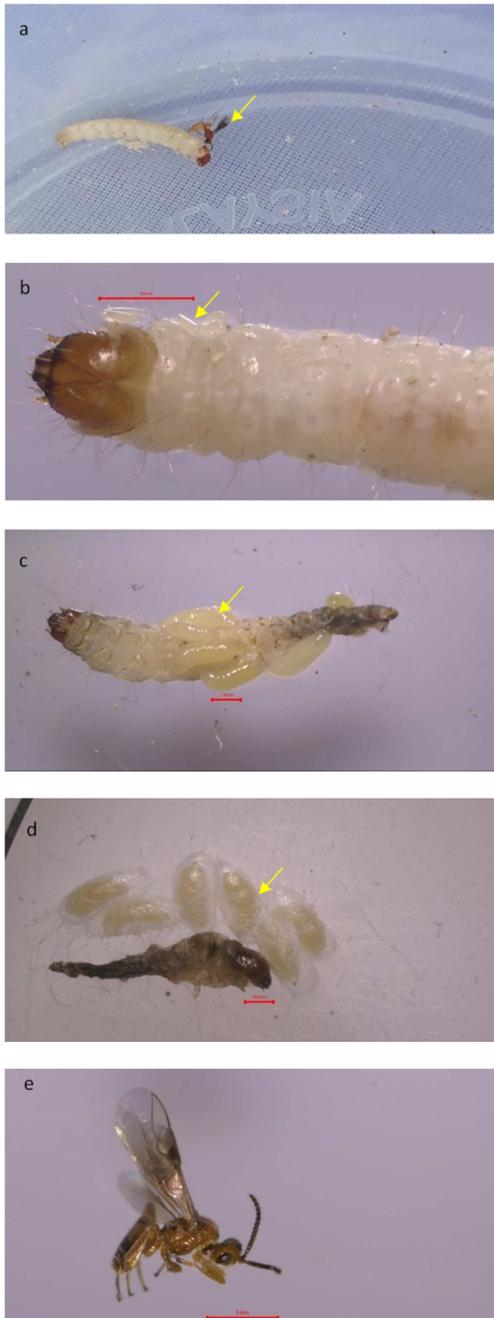


Figure 1. (a-e): Arrow showing a) Female of *B. brevicornis* parasitizing *C. cephalonica* larvae; b) Eggs of *B. brevicornis* on *C. cephalonica* larvae (2.0x mag.), c) Larvae of *B. brevicornis* fed on *C. cephalonica* larvae (0.6x mag.), d) Formation of *B. brevicornis* pupae (0.6x mag.), and e) Adult of *B. brevicornis* (1.5x mag).

Table 1. Mean number \pm Standard Errors of Mean (S.E) of eggs, larvae, pupae and adults of *B. brevicornis* in response to different sucrose concentrations. Lettering on means showing significant difference in treatments and similar letter showed no significant difference computed with $P = 0.05$.

Diets	†Means \pm S.E			
	Eggs	Larvae	Pupae	Adults
20% sucrose	82.4 \pm 0.9 ^a	82.4 \pm 0.9 ^a	74 \pm 0.9 ^a	66.6 \pm 0.8 ^a
30% sucrose	62 \pm 1.0 ^{ab}	61.4 \pm 1.0 ^{ab}	49.2 \pm 1.1 ^{ab}	47.6 \pm 1.1 ^{ab}
40% sucrose	37 \pm 0.1 ^{bc}	34.8 \pm 0.1 ^{bc}	32.4 \pm 0.1 ^{bc}	28.8 \pm 0.1 ^{bc}
Distilled water	22 \pm 0.2 ^c	18.6 \pm 0.2 ^c	16.6 \pm 0.3 ^c	13.4 \pm 0.3 ^c
P value	0.01*	0.003*	0.007*	0.004*

†Data were square-root transformed
*Significant at $\alpha = 0.05$

Table 2. Mean fecundity, hatchability, and fertility \pm Standard Errors of Mean (S.E) of *B. brevicornis* in response to different sucrose concentrations. Lettering on means showing significant difference in treatments and similar letter showed no significant difference computed with $P = 0.05$.

Diets	†Means \pm S.E		
	Fecundity	% Hatchability	% Fertility
20% sucrose	82.4 \pm 0.9 ^a	100 \pm 0 ^a	82.7 \pm 0.2 ^a
30% sucrose	62 \pm 1.0 ^{ab}	99 \pm 0.3 ^a	72.5 \pm 0.3 ^{ab}
40% sucrose	37 \pm 0.1 ^{bc}	94.2 \pm 0.1 ^a	80.4 \pm 0.2 ^a
Distilled water	22 \pm 0.2 ^c	84.6 \pm 0.2 ^b	60.4 \pm 0.4 ^b
P value	0.01*	0.0001*	0.01*

†Data were square-root transformed
*Significant at $\alpha = 0.05$

Table 3. Mean reproductive period (days) \pm Standard Errors of Mean (S.E) of *B. brevicornis* in response to different sucrose concentrations. Lettering on means showing significant difference in treatments and similar letter showed no significant difference computed with $P = 0.05$.

Diets	Means reproductive period (days) \pm S.E
20% sucrose	12.8 \pm 0.3 ^a
30% sucrose	10.0 \pm 0.4 ^b
40% sucrose	8.6 \pm 0.8 ^b
Distilled water	4.2 \pm 0.3 ^c
P value	<0.01*

*Significant at $\alpha = 0.05$

3.2. Longevity

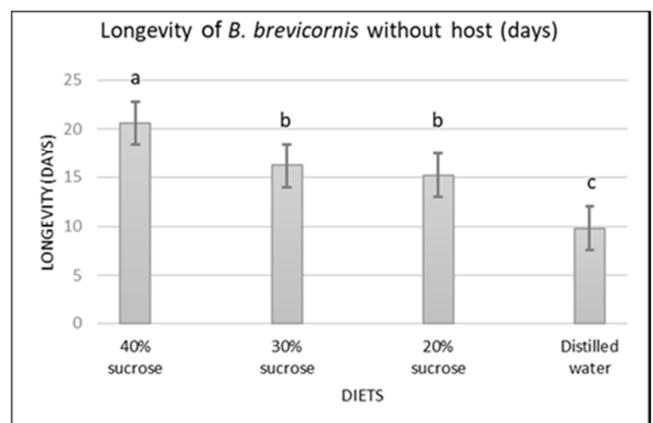


Figure 2. Mean longevity of *B. brevicornis* in response to different sucrose concentrations. Lettering on means showing significant difference in treatments and similar letter showed no significant difference computed with $P = 0.05$.

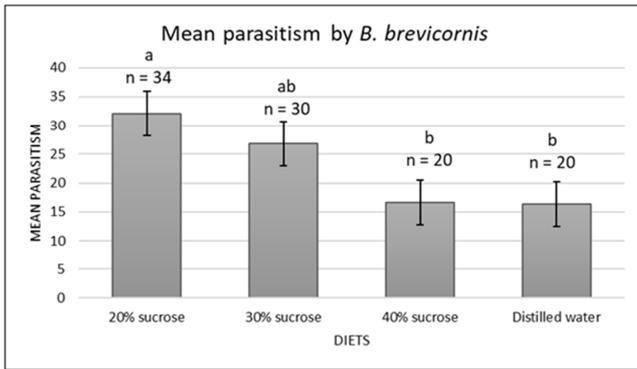


Figure 3. Mean parasitism by *B. brevicornis* adult females to *C. cephalonica* and *O. arenosella* in response to different sucrose concentrations. Lettering on means showing significant difference in treatments and similar letter showed no significant difference computed with $P = 0.05$. Letter n showing total number of hosts offered to *B. brevicornis*.

Results in Figure 2 reveal that diet with 40% sucrose was able to prolong *B. brevicornis* adults' longevity up to 20.6 days which is statistically different with those on 30% sucrose (16.2 days), 20% sucrose (15.2 days) and distilled water (9.8 days). Parasitised larvae of *C. cephalonica* normally show blackish crinkled appearance as a result of feeding activity by the *B. brevicornis* larvae. The parasitism rate by *B. brevicornis* (Figure 3) was also significantly affected by feeding on different sucrose concentrations with highest mean parasitism recorded when provided with 20% sucrose (33.4 ± 0.6) but not statistically different with 30% sucrose (27.8 ± 0.5). Mean parasitism by females fed on 40% sucrose (16.6 ± 0.1) shows similar result with 30% sucrose and distilled water (16.4 ± 0.1).

4. Discussions

Fecundity and fertility are important parameters in evaluating reproductive performance of parasitoids. According to Benelli et al., the parasitoid fertility and fecundity can be improved by providing optimal food sources in the right amount and quality [19]. In this study, 20% sucrose was found to be the parasitoid's ideal diet. This coincides with a study conducted by Huang et al., that *H. hebetor* produces the highest number of offspring when fed with 20% sucrose, using *P. interpunctella* as host [16]. Sucrose is a disaccharide made up of two types of sugar called glucose and fructose. Aside from playing a major role in inducing feeding stimulant effect on insects due to the palatability and nutritional value of fructose [20], this sugar also important in supplying energy to the parasitoids through presence of glucose for locomotion and somatic maintenance [21] as well as ovigenesis of synovigenic parasitoids [22] such as *B. brevicornis*.

When fed with sugar, the pattern of feeding lower sugar concentrations led to higher fecundity and fertility, which is most likely identical to its closely related species, *B. hebetor* [14]. The results also coincide with the finding by Salmah et al., in which *Apanteles metesae* (Hymenoptera: Ichneumonidae) recorded the highest fecundity when fed with 20% sucrose [23]. According to Basri et al., sucrose contributed the highest amount of sugar in extra-floral nectar

of two beneficial plants, *Cassia cobanensis* and *Crotalaria usaramoensis* which attract many parasitoids species [24]. Jervis, et al. [22] explained that lower mortality of idiobiont parasitoid progenies during development (i.e higher fertility in this case), reduces the need of the parasitoid to invest energy resources to fecundity, and in turn allows resource investment for each egg produced throughout its lifetime. This could also explain the longer reproductive period of *B. brevicornis* when fed with 20% sucrose, as it perceives the amount to be good quality of food and adequate for its reproduction. It is in agreement with Luo et al., who discovered that feeding with sucrose has prolong the reproductive period of *Microplitis mediator* (Hymenoptera: Braconidae) but at the slowest rate as compared to feeding with other sugars [25]. Aside from that, other factors could also contribute towards the prolonged reproductive period such as host species and temperature. According to Temerak, longer ovipositional period of *B. brevicornis* was observed when it was offered with fresh larvae of *S. cretica* daily, but no suggestion on type of diet given was mentioned [26].

High concentration of diet would probably give *B. brevicornis* the opportunity to store more energy for its various activities in longer period of time. According to Siekmann et al., parasitoid feeds smaller amount of higher diet concentrations, as a result of its higher viscosity [27]. This will slow down their feeding rate and able to acquire more reserved energy to live longer. This is also agreed by Reis et al., who found that sucrose supplementation had a strong positive effect on longevity of two Bracon species [15]. There are several studies that have been conducted on longevity of parasitoids which also resulted to increase in longevity with increasing diet concentrations, such as studies on *Aphidius ervi* (Hymenoptera: Braconidae) fed on glucose: fructose mixture [28], on *Encarsia formosa* (Hymenoptera: Aphelinidae) when fed with increasing concentrations of sucrose and trehalulose [29], on female of *B. hebetor* (Hymenoptera: Braconidae) when fed with different concentrations of glucose [30], and others. Moreover, Arrese and Soulages explained that excess calories which resulted in consumption of high-carbohydrate food i.e. sucrose, would be converted into glycogen and triglycerides as energy reserve [31]. Glycogen is vital in providing sufficient energy to sustain biological function for 24 hours, whereas the lipid, i.e. triglyceride, allow higher survival [32].

Parasitism is another energy-demanding activity which involves successful searching and recognizing of host before insertion of ovipositor. In the case of *B. brevicornis*, it also includes envenomation to paralyse the host [33]. All of these activities require physiological processes and locomotion of the parasitoid which involved the use of reserved energy converted from hydrolysed sugar, hence enzymatic breakdown of such sugars is indispensable for the conversion. According to Hausmann et al., parasitoid generally able to fully optimized most of disaccharides (that feature α -glucosidic bond) especially sucrose due to strong and specific α -glucosidase activity, which is an enzyme responsible for hydrolysing sucrose [34]. This could explain

the significant effect of parasitism on different sucrose concentrations, which is probably due to highly efficient sugar-energy conversion by the female who is fed on suitable concentration of sucrose. This is also supported by Tena *et al.*, in which a two-fold increase of parasitized hosts by *Aphytis melinus* in a citrus agroecosystem, was evident on trees supplied with sugar [35]. Apart from that, other factors such as host instar stage [36] and progenies development strategy such as gregarious parasitoid [37] could also affect the parasitism rate.

5. Conclusion

Studies to improve mass-rearing techniques are important to ensure optimum production of parasitoids before preparing for field release. From this study, we suggest that sucrose solution able to increase fertility and prolong the life-span of *B. brevicornis* when reared on *C. cephalonica* and 20% concentration could increase its fecundity and parasitism. However, the diet also needed to be tested for its effect on behaviours such as mating, and host searching in order to gain a better insight of the carbohydrate source that will optimise the efficiency of the biocontrol agent later in the field.

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