J. Crop Prot. *2016, 5 (1): 39-48*
doi: 10.18869/modares.jcp.5.1.39

Research Article

Mass rearing of *Bracon hebetor* (Hym.: Braconidae) on wax moth, *Galleria mellonella* (Lep.: Pyralidae) with varying density of parasitoid and the host

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Abstract: Rearing methods for *Bracon hebetor* (Say) (Hym., Braconidae) were investigated in the series of laboratory experiments designed to enhance the yield of the mass rearing of this parasitoid for biological control of lepidopteran field and stored product pests. In these experiments, the effects of parasitoid and host densities on fertility and sex ratio of *B. hebetor* were assessed. In parasitoid densities, 50 last-instar greater wax moth (GWM) *Galleria mellonella* (L.) larvae were placed per container and 1, 2, 4, 8 or10 pairs of *B. hebetor* (one male and one female) were released in each container. In host density study two pairs *B. hebetor* were introduce in six different densities (10, 20, 30, 40, 50 and 60) of host, GWM per container. A density of ten male-female pairs of *B. hebetor* produced a higher number of progeny (205 ± 7.07 adults) on 50 last instar larvae of GWM. Similarly, in a host density experiment, a density of 60 last instars GWM larvae produced a significantly higher number of parasitoid progeny (142.0 ± 8.75 adults), followed by 50 last instar larvae (141.0 ± 8.34 adults) among the tested host densities when two pairs of *B. hebetor* were used. The sex ratio of progenies was male-biased in all studies and there were no significant effects on sex ratio in various parasitoid and host densities. In mass rearing experiment, total number of emerged parasitoids per 200 wax moth larvae was 1091 ± 82.38 adults with mean parasitism rate of 98 ± 0.8%.

Keywords: Biological control, mass rearing, *Bracon hebetor,* parasitoid density, host density

Introduction

Bracon hebetor Say (Hymenoptera: Braconidae) is an ecto-parasitoid that attacks the 4th–and 5th stage of pyralid moth larvae, including the greater wax moth (GWM) *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Awadallah *et al*., 1985), *Plodia interpunctella* (Hübner) (Milonas, 2005), *Corcyra cephalonica* (Stainton) (Krombein *et al.,* 1979), *Ephestia kuehniella* Zeller (Darwish *et al*., 2003) and *Helicoverpa armigera* Hübner*, Heliothis virescens* (F.) (Attaran, 1996), that infest field crops as well as stored-products (Benson, 1974). The parasitoid is considered as a potential biological control agent of the lepidopteran stored product pests (Brower *et al*., 1996) and also some field insect pests.

The GWM is an important pest of the honey bee. The larval stage of the GWM feeds on the

Handling Editor: Ali Asghar Talebi

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Received: 27 October 2014, Accepted: 13 November 2015
Published online: 01 December 2015

honey, pollen and wax produced by honey bees (Nurullahoglu and Susurluk, 2001). GWM is preferred in entomological studies because of its nutritional needs, ecological adaptation and developmental characteristics. It is used as a host for rearing many hymenoptera species (Coskun *et al*., 2006).

For many years, the management of lepidopteran pests has traditionally involved the use of fumigants, aerosols and other chemical insecticides. However, these moth species have become resistant to insecticides (Zettler *et al.*, 1973). Moreover, insecticides pose a direct risk to human health and the environment due to the presence of their residue in food products and in processing facilities where workers are exposed (Fields and White, 2002). In recent years, interests have been focused for development of non-chemical strategies for insect control such as cultural, physical, biological, varietal, bio-rational and genetic control measures in place of conventional pesticides for the management of stored product insects as well as field pests. (Subramanyam and Hagsturm, 2000; Phillips, 2006). Of these strategies, the use of natural enemies, including parasitoids and predators is an important component of IPM and has many advantages over chemical control (Scholler *et al*. 1997; Scholler and Flinn, 2000).

Bracon hebetor females paralyze their host larvae first by stinging and then variable numbers of eggs are laid on or near the surface of paralyzed hosts (Antolin *et al*., 1995). The paralyzed larvae of host are then used as food sources for developing larvae and also for the adult females (Doten, 1911; Richards and Thomoson, 1932). Ghimire and Phillips (2010) studied the mass rearing of *B. hebetor* on *P. interpunctella* to observe the effects of host density, parasitoid density and the rearing containers size on adult progeny production and the sex ratio. They found that host density, parasitoid density and rearing container size had significant effect on adult progeny production but no effect on sex ratio. Al-Tememi, (2005) reared *B. hebetor* on GWM and *H. armigera* in the laboratory condition and successfully produced adult progeny which can easily be used in mass rearing for biological control. The fecundity and sex ratio of *B. hebetor* was studied on GWM and *E. kuehniella*. The fecundity of the female parasitoids was higher on GWM than *E. kuehniella* (Gunduz and Gulel, 2005). Since *B. hebetor* is considered an effective parasitoid, it can be used in the augmentation releases. For that reason easy and cost effective development of mass rearing protocol is very important. The present piece of research work was undertaken to develop the protocol for mass rearing of *B. hebetor* on GWM larvae as host under laboratory conditions.

Materials and Methods

Parasitoid and host density

Larvae of the greater wax moth (GWM) and *Bracon hebeto*r were obtained from IPM laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. Larvae of natural or wild GWM were released in 100 ml screw cap plastic containers. After 48 hours of emergence, adults of the parasitoid, *B. hebetor* were released into the containers and allowed to sting and oviposit for five consecutive days. In the parasitoid density study, 50 last-instar larvae of GWM were placed in each plastic container. Adults of the parasitoid *B. hebetor* were released in the containers at 1, 2, 4, 8 or 10 pairs (one male and one female) per container and closed with screw cap. In the host density experiments, two pairs *B. hebetor* were exposed to six different host densities. Last-instar larvae of GWM were released in 100 ml plastic containers at the rate of 10, 20, 30, 40, 50 or 60 per container.

The containers were placed in a growth chamber at 27 ± 2 °C temperature, 65 ± 10% RH and 14:10 hour (L: D) photoperiod. The emergence of parasitoids was monitored daily after one week of introduction until the adult emergence was ended (2-3 weeks). Observations were made on the number of adult parasitoids that emerged and the secondary sex ratio of the adults (proportion of females).

Mass rearing of *Bracon hebetor*

The study on mass rearing of *B. hebetor* parasitoid was undertaken in the IPM laboratory, Entomology Division. BARI, Gazipur. Last instar (5^{th}-6^{th} instars) larvae of GWM were released into the plastic 1000 ml jars at 200 larvae jar^{-1}. The full-fed larvae took position on the corrugated paper sheet placed in the jar for pupation. A total 50 adult of *B. habetor* (35 female and 15 male) were released in the plastic bottle with honey solution on cotton ball for their food. The opening of the jar was closed with black cloth. The wax moth larvae and *B. hebetor* were kept on shelf for 8-10 days for paralyzing, parasitizing, ovipositing and subsequently pupation followed by adult emergence of *B. hebetor*.

Data analysis

The numbers of adult parasitoid progeny and secondary sex ratio (%female) were used as response variables to assess the effect of parasitoid and host density. Host and parasitoid densities were used as independent variables for the analysis of response variables. Each treatment was replicated five times. Data on numbers of adult parasitoids and the secondary sex ratio were analyzed by one-way analysis of variance (ANOVA) and means were separated by Least Significant Differences (LSD) test, when the ANOVA was significant ($P < 0.05$), with MSTAT-C software. Relationship of adult progeny production with varying parasitoid and host density was measured by regression analysis. Excel version 2010 was used for drawing figures and graphs.

Results

Effect of parasitoid density on host

The highest number of *B. hebetor* adult progeny (205 ± 7.07) was recorded from the container having maximum density of 10 pairs of parasitoid which was statistically similar to container having 8 pairs of parasitoid. Effect of the two lowest densities was statistically similar and significantly higher compared to four higher densities of the parasitoid. Number of adult progeny was 185.00 ± 6.12, 158.60 ± 5.38 and 135.60 ± 5.04 at the densities of 4, 6 and 8 pairs of parasitoids respectively. There was no significant difference in total number of progeny production in 4, 6 and 8 pairs of *B. hebtor* density. The relationship between production of adult progeny and density of parasitoid was linear, positive and significant ($r = 0.982$). The relationship could be expressed by the regression equation, $y = 26.467\times + 51.933$, where 'y' represents number of progeny produced and 'X' represents pairs of *B. hebetor* released into the container bearing 50 last instar larvae of GWM. Values of coefficient of determination ($R^2 = 0.964$) reveals that the influence of parasitoid density on the variations of adult progeny production can be attributed to 96.4% (Fig. 1).

Numerically, the lowest number of females (45.25 / container) was produced at the lowest density (1 pair per container). The number of female progeny at densities of 2-10 pairs of the parasitoid varied from 49.60 - 52.12 per container. The variations were not significant ($F = 2.385$; $df = 4, 24$; $P = 0.0684$) (Fig. 2). The relationship of female population with density of the parasitoid was linear and positive and could be expressed by the regression equation $Y = 1.0437\times + 45.799$ (Fig. 2). The findings presented in Figure 1 show that the *B. hebetor* progeny produced in the containers were consistently male-biased (greater than 50% males) up to the density of 8 pairs of parasitoids released per container (Fig. 2).

Effect of host density

Total number of *B. hebetor* adults produced in container having host densities of 10 to 60 ranged from 43.80-142.00 per container. The number of progenies per container recorded from the highest and second highest levels of host density was statistically similar and significantly higher compared to four lower levels of host larvae ($F = 49.09$; $df = 5, 24$; $P < 0.0001$). Production of adult progeny at 30 and 40 host larvae per container was also statistically similar but significantly higher compared to only two lower levels. The lowest population of adult progeny was found in container having 10 host larvae / container which were statistically similar to the

[DOR: 20.1001.1.22519041.2016.5.1.11.0]

population recorded at 20 host larval densities in containers. There was a significant positive and linear relationship between progeny production and host densities (r = 0.9767). The relationship could be expressed by the regression equation Y = 21.740× + 17.08 (Fig. 3).

The percentage of female in total adult progeny (female sex ratio) of the parasitoid varied from 49.40 to 51.05%. The variations were not significant. There was linear and insignificant relationship between proportion of female and host densities (r = 0.246) (Fig. 4).

Mass rearing of *Bracon hebetor* on *Galleria mellonella* larvae

In replication-1, 98% of host GWM larvae were parasitized by the parasitoid (*B. hebetor*). The parasitism rate was 100% in jar-2 to jar-5. Days to adult emergence or total developmental time after parasitism was 9, 9, 11 and 10 in jars 2, 3, 4, and 5, respectively. Population of adult parasitoid emerged from 200 host larvae from replication 1, 2, 3, 4 and 5 was 1256, 1308, 1030, 968 and 875, respectively. The results of mass rearing of *B. hebetor* on GWM host larvae revealed that average parasitism rate of wax moth larvae by *B. hebetor* ranged from 98-100% with a mean of 98.8 ± 0.8%. Duration of parasitism to adult emergence varied from 8-11 days with a mean of 9.40 ± 0.51 days. Total adult parasitoid emergence per 200 wax moth larvae varied from 875 - 1308 with a mean of 1091 per 200 host larvae and mean adult parasitoid per larvae was 5.44. Longevity of adult *B. hebetor* in jars containing honey as food was 23.8 days (Table 1).

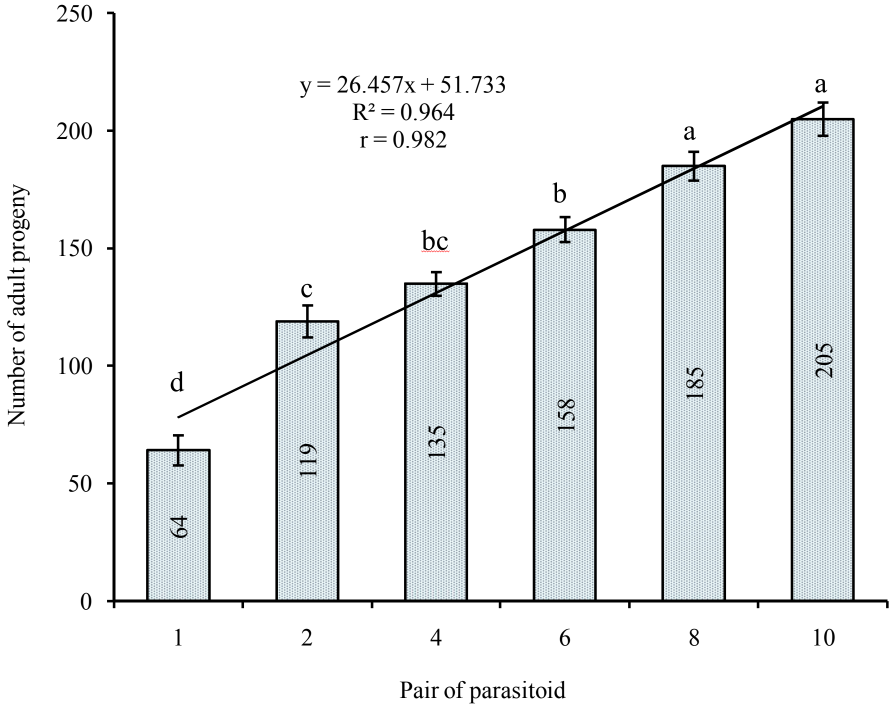

Figure 1 Effect of parasitoid density on progeny production of *Bracon hebetor* in plastic jars containing 50 last-instar *Galleria mellonella* larvae in each container. Bars followed by the same letters are not significantly different using Least Significant Differences tests (P < 0.05). Vertical lines in the bars show standard error of mean.

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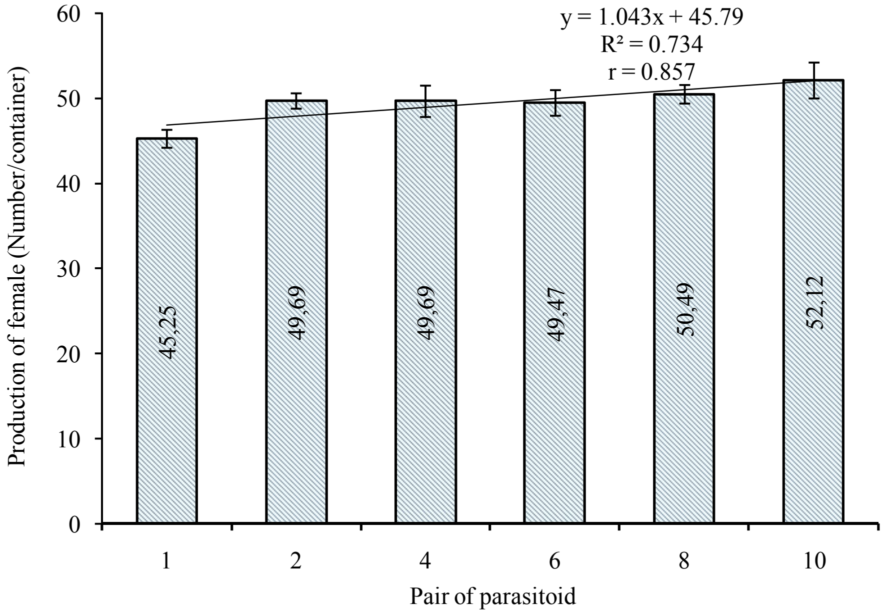

Figure 2 Percentage of adult female of parasitoid *Bracon hebetor* produced in plastic container having 50 last-instar GWM larvae at its different density level. Vertical lines in the bars show standard error of mean.

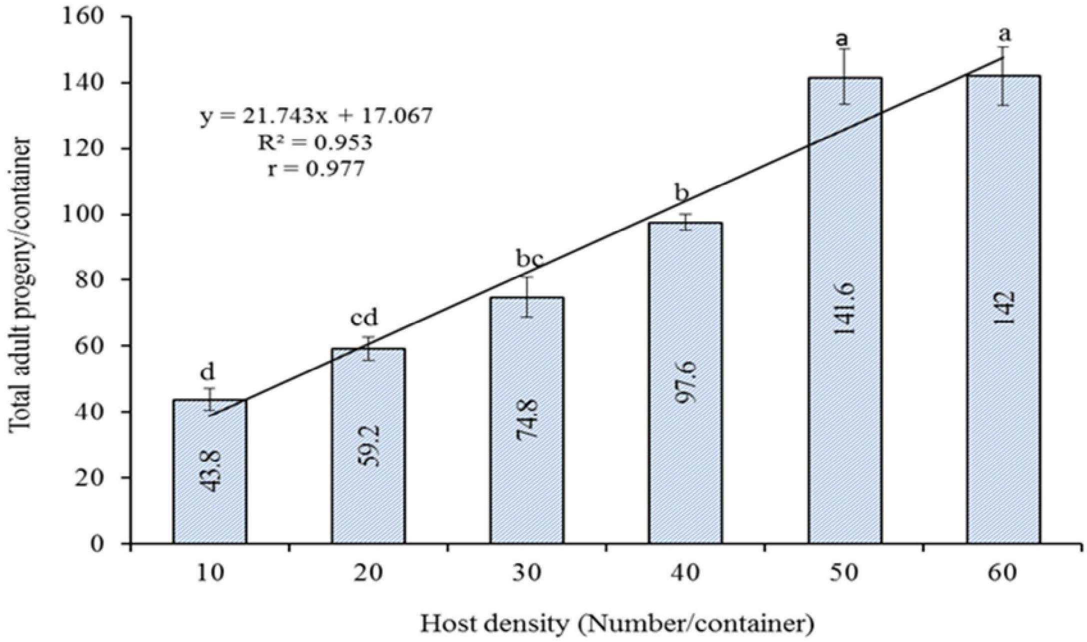

Figure 3 Total population of adult parasitoid *Bracon hebetor* progeny produced in containers containing different density levels of *Galleria mellonella.* Bars followed by the same letters are not significantly different using Least Significant Differences tests ($P < 0.05$). Vertical lines in the bars show standard error of mean.

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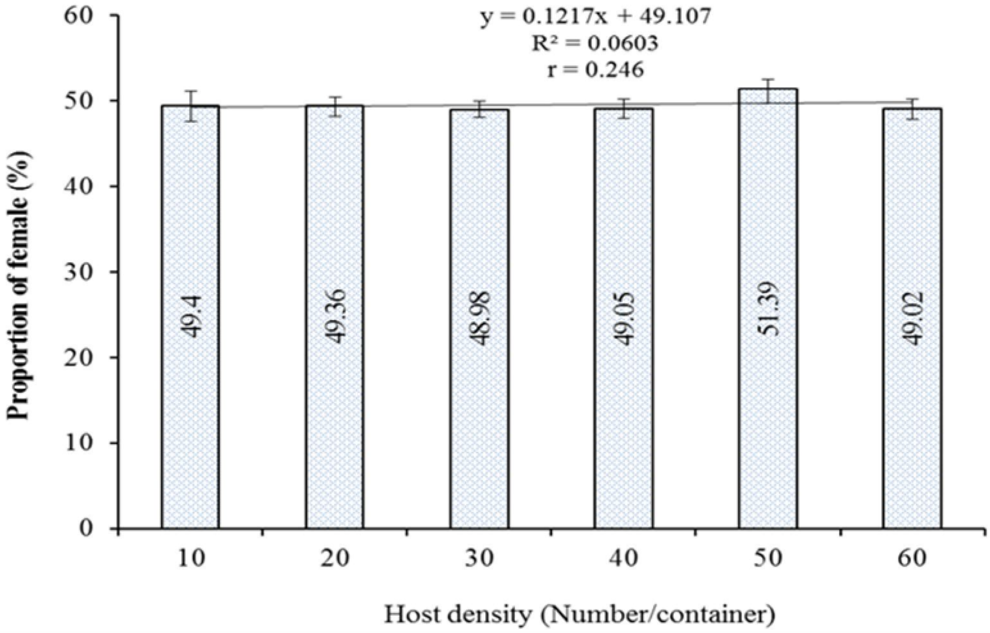

Figure 4 Percentage of female in total population of adult progeny of *Bracon hebetor* multiplied in containers having different density levels of last-instar *Galleria mellonella* larvae. Vertical lines in the bars show standard error of mean.

Table 1 Parasitism of *Galleria mellonella* larvae by *Bracon hebetor*. Adult emergence rate and adult longevity of *B. hebetor* in plastic jars under laboratory condition.

Replicates	Parasitism of host larvae by parasitoid (%)	Days between parasitism to adult emergence	Adult emergence (Number per 200 host larvae)	Adult longevity with honey as food (days)
1	98	8	1256	28
2	100	9	1308	24
3	96	9	1030	25
4	100	11	968	22
5	100	10	875	20
Mean ± SE	98 ± 0.8	9.4 ± 0.51	1091 ± 82.38	23.8 ± 1.36

Discussion

The results of the experiment of parasitoid (*B. hebetor*) density at a fixed number of hosts GWM larvae showed that production of total adult progeny of parasitoids increased gradually with the decrease in density of host larvae per container. Proportion of female also decreased with increasing parasitoid density at a fixed level of host larvae. Therefore, for multiplication of *B. hebetor* low level of density may be (8 pairs) recommended using at the time of infestation.

Findings of the experiment of host density on mass production of parasitoid (*B. hebetor*) revealed that population of the number of progenies increased linearly with the increased level of host density GWM larvae up to 50 larvae/container at fixed level of parasitoid (two pairs). The increase was not considerable when host density increased to 40 per container. Effect of host density on sex ratio of progeny

[DOR: 20.1001.1.22519041.2016.5.1.11.0]

was not significant. In another experiment, where 200 host larvae were released in 1000 ml plastic container and parasitized with 50 adult parasitoid (35 female and 15 male), average parasitism was 98%, period of parasitism to adult emergence was 8-11 days and average production of adult progeny was 1091 per container.

It may be possible that at high density of parasitoid, it may have suffered from the higher level of immature mortality due to competition for food as proposed by Benson (1973) and Yu *et al.* (2003). They reported that in case of *B. hebetor* parasitizing *Cadra* (= *Ephestia*) *cautella* (Walker) and *P. interpunctella*, larval mortality increased abruptly when the number of eggs on a host went beyond approximately 8 and 10, respectively, because of competition among the larval parasitoids. Secondly, *B. hebetor* females may avoid laying more eggs than could complete development on a host, as explained by Yu *et al.* (2003), in which *B. hebetor* females optimized oviposition and did not lay more than 7 or 12 eggs day^{-1} when they encountered only one host larva of the tortricid, *Adoxophyes orana* or the pyralid, *P. interpunctella*, respectively. Despite reduced progeny production per female observed at higher parasitoid introduction densities, the maximum number of progeny produced in this study came from containers having 10 male-female pairs of *B. hebetor*, which satisfied the objective of this study to develop a method to maximize production of wasp progeny in a mass rearing context. Additionally, a potential benefit of using a higher density of parental *B. hebetor* in a mass-rearing context was that the genetic variability and that "quality" of the progeny might be improved by promoting out-breeding and avoiding deleterious effects of inbreeding (Antolin *et al.*, 1995; Ode *et al.*, 1996) with a larger parental group of wasps in each container.

The present study showed that more adult parasitoid progeny were produced as host density increased. The results from this study were not in accordance with the earlier finding of Taylor (1988a, b) in which he reported the total numbers of eggs laid by *B. hebetor* was independent of the host density. The difference between these findings and Taylor's results could be due to a difference in the parasitoid populations or experimental conditions. The findings of present study agreed with more recent work of Yu *et al.* (2003), in which *B. hebetor* females were able to allocate eggs in relation to host density. Ghimire and Phillips (2010) studied the effects of host density, parasitoid density and the rearing containers size on adult progeny production and sex ratio of *B. hebetor*. In parasitoid density experiments, a density of eight male-female pairs of *B. hebetor* produced a higher number of progeny on *P. interpunctella* larvae than the densities of one and two pairs of *B. hebetor*. Similarly, in a host density study, significantly higher number of parasitoid progeny was produced among the tested host densities when two pairs of *B. hebetor* and a density of 50 last instar *P. interpunctella* larvae were used (Ghimire, 2008).

In conclusion, in parasitoid density experiments, a density of ten male-female pairs of *B. hebetor* produced a higher number of progeny on 50 last instar GWM larvae. Similarly, in a host density experiment, a density of 60 last instars GWM larvae produced a significantly higher number of parasitoid progeny followed by 50 last instar GWM larvae among the tested host densities when two pairs of *B. hebetor* were used. The sex ratio of the progenies was male-biased in these studies and there were no significant effects on sex ratio from variation in host density and parasitoid density.

Acknowledgements

This is a part of PhD work. This work has been financially supported by the Bangladesh Agricultural Research Institute strengthening fund phase-2, Ministry of Agriculture, The Government of the Peoples Republic of Bangladesh. The author is grateful to financial authority.

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پرورش انبوه زنبور *Bracon hebetor* (Hym.: Braconidae) روی شب‌پره موم، *Galleria mellonella* (Lep.: Pyralidae) در تراکم‌های مختلف پارازیتویید و میزبان

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دریافت: ۵ آبان ۱۳۹۳؛ پذیرش: ۲۲ آبان ۱۳۹۴

چکیده: روش‌های پرورش انبوه زنبور *Bracon hebetor* (say) (Hym., Braconidae) در مجموعه‌ای از مطالعات آزمایشگاهی به‌منظور افزایش عملکرد پرورش انبوه این پارازیتویید برای کنترل بیولوژیک آفات پروانه‌ای در مزرعه و انبار مورد بررسی قرار گرفت. در این آزمایشات تأثیر تراکم‌های مختلف پارازیتویید و میزبان روی باروری و نسبت جنسی زنبور *B. hebetor* ارزیابی شد. در تراکم‌های مختلف پارازیتویید، ۵۰ لارو سن آخر شب‌پره بزرگ موم، *Galleria mellonella* (L.) در هر ظرف قرار داده شد و تراکم‌های ۱، ۲، ۴، ۸ و ۱۰ جفت زنبور *B. hebetor* (یک زنبور نر و یک زنبور ماده) در هر ظرف رهاسازی شد. در آزمایش تأثیر تراکم میزبان، دو جفت زنبور پارازیتوئید در هر طرف محتوی تراکم‌های مختلف میزبان (۱۰، ۲۰، ۳۰، ۴۰، ۵۰ و ۶۰) رهاسازی شد. تراکم ۱۰ جفت زنبور پارازیتوئید نر و ماده تولید تعداد بیش‌تری نتاج (۷/۰۷ ± ۲۰۵ حشره کامل) در ۵۰ لارو سن آخر شب‌پره بزرگ موم نمود. به‌طور مشابه، در آزمایش تراکم میزبان که در آن دو جفت زنبور پارازیتوئید مورد استفاده قرار گرفت، تراکم ۶۰ لارو سن آخر میزبان در بین تراکم‌های مختلف به‌طور معنی‌دار تعداد بیش‌تری نتاج زنبور پارازیتویید (۸/۷۵ ± ۱۴۲ حشره کامل) تولید نمود و پس از آن تراکم ۵۰ لارو سن آخر میزبان (۸/۳۴ ± ۱۴۱ حشره کامل) قرار داشت. نسبت جنسی نتاج در کلیه آزمایشات تمایل به سمت نسبت بیش‌تر نتاج نر داشت ولی تفاوت معنی‌داری بین نسبت جنسی در تراکم‌های مختلف میزبان و پارازیتویید مشاهده نشد. در آزمایش تولید انبوه، مجموع تعداد پارازیتوییدهای ظاهر شده به‌ازای ۲۰۰ لارو شب‌پره بزرگ موم ۸۲/۳۸± ۱۰۹۱ حشره کامل با نرخ پارازیتیسم ۰/۸ ± ۹۸ درصد تعیین شد.

واژگان کلیدی: کنترل بیولوژیک، پرورش انبوه، *Bracon hebetor*، تراکم پارازیتویید، تراکم میزبان