

**EFFECT OF STORAGE DURATION ON THE STORED PUPAE OF  
PARASITOID *Bracon hebetor* (Say) AND ITS IMPACT ON  
PARASITOID QUALITY**

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**Abstract**

The ecto-endo larval parasitoid, *Bracon hebetor* (Say) is an important bio-control agent. Effective storage methods for *B. hebetor* are essential for raising its success as a commercial bio-control agent against lepidopteran pests. The study was undertaken to determine the effect of storage duration on the pupae of *Bracon hebetor* in terms of pupal survival, adult emergence, percent parasitism, female and male longevity, female fecundity and sex ratio. Three to four days old pupae were stored for 0, 1, 2, 3, 4, 5, 6, 7 and 8 weeks at  $4 \pm 1^\circ\text{C}$ . The ranges of time for adult emergence from stored pupae, production of total adult, survivability of pupae, parasitism of host larvae by the parasitoid, longevity of adult female and male and fecundity were 63.0 -7.5 days, 6.8-43.8/50 host larvae, 13.0-99.5%, 0.0 -97.5%, 0.00-20.75 days, 0.00-17.25 days and 0.00-73.00/50 female, respectively. The time of adult emergence and mortality of pupae increased but total number of adult emergence, survivability of pupae, longevity of adult female and male decreased gradually with the progress of storage period of *B. hebetor* pupae. The prevalence of male was always higher than that of female. Therefore, short-term storage of *B. hebetor* pupae could be stored for up to 4 weeks without disturbing the functioning of the parasitoid. It is important for sustaining and accumulating large numbers of parasitoids in mass rearing programs and subsequent use of field application.

Keywords: Cold storage, pupae, *Bracon hebetor*; biological control.

**Introduction**

The ecto-endo larval parasitoid, *Bracon hebetor* (Hymenoptera: Braconidae) is deemed as an important bio-control agent of different lepidopteran pests invading warehouse and field (Uwais *et al.*, 2006; Imam *et al.*, 2007; Garba and Gaoh, 2008). It is broadly used in studies of host parasitoid relations and biological control due to its high propagative rate, short cohort time and extensive range of host species (Asfaq *et al.*, 2011).

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The mass production of *B. hebetor* is restricted by the accessibility of host larvae. Moreover, host larvae parasitized by *B. hebetor* may be oversupplied and rejected when demand for parasitoids is low. As a result, finding effective storage methods for *B. hebetor* is necessary for increasing its success as a commercial biocontrol agent against lepidopteran pests. One of the major difficulties to the use of biological control agent as insect natural enemies is the breakdown to obtain adequate numbers required for release (Coudron *et al.*, 2007). As a result, the enhancement of storage methods for biocontrol agents is very important for efficient mass production of the parasitoid (Tezze and Botto, 2004; Greenberg *et al.*, 1996; Leopold, 1998). Cold storage is regarded as an important tool for storage of mass reared biocontrol agents (Chen *et al.*, 2011). For escalating the shelf-life of natural enemies and to make available a stable and enough number of parasitoids for biological control programs low temperature storage is an important technique. Storage at low temperature also permits coordinated field releases of natural enemies during the crucial stages of pest occurrences (Venkatesan *et al.*, 2000; Colinet and Boivin, 2011).

Adults and pupae have been accounted to be the most cold-hardy stages of *B. hebetor* (Franqui Rivera, 1995; Carrillo *et al.*, 2005). Very few studies have been done with cold storage on the performance of *B. hebetor*, and this study has focused on the adult stage (Al-Tememi and Ashfaq, 2005). Storage of *B. hebetor* pupae at 6°C for 3 weeks resulted in an 82.7% decrease in adult emergence (Al-Tememi and Ashfaq, 2005). According to Chen *et al.* (2011) more than 30 days of storage decreases parental parasitism but had no effect on parasitism of the F<sub>1</sub> generation. Parental durability and fecundity reduced after more than 20 days of storage. The present study was undertaken to determine the effect of storage duration of pupae of *B. hebetor* at 4±1 °C on some biological parameters of adults and their progeny.

## **Materials and Method**

### **Collection of host insect wax moth (*Galleria mellonella*)**

The target host used in the study was *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). It was originally collected from infested bee-hive and maintained on artificial diet in IPM laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. The dark bee wax was sterilized by boiling and put in 1000 ml glass jars. One hundred larvae of wax moth were kept in the glass jar. The adults of *G. mellonella* coming out from the wax in the jar were allowed to mate and egg laying. After egg hatching, the 1<sup>st</sup> and 2<sup>nd</sup> instar larvae of wax moth were released in jars containing artificial diet. The diet was prepared by mixing wheat flour, maize crush, milk, sugar, animal fat, wax and yeast and autoclaved at 120 °C under 1.0 Kg cm<sup>-2</sup> pressure for 70 minutes. Full grown final instar larvae were collected after 18 to 20 days and used as host for

rearing of *B. hebetor*. The ambient temperature of the rearing room was  $26.0 \pm 2^\circ\text{C}$ , relative humidity  $70 \pm 5\%$  and 12L: 12D photoperiod.

### **Collection of *Bracon hebetor***

*Bracon hebetor* was obtained from IPM laboratory, Entomology Division, BARI, Gazipur. It is used for many years for the biocontrol of lepidopteran pest and maintained on honey and last instar larvae of *G. mellonella*. Adult parasitoids were introduced into plastic jars (1000 ml) containing last instar larvae of *G. mellonella* and a cotton ball soaked with honey. Mouth of the jar was covered with black cloth. Environmental conditions of the rearing were the same as used for rearing the host *G. mellonella*. To obtain newly emerged parasitoids, 3 females and 2 males of *B. hebetor* adults were released into a plastic jar containing 10 *G. mellonella* final instars larvae and the whole container was covered with black cloth and kept for 3-4 days. To assess the parasitic activity of the *B. hebetor* in low temperature, 3 to 4 days old parasitoid pupae were isolated from the laboratory culture and placed in plastic jars. Before placing of pupae, the jars were sterilized in an electric oven. Each jar received 50 parasitoid pupae. The jars were stored in a refrigerator at  $4 \pm 1^\circ\text{C}$  and 60-70% RH for 1, 2, 3, 4, 5, 6, 7 and 8 weeks. Four jars (replications) were used for each treatment (storage period). Another four jars containing pupae of the parasitoid were stored at room temperature, which served as a control.

### **Effect of cold storage on *Bracon hebetor* parental generation**

At the end of each storage period, the pupae were moved to a chamber having ambient temperature of  $26 \pm 2^\circ\text{C}$  and RH  $70 \pm 5\%$ . Data on pupal survivability (adults emerged per parasitized pupae), time of adult emergence (days between the end of cold storage of the pupae until emergence of adult parasitoid) and sex ratio (adult females per total individuals) were recorded. The effects of storage time in low temperature storage on parasitism, longevity and fecundity of the parental generation were noted. To examine the capability and performance of a female *B. hebetor* after low temperature storage, a female and a male were taken where emerged from each cold storage period. Both male and female were placed in a plastic jar (1000 ml) having corrugated sheet containing 10 released larvae of *G. mellonella* and a cotton ball dipped in honey. The mouth of the jars was covered with black cloth and the whole container was covered with black cloth. The wax moth larvae and *B. hebetor* were placed on a rack for 8-10 days for parasitization, egg laying, pupation and adult emergence of *B. hebetor*. Data were expressed as % parasitism, fecundity and total adult progeny and sex ratio for each storage duration. Each treatment was replicated four times using completely randomized Design. There was also a control treatment maintaining room temperature  $26 \pm 2^\circ\text{C}$  and  $70 \pm 5\%$  RH.

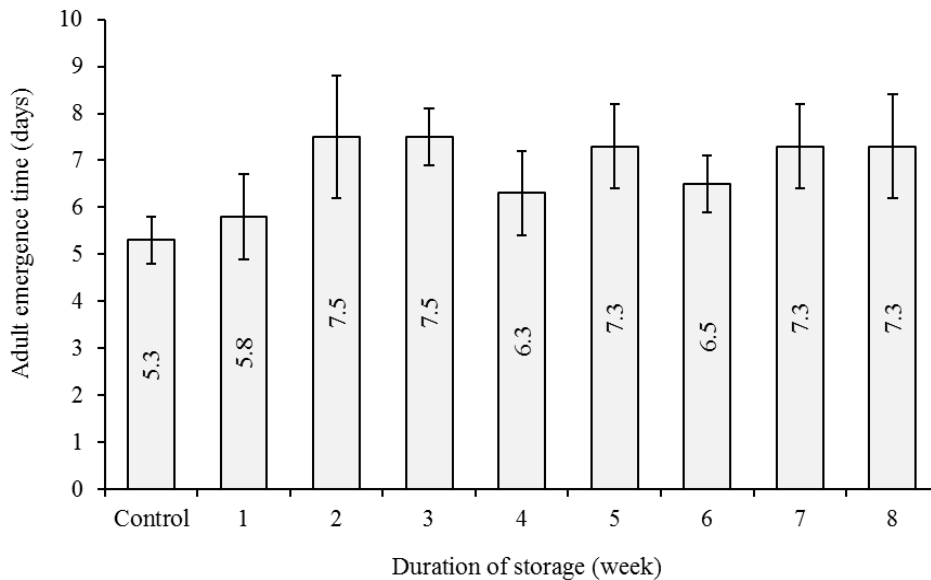
### Statistical analysis

Data were analyzed using SPSS 16 software and presented in mean  $\pm$  SE. Mean time of developmental, parasitism, longevity of adult, sex ratio, and daily and lifetime fecundity of the parasitoid were evaluated using one-way Analysis of Variance (ANOVA). Differences among the means were evaluated using Turkey's Honestly significant differences test. Before performing ANOVA, % parasitism, survival and mortality were transformed using arcsine transformation.

### Results and Discussion

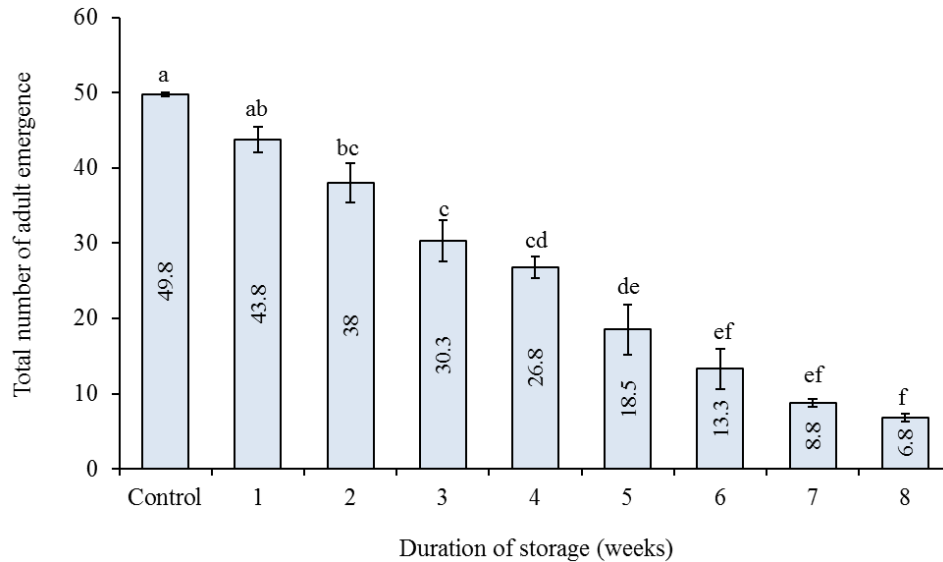
#### Effect of cold storage on *B. hebetor* parental generation

The minimum time of 5.3 days was required for adult's emergence from pupae under control. The time increased to 6.3-7.5 days when the pupae were stored in a refrigerator for 1 to 8 weeks. The increase in emergence time was significant over control when the pupae were stored for 2-8 weeks. The differences in adult emergence time from pupae stored for 2-8 weeks were not significant (Fig. 1).



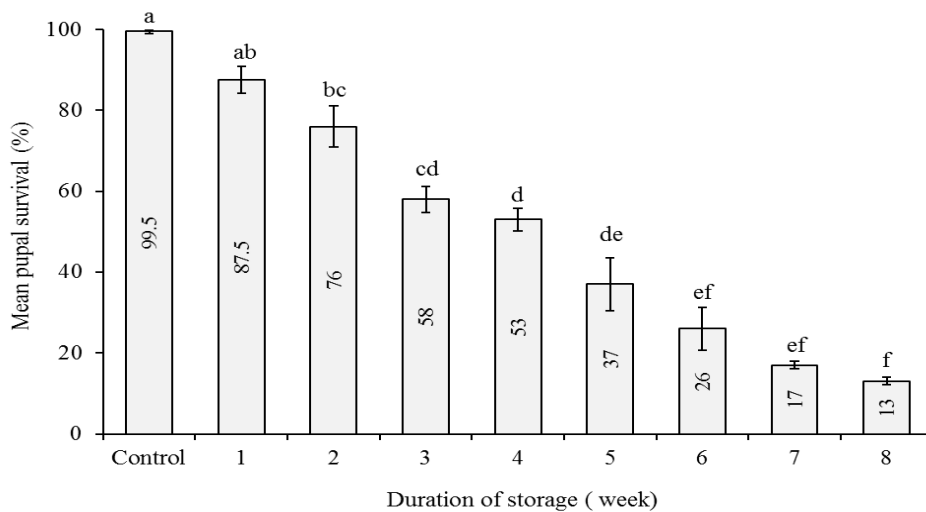
**Fig. 1. Adult emergence time (days) of *Bracon hebetor* from pupae stored at 4°C in a refrigerator for 1-8 weeks. Vertical line in the bar denotes the standard error of mean.**

Significantly the highest number of adult (49.8) was emerged in control. Adult emergence decreased 43.8 - 6.8  $50^{-1}$  pupae when the pupae were stored for 1-8 weeks. The lowest number of adults emergence was recorded from pupae stored for 8 weeks (Fig. 2).



**Fig. 2.** Total number of adult emergence from pupae of *Bracon hebetor* stored at 4°C in a refrigerator for 1 to 8 weeks. Vertical line in the bar denotes the standard error of mean. Bars indicating the same letter are not significantly different by Tukey's HSD test.

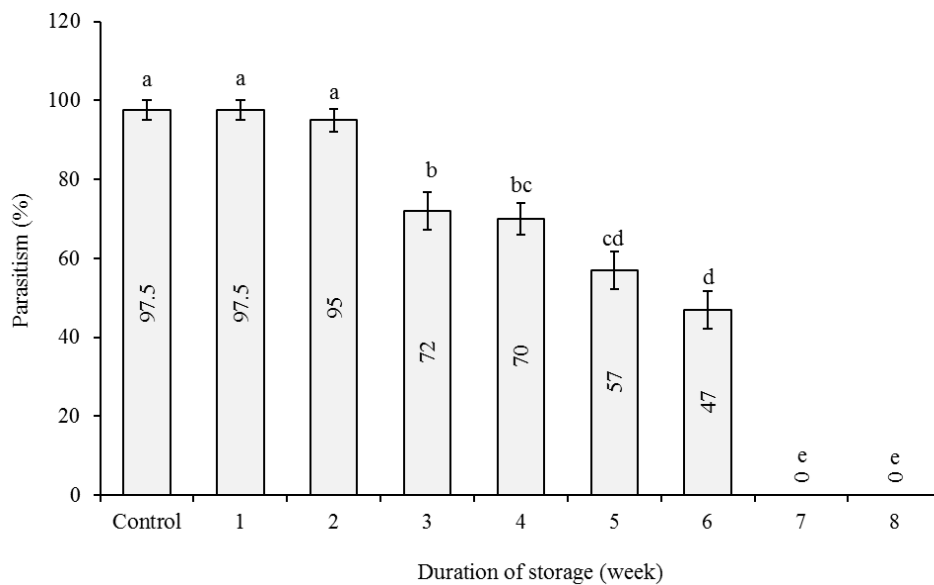
Significantly the highest percentage of survival (99.5%) of *B. hebetor* pupae was recorded in control. The survival rates were significant differences. The lowest survival rate (13.0%) was recorded after 8 weeks of storage (Fig. 3).



**Fig. 3.** Mean survival percentage of *Bracon hebetor* pupae stored at 4°C for 1-8 weeks. Vertical line in the bar denotes the standard error of mean. Bars followed by the same letter are not significantly different by Tukey's HSD test.

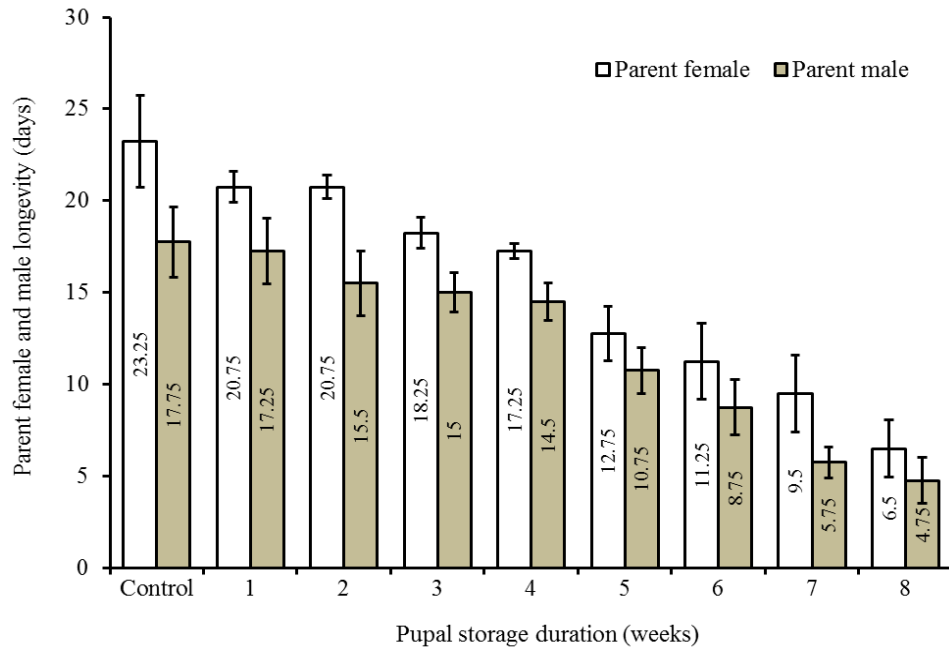
### Parasitism efficacy of *Bracon hebetor* after 1-8 weeks of storage

Percentage parasitism in 1 and 2 weeks were 97.5, 97.5 and 95.0%, which were statistically similar but significantly higher compared to other period of storage. After 2 weeks, the parasitism decreased gradually. The lowest parasitism was found after 6 weeks, which was statistically similar to 5 weeks of storage (Fig. 4). The pupae of *B. hebetor* stored for more than 6 weeks failed to parasitize the host larvae (Fig. 4).

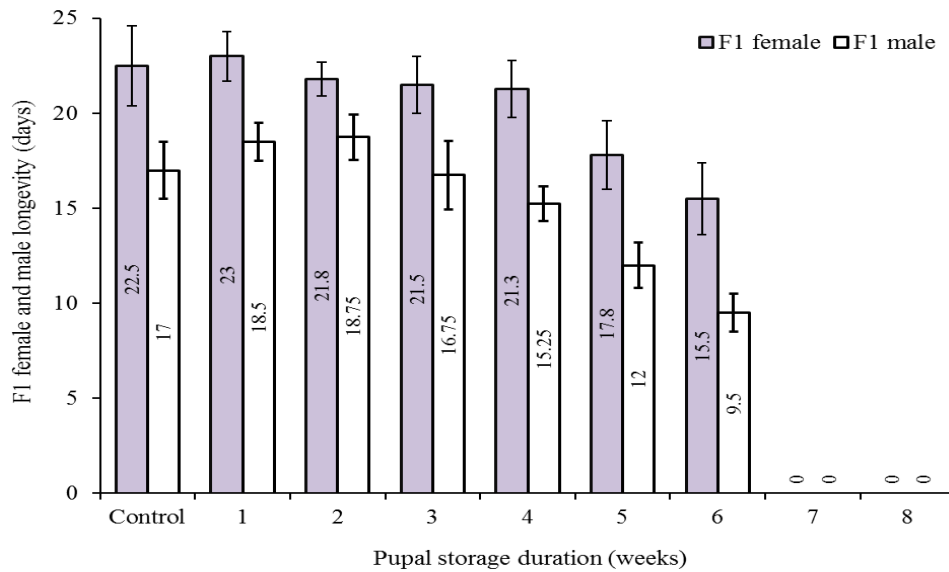


**Fig. 4.** Percentage of parasitized host (*G. mellonella*) larvae per female of *Bracon hebetor* emerged from pupae stored at 4°C for 1-8 weeks. Vertical line in the bar denotes the standard error of mean. Bars indicating the same letter are not significantly different by Tukey's HSD test.

The highest longevity of parent female was recorded from control, which was statistically similar to the females emerged from pupae stored for 1 and 2 weeks. The lowest longevity was found after 6 weeks of storage. The maximum survivability was found in parent male emerged from pupae stored at room temperature (control) followed by pupae stored at 4°C for 1, 2, 3 and 4 weeks (Fig. 5). The lowest longevity of F<sub>1</sub> male was recorded from pupae stored for 6 weeks followed by 5 weeks of storage (Fig. 6). Both F<sub>1</sub> female and male were recorded when the pupae were stored for 7 weeks or longer duration because no parasitism occurred. It is evident that the longevity of parents was generally lower than that of their F<sub>1</sub> offspring in both the cases of male and female.



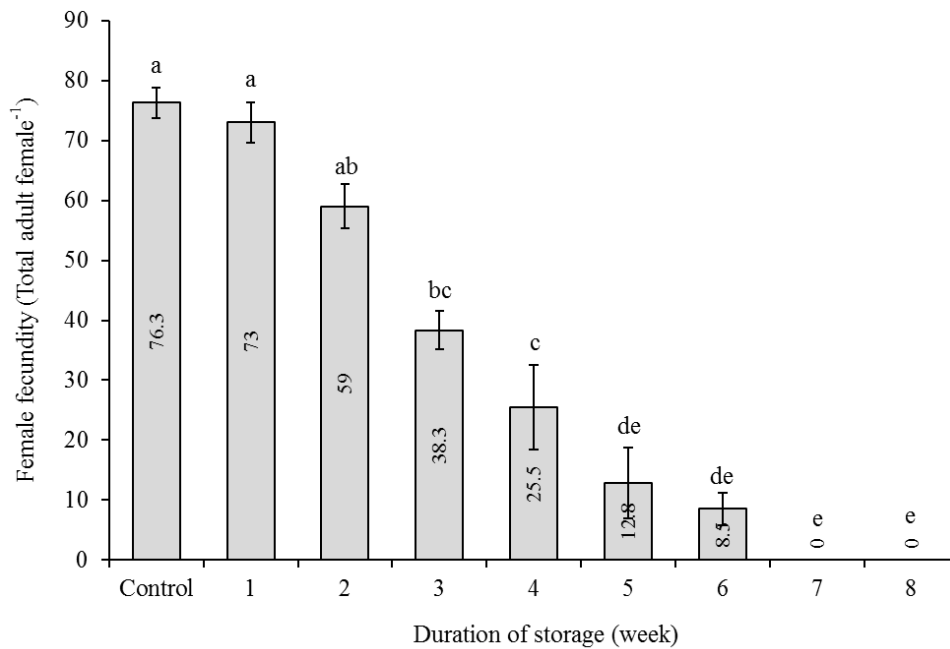
**Fig. 5.** Longevity of female and male parents emerged from pupae of *Bracon hebetor* stored at 4°C for 1-8 weeks. Vertical line in the bar denotes the standard error of mean.



**Fig. 6.** Longevity of F<sub>1</sub> female and male parents emerged from pupae of *Bracon hebetor* at 4°C for 1-8 weeks. Vertical line in the bar denotes the standard error of mean.

### Fecundity of parents

The highest fecundity (76.3 adults female<sup>-1</sup>) was recorded from pupae stored under room conditions, which was not significantly different from that of parents emerged from pupae stored for 1 week at 4°C. The fecundity of parents emerged from pupae stored at 4°C for 2, 3 and 4 weeks was different. The lowest fecundity was observed after 6 week of storage (8.5 adults female<sup>-1</sup>) followed by the storage duration of 5 weeks (Fig. 7).



**Fig. 7.** Fecundity (total adult female<sup>-1</sup>) of *Bracon hebetor* parents emerged from pupae stored at 4°C for 1-8 weeks. Vertical line in the bar denotes the standard error of mean. Bars indicating the same letter are not significantly different by Tukey's HSD test.

### Sex ratio of parent and F<sub>1</sub> progeny

The percentages of female and male emerged from pupae stored under control and at 4°C for 1-8 weeks ranged 26.97 – 40.43% with a mean of 40.44% and 51.40 – 59.57% with a mean of 59.57%, respectively. After each storage duration in refrigerator as well as under control, the prevalence of male was higher than that of female (Fig. 8). Like parent female and male sex ratio (%), the prevalence of male was higher compared to F<sub>1</sub> female. The percentage of F<sub>1</sub> female ranged 26.97 – 46.36% with a mean of 40.98% and that of male ranged 51.71 – 63.61% with mean 43.96% (Fig. 9).



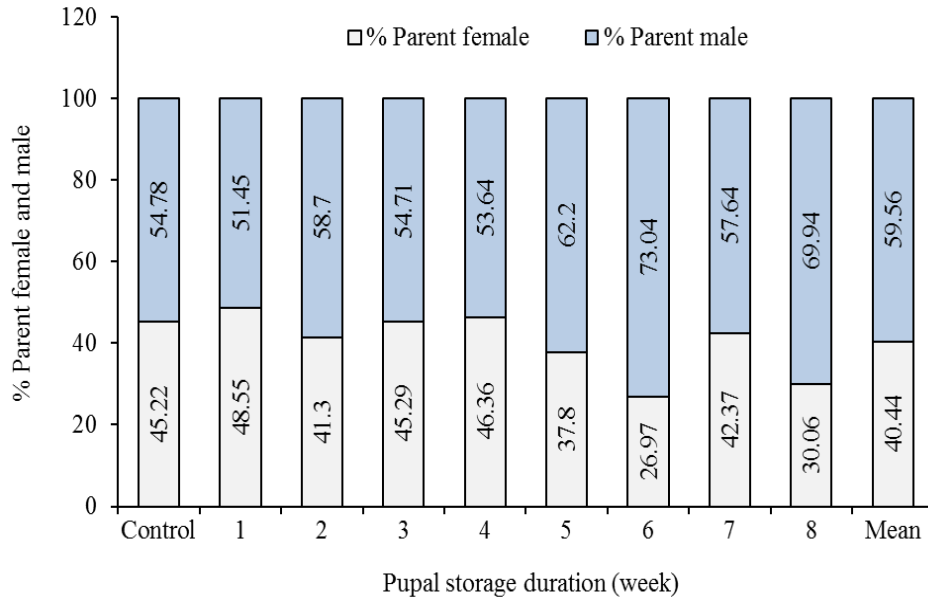


Fig. 8. Percentage of parent adult female and male emerged from pupae of *Bracon hebetor* stored at 4°C for 1-8 weeks.

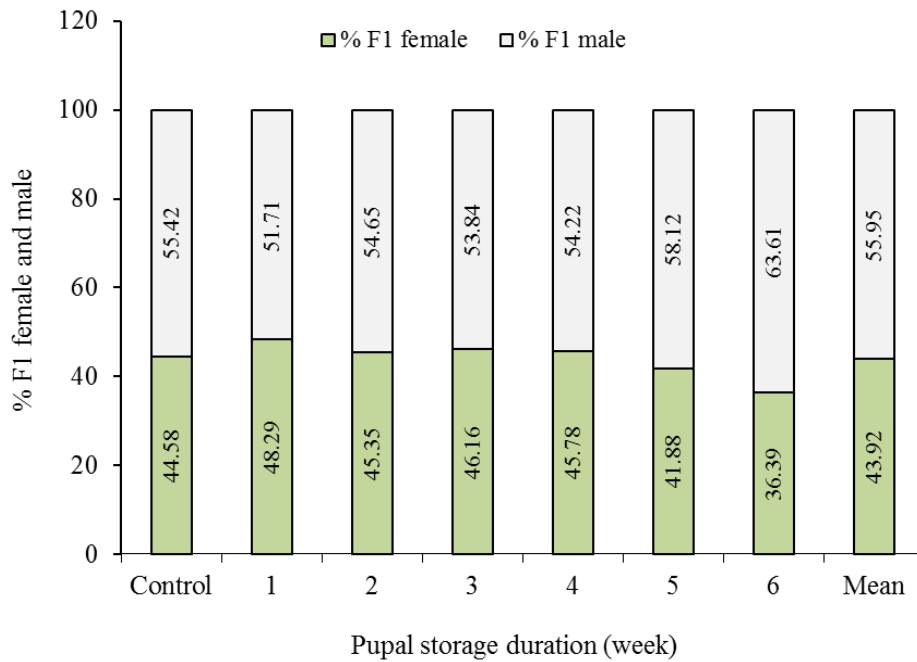


Fig. 9. Percentage of F<sub>1</sub> female and male of *Bracon hebetor* emerged from pupae stored under room conditions and at 4°C for 1 to 6 weeks.

Due to storage of *B. hebetor* pupae in a refrigerator at 4°C for 1- 8 weeks, the time of adult emergence and mortality of pupae increased but total number of adult emergence, survivability of pupae, longevity of adult female and male decreased gradually with the progress of storage period of *B. hebetor* pupae of the parasitoid. The prevalence of male is always higher compared to female. Similar findings have been reported by many other investigators (Al-Ramahi and Ali, 1983; Ahmed *et al.*, 1985). Adult emergence time is vital to realize the time needed for adult coming out after removing pupae from cool storage, particularly in order to synchronize adult emergence of parasitoid and availability of mature larvae of the pest in the field. Cold temperature prevented the embryonic growth of *Trichogramma nerudai* pupae (Tezze and Botto, 2004). In addition, in the existing observation the adult emergence time for cold stored pupae was 1 day higher than in the control group. This dissimilarity might be due to putting off in returning back to standard metabolic conditions at exposure after being cold stored for longer period. It is a correct statement that organisms placed at low, above zero temperatures have seasonal restructuring of metabolism as a way of cold-hardiness (Danks, 1978).

Mortality denotes the overall result of extended cold exposure. Moreover to cool damage, parasitoids stored as undeveloped may not have adequate energy to complete their growth and/or to come forward. In some solitary species, dissection of non-emerged parasitoids after cold storage has shown that mortality occurred mainly after metamorphosis in pharate adults (Colinet *et al.*, 2006; Colinet and Hance, 2009). There have been reported that the completely developed adults dying during the eclosion process (Levie *et al.*, 2005; Luczynski *et al.*, 2007). The procedure of coming out is energy-consuming as it needs strong muscle convulsion (Yocum *et al.*, 1994) and chilling is known to induce muscular nonfunctions (Kelty *et al.*, 1996). Emergence of pharate adults fail after cold storage due to lack of energy along with muscular perturbation. In another species, mortality withstands generally during pupation (Chen and Leopold, 2007).

Longevity of parents and their F<sub>1</sub> offspring declined with increase in cold storage period. Because living organisms have limited resources which are spent on growth, reproduction, and maintenance (Green and Rothstein, 1991), a possible explanation for the decline in longevity may be that increasingly larger amounts of resources are depleted by maintenance during longer periods of cold storage at 4°C thereby resulting in reduced performance and survival (Salt, 1961). In addition, accumulation of toxic metabolites also causes death or reduced fitness after prolonged exposures to cold (Storey and Storey, 1988).

Fecundity of parents declined with increase in cold storage period. Decline in fecundity with increase in cold storage period is probably due to the aforementioned reasons. The reproductive organs are particularly vulnerable to

low temperature effects (Denlinger and Lee, 1998). In *Euchalcidia caryobori* (Hanna), low temperature may either cause retardation of egg maturation or, in its extremes, malformation of reproductive organs in both sexes (Hanna, 1935). Numerous observations have been made on the reduction of parasitoid fecundity after cold storage. Like most of the traits affected by cold storage, the fecundity cost is generally proportional to both the temperature and the duration of exposure (Venkatesan *et al.*, 2000; Al-Tememi and Ashfaq, 2005).

Our present results demonstrated that the low temperature storage of the maternal age group did not seem to impact F<sub>1</sub> generation characters when cold storage periods were shorter than 35 days. This probably might be due to action of cold storage for the maternal generation only. The procedure for overtaking maternal effects of chilling injury to the F<sub>1</sub> generation is unidentified. One possible explanation is the property of eggs laid by females stored for a long time may decline. An alternative grounds for reduced fitness of the F<sub>1</sub> progeny might have been due to maternal aging (Hercus and Hoffmann, 2000; Opit and Throne, 2007).

Effect of low temperatures recurrently deform insect sex ratios (Denlinger and Lee, 1998). Hymenopteran parasitoids typically have a haplodiploid sex-determination system, as their females are able to decide the sex of their generation by regulating fertilization (Flanders, 1956). Deformation of sex ratio may appear from diverse origins. First alteration of the ratio of fertilized eggs oviposited (i.e. primary sex ratio) may be monitored following alteration of the female reproductive approach or the incapability of males to mate or inability to produce viable sperm after a cold treatment (Lacoume *et al.*, 2007). Sex ratio deformations can also result from a discrepancy of mortality between sexes when undeveloped stages are exposed to cold (i.e., secondary sex ratio). The results of the present study are similar to the findings of other studies (Ashfaq *et al.*, 2011; Shawkit *et al.*, 2000). They found that the ratio of adult-emergence from the cold-stored pupae of this parasitoid decreased with the increase of storage period. Findings denoted that the sex ratio of *B. hebetor* was 1:1.3 (F:M) (Al-Ramahi and Ali, 1983). Another study indicated that female: male sex ratio was 1:3.9 (Ahmed *et al.*, 1985). Male biased sex ratio was obtained in the present which explained that the male eggs hatched before than the female eggs, and the male larvae would have a better survivability due to sufficient food (Benson, 1973).

Our findings reveal that *B. hebetor* can be stored up to 4 weeks without affecting the performance of the parasitoid. However, short-term storage of *B. hebetor* pupae can be used for regulating and gathering huge numbers of parasitoids in mass production programs.

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