



## Research Article

# Cold storage of pupae of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) and its effect on key biological characters

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**ABSTRACT:** *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) is a solitary nymphal endoparasitoid of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), an important pest of cotton in Haryana. The present study on the effect of cold storage on some biological characteristics of *A. bambawalei* was carried out by storing one-day-old mummies of *P. solenopsis* (i.e. parasitoid in pupal stage) for 1 to 8 weeks at four storage temperatures viz., 5, 10, 15 and 20°C at 75 per cent relative humidity. Emergence of the parasitoid adults from mummies occurred within first week during storage at 20°C, hence, this temperature was judged unsuitable for cold storage. The parasitoid *A. bambawalei* could be stored for one week at 5°C and for two weeks each at 10 and 15°C, without any significant effect on emergence, longevity, fecundity and per cent females emerging in  $F_1$  progeny. At the above mentioned temperatures and durations these biological parameters of the parasitoid were comparable with the emergence (100%), longevity (19.60 and 33.20 days for males and females, respectively), fecundity (24.60 mealybugs parasitized per female in five days) and per cent females emerging in  $F_1$  progeny (63.12%) recorded in the control (27°C).

**KEY WORDS:** *Aenasius bambawalei*, biological parameters, cold storage, mummies, *Phenacoccus solenopsis*, temperature, storage duration

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## INTRODUCTION

*Aenasius bambawalei* Hayat, a solitary nymphal endoparasitoid, plays an important role in biological suppression of solenopsis mealybug, *Phenacoccus solenopsis* Tinsley infesting cotton and other host plants (Ram *et al.*, 2009; Ram and Saini, 2010). The parasitoid can be easily reared in the laboratory on 3<sup>rd</sup> instar *P. solenopsis* nymphs reared on potato tuber sprouts (Vijaya, 2011; Zain-ul-Abdin *et al.*, 2012). For inoculative releases of *A. bambawalei* against *P. solenopsis* in cotton crop, it is imperative to ensure that a large number of parasitoids are available at the appropriate time of release. Evidently, a technique to store *A. bambawalei* at lower temperatures would be helpful in achieving the desired objective. Earlier, the optimum storage temperature and duration was determined for different parasitoids, viz., *Trichogramma achaeae* Nagaraja and Nagarkatti (Jalali and Singh, 1992), *Venturia canescens* (Gravenhorst) (Press and Arbogast, 1991), *Sturmiopsis inferens* Townsend (Easwaramoorthy *et al.*, 2000), *Goniozus nephantidis* Muesebeck (Venkatesan *et al.*, 2000) and *Aphidius ervi* Haliday (Ismail *et al.*, 2010). Therefore, the present study was undertaken to find out the optimum period of storage of mummies of

*P. solenopsis* harbouring pupal stage of *A. bambawalei* at different low temperature regimes without affecting the key biological attributes viz., emergence, longevity, fecundity and per cent females emerging in  $F_1$  progeny of the parasitoid.

## MATERIAL AND METHODS

The present study on the effect of cold storage of *A. bambawalei*, during pupal stage, on its biological characteristics was carried out by storing one-day-old *P. solenopsis* mummies at four storage temperatures viz., 5, 10, 15 and 20°C for 1 to 8 weeks at 75 per cent relative humidity in refrigerator (for 5°C) and BOD incubators in the Biological Control Laboratory of the Department of Entomology, CCS Haryana Agricultural University, Hisar, during 2012-13. Mealybug mummies harbouring the parasitoid, *A. bambawalei* were obtained as a result of parasitization of the host insect, *P. solenopsis* by the parasitoid. *P. solenopsis* was reared on potato tuber sprouts. The potato tubers were first washed in formalin solution, air dried and then dipped into gibberellic acid solution for 24 hours to hasten the germination process. When the potato tuber sprouts were 2.5-5

cm in length, these were used for host rearing. The sprouted potatoes were then placed in plastic tubs (10-15 potato tubers/tub) of 15 cm diameter. Each tub was provided with filter paper at its base and the latter was regularly moistened to maintain turgidity of the sprouted tubers. The sprouts were then inoculated with gravid females with the help of a soft camel hair brush and also with leaves and twigs of *Abutilon* plant carrying egg sacs and first instar nymphs of *P. solenopsis*. Each tub was then covered with muslin cloth, kept at room temperature in the laboratory and left undisturbed till the establishment of mealybug colonies. The parasitoid, *A. bambawalei* was reared on *P. solenopsis* by releasing males and females of the parasitoid emerged from field collected mealybug mummies in a single hole wooden cage having third instar and adult mealybugs on potato tuber sprouts. The single hole wooden cage was constructed using 1.6 cm thick plywood with wooden base and wooden side-walls, back wall had a fine mesh for aeration, front wooden wall had a 6 inch diameter hole in centre and roof of cage was covered with a glass piece and sealed properly. A muslin fabric sleeve (20 × 6) was attached to the hole and fastened with rubber bands. The cage was painted from inside with a white exterior latex paint. The parasitoids were provided honey and water in rearing cage. The potato tuber sprouts were taken out regularly after parasitization of *P. solenopsis* and replaced with new sprouts having third instars and adults of *P. solenopsis* for further parasitization. The mealybug mummies formed were collected from the cage daily and used in the experiments.

Four hundred parasitoids in pupal stage in one-day-old mealybug mummies were stored at each of the four storage temperatures i.e. 5, 10, 15 and 20°C. At each temperature, the mummies were divided into eight batches, for eight storage durations (1 to 8 weeks), each consisting of 10 mummies replicated five times (i.e. 50 mummies) and were placed in small glass vials (7.5 × 1 cm). The glass vials carrying parasitoids were kept in glass jars in complete darkness and 75 per cent relative humidity was maintained with saturated sodium chloride solution in the BOD incubators and refrigerator (for 5°C). At the end of each storage period at each of the four temperatures, 50 mummies (i.e. one batch) were removed, transferred individually to small glass vials and placed at 27°C for recording adult emergence. For recording adult longevity, five female and five male parasitoids that emerged from each storage period were placed in

small vials and provided with honey streak on a paper piece. Five female parasitoids from every batch were also used to determine the effect of storage on their ability to produce offspring. Each female was paired with a male from same batch and provided with 25 3<sup>rd</sup> instar mealybug nymphs per day for five consecutive days after emergence for estimating fecundity and per cent females emerging in F<sub>1</sub> progeny of the parasitoid. Data collected on different parameters of cold stored parasitoids were compared with those reared at 27°C (control). The experiment was conducted in a Completely Randomized Design (CRD) with five replications. The data on adult longevity and fecundity were transformed using square root transformation, while percentage data on adult emergence and proportion of females in the F<sub>1</sub> progeny were transformed using angular transformation and analyzed with one factor CRD. Wherever there is 100 in the data it was replaced by 100-0.5=99.5. Instead of zeros, 0.5 was taken as the observation for angular transformation.

## RESULTS AND DISCUSSION

### Adult emergence

The data on the emergence of *A. bambawalei* adults from cold stored mummies of *P. solenopsis* are presented in Table 1. At 20°C, the parasitoid adults emerged within first week during storage, while at 5, 10 and 15°C adult emergence took place only when the mummies were kept at 27°C after removal from storage. Due to negligible or no adult emergence recorded after 4 weeks of storage at 5°C and 7 seven weeks of storage each at 10 and 15°C, the data on adult emergence and other key biological parameters of the parasitoid could not be recorded. Adult emergence decreased significantly as compared to the control as the storage duration increased from 1, 2 and 3 weeks onwards at 5, 10 and 15°C, respectively. Flanders (1938) reported that if storage was prolonged, the parasitoids would be deprived of sufficient nutrient material to complete their development because of scarce availability of fat cells in parasitoids refrigerated for two or more weeks, a probable reason for reduction in emergence when there was an increase in storage duration. At 5°C, maximum (100%) emergence was observed one week after storage and it was at par with the control (100%). The emergence of parasitoid adults declined significantly after 2 weeks of storage (76%). At 10°C, 100 per cent adult emergence was recorded from mummies stored up to two weeks and it was at par with the control.

**Table 1. Emergence of *Aenasius bambawalei* adults from cold stored mummies of *Phenacoccus solenopsis***

Storage period (weeks)	Adult emergence (%)			
	Temperature (°C)			
	5	10	15	20
1	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	-
2	76.00 (61.04)	100.00 (85.91)	100.00 (85.91)	-
3	22.00 (27.58)	60.00 (50.97)	98.00 (83.03)	-
4	12.00 (17.26)	42.00 (40.26)	68.00 (55.86)	-
5	0.00 (4.05)	38.00 (37.78)	44.00 (41.47)	-
6	0.00 (4.05)	24.00 (28.79)	32.00 (34.27)	-
7	0.00 (4.05)	0.00 (4.05)	8.00 (14.30)	-
8	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	-
Control (27°C)	100.00 (85.91)	100.00 (85.91)	100.00 (85.91)	-
CD $P=0.05$	(6.82)	(6.64)	(7.14)	-

Figures in parentheses are means of angular transformations

The adult emergence declined significantly after three weeks of storage (60%). At 15°C, high (98-100%) adult emergence was recorded when mummies were stored up to three weeks and there was significant decrease in the adult emergence after four weeks of storage (68%). Abdullah *et al.* (2009) who studied the storage of *Anaphes iole* Girault also found that adult emergence was better when eggs were stored at 10°C than at 4°C. Likewise, studies on cold storage of *Trichogramma* spp. clearly demonstrated that longer storage times accompanied with lower temperatures adversely influenced adult emergence (Jalali and Singh, 1992; Rundle *et al.*, 2004; Tezze and Botto, 2004). It was concluded that host mummies can be stored for 1, 2 and 3 weeks at 5, 10 and 15°C, respectively, without any adverse effect on parasitoid adult emergence.

#### Adult longevity

The data on longevity of *A. bambawalei* male and female adults that emerged from the cold stored mummies of *P. solenopsis* are presented in Table 2. Male longevity decreased significantly as compared to the control as the

storage duration increased from one week onwards at 5°C and three weeks onwards each at 10 and 15°C.

Similarly, longevity of females decreased significantly as compared to the control as the storage duration increased from one, three and two weeks at 5, 10 and 15°C, respectively. In general, female parasitoids lived longer than the males after removal from cold storage at all the temperatures and storage durations studied. Bernardo *et al.* (2008) who studied storage of *Thribiopus javae* (Girault) pupae at 5 and 10°C for 7, 14, 21 and 28 days reported that males were more susceptible than females and longevity of adults emerged from stored pupae reduced significantly after 14 days of storage. Similarly, Ayvaz *et al.* (2007) who studied the effect of cold storage at 10°C for 1 to 4 weeks duration on *Trichogramma evanescens* Westwood reported that the longevity of parasitoid adults decreased with an increase in length of the storage period. In the present study, maximum male longevity (19.60 days) and female longevity (33.20 days) were recorded in the control. Longevity of the males (12.60) and females (24.20) emerged from the mummies stored for two weeks at 5°C was worse affected and was

**Table 2. Longevity of *Aenasius bambawalei* adults emerged from cold stored mummies of *Phenacoccus solenopsis***

Storage period (weeks)	Male longevity (days)				Female longevity (days)			
	Temperature (°C)				Temperature (°C)			
	5	10	15	20	5	10	15	20
1	17.80 (4.28)	19.40 (4.48)	19.00 (4.45)	-	29.00 (5.45)	32.40 (5.77)	29.80 (5.53)	-
2	12.60 (3.66)	17.40 (4.25)	17.40 (4.25)	-	24.20 (4.98)	28.40 (5.40)	27.20 (5.28)	-
3	10.60 (3.33)	16.60 (4.17)	16.20 (4.12)	-	20.60 (4.63)	26.00 (5.18)	25.20 (5.09)	-
4	-	14.40 (3.88)	12.80 (3.70)	-	-	24.80 (5.06)	20.00 (4.55)	-
5	-	11.60 (3.52)	10.40 (3.36)	-	-	20.60 (4.62)	19.00 (4.45)	-
6	-	10.00 (3.30)	08.80 (3.11)	-	-	16.00 (4.06)	12.80 (3.66)	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
Control (27°C)	19.60 (4.52)	19.60 (4.52)	19.60 (4.52)	-	33.20 (5.84)	33.20 (5.84)	33.20 (5.84)	-
CD $P=0.05$	(0.82)	(0.62)	(0.57)	-	(0.69)	(0.66)	(0.74)	-

Figures in parentheses are means of square root transformations

significantly lower than the control. These findings are in agreement with Rundle *et al.* (2004) who studied the effects of cold storage on laboratory performance of *Trichogramma carverae* Oatman and Pinto at 4, 8 and 10°C for 1 to 8 weeks of storage duration and reported that lower storage temperatures and storage duration of three weeks or longer had a negative impact on adult longevity. Among the cold storage temperatures, maximum male and female longevity of 19.40 and 32.40 days, respectively, were recorded after one week of storage at 10°C. In general, males and females emerged from the *P. solenopsis* mummies stored at 10°C lived longer than those emerged from the mummies stored at 5 and 15°C. Hence, the parasitoid could be stored in the pupal stage for 1, 3 and 2 weeks at 5, 10 and 15°C, respectively, without significant effect on the adult longevity. Thus, with respect to adult longevity, storage of the parasitoid for 3 weeks at 10°C seemed to be the most suitable storage duration and temperature, respectively.

### Fecundity

The data on fecundity (parasitized hosts/female in five days) of *A. bambawalei* presented in Table 3 revealed that the fecundity (24.60 parasitized hosts/female in five days) was maximum in control. Fecundity decreased significantly as compared to control as the storage duration increased

from one week onwards at 5°C and two weeks onwards each at 10 and 15°C.

Reduction in fecundity has also been recorded in different parasitoids like *Trichogramma chilonis* Ishii, *Trichogramma eldanae* Viggiani and *Goniozus nephantidis* Muesebeck by Khosa and Brar (2000), Jalali and Singh (1992) and Venkatesan *et al.* (2000), respectively. Flanders (1938) reported that prolongation of storage period affected the organs involved in reproduction by insufficient availability of nutrient material, a probable reason for reduction in fecundity when there was an increase in storage duration. Fecundity (14.20 parasitized hosts/female in five days) was found significantly lower than the control after two weeks of storage at 5°C. At 10°C, maximum fecundity (24.40 parasitized hosts/female in five days) was recorded after one week of storage and it was at par with the fecundity recorded in control. At 15°C, maximum fecundity (22.20 parasitized hosts/female in five days) was recorded after one week of storage and it was at par with the respective fecundity recorded in control. Hence, fecundity was found significantly lower than the control after three weeks of storage at 10 and 15°C. Pitcher *et al.* (2002) during cold storage studies on *Trichogramma ostrinae* Pang and Chen reported that rate of parasitism by stored *Tricho-*

**Table 3. Fecundity of *Aenasius bambawalei* females during first five days after emergence from cold stored mummies of *Phenacoccus solenopsis***

Storage period (weeks)	Fecundity (number of parasitized hosts/female in five days)			
	Temperature (°C)			
	5	10	15	20
1	20.20 (4.58)	24.40 (5.01)	22.00 (4.79)	-
2	14.20 (3.89)	21.80 (4.76)	19.80 (4.55)	-
3	8.40 (3.04)	18.60 (4.42)	17.80 (4.33)	-
4	-	11.80 (3.54)	11.20 (3.48)	-
5	-	6.60 (2.75)	5.60 (2.50)	-
6	-	3.00 (1.98)	4.00 (2.21)	-
7	-	-	-	-
8	-	-	-	-
Control (27°C)	24.60 (5.04)	24.60 (5.04)	24.60 (5.04)	-
CD ( $P=0.05$ )	(0.56)	(0.55)	(0.52)	-

Figures in parentheses are means of square root transformations

*gramma* was generally similar to control after 2 to 4 weeks of storage at 9 and 12°C but declined at storage duration longer than 4 weeks. Similarly, Jalali and Singh (1992) also reported that fecundity of *Trichogramma acheae* Nagaraja and Nagarkatti declined drastically after storage for 14 days of storage at 2 and 5°C and after 21 days of storage at 10°C. In general, females emerged from the mummies stored at 10°C produced more progeny than the females emerged from the mummies stored at 15 and 5°C. Hence, the parasitoid could be stored safely at 5°C for one week and for two weeks each at 10 and 15°C, without significantly affecting its reproductive potential.

#### **Females emerging in $F_1$ progeny**

The data on proportion of females in  $F_1$  progeny of adults of *A. bambawalei* emerged from cold stored mummies of *P. solenopsis* are furnished in Table 4. The data showed that the highest proportion of *A. bambawalei* females emerged (63.12%) in control. In general, the proportion of females in  $F_1$  progeny declined as the storage duration increased at 5, 10 and 15°C. Pitcher *et al.* (2002) during cold storage studies on *Trichogramma ostrinae* reported that emergence of progeny of cold-stored females

was lower than control for all treatments and the percentage of female progeny from cold stored females was comparable to control up to 4 weeks of storage.

At 5°C, highest proportion of females in  $F_1$  progeny (48.62%) was recorded after one week of storage and it was at par with the control. The proportion of females in  $F_1$  progeny recorded after two (40.41%) and three (34.11%) weeks of storage was significantly lower than the control. At 10°C, highest proportion of females in  $F_1$  progeny (55.49%) was recorded after one week of storage and it was at par with that recorded after two (50.84%) and three weeks (41.95%) of storage as well as the control. After four weeks of storage, it decreased gradually and was significantly lower than the control. At 15°C, highest proportion of females in  $F_1$  progeny (53.60%) was recorded after one week of storage and it was at par with that recorded after two (48.62%) and three weeks (39.97%) of storage as well as the control. After four weeks of storage it was significantly lower than the control. It was concluded that the  $F_1$  progeny of the females stored at 5, 10 and 15°C for 1 to 8 weeks became male biased as the storage durations increased. Our findings are supported by Ballal *et al.* (1989) who reported that the progeny of parasitoids that emerged from cocoons



of *Allorhogas pyralophagus* Marsh stored at 10°C for 35 days consisted of only males. Likewise, Ismail *et al.* (2010) stored the pupae of *Aphidius ervi* and interpreted that the sex-ratio of the progeny was male biased after storage for

two weeks at 7°C. Hence, the parasitoid could be stored safely at 5°C for one week and at 10 and 15°C for three weeks without affecting the proportion of females emerging in the F<sub>1</sub> progeny.

**Table 4. Proportion of females in F<sub>1</sub> progeny of adults of *Aenasius bambawalei* emerged from cold stored mummies of *Phenacoccus solenopsis***

Storage period (Weeks)	Emergence of females in F <sub>1</sub> progeny (%)			
	Temperature (°C)			
	5	10	15	20
1	48.62 (44.20)	55.49 (48.15)	53.60 (47.07)	-
2	40.41 (39.32)	50.84 (45.48)	48.62 (44.20)	-
3	34.11 (35.51)	41.95 (40.22)	39.97 (39.16)	-
4	-	34.48 (35.83)	31.32 (31.74)	-
5	-	26.90 (29.08)	19.66 (22.51)	-
6	-	13.32 (16.52)	11.66 (15.48)	-
7	-	-	-	-
8	-	-	-	-
Control (27°C)	63.12 (52.63)	63.12 (52.63)	63.12 (52.63)	-
CD ( <i>P</i> =0.05)	(8.58)	(12.63)	(14.55)	-

Figures in parentheses are means of square root transformations

Thus, it can be concluded from the present studies that the parasitoid, *A. bambawalei* can be stored at low temperatures in pupal stage (i.e. in the mummies of *P. solenopsis*). The parasitoid can be stored in pupal stage for one week at 5°C and for two weeks each at 10 and 15°C, without any significant effect on the key biological parameters like emergence, longevity, fecundity and per cent females emerging in F<sub>1</sub> progeny. The results could be utilized for storage of mass produced parasitoids in the laboratory, aiming the biological control of solenopsis mealybug.

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