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Research Article

Response of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) adults to low temperature storage in relation to key biological parameters

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ABSTRACT: Studies were carried out to study the effect of storage of *Aenasius bambawalei* Hayat in adult stage at low temperatures *viz*., 5, 10, 15 and 20°C for different durations (1to 8 weeks) on the survival, longevity, fecundity and per cent females emerging in F1 progeny. One-day-old parasitoid adults were stored at each of the temperatures at 75 per cent relative humidity in full darkness. Key biological parameters of the adults surviving cold storage were studied and compared with that of unstored (control i.e. at 27°C) parasitoids. The survival of the adults was worse affected at 5°C, where the survival of the males and females was only 68 and 80 per cent, respectively, after one week of storage and complete mortality was observed after five weeks of storage. The best results were obtained at 10°C, where 100 per cent of the males and females survived storage up to three weeks and thereafter survival declined significantly being minimum of 20 and 32 per cent, respectively, after eighth week of storage. Adult longevity (19.20 and 33.00 days, respectively, for male and female), fecundity (24.00 parasitized hosts/female in five days) and per cent females in F1 progeny (48.10%) recorded after one week of storage at 10°C were comparable with the control. Thus, adults of the parasitoid, *A. bambawalei* could be stored only for 1 week at 10°C without any significant effect on the key biological parameters. Therefore current studies suggest that short-term storage could be used for maintaining and accumulating large number of parasitoids for inoculative releases against *Phenacoccus solenopsis* Tinsley.

KEY WORDS: *Aenasius bambawalei*, adults, biological parameters, cold storage, storage duration, temperature.

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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 also several other crops. The parasitoid, *A. bambawalei,* probably got introduced into the Indian subcontinent simultaneously along with *P. solenopsis* representing an example of fortuitous biological control (Ram *et al.,* 2009; Ram and Saini, 2010). The parasitoid can be easily reared in the laboratory on third instar solenopsis mealybug nymphs reared on potato tuber sprouts (Vijaya, 2011). 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* adults at lower temperatures would be helpful in achieving the desired objective. Therefore, the present studies were undertaken to find out the optimum period of storage of *A. bambawalei* adults at different low temperature regimes without affecting the key biological parameters *viz*., survival, longevity, fecundity and per cent females emerging in F_1 progeny of the parasitoid.

MATERIALS AND METHODS

The present studies were carried out by storing one-day-old *A. bambawalei* males and females at four storage temperatures *viz*., 5, 10, 15 and 20°C for 1 to 8 weeks and 75 per cent relative humidity in refrigerator (for 5°C) and 10, 15 & 20°C in BOD incubators in the Biological Control Laboratory of the Department of Entomology, CCS Haryana Agricultural University, Hisar, during 2013.

Host rearing

For rearing the host insect i.e. *P. solenopsis* small to medium sized healthy potato tubers were procured and brought to the laboratory. The potato tubers were washed in formalin solution and air dried in the laboratory. Later on, the potato tubers were dipped into gibberellic acid solution for 24 hours to hasten the germination process. Thereafter, the potato tubers were kept in dark room under laboratory conditions for sprouting. 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 and heath of the sprouted potato tubers. The potato sprouts were then inoculated with gravid females and leaves of *Abutilon* plant carrying first instar nymphs and egg sacs of mealybug. Each tub was then covered with muslin cloth, kept at room temperature in the laboratory and left undisturbed till the establishment of mealybug colonies. Depending upon the requirement of mealybugs for the experiment, more number of such tubs containing mealybug colonies were prepared and maintained regularly.

Parasitoid rearing

The parasitoid, *A. bambawalei* was reared on *P. solenopsis* by releasing males and females emerged from field collected mealybug mummies in a single hole wooden cage having third instar nymphs 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″ diameter hole in centre and top of the 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 rearing cage was painted from inside with a white exterior latex paint. The parasitoids were provided with honey on a piece of thermocol sheet and moistened filter paper in a petri dish as source of food and water, respectively, for the parasitoids in the cage. The potato tuber sprouts were taken out regularly after parasitization of *P. solenopsis* and replaced with new sprouts having third instar nymphs and adults of *P. solenopsis* for further parasitization. The mealybug mummies formed were collected from the cage daily and kept separately in the glass vials (7.5 × 1 cm) provided with honey streak on a paper strip as a source of food for the emerging adult parasitoids. Thus, freshly emerged male and female parasitoids were collected regularly and used in the experiments.

Cold storage technique and post storage record of biological parameters

Four hundred one-day-old parasitoid adults were observed at each of the four storage temperatures i.e. 5, 10, 15 and 20°C. At each temperature, the adults were divided into eight batches, for eight storage durations (1 to 8 weeks), each consisting of 10 adults (i.e. 5 males and 5 females) replicated five times (i.e. 50 adults) and were placed in small glass vials (7.5 × 1 cm). The parasitoids were provided with honey streak on a piece of paper. The glass vials carrying parasitoid adults were kept in glass jars in complete darkness at each of the temperature regime. At the end of each storage period at each storage temperature, 50 *A. bambawalei* adults were removed, transferred individually to small glass vials and placed at 27°C for recording different biological parameters of the parasitoid.

The number of live and dead males and females was recorded separately after removal from cold storage and adult survival was calculated. For recording adult longevity, five female and five male parasitoids that survived storage were placed in small vials, provided with honey streak on a paper piece. Five female parasitoids from each 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 third instar mealybug nymphs per day for five consecutive days after emergence for estimating fecundity and per cent females emerging in F_1 progeny of the parasitoid. Data collected on different biological parameters of cold stored parasitoids adults were compared with those observed 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 survival and proportion of females in the F_1 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 survival

In general, the data on survival of *A. bambawalei* adults during cold storage revealed that females were found to be less susceptible to low temperature storage as compared to males. Likewise, Archer *et al.,* (1976) who studied storage of *Aphelinus asychis* Walker adults for 15, 30, 60 and 90 days at temperatures of 10 and 4.4°C found that females survived storage longer than males. Jayanth and Nagarkatti (1985) stored adults of *Bracon brevicornis* Wesmael at 5°C for 30, 60 and 90 days and reported that with the increase in storage duration males were more susceptible than females. The resistance of females to low temperature was higher than that of males (Uckan and Gulel, 2001).

The data on survival of *A. bambawalei* males during cold storage revealed that most of the males were still alive after one week of storage at 10, 15 and 20°C (Table 1). The survival of the males was affected more during storage at 5°C, where only 68, 56, 48 and 20 per cent males survived after one, two, three and four weeks of storage. Similarly, in case of females only 80, 72, 64 and 24 per cent survived after one, two, three and four weeks of storage. Complete mortality of males and females was recorded after five weeks of storage at 5°C. Similarly, Bernardo *et al.,* (2008) reported that temperatures of storage lower than 15 and 10ºC had detrimental effects on adults of *Thribiopus javae* (Girault). Uckan and Gulel (2001) who studied the cold storage of *Apanteles galleriae* Wilkinson adults at 6°C also found that all the adults died within 15 days when parasitoid adults were stored at 6°C. The best results were obtained at 10°C, where 100 per cent of the males survived storage up to three weeks and thereafter survival of the males declined significantly being minimum of 20 per cent after eighth week of storage. Similarly, in case of females 100 per cent survival was recorded when stored up to three weeks at 10°C and it was at par with those surviving after four weeks of storage (88%) and thereafter survival of the females declined significantly. At 15°C, 100 per cent of the males survived after one week of storage and it was at par with those surviving after two weeks (88%). Thereafter survival of the males declined significantly being minimum of 8 per cent after eighth week of storage. Whereas 100 per cent of the females survived up to two weeks of storage and it was at par with those surviving after three weeks of storage (92%) at 15°C. Thereafter survival of the females declined significantly up to sixth week.

At 20°C, 92 per cent of the males survived after one week of storage and it was at par with those surviving after two weeks of storage (84%). Thereafter survival of the males declined significantly being minimum of 8 per cent after sixth week of storage. Complete mortality of males was recorded after seventh week of storage at 20°C. At 20°C, 100 per cent of the females survived after one week and it was at par with those surviving after two weeks of storage (88%). Thereafter survival of the females declined significantly being minimum (8%) after seventh week of storage. However, there were no surviving females after eighth week of storage at 20°C. Similarly, Chong and Oetting (2006) who stored adults of *Anagyrus* sp. nov. nr. *sinope* Noyes and Menezes for various storage durations (0, 1, 4, 7, 14 and 21 days) at 15 and 25ºC reported that parasitoids stored at 15ºC had higher survival as compared to those stored at 25ºC.

Table 1. Survival of *A. bambawalei* adults post cold storage

Storage period (weeks)	Adult survival post cold storage (%)							
	Temperature (°C)							
	5		10		15		20	
	♂	♀	♂	♀	♂	♀	♂	♀
1	68 (56.03)	80 (67.34)	100 (85.91)	100 (85.91)	100 (85.91)	100 (85.91)	92 (76.91)	100 (85.91)
2	56 (48.67)	72 (60.54)	100 (85.91)	100 (85.91)	88 (74.38)	100 (85.91)	84 (69.88)	88 (74.38)
3	48 (43.82)	64 (53.50)	100 (85.91)	100 (85.91)	72 (58.34)	92 (76.91)	68 (55.82)	76 (62.84)
4	20 (22.62)	24 (27.12)	80 (65.38)	88 (74.38)	52 (46.13)	60 (50.97)	48 (43.60)	56 (48.67)
5	0 (4.05)	0 (4.05)	64 (53.50)	68 (58.00)	44 (41.30)	48 (43.60)	24 (27.12)	32 (31.96)
6	0 (4.05)	0 (4.05)	48 (43.82)	56 (48.67)	32 (34.15)	36 (36.46)	8 (13.05)	20 (24.58)
7	0 (4.05)	0 (4.05)	36 (36.46)	40 (38.98)	16 (22.05)	28 (31.62)	0 (4.05)	8 (13.05)
8	0 (4.05)	0 (4.05)	20 (24.58)	32 (31.96)	8 (13.05)	20 (24.58)	0 (4.05)	0 (13.05)
CD (*P*=0.05)	(10.95)	(13.93)	(12.31)	(13.34)	(13.82)	(11.70)	(13.84)	(13.82)

Figures in parentheses are means of angular transformations

It is concluded that the adults can be stored for one and three weeks each at 5 and 10°C and for two weeks each at 15 and 20°C, respectively, without adversely affecting their survival during storage.

Adult longevity

Data on the longevity of *A. bambawalei* males and females that survived cold storage periods at different temperatures *viz*., 5, 10, 15 and 20°C are presented in Table 2. Maximum male and female longevity of 19.60 and 33.20 days, respectively, was recorded in control (27°C). In general, the male and female longevity decreased as the storage period increased at 5, 10, 15 and 20°C. Due to negligible or no adults surviving after 5th, 7th and 6th week of storage at 5, 15 and 20°C, respectively, the data on adult longevity could not be recorded. Reduction in longevity with the increase in storage duration in different parasitoids was also reported by Jayanth and Nagarkatti (1985), Ayvaz *et al.,* (2008) and Bernardo *et al.,* (2008). At 5°C, maximum male and female longevity of 17.60 and 30.60 days, respectively, were recorded after one week of storage and these was at par with the respective longevity recorded in control. After three weeks of storage, the male and female longevity decreased gradually and were significantly lower than the control. Bernardo *et al.,* (2008) also reported that temperatures of storage lower than 15 and 10°C had detrimental effects on adult longevity. Similarly, Chen *et al.,* (2013) investigated the effects of cold storage on *Habrobracon hebetor* Say and found that longevity was reduced after storage at low temperatures as compared with the culture females at optimum temperature. At 10°C, maximum male longevity was recorded after one week of storage (19.20 days) and it was at par with that recorded after two weeks (18.60 days), three weeks (17.20 days) and in the control (19.60 days).

Maximum female longevity was recorded after one week of storage (33.00 days) and it was at par with that recorded after two weeks (31.20 days), three weeks (27.80 days) and in the control (33.20 days). Thereafter, the male and female longevity decreased gradually and was significantly lower than the control. Likewise**,** Jalali *et al.,* (1990) studied the response of males and females of *Cotesia marginiventris* (Cresson) at low temperatures *viz*., 5 and 10°C and reported a significant reduction in adult longevity after 3 weeks of storage. Saleh *et al.,* (2010) studied the effect of cold storage on the adults of *Neochrysocharis formosa* (Westwood) and found that storage of parasitoid males and females at 10 ± 0.5°C, respectively, proved to be optimal. At 15°C, maximum male and female longevity of 18 and 29.40 days, respectively, was recorded after one week of storage and these was at par with the respective longevity recorded in control. After two weeks of storage, the male and female longevity decreased gradually and was significantly lower than the control. Likewise, Bernardo *et al.,* (2008) reported that residual longevity reduced significantly when adults of *Thripobius javae* were stored for more than 10 days at 15°C. Longevity of the males and females that survived storage at 20°C was significantly lower for all the storage weeks when compared with the control. So, 20°C was not found to be a suitable temperature for parasitoid storage in adult stage. It was concluded that both the male and female parasitoids could be stored at 5, 10 and 15°C for two, three and one week, respectively, without any adverse effect on the longevity. Longevity of female parasitoids was found to be more than the males at all the storage temperatures and durations studied. Similarly, Archer *et al.,* (1976) and Jackson (1986) found that females survived longer than males when stored at 7.2 and 10°C.

Table 2. Longevity of *A. bambawalei* adults after removal from cold storage

Storage period (weeks)	Adult longevity post cold storage (days)							
	Temperature (°C)							
	5		10		15		20	
	♂	♀	♂	♀	♂	♀	♂	♀
1	17.60 (4.29)	30.60 (5.60)	19.20 (4.48)	33.00 (5.80)	18.00 (4.34)	29.40 (5.51)	14.00 (3.85)	25.80 (5.16)
2	14.90 (3.98)	27.60 (5.32)	18.60 (4.41)	31.20 (5.65)	15.40 (4.04)	27.20 (5.30)	11.20 (3.48)	22.00 (4.78)
3	11.40 (3.50)	23.00 (4.88)	17.20 (4.24)	27.80 (5.33)	12.80 (3.70)	22.80 (4.86)	9.40 (3.20)	18.20 (4.37)
4	7.80 (2.95)	18.60 (4.40)	14.80 (3.96)	26.00 (5.01)	9.40 (3.21)	19.20 (4.84)	7.20 (2.84)	13.00 (3.73)
5	-	-	13.40 (3.78)	23.20 (4.91)	8.20 (3.02)	16.80 (4.20)	4.20 (2.26)	9.80 (3.28)
6	-	-	10.80 (3.42)	18.20 (4.36)	5.20 (2.46)	14.80 (3.94)	-	7.20 (2.85)
7	-	-	8.40 (3.05)	15.20 (4.04)	-	11.60 (3.53)	-	-
8	-	-	6.20 (2.67)	10.20 (3.34)	-	9.00 (3.14)	-	-
Control (27°C)	19.60 (4.52)	33.20 (5.84)	19.60 (4.52)	33.20 (5.84)	19.60 (4.52)	33.20 (5.84)	19.60 (4.52)	33.20 (5.84)
CD (*P*=0.05)	(0.54)	(0.53)	(0.46)	(0.64)	(0.45)	(0.52)	(0.48)	(0.44)

Figures in parentheses are means of square root transformations

Host parasitization

Fecundity (number of parasitized hosts/female in five days) of *A. bambawalei* females during first five days after removal from cold storage

Data on fecundity of *A. bambawalei* females during first five days after removal from different storage periods at different temperatures are presented in Table 3. Due to inadequate number of males and females required for pairing, after 5th, 7th and 6th week of storage at 5, 15 and 20°C, respectively, the data on fecundity could not be recorded. The data revealed that fecundity was maximum (24.60 mealybugs parasitized/female in five days) in control (27°C).

Fecundity of the females that survived during storage at 5, 15 and 20°C was significantly lower for all the storage weeks when compared with the control. At 10°C, maximum fecundity (24 mealybugs parasitized/female in five days) was recorded after one week of storage and it was at par with the control. Thereafter the fecundity decreased gradually and was significantly lower than the control. Thus, it can be concluded that the parasitoids can be stored for one week at 10°C, without any significant effect on the fecundity.

Table 3. Fecundity (number of parasitized hosts/female in five days) of *Aenasius bambawalei* females during first five days after removal from cold storage

Fecundity during first five days (number of parasitized hosts/female in five days)				
Storage period (weeks)	Temperature (°C)			
	5	10	15	20
1	17.20 (4.26)	24.00 (4.98)	20.20 (4.59)	17.00 (4.23)
2	13.80 (3.83)	19.60 (4.52)	15.60 (4.07)	13.20 (3.75)
3	6.60 (2.73)	15.40 (4.04)	11.40 (3.50)	10.40 (3.36)
4	2.40 (1.79)	10.80 (3.42)	8.60 (3.09)	6.40 (2.74)
5	-	7.20 (2.84)	5.40 (2.52)	4.00 (2.20)
6	-	6.40 (2.70)	4.60 (2.35)	-
7	-	5.20 (2.48)	-	-
8	-	3.20 (2.03)	-	-
Control (27°C)	24.60 (5.04)	24.60 (5.04)	24.60 (5.04)	24.60 (5.04)
CD (*P*=0.05)	(0.55)	(0.44)	(0.43)	(0.47)

Figures in parentheses are means of square root transformations

Similarly, Chen *et al.,* (2013) who investigated the effects of cold storage on *Habrobracon hebetor* found that fecundity reduced after storage at low temperatures as compared with the culture females. Similarly, Jayanth and Nagarkatti (1985), Jalali *et al.,* (1990), Venkatesan *et al.,* (2000) and Foerster and Doetzer (2006) reported that fecundity decreased as the storage period increased. Chen *et al.,* (2011) stored the newly emerged females of ectoparasitoid *Habrobracon hebetor* and reported that fecundity decreased after 20 days of storage at 5 ± 1°C. Bernardo *et al.,* (2008) reported that progeny production reduced significantly when adults of *Thripobius javae* were stored for more than 10 days at 15ºC. Similarly, Archer *et al.,* (1976) reported that adults stored for 15 days at 10°C reproduced at the same level as unstored adults, but reproduction declined as the length of storage increased. 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.

Proportion of females in the F_1 progeny of cold stored adults of *A. bambawalei*

It is interpreted from the data (Table 4) that the proportion of *A. bambawalei* females in the F_1 progeny of adults of *A. bambawalei* was maximum (63.12%) at 27°C. At 5 and 20°C, the proportion of females in the F_1 progeny for all the storage periods was significantly lower than the control. However, the proportion of females in the F_1 progeny after one week of storage at 10 and 15°C was comparable with the control, but decreased as the storage periods increased beyond two weeks. Chen *et al.,* (2013) who investigated the effects of cold storage for 1 to 8 weeks at 5°C on *Habrobracon hebetor* also reported that percentage of female F_1 offspring was always lower for cold stored insects than for the culture insects (36% vs. 52%). In general, the proportion of females in the F_1 progeny declined as the storage duration increased at 5, 10, 15 and 20°C. Proportion of females in the F_1 progeny of adults that survived storage at 10°C was more than the proportion of females in the F_1 progeny of adults that survived storage at 5, 15 and 20°C. It was concluded that the parasitoids can be stored for one week at 10 and 15°C, without significantly affecting the proportion of females in the F_1 progeny. The F_1 progeny of the females became male biased as the storage duration increased at 5, 10, 15 and 20°C. Saleh *et al.,* (2010) studied the effect of cold storage on the adults of *Neochrysocharis formosa* and found that sex-ratio shifted toward males. Saggara *et al.,* (2000), Uckan and Gulel (2001) and Almeida *et al.,* (2002) also concluded that longer storage durations had male preponderance in F_1 progeny.

Table 4. Proportion of females in the F1 progeny of cold stored adults of *Aenasius bambawalei*

Females emerging in F_1 progeny (%)				
Storage period (weeks)	Temperature (°C)			
	5	10	15	20
1	39.80 (38.722)	48.10 (43.86)	46.69 (43.65)	44.96 (42.05)
2	34.62 (35.88)	41.37 (39.98)	41.24 (39.81)	41.02 (39.75)
3	24.22 (27.31)	39.26 (38.73)	35.07 (36.27)	32.82 (34.82)
4	11.66 (15.48)	37.61 (37.78)	31.58 (34.12)	26.21 (30.62)
5	-	28.16 (31.90)	20.71 (25.13)	22.41 (26.33)
6	-	27.37 (31.37)	18.33 (21.72)	-
7	-	15.66 (19.98)	-	-
8	-	11.66 (15.48)	-	-
Control (27°C)	63.12 (52.63)	63.12 (52.63)	63.12 (52.63)	63.12 (52.63)
C.D. (*P*=0.05)	(13.48)	(10.44)	(11.03)	(8.77)

Figures in parentheses are means of angular transformations

Thus, it can be concluded from the present studies that the parasitoid, *A. bambawalei* can be stored at low temperatures in adult stage. The parasitoid adults can be stored only for 1 week at 10°C without any significant effect on the key biological parameters like survival, longevity, fecundity and per cent females emerging in F_1 progeny. The results could be utilized for storage of mass produced parasitoids in the laboratory and for the release of the adults during critical periods of solenopsis mealybug attack on cotton crop.

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Fig. 1. Potato tubers kept for sprouting.

Fig. 2. Mealybug colony on a potato tuber sprout.

Fig. 3. Single hole wooden cages used for rearing *A. bambawalei.*

Fig. 4. *A. bambawalei* male.

Fig. 5. *A. bambawalei* female.

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