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Research Article

Overwintering of *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat) (Hymenoptera: Encyrtidae) under screen house/semi-natural conditions

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ABSTRACT: Overwintering studies were carried out by placing newly emerged adults of the parasitoid, *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat) (Hymenoptera: Encyrtidae) and one day old mummies of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (i.e. parasitoid in pupal stage) during winter season under screen house conditions in 2012-13. Maximum mean pupal period of male and female parasitoids was 86.47 and 92.67 days, respectively, when mummies were exposed on 5th December followed by 73.69 and 82.83 days, respectively. When exposed on 20th December the emergence of parasitoid adults from the overwintering mummies seemed to be well synchronized with ambient weather conditions. This implies that there is no diapause in *A. arizonensis.* There was no adult emergence until 10th standard week due to prevailing low temperature conditions during December and January with average maximum and minimum temperatures of 20.9, 6.4°C and 17.1, 4.1°C, respectively. Emergence of parasitoid adults from overwintering mummies of different exposure dates *viz.,* December 05 and December 20, 2012; January 04, January 19, February 03 and February 18, 2013 started from 11th standard week (first week of February), reached maximum in 14th standard week (first week of March) and continued until 16th standard week (third week of March). Maximum temperature ranged between 20.3 and 29.6°C and minimum between 6.6 and 12.5°C during the period of adult emergence. Adult parasitoids successfully overwintered when provided with honey. Males survived for 52-84 days (69.33 days) and females survived for 69-103 days (83.40 days) during winter season in absence of host, when exposed on 5th December, 2012. Our results demonstrate that the parasitoid is able to successfully overwinter as pupa and adult via combination of slow development and increased adult longevity. This information seems to be useful in enhancing population of this parasitoid in spring when *A. arizonensis* can suppress solenopsis mealybug on cotton crop.

KEY WORDS: *Aenasius arizonensis*, adult, mummies, overwintering, *Phenacoccus solenopsis*, pupal period

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INTRODUCTION

Solenopsis mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), an exotic species, is a serious sucking pest of cotton in Haryana. The parasitoid, *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat) (Hymenoptera: Encyrtidae) was fortuitously found parasitizing cotton mealybug in India in 2008. Now, the solenopsis mealybug is heavily parasitized (37.6-72.3%) by A. arizonensis on cotton crop in Haryana (Ram *et al*., 2009). This is the most successful example of biological control of mealybug (Tanwar *et al*., 2011). Fallahzadeh *et al*. (2014) synonymised with *A. arizonensis* as they found the type of *A. arizonensis* at the Smithsonian Institution National Museum of Natural History (SI-NMNH) collection was identical to Hayat's description of *A. bambawalei*. The fortuitous biological control is not only as an accidental but also a fortunate phenomenon since the pests carry along with them the beneficial species to the new areas (Nechols, 2003). Survival of parasitoids during winter season particularly in the absence or shortage of hosts is an important aspect to continue the life cycle. Mansingh (1971) stated that overwintering strategies allow insects to survive in environments unfavourable for continuous reproduction and normal metabolic functions. Overwintering parasitoids can be used to initiate mass-culture for use in biological control programmes against insect pests. Most of the parasitoids generally overwinter as immature stages within their host. Unlike other insects, parasitoids are unable to directly choose protected locations for overwintering and rely on physiological changes to successfully survive unfourable environment conditions (Boivin, 1994). These changes may involve a state of dormancy that involves diapause or quiescence (Tauber *et al*., 1986).

Most studies on overwintering in parasitoids have focused on the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) (Lopez and Morrison, 1980; Rundle and Hoffman 2003; Ozder and Saglam, 2005). But no information is available on the overwintering strategies of *A. arizonensis*. Thus, the present study was planned to unravel the overwintering ability of the parasitoid by considering adult survival and temperature dependent development of the immature stages, thereby testing whether diapause occurs in *A. arizonensis* when held in screen house conditions during winter season.

MATERIALS AND METHODS

Survival of *Aenasius arizonensis* pupae under screen house conditions during winter season

Survival of *A. arizonensis* under screen house conditions from December, 2012 to February, 2013 was recorded by placing 10 parasitoid pupae (one day old mummies) replicated three times, in glass vials at 15 days interval. The parasitoid pupae were placed in six batches (30 mummies/batch) at respective exposure dates *viz.,* December 05 and December 20, 2012; January 04, January 19, February 03 and February 18, 2013. The glass vials carrying pupae were plugged and placed in the screen house in shady area avoiding direct exposure from rain and solar radiation. The pupae were examined daily for adult emergence. Observations were recorded on time taken to emerge from mummies both for males and females (days), per cent emergence, emergence pattern and sex of emerged individual. The mummies from which parasitoids failed to emerge were kept in petri dishes provided with moist filter paper at the base for 24 hours for softening and then dissected under stereo zoom binocular microscope to record the stage of the parasitoid (pupa or adult) as a measure of mortality. Data on temperature, relative humidity, rainfall etc., were obtained from the Meteorological Observatory of the Department of Agrometeorology, CCS HAU, Hisar and used to calculate the effect of temperature on survival and emergence of overwintering parasitoids. The weekly data recorded for the purpose are given below:

Survival of *Aenasius arizonensis* adults under screen house conditions during winter season

Likewise, survival of *A. arizonensis* during adult stage was recorded by placing 10 parasitoid adults (i.e. 5 males and 5 females) in glass vials, replicated three times, at 15 days interval from December, 2012 to February, 2013. The parasitoid adults were placed in six batches (30 adults/batch) at respective exposure dates *viz*., December 05 and December 20, 2012; January 04, January 19, February 03 and February 18, 2013. The adults were provided honey streak as food on a piece of paper which was replaced every third day. The glass vials carrying adults were plugged and placed in the screen house in shady area avoiding direct

Table 1. Average weather data of Hisar during the experiment season 2012-2013

Standard Week (SW)	Temperature (°C)			Relative Humidity (%)			Total Rain-fall (mm)
	Max.	Min.	Avg.	Morn.	Even.	Avg.	
1. (Nov. 26-Dec. 02)	24.2	06.5	15.4	92	46	69.0	0.0
2. (Dec. 03-09)	24.5	05.4	15.0	92	47	69.5	0.0
3. (Dec. 10-16)	22.1	10.4	16.3	92	62	77.0	5.5
4. (Dec. 17-23)	22.1	04.4	13.3	93	50	71.5	0.0
5. (Dec. 24-31)	15.0	04.5	09.7	95	75	85.0	0.0
6. (Jan. 01-07)	11.6	01.6	06.6	98	82	90.0	0.0
7. (Jan. 08-14)	18.8	04.0	11.4	96	48	72.0	0.0
8. (Jan. 15-21)	18.4	07.2	12.8	95	63	79.0	6.1
9. (Jan. 22-28)	19.7	03.5	11.6	92	42	67.0	0.0
10. (Jan. 29-Feb. 04)	22.6	08.1	15.4	95	59	77.0	0.6
11. (Feb. 05-11)	20.3	06.6	13.5	98	53	75.5	1.5
12. (Feb. 12-18)	21.4	08.9	15.2	96	63	79.5	0.9
13. (Feb. 19-25)	21.1	09.7	15.4	97	67	82.0	1.7
14. (Feb. 26-Mar. 04)	24.3	08.5	16.4	92	43	67.5	0.0
15. (Mar. 05-11)	29.6	10.9	20.3	90	41	65.5	0.0
16. (Mar. 12-18)	28.2	12.5	20.4	98	58	77.0	4.4
17. (Mar. 19-25)	30.3	14.6	22.5	88	45	66.5	0.0

exposure from solar radiations and rain. The adults were examined till their death for recording male and female longevity (days).

Statistical analysis

The data obtained were tabulated and subjected to analysis of variance and standard error by using one factor completely randomized design (CRD). The differences were compared using critical difference (CD) at P=0.05 level of significance. Wherever necessary, the data were subjected to angular or square root transformation to approach normality and homogeneity of variance. 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

Survival during pupal stage

Pupal period of *Aenasius arizonensis* in the overwintering mummies of *Phenacoccus solenopsis*

The data revealed that there were significant differences in the pupal period of both male and female parasitoids (Table 2). Pupal period of *A. arizonensis* decreased significantly as the exposure duration decreased. It was found that female parasitoids took longer time to emerge out from the overwintering mummies than the male parasitoids. Maximum pupal period of male and female parasitoids was 86.47 and 92.67 days, respectively, when one day old mummies were exposed on 5th December followed by 73.69 and 82.83 days, respectively, when one day old mummies were exposed on 20th December. In line with the findings, Rundle and Hoffman (2003) reported that maximum and minimum mean development times were 84 and 63.6 days, respectively, for eggs parasitized by overwintering *Trichogramma funiculatum* Carver in early winter and 72.6 and 51.5 days, respectively, for eggs parasitized in mid-winter. The pupal period was shortest for both males and females i.e. 5.61 and 8.75 days, respectively, at 27°C (control). Hence it was found that *A. arizonensis* survived in pupal stage in mummies of *P. solenopsis* during winter season.

Similarly, Jones *et al*. (2005) and Santolamazza-Carbon *et al,* (2008) studied the overwintering abilities of *Lysiphlebus testaceipes* (Cresson) and *Anaphes nitens* Girault, respectively and reported that these parasitoids overwinter as pupae inside their hosts. In addition, Curl and Burbutis (1977) found that *Trichogramma nubilale* Ertle and Davis overwinters in pupal stage in eggs of the European corn borer *Ostrinia nubilalis* (Hubner).

Table 2. Pupal period of *Aenasius arizonensis* in the overwintering mummies of *Phenacoccus solenopsis*

Date of Exposure	Pupal period (days)			
	Male		Female	
	Mean	Range	Mean	Range
Dec. 05, 2012	86.47 (9.35)	71-93	92.67 (9.67)	85-95
Dec. 20, 2012	73.69 (8.64)	60-78	82.83 (9.15)	76-85
Jan. 04, 2013	50.69 (7.18)	44-58	56.92 (7.61)	54-62
Jan. 19, 2013	27.88 (5.36)	16-39	35.50 (6.04)	21-42
Feb. 03, 2013	18.83 (4.45)	08-28	21.68 (4.75)	11-29
Feb. 18, 2013	12.39 (3.65)	06-19	16.25 (4.15)	09-20
Control (27°C)	05.61 (2.56)	04-08	08.67 (3.10)	06-10
CD (P=0.05)	(0.42)		(0.37)	

Figures in parentheses are means of square root transformations

Emergence of *Aenasius arizonensis* adults from the overwintering mummies of *Phenacoccus solenopsis*

High emergence of parasitoid adults from overwintering mummies indicated that the parasitoid survived during winter season in the pupal stage by decreased temperature dependent development inside the host mummies which is supported by the work of Keller (1986) on *Trichogramma exiguum* Pinto and Platner. Among different exposure dates, per cent adult emergence increased as the exposure duration decreased. Least emergence of the parasitoid adults occurred from the mummies exposed to winter conditions on 5th December (76.67%) followed by those exposed on 20th December (90%).

Table 3. Emergence of *Aenasius arizonensis* adults from the overwintering mummies of *Phenacoccus solenopsis*

Date of Exposure	Emergence (%)
Dec. 05, 2012	76.67 (61.20)
Dec. 20, 2012	90 (73.61)
Jan. 04, 2013	100 (85.91)
Jan. 19, 2013	100 (85.91)
Feb. 03, 2013	100 (85.91)
Feb. 18, 2013	100 (85.91)
Control (27°C)	100 (85.91)
CD (P=0.05)	(08.03)

Figures in parentheses are means of angular transformations

The emergence of the parasitoid adults from mummies exposed on dates 4th January, 19th January, 3rd February and 18th February was 100 per cent and comparable with the control. Similarly, Daane *et al.* (2004) and Ozder and Saglam (2005) reported that per cent adult emergence increased as the exposure dates advanced because of rise in average temperature (Table 3).

Emergence of *Aenasius arizonensis* adults from overwintering mummies of *Phenacoccus solenopsis* with respect to the exposure dates

Emergence of the parasitoid adults from the overwintering mummies of *P. solenopsis* occurred between 11th standard week and 16th standard week. Similarly, Ozder and Saglam (2005) reported that emergence of *Trichogramma brassicae* Bezd. and *Trichogramma cacoeciae* Marchall for the five exposure dates occurred during spring months in the same year. From the mummies that were exposed on 5th December, 20th December, 4th January and 19th January, emergence of the parasitoids occurred during 12th standard week to 16th standard week and the adult emergence was at peak during 14th standard week. From the mummies that were exposed on 3rd and 18th February, adults emerged much earlier than those exposed during December-January because of increase in temperature and hence faster development (Fig. 1).

Emergence of *Aenasius arizonensis* adults from the overwintering mummies of *Phenacoccus solenopsis* irrespective of exposure dates

The emergence of parasitoid adults from the overwintering mummies of *P. solenopsis* (irrespective of the dates when they were exposed to outdoor winter conditions) seemed to be well synchronized with ambient weather conditions. Similarly, Daane *et al.,* (2004) during his studies on *Anagyrus pseudococci* (Girault) found that the emergence of *A. pseudococci* from the overwintering mummies was concentrated over a 15 day period in early May, regardless of when vine mealybugs were exposed. There was no adult emergence until 10th standard week due to prevailing low temperature conditions during December and January with average maximum and minimum temperatures of 20.9, 6.4°C and 17.1, 4.1°C, respectively. Emergence of parasitoid adults started from 11th standard week, attained peak in 14th standard week and continued until 16th standard week when all the parasitoid adults had emerged. Maximum temperature ranged between 20.3 and 29.6°C and minimum between 6.6 and 12.5°C (average 13.5 to 20.4°C) during the period of adult emergence.

Therefore, parasitoids emerged during 11th standard week and 16th standard week because of increase in the average minimum and average maximum temperature during this period as compared to prevailing low temperature

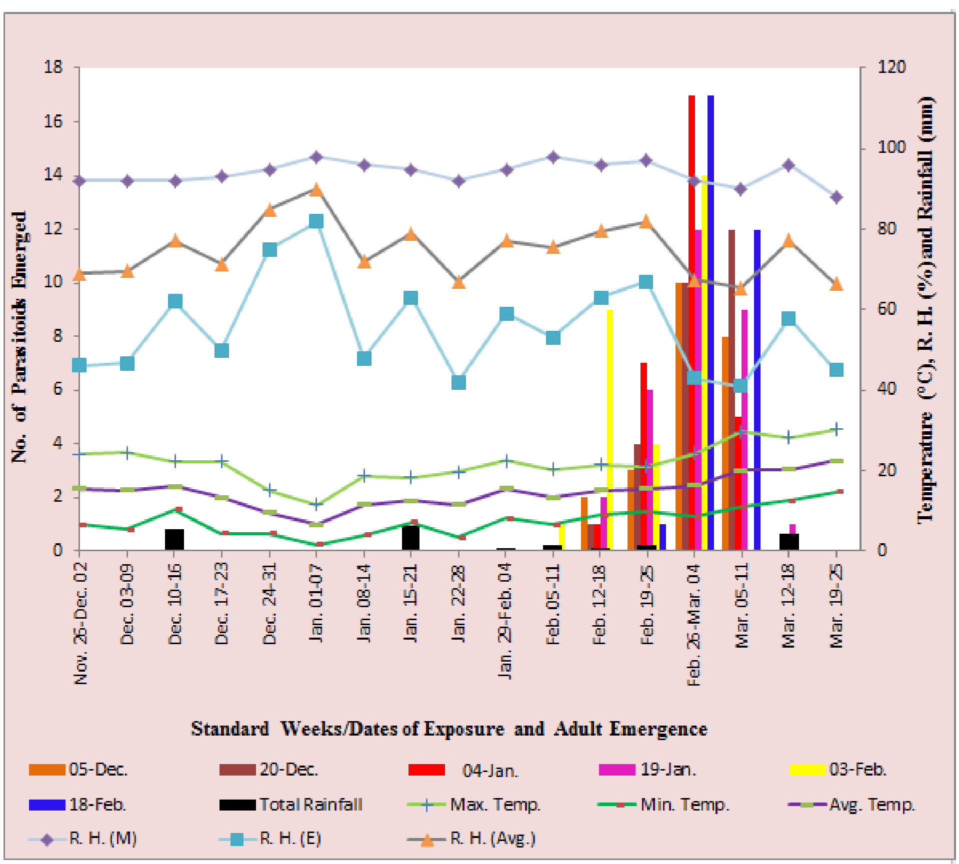

Fig. 1. Emergence of *Aenasius arizonensis* adults from overwintering mummies of *Phenacoccus solenopsis* with respect to the exposure dates.

conditions (average maximum and average minimum) during December (20.9, 6.4°C) and January (17.1, 4.1°C), respectively (Fig. 2). Our findings are supported by Lopez and Morrison (1980) who reported that emergence of overwintering *Trichogramma pretiosum* Riley from parasitized eggs of *Siiotroga cerealella* (Olivier) was least in January but maximum in February and March because of increase in temperature that regulated faster development and adult emergence.

Survival during adult stage

Longevity of overwintering *Aenasius arizonensis* adults

The data on the longevity of male and female parasitoids of *A. arizonensis* when exposed for different durations under screen house conditions revealed that the parasitoid also survives in adult stage during the winter season (Table 4). In line with the findings, Keller (1986), Rundle and Hoffman (2003) and Jones *et al.* (2005) investigated that parasitoids could overwinter by prolonged adult longevity. Longevity of the adult parasitoids decreased as the exposure period increased. Longevity of the adult parasitoids exposed at different durations under winter conditions was higher as compared to the control. In general, males were short-lived (69.33-18.43 days). Among the exposure dates, longest male longevity was observed when the males were exposed on 5th December (69.33 days) while shortest was observed when males were exposed on 18th February (36.93 days).

Table 4. Longevity of overwintering male and female *Aenasius arizonensis* parasitoids

Date of Exposure	Longevity (days)			
	Male		Female	
	Mean	Range	Mean	Range
Dec. 05, 2012	69.33 (8.38)	52-84	83.40 (9.18)	69-103
Dec. 20, 2012	67.33 (8.26)	47-81	80.60 (9.03)	65-100
Jan. 04, 2013	59.47 (7.64)	39-71	68.20 (8.32)	62-077
Jan. 19, 2013	51.26 (7.36)	37-68	61.70 (7.92)	51-068
Feb. 03, 2013	43.40 (6.88)	32-55	55.20 (7.49)	40-067
Feb. 18, 2013	36.93 (6.15)	21-45	40.13 (6.41)	33-054
Control (27°C)	18.43 (4.39)	7-22	32.50 (5.78)	14-038
CD (*P*=0.05)	(0.38)		(0.29)	

Figures in parentheses are means of square root transformations

Similarly, for females, among the exposure dates longest female longevity was observed when the females were exposed on 5th December (83.40 days) while shortest was observed for females exposed on 18th February (40.13 days). Similarly, Santolamazza-Carbon *et al.* (2008) investigated that honey-fed (without hosts) females of *Anaphes nitens* had the highest longevity (53 days). No significant differences were observed in male longevity for exposure dates 5th December (69.33 days) and 20th December (67.33 days). Differences were also not observed in female longevity for exposure dates 5th December (83.40 days) and 20th December (80.60 days). Both males and females were short-lived in the control (27°C) i.e. for 18.43 and 32.50 days, respectively.

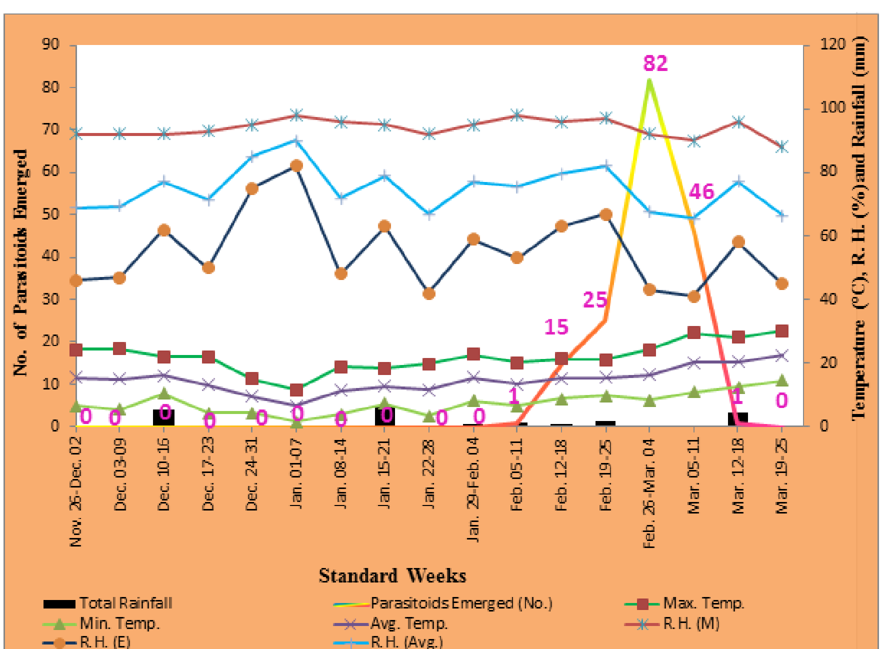

Fig. 2. Emergence of *Aenasius arizonensis* adults from the overwintering mummies of *Phenacoccus solenopsis* irrespective of exposure dates.

Thus, it can be concluded from the present studies that *A. arizonensis* overwinter as pupae as well as adults by a combination of sluggish development in the host mummies and prolonged adult longevity, respectively, under cold conditions during winter season. The capability of overwintering might ensure a greater profitability for survival under variable and often extreme winter environment conditions.

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