



## EFFECT OF ELEVATED TEMPERATURE ON THE BIONOMICS OF *AENASIUS ARIZONENSIS* GIRAULT

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### ABSTRACT

An experiment was conducted to know the effect of thermal stress on the biological attributes of the parasitoid *Aenasius arizonensis* Girault of the cotton mealybug *Phenacoccus solenopsis* Tinsley. The results revealed that temperature showed a significant effect on the biological fitness traits of the *A. arizonensis*; when exposed to 32°C, parasitisation, fecundity, female sex ratio and adult longevity. At 32°C these viz., parasitisation (77%), sex ratio (63.46) and days taken from oviposition to mummy formation (6.4) were found to be the highest. Other fitness traits like fecundity (142.8) and developmental duration (11.2 - males, 12.6 - females) were found to be the highest at 27°C. These parameters gradually decreased with increasing temperature, indicating a negative relationship. Morphometrics of adults revealed that mean length and breadth of mummies, total length and breadth of adult male and females, total width of head, total length of antenna and total hind tibial length were maximum at 27°C as compared to those observed with high temperature treatments. Thus high temperature was observed to have an adverse effect on the growth and development of the parasitoid.

**Key words:** *Phenacoccus solenopsis*, *Aenasius arizonensis*, elevated temperature, bionomics, cotton, parasitoid, mummies, biological control, morphometrics, length, breadth, head, antenna, hind tibial, parasitisation, fecundity, longevity, sex ratio

After the introduction of Bt cotton in 2002, the sucking pest problem is the main reason for the dwindling production of cotton in India. One among them is the solenopsis mealybug *Phenacoccus solenopsis* Tinsley (Pseudococcidae: Hemiptera) which has invaded and colonized in more than 23 countries (Ahmed et al., 2015). The pest is reported to feed on 202 host plant species that encompasses field crops, ornamentals, trees, and vegetables (CABI, 2015). Biological control agents are the ecofriendly options for sustainable IPM in agri-horticultural crops. Biological control has been demonstrated by the recent success of fortuitous biological control of *P. solenopsis* (Gautam et al., 2009). The cotton mealy bug parasitoid *Aenasius arizonensis* Girault is an endophagous nymphal parasitoid causing 80% parasitization under field conditions. However, its sustainability largely depends on the abiotic factors and the temperature is one of the most important (Huffaker et al., 1999). Several studies suggest that the host insect and temperature may affect the rate of development and longevity of parasitoids (Deng and Tsai, 1998). Temperature is a key abiotic factor that regulates insect population dynamics, developmental rates, and seasonal occurrence (Campbell et al., 1974; Logan et al., 1976). The ability of an insect to tolerate the temperature stress without compromising its growth and development is an

important adoption (Mizell and Nebeker, 1978). Insects require a certain amount of heat units (degree-days) to develop from one life stage to the next (Gordan, 1999). Extreme temperatures adversely affect the survival and abundance of mealybug and its parasitoids (Amarasekare et al., 2008; Vennila and Agarwal, 2013). The present study explores the influence of temperature regimes on *A. arizonensis*.

### MATERIALS AND METHODS

The *P. solenopsis* culture was maintained in the laboratory by rearing them on sprouted potato tubers following Geethu et al. (2020) and Sweetee Kumari et al. (2021). The parasitoid (*A. arizonensis*) mummies were collected from the mealybug infested cotton and *Hibiscus rosa-sinensis* plants and placed in glass cylinder (10x4 cm) for emergence (Suroshe et al., 2013). From these adults of *A. arizonensis* were transferred to plastic jars (22.5 x 14 cm) having mealybug culture. The pure mealybug and parasitoid culture was further used. Studies on the effect of temperature were conducted at four temperatures viz., 27, 32, 35 and 38± 1°C in BOD incubators. For this purpose, thirty III instar nymphs were taken from the main culture jars and released on the fresh sprouted potatoes in the small

plastic jar (10 x 7 cm) using the camel hair brush. Then the mated one day old female of *A. arizonensis* was released. After 24 hr the nymphs were transferred on the cotton leaves placed in the petri dish (200 x 20 mm) having its petiole inserted in the solidified agar solution (2%) for maintaining the leaves fresh and tender (Venkanna et al., 2020). After every 24 hr the new batch of III instar mealybug was released in the plastic jar having sprouted potatoes till the released female parasitoid dies. Petri dish having cotton leaves and agar solution was replaced as and when required and the parasitized mealybugs were transferred on it as per the need. Mealybugs which turned to mummies were individually reared in the homeopathy vials (7 x 1.5 cm) for adult parasitoid emergence (Suroshe et al., 2013). The observation on parasitism %, days to mummy formation, fecundity, longevity, developmental time and sex ratio was recorded. Parasitisation % was recorded at 24 hr after parasitisation, whereas, the fecundity was recorded based on the number of mealybugs parasitized till the death of female. The stages of mealubugs and its mummies were critically observed before and after emergence under Nikon stereozoom microscope with camera attachment using software “Vimage-2016” for morphometrics analysis. Observation on the morphological characters was taken following Aga et al. (2016).

## RESULTS AND DISCUSSION

The results revealed that temperature had a significant effect on the parasitism by *A. arizonensis*-highest (77) and the lowest (55) parasitism were observed at 32 and 38°C, respectively; it significantly increased with temperatures from 27(63.2 %) to 32°C (77 %), however it dropped when the temperature exceeded from 32 to 38°C. Parasitism at 35°C was found to be at par with the parasitism at 27 and 38°C (Table 1). The parasitoid, *A. arizonensis* is basically solitary endoparasitoid. Therefore a parasitized host has been considered as criteria for its fecundity. Effect of temperature on fecundity and mummy formation was found statistically significant; increasing temperature decreased the fecundity; maximum (148.8± 10.52) was at 27°C followed by 32°C (113.6± 8.64), 35°C (77.2± 7.66) and 38°C (39.2± 9.52). Temperature had significant effect on the developmental duration of both male and female; male progeny lasted less compared to female at all temperature regimes. The highest (11.2± 0.836 days) developmental period was observed at 27°C and the lowest (0.8± 0.707 days) at 38°C in male; with female the highest duration was observed at 27°C

Table 1. Effect of temperature regimes on biological fitness traits, developmental duration, longevity and sex ratio (females) of *A. arizonensis*

Temperature	Fecundity (Mean± SE)* (Numbers)	Egg to mummy formation (Mean± SE)* (Days)	Parasitisation % (Mean± SE)*		Devt. duration (Mean± SE)* (Days)		Longevity (Mean± SE)* (Days)		Sex ratio (Mean± SE)*
			Female	Male	Female	Male	Female	Male	
27°C	148.8± 10.521 <sup>a</sup>	6.2± 0.836 <sup>a</sup>	63.2± 9.03 (52.78) <sup>b</sup>	11.2± 0.836 <sup>a</sup>	12.6± 0.547 <sup>a</sup>	9± 0.707 <sup>a</sup>	16± 1.22 <sup>a</sup>	55.33 (48.075) <sup>b</sup>	
32°C	113.6± 8.648 <sup>b</sup>	6.4± 0.547 <sup>a</sup>	77± 5.58 (61.55) <sup>a</sup>	9.2± 0.836 <sup>b</sup>	10.6± 0.894 <sup>b</sup>	7.4 0.547 <sup>b</sup>	12.8± 0.447 <sup>b</sup>	63.46 (52.769) <sup>a</sup>	
35°C	77.2± 7.661 <sup>c</sup>	3.8± 0.836 <sup>b</sup>	60.2± 5.23 (50.94) <sup>b</sup>	8.4± 0.547 <sup>bc</sup>	9.2± 0.836 <sup>c</sup>	6.8± 1.643 <sup>b</sup>	9.4± 0.547 <sup>c</sup>	55.65 (48.075) <sup>b</sup>	
38°C	39.2± 9.523 <sup>d</sup>	3.2± 0.836 <sup>b</sup>	55± 5.96 (47.93) <sup>b</sup>	8.0± 0.7 <sup>c</sup>	8.8± 0.836 <sup>c</sup>	5± 1 <sup>c</sup>	7.2± 1.095 <sup>d</sup>	44.66 (41.927) <sup>c</sup>	
S.Em. ±	4.09	0.34	1.93	0.33	0.35	0.47	0.4	1.223	
C D (p≤0.05)	12.26	1.03	5.79	0.99	1.06	1.42	1.19	3.66	

\*Mean of five replications; Data in parentheses arc sine transformed values.

( $12.6 \pm 0.547$  days) and at  $38^\circ\text{C}$  was and the lowest  $8.8 \pm 0.83$  days. Temperature had a significant effect on the adult longevity and it decreased with increase in temperature. Usually, male longevity remains shorter than the female. Male lived longest at  $27^\circ\text{C}$  ( $9 \pm 0.707$  days) and shortest at  $38^\circ\text{C}$  ( $5 \pm 1$  days) whereas female lived longest at  $27^\circ\text{C}$  ( $16 \pm 1.22$ ) and shortest at  $38^\circ\text{C}$  ( $7.2 \pm 1.095$  days). Observed sex ratio at all temperature treatments were found to be statistically significant with a female of 55.3, 63.46, 55.65 and 44.6% at  $27^\circ\text{C}$ ,  $32^\circ\text{C}$ ,  $35^\circ\text{C}$  and  $38^\circ\text{C}$ , respectively (Table 1).

Natural enemies get exposed to different environmental stresses, such as temperature, might affect their biological traits (Fand and Suroshe, 2015). The successful mass rearing and release of parasitoids depends on the suitable temperature condition (Dhillon and Sharma, 2009). Thus, it is necessary to ascertain the effect of temperature on the biology of parasitoids. Optimum range of temperature is required for development of parasitoids to promote parasitisation and survival (Tunca et al., 2010; Qiu et al., 2012). In the present study the highest parasitism % was at  $32^\circ\text{C}$  and increased from 27 to  $32^\circ\text{C}$ , but dropped when temperature exceeds  $32^\circ\text{C}$ . Similar results were observed by He et al. (2018) but in contrast to Kahya et al. (2021) where they reported highest parasitization rate at  $25^\circ\text{C}$ . Fecundity of female parasitoid during its longevity was the highest at  $27^\circ\text{C}$  and it decreased with temperature. These, results are in line with Hira et al. (2023). Iqbal et al. (2016), studied the effect of parasitoid age on fecundity of *A. bambawalei* at  $27 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  RH. The results revealed that the fecundity did not change significantly during the first five days of age; thereafter it declined significantly under laboratory condition. Sex ratio of female was the highest at  $32^\circ\text{C}$ , and as the temperature increased further, it decreased significantly under laboratory condition. This implies that, females prefer not to parasitize their hosts at higher temperatures. These results are partially corroborate with Zhang et al., 2016, on *A. bambawalei*. The developmental duration of male and female decreased with temperature; lower temperature, may reduce the physiological and metabolic rates of insects by prolonging the hatching of eggs, larval development and pupal periods and delaying adult emergence. Similar results were obtained in *A. arizonensis* when exposed to temperature stresses (H et al., 2018; Kahya et al., 2021; Hira et al., 2023). The survival rates of the insects have already been shown to be often affected by temperature (Huffaker et al., 1999). Results revealed that the longevity of adult female and male parasitoids

had an inverse relationship with temperature; maximum longevity of both adult female and male was at  $27^\circ\text{C}$  and it decreased with temperature. Soumia et al. (2011) also reported that the longevity of female and male adults of *A. bambawalei* decreases with increases in temperature. The parasitoids *Microplitis manila*, *Eretmocerus hayati* and the predaceous pentatomid *Arma chinensis* (Fallou) survival rates also decreased with temperature (Qiu et al., 2012; Zhang et al., 2019; Xia et al., 2022)

Both adult male and females are blackish with female conspicuously larger; apex of antennae clubbed like and pale black. The mummies barrel shaped and dark brown measuring length and breadth as  $2.81 \pm 0.36$  mm and  $1.61 \pm 0.44$  mm, respectively at  $27^\circ\text{C}$ ; these became shorter ( $2.39 \pm 0.35$  and  $1.52 \pm 0.37$ ) at  $38^\circ\text{C}$  (Table 2). Adults emerged out through circular hole made on the mummies after completion of pupal period. The maximum total length and breadth of female varied from 1.29 to 2.97 mm and 0.36 to 0.98 mm, respectively. In male it varied from 0.7 to 1.78 mm and 0.37 to 0.72 mm, respectively at  $27^\circ\text{C}$ ; at  $38^\circ\text{C}$  these became from 0.72 to 2.1 mm and 0.3 to 1.2 mm (female) and 0.52 to 1.51 mm and 0.27 to 0.72 mm (male), respectively. Head size varied between male and female was larger ( $0.85 \pm 0.22$ ) at  $27^\circ\text{C}$  and the lowest at  $38^\circ\text{C}$  ( $0.37 \pm 0.08$  mm); in male it was found to be  $0.50 \pm 0.11$  mm at  $27^\circ\text{C}$  and  $0.40 \pm 0.11$  mm at  $38^\circ\text{C}$ . Antenna is used for differentiating the male and female and it was measured  $0.85 \pm 0.19$  and  $0.82 \pm 0.10$  mm, respectively at  $27^\circ\text{C}$ ; and  $0.67 \pm 0.21$  and  $0.61 \pm 0.09$  mm, respectively at  $38^\circ\text{C}$ . The maximum hind tibial length of the female and male parasitoid was observed as  $0.51 \pm 0.06$  mm and  $0.33 \pm 0.5$  mm, respectively at  $27^\circ\text{C}$ . The minimum hind tibial length of the female and male parasitoid was noticed as  $0.35 \pm 0.034$  mm and  $0.26 \pm 0.05$  mm, respectively at  $38^\circ\text{C}$  (Table 2).

The above morphometrics observed are in accordance with previous studies (Aga et al., 2016; Poorani, 2009; Hayat, 2009). Mummies of the parasitoids were barrel shaped and dark brown; size varied with temperature and the maximum length ( $2.81 \pm 0.36$  mm) and breadth ( $1.61 \pm 0.44$  mm) of mummies were observed at  $27^\circ\text{C}$  and the minimum length ( $2.39 \pm 0.35$  mm) and breadth ( $1.52 \pm 0.37$  mm) was observed at  $38^\circ\text{C}$ . Total length and breadth, head breadth and length of antenna were found to vary with the increasing temperature and the highest mean value was recorded at  $27^\circ\text{C}$  and the lowest value at  $38^\circ\text{C}$ . This suggests that, the temperatures ranging between 27 to  $32^\circ\text{C}$  is the best suited for the growth and development of parasitoids under laboratory

Table 2. Morphometrics of *A. arizonensis* at different temperatures

Sl. No.	Morphological characters*	27°C			32°C			35°C			38°C				
		Male		Female	Male		Female	Male		Female	Male		Female		
		Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range		
1	Total length	1.27±0.34	0.7-1.78	1.73±0.30	1.29-2.97	1.39±0.45	0.92-2.01	0.86±0.36	0.59-1.51	1.3±0.48	0.79-2	0.84±0.38	0.52-1.51	1.27±0.55	0.72-2.1
2	Total breadth	0.52±0.11	0.37-0.72	0.72±0.26	0.36-0.98	0.47±0.18	0.28-0.68	0.4±0.08	0.27-0.5	0.58±0.37	0.31-1.2	0.53±0.19	0.27-0.72	0.60±0.38	0.3-1.2
3	Head breadth	0.50±0.11	0.38-0.69	0.85±0.22	0.56-1.2	0.47±0.18	0.28-0.68	0.44±0.15	0.32-0.62	0.38±0.11	0.27-0.56	0.40±0.11	0.31-0.61	0.37±0.08	0.27-0.5
4	Length of antenna	0.82±0.10	0.68-0.97	0.85±0.19	0.59-1.14	0.83±0.25	0.54-1.09	0.70±0.08	0.58-0.79	0.76±0.24	0.49-1.01	0.61±0.09	0.48-0.72	0.67±0.21	0.42-0.9
5	Length of hind tibia	0.33±0.5	0.26-0.39	0.51±0.06	0.45-0.59	0.4±0.015	0.38-0.42	0.30±0.032	0.28-0.41	0.38±0.021	0.36-0.41	0.26±0.055	0.2-0.32	0.35±0.034	0.32-0.4
6	Length	Mummies* (Mean±SE)													
7	Breadth	2.81±0.36											2.55±0.65	2.49±0.40	2.39±0.35
		1.61±0.44											1.37±0.40	1.56±0.38	1.52±0.37

\*Mean of ten replications. All observations in mm.

condition. Aga et al. (2016) observed the mean length and breadth of mummies of *A. arizonensis* as  $3.74 \pm 0.13$  mm and  $2.05 \pm 0.13$  mm, respectively at  $27^\circ\text{C}$ ; and externally it appeared barrel shaped and dull brown. The adult female length was  $1.83 \pm 0.08$  mm and male was  $1.38 \pm 0.06$  mm. The mean length and breadth of adult female fore, middle and hind legs were  $1.35 \pm 0.04$ ,  $1.55 \pm 0.04$  and  $1.89 \pm 0.05$  mm, respectively. The mean length of adult female antennae was  $0.93 \pm 0.05$  mm. Sangle et al. (2013) with *A. bambawalei* observed the same trend. Present observations revealed that the optimum parasitization, longevity, sex ratio and developmental durations of *A. arizonensis* could be achieved between 27 to  $32^\circ\text{C}$  without compromising its growth and development. Thus, 27 to  $32^\circ\text{C}$  is the optimum temperature for mass rearing of parasitoid in laboratory condition.

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#### AUTHOR CONTRIBUTION STATEMENT

SSS designed the experiments and reviewed the manuscript; TMN performed the experiment and wrote the manuscript; KGN reviewed and incorporated the corrections in the manuscript.

#### CONFLICT OF INTEREST

No conflict of interest.

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