Journal of Biological Control, 30(4): 210-216, 2016, DOI: 10.18311/jbc/2016/15473

Research Article

Influence of temperature on the biology of *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae), a parasitoid of solenopsis mealybug, *Phenacoccus solenopsis* Tinsley

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ABSTRACT: Effect of temperature on the biology of *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat) (Hymenoptera: Encyrtidae), a parasitoid of solenopsis mealybug, *Phenacoccus solenopsis* Tinsley, was studied at four constant temperatures *viz*. 20±1, 25±1, 30±1 and 35±1^{0}C. Mealybugs were reared on sprouted potatoes. The parasitoid completed its development at all the temperatures studied and males developed faster than the females. For males and females, respectively, total development period was 30.56, 24.16, 13.40 and 11.60 days and 34.40, 26.20, 14.88, and 12.04 days at 20±1, 25±1, 30±1 and 35±1^{0}C, respectively. The daily and total fecundity (number of hosts parasitised) was 1.42, 1.82, 3.17, 2.16 and 57.13, 63.15, 65.60, 37.46 at 20±1, 25±1, 30±1 and 35±1^{0}C, respectively. Female parasitoids parasitised mealy bugs on the day of emergence at all the temperatures. At respective temperatures oviposition and post-oviposition periods were 35.86, 33.60, 18.93, 15.53 days and 2.86, 2.26, 2.60 and 2.33 days, respectively. Parasitoid males were short lived as compared to females at all the temperatures. The male and female longevity, respectively, was 23.06, 17.26, 13.20, 10.33 days and 38.66, 34.53, 20.86, 17.86 days at 20±1, 25±1, 30±1 and 35±1^{0}C, respectively. Sex-ratio (male: female) was in favour of female parasitoids (1: 1.1 to 1: 1.2) at all the temperatures. Net reproductive rate (36.41), intrinsic rate of increase (0.155) and finite rate of increase (1.168) were maximum at 30±1^{0}C indicating this temperature to be the most favourable for the development and reproduction of the parasitoid. Males emerged out from the small sized mummies (2.28×1.20mm) whereas; large sized mummies (2.90×1.59mm) yielded mostly the female parasitoids. Males were generally smaller in size (1.26×0.58mm) as compared to female parasitoids (1.84×0.89mm). The results of the present studies could be useful in establishing a mass rearing programme for the parasitoid.

KEY WORDS: *Aenasius arizonensis,* biology*, Phenacoccus solenopsis,* temperature

(Article chronicle: Received: 21-09-2016; Revised: 11-11-2016; Accepted 02-12-2016)

INTRODUCTION

Aenasius arizonensis (Girault) (=*Aenasius bambawalei* Hayat) (Hymenoptera: Encyrtidae) has been found to parasitize solenopsis mealybug in the Indian subcontinent (Mahmood, 2008; Tanwar *et al*., 2008, Anonymous, 2009, Ram *et al*., 2009). Wasps from the family Encyrtidae have been acknowledged as potential parasitoids of mealybugs and are known to play a role in biological control of various mealybug species (Bertschy *et al*., 2000, Dorn *et al*., 2001). Surveys of cotton growing areas of Haryana have showed 23.7 to 76.6 per cent parasitisation of mealybugs by *A. arizonensis* on cotton (Ram and Saini, 2010). Studies conducted on the biology of *A. arizonensis* revealed that the parasitoid has great potential for biological control of solenopsis mealybug as it has shorter development period (12-14 days), high fecundity (100.8 parasitised hosts/female) and high female longevity (24.9 days) (Vijaya *et al*. 2011). Efforts have also been made to study the off-season survival (Rathee and Ram, 2016b), effect of cold storage (Rathee *et al*., 2015; Rathee and Ram, 2014; 2016a) and side-effects of commonly used insecticides (Meenu and Ram, 2014; 2015) on this parasitoid. However, little information is available on the effect of temperature on the biology of this parasitoid. Therefore, in view of the importance of this parasitoid for the biological control of the solenopsis mealybug, the present study was planned to study the effect of different temperatures on the biology of *A. arizonensis* with the objective to find optimum temperature for mass rearing of the parasitoid.

MATERIALS AND METHODS

Host Rearing

Small to medium sized potatoes were procured from the market. The potatoes were washed, dried and placed in dark at room temperature for sprouting. When the sprouts were about 2.5cm in length, these were used for mealybugs rearing (Fig. 1). Each sprout was inoculated with a gravid female mealybug with the help of a soft camel hair brush. The infested sprouted potatoes were kept in glass jars (20×15 cm) covered with muslin cloth at room temperature (23.2-33.3^{0}C) in the laboratory.

Parasitoid Rearing

The parasitoid, *A. arizonensis,* was reared on *P. solenopsis* colonies on sprouted potatoes kept in separate glass jars by placing the field collected mummies (mealybugs transformed into dark red or brown coloured hard masses after 5-6 days of parasitisation) into the glass jars initially. The mummies formed thereafter were collected and kept separately in glass vials (7.5 x 1 cm) provided with honey streak on a paper strip as a source of food for the adult parasitoids emerging out of the mummies (Fig. 2). The freshly emerged or 24 hour old adult parasitoids emerging out of the mummies were used in experiments.

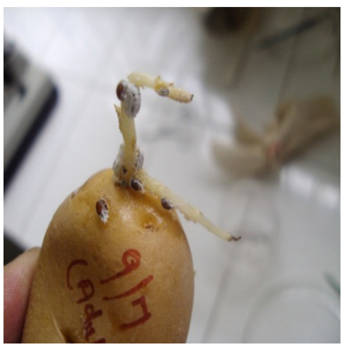

Fig. 1. Colony of *Phenacoccus solenopsis* on a potato sprout.

Fig. 2. Mummies of *Phenacoccus solenopsis* on a potato sprout.

Biological Characteristics

For studying life cycle of *A. arizonensis*, freshly emerged adult parasitoids were paired by identifying male (Fig. 3) and female (Fig. 4) on the basis of their antennal structure and size. The parasitoids were kept in a glass jar covered with a muslin cloth along with a streak of honey on the paper strip as a source of food. A sprouted potato infested with 50 third instar mealybugs was carefully placed in the jar for parasitization for the next 24 hours. After 24 hours, the parasitoid pair was removed and shifted to another new set of mealybug instars in a separate glass jar and the same procedure was repeated after every 24 hours until the death of the parasitoid female. The experiment was conducted at four constant temperatures of 20±1, 25±1, 30±1 and 35±1^{0}C with 65±5 per cent relative humidity (R.H.). The constant temperatures were maintained in the B.O.D. incubator. The observations on different biological parameters were recorded on 15 pairs of parasitoids. Development period from oviposition to host mummy formation and from host mummy formation to adult emergence was recorded on 25 parasitized hosts at each temperature and total developmental period was also calculated. The other biological parameters observed were: pre-oviposition period, oviposition period, post-oviposition period, daily and total fecundity (number of parasitized hosts per female), male and female adult longevity and sex-ratio.

Fig. 3. Male of *Aenasius arizonensis.*

Fig. 4. Female of *Aenasius arizonensis.*

Biological parameters like developmental period, fecundity, longevity and per cent females in the progeny were used to construct the life tables for *A. arizonensis* as per the method worked out by Carey (1993).

The adult parasitoids and the mummies (from which the adults emerged) reared at room temperature (23.2-33.3^{0}C) were also preserved in 70 per cent alcohol for measurements of morphological parameters. Body length and head width across eyes of both male and female adults and length and breadth of the mummies were measured on 25 individuals under stereozoom microscope with the help of ocular and stage micrometer. Correlation between mummy size and parasitoid adult size was also worked out.

RESULTS AND DISCUSSION

Development Period

The parasitoid completed its development at all the temperatures studied. Development period of the parasitoid from oviposition to host mummy formation decreased as the temperature increased. There was no difference in the development period of male and female parasitoids from oviposition to host mummy formation stage i.e. both males and females developed at a similar rate at all the temperatures (Table 1). Longest period to mummy formation (15.60 days) was observed at 20±1^{0}C while shortest (6.08 days) was observed at 35±1^{0}C both for male and female parasitoids. For both males and females there were significant differences in the days to mummy formation at 20±1^{0}C and 25±1^{0}C while no differences were observed at 30±1^{0}C and 35±1^{0}C.

Development period of the parasitoid from host mummy formation to adult emergence, in both male and female parasitoids, also decreased as the temperature increased (Table 1). Female parasitoids took longer time to emerge out of the host mummies as compared to males. Longest development period in male parasitoids, from mummy formation to adult emergence, was of 21.2 days at 20±1^{0}C while it was shortest (4.84 days) at 35±1^{0}C (Table 2). There were significant differences in the development period of male parasitoids at 20±1^{0}C (21.2 days) and 25±1^{0}C (15.8 days). In case of female parasitoids, no difference was found in the development period at higher temperatures of 30±1^{0}C (7.72 days) and 35±1^{0}C (6.40 days) whereas, at lower temperatures of 20±1^{0}C (23.1 days) and 25±1^{0}C (17.80 days) the differences were significant. Longest development period (23.1 days) was observed at 20±1^{0}C while shortest period of 6.40 days was observed at 35±1^{0}C.

The total development period of both male and female parasitoids decreased as the temperature increased (Table 1). Males developed faster than the females at all the temperature regimes. For males, development period was

Table 1. Development period of Aenasius arizonensis at different temperatures

Temperature (^{0}C)	Development period (Days)					
	Days to host mummy formation		Days to adult emergence		Total development period	
	Male	Female	Male	Female	Male	Female
20±1	15.60^{a}	15.60^{a}	21.2^{a}	23.1^{a}	30.56^{a}	34.40^{a}
25±1	11.44^{b}	11.44^{b}	15.8^{b}	17.80^{b}	24.16^{b}	26.20^{b}
30±1	6.68^{c}	6.68^{c}	5.88^{c}	7.72^{c}	13.40^{c}	14.88^{c}
35±1	6.08^{c}	6.08^{c}	4.84^{c}	6.40^{c}	11.60^{d}	12.04^{d}

Means followed by the same letters in a column do not differ significantly to the level of 5% probability

Table 2. Fecundity, oviposition and post-oviposition period, adult longevity and sex-ratio of *Aenasius arizonensis* at different temperatures

Biological parameter	Temperature (^{0}C)			
	20±1	25±1	30±1	35±1
Daily fecundity (number of parasitized hosts/female)	1.42^{c}	1.82^{bc}	3.17^{a}	2.16^{b}
Total fecundity (number of parasitized hosts/female)	57.13^{bc}	63.15^{b}	65.60^{b}	37.46^{c}
Oviposition period (Days)	35.86^{a}	33.60^{a}	18.93^{b}	15.53^{b}
Post-oviposition period (Days)	2.86^{a}	2.26^{a}	2.60^{a}	2.33^{a}
Male longevity (Days)	23.06^{a}	17.26^{b}	13.20^{bc}	10.33^{c}
Female longevity (Days)	38.66^{a}	34.53^{a}	20.86^{b}	17.86^{b}
Sex-ratio (Male: Female)	1:1.1	1:1.1	1:1.2	1:1.2

Means followed by the same letters in a column do not differ significantly to the level of 5% probability longest (30.56 days) at 20±1^{0}C and shortest (11.60 days) at 35±1^{0}C. Similarly, for females development period was longest (34.40 days) at 20±1^{0}C and shortest (12.04 days) at 35±1^{0}C. For males, total development period was 30.56, 24.16, 13.40, and 11.60 days at 20±1^{0}C, 25±1^{0}C, 30±1^{0}C and 35±1^{0}C, respectively. Similarly, for females it was 34.40, 26.20, 14.88 and 12.04 days at 20±1^{0}C, 25±1^{0}C, 30±1^{0}C and 35±1^{0}C, respectively. Similar effects of temperature on development period have also been recorded in other parasitoids (Reitz, 1996; Forester and Doetzer, 2002; Malina and Praslicka, 2008).

Pre-oviposition, Oviposition and Post-oviposition period

Parasitoid adults mated soon after emergence and the mealybugs were parasitised on the day of emergence at all the temperatures. Oviposition period decreased as the temperature increased. Oviposition period was 35.86, 33.60, 18.93 and 15.53 days at 20±1^{0}C, 25±1^{0}C, 30±1^{0}C and 35±1^{0}C, respectively (Table 2). There was no difference in the oviposition period at 30±1^{0}C (18.93 days) and 35±1^{0}C (15.53 days) while significant differences were observed at 20±1^{0}C (35.86 days) and 25±1^{0}C (33.60 days). There was no difference in the post-oviposition period at all the temperatures studied. The post-oviposition period was 2.86, 2.26, 2.60 and 2.33 days at 20±1^{0}C, 25±1^{0}C, 30±1^{0}C and 35±1^{0}C, respectively. Sharaf and Batta (1996) studied the effect of temperature on the life history of *Eretmocerus mundus* Meret*,* a parasitoid of *Bemisia tabaci* Genn. and reported that pre-oviposition period of the parasitoid increased from 1.6 days to 2.8 days with the decrease in temperature from 25-14^{0}C.

Fecundity

The female parasitoid parasitized mealybugs at all the temperature regimes studied (Table 2). Daily fecundity was significantly higher at 30±1^{0}C (3.17 parasitized hosts per female) and 35±1^{0}C (2.16 parasitized hosts per female). Lowest daily fecundity was recorded at 20±1^{0}C (1.42 parasitized hosts per female) and 25±1^{0}C (1.82 parasitized hosts per female). Total fecundity was minimum (37.46 parasitized hosts per female) at 35±1^{0}C. The total fecundity at 20±1^{0}C (57.13 parasitized hosts per female), 25±1^{0}C (63.15 parasitized hosts per female) and at 30±1^{0}C (65.60 parasitized hosts per female) was at par. Similar observations were made by Emana (2007) in *Cotesia flavipes* (Cameron) where lowest fecundity of 23.8 eggs was seen at 20^{0}C in comparison to 41.4 eggs laid at 40^{0}C. Thanavendan and Jeyarani (2010) also reported minimum fecundity at 20^{0}C in *Bracon brevicornis* Wesmael, a parasitoid against fruit borers of *Abelmoschus esculentus* (L.).

Adult Longevity and Sex-ratio

Longevity of the parasitoids decreased as the temperature increased (Table 2). Males were short lived in comparison to female parasitoids at all the temperatures studied. Shortest male longevity (10.33 days) was observed at 35±1^{0}C while the longest of 23.06 days was observed at 20±1^{0}C. There were significant differences in male longevity at 20±1^{0}C (23.06 days) and 25±1^{0}C (17.26 days), however, no differences were observed in male longevity at 30±1^{0}C (13.26 days) and 35±1^{0}C (10.33 days). Male longevity was on par at 25±1^{0}C (17.26 days) and 30±1^{0}C (13.20 days). Similar pattern was observed in longevity of female parasitoid adults also. Longest duration of 38.66 days was found at 20±1^{0}C while it was shortest (17.86 days) at 35±1^{0}C. Significant differences were observed in the female longevity at 20±1^{0}C (38.66 days) and 25±1^{0}C (34.53 days) whereas, no differences were found at higher temperatures of 30±1^{0}C (20.86 days) and 35±1^{0}C (17.86 days. Al-Maliky and Al-Izzi (1990) also stated that there was an inverse relationship between temperature and longevity of *Apanteles* sp. Group *ultor,* a parasitoid of *Ectomyelois ceratoniae* (Zeller). Sharaf and Batta (1996) reported that in *Eretmocerus mundus,* a parasitoid of *Bemisia tabaci*, as the temperature increased from 14-25^{0}C, the adult longevity decreased and males were short-lived as compared to females at both temperatures. Similarly, Wang *et al*. (1999) reported that longevity of females of *Oomyzus sokolowskii* (Kurdj.), a parasitoid of *Plutella xylostella* (L.) decreased from 28.3 days to 6.6 days as the temperature increased from 20-30^{0}C.

Sex-ratio (male: female) was in favour of female parasitoids (1: 1.1 to 1: 1.2) at all the temperatures studied. It was 1:1.1 at 20 and 25^{0}C to 1:1.2 at 30 and 35^{0}C. Sharaf and Batta (1996) studied the effect of temperature on the life history of *Eretmocerus mundus,* a parasitoid of *B. tabaci* and found that more females were produced in the progeny at 25^{0}C as compared to lower temperature of 14^{0}C.

Life Table

The life table parameters of the parasitoid, *A. arizonensis* studied at different temperature regimes are presented in Table 3. The net reproductive rate (R_O) increased from 29.60 to 36.41 as the temperature increased from 20±1^{0}C to 30±1^{0}C, however, at high temperature of 35±1^{0}C it was decreased to 20.32. The net generation time (T) decreased as the temperature increased. It was longest (51.96 days) at 20±1^{0}C and shortest (19.38 days) at 35±1^{0}C. The intrinsic rate of increase (r_m) and finite rate of increase (λ) also increased as the temperature increased. Lowest intrinsic rate of increase of 0.065 and minimum finite rate of increase of

Table 3. Life table parameters of *Aenasius arizonensis* at different temperatures

Temperature (^{0}C)	Life table parameters			
	Net reproductive rate (R_O)	Net generation time (T)	Intrinsic rate of increase (r_m)	Finite rate of increase (λ)
20±1	29.60	51.96	0.065	1.067
25±1	33.93	42.27	0.083	1.086
30±1	36.41	23.41	0.153	1.165
35±1	20.32	19.38	0.155	1.168

1.067 were observed at 20±1^{0}C while maximum intrinsic rate of increase (0.155) and maximum finite rate of increase (1.168) was found at 35±1^{0}C. Net reproductive rate of 36.41 was maximum 30±1^{0}C indicating this temperature to be the most favourable for the development and reproduction of the parasitoid. Wang *et al*. (1999) studied the effect of temperature (20, 25 and 30^{0}C) on the population growth of *Oomyzus. sokolowskii*, a parasitoid of *P. xylostella* and found that the parasitoid had the highest R_o at 25^{0}C. However, the highest r_m was found at 30^{0}C in the mean generation time of 14.5 days at this temperature as compared to that at 20 and 25^{0}C, suggesting that the parasitoid had the highest potential for population growth at relatively high temperatures. R_m was found increasing from 0.082 to 0.263 females per day as the temperature increased from 20-30^{0}C.

Size of Mummies and Parasitoids

Males emerged out from the small sized mummies (2.28×1.20mm) whereas; large sized mummies (2.90×1.59mm) yielded mostly the female parasitoids (Table 4). There were differences in the size of male and female parasitoids and it was observed that males were generally smaller in size (1.26×0.58mm) in comparison to female parasitoids (1.84×0.89mm) (Table 5).

In both male and female parasitoids, correlation of body length with length of host mummy from which it emerged was found non-significant, however, there was significant positive correlation (r=0.52) between body length of male parasitoid and breadth of host mummy (Table 6). There was also significant positive correlation between width across head of both male and female parasitoids and the length and breadth of host mummy. It was concluded that male parasitoids emerged out from the small sized mummies whereas large sized mummies yielded mostly female parasitoids. Males were generally smaller in size in comparison to female parasitoids. In other parasitoids, Jong and Alphen (1989) also reported that females of *Leptomastix dactylopii*

Table 4. Size of *Phenacoccus solenopsis* mummies from which male and female parasitoid adults of *Aenasius arizonensis* emerged

Parasitoids	Mummy size (mm) (n=25)			
	Length (Mean ± S.D.)	Range	Breadth (Mean ± S.D.)	Range
Male	2.28±0.27	1.88-2.97	1.20±0.20	0.9-1.65
Female	2.90±0.25	2.33-3.20	1.59±0.23	1.2-2.18

Table 5. Size of male and female parasitoid adults of *Aenasius arizonensis*

Morphological parameter	Parasitoid adults (n=25)			
	Male		Female	
	Mean ± S.D.	Range	Mean ± S.D.	Range
Body length (mm)	1.26±0.17	1.01-1.80	1.84±0.48	1.31-3.76
Head width across eyes (mm)	0.58±0.07	0.45-0.75	0.89±0.09	0.9-1.09

Table 6. Correlation coefficient values (r) between size of adult parasitoids of *Aenasius arizonensis* and mummies of *Phenacoccus solenopsis* from which parasitoids emerged

Morphological parameter of parasitoid	Mummies from which females emerged		Mummies from which males emerged	
	Mummy length	Mummy breadth	Mummy length	Mummy breadth
Body length	0.31	0.41	0.25	0.52
Head width across eyes	0.71	0.65	0.69	0.54

Howard were larger in size than males emerging from small size class of *Planococcus citri* (Risso). Bokonon-Ghanta *et al*. (1995) also found that females of *Anagyrus mangicola* emerging from any size of *Rastrococcus invadens* were always larger than the corresponding male parasitoids.

The data recorded on various biological parameters of *A. arizonensis* at four constant temperatures revealed that a temperature of 30±1^{0}C can be considered as optimum for rearing this parasitoid as key biological parameters of the parasitoid like development period, fecundity, female longevity and sex-ratio were better at this temperature.

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