Journal of Animal & Plant Sciences, 33(3): 2023, Page: 553-561
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.3.0648

EFFECTS OF VARYING TEMPERATURE ON THE REPRODUCTION AND SURVIVAL OF MEALYBUG PARASITOID, *AENASIUS ARIZONENSIS* (HYMENOPTERA: ENCYRTIDAE)

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ABSTRACT

Host parasitoid interactions in insects offer some innovative opportunities for the development of successful biocontrol programmes in field crops. *Aenasius arizonensis* (Girault) is a species specific, solitary endoparasitic wasp of the cotton mealybug, *Phenacoccus solenopsis* Tinsley and a potential insect control tool. Effects of temperature on different biological traits of *A. arizonensis* were studied at different constant temperatures i.e., 20±2℃, 25±2℃, 30±2℃ and 35±2℃ with a relative humidity of 65±5%. Host insects/mealybugs were reared on sprouted potatoes or pumpkins. Total developmental period of the parasitoid at different constant temperatures was recorded with the daily parasitization rate (number of host insects parasitized). At respective temperatures, oviposition and post-oviposition periods were also observed along with the longevity of the male and female wasps. Sex ratio (male: female), reproductive rate, intrinsic rate of increase, and finite rate of increase were also determined at all temperatures. The most favorable temperature for the development and reproduction of the parasitoid was observed as 30±1 ºC. The information obtained from this preliminary study will be helpful in establishing a mass rearing programme for the parasitoid leading toward sustainable insect pest management of cotton mealybugs in economically important crops.

Keywords: Temperature; Mealybug; Development; Biology; Oviposition; Fitness.

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Published first online February 10, 2023 Published final June 18, 2023

INTRODUCTION

Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is one of the most damaging pests of cotton throughout the world (Nagrare *et al.,* 2009). The species was described from New Mexico, USA in 1898 (Tinsley 1898a, b), and is considered native to North America. However, it has also been found in areas outside of North America including South America, Central America, Hawaii, the Caribbean Islands, Africa, Oceania and Asia (Wasiams and Granara de Wasink 1992; Watson and Chandler 2000; Kumashiro *et al.,* 2001; Hodgson *et al.,* 2008). It was first observed in Asia in 2005 from Pakistan (Abbas *et al.,* 2005) and afterward from India (Yousuf *et al.,* 2007), Vietnam (Nguyễn and Huỳnh 2008), Thailand (Hodgson *et al.,* 2008), China (Wu and Zhang, 2009), Sri Lanka (Sirisena *et al.,* 2012), and Malaysia (Sartiami *et al.,* 2016). The cotton mealybug has emerged as a serious threat to cotton in Pakistan and India, resulting in severe economic losses (Wang *et al.,* 2010) and has spread to almost all cotton growing regions of India (Nagrare *et al.,* 2009). CLIMEX models predict that the mealybug could establish worldwide in over 100 tropical and subtropical countries (Wang *et al.,* 2010)

Control of the mealybug relies heavily on the use of synthetic insecticides, but their widespread use has led to some serious problems including the development of strains resistant to insecticides (Zettler and Cuperus, 1990; Ribeiro *et al.,* 2003). Resistance in insects to several types of insecticides including neonicotinoids, organophosphates, pyrethroids, avermectins and insect growth regulators has been observed in Pakistan and other countries (Saddiq *et al.,* 2014, 2015; Ahmad and Akhtar, 2016). Moreover, due to the waxy covering of the mealybug body, insecticides tend to be less effective against mealybugs. Natural enemies, which are present in the crop environment help to control the mealybug but due to excessive use of pesticides, natural enemy effectiveness is usually decreased. Parasitoids and predators include a diverse array of biological control agents, which attack host pests (Quick, 1997; Rehnault *et al.,* 2005). Parasitoids are generally divided into 2 groups *i.e.,* ecto- and endoparasitoids: ectoparasitoids lay their eggs on external surface/skin of the host body while endoparasitoids insert their eggs to the interior of the host (Asgari and Rivers, 2011). Ectoparasitoids are able to

parasitize the victim thanks to their venom (Scieuzo *et al.,* 2021) while Hymenopteran endoparasitoids have combined strategies of parasitization, induced by female secretions (venom and ovarian protein and sometimes polydnaviruses) (Varricchio *et al.,* 1999; Malva *et al.,* 2004; Laurino *et al.,* 2016; Salvia *et al.,* 2021)

Aenasius arizonensis (Girault) (Hymenoptera: Encyrtidae) is an important solitary nymphal endoparasitic wasp of *P. solenopsis* in India (Hayat, 2009), Pakistan (Mahmood, 2008), China (Chen *et al.,* 2010), Iran (Abdin *et al.,* 2012) and Australia (Khan *et al.,* 2012). It is a key mortality factor of mealybugs under field conditions (Ram *et.al*., 2009). Field parasitism of 95% by *A. arizonensis* has been recorded in *P. solenopsis* (Khuhro *et al*., 2011). It is considered as an important biological control agent beause of its high-host searching capacity, fast multiplication ability, environmental adaptability, ease of culturing in the laboratory, female-biased sex ratio, high-dispersal capacity, and synchronization in life-cycle/biology with the host (Ram *et al.,* 2009). *Aenasius arizonensis* has a high parasitization rate (37.6-72.3%) of its host mealybug on cotton in Haryana (Ram *et al.,* 2009).

Temperature, and the ability to adapt to it, has significant impact on the life traits and population expansion of insects (Emana, G.D. 2007). Information related to the response of invasive species to temperature changes is needed to elucidate patterns and practices of invasion (Kelley, 2014). An extensive knowledge has been gathered on relations between temperature and the survival, reproduction, development and population development of *P. solenopsis* (Lu *et al.,* 2011; Prasad *et al.,* 2012; Wang *et al.,* 2012; Ali *et al.,* 2012; Sreedevi *et al.,* 2013; Kumar *et al.,* 2013; Fand *et al.,* 2014; Chen *et al.,* 2015). *Phenacoccus solenopsis* completes immature development at temperatures between 15 and 35°C, reproduces efficiently at 22-35°C (Kumar *et al.,* 2013; Sreedevi *et al.,* 2013; Fand *et al.,* 2014); its upper developmental threshold (39.0–40.7°C), enables it to survive at extreme temperatures in tropics and subtropical areas (Prasad *et al.,* 2012; Sreedevi *et al.,* 2013).

Studies reveal that temperature can significantly affect development, parasitism rates and fecundity rates of parasitic wasps (Mann *et al.,* 1990; Qiu *et al.,* 2012). In general, the development, parasitism, fecundity and survival of parasitoids are more efficient at optimum temperature ranges compared to other temperature ranges (Flinn, 1991; Oliveira *et al.,* 1998; Malina and Praslicka, 2008; Qiu *et al.,* 2012; Carcamo *et al.,* 2013). Understanding temperature needs of parasitic wasps will provide basic knowledge to aid in culturing and maintenance of laboratory populations (Gould and Elkinton, 1990; Tillman and Powell, 1991; Oliveira *et al.,* 1998; Reznik *et al.,* 2009), which can be manipulated to understand the optimum temperatures for wasp development and to synchronize the rearing of parasitoids and their hosts. This understanding can also provide information on the population dynamics of the parasitic wasps in their surroundings (Tunca *et al.,* 2010; Bueno *et al.,* 2012; Perveen *et al.,* 2012; Qiu *et al.,* 2012).

This study was conducted to find the optimum temperature range for rearing and development of the parasitoid, *Aenasius arizonensis*. We observed different biological parameters under the effect of four constant temperature ranges in the laboratory. The results may provide valuable information regarding optimum temperature conditions for mass rearing of the parasitic wasp in the insectary leading towards its use as a successful biological control agent of *P. solenopsis*.

MATERIALS AND METHODS

Experimental insects: The experiment was conducted in Insect Molecular Biology Lab. University of Agriculture, Faisalabad, Pakistan in 2019. The endophagous encyrtid parasitic wasp *Aenasius arizonensis* was reared on colonies of its regular host mealybug, *Phenacoccus solenopsis*. Parasitized host/mealybug dead bodies called "mummies" were collected in plastic containers from fields of cotton and vegetables (e.g., okra, tomato, eggplant, and pumpkin) situated in the experimental fields of the University of Agriculture, Faisalabad. A rearing culture of *A. arizonensis* was developed from mummies of parasitized mealybugs. Small to medium sized potatoes were provided as a food for the mealybug. Potatoes were washed and dried and then held at room temperature in the dark. When potatoes sprouted to approximately 2.5 cm, they were used for mealybug food. Another plant used for host rearing was an average sized green bottle gourd. Gravid females were shifted onto a new food with a camel hair brush. Sprouted potatoes were put into approximately 20x15 cm glass bottles and covered with fine mesh cloth. Before studies, the wasp was reared on mealybugs reared on the green bottle gourd. First instar mealybug nymphs were placed on leaves of green bottle gourd and reared for a few generations. Third instar nymphs were utilized for experimental studies. Mealybugs and parasitoids were kept in glass containers at 27±2℃, 65±5% relative humidity (RH) and a 18/6 hr light and dark photoperiod by following a modified methodology of Shaina *et al.,* 2016. Credulous/virgin parasitoid adults were acquired from mealybug mummies and given Honey and water as a nourishment source.

Biological parameters at different temperatures: In order to study the life cycle of *A. arizonensis*, newly developed adult parasitoids were paired; males and females were separated based on antennal size and ovipositor structure. Parasitoids were kept in a glass container secured with a muslin cloth along and a honey solution-soaked cotton swab was added as a food source.

A grown potato or green bottle gourd infested with fifty 3rd instars mealy-bugs was placed into the glass jar for 24 hours. Parasitoids pairs were continually moved to a new arrangement of mealy-bug instars in a different glass container and a similar strategy was repeated until the death of the parasitoid female. The test was conducted at four different temperatures of 20±2, 25±2, 30±2 and 35±2 ℃ with 65±5% relative humidity (RH).

Constant temperatures were maintained in the insect growth chambers. Development time from egg to mummification, mummification to adult emergence, total developmental time, and number of adults emerged was recorded. Other parameters examined were pre-oviposition period, post-oviposition period (the period following oviposition for which the adult wasp stops egg laying until it is expired), daily and total fecundity (fecundity was calculated through observing number of parasitized hosts on daily basis for whole life of a single mated female wasp), male and female adult lifespan, and sex ratio. Parameters including developmental period, fecundity, life span and percent females in the offspring were utilized to build life tables for *A. arizonensis*.

Adult parasitoids and mummies (from which the adults emerged) reared at room temperature were saved in 70 percent ethanol for estimations of morphological parameters. Head width and body length for both both male and female adults and the size and length of mummies were recorded for 20 specimens from each temperature.

Data analysis: A Completely randomizd design was used for planning of the experiment and all temperature treatments were replicated three times. Data was analyzed using Statistix 8.1 Software and subjected to analysis of variance under a completely randomized design. Means were separated using Tukey HSD testing using a significance level of P<0.05.

RESULTS

This study was conducted to observe different biological parameters of the parasitic wasp, *Aenasius arizonensis,* during parasitization and reproduction of the cotton mealybug host at differing constant temperatures. The results indicate that temperature has significant effects on the recorded specific to wasp development period, number of progeny emerged, and sex ratio.

For example, the number of male and female progeny decreased gradually with increasing temperature from 20±2 ℃ to 35±2 ℃ (Fig. 1). However, the number of females emerged was always greater than male emergence at all temperatures; 36 and 34 females emerged as compared to 22 and 16 males at 20±2 and 25±2 ℃, respectively. The same was observed for other temperatures.

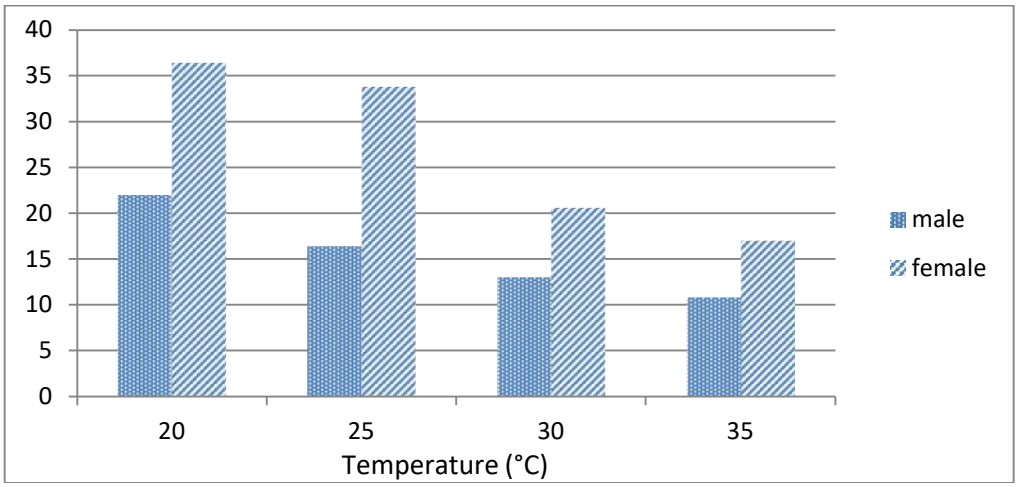

Fig. 1. The number of male and female parasitoids emerging at different temperatures.

Table 1. Mean comparisons of daily fecundity and total fecundity of *Aenasius arizonensis* when exposed to different temperatures

Temperature (℃)	Total fecundity (Means±SE)	Daily fecundity (Means±SE)
20	54.200± 1.428 B	1.4000± 0.244 A
25	64.000± 3.492 A	2.0000± 0.316 A
30	63.600± 3.549 A	2.0000± 0.316 A
35	36.400± 0.509 C	1.6000± 0.244 A

Means followed by different letter(s) within each column (denoted by upper-case letters) are significantly different by Tukey HSD test at P <0.05.

In the case of fecundity (number of parasitized host), daily fecundity was markedly higher at 25±2℃ (2 parasitized hosts per female) and 30±2℃ (2 parasitized hosts per female) as shown in Table 1. Minimum daily fecundity was observed at 20±2℃ (1.40 parasitized hosts per female) and 35±2℃ (1.6 parasitized hosts per female). Total fecundity was highest (64 parasitized hosts per female) at 25±2℃. Likewise, total fecundity at 20±2℃ (54.2 parasitized hosts/ female) and 30±2℃ (63.6 parasitized hosts/ female) was also higher but lowest at 35±2℃ (36.4 parasitized hosts/ female), so, there is a significant effect of temperature on daily and total fecundity

Table 2. Means for total oviposition and post-oviposition period under different temperatures.

Temperature (°C)	Post-oviposition period (days) (Means±SE)	Total oviposition period (days) (Means±SE)
20	2.0000± 0.316 a	32.600± 1.248 a
25	2.0000± 0.316 a	31.800± 0.583 a
30	2.0000± 0.316 a	17.000± 0.894 b
35	2.0000± 0.316 a	14.600± 0.509 b

Means followed by different letter(s) within each column (denoted by upper-case letters) are significantly different by Tukey HSD test at P <0.05

Oviposition period decreased with increasing temperature: it was 32.6, 31.8, 17 and 14.6 days at 20±2 ºC, 25±2 ºC, 30±2 ºC and 35±2 ºC, respectively. There was no difference in the oviposition period at 30±2 ºC (17 days) and 35±2 ºC (14.6 days) while significant differences were observed at 20±2 ºC (32.6 days) and 25±2 ºC (31.8 days) as shown in Table 2. No significant difference was observed in the post-oviposition period at the different temperatures studied. The post-oviposition period was 2, 2, 2 and 2 days at 20±2 ºC, 25±2 ºC, 30±2 ºC and 35±2 ºC, respectively.

Table 3. Mean comparison values for the development period (days) of the parasitoid from egg stage to pupation/host mummification under different temperatures

Temperature (℃)	Male mummy development period (days) (Means±SE)	Female mummy development period (days) (Means±SE)
20	14.000± 0.707 A	15.000± 0.447 A
25	11.000± 0.583 A	9.8000± 0.583 B
30	5.8000± 0.860 B	6.2000± 0.860 C
35	5.4000± 1.122 B	6.0000± 1.140 C

Means followed by different letter(s) within each column (denoted by upper-case letters) are significantly different by Tukey HSD test at P <0.05

The development time for male mummies formation was shorter as compared to the female ones, Moreover, emergence of males was observed later on from smaller sized mummies while larger sized mummies produced mostly female wasps, so. difference in sizes of male vs female mummies was also clearly noticed (Table 3).

Female wasp emergence took longer as compared to males. The longest development time for males from mummy formation to adult emergence was of 20.4 days at 20±2℃, and shortest at 3.8 days at 35±2℃. A significant difference was found in the developmental period of male wasps at 20±2℃ (20.4 days) and 25±2℃ (14.4 days). In case of female wasps, there was no significant difference in the developmental time at the higher temperatures of 30±2℃ (6.4 days) and 35±2℃ (4.8 days). At the lower temperatures of 20±2℃ (22.4 days) and 25±2℃ (16.6 days) the differences were significant. A longer development period (22.4 days) was observed at 20±2℃ while the shortest period of 4.8 days was observed at 35±2℃ (Fig. 2). Overall, the temperature was shown to have a significant effect on the adult emergence in both female and male wasps.

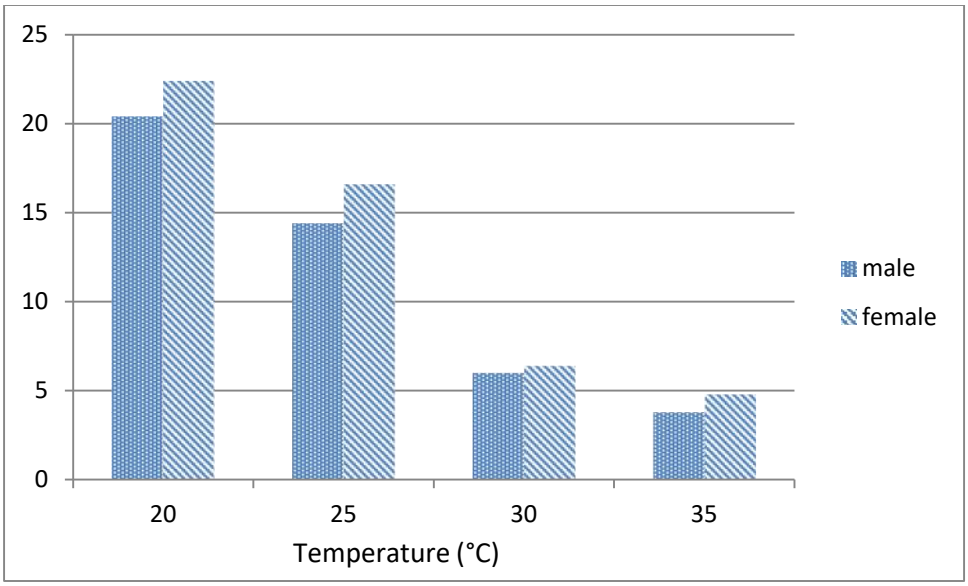

Fig. 2. The development time of parasitoid from mummy to adult emergence under varied temperatures

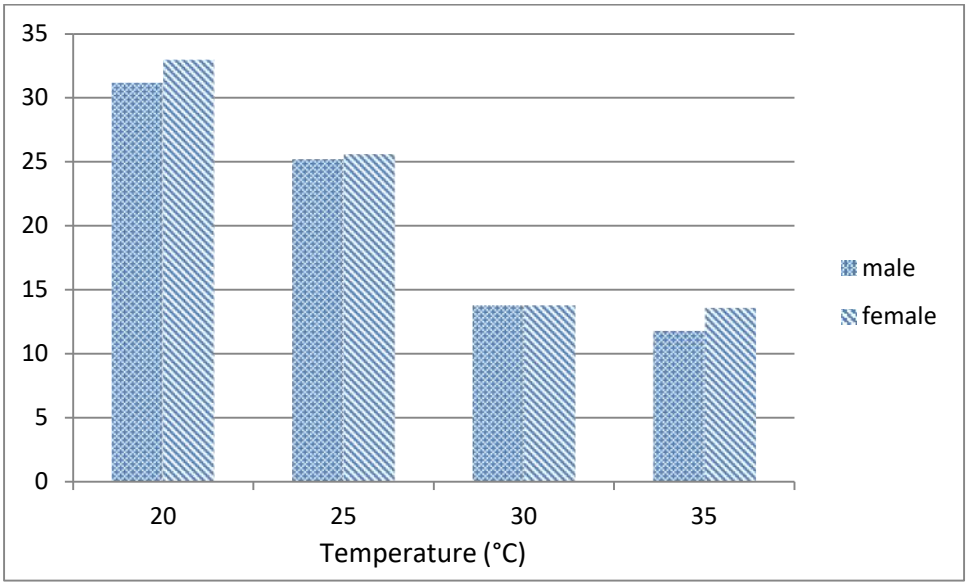

Fig. 3. Total development period (days) of the parasitic wasp under different temperature exposures.

The total development time (days) of both male and female parasitic wasps decreased with increasing temperature. Development of males was faster as compared to females at all the given temperatures. Development period was longest in males (31.2 days) at 20±2℃ and shortest (11.8 days) at 35±2℃. Likewise, development period for females was longest (33 days) at 20±2℃ and shortest (13.6 days) at 35±2℃. Total development period for males was 31.2, 25.2, 13.8, and 11.8 days at 20±2℃, 25±2℃, 30±2℃ and 35±2℃, respectively. Similarly, for females it was 33, 25.6, 13.8 and 13.6 days at 20±2℃, 25±2℃, 30±2℃ and 35±2℃, respectively (Fig. 3). Temperature also influenced the total development period significantly.

DISCUSSION

Specific parameters of the developmental period of the parasitic wasp, *A. arizonensis,* at differing constant temperatures were studied. Results of this study indicate that parasitoid development time from egg insertion to host mummy formation decreased with increasing temperature. No significant difference was observed in the developmental time of male and female wasps from oviposition to mummy formation, both female and male wasps developed at the same rate at all the given temperatures. Mummy formation took a long period (15.6 days) at 20℃ and shorter (6.08 days) at 35℃ for both the male and female wasps. Significant differences for the

egg to mummy formation for both sexes were found at 20 and 25℃, whereas no marked differences were found at 30℃ and 35℃. Male wasps were usually smaller in size as compared to female wasps. Jong and Alphen (1989) reported that female wasps of another parasitoid, *Leptomastix dactylopii* Howard, were larger in size as compared to males emerging from their host, *Planococcus citri* (Risso). Females of *Anagyrus mangicola* (Bokonon-Ghanta) were also found larger as compared to male wasps (Bokonon-Ghanta *et al.* 1995)

Development time of the parasitic wasp from mummy formation to adult emergence, in both sexes, decreased with increasing temperature. Female wasps took longer to emerge from host mummies as compared to males. The longest development time in males, from mummy to adult emergence was at 20℃ (20.4 days) and shortest at 35℃ (3.8 days).

The total development time of both female and male wasps decreased with increasing temperature. Development of males was faster compared to females at all temperatures. For males and females, development period was longest at 20℃; 31.2 and 33 days, respectively and shortest at 35℃, 11.8 and 13.6 days, respectively. The temperature also influenced the total development period significantly; our findings are in agreement with Malina and Praslicka (2008).

Adult parasitoids mated instantly after emergence and mealybugs parasitization began on the day of emergence at all temperatures. Oviposition period decreased as the temperature increased, oviposition period was 32.6, 31.8, 17 and 14.6 days at 20, 25, 30 and 35 ºC, respectively. There was no marked difference in the egg laying period at 30 ºC and 35 ºC, whereas, significant differences were observed at 20 ºC (32.6 days) and 25 ºC (31.8 days), respectively. Similarly, in the post-oviposition period, no difference was observed at any tested temperature. Our study agrees with Sharaf and Batta (1996) who observed the effect of temperature on the biology of *Eretmocerus mundus* Meret, a parasitic wasp of *Bemisia tabaci Genn*. and concluded that pre-oviposition period of the wasp increased from 1.6 days to 2.8 days with the decreasing temperature of 25-14 ℃.

Daily fecundity was markedly higher at 30℃ (2.00 parasitized hosts per female) and 25℃ (2 parasitized hosts per female). Lowest daily fecundity was recorded at 20℃ (1.40 parasitized hosts per female) and 35 ℃. The work done by Emana (2007) states that in *Cotesia flavipes* (Cameron), minimum fecundity of 23.8 eggs was observed at 20 ℃ compared with 41.4 eggs at 40℃. Likewise, lowest fecundity in *Bracon brevicornis* Wesmael, a parasitic wasp of fruit borers of *Abelmoschus esculentus* (L.) was observed at 20℃ (Thanavendan and Jeyarani, 2010).

The longevity of parasitic wasps decreased with increasing temperature. The longevity of males was shorter compared to females at all tested temperatures. Male longevity was shortest at the high temperature of 35 ℃ while longest at 20℃. Significant differences in longevity of both male and female wasps were observed at 20 and 25℃, respectively, but no marked differences were observed at higher temperatures. Female longevity was 38.66 days at 20℃ and 34.53 days at 25℃. These results are supported by Al-Maliky and Al-Izzi (1990) who found that there was an inverse impact of temperature over longevity of *Apanteles* sp.; longevity was decreased at increasing temperature.

Sharaf and Batta (1996) found that in *Eretmocerus mundus*, a parasitic wasp of *Bemisia tabaci*, adult longevity decreased with increasing temperature from 14 to 25℃, males lived shortest compared to female wasps at both temperatures. An investigation of adult age of females of *Oomyzus sokolowskii* (Kurdj.), a parasitic wasp of *Plutella xylostella* (L.), found a decrease from 28.3 d to 6.6 d with increasing temperature of 20 to 30℃, respectively. Moreover, the sex ratio of the wasps was female biased at all the tested temperatures. It was 1:1.1 at 20-25 ℃ and 1:1.2 at 30-35℃, respectively (Wang *et al.,* 1999).

Conclusions: Results of this study indicate that a temperature of 30±2℃ from all the provided temperatures could be considered a suitable condition for the efficient growth, multiplication, and rearing of *A. arizonensis* populations, which may lead to the development of a successful biocontrol programme for the management of mealybug pests in cotton crop.

Conflict of Interest: No conflicts of interest among the authors exist.

Author's contribution: H.Hira wrote the paper out of her M.Phil Thesis work, Zain ul Abdin (supervised the research work and edited the paper), M.Tayyib provided inputs for taxonomic identification, M.Arshad, preliminary reviewed, F.Hussain and S.K.Abbas helped out in statistical analysis and journal selection.

Acknowledgments: We are grateful to HEC (Higher Education Commission) No. 20-1455/R&D/2009/223, No. 20-3247/NRPU/R&D/HEC/13727 for providing financial support.

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