

# Seasonal and biological interactions between the parasitoid, *Aenasius arizonensis* (Girault) and its host, *Phenacoccus solenopsis* Tinsley on cotton

Prasun Karmakar · P.S. Shera 

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**Abstract** *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae) is an important solitary endoparasitoid of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Studies on seasonal and biological interactions between host and parasitoid are important to optimize the mass production and field release of high-quality females. We investigated the influence of different crop seasons viz., pre-monsoon, monsoon and post-monsoon and host stages viz., three nymphal instars (1st, 2nd and 3rd) and adult stage of *P. solenopsis* on the development, longevity, fecundity and sex ratio of its parasitoid, *A. arizonensis*. *A. arizonensis* female parasitized all life stages of its host except the 1st instar nymphs. Only males emerged from parasitized 2nd instar nymphs, while the sex ratio in the later host instars was strongly female-biased. The adult host stage was most suitable for *A. arizonensis* in terms of shorter development time, while the 3rd instar nymphal stage was most suitable with respect to higher fecundity, proportion of adults emerged and more females in the progeny. Among different seasons, post-monsoon season (September–October) with temperature (25 to 29 °C) and relative humidity (68 to 73%) was most suitable for the overall fitness of the parasitoid. The findings of this study have implications in designing mass rearing of this parasitoid and devising an effective biological control strategy for *P. solenopsis* on cotton.

**Keywords** *Aenasius arizonensis*. *Phenacoccus solenopsis*. Parasitism. Development. Fitness. Season

## Introduction

The invasive mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) was first documented as a pest of cotton in Texas, United States (Fuchs et al. 1991) and now widely distributed in various ecological zones of the globe (García Morales et al. 2016). During the last decade, this mealybug species has emerged as a serious threat to cotton in India and Pakistan, causing severe economic losses (Wang et al. 2010) and is now widespread in almost all cotton growing states of India (Nagrare et al. 2009). Since it is an exotic pest, biological control must be attempted as an effective means of its management (Ram and Saini 2010).

Encyrtids are the most successful natural enemies used in the biological control programs against mealybugs (Noyes and Hayat 1994). *Aenasius arizonensis* (Girault) is an important koinobiont nymphal endoparasitoid of *P. solenopsis* reported 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 *P. solenopsis* under field conditions (Ram et al. 2009). Field parasitism by *A. arizonensis* to an extent of 95% has been recorded in *P. solenopsis* (Khuhro et al. 2011). It was first time reported by Mahmood (2008) in Pakistan and later, was described and named as *A. bambawalei* by Hayat (2009). However, Fallahzadeh

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P. Karmakar · P. Shera (✉)  
Department of Entomology, Punjab Agricultural University,  
Ludhiana, Punjab 141004, India  
e-mail: psshera@pau.edu

et al. (2014) from Iran reported that this species is closer to *Aenasius arizonensis* (Girault) and reported on *A. bambawalei* as a junior synonym of *A. arizonensis*.

Host life stage is one of the key factors influencing oviposition preference, sex allocation and offspring fitness in solitary parasitoids (King 1989; Hagvar and Hofsvang 1991; Harvey et al. 1994; Kant et al. 2012). Furthermore, the progeny fitness is positively related to host resources; consequently, the parasitoid tends to allocate the offspring sexes according to host-size-dependent sex allocation principle (Charnov 1982; Godfray 1994; West and Sheldon 2002; West 2009). Parasitoids make a series of oviposition decisions while locating their hosts to achieve maximum fitness gains which include the number of eggs laid, the ratio of fertilized to unfertilized eggs and whether to superparasitize the host or not. Such decisions are relatively influenced by the quality of hosts encountered by the parasitoids (Kant et al. 2012). Earlier studies by many workers on different parasitoids have reported that host stage at the time of parasitism affects their reproductive fitness, resulting in altered developmental time (Sequeira and Mackauer 1993), body size (Liu 1985; Lampson et al. 1996) and progeny sex ratio (Godfray 1994; Napoleon and King 1999).

Most of the studies on host stage preference of *A. arizonensis* (= *A. bambawalei*) have been carried out by earlier workers on the basis of parasitization rate under choice and no choice tests. (Fand et al. 2011; Abdin et al. 2012; Vijaya and Ram 2013; Badshah et al. 2016; Iqbal et al. 2016; Zhang et al. 2016). Furthermore, the biology of *A. arizonensis* has been studied earlier on one host stage only, either on 3rd instar nymphs or adult host stage (Poorani et al. 2009; Fand et al. 2011; Abdin et al. 2013; Vijaya and Ram 2013). Information about whether and to what extent different host stages of *P. solenopsis* affect the detailed biological parameters of this solitary endoparasitoid is still lacking. Moreover, comprehensive biology of *A. arizonensis* in cotton crop seasons with varying temperature and humidity conditions is not available. The aim of this study was to investigate the effect of different crop seasons and host stages on the overall development, reproductive fitness and offspring sex allocation by *A. arizonensis* on cotton. This information could be helpful in developing an optimal mass rearing program for this parasitoid and devising an effective biological control strategy for *P. solenopsis* in cotton ecosystem.

## Materials and methods

The biological parameters of *A. arizonensis* were studied on three nymphal instars (1st, 2nd and 3rd) and adult stage of mealybug, *P. solenopsis* in the screen house (4 m × 3 m × 2 m) fitted with 30 mesh, galvanized wire gauge, at the Entomological Research Farm, Punjab Agricultural University (PAU), Ludhiana during the pre-monsoon (May–June), monsoon (July–August) and post-monsoon (September–October) seasons during 2015. The meteorological data were recorded using digital thermohygrometer (HTC Instruments, India). The mean temperature ranged between 25.2 to 28.7 °C in the post-monsoon (September–October), 29.9 to 30.3 °C in the monsoon (July–August) and 31.7 to 31.8 °C in the pre-monsoon (May–June) season. The mean relative humidity during the post-monsoon season was 68.0 to 72.9%, while it was 74.5 to 76.5% in the monsoon season and 31.5 to 49.5% in the pre-monsoon season.

### Raising of host plant

The seeds of Bt cotton hybrid (NCS 855 BG II) were sown in earthen pots filled with soil and farm yard manure (1:1) and plants were raised without any plant protection measures (Anonymous 2015). These pots were placed in sunlight till germination. After germination, pots were transferred to screen house to avoid any pest infestation. Only one healthy seedling was retained in each pot for conducting experiments.

### Rearing of *P. solenopsis*

*P. solenopsis* was reared on sprouted potatoes (*Solanum tuberosum* L.) according to the procedures outlined by Nagrare et al. (2011). Small to medium sized potato tubers were washed, air dried and held at room temperature in the dark until sprouting. When the potato sprouts were 2.5–5 cm in length, they were placed in glass jars (4–5 tubers/ jar) of size 20 × 15 cm, with moist soil at the base to maintain turgidity of the sprouted tubers. The sprouts were inoculated with gravid females with the help of a soft camel hair brush. The jars were then covered with muslin for the establishment of mealybug colonies. The culture of *P. solenopsis* was maintained for multiple generations, which was used for rearing of *A. arizonensis* and for the experiments.

## Rearing of *A. arizonensis*

Parasitized mealybugs (mummies) were collected from mealybug infested cotton, *Hibiscus* sp., and weed hosts (*Abutilon* sp., *Sida* sp. and *Parthenium* sp.) and brought to the laboratory. Naive/virgin *A. arizonensis* parasitoid adults obtained from mummies of mealybug were sexed (Hayat 2009) and were released in glass jars containing healthy colonies of mealybug maintained on sprouted potatoes. After parasitism, the mummies were collected and kept in glass vials plugged with cotton wool. These glass vials were provided with the honey streak on a paper strip as a source of food for the emerging adult parasitoids. The newly emerged adult parasitoids were used to study various biological parameters.

Biological parameters of *A. arizonensis* on different stages of mealybug, *P. solenopsis* during pre-monsoon, monsoon and post-monsoon seasons

Twenty individuals (5 replicates;  $n = 100$ ), from each of the three nymphal instars (1st, 2nd and 3rd) and adult stage of *P. solenopsis* were transferred from the maintained insect culture on different *Bt* cotton plants (NCS 855 BG II) with a soft camel hair brush. They were kept in separate wooden screen cages (15 cm × 15 cm × 20 cm). A pair of freshly emerged adults of the parasitoid was released in each cage for 48 h and provided with cotton swab dipped in 10% honey solution as a source of food for *A. arizonensis* adults. After parasitism, mummies were collected and kept in separate plastic vials (45 mm diameter × 60 mm height) provided with the honey streak on a paper strip as a source of food for the emerging adult wasps. Observations were recorded on the total immature period (oviposition to adult emergence), proportion of adults emerged from mummies of *P. solenopsis* in progeny (adult emergence), sex ratio (proportion female), adult longevity of the parasitoid. The adult emergence (proportion) was worked out by dividing the number of adults emerged with number of mummified mealybugs.

$$\text{Adult emergence (proportion)} = \frac{\text{Number of adult parasitoids emerged}}{\text{Total number of mummies}}$$

The oviposition period was recorded by releasing a couple of 1 day-old *A. arizonensis* adults in separate aluminium screen cages (10 cm × 10 cm × 10 cm) having 30 individuals (5 replicates;  $n = 150$ ), from each of the three nymphal instars (1st, 2nd and 3rd) and adult stage

of *P. solenopsis*. All the experiments were replicated five times. After every 24 h, these adults were removed and transferred to new jars for determining daily fecundity. The total number of mummies and un-mummified mealybugs were recorded daily. Un-mummified mealybugs were subjected to xylene treatment (Souissi and Le Ru 1997). Total fecundity per female was calculated by adding the number of mummies and the number of un-mummified mealybugs showing signs of encapsulation. A cotton swab dipped in 10% honey solution was hung in each cage as a source of food for *A. arizonensis* adults.

## Statistical analysis

Data are presented as mean ± standard error of the mean (SE). The data on the proportion of adults emerged and sex-ratio (expressed as proportion of females) in progeny were analysed by means of a logistic ANOVA model: Generalized Linear Models (using a logit link function) assuming quasi binomially distributed error variances with significance assessed through model simplification (Crawley 1993; Wilson and Hardy 2002). Total fecundity of *A. arizonensis* was analyzed using a Generalized Linear Model with a poisson error distribution (using a log link function) (Yamane et al. 2015). The data on total development period of immature *A. arizonensis*, female longevity and ovipositional period of *A. arizonensis* adults were analyzed using Generalized Linear Models with normal error distributions (using an identity link function). Generalized Linear Models results are presented in the form of Wald Chi-square  $\chi^2$  test and  $p$  values. Pair-wise tests were performed by choosing appropriate contrasts, and an overall significance level of 5% was maintained using a modified Bonferroni procedure (Hochberg 1988). All statistical tests were carried out using IBM SPSS 22.0 for Windows (IBM Corporation, Armonk, New York, USA).

## Results

The mean temperature in the post-monsoon (26.9 °C) differed significantly from the mean temperature in the monsoon (30.1 °C) and the pre-monsoon (31.8 °C) season (Wald  $\chi^2 = 136.55$ ;  $df = 2$ ;  $p = <0.0001$ ). The mean relative humidity in the post-monsoon (70.3%) also differed significantly from the mean relative humidity in monsoon (75.4%) and pre-monsoon (43.5%) season (Wald  $\chi^2 = 361.41$ ;  $df = 2$ ;  $p = <0.0001$ ).

## Total immature period

*A. arizonensis* females parasitized all the stages of mealybug, *P. solenopsis* except the 1st instar nymphs. For both sexes, total immature period, i.e. from oviposition to adult emergence also showed significant differences among different seasons as well as host stages. Among different seasons, the period was significantly shorter (11.87 days) in the post-monsoon season, followed by monsoon season (13.02 days), whereas, it was longest in pre-monsoon season (14.51 days) for male parasitoids (Table 1; Wald  $\chi^2 = 45.58$ ;  $df = 2$ ;  $p < 0.0001$ ). For female parasitoids, the total immature period was significantly shorter (13.56 days) in post-monsoon season; and it was not statistically different from the monsoon season (13.74 days) (Wald  $\chi^2 = 18.69$ ;  $df = 2$ ;  $p = 0.0004$ ). For both sexes, developmental time decreased with increase in host age and males developed within a shorter period of time as compared to females. The total immature period of *A. arizonensis* males was significantly longer (15.01 days) in 2nd instar followed by 3rd instar nymphs (13.20 days), while it was least (11.19 days) in adult host stage (Wald  $\chi^2 = 37.47$ ;  $df = 2$ ;  $p < 0.0001$ ). For females also, it was significantly shorter in adult host stage as compared to those recorded in 3rd instar host stage (Table 1; Wald  $\chi^2 = 23.56$ ;  $df = 1$ ;  $p < 0.0001$ ). The interaction analysis for the total immature period between different seasons and host stages was found to be significant for male (Wald  $\chi^2 = 59.03$ ;  $df = 4$ ;

$p < 0.0001$ ), and female (Wald  $\chi^2 = 43.58$ ;  $df = 2$ ;  $p < 0.0001$ ) parasitoids.

## Proportion of adults emerged in progeny and sex ratio (proportion female)

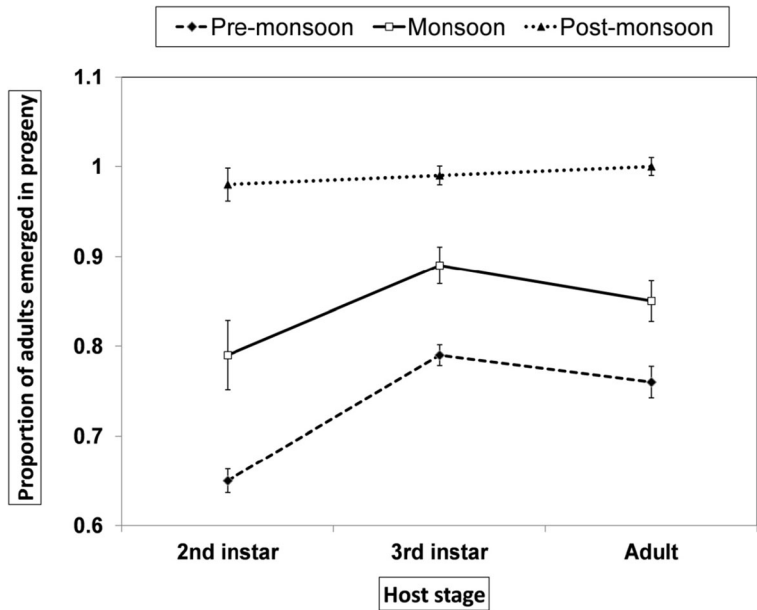
The proportion of *A. arizonensis* adults emerged from *P. solenopsis* mummies was significantly influenced by seasons (Fig. 1; Wald  $\chi^2 = 41.37$ ;  $df = 2$ ;  $p < 0.0001$ ) but no significant differences were observed in proportion of *A. arizonensis* adults emerged from different host stages of *P. solenopsis* (Wald  $\chi^2 = 2.84$ ;  $df = 2$ ;  $p = 0.200$ ). Moreover, the interaction between different seasons and host stages was also non-significant for proportion of adults emerged in progeny (Wald  $\chi^2 = 0.06$ ;  $df = 4$ ;  $p = 0.786$ ). Significantly higher proportion of *A. arizonensis* adults (0.99) emerged from mummies in the post-monsoon season, followed by monsoon (0.84) and pre-monsoon seasons (0.74). Among host stages, it was highest (0.89) from mummies of 3rd nymphal instar and it was not statistically different from those which emerged from adult hosts (0.87) (Fig. 1). Only males emerged from parasitized 2nd instar *P. solenopsis* nymphs, whereas, it was female-biased in the later host stages. Between the two stages, the relatively high proportion of *A. arizonensis* female wasps emerged from 3rd instar *P. solenopsis* nymphs (0.59) as compared to adult host stage (0.57) (Fig. 2). However, there was no significant interaction between season and host stage on the

**Table 1** Effect of different seasons and *P. solenopsis* stages on the total immature period (oviposition to adult emergence) of *A. arizonensis*

| Host stage at parasitism | Total immature period of <i>A. arizonensis</i> males (days) |                               |                               |                               | Total immature period of <i>A. arizonensis</i> females (days) |                               |                               |                               |
|--------------------------|---|-------------------------------|-------------------------------|-------------------------------|---|-------------------------------|-------------------------------|-------------------------------|
|                          | Pre-monsoon season  | Monsoon season                | Post-monsoon Season           | Mean $\pm$ SE                 | Pre-monsoon season  | Monsoon season                | Post-monsoon Season           | Mean $\pm$ SE                 |
| 1st instar               | *   | *                             | *                             | *                             | *   | *                             | *                             | *                             |
| 2nd instar               | 17.40 $\pm$ 0.32  | 14.58 $\pm$ 0.07              | 13.05 $\pm$ 0.13              | 15.01 $\pm$ 0.49 <sup>c</sup> | **  | **                            | **                            | **                            |
| 3rd instar               | 14.40 $\pm$ 0.30  | 13.27 $\pm$ 0.16              | 11.92 $\pm$ 0.13              | 13.20 $\pm$ 0.29 <sup>b</sup> | 15.63 $\pm$ 0.14  | 14.87 $\pm$ 0.14              | 14.64 $\pm$ 0.13              | 15.05 $\pm$ 0.13 <sup>b</sup> |
| Adult                    | 11.72 $\pm$ 0.20  | 11.22 $\pm$ 0.19              | 10.65 $\pm$ 0.10              | 11.19 $\pm$ 0.15 <sup>a</sup> | 13.55 $\pm$ 0.51  | 12.61 $\pm$ 0.13              | 12.48 $\pm$ 0.08              | 12.88 $\pm$ 0.21 <sup>a</sup> |
| Mean                     | 14.51 $\pm$ 0.64 <sup>c</sup>                               | 13.02 $\pm$ 0.38 <sup>b</sup> | 11.87 $\pm$ 0.27 <sup>a</sup> | –                             | 14.59 $\pm$ 0.43 <sup>b</sup>                                 | 13.74 $\pm$ 0.39 <sup>a</sup> | 13.56 $\pm$ 0.37 <sup>a</sup> | –                             |
|                          | Season (S)  | Host stage (H)                | S x H                         |                               | Season (S)  | Host stage (H)                | S x H                         |                               |
| $\chi^2$                 | 45.58   | 37.47                         | 59.03                         |                               | 18.69   | 23.56                         | 43.58                         |                               |
| <i>p</i> value           | <0.0001   | <0.0001                       | <0.0001                       |                               | 0.0004  | <0.0001                       | <0.0001                       |                               |
| <i>df</i>                | 2   | 2                             | 4                             |                               | 2   | 1                             | 2                             |                               |

\* No successful parasitism occurred on 1st instar nymphs; \*\*only male parasitoids emerged from mummies of 2nd instar *P. solenopsis*; Data are presented as the mean  $\pm$  standard error (SE) computed on five replications. Mean values followed by the different letters are significantly different ( $P < 0.05$ ) from each other. Pre-monsoon season - May-June; Monsoon season - July-August; Post-monsoon season - September-October

**Fig. 1** Proportion of *A. arizonensis* adults emerged from different stages of *P. solenopsis* during different crop seasons



proportion of *A. arizonensis* females in progeny (Wald  $\chi^2 = 0.27$ ;  $df = 4$ ;  $p = 0.801$ ).

**Adult longevity**

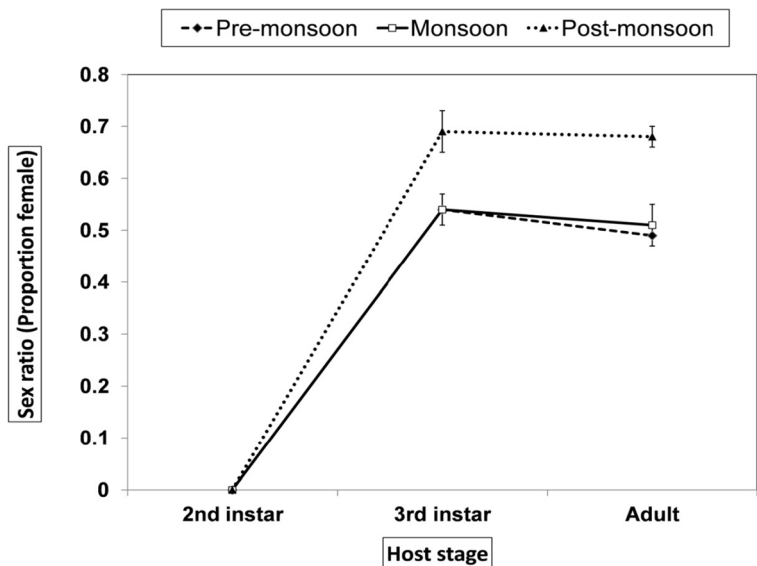
The longevity of female adults emerged from *P. solenopsis* mummies was significantly influenced by seasons (Table 2; Wald  $\chi^2 = 55.51$ ;  $df = 2$ ;  $p < 0.0001$ ) and host stages of *P. solenopsis* (Wald  $\chi^2 = 34.21$ ;  $df = 1$ ;  $p < 0.0001$ ). Moreover, the interaction between seasons and host stages was also significant for longevity of

female adults (Table 2; Wald  $\chi^2 = 51.05$ ;  $df = 2$ ;  $p < 0.0001$ ).

**Oviposition period**

The oviposition period of *A. arizonensis* females was significantly higher in the post-monsoon season (13.33 days), followed by the monsoon season (6.53 days), while it was lowest (5.93 days) in pre-monsoon season (Table 3; Wald  $\chi^2 = 42.20$ ;  $df = 2$ ;  $p < 0.0001$ ). Among the host stages, the oviposition

**Fig. 2** Proportion of *A. arizonensis* females emerged from different stages of *P. solenopsis* during different crop seasons. \*only males emerged from the progeny of 2nd instar *P. solenopsis* nymphs



**Table 2** Effect of different seasons and *P. solenopsis* stages on the longevity of *A. arizonensis* females

| Host stage at parasitism | Adult longevity (days)   |                          |                           | Mean                      |
|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|
|                          | Pre-monsoon season       | Monsoon Season           | Post-monsoon season       |                           |
| 1st instar               | *                        | *                        | *                         | *                         |
| 2nd instar               | **                       | **                       | **                        | **                        |
| 3rd instar               | 6.67 ± 0.16              | 7.42 ± 0.11              | 14.99 ± 0.14              | 9.69 ± 1.01 <sup>b</sup>  |
| Adult                    | 7.52 ± 0.10              | 8.61 ± 0.15              | 16.59 ± 0.11              | 10.91 ± 1.08 <sup>a</sup> |
| Mean                     | 7.10 ± 0.17 <sup>c</sup> | 8.02 ± 0.22 <sup>b</sup> | 15.79 ± 0.28 <sup>a</sup> | –                         |
|                          | Season (S)               | Host stage (H)           | S x H                     |                           |
| $\chi^2$                 | 55.51                    | 34.21                    | 51.05                     |                           |
| <i>p</i> value           | <0.0001                  | <0.0001                  | <0.0001                   |                           |
| <i>df</i>                | 2                        | 1                        | 2                         |                           |

\* No successful parasitism occurred on 1st instar nymphs; \*\* only male parasitoids emerged from mummies of 2nd instar *P. solenopsis*; Data are presented as the mean ± standard error (SE) computed on five replications. Mean values by different letters are significantly different ( $P < 0.05$ ) from each other. Pre-monsoon season - May-June; Monsoon season - July-August; Post-monsoon season - September-October

period of *A. arizonensis* females was significantly higher (9.67 days) in adult host stage, followed by 3rd instar (8.93 days) and 2nd instar (7.20 days) host stages

**Table 3** Effect of different seasons and *P. solenopsis* stages on the oviposition period of *A. arizonensis* females

| Host stage at parasitism | Oviposition period (days) |                          |                           | Mean                     |
|--------------------------|---------------------------|--------------------------|---------------------------|--------------------------|
|                          | Pre-monsoon season        | Monsoon Season           | Post-monsoon season       |                          |
| 1st instar               | *                         | *                        | *                         | *                        |
| 2nd instar               | 5.00 ± 0.32               | 5.60 ± 0.24              | 11.00 ± 0.32              | 7.20 ± 0.74 <sup>c</sup> |
| 3rd instar               | 6.60 ± 0.24               | 6.60 ± 0.24              | 13.60 ± 0.24              | 8.93 ± 0.89 <sup>b</sup> |
| Adult                    | 6.20 ± 0.20               | 7.40 ± 0.24              | 15.40 ± 0.24              | 9.67 ± 1.10 <sup>a</sup> |
| Mean                     | 5.93 ± 0.23 <sup>c</sup>  | 6.53 ± 0.24 <sup>b</sup> | 13.33 ± 0.50 <sup>a</sup> | –                        |
|                          | Season (S)                | Host stage (H)           | S x H                     |                          |
| $\chi^2$                 | 42.20                     | 32.90                    | 63.69                     |                          |
| <i>p</i> value           | <0.0001                   | <0.0001                  | <0.0001                   |                          |
| <i>df</i>                | 2                         | 2                        | 4                         |                          |

\* No successful parasitism occurred on 1st instar nymphs; Data are presented as the mean ± standard error (SE) computed on five replications. Mean values followed by the different letters are significantly different ( $P < 0.05$ ) from each other. Pre-monsoon season - May-June; Monsoon season - July-August; Post-monsoon season - September-October

(Wald  $\chi^2 = 32.90$ ;  $df = 2$ ;  $p < 0.0001$ ). The interaction between seasons and host stages was also significant for the oviposition period of female adults (Table 3; Wald  $\chi^2 = 63.69$ ;  $df = 4$ ;  $p < 0.0001$ ).

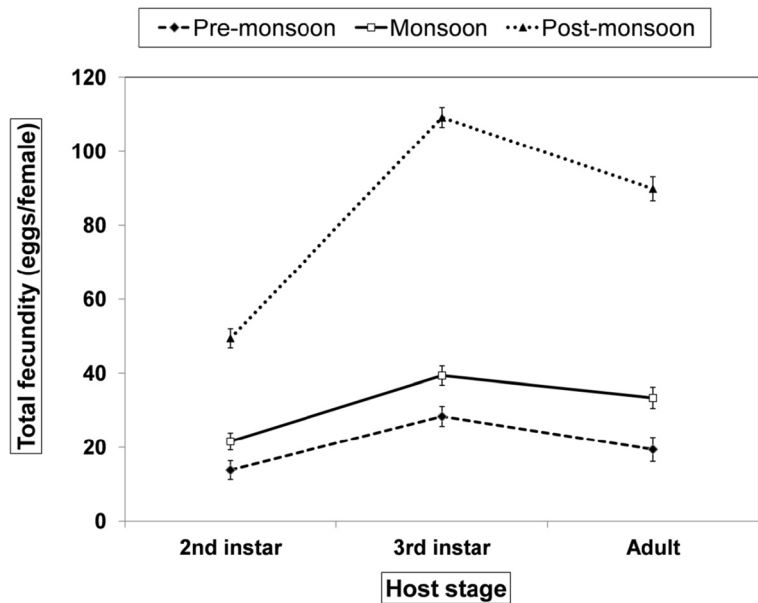
### Total fecundity

Total fecundity of *A. arizonensis* females showed significant differences among different seasons (Wald  $\chi^2 = 63.15$ ;  $df = 2$ ;  $p < 0.0001$ ), host stages (Wald  $\chi^2 = 46.27$ ;  $df = 2$ ;  $p < 0.0001$ ) and their interaction (Fig. 3; Wald  $\chi^2 = 72.27$ ;  $df = 4$ ;  $p < 0.0001$ ). It was significantly higher (82.80 eggs/female) in the post-monsoon season, followed by monsoon (31.47 eggs/female) and pre-monsoon seasons (20.53 eggs/female). Among the host stages, the fecundity was significantly higher (59 eggs/female) when females were offered 3rd instar *P. solenopsis* nymphs, followed by adult host stage (47.53 eggs/female); however, it was lowest in 2nd instar nymphs (28.27 eggs/female) (Fig. 3).

### Discussion

The amount of nutritional resources available during larval development is a key determinant for progeny fitness in parasitoids (Jervis et al. 2008; López et al. 2009). Host size varies due to nutritional resources which are considered to be an index of host quality (Sequeira and Mackauer 1992; Godfray 1994). Furthermore, parasitoids may differ in their strategy for utilizing/exploiting the host body resources in accordance with the host size or host stage. Our studies showed that *A. arizonensis* parasitized all the stages of its host *P. solenopsis* except 1st instar nymphs. No parasitism in 1st instar nymphs may be due to inadequate resources available for the parasitoid at this stage for their survival and progeny development (Hu et al. 2003; Colinet et al. 2005; Chong and Oetting 2006). Our results are consistent with the findings of earlier workers (Fand et al. 2011; Abdin et al. 2012; Vijaya and Ram 2013; He et al. 2015; Iqbal et al. 2016; Zhang et al. 2016). Less preference for younger host stages have also been reported in other parasitoid species like *Aenasius vexans* Kerrich on *Leptomastix dactylopii* Howard and *Planococcus citri* (Risso) (Jong De and van Alphen 1989), *A. vexans* on *Phenacoccus herreni* Cox and Williams (Bertschy et al. 2000) and *Anagyrus loecki*

**Fig. 3** Total fecundity of *A. arizonensis* females on different stages of *P. solenopsis* during different crop seasons



Noyes and Menezes on *Phenacoccus madeirensis* Green (Chong and Oetting 2006).

Our findings showed that development period of *A. arizonensis* from oviposition to adult emergence was longer in younger stages and decreased with increase in host age for both sexes. This can be attributed to inadequate resources available at younger stages of the host for the immature stages of the parasitoid (Hu et al. 2003; Colinet et al. 2005; Chong and Oetting 2006) which might have delayed the duration. The present results corroborate with the findings of Fand et al. (2011), Abdin et al. (2013) and Vijaya and Ram (2013) who also found that total developmental period of *A. bambawalei* (= *A. arizonensis*) was relatively shorter in adult host stage as compared to 3rd and 2nd instar nymphal stages. Godfray (1994) also concluded that higher fitness costs were involved in the parasitism of small hosts by koinobiont parasitoids, as the developmental time is often lengthened and survival is often reduced. Decreased developmental time in larger hosts has also been reported to be a common feature in the development of different parasitoid species (Karamaouna and Copland 2000; Neveu et al. 2000).

We report here that the progeny of *A. arizonensis* that emerged from older host stages of *P. solenopsis* was female-biased which corroborate with the studies conducted by Fand et al. (2011) and Abdin et al. (2013). Similar findings have also been reported in other parasitoid species like *Leptomastix epona* (Walker) on *Pseudococcus viburni* (Signoret) (Karamaouna and

Copland 2000), *Aphidius ervi* (Haliday) on pea aphids (Sequeira and Mackauer 1993) and *A. vexans* on *P. herreni* (Bertschy et al. 2000). Fand et al. (2011) and Abdin et al. (2013) reported that the progeny of *A. bambawalei* (= *A. arizonensis*) from 2nd instar nymphs produced primarily males, however in the present studies, progeny from 2nd instar *P. solenopsis* nymphs yielded only males as no females emerged from 2nd instar host stage.

Our results on sex allocation suggest an innate ability of *A. arizonensis* to recognise second instar hosts as less suitable for females. Males can become more abundant due to differential mortality, even when females invest equally in both sexes during oviposition (Hardy 1992; King 1993; Hardy et al. 1998; Ode and Hardy 2008). Haplodiploidy in parasitoids allows females to manipulate investment in each sex through the decision of whether or not to fertilize an egg; hence females can adjust the sex ratio of their offspring at the time of oviposition. More female progeny in older stages can be explained as per host size-dependent sex allocation model given by Charnov et al. (1981), which suggested that a higher proportion of females may be produced from larger size hosts due to greater nutritional requirement and reproductive benefits for the female progeny. Females of the hymenopteran parasitoids are known to control the sex of their offspring by a haplodiploid sex determination mechanism and they preferentially lay female (diploid) eggs in larger hosts and male (haploid) eggs in smaller hosts (King 1993).

The size of female wasps is an important measure of parasitoid fitness and is known to influence other fitness parameters like mating capacity, longevity, fecundity and reproductive rate (Jervis and Copland 1996; Harvey 2005). Large sized insects tend to live longer than small ones (Godfray 1994). This positive relationship between adult body size and their longevity has been reported for several Hymenopteran parasitoid species (Liu et al. 2001; Babendreier 2007; Gulec et al. 2007). For both sexes, adults of *A. arizonensis* that emerged from larger hosts lived longer as compared to those which came out from younger host stages. This may be attributed to inadequate/reduced body resources available at younger stages of the host to the parasitoid progeny (Colinet et al. 2005; Chong and Oetting 2006). Godfray (1994) also showed that higher fitness costs were involved in the parasitizing of small hosts by koinobiont parasitoids, as the survival is often reduced. However, Fand et al. (2011) have reported that adult longevity was significantly higher in 3rd instar *P. solenopsis* nymphs followed by adult and 2nd instar host stages. The development time of immature stages was longer for females than males. Likewise, female adults live longer as compared to male adults.

Most of the earlier studies on parasitism and sex allocation were conducted under constant (Fand et al. 2011; Abdin et al. 2013) and different (Zhang et al. 2016) temperature conditions under laboratory conditions. In this study, we tested the effect of different crop seasons with varying temperature and humidity conditions on the *A. arizonensis* under screen house conditions. Our results demonstrate that post-monsoon season (September–October months) with mean temperatures varying from 25 to 29 °C along with 68 to 73% relative humidity was the best season for the parasitoid in terms of the shorter development period, higher adult emergence, and more fecundity. Moreover, the parasitism in this season also yielded more females in the progeny. The prevailing weather conditions particularly temperature and humidity during September–October months might have contributed to higher fitness of the parasitoid. The mean temperature ranged between 25.2 to 28.7 °C in the post-monsoon (September–October), 29.9 to 30.3 °C in the monsoon (July–August) and 31.7 to 31.8 °C in the pre-monsoon (May–June) season. The mean relative humidity during the post-monsoon season was 68.0 to 72.9%, while it was 74.5 to 76.5% in the monsoon season and 31.5 to 49.5% in the pre-monsoon season. The parasitism rate and biological attributes of

*A. arizonensis* were severely affected during the pre-monsoon season under heat stress. Our results are consistent with the findings of He et al. (2018) who reported that emergence rate of *A. arizonensis* adults increased significantly with an increase in temperature from 19 to 31 °C, but dropped when the temperature exceeded 31 °C. The emergence of the parasitoid adults could be affected by high temperatures due to increasing rates of evaporation from host egg leading to desiccation and killing the parasitoid progeny inside (Mohammad et al. 2015). However, Zhang et al. (2016) studied the parasitism of *A. bambawalei* (= *A. arizonensis*) on *P. solenopsis* at different temperature conditions ranging from 21 °C to 39 °C under laboratory conditions and found the more beneficial effect in the temperature range of 30–36 °C.

The third instar *P. solenopsis* nymphs appear to be the most appropriate hosts for a mass-rearing program because the parasitoid gains greater fitness return and yields more female progeny which is highly desirable for increasing the efficiency of any biological control program. Since the parasitoid had the higher parasitism, emergence rate and female ratio in the post-monsoon season (September–October), we conclude that the temperature varying from 25 to 29 °C and relative humidity from 68 to 73% are optimum for the mass rearing of *A. arizonensis*. The findings of the study will help in mass rearing of the parasitoid vis-à-vis biological management of *P. solenopsis*.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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