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Determining some biological parameters of *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae) on cotton mealybug and the rate of parasitism in field conditions

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Abstract: *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae) is the primary parasitoid of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Some biological parameters of this parasitoid have not been fully understood. Therefore, this study was conducted to determine biological parameters of *A.arizonensis* at 25 °C, 65% R.H, 16 : 8 L : D with age-stage two-sex life table and effectiveness of this parasitoid in field conditions in Turkey. The results showed that pre-oviposition, oviposition and post-oviposition periods of *A. arizonensis* were calculated as 1.5, 26, and 3.1 days, respectively. In addition, total mean longevity was determined as 31 days for female and 18 days for male individuals. Moreover, total mean fecundity and daily mean fecundity were also found as 83 mealybugs/female, and 4.1 mealybugs/female/day, respectively. The intrinsic rate of increase (r), net reproduction rate (R_0), finite rate of increase (λ), gross reproductive rate (GRR), and mean generation time (T) were found 0.135, 41.25, 1.144, 60.90, and 27.57, respectively. Field studies have been conducted to determine the efficacy of this parasitoid in 2018 in Adana and Mersin / Turkey. The parasitism rate was risen to 78%–80% in September, both in Adana and Mersin provinces, attributed to early releases in August, 2018. In conclusion, higher parasitism rates showed that this parasitoid can be successfully used against cotton mealybug as an effective biological control agent.

Key words: *Aenasius arizonensis*, *Phenacoccus solenopsis*, biological control, biological parameters, field study, parasitism rate, two-sex life table

1. Introduction

Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) is known as one of the most important invasive species around the world. Initially, this species was reported from North America but has quickly spread to all parts of the globe (Fand and Suroshe, 2015). Host plant range of *P. solenopsis* includes 204 species from 64 families (Garcia Morales et al., 2016). This species was also reported in Turkey in 2012 (Kaydan et al., 2013). In addition, Çalışkan and Ulusoy (2018) reported 72 host plants from 55 families for *P. solenopsis* during 2012–2017. Due to insufficient control strategies, *P.solenopsis*, cotton mealybug, cause economically important damages (30%–80%) on cotton in India (Nalwar et al., 2009). Keeping its damage potential and wide host range in mind, other researchers have determined biology of this mealybug at different temperatures and on different host plants (Fand et al., 2011; Wang et al., 2012; Kumar et al., 2013; Çalışkan-Keçe, 2019).

Generally, mealybugs have many natural enemies, i.e. parasitoids and predators that are commonly used against these species (Franco et al., 2009; Ram and Saini, 2010). There are many parasitoids and predators recorded for *P. solenopsis*, especially parasitoids can be used as an effective biological control agent (Ram et al., 2009). *Aenasius arizonensis* (Girault) is one of the most important and effective parasitoids of *P. solenopsis*. Hayat (2009) firstly described this species as *Aenasius bambawalei* Hayat and *A. bambawalei* was determined a junior synonym of *A. arizonensis* by Fallazadeh et al (2014). This solitary nymphal-adult endoparasitoid (Rathee et al., 2015) was also first reported in Turkey in 2018 (Çalışkan-Keçe et al., 2018).

Some of the biological characteristics and parasitism potential of *A. arizonensis* have been studied by foreign researchers (Vijaya, 2011; Rathee and Ram, 2014; Rathee et al., 2015; Vijaya and Singh, 2018). Vijaya and Ram (2013), Badshah et al (2016) and Kahya et al (2019) studied

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host-stage preference of *A.arizonensis* and this parasitoid parasitize only third nymphal stage and adult female stage of *P. solenopsis*. In addition, Aga et al. (2016) found that third nymphal stage and adult female stages of *P. solenopsis* were preferred by *A. arizonensis*. Moreover, the longevity of female (26.2 ± 1.72 days) and male (16.3 ± 1.41 days) *A. arizonensis* individuals was determined by Aga et al. (2016). Moreover, Vijaya and Ram (2016) have studied the biology of *A. arizonensis* at 20 °C, 25 °C, 30 °C, and 35 °C. Previously, some other researchers have also studied the biological characteristics of *A. bambawalei* (Poorani et al., 2009; Ram and Saini, 2011; Solangi and Mahmood, 2011; Arif et al., 2012), but studies on life-table analysis are scanty. In one of the few studies, Joodaki et al. (2020) computed the life table parameters of *A. bambalawei* at different temperatures (25, 30, and 35 °C) with age-stage two-sex life table analysis.

Field studies about *A. arizonensis* have been done to determine the effectiveness of this parasitoid against *P.solenopsis*. Ram et al. (2009) studied field parasitism of *A. arizonensis* on cotton between 2008–2009. According to their study, July and August can be more suitable for mass-release of *A. arizonensis*. The natural parasitism of *P. solenopsis* by *A. bambawlaei* ranging from 46%–64% has been recorded on cotton crop (Kumar et al., 2009). Moreover, effects of different host plants on parasitism rate of *A.arizonensis* in field and greenhouse conditions have been studied by Vijaya and Singh (2018) and Shete & Ram (2018), respectively. As per Li et al. (2020), when *P. solenopsis* was fed with different host plants, parasitism rate of *A. bambawalei* varied significantly.

A. arizonensis is one of the most effective parasitoid of *P. solenopsis*. Studies on the biological and life table parameters of this parasitoid may help to rear, multiply and utilize it in biological control strategies against cotton mealybug. In addition, field studies on parasitization potential of *A.arizonensis* may be alternative control method against *P. solenopsis* instead of chemical control in parks and recreation areas. This study was aimed to determine the biology of *A. arizonensis* in laboratory conditions with two-sex life table analysis. In addition, the release studies of *A. arizonensis* have been done to detect parasitism rate in field conditions on *H.rosa-sinesis* L. in Adana and Mersin provinces in Turkey in 2018.

2. Materials and methods

Experiments have been conducted at Çukurova University, Agriculture Faculty, Plant Protection Department, Nedim Uygun Biological Control Laboratory and Biological Control Research Institute in Adana /Turkey in 2018. The biology of *A. arizonensis* has been carried out at 25 °C, 65 ± 10% relative humidity (R.H.) and 16 : 8 (L : D) in climate cabinets. Pre-oviposition, oviposition and postoviposition period, fecundity, longevity, and life table parameters were calculated within this study. The stock cultures of *A. arizonensis* and *P. solenopsis* for the experiments were achieved in climate rooms at Biological Control Research Institute.

Field studies have been done in Adana and Mersin in 2018. Two locations from Adana (Kıyıboyu [37.00989 ; 35.31865] and Balcalı [37.050255 ; 35.356334]) and two locations from Mersin (Tömük [36.66854 ; 34;40249] and Merkez [36.78399 ; 34.60068]) were determined for the release of *A. arizonensis*. Parasitism rate in each location was checked and recorded regularly. Parasitized and unparasitized mealybug mummies were counted, and parasitism rate was calculated and analysed during this study.

2.1. Biological parameters of *Aenasius arizonensis* under laboratory conditions

Firstly, one day hatched *A. arizonensis* individuals were obtained from the stock-culture. These individuals were placed into 9 mm Petri dishes with cotton leaves and adult *P. solenopsis*. One female and 2 male *A. arizonensis* individuals were released into each Petri dishes and ten *P. solenopsis* (adult female) were added every day for *A.arizonensis* (Figure 1 and Figure 2). These experiments continued until every individual of *A.arizonensis* died. In these experiments, total developmental period of immature (pre-adult) stage, pre-oviposition, oviposition and post-oviposition period, the number of daily and total parasitized mealybugs/female, male and female longevity were determined. These experiments were done with 20 replications at 25 °C, 65 ± 10% R.H. and 16 : 8 (L: D) in climate cabinets.

2.2. Life table parameters of *Aenasius arizonensis* under laboratory conditions

Life table parameters of *A. arizonensis* were also done with 20 replications at 25 °C, 65 ± 10% R.H. and 16 : 8 (L : D) in climate cabinets. Age-stage specific survival rate (S_{xj}), age-specific survivorship (l_x), age-specific fecundity (m_x) were calculated and depicted in Figures 3 and 4. Moreover, the intrinsic rate of increase (r), net reproduction rate (R_0), the number of female adults (N_f), finite rate of increase (λ), gross reproductive rate (GRR), and mean generation time (T) were calculated during this study. Biological and life table parameters of *A. arizonensis* were analysed with "TWO SEX-MS Chart" programme (Chi, 1988[1]; Chi, 2018). In addition, 100000 bootstrap was performed for the calculation of S.E. (standard error) of parameters.

[1] Chi H (2018). Website http://140.120.197.173/Ecology/prod02.htm [accessed 20 October 2018].

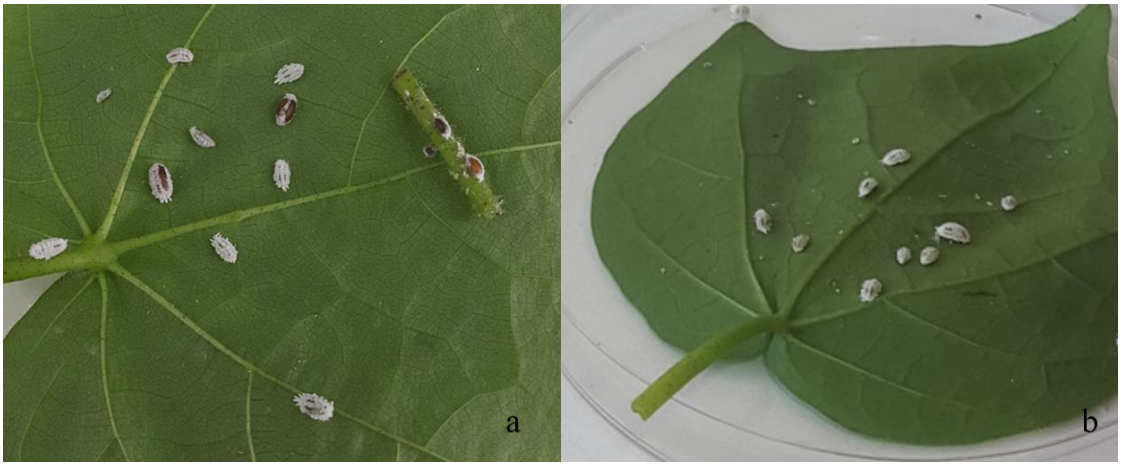

Figure 1. a) Parasitized *Phenacoccus solenopsis* individuals on cotton leaves (Adult female), b) *Phenacoccus solenopsis* individuals on cotton during laboratory studies (adult female).

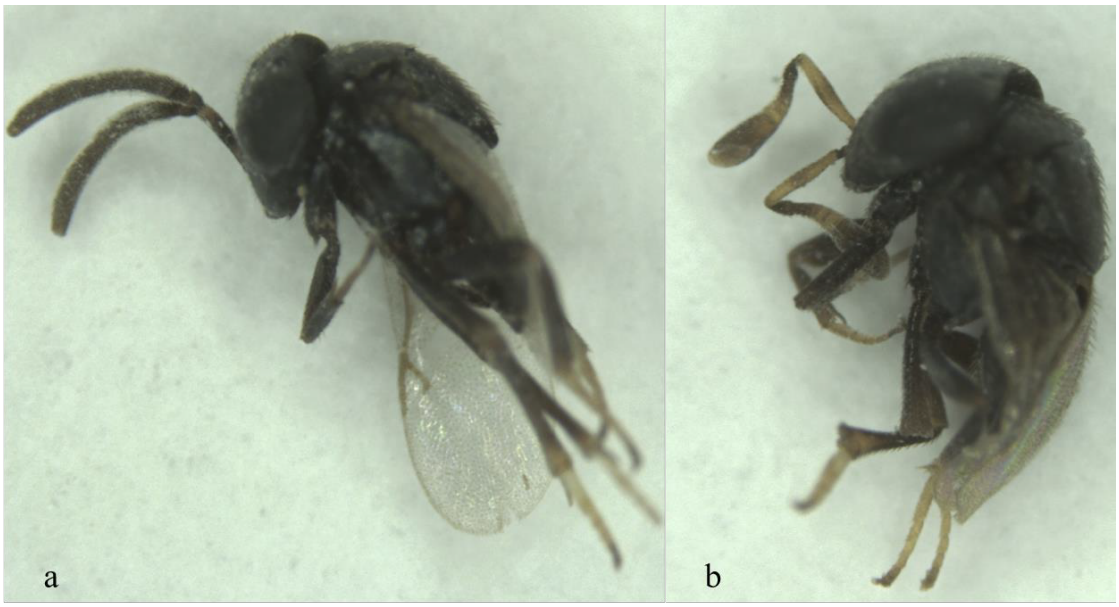

Figure 2. a) Male of *Aenasius arizonensis*, b) Female of *Aenasius arizonensis*.

These parameters were calculated with below mentioned formula: TWO SEX-MS Chart programme (Chi, 1988; Chi et al., 2020):

$$R_0 = \frac{N_f}{N}$$

$$T = \frac{lnR_0}{r}$$

$$\lambda = e^r$$

N_f: the number of female adults
R_0: net reproduction rate
T: mean generation time
r: the intrinsic rate of increase
λ: finite rate of increase

2.3. The Release study of *Aenasius arizonensis* on *Hibiscus rosa-sinensis* in field conditions in Adana and Mersin provinces

This part of study was conducted in field conditions. Firstly, 2 different parks were determined to initiate the releases. Totally, four *H. rosa-sinensis* trees (naturally infested with *P. solenopsis)* were selected for each location in Adana and Mersin provinces. Firstly, initial parasitization of mealybugs in trees was checked, and parasitism rate were recorded just before the release of parasitoids. A total of 100 *A. arizonensis* parasitoid adults (50 females, 50 male) were released for each *H. rosa-sinensis* tree on 07.08.2018 in Adana and Mersin. Parasitized and unparasitized mealybugs were counted weekly from each tree to

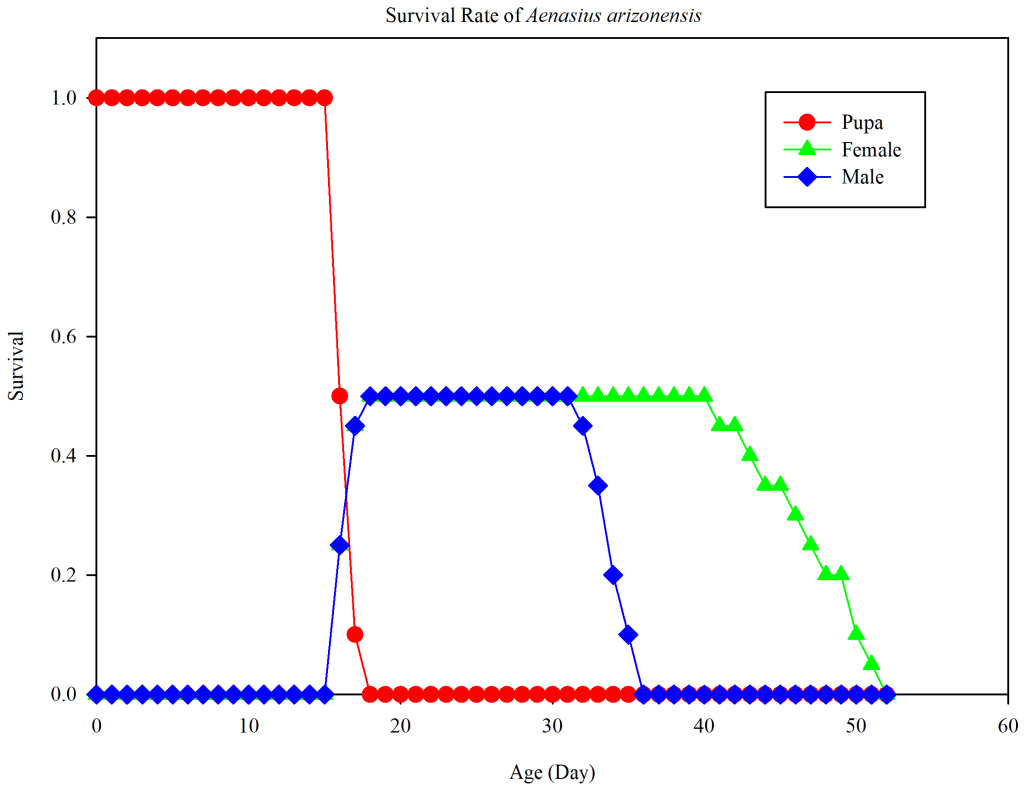

Figure 3. The longevity of immature and mature stages of *A.arizonensis*.Y axis represents age-stage specific survival rate (S_{xj}), X axis indicates the age of parasitoid.

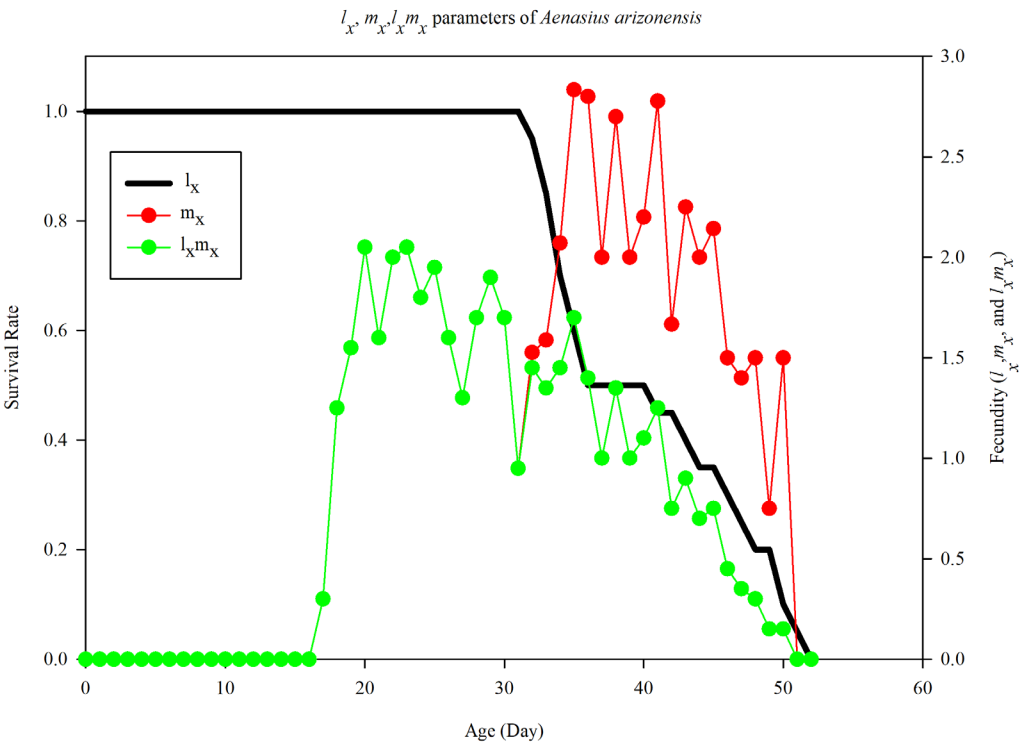

Figure 4. Age-specific survival rate (l_x), age-specific fecundity (m_x), and age-specific maternity (lxm_x) of *Aenasius arizonensis*.

calculate parasitism rate in Adana and Mersin provinces. One-way ANOVA and Duncan multiple comparison tests were applied to determine the statistical differences of parasitism rate.

3. Results and Discussion

3.1. Biological parameters of *Aenasius arizonensis* under laboratory conditions

The total development period of immature stages, pre-oviposition, oviposition and post-oviposition periods, female-male longevity, daily and total fecundity of *A. arizonensis* were determined during this study. Mean hatching period of this parasitoid was 17 ± 0.2 days (Min: 16; Max: 18 days). Adult female and male *A. arizonensis* longevity was detected as 31 ± 1.0 days (Min: 27; Max: 35 days), 18 ± 0.4 days (Min: 16; Max: 19 days), respectively (Table 1 and Figure 3).

Pre-oviposition, oviposition and post-oviposition periods of *A.arizonensis* were calculated as 1.5 ± 0.26 days, 26 ± 1.6, and 3.1 ± 0.3 days, respectively. In addition, the mean total and daily fecundity of *A. arizonensis* was determined as 83 ± 7.7 parasitized mealybug adults/female (Max:115), and 4.1 ± 0.1 parasitized mealybug adults/female/day within this study (Table 2).

Aga et al. (2016) have studied some biological parameters of *A. arizonensis* and found 26.2 ± 1.72 and 16.3 ± 1.41 days for female and male longevity. In addition, pre-oviposition, oviposition and post-oviposition periods of *A. arizonensis* were detected as <1, 23.1 ± 1.66, and 3.1 ± 0.55 days, respectively. Aga and co-workers further reported, total and daily fecundity of 100.5 ± 11.57 parasitized mealybug adults/female, and 4.24 ± 0.20 parasitized mealybug adults/female/day, respectively. Arif et al. (2012) determined biological characteristics of *A.bambawlaei* (junior synonym of *A. arizonensis*) at 28°C ± 1, 70 ± 5% (R.H.) and 18 / 6 (L / D), and, according to their study, hatching process of this parasitoid completed within 12–17 days, and longevity of the female parasitoids ranged from 15 to 32 days. Likewise, Ram and Saini (2011) found oviposition and post-oviposition period of *A. bambawalei* to be 24.33 and 3.33 days. Joodaki et al (2020) found 15.92 days for total pre-adult period and 33.35 days for oviposition at 25 °C, and latter decreased with increase temperatures from 25 to 35 °C.

3.2. Life table parameters of *Aenasius arizonensis* under laboratory conditions

According to results of life table parameters of *A. arizonensis*, the intrinsic rate of increase (r), net reproduction rate (R_0), finite rate of increase (λ), gross reproductive rate (GRR), and mean generation time (T) were found 0.135 day^{-1}, 41.25 offspring/female, 1.144 day^{-1}, 60.90 offspring /female, and 27.57 days, respectively (Table 3). Figure 4 showed that mortality started for male individuals 17 days after hatching period. The survival rate of female individuals decreased 31 days after hatching. In addition, age-specific fecundity (m_x) started to increase for a female 3 days after hatching and started to decrease on the 27th day.

Table 1. The longevity of immature and mature stages of *Aenasius arizonensis.*

	Developmental period of total pre-adult parasitoid (Day/Mean ± SE)	The longevity of female adult parasitoid ((Day/Mean ± SE)	The longevity of male adult parasitoid (Day/Mean ± SE)
Aenasius arizonensis	17 ± 0.2	31 ± 1.0	18 ± 0.4

Table 2. Preovipoisiton, oviposition, postoviposition period, total and daily fecundity of *Aenasius arizonensis.*

	Adult preoviposition period of female (APOP) (Day/Mean ± SE)	Oviposition period of female (Day/Mean ± SE)	Post-oviposition period of female (Day/Mean ± SE)	Total fecundity of female (Mean ± SE)	Daily fecundity of female (Mean ± SE)
Aenasius arizonensis	1.5 ± 0.26	26 ± 1.6	3.1 ± 0.3	83 ± 7.7	4.1 ± 0.1

Table 3. Life table parameters of *Aenasius arizonensis* (Mean ±SE).

	The intrinsic rate of increase (r) (day^{-1})	Net reproduction rate (R_0) (offspring/female)	Finite rate of increase (λ) (day^{-1})	Gross reproductive rate (GRR) (offspring/female)	Mean generation time (T) (day)
Aenasius arizonensis	0.135 ± 0.010	41.25 ± 9.89	1.144 ± 0.011	60.90 ± 8.017	27.57 ± 0.476

Vijaya and Ram (2016) studied the biology of *A. arizonensis* at different temperatures and recorded net reproductive rate (R_0), the intrinsic rate of increase (r_m), finite rate of increase (λ), and net generation time (T) was 33.93, 0.083, 1.086, and 42.27, respectively, at 25 °C. Joodaki et al (2020) found r, R_0, λ, and T values as 0.1192 day^{-1}, 38.04 offspring/female, 1.126 day^{-1}, and 30.52 days, respectively at 25 °C with age-stage two-sex life table during their study. Two-sex (male and female) life table theory included both sex populations, stage differentiation and variable developmental rates in the theory put forth by Chi (1988). As can be seen in present and previous studies about life table parameters, especially r (the intrinsic rate of increase) value, which affects fertility and mortality, was found higher in two-sex life table analysis. In addition, R_0 (net reproductive rate) was found higher in two-sex life table at 25 °C.

3.3. The Release of *Aenasius arizonensis* on *Hibiscus rosa-sinensis* in field conditions in Adana and Mersin provinces

The cotton mealybug, *P. solenopsis* cause severe damages on *H. rosa-sinensis* in parks and recreational areas in Adana and Mersin provinces. Owing to heavy infestation of *P. solenopsis*, parasitoid's population increased in the study area. The parasitism rate was determined in selected parks in Adana and Mersin in 2018 during this study. Firstly, parasitized and unparasitized mealybugs were counted before the release of *A. arizonensis*. The parasitized mealybug mummies were recorded and parasitism rate was calculated after the scheduled releases as well.

The results of this study showed that parasitism rate at first (Kıyıboyu) and second (Balcalı) location started with 2% and 8%, and increased to 80% and 78% for two locations, respectively in Adana (Figure 5 and Figure 6). In addition, the parasitism rate for two different locations (Tomuk and Merkez) in Mersin increased from 7% and 5% to 82% and 88%, respectively (Figure 7 and 8). Generally, Figure 5, 6, 7, and 8 showed that higher temperatures increased the effectiveness and parasitism rate of this parasitoid. Early releases of *A. arizonensis* (August, 2018) showed higher parasitism rate (above 75%), both in Adana and Mersin provinces. Differences between weekly parasitism rate were found statistically significant in Adana and Mersin in this study (Figures 5, 6, 7, and 8) ($p < 0.05$).

There is no specific study about parasitism situation of *A. arizonensis* on *H. rosa-sinensis* in field conditions. However, *A. bambawalei*, the junior synonym of *A. arizonensis*, has been studied by some researchers in field conditions. Ram et al. (2009) studied the parasitism situation of *A. bambawalei* on cotton between 2008 and 2009 and found that parasitism rate of this parasitoid started to increase in August and continue until September (between 54.5%–76.6%) during the cotton growing season. Kumar et al. (2009) have studied natural parasitization of *A. bambawalei* against *P. solenopsis*, according to results of their study, parasitism rate of *A. bambawalei* changed between 46%–64% on cotton. Nahiyoon et al. (2016) also studied parasitism situation of *A. bambawalei* against *P. solenopsis* on 5 different plants between 2010 and 2011, and parasitism rate was between 79%–82% on cotton,

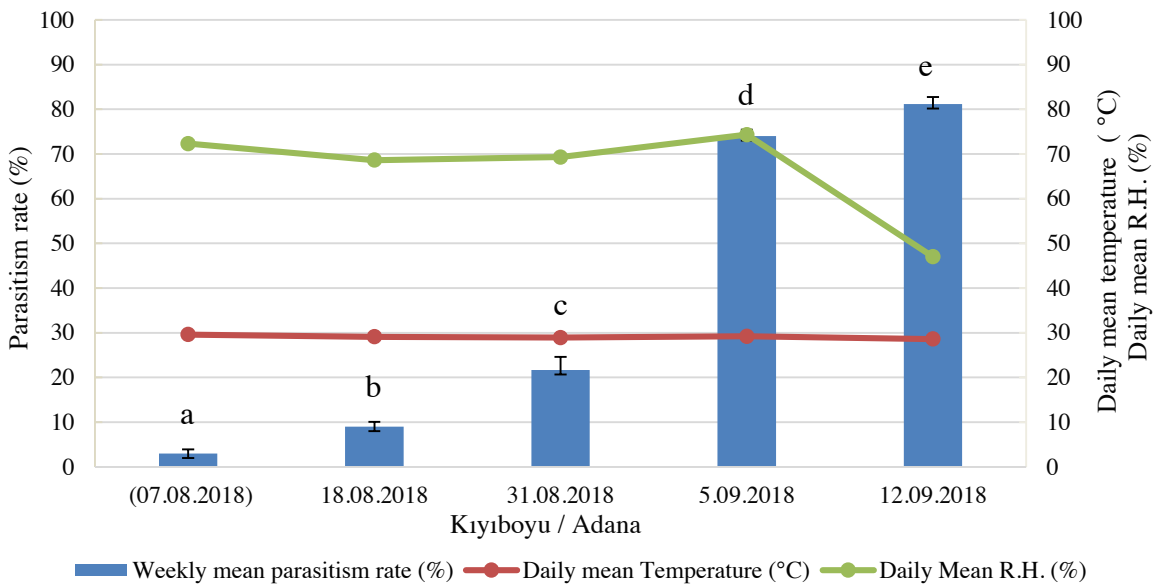

Figure 5. Parasitism rate of *Aenasius arizonensis* with Daily mean temperature and mean R.H.in 2018 for Kıyıboyu / Adana.

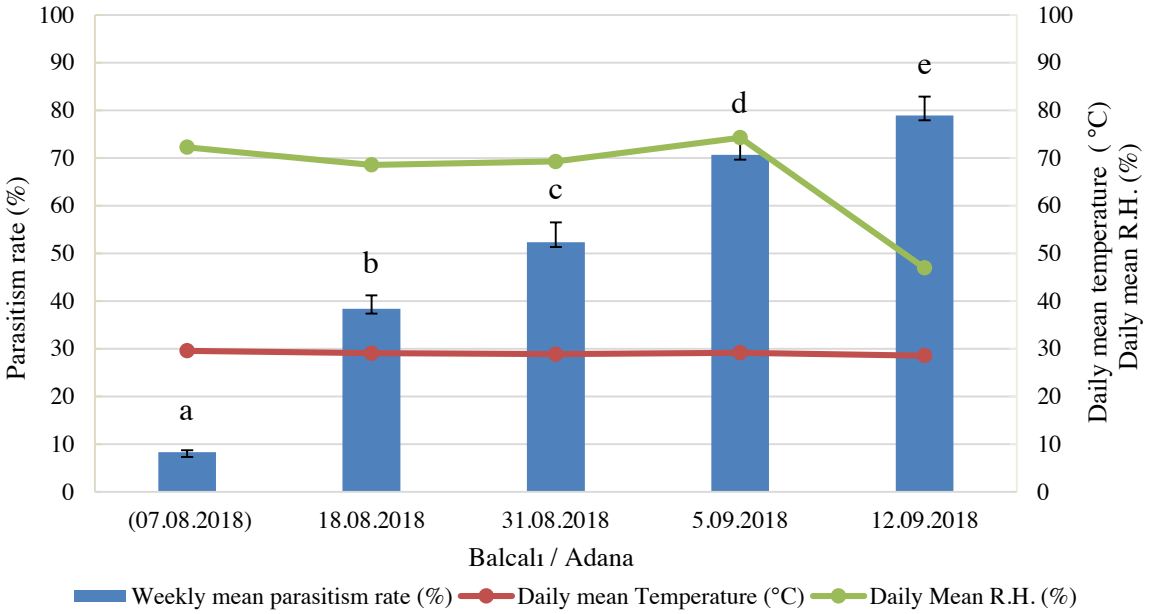

Figure 6. Parasitism rate of *Aenasius arizonensis* with Daily mean temperature and mean R.H.in 2018 for Balcalı /Adana.

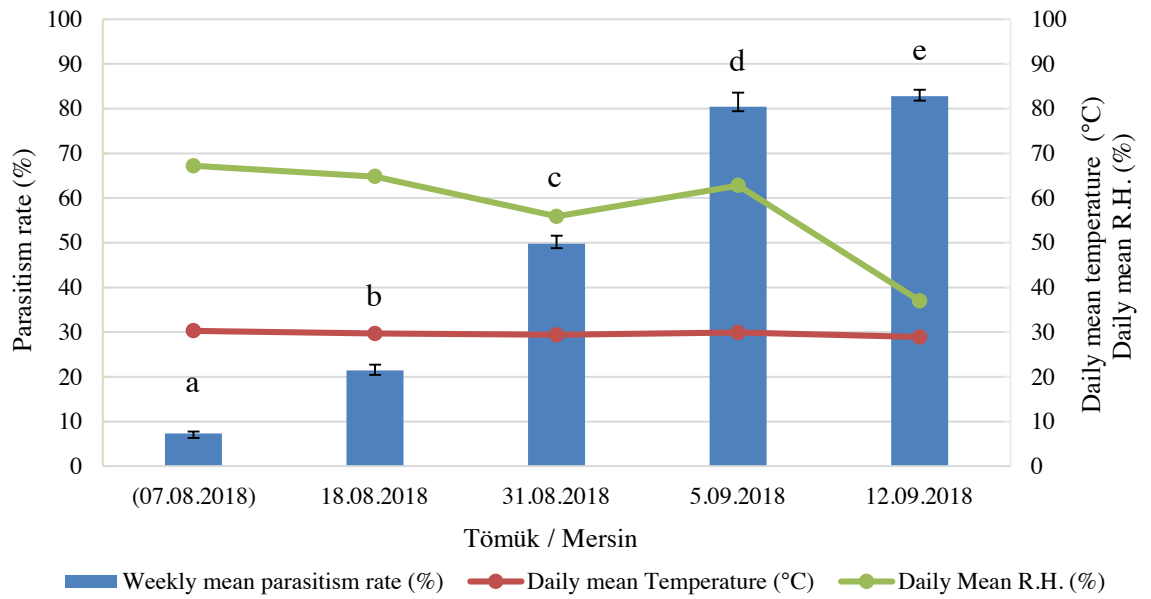

Figure 7. Parasitism rate of *Aenasius arizonensis* with Daily mean temperature and mean R.H.in 2018 for Tömük / Mersin.

76% on tomato, and 74%–81% on China rose. Moreover, Solangi and Mahmood (2011) studied the parasitism rate of *A. bambawalei* in pesticide-free farms and found 79%–93% parasitism against *P. solenopsis* on cotton. Shete and Ram (2018) studied different host plants to determine parasitism rate of *A.arizonensis* and found 60% parasitism rate on *H.rosa-sinenis* in screen-house. Highest parasitism rate was observed as 65% on *Abutilon indicum* (Link) Sweet during their study. Li et al. (2020) used different host plant as food for *P.solenopsis* and released *A. bamabawalei* to determine effects of host plant on parasitism rate. The higher parasitism rate (55.6%) was obtained when *P. solenopsis* fed with *H.rosa-sinensis*. These studies showed that *H. rosa-sinensis* is one of the most important host plants of *P. solenopsis* and higher parasitism rate conceivably be obtained for *A.arizonensis* in suitable conditions.

4. Conclusion

This study was conducted to determine some biological parameters and life table parameters of *A. arizonensis*. In

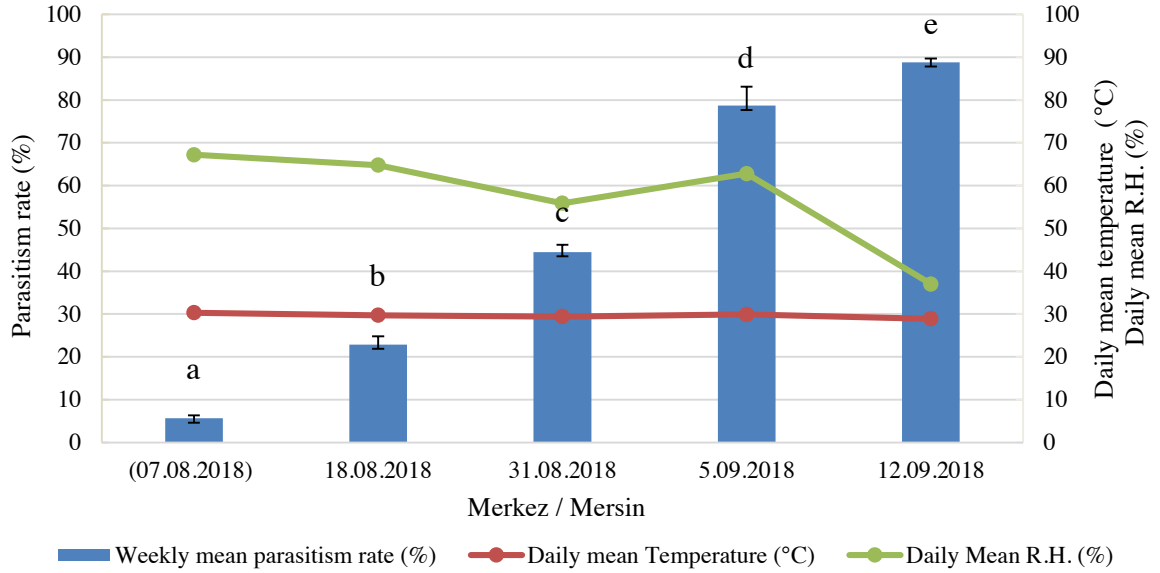

Figure 8. Parasitism rate of *Aenasius arizonensis* with Daily mean temperature and mean R.H.in 2018 for Merkez / Mersin.

addition, the parasitism rate of this parasitoid was detected in field conditions in Adana and Mersin provinces in the Mediterranean region of Turkey. According to the results of laboratory and field studies, *A. arizonensis* can be used as an effective biological control agent against *P. solenopsis*. Especially, damage caused by *P.solenopsis* can be reduced by using this parasitoid in summer and early autumn seasons for Turkey. The mass-release of *A. arizonensis* on *H.rosasinensis* may prevent the spread of *P. solenopsis* in city parks and recreation areas without using any insecticides.

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