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Original article

The determination of developmental time, parasitism rate at different temperatures and parasitism behavior at constant temperature of *Aenasius arizonensis* Girault 1915 (Hymenoptera: Encyrtidae)

Aenasius arizonensis Girault 1915 (Hymenoptera: Encyrtidae)'in farklı sıcaklıklarda gelişme süresi, parazitleme oranı ve sabit sıcaklıkta parazitleme davranışının belirlenmesi

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ABSTRACT

Aenasius arizonensis Girault, 1915 (Hymenoptera: Encyrtidae) is a primary parasitoid species of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Some biological characteristics of *A. arizonensis* at different temperatures were studied to evaluate its potential role against *P. solenopsis* in biological control. Six temperatures (15, 20, 25, 30, 35±2, and 25/35±2 °C) were studied to determine the most suitable temperature for parasitism. The results showed that the highest parasitism rate (79%) and the optimum developmental time from parasitism to adult emergence (16-17 days) were recorded at 25 °C. Although developmental periods were shorter at 30 and 35 °C, parasitism rate was lower at higher temperatures. The longest developmental period and lowest parasitism rate were recorded at 20 °C.The host preference of *A. arizonensis* was studied on different mealybug species and this parasitoid parasitized only *P. solenopsis*. The parasitism behavior of *A. arizonensis* based on the increasing the number of the host was carried out at 25 °C, where the best results were obtained in terms of parasitism rate during this study. The regression curve showed that *A.arizonensis* can be classified as Holling Type II. In conclusion, this study revealed that *A. arizonensis* can be an effective biological control agent to use against *P. solenopsis* and 25 °C is an optimum temperature for the mass-rearing of this parasitoid.

INTRODUCTION

Mealybugs are known as one of the most important pests in terms of agriculture. They can damage all parts of plants causing economic losses to the crops (Nagrare et al. 2009). Due to climate changes, invasive mealybug species became more important pests throughout the world (Mani and Shivaraju 2016). Generally, the *Phenacoccus* genus has been recorded in the Mediterranean region for 10-15 years (Kaydan et al. 2013, Mendel et al. 2016). This genus includes

approximately 180 species. Among them, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is one of the most dangerous invasive mealybug species, recorded in Turkey in 2012 (Kaydan et al. 2013). *P. solenopsis* has a wide-range of host plant spectrum (202 host plants from 55 families) (Garcia et al. 2016). The species has been reported on 72 host plants from 55 families in Turkey (Çalışkan and Ulusoy 2018).

Biological control of Cotton mealybug can be potential prevent the spread of this pest. There are many studies have been conducted to determine parasitoids and predators of *P. solenopsis* (Ben-Dov et al. 2012, Çalışkan et al. 2016, Çalışkan-Keçe et al. 2018, Hayat 2009). *Aenasius arizonensis* Girault (Hymenoptera: Encyrtidae) is one of the most effective solitary endoparasitoids and reported in Turkey in 2018 (Çalışkan-Keçe et al. 2018, Hayat 2009). This parasitoid is specialized in *P. solenopsis* and does not parasitize other mealybug species (Shera et al. 2017).

Aenasius arizonensis represented 95% of the parasitoids under field conditions (Solangi and Mahmood 2011). The efficacy of *A. arizonensis* has been studied in both laboratory and field conditions. Ram and Saini (2009) reported that field parasitism with this parasitoid species ranged between 37.6-72.3%. Aga et al. (2016) and Kahya et al. (2019) found that third nymphal instar and adult stage of *P. solenopsis* were preferred by *A. arizonensis*. Moreover, many studies were conducted about *A. arizonensis* and its parasitism behavior against *P. solenopsis* (Bodlah et al. 2010, Prasad et al. 2011, Spargo et al. 2013). The effect of different temperatures on *A. arizonensis* parasitism has been studied and minimum-maximum thresholds were determined as 11.5 and 11.2 °C by He et al. (2017). Joodaki et al. (2018) studied the functional response of *A. arizonensis* at different temperatures and the maximum parasitism rate was found at 25, 30, and 35 °C during their study. Moreover, effects of different temperatures on *A. bambawalei* Hayat (=*Aenasius arizonensis* Girault) (Hymenoptera: Encyrtidae) were studied by Zhang et al. (2016) and found that higher temperatures have negative effects on the reproductive capacity of *A. bambawalei*.

This study carried out to determine developmental period (from parasitism to adult emergence) and parasitism rate at 15, 20, 25, 30, 35, and 25/35 °C. In addition, parasitism behavior of *A. arizonensis* based on different hosts species and the number of the increasing host were studied within this study. The main objective is to detect the most suitable temperature and the host consumption capacity of *A. arizonensis* for mass-rearing under controlled conditions.

MATERIALS AND METHODS

The study was carried out at Nedim Uygun Biological Control Laboratory (Çukurova University Agriculture Faculty Plant Protection Department/Adana/Turkey) and Biological Control Research Institute in Adana/Turkey. Six different temperatures (15, 20, 25, 30, 35±2 °C and 25/35±2 °C) were tested in climate cabinets for finding out the most suitable temperature for parasitism. In addition, parasitism behavior of *A.arizonensis* depending on the number of the host was estimated at 25 °C, 65% ± 10 RH, and 16:8 (L:D). Moreover, parasitism behavior of *A. arizonensis* on the different host was found during this study. Stock culture of *A. arizonensis* and *P. solenopsis* were mass-reared at Biological Control Research Institute and individuals, which were used in these experiments, were obtained from the stock cultures.

Developmental time and parasitism rate of Aenasius arizonensis at different temperatures

Experiments were conducted using adults of *P. solenopsis* and *A.arizonensis*. Cotton leaves were placed in Petri dishes (9 mm) including 10 *P. solenopsis* adult females. Moreover, one female and 2 males of *A. arizonensis* were released into each Petri dish for 24 hours then removed. Experiments were done at 15, 20, 25, 30, 35±2 °C and 25/35±2 °C and 10 replicates were used for each temperature. Experiments were examined daily. Mean total developmental period (from parasitism to adult emergence) and parasitism rate at each temperature were calculated and recorded.

Parasitism behavior of Aenasius arizonensis on different hosts

Parasitism behavior of *A. arizonensis* on different hosts was studied. *Planococcus citri* Risso (Hemiptera: Pseudoccocidae) and *Phenacoccus madeirensis* Green (Hemiptera: Pseudoccocidae) were used as a host during this experiment. These mealybug species were cultured in climate rooms in Nedim Uygun Biological Control Laboratory (Cukurova University Agriculture Faculty Plant Protection Department/Adana/Turkey) and Biological Control Research Institute in Adana/Turkey. Totally, 100 mixed individuals of *P. citri* and *P. madeirensis* were placed into different plastic boxes with sprout potatoes. After that, 10 females and 20 males of *A. arizonensis* were released into these boxes for 24 hours then removed. Experiments were conducted at 25 °C, 65% ±10 RH and 16:8 (L:D) in climate cabinets and controlled daily.

Parasitism behavior of Aenasius arizonensis with the increasing number of host (Phenacoccus solenopsis)

According to results of parasitism rate results at different temperatures, 25 °C was found the most suitable temperature for parasitism and these experiments were conducted at

25 °C. *P. solenopsis* individuals adapted to cotton leaves were placed into Petri dishes where 1 female, 2 males of *A. arizonensis* were released into the Petri dishes for 24 hours then removed. Each Petri dish included 5, 10, 20, 40 and 80 individuals (the number of hosts were determined according to the geometric increase) of *P. solenopsis*. This experiment was carried out through 10 replicates at 25 °C, 65%±10 RH and 16:8 (L:D). Results were controlled and recorded daily.

Statistical analysis

Analysis were done with SPSS 23 statistic program. One-way ANOVA and Duncan multiple comparison tests ($p<0.05$) were used for the determination statistical differences between developmental time and parasitism rate of *A. arizonensis* at different temperatures. The logistic regression test was applied to detect the relationship between parasitoid and increasing the number of host. R, R2, and p values were calculated by SPSS 23 statistic program. According to Holling (1959), the type of the parasitoid was determined through the above tests.

RESULTS AND DISCUSSION

Developmental time of Aenasius arizonensis at different temperatures

The longest mean total developmental period of *Aenasius arizonensis* from parasitism to adults emergence was obtained at 20 °C (43.9±0.8 days) for female, (42.8±1.4 days) for male, while the shortest one was found at 30 °C (13.6±0.4 days) for female and (14.4±0.8 days) for male. Optimum values were found at 25 °C for both developmental periods (16.7±0.3 and 17.0±0.5 days) for females and males, respectively (Table 1). The developmental periods of *A. arizonensis* at different temperatures were estimated as a whole (from parasitism to adults emergence), as the immature stages of such endo-parasitoid are completely internal of the host (*P. solenopsis*). This parasitoid lays their eggs into the mealybugs and eggs, larval and pupal stages are developed inside host (*P. solenopsis*). According to visual observation under the stereomicroscope, mealybugs were paralyzed after parasitism in 24 hours. It was estimated that egg hatching occurred within 72 hours inside a host, and also 5-7 days later, the colour of parasitized mealybug individuals started to change (mostly brownish black colour) and powdery was vanished (Figure 1). Consequently, *P. solenopsis* are paralyzed after parasitism and parasitized mealybug individuals are enlarged and turn into brownish black in 5- 7 days. Finally, *A. arizonensis* individuals became adults (from parasitism to adults emergence) within 15-17 days at 25 °C, 65% ± 10 RH and 16:8 (L:D). Although the shorter developmental periods from parasitism to adults' emergence were detected at 30 and 35 °C, the parasitism rate was reduced from 79% to 50% above 25 °C. The changeable temperature 25/35±2 °C was not suitable for *A. arizonensis* because the developmental period increased to 18.0±0.8 days (female), and 17.1±0.7 days (male). Significant differences were found between temperatures and the developmental periods from parasitism to emergence of males and females ($p<0.05$). Ram (2016) studied developmental period of *A. arizonensis* at different temperatures (20, 25, 30, 35±2 °C) and found that the adult emergence of *A. arizonensis* from parasitisation was completed in 15.8 days for male and 17.8 days for female at 25 °C. In addition, Vijaya (2011) studied some biological characteristics of *A. bambawalei* at different temperatures and stated that the parasitoid completed its development between 20 and 35 °C and maximum fecundity was obtained at 23-33 °C. Abdin et al. (2012) reported that adult emergence from pupa occurred after 12-17 days at 28 °C, 70% RH, 18:6 h (L:D). He et al. (2017) found that 19-37 °C was the suitable temperature for the development of *A. arizonensis*.

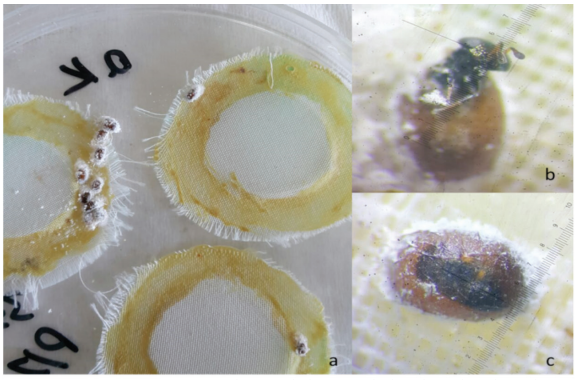

Figure 1. a) parasitized mealybug adults b) the hatching of Aenasius arizonensis, c) before hatching of Aenasius arizonensis

Table 1. Developmental period and parasitism rate of Aenasius arizonensis at different temperatures

Temperature (°C)	Parasitism rate (%mean±SE)	Developmental period of female (pupa to adult) (mean/day±SE)	Developmental period of male (pupa to adult) (mean/day±SE)
15	0	0	0
20	24.0±0.5*c	43.9±0.8c	42.8±1.4c
25	79.0±0.3a	16.7±0.3b	17.0±0.5b
30	61.0±0.5ab	13.6±0.4a	14.4±0.8a
35	54.0±0.7b	15.1±0.9a	14.3±0.9a
25/35	46.0±0.8b	18.0±0.8b	17.1±0.7b

*Each column showed that same letters are not statistically significant (Duncan test, $p<0.05$)

As shown in Table 1, the parasitism rate was determined at the 6 different temperatures. Maximum parasitism rate was obtained at 25 °C as it increased to 79.0%, while it ranged between 54.0-61.0% at 30 and 35 °C. Lower parasitism rate was also obtained at the changeable temperature (25/35±2 °C). The lowest parasitism rate was found as 24.0% at 20 °C. At 15 °C, no parasitization was determined. Significant differences were determined among temperatures in terms of parasitism rate for male and female ($p<0.05$). He et al. (2017) found that parasitism rate tendency increased from 19 to 31 °C. However, high temperatures (higher than 31 °C) had negative effects on parasitism rate of *A. arizonensis*. Moreover, Zhang et al. (2016) studied the parasitism rate of *A. bambawalei* at different temperatures, and demonstrated that when the temperature rose to 36 °C, the parasitism rate decreased to 52.0%. Thimmegowda (2017) recorded temperature's tolerance of *A. arizonensis* at 27, 32, 35 and 38 °C, and the optimum developmental duration from oviposition to pupal stage and fecundity values were obtained at 27 °C. Many researchers have also studied the effects of temperature on the parasitism rate. For example, Daane et al. (2004) found that 24.7 °C was the optimum temperature of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) for parasitism on *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae). As shown in the present study, parasitism rate affected easily by higher temperature.

Parasitism behavior of Aenasius arizonensis on different hosts

Parasitism behavior of *Aenasius arizonensis* on different host were determined during this study. According to results of this experiment, *A. arizonensis* did not lay eggs on *P. citri* and *P. madirensis*. Shera et al. (2017) used 6 different mealybug species for parasitism situation of this parasitoid and *P. solenoopsis* were found only host of *A. arizonesis*.

Parasitism behavior of Aenasius arizonensis with increasing the number of host (Phenacoccus solenopsis)

Parasitism behavior of *Aenasius arizonensis* was determined at 25 °C. The results showed that there was a strong correlation between *P. solenopsis* and *A. arizonensis* (R=0.83, R^2=0.97). Besides, the parasitism increased as the number of host until 40 individuals. This situation suggested that parasitism rate increased with host density directly proportional. However, the breaking point was 40 prey/individuals for *A. arizonensis* (Table 2 and Figure 2).

Table 2. Functional and numerical response of *Aenasius arizonensis*

Number of hosts Phenacoccus solenopsis (Mean)	Number of parasitized mealybug individuals (Mean±SE)
5	3.0±0.1
10	7.0±0.2
20	9.0±0.2
40	16.0±0.2
80	15.0±0.2

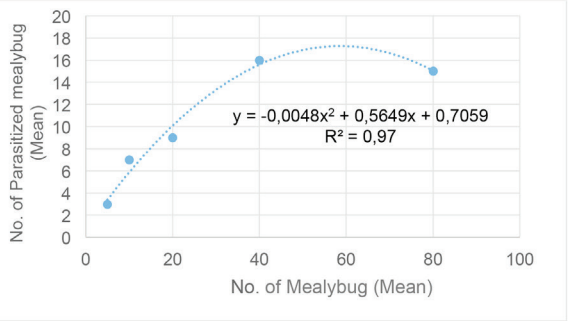

Figure 1. Quadratic regression curve of *Aenasius arizonensis*

Accordingly, *Aenasius arizonensis* can be classified as Type II model (Holling 1959).The R and R^2 values were found as 0.83 and 0.97, respectively (Figure 2). This solitary parasitoid parasitized an adult female of *P. solenopsis* with higher parasitism rates at 25 °C than 1st , 2nd and 3rd nymphal stages of this mealybug. Although Type II model seems less suitable than Type III model in terms of functional response, Type II functional response is generally fitted for parasitoid species (Fernandez-Archex and Corely 2003, Holling 1965). Joodaki et al. (2018) studied the numerical and functional response of *A. bambawalei* and classified it as Type II model Holling (1959). In addition, Chong and Oetting (2006) studied Madeira mealybug parasitoid, *Anagyrus* sp. nov.nr. *Sinope* and classified the parasitoid as type II functional response when opposed to the different numbers of *P. madeirensis*.

Phenacoccus solenopsis, Cotton mealybug, has been cause economically important damages on crops in Turkey since 2012. *Aenasius arizonensis* is one of the most effective parasitoids of this pest and has been detected in Turkey since 2018. This study aimed to find out some biological characteristic of this parasitoid in laboratory conditions and to enhance the mass-rearing opportunities of *A. arizonensis*. According to results of this study, *A. arizonensis* can be mass-produced at 25 °C successfully and can be used as an effective biological control agent properly against *P. solenopsis* in biological control studies.

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ÖZET

Aenasius arizonensis Girault, 1915 (Hymenoptera: Encyrtidae) *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)'in önemli parazitoitlerinden biri olarak bilinmektedir. Yapılan bu çalışmada *A. arizonensis*'in farklı sıcaklıklarda bazı biyolojik özellikleri belirlenerek *P. solenopsis*'e karşı biyolojik mücadelede kullanım potansiyeli değerlendirilmiştir. Bu parazitoit için en uygun parazitleme sıcaklığını belirlemek için 6 farklı sıcaklık (15, 20, 25, 30, 35±2 ve 25/35±2 °C) çalışılmıştır. Elde edilen bulgulara göre parazitleme ile ergin çıkışı arasında en iyi gelişme süresi (16-17 gün) ve en yüksek parazitleme oranı (79%) 25 °C'de elde edilmiştir. 30 ve 35 °C'de daha kısa gelişme süresi bulunmasına rağmen parazitleme oranının yüksek sıcaklıklarda düştüğü tespit edilmiştir. En uzun gelişme süresi ve en düşük parazitleme oranı 20 °C'de bulunmuştur. *A. arizonensis*'in parazitlemede farklı konukçuları tercih çalışmaları yapılmış ve sadece *P. solenopsis*'i parazitlediği belirlenmiştir. En iyi parazitlemenin elde edildiği 25 °C'de A. arizonensis'in artan konukçu yoğunluğuna bağlı parazitleme davranışı çalışılmıştır. Elde edilen regresyon eğrisine göre bu parazitoit Holling Tip II olarak sınıflandırılmıştır. Sonuç olarak, *A. arizonensis*, *P. solenopsis* ile mücadelede etkili bir parazitoit olarak kullanılabileceği ve bu parazitoitin kitle üretiminin 25 °C'de başarılı bir şekilde gerçekleştirilebileceği sonucuna ulaşılmıştır.

Anahtar kelimeler: *Aenasius arizonensis*, farklı sıcaklık, parazitleme davranışı, *Phenacoccus solenopsis*

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