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***Orijinal araştırma* (*Original article*)**

The determination of the biological stages of the host, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), preferred by the parasitoid, *Aenasius arizonensis* Girault (Hymenoptera: Encyrtidae) [1]

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Aenasius arizonensis Girault (Hymenoptera: Encyrtidae)'in *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)'i parazitlemede tercih ettiği biyolojik dönemlerin belirlenmesi

Öz: *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)'in etkili parazitoiti olan *Aenasius arizonensis* Girault (Hymenmoptera: Encyrtidae)'in konukçusunu parazitlemede hangi biyolojik dönemlerini tercih ettiğini belirlemek amacıyla Seçimsiz (No-choice) ve Seçilimli (Choice) olarak denemeler kurulmuştur. Sonuç olarak; *A. arizonensis*' in seçimsiz ve seçimli olarak yürütülen her iki denemede de *P. solenopsis*'in üçüncü nimf dönemini % 30'un üzerinde, ergin dişilerini %70'in üzerinde parazitlediği saptanmıştır. *A. arizonensis* 'in konukçusunun birinici ve ikinci nimf dönemlerini, hareketli ve daha küçük yapıda olmaları nedeniyle tercih etmediği tespit edilmiştir.

Anahtar kelimeler: *Aenasius arizonensis¸ Phenacoccus solenopsis*, Biyolojik Mücadele, Konukçu-dönem tercihi

Abstract: This study was conducted to determine the host stage preference of the parasitoid, *Aenasius arizonensis* Girault (Hymenoptera: Encyrtidae), on *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). No-choice and choice experiments were carried out. There was approximately 30% parasitism of the third nymphal stage and more than 70% parasitism of female adults. This parasitoid does not parasite the first and second nymphal stage of *P. solenopsis* because these stages are smaller and more mobile than the third nymphal stage and adult females.

Keywords: *Aenasius arizonensis¸ Phenacoccus solenopsis,* Biological Control, Host-stage preference

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Introduction

Mealybugs are one of the most important insect species in agriculture. Approximately 5000 mealybug species have been identified on 246 host plants across the world (Ben Dov, 1994). *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), the cotton mealybug, one of the most widespread mealybug species, causes damage to ornamentals, numerous crops and weeds (Hodgson et al, 2008; Arif et al, 2009). The first record of this mealybug is from the USA but it has been spreading to other regions since the middle of the 20th century (Wang et al, 2010; Pellizzari & Germain, 2010). This species was first reported in Turkey in 2012 (Kaydan et al, 2013) and since then has been seen on 202 host plants from 55 families (Çalışkan & Ulusoy, 2018). Natural enemies of this mealybug have also been reported in Turkey (Çalışkan et al, 2016).

This mealybug, which has been reported from more than 100 countries, has the potential to cause damage in tropical and subtropical regions (Wang et al, 2010). *Aenasius arizonensis* Girault (Hymenoptera: Encyrtidae), which was described in 2009, is a solitary endoparasitoid effective against *P. solenopsis* under natural conditions (Hayat, 2009). Ram et al (2010) reported parasitism rates for this parasitoid that varied from 37.6% to 72.3% in India. The presence of this parasitoid in Turkey was first reported by Çalışkan-Keçe et al (2018).

The biological control of the cotton mealybug plays an important role in preventing the spread of this species. Many researchers have studied the effectiveness of *A. arizonensis* (Kumar et al, 2009; Aga et al, 2016). Kumar et al (2009) reported natural parasitism in the range of 46% to 64% under field conditions. Kumar et al (2009) further reported that pesticides reduced the natural parasitism rate after inundative release from 70% to 57.52%. Aga et al (2016) studied the host preference of *A. arizonensis* and reported that adult female and 3rd nymphal instars were the most parasitized stages.

The mentioned studies show that biological control can be used as an effective control method against cotton mealybug. The present study aimed to determine the host stage preference of *A. arizonensis* under laboratory conditions. The main objective of this study was to prevent the spread of the cotton mealybug, *P. solenopsis*, by investigating the potential for its biological control in Turkey. However, host stage preference and biological parameters should first be determined. Therefore, this study focused on the host stage preference of *A. arizonensis* by applying choice and no-choice tests.

Materials and Methods

This study was conducted to determine the host stage preference of *A. arizonensis* with choice and no-choice tests in climate control cabinets at Çukurova University Agriculture Faculty Plant Protection Department and the Biological Control

Research Institute. Experiments were carried out at 25ºC, 65±10% relative humidity and 16: 8 (L: D). The host and parasitoid stock cultures were from the Biological Control Research Institute.

No choice testing

This test determined the most suitable stage of *P. solenopsis* for parasitism by *A. arizonensis*. *P. solenopsis* individuals pre-adapted to feed on cotton leaves were used in the experiment. Firstly, water agar was added to nine millimetre Petri dishes and cotton leaves were then placed on the agar. Ten individuals of each stage of *P. solenopsis* (1st, 2nd, 3rd and female adult stages) were placed in separate dishes. One female and two male *A. arizonensis* individuals from the stock culture were then released to each Petri dish. Experiments for each mealybug stage were replicated 10 times. That meant that during no choice testing, a total of 100 individuals of each stage of the host were used, and 10 females and 20 males of the parasitoid were used.

Choice Testing

The principle of the 'no-choice test' is to not give an opportunity to choose the prey stage but in the 'choice' test the parasitoid can choose the most suitable prey. The testing was as per the 'no choice' test except that 10 individuals of each stage of *P. solenopsis* (1st, 2nd, 3rd and adult, female stages) individuals were placed in the same Petri dish and 1 female adult and 2 males adults of *A. arizonensis* were then released with them. A total of 400 *P. solenopsis* individuals, and 10 female and 20 male *A. arizonensis* individuals, were used in choice tests during this study.

Statistical Analysis

Aenasius arizonensis host stage preference, the difference between 1st, 2nd, 3rd and adult, female stages of *P. solenopsis* were analyzed with One-way ANOVA and the Duncan's multiple range test. The analyses were done with IBM SPSS 23. In addition, the parasitism percentage for each stage was calculated for the No-choice and choice tests separately with Excel 2016.

Results and Discussion

The data from No choice and Choice testing of the parasitism of *Phenacoccus solenopsis* by *Aenasius arizonensis* were evaluated separately (Table 1)

No-choice testing

In the No-choice test, only the 3rd nymphal stage and adult female individuals were parasitised by *A. arizonensis.* The parasitism percentage was 34.7% for the 3rd nymphal stage. However, the female adult of *P. solenopsis* was the host stage most prefered for (70.7%) (Figure 1). There was a significant difference in the mean number of parasitized individuals between each stage of *P. solenopsis*, except between the 1st and 2nd stages ($p<0.05$) (Table 1).

Table 1. No choice and choice testing results for the parasitism of *Phenacoccus solenopsis* by *Aenasius arizonensis*

Stage of *Phenacoccus solenopsis*	Number parasitized (No choice) (mean±SE)	Number parasitized (Choice) (mean±SE)
1st nymphal stage	0.0±0.00 a*	0.0±0.00 a*
2nd nymphal stage	0.0±0.00 a	0.0±0.00 a
3rd nymphal stage	3.5±0.22 b	4.0±0.26 b
Adult (Female)	**7.1±0.56 c**	**7.2±0.20 c**

*Means in the same column followed by a different letter are significantly different ($p<0.05$)

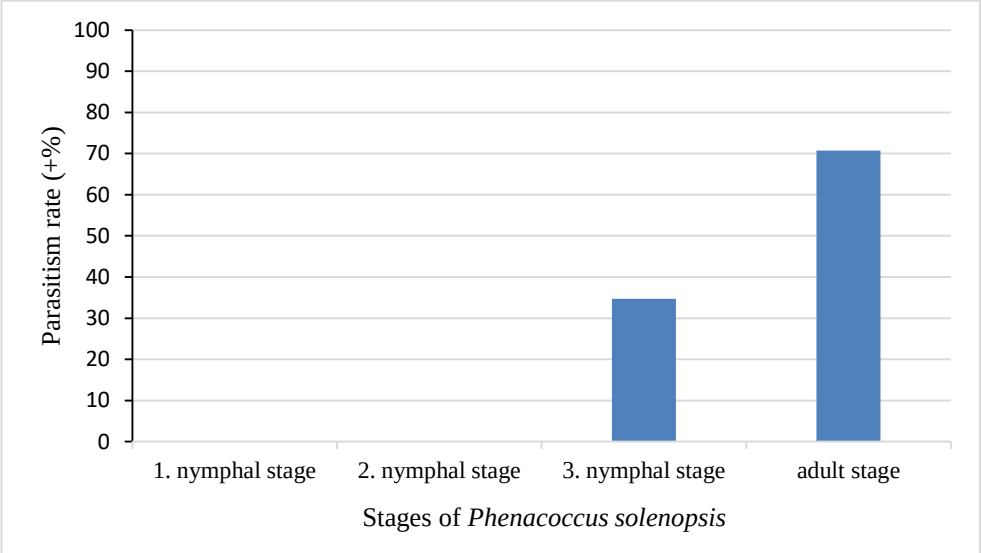

Figure 1. Parasitism of *Phenacoccus solenopsis by Aenasius arizonensis* in 'No-Choice' testing

Choice testing

The Choice test was applied after the No choice test. The main objective of this test was to determine the most suitable stage for parasitism. The third nymphal and adult female stages were parasitised by *A. arizonensis*. The parasitism rates for the 3rd nymphal and adult female stages were 40 % and 71.6 %, respectively (Figure 2). The mean number of parasitized mealybug individuals for each stage was compared and there was a significant difference between the different stages *P. solenopsis*, except for 1st and 2nd stages ($p<0.05$) (Table 1).

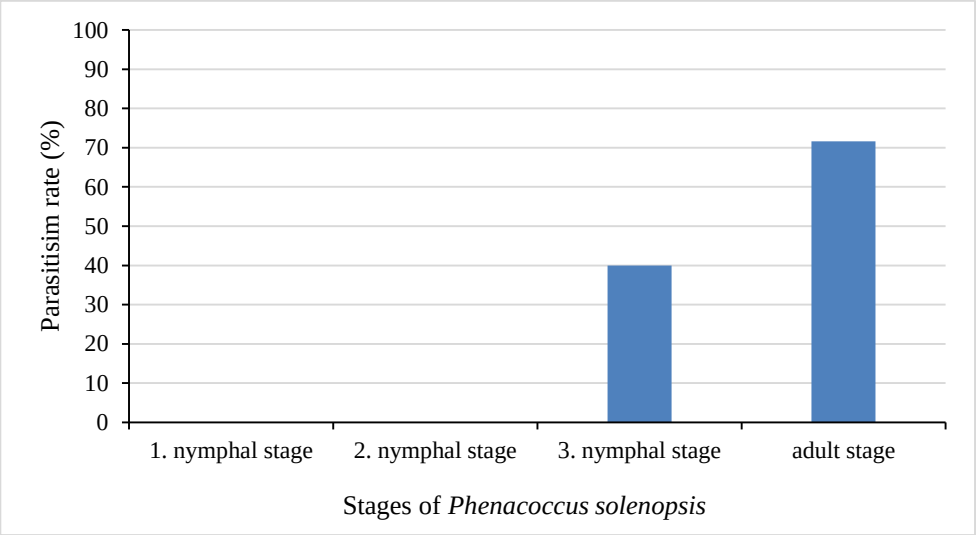

Figure 2. Parasitism of *Phenacoccus solenopsis by Aenasius arizonensis* in'Choice'testing

The No-choice and Choice experiments showed that the 3rd nymphal stage and adult female individuals are the only parasitized stages. *Aenasius arizonensis* can be mass-produced successfully by using a mixed culture of these stages. Chong & Oetting (2006) stated that the parasitoid-host relationship can be age specific. Our results indicate that age and size of the host are two of the most important factors to consider in mass-rearing.

Generally, *A. arizonensis* always chose the 3rd nymphal and adult stages for parasitism because these stages are bigger and less mobile than the first and second nymphal stages. Aga et al (2016) reported that the most suitable *P. solenopsis* stage for parasitism was the adult. Fand et al (2015) stated that the size of the host is one of the most important factors in determining the level of parasitism, and that the parasitism rate increased directly with the size of *P. solenopsis*. Badshah et al (2016) reported that the 3rd nymphal stage and adult stage of *P. solenopsis* are chosen by *A. bambawalei* for parasitism. In addition, the same stated that the parasitoid cannot develop in the first and second nymphal stages. Abidin et al (2012) reported that although the 3rd stage of *P. solenopsis* was parasitized by *A. bambawalei,* maximum parasitism occurred in the adult stage.

Conclusions

Phenacoccus solenopsis, which is one of the most important invasive species in Turkey, has caused substantial damage to agricultural crops since 2012. The biological control of this pest has become more realistic because the presence of one of the most effective known parasitoids, *A. arizonensis,* was reported in Turkey in 2016 (Çalışkan-Keçe et al, 2018). The current study focused on the host-stage

preference of *A. arizonensis* by employing No-choice and Choice experiments. In the experiments, *A. arizonensis* parasitized in the range of 30% to 40% of the 3rd nymphal stage, and 70% to 80% of the adult stage, of *P. solenopsis.* These results suggest that *A. arizonensis* could be a successful candidate for mass rearing and use as a natural enemy against *P. solenopsis*. In addition, the mass rearing of *A. arizonesis* should be done with a mixture of the 3rd nymphal and adult stages of *P. solenopsis* to maximize the efficiency of the rearing process.

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