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Influence of the Different Densities of *Phenacoccus solenopsis* Tinsley on the Parasitism Ratio of *Aenasius arizonensis* (Girault) Under Laboratory Conditions

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ABSTRACT

The cotton mealybug, *Phenacoccus solenopsis* Tinsley is an important pest that infested many vegetable crops. *Aenasius arizonensis* (Girault) was found associated with this pest. In this study, the effect of different densities of *P. solenopsis* on the parasitism ratio of *A. arizonensis* females and calculation of the parasitoid to host ratios mathematically (PHR) was carried out under laboratory constant conditions of 25±1˚C, 70±5% RH and 12:12 (L:D) hrs. The densities of 10, 20, 30, and 40 individuals of the third nymphal instar were tested: 10, 20, 30, and 40 nymphs. The obtained results showed that there were significant differences between the percentage of parasitism by mated and unmated *A. arizonensis* females and the density of the third nymphal instar of the cotton mealybug whereas LSD values were 10.75 and 6.66, respectively. The highest parasitism percentage was recorded with the lowest value of host density in all the treatments. Parasitoid to host ratio (PHR) was calculated mathematically at 50 % (PHR_{50}) equaled 21.55 individuals and PHR_{25} equaled35.92 individuals for mated females, but PHR_{25} was 22.29 individuals for unmated females. The obtained results are useful in developing (IPM) programs against the previously mentioned pest through the effect of host density on the associated parasitoid efficacy for choosing the best time to release parasitoid. The present study suggests the possibility of utilization of *A. arizonensis* as an ideal biocontrol agent to reduce *P. solenopsis* populations.

INTRODUCTION

The cotton mealybug, *Phenacoccus solenopsis* Tinsley is a mostly polyphagous insect that infested a huge number of field and horticultural crops. Mealybug does not only weaken the host plant by sucking the sap but also it is responsible for transmitting diseases. (Bertin *et al*., 2010). Furthermore, it excreted honeydew on surfaces of plants which provides a medium for the development of black mold which reduces the photosynthesis efficiency of the plant (Buss and Turner ,2006 and Nabil and Hegab, 2019). The outbreaks often occur when mealybugs get introduced into new locations in the absence of their natural enemies Dhaliwal (2010). Therefore, the parasitoid has been played as a good option in IPM programs in reducing the populations of mealybug where it is more sensitive to the chemicals than the target pests. (Bokonon-Ganta *et al*., 2002; Muniappan *et al.*, 2006; Roltsch *et al*., 2006 and Shylesha *et al*., 2010). In this study, it was found that the effect of the different third nymphal stage densities of *P. solenopsis* on the parasitism ratio of

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Aenasius arizonensis (Girault)females to find out the suitable time to release parasitoid that can be beneficial when developing an integrated program for this pest. Thus, a new theory can be reached to expand the use of parasitoids parasites associated with this pest, which makes progress in the integrated control of the pest safely without the use of pesticides that may pollute the environment.

MATERIALS AND METHODS

This study was carried out under constant laboratory conditions of 25±1ºC, 70±5% RH and 12 hours of photoperiod at Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt.

Collection of Insects:

The mummies of the cotton mealybug, *P. solenopsis* and its associated parasitoid, *A. arizonensis* were collected from okra plants, *Abelmoschusesculentus* L. (Malvaceae) at Hihya district, Sharkia Governorate, Egypt.

Effect of the Cotton Mealybug Density on The Parasitism Ratio of *Aenasius arizonensis* (Girault):

The mummies were separated individually in test tubes as female and male mummies and were kept till the emergence. Five replicates of different numbers of the third nymphal instar of *P. solenopsis* including 10, 20, 30 and 40 nymphs were offered to newly emerged mated and unmated females on potato sprouts at glass lamp container (15cm long and 10cm diameter) covered with a muslin cloth. The duration of the experiment was 48 hrs. and then the parasitoids were removed and the containers containing mealybug nymphs were kept in incubators at the same constant conditions. When the mummies appeared on the potatoes, their numbers were recorded to calculate the parasitism percentages and sex ratios.

Statistical Analysis:

Data were subjected to analysis of variance (ANOVA) using a software package, CoStat Statistical Software (2005), a product of Cohort Software, Monterey, California. Means were compared by calculated least significant differences at $P \leq 0.05$ level of probability. To calculate the Parasitoid to Host Ratio (PHR) at 25 and 50%, the partial regression formula was used according to Hendi (1969) and Nabil (2003), the dependence variance (Y) represented the percentage of parasitism and the independent variance (X) was the host density. The partial regression was used to show the variability in the percentage of parasitism that is caused by the variable in host density. The slop (b) of the straight regression line was calculated to obtain the corrected values of the percentage of parasitism by using the formula: $Y = a \pm bX$.

RESULTS AND DISCUSSION

Effects of the Cotton Mealybug Density on The Parasitism Ratio of *Aenasius arizonensis* (Girault)

Data tabulated in Tables (1 and 2) showed that the percentage of parasitism by mated and unmated *A. arizonensis* females were significantly affected by different densities of the host, third nymphal instar of *P. solenopsis.*

The highest parasitism percentage of mated and unmated *A. arizonensis* females were 82.00% and 44.00% when the host density was 10 individuals per treatment, followed by 36.00 & 21.00% and 32.67&13.33% for 20 and 30 individuals of third nymphal instar, respectively. While the lowest percentages of parasitism were 25.00% and 9.50% for 40 individuals of the third nymphal stage.

The statistical analysis showed that there were significant differences between the percentage of parasitism and the host densities of the third nymphal stage of the cotton mealybug whereas the LSD values were 10.75 and 6.66for mated and unmated females, successively.

The sex ratios by mated females were almost to 1:1 in all over the treatment while it was 0:1 when females did not mate as the new offspring were males only (Table 3).

As shown in Figs. (1 and 2) to evaluate the Parasitoid to Host Ratio (PHR), the straight-line formula between mated and unmated *A. arizonensis* females and different densities of the host, third nymphal instar of *P. solenopsis* Y= 87.50 – 1.74 X and Y= 49.75 – 1.11 X were applied, conclusively. The Parasitoid to Host Ratio at 50 and 25% of parasitism (PHR_{50} and PHR_{25}). $PHR_{50}$was 21.55 individuals and PHR_{25} equaled 35.92 individuals. That means in IPM programs especially parasitoid release when the reduction or parasitism ratio needs to be between 25 to 50%, the host density must be from 22 to 36 individuals to every mated female. While for unmated females the PHR_{25} was 22.29 individuals. That means the parasitism ratio needs to be 25 % the host density must be from 22.29 individuals.

Table 1. Parasitism percentage of mated *Aenasius arizonensis* (Girault) females on different third nymphal instar densities of *Phenacoccus solenopsis* Tinsley under laboratory constant conditions of 25±1˚C, 70±5% RH and a photoperiod of 12:12 (L:D) hrs.

Host density	Rep.	No. of parazitied individuals	Parasitism %	Mean%± SE
10	1	8	80.00	82.00^{a}±6.62%
	2	8	80.00	
	3	10	100.00	
	4	6	60.00	
	5	9	90.00	
20	1	7	35.00	36.00^{b}±1.87%
	2	6	30.00	
	3	7	35.00	
	4	8	40.00	
	5	8	40.00	
30	1	10	33.33	32.67^{bc}±1.63%
	2	11	36.67	
	3	10	33.33	
	4	8	26.67	
	5	10	33.33	
40	1	11	27.50	25.00^{c}±1.12%
	2	9	22.50	
	3	10	25.00	
	4	11	27.50	
	5	9	22.50	
P	< 0.0001**			
LSD	10.75			

Means in column with the same letter are not significantly different at 0.05 levels

Table 2. Parasitism percentage of unmated *Aenasius arizonensis* (Girault) females on different third nymphal instar densities of *Phenacoccus solenopsis* Tinsley under laboratory constant conditions of 25±1˚C, 70±5% RH and a photoperiod of 12:12 (L:D) hrs.

Host density	Rep.	No. of parazitied individuals	Parasitism %	Mean%± SE
10	1	4	40.00	44.00^{a}±3.99%
	2	5	50.00	
	3	5	50.00	
	4	3	30.00	
	5	5	50.00	
20	1	5	25.00	21.00^{b}±1.87%
	2	5	25.00	
	3	4	20.00	
	4	4	20.00	
	5	3	15.00	
30	1	4	13.33	13.33^{c}±00%
	2	4	13.33	
	3	4	13.33	
	4	4	13.33	
	5	4	13.33	
40	1	4	10.00	9.50^{c}±0.50%
	2	4	10.00	
	3	4	10.00	
	4	4	10.00	
	5	3	7.50	
P	< 0.0001**			
LSD	6.66			

Means in column with the same letter are not significantly different at 0.05 levels

Table 3. Sex ratio of the progeny of mated and unmated *Aenasius arizonensis* (Girault) females on different nymphal instar densities of *Phenacoccus solenopsis* Tinsley under laboratory constant conditions of 25±1˚C, 70±5% RH and a photoperiod of 12:12 (L:D) hrs.

Host density	Mated females			Unmated females		
	No. of emerged parasitoids		Sex ratio	No. of emerged parasitoids		Sex ratio
	♀	♂		♀	♂	
10	20	19	1.05:1	0	22	0:1
20	18	18	1:1.1	0	21	0:1
30	24	25	1:1.04	0	20	0:1
40	25	25	1:1	0	19	0:1
Total	87	87		0	82	
Mean			1:1			0:1

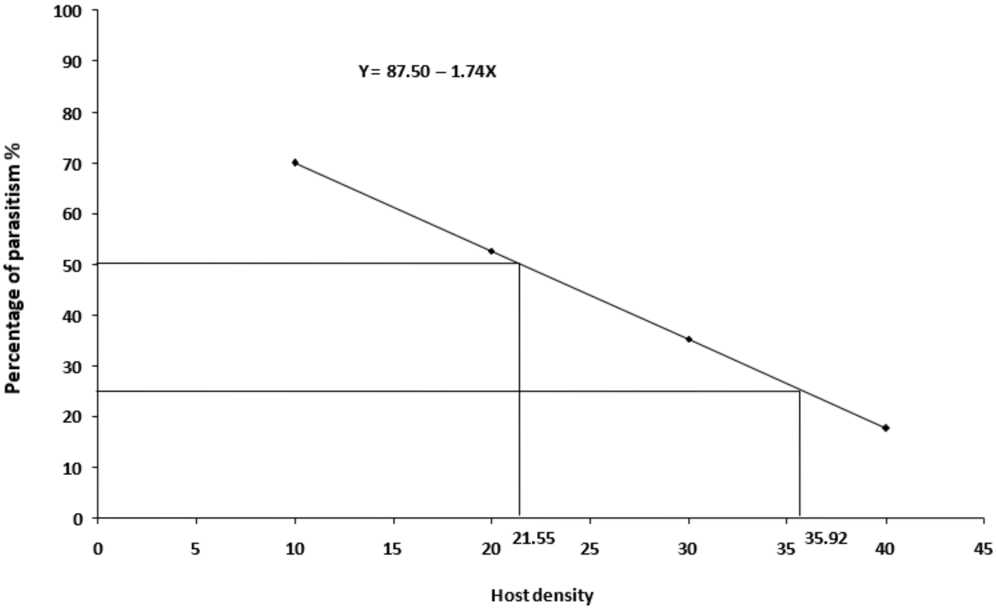

Fig.1. The calculated Parasitoid to Host Ratio at 50 and 25% (PHR_{50} and PHR_{25}) of parasitism between mated *Aenasius arizonensis* (Girault) females on different third nymphal instar densities of *Phenacoccus solenopsis* Tinsley under laboratory conditions

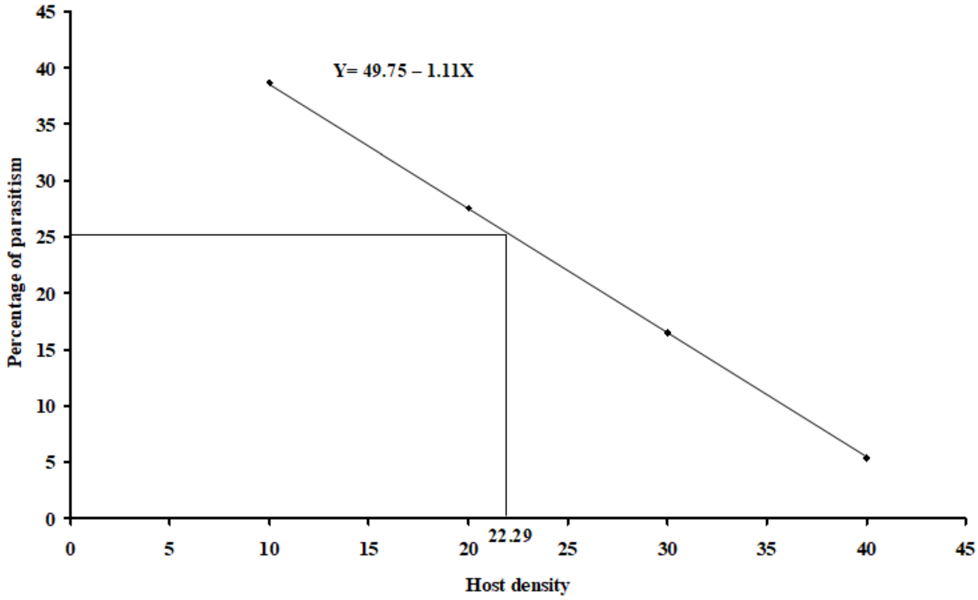

Fig. 2: The calculated Parasitoid to Host Ratio 25% (PHR_{25}) of parasitism between unmated *Aenasius arizonensis* (Girault) females on different third nymphal instar densities of *Phenacoccus solenopsis* Tinsley under laboratory conditions.

The results showed that the parasitism ability of mated and unmated *A.arizonensis* females was enhanced along with the third nymphal instar stage density decreasing whereas the host density played a significant role in parasitism of *A. ariozonensis*. The mean numbers of parasitized nymphs were obviously decreased with increasing the number of host densities from 10 to 40 host densities, which provided to this parasitoid. These results are similar to parasitic behavior observed by Fen He *et al.* (2015) in China**,** who reported that the mated parasitoid females of *Aenasius bambawalei* Hayat of *Phenacoccus solenopsis* Tinsley could oviposit fertilized eggs within 24 hrs. Their offspring included males and females, and the female fraction of the progeny adults was 0.9. Unmated parasitoid females could oviposit unfertilized eggs within 24 hrs. but all their offspring were males. **Aga *et al.* (2016)** in India, investigated some biological aspects of *A. bambawalei on P. solenopsis* under laboratory conditions and recorded that the sex ratio was skewed towards females and recorded as 1:2. maximum. Also, the percentage of parasitization that occurred in the three-nymph instar with 73.33 0% whereas the 3rd instar nymph was the most suitable stage for mass multiplication of the parasitoid. Badshah *et al.* (2016) and Shahzad *et al.* (2016)in Pakistan, mentioned that the 3rd nymphal instar of *P. solenopsis* was the most suitable stage for mass multiplication of *A. bambawalei* and confirmed that *A. bambawalei* showed no attraction to male mealybugs and no-host feeding on any host stage was recorded. This basic research regarding the reproduction of the parasitized host mealybugs would be very helpful in devising sustainable biological control strategies for cotton mealybug. Joodaki *et al.* (2018) in Iran, studied the parasitism response of *A. bambawalei* to different population densities of the cotton mealybug and reported that the mean number of parasitized nymphs increased significantly with increasing the number of host individuals offered. However, the proportion of hosts parasitized relative to the initial host density decreased as host density increased. Karmakar and Shera (2018) in India, mentioned that *A. arizonensis* females parasitized all the host stages except the first nymphal instar and the parasitoid females preferred 3rd nymphal instar with high parasitism (74.0–84.0%) and produced more females compared to other host stages. They added these data could be used in the laboratory to mass rearing this parasitoid as biological control of *P. solenopsis.* Rind *et al.* (2019) in Pakistan, studied the host stage preference of the parasitoid, *A. arizonensis* on *P. solenopsis* and recorded that the highest host instar specific parasitism was the third nymphal instar of mealybugs under choice and non-choice-based experiments.

Conclusion

Biological control by using parasitoids is considered the best alternative to chemical control as the expansion in the use the pesticides lead to the elimination of many numbers of it and the ability of *P. solenopsis* to resistance against new pesticides. So scientists necessitate applying biological control as an eco-friendly control method. Generally, this study strongly suggests the possibility of utilization of this parasitoid, *A. arizonensis* as an ideal biocontrol agent to reduce populations of the cotton mealybug be optimized by multiplying at the third nymphal instar of this host in the laboratory and thereafter inoculative release into the field. However, further investigation is needed to determine more information about parasitoid-host interaction, the effect of different biological and ecological factors that may be involved in order to apply this species under protected cultivations which expanded so far greatly in Egypt.

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ARABIC SUMMARY

تأثير الكثافات المختلفه من بق القطن الدقيقى *Phenacoccussolenopsis* Tinsley على نسبة التطفل للطفيل *Aenasiusarizonensis* (Girault) تحت الظروف المعملية

محمد على مرسى حجاب، على عبد الحميد شاهين، كامل عبد اللطيف حماد وشادية احمد نبيل حسن مصطفى
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يعتبر حشرة بق القطن الدقيقي Phenacoccus *solenopsis* Tinsley من الأفات الهامة التى تصيب العديد من محاصيل الخضر. وجد الطفيل *Aenasius arizonensis* (Girault) مرتبطاً بهذه الآفة. فكان الهدف من هذه الدراسة هو تقدير تأثير كثافات مختلفة من العائل *P. solenopsis*على نسبة التطفل لإناث الطفيل *A.arizonensis* وحساب نسب الطفيل إلى العائل رياضيًا (PHR) تحت الظروف المعملية عند 25 ± 1 درجة مئوية مع 70 ± 5٪ رطوبه نسبية و 12ساعة فترة ضوئية. تم اختبار الكثافات المختلفة التالية من العمر الحوري الثالث: 10 و20 و 30 و 40 حورية. أظهرت النتائج المتحصل عليها إلى وجود فروق معنوية بين نسب التطفل لإناث الطفيل المتزاوجة وغير المتزاوجة *A.arizonensis* و الكثافات المختلفه من العمر الحورى الثالث لبق القطن الدقيقي حيث كانت قيم LSD(إقل فروق معنوية) تساوى 10.75و 6.66 على التوالي. حيث سجلت أعلى نسبة تطفل مع أقل قيمة من كثافة العائل في جميع المعاملات. كذلك تم حساب نسبة العائل إلى الطفيل رياضياً والتى تعطى 50% و 25% تطفل تساوى 21,55فردًا و35,92 فردًا لكل إنثى متزوجة، لكن بالنسبة للإناث الغير متزاوجة فكانت نسبة العائل إلى الطفيل والتى تعطى 25% تطفل تساوى 22,29 فردًا لكل إنثى. يمكن الاستفادة من النتائج التي تم الحصول عليها في تطوير برامج (IPM) ضد الآفة المذكورة سابقاً من خلال تأثير كثافة العائل على فعالية نسبة التطفل للطفيليات المصاحبة لإختيار أفضل وقت لإطلاق الطفيل. لذا تقترح هذة الدراسة إمكانية أستخدام الطفيل *A. arizonensis* كعامل تحكم بيولوجي مثالي لتقليل تعدادات بق القطن الدقيقى.