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Effectiveness of Endoparsitoid Wasp *Aenasius Arizonensis* (Girault) as a Successful Bio-Control of Cotton Mealy Bug, *Phenacoccus Solenopsis* Tinsley, in Khartoum State, Sudan.

Nawal Ahmed Mohamed[1], Awad KhalafAlla Taha[2] , Abubaker Haroun Mohamed Adam[3,*]

[1]Ministry of Agriculture, Animal Wealth and Irrigation, Khartoum State, Sudan

[2]Department of Plant Protection, College of Agricultural Studies - Shambat, Sudan University of Science andTechnology, Sudan

[3]Department of Crop Science, College of Agriculture, University of Bahri, AlKadaru, Sudan.

Abstract

The objective of this study was to evaluate the effectiveness of endophagous Encyrtid parasitic wasp *Aenasius arizonensis* (Girault) (Hymenoptera, Chalcidoidea), as a natural enemy for controlling the Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Where a Randomized Complete Block Design (RCBD) was adopted. Both, field survey and Laboratory experiments were conducted to study some biological characteristics of the parasitoid *A. arizonensis*. The results of field study revealed that, the parasitioid was available most of the year, and was highly effective on adults and last larval instar of the mealy bug, *P. Solenopsis,* with a total percentage of parasitism of 31.26%. While the laboratory results showed that, the duration from oviposition to adult emergence were similar for both male and female. Longevity of male was 23± 2.33 days and female 23.37±1.89 days. Mean daily fecundity in terms of number of Parasitized hosts /female / day ranged from 20 to 24. The sex ratio was 1:1.6 for the male and female in the progeny. Generally, a mass rearing of this parasitoid, with a conservation and augmentation releases could help in the application of an effective management strategy for the control of the cotton mealy bug in the field.

Corresponding author: Abubaker Haroun Mohamed Adam, Department of Crop Science, College of Agriculture, University of Bahri, AlKadaru, Sudan, Email: abubakerharoun@gmail.com

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Introduction

The species, of Cotton Mealy bug *(Phenacoccus solenopsis*) , has recently emerged as a serious insect pest of Cotton, and several plant species [1, 2]. It is native to the Nearctic, originated in New Mexico, USA [3]. Now it is found in numerous regions including Central and South America, Africa and Asia.

Several researchers' recorded Cotton Mealy bug in Sudan for the first time, after the outbreak in different States of the country during the season 2012/ 2013 [4]. Then specimens were sent to Plant diagnostic Centre, Sacramento, CA, USA, where it was identified as Phenacoccus solenopsis Tinsley, which attacks at least 26 host plant species belonging to 16 plant families.

The chemical control of Mealy bugs is not only expensive, but it also disturbs the habitats of natural enemies and has negative impacts on different ecosystems and human health [5, 6]. To avoid such problems, several parasitoids and predators were used to control the spread and damage that can be caused by Mealy bugs and keep the pest populations below the economic injury level [7].

Family Encyrtidae (Hymenoptera, Chalcidoidea) is an important Entomophagous group of insects that are parasitoids of a wide range of insects and other Arthropods. Several species of the family have been successfully used as biological control agents in some parts of the world [8].

In the United States of America, the parasitoid, Aenasius arizonensis (Girault), Family Encyrtidae (Hymenoptera, Chalcidoidea) was described as Chalcaspis arizonensis [9], and later transferred to Aenasius [10]. It is a solitary aggressive endoparasitoid of *P. solenopsis* under natural conditions, and a most successful example of many biological control agents[11, 12]. This is due to the fact that, this parasitoid has some characteristics of adaptation to different environmental conditions, multiply faster than the host, short life Cycle (17-20 days), high host searching capacity and synchronise life cycle with host, with a parasitism ranging from 5-100% [8]. The parasitoid was recorded from USA, India, Pakistan, China, Iran, Egypt, Turkey and Iraq [9, 13]

One of the researchers [14] studied the host specificity of the parasitoid, Aenasius bambawalei (syn. of A. Arizonensis) to six species of Mealy bugs and their suitability for development of parasitoid. Only Phenacoccus solenopsis was found to be suitable for development and none of the other species of Mealy bugs were parasitizing. This means that the host specificity is confined to P. solenopsis.

In Sudan, the first record of the parasitoid, A. arizonensis was made by researchers [15] during the monthly survey in Gezira Research Station. They observed the presence of Mealy bug mummies containing parasitoid or its emergence holes on Hambouk (Abutilon spp), Datura (Datura stramonium) and Okra (Hibiscus esculentus).

Many studies revealed that ants are commonly found in association with Mealy bugs infestation, feeding on honey dew secretions and have long been known to aggravate Mealy bug populations and other honey dew producing insect species by disturbing the natural biological control on these species [15, 16] which could be a delimiting factor and need to be considered in mass rearing of the wasp.

The present study was carried out to evaluate some of the biological characteristics of this parasitoid, to be applied as a bio-control agent for cotton Mealy bug, *P. solenopsis in* Sudan.

Materials & Methods

A field survey was made to determine the seasonality of Mealy bug, P. Solenopsis and its parasitoid, Aenasius arizonensis. Records were made during the season 2015 - 2016, at two weeks intervals, in three Agricultural Schemes namely; Soba, Gommueia and Selait, which were infested with Mealy bug, P. Solenopsis. The species were identified based on the morphometric features described by the researchers [17, 18]

Another field survey was carried out in April, 2019 to study the Infestation of P. solenopsis and parasitism by A. arizonensis at Soba Agricultural Scheme. Where the infestation of P. solenopsis and its parasitized mummies were observed on three different families of weeds, which are locally known as Raba (Trianthema brasilicum), Lissan Al-tair (Amaranthus virids), Hambouk (Abutilon spp). To determine the parasitisation percentage, 50 apical shoots of Hambouk,

each of 30 cm in length were collected randomly from five sites.

For the laboratory studies, the Mealy bug mummies were collected in plastic jars from ornamental plants, Lantana (Lantana camara) and hibiscus (Hibiscus rosa - sinensis) from Horticulture orchard at the Faculty of Agriculture, Khartoum University, Shambat. They were reared in four transparent plastic jars under laboratory conditions of 28ºC temperature and 45% relative humidity so that to allow the emergence of the parasitoid. Breeding colonies of A. arizonensis were established from individuals emerged from the dark mummies.

One day old males and females of the emerged parasitoid were allowed to mate for 24 hours. Then, they were transferred by an aspirator to a culture of 30 adult females of the Mealy bugs, reared on Hibiscus *rosa -sinensis* in a plastic jar. A solution of 2% sugar mixed with water was supplied as a food source for the parasitoid adults by wetting a Cotton swab, tied with thread, kept hanging in the jar.

After 24 hours, the pair of the parasitoid was taken out from the plastic jar. The exposed Mealy bugs were checked daily until adult parasitoids emerged from the mummified Mealy bugs. Then the following parameters were recorded: mean developmental periods from oviposition to mummy formation, mummy formation to adult emergence, total life span of males and females, sex ratio and mean daily parasitisation. To get more accurate information, the experiment was repeated three (3) times as prescribed by some researchers [19].

Statistical Analysis

Data regarding the biology and parasitization of Aenasius arizonensis in the field and the laboratory were statistically analyzed by using Software excel version 2007 and Statistix 8 (Analytical Soft Ware, 2003). Then they were subjected to analysis of variance (ANOVA) under completely randomized design. Means were compared following the least significant difference test (LSD test) at probability level of 5% to get the mean values of the observations, S.E., parasitism and the Sex ratio from the recorded data.

Results and Discussion

From table (1), the preliminary survey of the parasitoid, A. arizonensis showed that, it is available most of the year around on some host plants. The biology of the wasp was studied under laboratory conditions. The observations showed that, after mating, the females oviposit fertilized the eggs within 24 hours inside the Mealy bug. The parasitized Mealy bug shed its wax,

Table 1. Seasonality of the parasitoid, *A. arizonensis* on different host plants under field conditions- Khartoum State-Sudan (2015- 2016)

No.	Month	Host plant species
1	October	Okra
2	November	Okra
3	December	Okra - Lantana ((Figure 1)
4	January	Okra- Hambouk
5	February	China rose (Figure 2) – Hambouk
6	March	Hambouk
7	April	Hambouk
8	May	Ramtouk- Hambouk
9	June	Datura- Hambouk- china rose
10	July	China rose- hambouk
11	August	-
12	September	-

swollen and showed very poor movement after 2-4 days of oviposition, then hardens into a leathery; brown colored structure, which transformed into a barrel shaped; dark brown mummy within a week. This agrees with the findings by some researchers [14, 20] who showed that, the parasitized Mealy bug took a week to transform into mummy feature (Fig.1 and Fig2).

It is observed that, the adults emerge from mummies by cutting a circular hole after a pupal period. The emergence holes were found on the posterior– dorsal part of the dorsum of the mummified P. Solenopsis (Fig.3). Only one parasitoid adult emerges from each mummy of the host (Fig.4). The parasitoid female lays a single egg in its host; which agrees with other findings (14). The offspring includes males and females, with a sex ratio of 1:1.6, respectively.

Considering the table (2), the results of the study indicated that no difference in the mean developmental periods from ovipostion to emergence of the parasitoid females (13.09 ± 1.02 days) and males (13.29± 1.12 days), with a range of 10-17 and 10-16 days respectively. The males died within 9.71± 1.21 days from emergence, with a range of 5-11 days and a pre-oviposition period less than one day, which is similar to other research findings [6, 18].

As far as the mean longevity period (from egg to death) for females concern; was 23.37 ±1.89 days and 23± 2.33 days for males, with a range of 13-29 and 13-25 days, for females and males, respectively, which corresponds to the findings of some researchers (2012). The number of parastized Mealy bugs per day ranged from 20- 24, with a mean of 22.5± 0.96 (Table 2), while the findings of other researches [18] stated that the parastized Mealy bugs per day ranged from 2- 19

During the field surveys, high infestation of *Phenacoccus solenopsis* and its mummies of the parasitoid were observed on Hambouk, attacking the 3rd nymphal instar and adult females of *P. Solenopsis*. The results (Table 3) revealed a significant difference between the mean number of female's mummies per 30 cm apical shoot (17.9± 0.85) and that of 3rd instars mummies (4.24± 0.85).

Regarding table (3); the results showed the total parasitism on Hambouk was 31.26%, which expressed as 25.02% on adult females and 6.24% on the 3rd instar. No parasitism was observed on the first and second instars nymphs, This is supported by a group of researchers [21, 22, 18], who recorded that, the parasitoid preferred large, late stage host for development, which suffer lower mortality and contain adequate food sources to generate superior off springs

During this study, activities of ants as predators of mummified mealy bugs were observed and also observed carrying about young mealy bug crawlers, aiding in their dispersal (Figure 5).

Conclusion & Recommendation

Table 2. The developmental parameters of A. arizonensis on P. solenopsis under laboratory conditions- Khartoum State-Sudan (2015-2016).

Biological characteristics	**Mean ± S.E (days)**	**Range (days)**
Oviposition to mummy formation	3 ± 1.16	2- 4
Adult male emergence after oviposition	13.29 ± 1.12	10- 16
Adult female emergence after oviposition	13.09 ± 1.02	10-17
Emergence to death for male	9.71 ± 1.21	5- 11
Emergence to death for female	10.28 ± 0.87	9- 14
Pre- oviposition period	<1	<1
Male longevity	23 ± 2.33	13- 25
Female longevity	23.37 ± 1.89	13- 29
Mean of Parasitization (no. of parasitized host / female/ day).	22.5 ± 0.96	20- 24
Sex ratio of progeny (male: female)	1: 1.6	

Figure 1. *Aenasius arizonensis* parasitized mummies of *P. solenopsis* on Lantana

Figure 2. *A. arizonensis* parasitized mummies of *P. solenopsis* on China rose

Figure 3. Pupae of *A. Arizonensis*

Figure 4. Emerging Adult female of *A.Arizonensis*

Table 3. Mean numbers of parasitoid mummies and percentage parasitism on different stages of *P.solenopsis* under field conditions, Khartoum State- Sudan (April 2019)

Stage	Mean no. of mummies per 30cm shoot	% parasitism	Total % parasitism
Female	17.908 A	25.02	31.26
Third Nymphal instar	4.24 B	6.24	
CV	54.14		
SE	0.8479		
LSD	1.986		

Figure 5. Active ants seeking the honey dew on a Colony of Cotton Mealy bug, P. solenopsis

Wasp, *Aenasius arizonensis* is an aggressive parasitoid, and is considered the main factor for declining the Mealy bug populations in many areas of its distribution. This study provides a new; environmentally friend approach for controlling Mealy bug. Therefore, a programme of mass rearing of this wasp, in addition to population conservation and augmentation releases can help in effective strategic management *P. Solenopsis* in the field. Further extensive study is recommended.

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