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Biology of cotton mealy bug (*Phenacoccus solenopsis* (Tinsley) on cotton plants under the laboratory conditions

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Phenacoccus solenopsis **Tinsley (*Hemiptera: Pseudococcidae*) is one of the invasive insect species, which appeared in Ethiopia 2012, and then widely spread all over cotton growing areas of the country within few years. The current study was done under laboratory condition to study the biology of *Phenacoccus solenopsis* in Ethiopia by using cotton leaves placed in Petri plates as a feed for the insect. *Phenacoccus solenopsis* developmental stage and reproduction were observed in detailed at mean room temperature of 26.7-30.8 and relative humidity of 45.3-57.3%. The result revealed that P. solenopsis possess three instars for females and four instars for males. Among these the third instar required longer developmental period (5.3±0.1 day) and the first instar and second instar had nearly similar developmental periods 3.7±0.1, 4.3±0.1 days respectively. The second instar had lowest survival percentage (53.7%) than first and third instar (72.7%, 77.8%) respectively. Female P. solenopsis were differentiated from male at third instar where, male developed cocoon and wing whereas the female remained wingless and its body covered with white dusty. The female has three molting stages and the male has four. Females have fecundity range of (103 to 848) during its reproductive period. The male total life span was (13 to 20 days) shorter than female (30 to 53 days). The present study indicates that management interventions should be taken before third instar developmental period for effective management of this insect.**

Keywords: Cotton mealbug, crawlers, developmental period.

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INTRODUCTION

Cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is one of the most devastating insect pests of cotton, ornamentals and vegetable crops in different parts of the world.

Phenacoccus solenopsis firstly originated in America 1897 (Tinsley 1898) and then widely distributed from its

origin to other part of the world in Chile 2002 (Larrain, 2002), Argentina 2003 (Granara, 2005), Brazil 2002 (Culik and Gullan, 2005), Pakistan 2005 (Abbas*, et al,* 2007; Hodgson *et al.,* 2008), India 2005 (Karar *et al.* 2008) and China 2008 (Wang, *et al*. 2009). *Phenacoccus solenopsis* was first reported in Eastern part of Ethiopia in 2011 (Miesso *et al*, 2012).

Phenacoccus solenopsis is sexually dimorphic, having short lived, winged males and longer-lived, wingless form of females (Vennila *et al.,* 2010 and Nagrare *et al.,* 2011). *P. solenopsis* possess paired dark spots on dorsal side, oval shaped body, antennae with 9 segments, legs well developed; claw with a pair of digitule, translucent pores on the apex of the hind femur and tibia, large and flaccid circulus and multilocular disc pores more concentrated near the region of vulva or restricted in the segments (Nagrare *et al*., 2011).

Phenacoccus solenopsis has shown sexual reproduction, producing live young ones instead of laying eggs by a phenomenon of ovoviviparity (Abbas, *et al* 2007). Mealy bug, soft body insect, also reproduces mostly parthenogenetically, female lays eggs in ovisacs containing 150-600 eggs. Hatching takes place in 3-9 days into nymphs which lasts for 22-25 days finally growing into adults in 25-30 days under optimum condition (Joshi *et al.* 2010). *Phenacoccus solenopsis* produce 128–812 crawlers per female (Vennila *et al.,* 2010). Akintola and Ande, (2008) observed that *P. solenopsis* required six, eight and ten days for the development of first, second and third instar on China rose, respectively.

Weather conditions have positive and negative effect on the insect biology and their incidence in field (Dhawan and Saini, 2009). Temperature and relative humidity of 23.3 -30.2°C and 40.5-92.5% RH, respectively were optimum condition for *P. solenopsis* reproduction and development under laboratory conditions (Vennila *et al.,* 2010). At such room temperature high *P. solenopsis* fecundity (128 to 812 crawlers per female) were recorded by Vennila *et al*., 2010. Under laboratory conditions the second instar of *P. solenopsis* had longer developmental period than other instars (Chong *et al., 2*003; Vennila *et al.,* 2010). *P. solenopsis* also required six, eight and ten days for the development of first, second and third instar on China rose, respectively (Akintola and Ande, 2008).

P. solenopsis is a polyphagous insect pest, which has been recorded on a number of cultivated and on weedy species in Ethiopia reported by Sharew and Bayeh, 2014 (unpublished data). *P. solenopsis* is greatly damage cotton crop and thus reduce yield considerably both in terms of quantity and quality (Fuchs *et al* 1991). Particularly incidence of cotton *P. solenopsis* to Ethiopia eight years ago became challenge of cotton production. Although few activities which include chemical screening and survey have been done but some basic information like biology of the pest in the context of Ethiopia is lucking and should be studded (Miesso *et al.,* 2012). Therefore, the present study was done on biology of *P. solenopsis* in the laboratory condition to generate information on its developmental stages, mortality and fecundity that may be used to design possible management strategy of the pest.

MATERIAL AND METHODS

Description of the Study Area

The study was conducted under laboratory conditions of Werer Agricultural Research Center (WARC), Ethiopian Institute of Agricultural Research (EIAR). WARC is located 278 km to the East of Addis Ababa at an altitude of 740 m.a.s.l, latitude of $9^0 16^0$N and longitude of 40^0 9^0E. The study area is characterized by having mean annual rainfall of 540mm and mean maximum and minimum environmental temperatures of 34.4^0c and 19.6^0c, respectively.

Mealy bug collection, cotton leaves preparation and rearing

The study was done for two consecutive years (20162017) July to September under the laboratory at WARC. Cotton plants (*Gossypium hirsutum*) were raised in lath house on flat seed bed a month before starting the experiment, this cotton plant used as source of food for mealy bug. Fresh cotton leaves with petioles were collected from similar position of the cotton plants grown in lath house washed with tap water to remove pathogens contamination, dusts and insect pests. The base of the petiole of individual leaves was covered with a water soaked cotton swab to prevent desiccation of the leaf (figure 1)

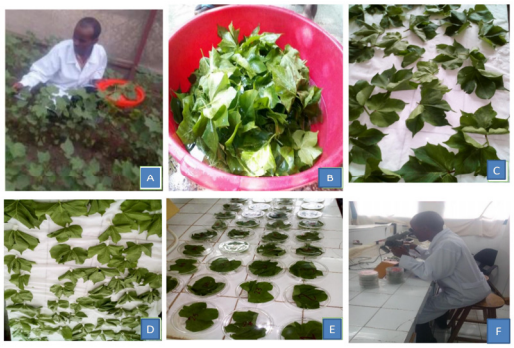

Figure 1. Steps in study on mealy bug biology: A. Collecting leaves, B. Washing of leaves, C. Drying, D. Covering base of the petiole with cotton swab, E. Release of mealy bug on cotton leaf in Petri plate, F. Follow up molting

Adult females of *P. solenopsis* with twigs of cotton plants were collected from the cotton field and brought to the laboratory to produce initial crawlers (Figure 1). Individual neonate crawlers emerged from adult females fed on cotton leaves in Petri plates in the laboratory was used to start the study. Crawlers drawn from different females but laid on the same day individually were transferred to separate glass Petri plates. A total of 150 crawlers drawn from different females but laid on the same day were individually transferred to separate glass Petri plates each containing a cotton leaf. During the study each successfully completed stage to be used as initial for the next stage observation. Daily room temperature and relative humidity were measured for monitoring purpose.

Data collection

Observations on survival and molt of the crawlers were recorded daily under stereoscopic microscope until they became adults (Figure 1). Unless the crawlers were in a pre-molt stage, Petri plates along with cotton leaves were changed on alternate days or else transferred after the molt using a camel hair brush. The developmental time of each instar was recorded based on an observed exuvia.

The moulting was confirmed by the presence of exuivum on cotton leaf or on the posterior end of nymphs. Daily counting of crawlers was done until they stopped further molting and reached pre-ovipositional adult stage. As the eggs or neonate crawlers were counted and discarded, the individual adults that had produced them were transferred to new Petri plates for further observations. During the study eggs were separated along with the leaf and observed until they hatched (Figure 2). Also during the study pre-reproduction, reproduction and postreproduction periods were studied. The female laid their eggs in ovisac located at posterior end of its abdomen (Figure 2), the ovisacs were collected during the oviposition period and counted the number of eggs in each ovisac for calculating fecundity.

Data analysis

Data was analyzed using SPSS statistics software (Version: 20): The cotton mealy bug (CMB), biology parameter were calculated using SPSS package. The range and mean values for the developmental period of the three instar nymphal stage, pre-reproductive, reproductive, non-reproductive periods, fecundity, and longevity of females and males were calculated based on the total number of days made. The number of females and males out of the total population that survived to adult stage was calculated

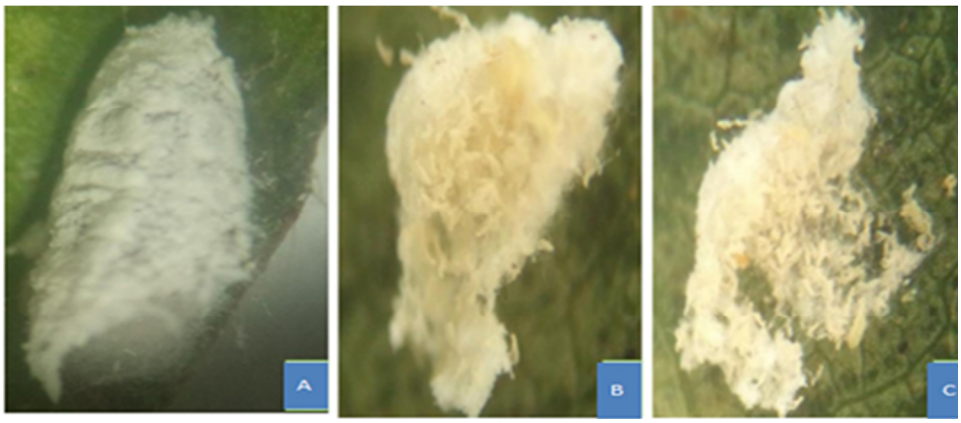

Figure 2. Steps of separating eggs from female mealy bug for getting initial crawlers A. Female mealy bug laying eggs in ovisac, B. separating eggs with ovisac, C. eggs hatched from ovisac

RESULTS AND DISCUSSION

Biology of *Phenacoccus solenopsis*

Phenacoccus solenopsis biology was studied for two consecutive years at Werer Agricultural research center under laboratory condition at a mean room temperature 26.7-30.8 C^0 relative humidity 45.4-57.3%. The experiment was started with a total of 150 numbers of crawlers for each year. The developmental periods of male and female *P. solenopsis* varied under laboratory condition. The crawlers with three molting stage were developed to female with the overall mean up to oviposition period was 19.1±0.3 days and those with four molting stage developed to males in 13.9±0.3 days (Table 3).

Table 1. Mean duration of development stages of *P. solenopsis* under laboratory condition at Werer, 2016

		Duration (days)		Survival%
Development stages	Observation(n1)	Range	Mean ±SE	
First instar	91	3-6	3.8±0.1	60.7
Second instar	46	3-6	4.1±0.2	50.5
Third instar	33	4-9	5.4±0.2	71.7
Male pupation	7	5-8	6.1±0.5	-
Adult				
Male	5	13-16	14.2±0.5	71.4
Female	26	15-25.	19.2±0.5	90.2
Pre reproductive period	26	4-9	5.7±0.3	100
Reproductive period	24	10-16	14.0±0.4	76.1
post reproductive period	21	8-11	9.4±0.2	0
Female total life span	21	30-51	42.7±1.0	-
Adult male longevity	5	1-2.	1.8±0.2	-
Male total life span	5	15-18	16.0±0.5	-
Fecundity	21	103-695	319±31.7	-

*n1=number of observation carried out in each development stage of first year

Developmental period of first, second and third instar *P. solenopsis* were ranged 3-6, 3-6 and 4-9 mean of 3.8±0.1, 4.1±0.2 and 5.4±0.2 respectively. The first and third instars has with high survival percentage 60.7% and 71.7%, the second instar has only 50.5% survival and the adult female and male survival percentage was (90.2% and 71.4%) respectively. Female adults have better survival than male adults (Table 1). This result is supported by Vennila *et al.,* 2010 who reports survival of second instars was lower than the first and the third instars. *P. solenopsis* nymphs were light yellowish in colour at hatching and a day after hatching the body was covered with white dusty. The female passed through three nymphal instar stages, while male has four nymphal instar stages. The crawlers per female were ranged from 103-695, with mean of 319±31.7 during the first year experiment (Table 1).

Table 2. Mean duration of development stages of *Phenacoccus solenopsis* under laboratory condition at Werer, 2017

		Duration (days)		Survival%
Development stages	Observation(n2)	Range	Mean ±SE	
First instar	127	3-6.	3.6±0.6	84.7
Second instar	71	3-6	4.4±0.2	55.9
Third instar	58	4-8	5.2±0.1	81.7
Male pupation	18	5-8	5.9±0.3	-
Adult				
Male	11	12-18	13.8±0.4	61.1
Female	46	14-24	19.0±0.4	90.2
Pre reproductive period	46	4-8	5.6±0.2	100
Reproductive period	35	10-17	14.2±0.4	76.1
post reproductive period	35	6-12	9.0±0.4	0
Female total life span	35	33-53	42.1±0.7	-
Adult male longevity	11	1-2.	1.6±0.1	-
Male total life span	11	13-20.	15.4±0.4	-
Fecundity	35	113-848	330.9±36.2	-

*n2= Number of observation carried out in each development stage in second years

Developmental period of the first, second and third instar nymphs were ranged 3-6, 3-6 and 4-8 with an average of 3.6±0.6, 4.4±0.2 and 5.2±0.1 days, respectively (Table 2). The total minimum and maximum nymphal developmental period was 10 and 18 days respectively with an average of 13.3±1.7 days. At early stage, the crawlers were more mobile than an adult stage. During this stage they actvelt search for suitable site of food; they mostly settle near by the leaf veins. *P. solenopsis* under laboratory conditions had intermediate developmental periods. This finding is in line with (Vennila *et al.,* 2010). The crawlers per female were ranged from 113-848, with mean of 330±36.2 during the second year experiment (Table 2).

Table 3. Overall mean duration of development stages of *Phenacoccus solenopsis under laboratory condition at Werer, 2016 and 2017*

		Duration (days)		Survival%
Development stages	Observation(n1+n2)	Range	Mean ±SE	
First instar	218	3-6	3.7±0.1	72.7
Second instar	117	3-6	4.3±0.1	53.7
Third instar	91	4-8	5.3±0.1	77.8
Male pupation	25	5-8	6.0±0.2	-
Adult				
Male	16	12-18	13.9±0.3	64
Female	72	15-25	19.1±0.3	79.1
Pre reproductive period	59	4-9	5.6±0.2	81.9
Reproductive period	56	10-17	14.1±0.3	94.9
post reproductive period	56	6-12	9.2±0.2	0
Female total life span	56	30-53	42.4±0.6	-
Adult male longevity	16	1-2.	1.7±0.1	-
Male total life span	16	13-20	15.5±0.3	-
Fecundity	56	103-848	325.8±24.5	-

*n1+n2= Sum number of observation used in both development period of the years

The first instar nymphal development period has the shortest and while the third instar has the longest (Table 3). The result contrasts the finding of Chong *et al.* (2003) and Vennila *et al*. (2010), they observed under laboratory conditions that the second instar of *P. solenopsis* had longer developmental period than other instars. The first instar nymphs were similar to that of second instar nymphs in general appearance and morphological features, except their size. Also the first instar and the third instar had nearly similar developmental period. Similar morphology was noticed by Vennila *et al.* (2010) and Muthulingam and Vinobaba, 2013. Nymphs were secreted white waxy powder and waxy fibers on dorsal side a day after first moult. The exuvium of the instar was seen near the posterior end of the abdomen.

Biology and Morphology of female *P. solenopsis*

Adult females of *P. solenopsis* is oblong in shape and light to dark yellow in colour, having two pairs of black spots on dorsal side of body with winglss (apterous), soft bodied and covered with white waxy. It also possessed a pair of nine segmented filiform antennae and three pairs of legs. Adult females emerged after the last moult of third instar.

Reproduction of *P. solenopsis* was parthenogenetic and offspring produced as crawlers and eggs through ovoviviparity (eggs retained inside female, sufficient yolk developed in embryo and nymphs hatched very soon after egg laid) and oviparity (laying eggs). The female laid eggs in silken ovisac of thin white cushiony pocket at posterior part of the abdomen. The eggs were minute, oval in shape and light yellow in colour. The adult female was covered with white swollen waxy coating layer and had two-dark stripes on the body.

Figure 3. Female life stage A. Egg in ovisac B. First moult C. Second moult D. Third moult E. Adult F. Oviposition

Total life span of female ranged from 30 to 53 days with an average of 42.4±0.6 days. Females after the final moult took about 4-9 days for reproduction with a mean pre reproductive period of 5.6±0.2 days. Reproduction and non-reproduction periods of *P. solenopsis* revealed that it varied from 10 to 17 and 6 to 12 days with an average of 14.1±0.3 days and 9.2±0.2 days respectively (Table 3). Female average developmental period from crawlers to oviposition period was taken 19.1±0.3 day, at mean temperature of 26.7-30.8°C and relative humidity 45.4-57.3%. Females showed variable patterns of fecundity with a number of crawlers produced per female ranging between 103 and 848, with a mean of 325.8±24.5. This result is supported by Vennila *et al*., (2010) who reported that 128– 812 crawlers per female at temperature of 23.3–30.2°C and relative humidity of 40.5–92.5%. Time of changing leaf in petri plates has a great influence on growth, development and reproduction of *P. solenopsis*.

Biology of male *P. solenopsis*

Adult male *P. solenopsis* nymphs formed a white silken cocoon at their third moult. The white silken fiber structure initially started from the head part and within 24hr the body totally covered. Male and females of *P. solenopsis* nymphs can be differentiated at third instar, the male secrete fiber like structure and female produced white waxy coating that used to protect from any danger including biotic and abiotic factors. Similar finding was reported by Vennila *et al.* (2010). Adult male was winged (alate) milky whitish in colour and it does not feed anything throughout its life. A pair of wings was observed in male adult with two tails like appendages on tip of the abdomen were observed (Figure 4).

The developmental period of males are longer than females, this is due to an additional molting and prepupal processes in male and the pupation period was 5-8 days with average of 6.0±0.2 days (Table 3). The male nymphs moulted four times and the female three times. The proportion of males and females are (9.8% and 90.2%) respectively from a total population used in study. They lived minimum of 1 days and maximum of 2 days with mean of 1.7±0.5 (table 1). Males are quiet small body size and antennae were ten segmented and found to be much longer than that of female antennae. The body cover with few setae and also legs and antennae covered with hair. Similar investigation carried out by Vennila *et al.* (2010) and Muthulingam and Vinobaba, 2013.

Figure 4. Male life stage: A. First instar moult, B. Second instar moulted and soon coccon developed, C. Third moult, D. Pupation, E. Adult ready to emergence F. Adult male

CONCLUSION

From this study it can be concluded that the developmental period of crawlers of *P. solenopsis* was shorter in first and second instar and longer for the third instar. The first, second and third instar nymphs body were not covered with dense waxy coating as adult *P. solenopsis*. Appling insecticide before developing dense waxy coating at pre-reproduction period is the ideal management stage since it is not covered by waxy coating. Specially during reproduction period adult female developed highly dense waxy probably to protect itself from biotic and abiotic factors.

Higher mortality occurred during crawlers than adult stage. High percent mortality was recorded from second instars; this may be due to high mobility of nymphs at this stage. This stage is also the period at which it settles on suitable positions of plants, to get suitable food sources during adult stage. So that knowing biology *P. solenopsis* possibly has a great advantage in its management. Male had lower population number than adult female and shorter life span, this revealed that they have a minor role in reproduction, although under field conditions sexual reproduction could be a possibility but in laboratory such condition did not take place. On the other hand the females of *P. solenopsis* had a great role in agricultural crop damage because they lay large number of eggs throughout parthenogenesis and both the nymphs and adults are feeding in all stage. Thus *P. solenopsis* management interventions should be focused against reproducing adult females rather than crawlers to prevent the multiplication and spread of the pest.

This study filled knowledge gaps on biology and morphology of *P. solenopsis* which is basic to identify possible vulnerable stage of the pest and its potential multiplication and thus design effective control measure. Yet, there are knowledge gaps on the role of *P.solenopsis* morphology and anatomy in resisting pesticides and assuring its survival. Thus, future studies are required on *the* anatomy, and physiology of this pest. Studying factors influencing *P. solenopsis* development and reproduction at field condition and evaluation of insecticides resistance in relation to *P. solenopsis* development period are also important to manage this pest.

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