Research Article

# Evaluation of Selected Host Plants for Basic Life Cycle Parameters of Cotton Mealybug *Phenacoccus solenopsis* Tinsley under Laboratory Conditions

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**Abstract** | In order to establish a good mass-rearing production techniques of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) (a parasitoid of the cotton mealybug) in the Laboratory, it is necessary to know the development capacity of the cotton mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae), on different host plants. Such studies would identify the best host plant for its efficient rearing. The present study investigated some basic life table parameters i.e. female life span, adult female weight, longevity and reproductive potential of *P. solenopsis* on okra (*Abelmoschus esculentus* Linn.), brinjal (*Solanum melongena* Linn.), potato (*Solanum tuberosum* Linn.), China rose (*Hibiscus rosa-sinensis* Linn) and tomato (*Lycopersicon esculentum* Mill.). The experiment was conducted under Laboratory conditions (25-32˚C, 55-65% R.H. and 12:12 light: dark, photoperiod) during 2012. Results revealed that *P. solenopsis* successfully and significantly ($P<0.05$) completed its life cycle along with highest body weight gain on China rose followed by potato. Moreover, *P. solenopsis* had the best survival (34.2 ± 3.8 days) and reproduction (7.8 ± 0.4 nymphs/adult female) when separated and kept without food from their respective host plants. China rose therefore seems to have the potential as suitable host for the rearing of *P. solenopsis* under laboratory conditions for the mass scale production of *Aenasius bambawalei*. However, further study is suggested to identify if there are any detrimental effects on the reared parasitoid i.e. a tri-trophic effects on parasitoid- *Aenasius bambawalei*.

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## Introduction

In Pakistan, the mealybug *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) was detected for the first time in 2005 on cotton and other cultivated plants in Vehari (Punjab) and Sanghar (Sindh) (Muhammad, 2007). It is a polyphagous insect feeding on a large variety of plants species including fruits, vegetables, other crops and a few ornamentals belonging to Malvaceae, Solanaceae and Leguminaceae families (Arif et al., 2009). Being a polyphagous *P. solenopsis* can be considered in Pakistan as an all-time pest on most of economic crops, vegetables and other plants cultivated in that region (Abbas et al., 2010). In addition to the direct losses that the insects can cause by sucking the phloem sap, its feeding secretions (honeydew) cause additional losses to the plants by disturbing the photosynthesis activity and induc-

ing fungal contaminations (Arif et al., 2012).

Farmers mostly rely on insecticides for the control of insect pests including mealybug (Saeed et al., 2007). However, due to inadequate public awareness on the dangers of pesticides and insufficient end-user protection; the use of pesticides is often unsophisticated and abusive causing severe human health problems, insect resistance and environmental pollution (Nadeem et al., 2014). Therefore, biological control approach was adopted which resulted in a better success to control mealybug (Kairo et al., 1997). Moreover, other studies also show that the biological control of mealybugs in the field is successful after using natural enemies previously mass-reared under laboratory conditions (Walton and Pringle, 2004).

In cotton growing areas the presence of natural enemies of *P. solenopsis* remained low due to unsophisticated and abusive insecticidal application on cotton and other crops and in India, in some cotton growing areas an accidentally introduced parasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) was discovered in 2008 (Hayat, 2009). The same parasitoid was reported as *Aenasius* sp. nov. *longiscapus* (Hymenoptera: Encyrtidae) in Pakistan in 2007 and then *Aenasius bambawalei* Hayat in 2008, it showed to be efficient to control *P. solenopsis* on cotton and other vegetables (Bodlah et al., 2010). This parasitoid was reported to be an efficient natural enemy of *P. solenopsis* associated with cotton and other vegetables (Ram et al., 2009; Mohindru et al., 2009). Bodlah et al. (2010) also reported *A. bambawalei* an aggressive parasitoid and was proven to be effective for controlling the population of *P. solenopsis* in cotton growing areas of Punjab, Sindh and Baluchistan province of Pakistan.

In developed countries, biological control has proved to be effective but in developing countries like in Pakistan, very little attention has been given to such environmental friendly technique (Ahmad et al., 2011). Also, very little is known in Pakistan about the rearing of *P. solenopsis* and its associated parasitoids, *Aenasius bambawalei.* Therefore, the knowledge regarding the life cycle of *P. solenopsis* on host plants can serve as an important basis to identify, under laboratory conditions, which plant species can be the best host to multiply efficiently *P. solenopsis* and simultaneously its associated parasitoid, *A. bambawalei,* as a biological control agent. In this context, the purpose of this study was to check the life cycle parameters of *P. solenopsis* on five host plant species namely china rose, potato, okra, brinjal and tomato, commonly found natural hosts of *P. solenopsis* in the field. All these host plant species tested were collected from the same locality within Peshawar city (Pakistan).

## Material and Methods

### Potato culture and mealybugs rearing

Potatoes' tubers were washed thoroughly in distilled water; air dried and put on moist gunny bag. Water was sprinkled daily to keep the bag moistened to encourage sprouting. After 28-30 days potatoes produced sprouts of 5-7 cm, a suitable plant stage for mealybugs rearing. The mealybugs were reared according to the procedures of Venilla et al., (2010). The sprouted potatoes were placed in plastic cages (70 cm length x 40 cm width x 40 cm height) and for aeration 0.02 mm mesh was used on both sides of the cage. For mealybug inoculation, China rose infested plant shoot containing female mealybugs (already confirmed to be *Phenacoccus solenopsis* Tinsley through taxonomic keys of Hodgson et al. (2008)) with nymphs sacs were put in the middle of the cage. After a while all the hatched and active crawlers started moving from the twig to the uninfested potato sprouts. After 40 minutes, the twigs were removed and the cage was fully closed. After 30 days, we obtained a full culture of the mealybug having each developmental stage and were used for all experiments. The insects were kept at 25-32°C, 55-65±5% R.H. and 12:12 light: dark photoperiod. The experiments were conducted in Entomology Section, Agricultural Research Institute Tarnab Peshawar-Pakistan.

The experiment was conducted following the procedure described by Aheer et al. (2009) and Venilla et al. (2010). One washed and air-dried young leaf of the 5 host plants (Table 1) (a single leaf per plant species used for each Petri dish) with the petiole covered by cotton swab soaked previously in distilled water was kept in the middle of each Petri dish (12.5 cm diameter). The ovisacs/nymph-sacs from the mass-reared colony of mature females were removed carefully with soft camel hair brush and left in a small container to collect thereafter the newly hatched crawlers. Fifty newly hatched mealybugs were deposited on each leaf of each host per Petri dish and replicated 5 times per host plant species tested (n=5). For each Petri dish, each host plant was changed every two days to provide fresh food to the mealybugs. Then, the developmental

**Table 1:** *Effect of different host plants on different life periods of Phenacoccus solenopsis females (means[1] ± SE, n=5)*

| Host plants | Total nymphal and pre-reproductive period (days) | Reproductive period (days) | Post-reproductive period (days) | Total life span (days) |
|---|---|---|---|---|
| Okra | 18.8 ± 0.4ab | 17.0 ± 0.4cd | 1.8 ± 0.4a | 37.6 ± 0.9c |
| Tomato | 19.6 ± 0.5a | 16.2 ± 0.4d | 2.0 ± 0.3a | 37.8 ± 0.8c |
| Potato | 18.0 ± 0.4bc | 20.8 ± 0.6b | 2.2 ± 0.4a | 41.0 ± 0.71ab |
| Brinjal | 18.2 ± 0.4bc | 18.2 ± 0.4c | 2.4 ± 0.4a | 38.8 ± 0.9bc |
| China rose | 17.4 ± 0.5c | 22.2 ± 0.4a | 2.4 ± 0.2a | 42.0 ± 0.9a |

*[1]Means in each column followed by the same letters are non-significant at 5% level, using LSD test following ANOVA.*

time (days was recorded daily under binocular microscope. The following parameters were then recorded: duration of the pre-reproductive period (days), duration of the reproductive period (days), duration of the post reproductive period (days) for females. This procedure was repeated 5 times for each host plant (n=5). Similarly, before turning into adults, two female mealybugs per Petri dish were separated (i.e. a total of 10 females from each host plant tested) and weighed individually with a micro balance (Libror AEG-120. Shimadzu-Japan).

### Female fecundity potential

To determine the reproductive potential, 50 newly hatched nymphs were placed on a single leaf of each host plant species separately in a Petri dish and before entering to pre-reproductive stage, five individuals per host plant were selected and reared separately on the same host plant to the adult stage and then dissected one by one under a binocular loupe into 2% saline water. The female fecundity was estimated by counting the total number of developed eggs inside the ovaries.

### Female's fecundity and longevity without food

From each host plant, five female mealybugs when moulting to third instars were selected and kept individually in Petri dishes (5 cm diameter) without any plant leaf or food. The insects were observed daily under the binocular microscope to check their development and any ovi-sac/nymph-sac formation.

### Statistical analysis

Data obtained where needed were log-transformed for normalization and then subjected to analysis of variance (ANOVA) through CRD (Completely Randomized Design). Untransformed results are presented in the tables. All means were separated by LSD (Least Significance Difference) at 5% level. All statistical tests were done using STATISTIX 8.1 package.

## Results and Discussion

### Female life span

The host plants tested influenced significantly the pre-reproductive, reproductive periods and total longevity (life span) of *P. solenopsis* females (Table 1). The results indicated that mealybugs exhibited the highest pre-reproductive period-nymphal period (total nymphal period) on tomato and the lowest on China rose (F=3.50 and p=0.0254). Similarly the reproductive period was significantly high on potato and China rose followed by brinjal and lowest on tomato and okra (F= 33.70; p= 0.0001). The post reproductive periods did not differ significantly between plant species (F= 0.57; p= 0.6882) while life span differed significantly among host plants where the longest life span was recorded on China rose and potato followed by brinjal and the shortest on okra and tomato (F= 5.31; p= 0.0044 for the total life span).

Host plant species are an important factor in the development of Mealybugs; the development of the mealybugs were taken as a sign of the quality of that host plant to serve as a food source for its development. Most of our results are in agreement with Patil et al. (2011) and Rashid et al. (2012) but the life cycle duration of both females and males in our study lasted longer than their studies. This is probably because our experiments were conducted at lower temperatures (25 to 32°C) that those of Rashid et al. (2012) which was, for example, around 40°C. It is well known that at lower temperatures developments and growth rates occur at slower rate than at high temperature (Jarosik et al., 2004). Similarly Patil et al. (2011) demonstrated that the most suitable rearing temperature for *M. hirsutus* was 38°C while Rashid et al. got maximum results on 40°C, the difference of 2°C could be the different mealybug spp used in both the experiments. It has been also recorded in nature that an increase of temperature of 2°C can induce one to five extra gen-

erations of mealybugs per season (Patil et al., 2011; Yamamura and Kiritani, 1998). Moreover, at lower temperatures the mealybugs crawlers or immature stages cannot convert completely their food into the body tissues, then causing a decrease of the growth rate which consequently increase their developmental time (Atkinson and Sibly, 1997). Females *M. hirsutus* reared at 25°C produced significantly fewer eggs and reduced numbers of crawlers (Patil et al., 2011).

### Adult female weight

Similarly, female reared on different hosts exhibited different trends for weight gain i.e. female adult gain significantly high weight on China rose and lowest on tomato (F=26.7; p=0.0001) statistical analysis showed a positive correlation (r = 0.7085) between fecundity (number of eggs per female dissected and the weight gain of the mealybug reared on different host plants (Table 2).

**Table 2:** *Effects of host plants on Phenacoccus solenopsis adult female weight (mg), no of eggs and percent female reared on different host plants*

| Host plants | Female weight (mg) | No. of eggs per mealybug dissected | Female (%) |
|---|---|---|---|
| Okra | 3.40 ± 0.34c | 168.8 ± 16.9b | 58.26 ± 2.89 b |
| Tomato | 2.71 ± 0.23c | 106.4 ± 5.5c | 65.36 ± 2.77 ab |
| Potato | 5.78 ± 0.46b | 177.2 ± 13.8b | 67.60 ± 2.39 a |
| Brinjal | 5.70 ± 0.47b | 131.4 ± 8.0bc | 69.38 ± 2.61 a |
| China rose | 0.084 ± 0.07a | 297.8 ± 26.3a | 66.26 ± 1.81 a |

[1]*Means in each column followed by the same letters are non-significant at 5% level, using LSD test following ANOVA*

### Longevity and reproduction without food

The longevity of adult mealybugs without food differed significantly according to host plant from which the insect is coming from. Figure 1 shows the longevity of females surviving without food when they have been removed from their respective hosts. It indicates that mealybug reared previously on China rose survived significantly longer than mealybugs reared before in another plant species (F=5.12; p=0.0052). In contrast, the mealybugs coming from tomato survived significantly less. Moreover, the total number of crawlers/nymphs produced by such mealybugs (without food) also differed significantly between different types of mealybugs according to their host plant origin (F=08.77; p=0.0003). The starved mealybugs lived significantly longer when they came from China rose than from other plant species. A similar influence of the host plant origin on starved mealybugs longevity was reported by Aheer et al. (2009). Similarly, in another such study Patil et al. (2011) also found that mealybugs *Maconellicoccus hirsutus* reared on China rose exhibited greater adult longevity. Similarly Table 2 shows the reproductive potential of *P. solenopsis* females reared on different host plants. The mealybugs reared on China rose exhibited significantly F=22.00; p=0.0000 highest reproduction in terms of number of eggs per female dissected. In contrast, the mealybugs exhibited a significantly lowest reproductive capacity on tomato. In the same way female sex ratio of mealybug was also significantly F=2.87; p=0.0496 differed among different host plants i.e. mealybugs reared on okra have significantly lowest female ratio followed by tomato while the highest females were recorded on brinjal but statistically not different from the female ratio recorded on china rose, potato and tomato among different host plants. Similar study also reported by Boavida et al. (1995) that Mealybug *Rastrococcus invadens* (Sternorrhyncha: Pseudococcidae) field sex ratios were observed as highly variable on different host plants when studied during 1988-1992. Considering all the parameters evaluated in our study, we observed that China rose were found to be the most suitable host plant.

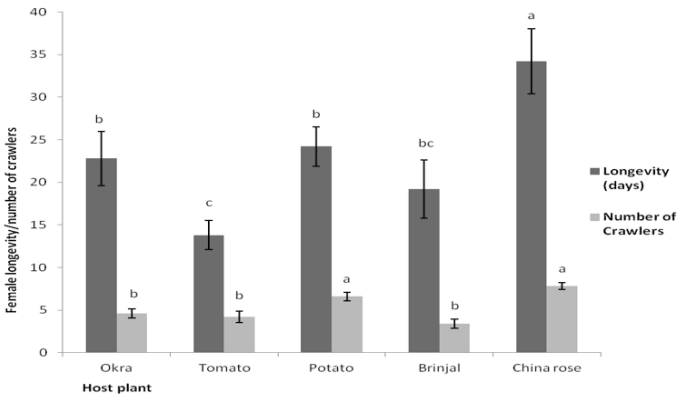


**Figure 1:** *Female longevity and number of crawlers (±SEM) produced by females without food after separating from their different host plants. Bars topped up by the same letters are non-significant at 5% level, using LSD test following ANOVA*

## Conclusions

Based upon the optimal values for the life cycles parameters evaluated for *P. solenopsis*, the availability in the natural environment and for prolonged durability the China rose seems to be a potential host for mass-rearing purposes of *P. solenopsis* under laboratory and green house conditions. However, for the con-

firmation of this study further research is suggested on these hosts to exploit that which factors i.e. chemicals of these host plants contribute more to these life cycles parameters and also for other biological and reproductive traits of mealybugs for its efficient rearing and for mass scale production of biological control agents that could be used against the mealybug in the field. Moreover, the field performance of biological control agents reared from mealybugs maintained on different plant species needs to be evaluated before mass production systems are established.

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