



EFFECT OF DIFFERENT MATING EXPOSURE TIMINGS ON THE REPRODUCTIVE PARAMETERS OF PAPAYA MEALYBUG, *PARACOCCLUS MARGINATUS* (HEMIPTERA: PSEUDOCOCCIDAE)

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ABSTRACT

The papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) is a notorious, widespread and polyphagous insect pest. Recently, its infestation has been reported in the coastal areas of Pakistan, especially on papaya, *Carica papaya*. Considering its potential threat to fruits and vegetables all over Pakistan, the study was undertaken to evaluate the effect of various mating exposure timings i.e., 24 hour, 48 hour and 72 hour on its reproductive parameters. Results obtained showed that fecundity rate of *P. marginatus* after 24, 48 and 72 hours mating durations were 112.60 ± 3.87 eggs/female, 150.60 ± 7.92 eggs / female and 170.80 ± 12.43 eggs/ female, respectively. Oviposition and post-oviposition duration was comparatively higher at maximum mating exposure timings, whereas no effect of exposure timings was recorded on pre-oviposition duration. Females and male went through three and four nymphal instars to become adult, where female live longer than males. The information obtained in the study could provide a base to understand biology of *P. marginatus* to restrict their spread and damage in Pakistan.

Keywords: Mating, Exposure timing, Fecundity, Papaya mealybug, *P. marginatus*

INTRODUCTION

Papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink 1992 (Hemiptera: Pseudococcidae) is native to Mexico. Afterwards, it distributed to the Caribbean in 1994 (Walker *et al.*, 2006) and Florida, USA in 1998 (Walker *et al.*, 2006). In 2002, it was reported in the Pacific region from Guam (Meyerdirk *et al.*, 2004), Palau in 2003 (Muniappan *et al.*, 2006) and from the Island of Maui, Hawaii in 2004 (Heu *et al.*, 2007). Within short duration, papaya mealybug was found in many Asian countries including India, Sri Lanka, Malaysia, China and others (Hettiarachchi, 2009; Hettiarachchi and Silva, 2009; Muniappan *et al.*, 2009, 2011; Chen *et al.*, 2011; Mastoi *et al.*, 2011; Shylesha *et al.*, 2011; Meyerdirk *et al.*, 2004).

Papaya mealybug is a small, yellowish polyphagous insect pest (Ben-Dov *et al.*, 2015). Among host plants, papaya mealybug caused very serious damage to papaya, *Carica papaya* in many countries of the world (Helemul, 2013). It

caused damage to papaya by sucking the cell sap from plant tissues using its piercing and sucking mouth parts (Sharma, 2013). Due to heavy infestation of mealybug, leaf become yellow, curl and fall off, deformation of buds, and falling of unripened fruits (Tanwar *et al.*, 2010; Suganthi *et al.*, 2012; Kirsur *et al.*, 2014; Ben-Dov *et al.*, 2015). Papaya mealybug also excrete honeydew which promote the development of sooty mold, which cover the essential plant parts (Leaf, Stem, Fruit) impeding photosynthesis. This resulted in chlorosis, leaf deformation, and dropping of leaves along with development of thick white wax on fruits, stems and leaves that become inedible (Muniappan, 2011). Papaya mealybug reproduces bisexually as clusters of cotton-like masses on the above-ground portion of plants (Tanwar, 2010).

Recent invasion of papaya mealybug in Pakistan, create serious concern for papaya and other fruit crops. Therefore, it has become impetuous to understand the biology of this pest under local environment. Accordingly, this laboratory study was carried out to understand the effect of various mating

exposure timings on the reproductive potential i.e., pre-oviposition, oviposition and post-oviposition periods along with fecundity. The results of the study could provide a base to growers and researchers to control the spread and damage of this invasive mealybug.

MATERIALS AND METHODS

Study site

The research was carried out in the laboratory of the Insect Pest Management Programme (IPMP), National Agricultural Research Center, Islamabad, Pakistan (NARC).

Collection of papaya mealybug, *P. marginatus*

Papaya mealybug was collected from during survey of NARC. Freshly emerged males, females and their eggs were collected from their preferred hosts.

Rearing of Papaya mealybug, *P. marginatus*

The laboratory culture of *P. marginatus* was maintained on Pumpkins. Pumpkins was thoroughly washed with tap water and air dried. Air dried pumpkins were transferred to the trays containing sterilized sand and were infested with five to six ovisacs of *P. marginatus*. To maintain the colony of *P. marginatus*, 5-10 pumpkins were infested every week with ovisacs of *P. marginatus*. During rearing, temperature and relative humidity were maintained at $25\pm 2^{\circ}\text{C}$ and $60\pm 5\%$, respectively with photoperiod of 12:12 (L:D).

Experiment setup

Selection of host plant

Brinjal were selected as host plant and 20 petiolated Brinjal leaves were removed and kept in petridishes. A hole was made in each petridish so that the petiole can pass in and dipped into the water to prevent the desiccation of leaf. Three treatments i.e. T1 (24 hour), T2 (48 hour) and T3 (72hour) were maintained during the experiment. Each treatment was replicated with five times.

Treatment structure

Each leaf of brinjal was inoculated with 20 eggs of *P. marginatus* using a camel hair brush. The eggs used in all four treatments were collected from rearing culture of *P. marginatus*. All treatments were observed daily for the hatching of eggs. The intervals between each molt were observed via exuvia (shedding skin) on the leaves using microscope (40 X magnification). Exuvia were removed after each molt to check the next molt. Morphological examining of all instars was done under compound microscope. The number of days in each stage was counted to construct the individual life cycle of male and female *P. marginatus*.

After adult emergence, mealybugs were transferred to new Petri dishes (containing leaves lined with water) at the rate of 5 males and 5 females for 24 hours (T1), 48 hours (T2) and 72 hours (T3) for monitoring the procreative periods (Pre-oviposition, Oviposition, and Post-oviposition periods).

After the respective exposure timings, males were removed from the Petri dishes to observe the ovipositional behavior of female *P. marginatus*. Number of eggs laid by each female in individual treatments were counted until the death of females to calculate the fecundity under the influence different

exposure timings.

The data obtained in the experiment was analyzed by Analysis of Variance (ANOVA) whereas means with significant differences were compared using the Least Square Difference (LSD). All analyses were performed using SAS 9.4 Statistical software.

RESULTS AND DISCUSSION

Life cycle of papaya mealybug, *P. marginatus*

Summarized life cycle of *P. marginatus* is given in Table 1.

Eggs: The eggs were rhombus in shape and greenish yellow in color (Fig. 1a). The females laid eggs in an ovisac on the ventral side of the body. Incubation period of eggs was recorded 4.9 ± 1.50 days.

First Instars Crawlers: Freshly emerged first instar nymph were rhombus in shape and light yellow in color (Fig. 1b). Developmental time of first instar on brinjal was calculated as 4.1 ± 1.2 days.

Second Instars Female: Second instar female nymph were rhombus shaped and yellow colored (Fig. 1c). second instar female nymph took 4.4 ± 1.07 days to convert itself to third instar.

Third Instars Female: Third instar females (Fig. 1d) were also rhombus in shape and yellow in color and complete its development within 6.2 ± 1.3 days.

Adult female: The color of adult female was yellow and it was covered with white waxy coat. It also contained a series of waxy caudal filaments of small size around its body. The female longevity was recorded as 19.6 ± 0.18 days.



Fig. 1

Papaya mealybug, (a) eggs; (b) first instar; (c) second instar female; (d) third instar female

Second Instars Male: The second instar male nymph of *P. marginatus* (Fig. 2a) was pinkish in color with yellowish pattern. It completed its development in 4.3 ± 1.9 days.

Third Instars Male (Pre-Pupa): Third instar male *P. marginatus* nymphs (Fig. 2b) were pink in colored and diamond shaped. Duration of third instar male was recorded 3.2 ± 0.95 days.

Fourth Instars Male (Pupa): At the end of developmental period of third instar, males nymph started making cocoons which are cylindrical in shape and white in color (Fig. 2c). It took 5.6 ± 0.43 days to change into matured adult.



Fig. 2
Male papaya mealybug; (a) second instar; (b) third instar (Prepupa); (c) fourth instar (Pupa); (d) adult.

Table 1

Life stages of *P. marginatus* from egg to adult.

Sex	Eggs (Days)	First instar (Days)	Second instar (Days)	Third instar (Days)	Fourth instar (Days)	Adult (Days)	Total life cycle duration(Days)
Female	4.9±1.5	4.1±1.2	4.4±1.07	6.2±1.3	-	19.6±0.18	39.2±1.12
Male			4.3±1.9	3.2±0.95	5.6±0.43	2.5±0.5	24.6±1.03

The description of various nymphal male and female instars and adult male and females are in accordance with those given by Miller and Miller (2002), Tanwar (2010) and Suganthy *et al.* (2012). In this study, male and females completed their life cycle in 39.2±1.12 and 24.6±1.03 days, respectively. Suganthy *et al.* (2012) reported that female *P. marginatus* took 39.33 ± 2.53 days to complete its entire life cycle, whereas 24.00 ± 1.73 days were taken by males to complete its life cycle. The nymphal duration recorded for 1st, 2nd and 3rd instars were observed as 4.00 ± 1.00, 3.67 ± 0.58 and 5.00 ± 1.00 days, respectively. Oviposition period of 4.8±0.9 days along with nymphal duration of 11-15 days for females and 17-23 days for males has also been reported (Mishra, 2011). Moreover, comparatively longer life cycle duration has been reported for female *P. marginatus* than males (Amarasekare *et al.*, 2008; Mishra, 2011, Suganthy *et al.*, 2012) and all above mentioned findings confirmed the findings of this study.

Reproductive parameters of *P. marginatus* at different mating exposure timings

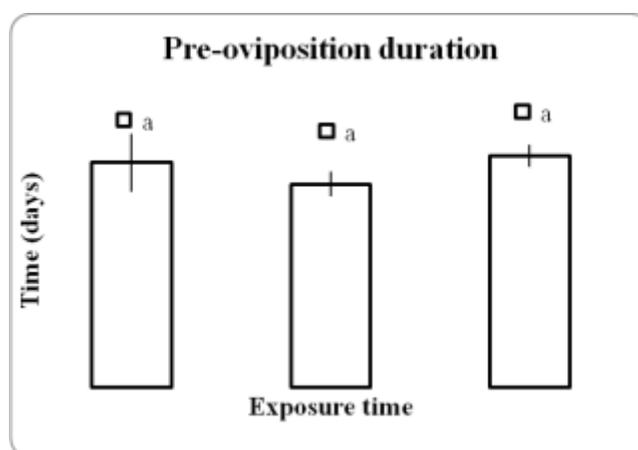
Pre-Oviposition duration

No significant effect ($P > 0.05$) of exposure timings was recorded on the pre-oviposition period of female *P. marginatus* (Fig. 3). However, females took relatively higher time to start oviposition in 72 hours exposure time for mating (4.74±0.22 days) in comparison to 24hours (4.60±0.60 days) and 48 hours (4.16±0.26 days).

In this study, females and males completed their life cycle within 39.2±1.12 and 24.6±1.30 days, respectively.

Adult male:

In comparison to females, male *P. marginatus* has an elongated body around 1 mm in length. The wings in males are about the same length as their body with small basal vein (Fig. 2d). Male remained alive only 2.5±0.5days.



Oviposition duration

Various exposure timings of mating showed a significant impact ($P < 0.05$; Fig. 4) on the ovipositional duration of *P. marginatus* as the highest oviposition duration was recorded in the highest exposure time i.e., 72hour (7.84±0.40 days), followed by 48hour (7.36±0.39 days). The least oviposition duration was recorded in 24hour (5.25±0.29 days), that was the least duration allowed to *P. marginatus* for mating. Findings of studies of Suganthy *et al.* (2012) confirmed that *P. marginatus* took 7.33 ± 0.58 days for its oviposition and it is in accordance with the findings of this study.

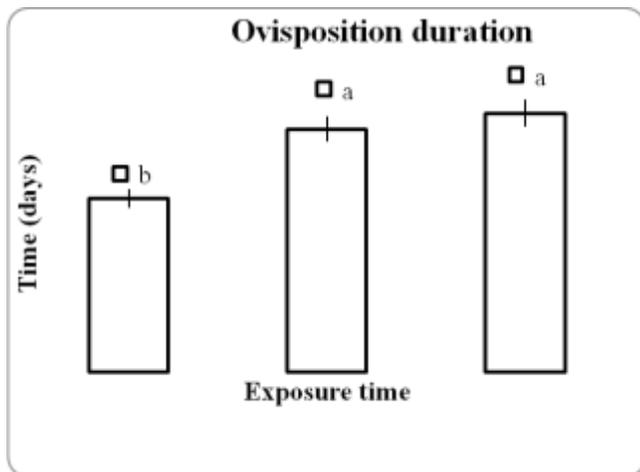


Fig. 4

Effect of different exposure timings of mating on the ovisposition duration of papaya mealybug, *P. marginatus*

Post-oviposition duration

Similar to oviposition duration, exposure timings also showed significant impact ($P < 0.050$) on the post-oviposition duration of female *P. marginatus* (Fig. 5). They highest post-oviposition duration of female *P. marginatus* was recorded in 48hour exposure timings (4.24 ± 0.71 days) followed by 72hour (3.40 ± 0.37 days) and 24hour (3.08 ± 0.44 days) exposure timings.

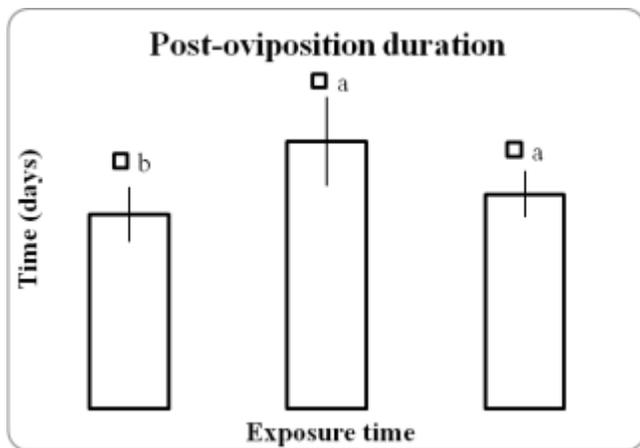


Fig. 5

Effect of different exposure timings of mating on the post-oviposition duration of papaya mealybug, *P. marginatus*.

Fecundity

Fecundity of female *P. marginatus* was significantly affected ($P < 0.05$) by the different exposure timings of mating (Fig. 6) as the highest fecundity (170.80 ± 12.43 eggs / female) were recorded in the highest exposure timing of mating i.e., 72 hour. The least exposure timings of 24hour showed the minimum fecundity of female *P. marginatus* (112.60 ± 3.87 eggs / female) followed by 48 hour (150.60 ± 7.92 eggs /

female). Suganthy *et al.* (2012) observed fecundity of *P. marginatus* at 329.33 ± 20.03 eggs. Another study by Mishra (2011) also confirmed the fecundity of *P. marginatus* between 197 to 436 eggs per female inside ovisacs. Therefore, except Suganthy *et al.*, (2012), other studies endorsed the findings of this study. However, the variable pattern of fecundity in *P. marginatus* may be due to different host plant species and variable environmental conditions (Amarasekare *et al.*, 2008).

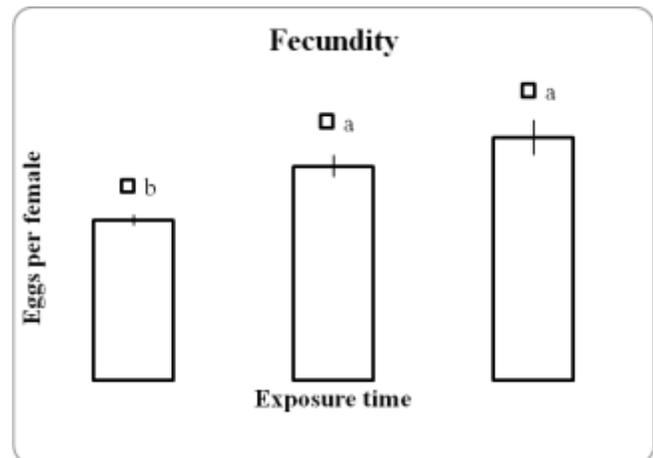


Fig. 6

Effect of different exposure timings of mating on the fecundity of papaya mealybug, *P. marginatus*

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