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EFFICIENCY OF *Acerophagus papayae* ON DIFFERENT HOST STAGE COMBINATIONS OF PAPAYA MEALYBUG, *Paracoccus marginatus*

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Acerophagus papayae is a koinobiont endoparasitoid of the invasive papaya mealybug, *Paracoccus marginatus* and has been introduced as a classical bio-control agent of the mealybug in many countries. Considering the importance, parasitism efficiency of *A. papayae* against various life stages of *P. marginatus* was conducted in choice experiments. Results suggested that *A. papayae* showed higher parasitism efficiency towards third instar female nymphs and adult female *P. marginatus* in comparison to second instar male. However, second instar male nymphs were more preferred in comparison to second instar female nymphs. *Acerophagus papayae* exhibited a highly significant sex-biased development ratio as a female dominant progeny emerged while feeding on female hosts and vice versa. No difference was recorded in the developmental time of male and female *A. papayae*, however, females matured one day later than males. The only gregarious behaviour of *A. papayae* was recorded on adult female *P. marginatus* as more than one adult parassitoid emerged from a single host. Study results suggested that *A. papayae* has a tremendous potential to be utilized in classical biological control programs against *P. marginatus* as it showed more preference towards female hosts; hence not only reducing available pest population but also will affect the pest population of next generation.
Keywords: *Acerophagu spapayae*, papaya, mealybug, *Paracoccus marginatus*, parasitism

INTRODUCTION

Acerophagus papayae Noyes and Schauff, 2003 (Hymenoptera: Chalcidoidea: Encyrtidae) is a koinobiont endoparasitoid of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink 1992 (Hemiptera: Pseudococcidae). It is native to same of Mexico from where *P. marginatus* originated (Noyes and Schauff, 2003). In 2008, a team of IPM CRSP researchers first time reported *P. marginatus* from Indonesia and India, causing serious damage to papaya and warned about its potential presence and spreading in the neighboring countries (Muniappan *et al.*, 2008). However, (Mastoi *et al.*, 2011) first time confirmed the presence of *P. marginatus* in papaya orchards of Malaysia along with its parasitoids i.e., *A. papayae, Chartocerus sp. (*Signophoridae: Hymenoptera), *Marietta leopardine* (Aphelinidae: Hymenoptera) and *Cheiloneurus sp.* (Encyrtidae: Hymenoptera).During last decades, *A. papayae* is widely introduced in many countries of the world i.e., Guam, Palau, Puerto Rico, Sri Lanka, Dominican Republic and India to manage the populations of invasive *P. marginatus* (Walker *et al.*, 2003; Meyerdirk *et al.*, 2004; Muniappan *et al.*, 2006; Shylesha *et al.*, 2010; Galanihe *et al.*, 2010). However, for continuous and successful augmentative biological control programme of *P. marginatus* requires mass rearing of *A. papaya* in large enough populations to suppress mealybug outbreaks. Accordingly, knowledge of the most suitable host stage to support growth and multiplication of the parasitoid is vital for the mass rearing (Rehman and Powell, 2010).

Large hosts are considered of better quality as they contain more food resources to support many parasitoid offspring, whereas only a single parasitoid can survive in a small host (Vinson, 1976). Moreover, a female parasitoid from the Hymenoptera has the ability to influence offspring sex ratio at oviposition considering the size of host as larger hosts are supposed to support a female biased offspring ratio (King, 1987). Although mealybug biological stages usually overlap in the field, data on host stage preference of *A. papayae* and dependent sex-ratios will ensure synchronization with the most preferable host stage availability/abundance at the time of release and thus the optimum parasitoid offspring fitness.

Previous studies on parasitism of *A. papaya* on *P. marginatus* were conducted without differentiating the male and female mealybug instar nymphs (Amarasekare *et al.*, 2009, 2010, 2012). Thus, no information is available on the relative parasitism and gregarious behaviour of *A. papayae*on male instar nymphs of *P. marginatus* except Mastoi *et al.* (2014a), who studied percent parasitism and sex ratio of *A. papaya* on various male and female stages of *P. marginatus.* Better understanding of host stages of prey for parasitism, sex ratio, developmental time and gregariousness of *A. papayae* will help to understand population dynamics of both host and its parasitoid. Therefore, studies were undertaken to evaluate relative preference of different male and female *P. marginatus* stages under choice condition to find out the best host stage to support development and mass rearing of *A. papayae*

MATERIALS AND METHODS

***Study Site*:** The experiment was conducted at Entomology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia at an ambient environment of 26 ± 2 °C, 60±5% relative humidity with 12:12 (LD) photoperiod.

***Rearing of P. marginatus*:** Un-ripe green papaya fruits were used to maintain the culture of *P. marginatus* in rearing plastic cages (24h"x12l"x12w"). Adult gravid females (10-12 females per papaya fruit) were in the containers introduced for the rearing of *P. marginatus.*

***Rearing of A. papayae*:** Initial collection of *A. papayae* was done from mealybug mummies collected from infested papaya plants, *Ladang*-2 and 10, Universiti Putra Malaysia. Only highly infested papaya leaves were brought to Entomology Laboratory and placed in muslin cloth covered plastic cages for the emergence of adult parasitoids. Adult *A. papayae* emerged were separated with the help of insect aspirator for further rearing. Second and third instar *P. marginatus* nymphs were offered as host to *A. papayae*to get pure laboratory culture of parasitoid. Four to five stripes of 80% honey solution were provided to *A. papaya* to enhance its longevity and fecundity. Mealybug nymphs mummified with *A. papaya* were collected every week and placed in glass vials for the emergence of adults.

***Experimental setup*:** The experiment was conducted in a choice situation by offering three different combinations of *P. marginatus* to *A. papayae.* Host stages offered in choice experiment were (1) second instar male with second instar female; (2) second instar male with third instar female; and (3) second instar male with adult female. The identification of second instar male and female mealybugs were based on their color as those who changed their color to pinkish were separated as males, while who those did not change color were separated as females. Moreover, according to Miller and Miller (2002), the average size of second instar male nymph was 0.6 mm and for second instar female nymph it was 0.7 mm. In a Petri dish, five individuals of each combination stage were introduced on hibiscus leaf to settle down. Hibiscus leaf was placed on a cup of water so that the petiole to be immersed for maintaining leaf freshness. A single mated female *A. papayae* was then released for 24 hours. 80% honey solution was offered as food to parasitoid. To avoid the escape of mealybugs or parasitoid, sides of Petri dish were sealed using parafilm. After 24 hours, individuals of each host stage were separated to two different Petri dishes for further development. Individuals of each host stage were examined daily and upon mummification, the mummies were collected and isolated in separate vials until adult parasitoid emerged. The parasitism rate, sex ratio, developmental time from egg deposition to adult eclosion, and gregariousness behaviour of *A. papayae* on each combination of host stages of *P. marginatus* was recorded.

***Data analysis*:** The experiment was conducted in a complete randomized design with each treatment replicated ten times. Data collected for different parameters of parasitism rate, sex ratio, developmental time and gregariousness of parasitoid were analysed using student t-test. All the statistical analyses were done using SAS 9.4 (SAS Institute Inc. 2013).

RESULTS

***Percent parasitism*:** Results of the percent parasitism are given in Table 1. In choice experiment of second instar male vs. second instar female nymphs, significantly higher (P ≤ 0.05) percent parasitism of *A. papaya* was recorded on second instar male. *Acerophagus papayae* showed significantly higher percentage parasitism (P ≤ 0.001) in female third instar nymphs compared with male second instar nymphs. No significant difference (P ≥ 0.05) was recorded in percent parasitism of *A. papaya* in choice between second instar male nymphs and adult female.

Table 1. Percent parasitism of *A. papayae* in combination of two host stages of *P. marginatus*.

Host stage	Mean ± SE	t-value	Significance level
Second Instar Male	57.10±4.17 a	2.40	0.027
Second Instar Female	42.90±4.17 b		
Second Instar Male	42.40±2.00 b	-5.38	< 0.000
Third Instar Female	57.60±2.00 a		
Second Instar Male	45.05±3.88 a	-1.81	0.088
Adult Female	54.95±3.88 a		

***Sex ratio of A. papayae*:** Results of the sex ratio indicated a significantly higher (P ≤ 0.05) sex based adult emergence of *A. papaya* from the respective male and female hosts of *P. marginatus*. A higher male parasitoid emergence was observed from second instar male of *P. marginatus*; however, female *A. papayae* emergence was higher from second instar female nymphs of the mealybug. Only 12.33 ± 6.40% males of *A. papayae* were recorded from second instar female nymphs, whereas female parasitoids which emerged from second instar male nymphs were 21.67±6.35%. Similar results were obtained in combination of second instar male vs. third instar female and second instar male vs. adult female *P. marginatus*, as significantly higher males emerged from second instar male (Table 2).

Table 2. Sex ratio (male: female) of *A. papayae* in combination of two host stages of *P. marginatus*.

Host stage	Mean ± SE (male: female) (n=5:5) resultant parasitoids	t-value	Significance level
Second Instar Male	78.33a :21.67b ±6.35	7.32	< 0.000
Second Instar Female	12.33b:87.67a±6.40		
Second Instar Male	96.67a:3.33b ±3.33	19.44	< 0.000
Third Instar Female	5.00b:95.00a ±3.33		
Second Instar Male	88.33a:11.67b ±6.11	8.65	< 0.000
Adult Female	21.92b:78.08a ±4.65		

*Different letters in the same row indicate significant difference (P < 0.05) in male and female emergence of *A. papayae*from the particular host

***Developmental time of A. papayae*:** No significant (P ≥ 0.05) difference was observed in the developmental time of both male and female *A. papayae* to attain maturity in different combinations of male and female hosts . However, females took comparatively more developmental time (14.33-14.57 days) to mature as compared to males (13.47-13.67 days).

Table 3. Developmental time of male *A. papayae* in combination of two host stages of *P. marginatus*.

Host stage	Mean±SE (days)	t-value	Significance level
Second Instar Male	13.47±0.18a	-0.518	0.615
Second Instar Female	13.67±0.33a		
Second Instar Male	13.02±0.10a	-1.67	0.12
Third Instar Female	13.50±0.50a		
Second Instar Male	13.01±0.19a	-1.388	0.184
Adult Female	13.39±0.19a		

Table 4. Developmental time of female *A. papayae* in combination of two host stages of *P. marginatus*.

Host stage	Mean ± SE (days)	t-value	Significance level
Second Instar Male	14.33±0.21a	-1.19	0.254
Second Instar Female	14.57±0.09a		
Second Instar Male	14.00±0.00a	-0.99	0.346
Third Instar Female	14.33±0.10a		
Second Instar Male	14.00±0.00a	-0.592	0.566
Adult Female	14.14±0.12a		

***Gregarious behaviour of A. papayae*:** The observations on gregariousness of *A. papayae* exhibited that it only showed gregarious behaviour in adult female *P. marginatus* where two parasitoids emerged from a single female. Among remaining treatments, *A. papaya* showed a solitary behaviour .

Table 5. Gregarious behaviour of *A. papayae* in combination of two host stages of *P. marginatus*.

Host stage	Mean ± SE	t-value	Significance level
Second Instar Male	1.00±0.00a	-	-
Second Instar Female	1.00±0.00a		
Second Instar Male	1.00±0.00a	-	-
Third Instar Female	1.00±0.00a		
Second Instar Male	1.00±0.00b	21.09	< 0.001
Adult Female	2.00±0.21a		

DISCUSSION

The selection of any parasitoid for biological control programs primarily depend on the efficiency of parasitizing various host stages while maintaining their sex ratio (Vinson, 1976). In this study, third instar female and adult female of *P. marginatus* were more preferred by the *A. papaya* indicating that the parasitoid preferred the larger sized host. However, Amarasekare *et al.* (2010) have reported the highest percent parasitism of *A. papaya* on second instar *P. marginatus* without sex differentiation, whereas the least parasitism was recorded in females. Studies have shown that second instar males and females *P. marginatus* comparatively possessed small body sizes, hence cannot provide enough nutrients to young ones of their parasitoids for their survival and growth (Miller and Miller (2002). The lower parasitism rate of *A. papaya* on *P. marginatus* females may be due to their defensive behaviour and accordingly, parasitoid needs more time to handle the bigger hosts (Bertschy *et al.*, 2000).

The successful establishment of any parasitoid is dependent on its life-long fertility and female based sex ratio (King, 1987). Findings of this study reported that the sex ratio of *A. papayae* depends on the host stage as male-biased sex ratio was found in second instar male *P. marginatus*, while, female biased sex-ratio was recorded in all female treatments of *P. marginatus* used. Amarasekare *et al.* (2010) also recorded higher proportion of progeny females of *A. papayae* in third instar female and adult female *P. marginatus*. In a no-choice experiment conducted by Mastoi*et al.* (2014b), a highly sex based male and female ratio was recorded from second instar male *P. marginatus* and third instar female nymphs and adult females of *P. marginatus*, respectively. Another parasitism study of *A. bambawalei* on mealybug, *P. solenopsis* showed higher emergence of females and male parasitoids from third and second instar nymphs, respectively (Fand *et al.*, 2010). Many previous studies also highlighted that smaller hosts support a male based progeny, whereas larger hosts support a female biased progeny (King, 1987; Karamaouna and Copland, 2000; Amarasekare *et al.*, 2010).

The results of this study also highlight that on average, female *A. papayae* completed their life cycle one day later than males. Similar results have been obtained by Amarasekare *et al.* (2010). Fand*et al.* (2010) also reported that the developmental time of female *A. bambawalei* was longer than male on mealybug, *P. solenopsis*. Studies also suggested that shorter developmental time for parasitoids especially females in comparison to their host is an important factor in success of any biological control program (Greathead, 1986). Accordingly, developmental time of female *A. papaya* recorded in this study is much shorter than to its host, *P. marginatus* that averagely completed its life cycle in 25.9 days (Amarasekare *et al.*, 2008).

The parasitoid, *A. papaya* showed gregarious behaviour only on adult females as more than one adult emerged from a single host. Mastoi *et al.* (2014b) also reported the gregarious behaviour of *A. papaya* from the female *P. marginatus*in no-choice studies. The gregarious behaviour is common among many parasitoids to produce females based progenies with fewer resources utilized (Kraft and Van Nouhuys, 2013). Among other reasons of gregariousness in parasitoids includes shortage of hosts (Takagi, 1987), to avoid the immune responses of the hosts (Hegazi and Khafagi, 2008) or many other attributes of the hosts (Dorn and Beckage, 2007). Studies conducted on parasitoid *Pteromalus apum* indicated that it showed gregariousness on two of its hosts, *Melitaea cinxia* and *Melitaea athalia* in the field conditions due to their lower densities (Kraft and Van Nouhuys, 2013).

***Conclusion*:** Comparatively higher parasitism of *A. papaya* was recorded on third instar female nymphs and adult females as compared to their corresponding second instar male nymphs. Male-biased sex ratio was observed in second instar male while, female biased sex ratios were found in second instar female, third instar female and adult female *P. marginatus.* Comparatively longer developmental period was recorded for females than males. *Acerophagus papayae* showed gregarious behaviour in adult female while, solitary in second instar male, second instar female and third instar female *P. marginatus*. Thus, this study also confirmed the potential of *A. papayae* as one of the key and efficient parasitoid in managing the *P. marginatus* populations below threshold levels in Malaysia because of its ability not only to parasitize nymphal stages of *P. marginatus* as solitary parasitoid but also behaved as gregarious on adult females.

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