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RESEARCH ARTICLE

HOST PREFERENCE BETWEEN SYMBIOTIC AND APOSYMBIOTIC *PARACOCCUS MARGINATUS*, BY THE PARASITOID, *ACEROPHAGOUS PAPAYAE*

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ABSTRACT

Acerophagus papayae Noyes and Schauff (Hymenoptera: Chalcidoidea: Encyrtidae), an introduced solitary koinobiont endoparasitoid is considered as one of the efficient parasitoids for the suppression of Papaya mealybug (Muniappan *et al.,* 2006). Disruption of microbial symbiosis demanded by insect pest and symbionts-mediated manipulation of insect pest traits are the two emerging trends in insect pest control. The present study was aimed to understand the parasitic potential, emergence rate and development time of parasitoid *A. papayae* against *P. marginatus* reared on four host plants *viz*., papaya (*Carica papaya* L.), mulberry (*Morus alba* L.), brinjal (*Solanum melongena* L.) and tapioca (*Manihot esculenta* Crantz). Results revealed that invariable of the host plants, both per cent parasitization and per cent emergence were maximum in aposymbiotic ones than in endosymbiotic mealybugs. And also the parasitoids takes longer time to emerge as an adult from aposymbiotic population whereas, from endosymbiotic mealybugs comparatively it emerges soon. Yet, further study on the assessment of parasitoid fitness over several generations and simultaneous metagenomic analysis of changes in the endosymbiotic profile of mealybugs over generation after antibiotic treatment will help to explain the degree to which the essential endosymbiont adds to the accomplishment of parasitism in papaya mealybugs.

INTRODUCTION

Acerophagus papayae Noyes and Schauff (Hymenoptera: Chalcidoidea: Encyrtidae), an introduced solitary koinobiont endoparasitoid is considered as one of the efficient parasitoids for the suppression of Papaya mealybug (Muniappan *et al.,* 2006). The mealybugs are relying on endosymbionts for the essential amino acids which were found to be deficit in the sap of its host plants (Scarborough *et al.,* 2005). Mealybugs harbour a beta-proteobacterial maternally inherited primary endosymbiont, *Tremblaya princeps* and an additional gamma-proteobacterial secondary endosymbiont (Fukatsu and Nikoh 1998; von Dohlen *et al*. 2001; Thao *et al*. 2002; Kono *et al*., 2008; McCutcheon and von Dohlen 2011; Gatehouse *et al*., 2012). Our previous study on isolation of culturable endosymbionts from papaya mealybug (PMB) *Paracoccus marginatus,* using Nutrient Agar and Luria Bertani agar media yielded 19 isolates in total, belonging to five species *viz., Bacillus cluasii, B. altitudinis*, *B. siamensis*, *Serratia marcescens* and *Stenotrophomonas maltophilia* (unpublished data). Defensive role of secondary endosymbionts (whose presence is not obligate for survival and reproduction of host insect) like *Regiella insecticola* which increases the resistance to fungal pathogens in the pea aphid (Scarborough *et al.*, 2005) and *Hamiltonella defensa* that protect aphid against parasitoid wasps are very well studied (Ferrari *et al.*, 2004; Oliver et al. 2005; Vorburger et al. 2009). Researchers found that protection by *H. defense* is influenced by the presence of lysogenic bacteriophages called APSE within the *H. defensa* genome (Moran *et al.*, 2005; Degnan & Moran, 2008; Oliver *et al.*, 2008, Oliver *et al.*, 2009). There is documentation that different strains of a secondary endosymbiont may provide varying degrees of protection against parasitoids (Oliver *et al.*, 2005). Disruption of microbial symbiosis demanded by insect pest and symbionts-mediated manipulation of insect pest traits are the two emerging trends in insect pest control. Reports are stating that the complete elimination of endosymbiotic organisms by antibiotics will lead to reduced life span and insect population suppression (Douglas, 2007). Hence, it is essential to study the effect of antibiotic disruption of mealybug endosymbionts on the fitness of its parasitoid. Therefore, the present study was aimed to understand the parasitic potential, emergence rate and development time of parasitoid *A. papayae* against *P. marginatus* reared on four host plants *viz*., papaya (*Carica papaya* L.), mulberry (*Morus alba* L.), brinjal (*Solanum melongena* L.) and tapioca (*Manihot esculenta* Crantz).

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MATERIALS AND METHODS

Culturing of host insect *Paracoccus marginatus:* *Paracoccus marginatus* were collected from different host plants (papaya, mulberry, brinjal, cassava and congress grass) in farmers filed located at 11°37'35.9"N 78°28'41.1"E.

Host plants except papaya were raised in plastic pots of 5kg capacity (containing pot mixture, loamy sand, soil and vermicompost in the ratio of 1:2:1) and kept in the metallic cages and the collected mealybugs were released on to their respective host plants using camel hairbrush at the rate of three to five gravid females per plant. Two medium-sized un-ripen papaya fruits were placed in metallic cages, and three to five gravid females per papaya fruit were introduced for multiplication of culture. Colonies of *P. marginatus* was maintained at an ambient environment of 26 ± 2 °C, 60±5% relative humidity with 12:12 (L: D) photoperiod and mealybugs *en masse* obtained within 25 to 30 days of release.

Development of aposymbiotic mealybug: Aposymbiotic mealybugs were developed by adapting the method of Douglas (1996). The roots of 5 leaf stage host plants were cleaned of excess pot mixture by careful washing in tap water and the plants were transferred to distilled water containing antibiotic (Ofloxacin @ 50mg/25ml) and plants in antibiotic-free distilled water will serve as the control. One day later, the second instar mealybug nymphs were released to the plants: those on the ofloxacin-treated plants are described as aposymbiotic mealybugs and those on control plants as symbiotic mealybugs. After 2 days of treatment, all mealybugs were transferred to plants in pots under the standard culture conditions. This treatment was fully effective in disrupting the symbiotic bacteria as indicated by the absence of bacterial colonies in *in-vitro* isolation experiment. As antibiotic treatment resulted in the reproduction failure of aposymbiotic mealybug, vitamin B (100µg/ml) was supplemented to get next-generation aposymbiotic mealybug population. F1 offspring from populations under control condition and antibiotic-treated population were used for parasitization studies.

Source of parasitoid *Acerophagous papayae:* A pure culture of *A. papayae* cultured on potato sprouts was obtained from Biological control Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

Assessment of the parasitic potential of *Acerophagous papayae:* A study on the parasitic potential of *A. papayae* was carried out with different nymphal instars and adults of *P. marginatus*. The experimental arena for the study consisted of a plastic container. One side of the container was cut in the centre in a square shape (5 cm dia.) and was covered with muslin cloth for air circulation inside the container. A tender leaf of five different host plants along with a 4-5 cm long petiole was placed in each plastic container with the petiole inserted through the moist cotton kept in glass vials to keep the leaf fresh. Fifty individuals of second instar nymphs were released per container. Ten numbers of one day old single mated female of *A. papayae* was released for 24 hours to each plastic container. Cotton swab soaked with 80 per cent honey solution was also offered to parasitoids as adult feed. The experiment was conducted in a completely randomized design (CRD) with five replications and maintained at an ambient environment of 33 ± 2 ∘ C, 40–50% relative humidity and a 14 h light/10 h dark photoperiod. The parasitoid was removed after 24 hours and the mealybug cohort retained in the same container to continue its development. The mealybug cohort was examined daily and upon mummification, the mummies were collected and isolated in separate vials until eclosion of adult parasitoids. The number of parasitoids emerging from the mummified mealybugs was recorded for each host plant. The parasitism rate, developmental time and adult emergence rate of *A. papayae* on each host plant were recorded. The parasitism rate was calculated using the formula

RESULTS

The parasitic potential of *Acerophagous papayae:* Significant differences in the parasitic potential of *A. papayae* on endosymbiotic second instars mealybugs of four different host plants were recorded. The maximum parasitism rate of 74.67 and 73.33 per cent was found in papaya and mulberry, respectively followed by tapioca and brinjal with 48.67 and 33.33 per cent parasitization, respectively. As well, in aposymbiotic second instar mealybugs the maximum parasitism rate of 85.33 and 80.67 per cent was found in papaya and mulberry, respectively followed by tapioca and brinjal with 56 and 46.67 per cent parasitization, respectively (Table 1) (Figure 1).

Emergence rate of *Acerophagous papaya:* The emergence rate showed significant differences among the host plants. In endosymbiotic population, the maximum emergence rate of 83.56 per cent and 79.22 was found in papaya and mulberry followed by brinjal and tapioca with 69.66 and 68.07 per cent emergence, respectively. In aposymbiotic population also the same trend was observed *i.e.,* the maximum emergence rate of 86.07 per cent and 83.57 was found in papaya and mulberry followed by brinjal and tapioca with 66.22 and 70.27 per cent emergence, respectively (Table 1) (Figure 1).

Development time: In both endosymbiotic and aposymbiotic population, a significant difference between development times was observed. In endosymbiotic population, parasitoid took 14.6, 15.2, 16.8 and 16.2 days to emerge as an adult and in aposymbiotic population, it was 17.6, 18.2, 19.8 and 20.4 days from papaya mealybug on papaya, mulberry, brinjal and tapioca host plant, respectively (Table 1) (Figure 1).

DISCUSSION

Growth and development of *Acrythosiphon pisum* parasitoid on *Aphidius ervi* larvae is closely linked to the activity of the primary endosymbiont (Pennachio et al. 1999; Rahbé et al. 2002; Cloutier and Douglas 2003) and to the presence of any detrimental secondary endosymbionts (Ferrari et al. 2004; liver et al. 2005). The reproductive success of female parasitoids is subject to their capacity to precisely survey the suitability of a host for progeny development at the time of oviposition.

For an idiobiont parasitoid that stops any further host growth after oviposition, the choice to parasitize a host ought to be founded on whether the quality and amount of the supplements accessible exceed the expenses as far as time and vitality (Rivero 2000). Interestingly, for most koinobiont parasitoids the possibility to evaluate host suitability is constrained by the unpredictability of future host development and survivorship following oviposition (Harvey 2005). Mealybugs vary from other phytophagous insects, by having harmonious microscopic organisms in it, and if these microorganisms give prompts that can be utilized in the appraisal of host quality, it is expected that endosymbiotic mealybugs would be exposed to a more noteworthy number of powerful stings than their aposymbiotic partners.

Table 1. Parasitisation rate, total developmental time and emergence rate of *Acerophagous papayae* on second instar *Paracoccus marginatus* nymph from different host plants

Host plants	Parasitization (%)		Emergence (%)		Development time (days)	
	Endosymbiotic	Aposymbiotic	Endosymbiotic	Aposymbiotic	Endosymbiotic	Aposymbiotic
Brinjal	33.33	46.67	69.66	66.22	16.8^{a}	19.8^{a}
	(35.25)^{c}	(43.07)^{b}	(56.74)^{b}	(54.70)^{b}		
Tapioca	48.67	56	68.07	70.27	16.2^{ab}	20.4^{a}
	(44.23)^{b}	(48.48)^{b}	(55.66)^{b}	(56.96)^{b}		
Papaya	74.67	85.33	83.56	86.07	14.6^{c}	17.6^{b}
	(60.45)^{a}	(67.97)^{a}	(66.34)^{a}	(68.35)^{a}		
Mulberry	73.33	80.67	79.22	83.57	15.2^{bc}	18.2^{b}
	(58.94)^{a}	(64.36)^{a}	(6.06)^{a}	(66.17)^{a}		
CD (p=0.05)	6.27	6.66	6.08	5.59	1.14	1.42
SE	1.17	1.24	1.13	1.04	0.21	0.27
CV (%)	9.41	8.88	7.51	6.77	5.42	5.58

Figures in the parentheses are Arcsine transformed values, Means followed by the same alphabets are not significantly different at 5 % level by DMRT

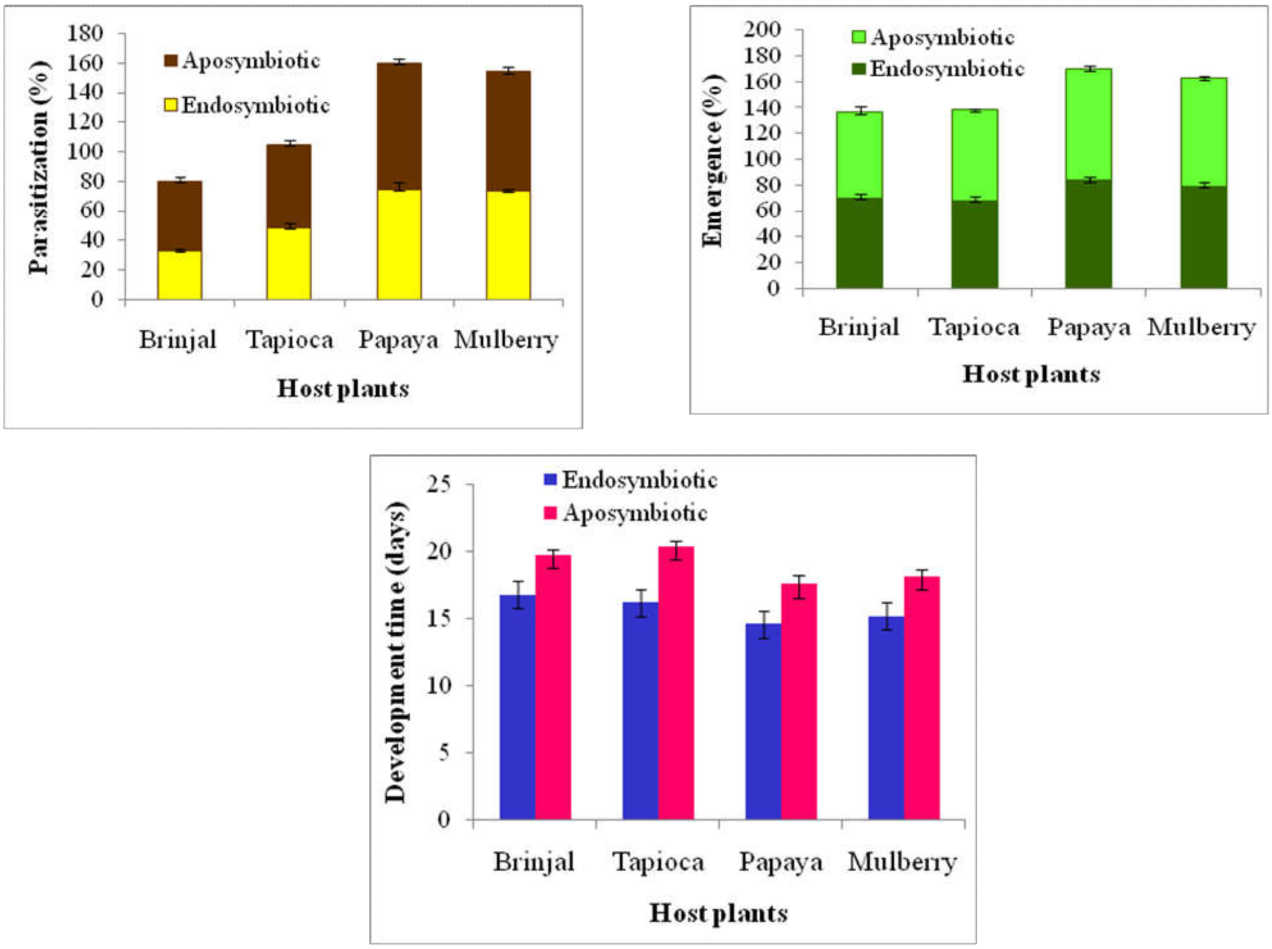

Fig. 1. Per cent parasitization, emergence and development time of parasitoid *Acerophagous papayae* on endosymbiotic and aposymbiotic populations of Papaya mealybug reared on four host plants

In the present study invariable of the host plants, both per cent parasitization and per cent emergence were maximum in aposymbiotic ones than in endosymbiotic mealybugs. And also the parasitoids takes longer time to emerge as an adult from aposymbiotic population whereas, from endosymbiotic mealybugs comparatively it emerges soon. It may be due to that the parasitoid descendants survivorship was not altogether affected by disturbance of the essential endosymbiont in aposymbiotic mealybugs (Cloutier and Douglas, 2003; (Rahbe *et al*., 2002). Along these lines, there is potential for variation in the impact of aposymbiosis in aphids on the accomplishment of parasitism because of the obscure quantitative commitment of the essential symbiont to aphid success (Brinza et al. 2009).

Conclusion

It may be concluded that the parasitism of *P. marginatus* by A. *papayae* is influenced by the activity symbionts in their host insect. Yet, further study on the assessment of parasitoid fitness over several generations and simultaneous metagenomic analysis of changes in endosymbiotic profile of mealybugs over generation after antibiotic treatment will help to explain the degree to which the essential endosymbiont adds to the accomplishment of parasitism in papaya mealybugs.

Conflict of interest: The authors declare that there is no conflict of interest.

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