



EFFECT OF FOOD, HOST DENSITIES AND MATING STATUS ON PARASITIZATION OF *AENASIUS BAMBAWALEI* HAYAT AND *AENASIUS ADVENA* COMPERE (HYMENOPTERA : ENCYRTIDAE)

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Abstract

Studies were conducted to observe the effect of food, host densities and mating status on parasitization of *Aenasius bambawalei* and *Aenasius advena* in the Parasitoid Taxonomy and Biocontrol Laboratory, Department of Entomology, Faculty of Agriculture, Annamalai University during 2009-10. The study shows that longevity was 30.00d, 19.40d and 25.00d, 14.80d when fed with 100 per cent honey for *A. bambawalei* (female, male) and *A. advena* (female, male) respectively, which was higher than 50 per cent honey, sucrose 1 M, glucose 1 M, fructose 1 M, distilled water and control with no food. Highest per cent parasitism was found in the host density with 50 (68.40 and 61.20 per cent) in *A. bambawalei* and *A. advena*. Mated females of *A. bambawalei* and *A. advena* alone produced female progenies and registered higher per cent parasitism (61.67 and 51.67 per cent) whereas virgin females produced only male progenies in both parasitoid species.

Key words : Food, host densities, mating status, parasitization, *A. bambawalei*, *A. advena*.

Introduction

The family Encyrtidae (Hymenoptera : Chalcidoidea) is one of the largest of the chalcidoid families. This family currently includes nearly 4,000 described species and represents one of the most successful groups used in the biological control of agricultural pests worldwide (Noyes, 1985 and Greathead, 1986), especially the biocontrol of mealybugs (Noyes and Hayat, 1994). The importance of the Encyrtidae in mealybug management is not surprising since almost all the members of the family are primary parasitoids of mealybugs; with the vast majority of these species belonging to the subfamily Tetracneminae (Noyes, 2000). In India, 143 genera (ten genera without included species) and 500 species (including 3 introduced species, and at least 15 species, which are of doubtful position or of doubtful validity) of Encyrtidae are known (Hayat, 2006, 2009). Mealybugs (Homoptera : Pseudococcidae) are small, soft-bodied insects that feed by sucking plant sap. Adult females and nymphs are wingless and frequently covered in a white, powdery or mealy wax secretion. In addition, the margin of the body often has a series of white, lateral wax filaments that typically are most prominent posteriorly. Adult males, if present, are

short-lived, non-feeding and rarely collected. Some mealybug species cause considerable economic damage to agricultural and horticultural crops (McKenzie, 1967; Williams and Granara de Willink, 1992; Miller *et al.*, 2002, 2005). Crop damage by mealybugs results from the direct effects of sap removal and injection of toxins, as well as indirectly by honeydew contamination and associated sooty mold growth that decreases photosynthesis (Mibey, 1997) and occasionally from the effects of transmitted plant viruses. Feeding damage may cause leaf yellowing, defoliation, reduced plant growth and death of plants. The occurrence of honeydew and sooty mold may reduce the marketability of plant products such as fruits (Culik *et al.*, 2006).

Earlier, two tailed mealybug, *Ferrisia virgata* (Cockerell) and pink mealybug, *Maconellicoccus hirsutus* (Green) were considered as polyphagous major coccid pests in India. In addition, citrus mealybug, *Planococcus citri* (Risso) and long tailed mealybug, *Pseudococcus longispinus* (Tag-Tazz.) were also recorded on few fruit crops and on coconut. But during 2004-05, there was a severe incidence of mealybug on cotton in Haryana and subsequently in Punjab, Gujarat,

Maharashtra and Karnataka. Initially, it was considered as two species *viz.*, solanum mealybug, *Phenacoccus solani* Ferris and solenopsis mealybug, *Phenacoccus solenopsis* Tinsely (Suresh and Kavitha, 2007). However, detailed studies revealed that both of them are one and the same species (Hodgson *et al.*, 2008). In Tamil Nadu, its incidence was quite severe only during 2006-07 season attacking cotton, sunflower, many vegetable crops and weed hosts resulting in heavy yield loss. In 2008, another species of mealybug, *Paracoccus marginatus* Williams and Granara de Willink was recorded in papaya for the first time in Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India (Suresh *et al.*, 2010).

Materials and Methods

Culturing of *Phenacoccus solenopsis* and *Ferrisia virgata*

The cultures of *P. solenopsis* and *F. virgata* were established in the laboratory from individuals collected from the fields. Experiments were conducted in the Parasitoid Taxonomy and Biocontrol Laboratory, Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar (Tamil Nadu), India. Both mealybug cultures were maintained separately in two incubators at $32\pm 2^{\circ}\text{C}$, 65 ± 5 per cent RH and L10:D14 on potato sprouts kept in plastic tubs (10cm high and 33cm dia.), with fine sand of 5cm depth. Six to twelve potato sprouts were placed (depending upon size of potatoes) per tub and moistened regularly. Two adult females of *P. solenopsis* and *F. virgata* were released per potato separately. After one to two weeks (*P. solenopsis*) and two to three weeks (*F. virgata*) of inoculation, the potato sprouts had second and third instars. This inoculation was repeated on fresh potato sprouts every three weeks to get a continuous supply of different mealybug instars throughout the study period.

Culturing of *Aenasius bambawalei* and *A. advena*

The cultures of *Aenasius bambawalei* Hayat and *Aenasius advena* Compere were established in the laboratory from the mummies of the respective mealybugs collected from the fields. The parasitoids emerged were identified and cultured on *P. solenopsis* and *F. virgata*, respectively. Adult parasitoids (five pairs) were released in to a glass container (15cm long and 10cm dia) covered with khadda cloth containing 1-2 potato sprouts supporting second and third instars of *P. solenopsis* and *F. virgata* and removed after 48h of oviposition. A streak of 100 per cent honey was placed on the inside wall of glass container. Ten to fifteen days after parasitization adults started emerging out from the mealybug mummies. Both

parasitoid cultures were kept separately in two incubators and maintained at $32\pm 2^{\circ}\text{C}$, 65 ± 5 per cent RH and L10:D14.

Effect of food resources on longevity of *Aenasius bambawalei* and *A. advena*

Mummies of *P. solenopsis* and *F. virgata* were collected from the cultures of *A. bambawalei* and *A. advena* maintained respectively on potato sprouts. They were placed in glass container (15cm long and 10cm dia) covered with khadda cloth and observed daily for the emergence of adults. Newly emerged individual female and male parasitoids were transferred to a separate glass container covered with khadda cloth and allowed for 24h mating and were used in the experiment. Six food sources *viz.*, Honey (100 per cent commercial Dabur honey), honey diluted in distilled water (50 per cent), sucrose, glucose, fructose, distilled water and a control with no food served as treatments with five replications. Each replication had five females which were released in to glass containers with treatments. For the sugar (HiMedia) treatments, 1 M solution of each sugar was prepared in distilled water. Food resources were placed on the inside of the glass container wall as a fine streak. The food provision was repeated as and when exhausted till the death of parasitoids and thus the longevity was noted. The same protocol was followed for the male parasitoids also.

Effect of host density on sex ratio and parasitism of *A. bambawalei* and *A. advena* after mummification

Third instar *P. solenopsis* at four different densities (5, 15, 25, 50/ potato sprout) collected from the cultures maintained in the laboratory were tested. Each density of *P. solenopsis* was transferred to one to two potato sprouts (depending on their size) in glass containers (15cm long and 10cm dia) covered with khadda cloth. A streak of 100 per cent honey was placed inside; one fresh mated female parasitoid from the cultures maintained in the laboratory was introduced into the glass containers to lay eggs on the mealybugs for 24h and kept in incubator. The mealybugs of each density were considered as treatments and replicated five times. After mummification, hosts were transferred individually into 5ml glass vials, incubated and observed daily for the emergence of parasitoid offsprings from 10 days after oviposition. The same protocol was followed for the *A. advena* except that third instar *F. virgata* was used instead of *P. solenopsis*. The number of progeny was noted, sex ratio (females) and per cent parasitism were calculated for the two parasitoids.

$$\text{Per cent parasitism} = \frac{\text{Number of encyrtids emerged} \times 100}{\text{Total number of mealybugs}}$$

Influence of mating status on sex ratio and parasitism of *Aenasius bambawalei* and *A. advena*

Phenacoccus solenopsis mummies were isolated individually from the culture maintained in the laboratory, in 5ml glass vials. Female parasitoids emerged were allowed to mate with newly emerged males for 24h. Ten mealybugs of third instar *P. solenopsis* collected from the cultures maintained in the laboratory were transferred to the glass container with one to two potato sprouts covered with khadda cloth. A streak of 100 per cent honey was placed inside. Mated and virgin females were considered as treatments. Six replicates were maintained for each treatment. One mated or virgin female of *A. bambawalei* was introduced individually into glass container for 24h. After mummification, hosts were transferred individually into 5ml glass vials, incubated and observed daily for the emergence of parasitoid offsprings from 10 days after oviposition. The emerged parasitoids were counted, sex ratio (females) and per cent parasitism were calculated for the two parasitoids. The same protocol was followed for the *A. advena* except that *F. virgata* was used instead of *P. solenopsis*.

Results and Discussion

Effect of food resources on longevity of *Aenasius bambawalei* and *A. advena*

The effect of food on adult longevity of both the sexes of *A. bambawalei* and *A. advena* (table 1) were examined. The longevity was 30.00, 19.40 and 25.00, 14.80d when fed with 100 per cent honey for *A. bambawalei* (female, male) and *A. advena* (female, male) respectively. While feeding 50 per cent honey, the longevity was 15.00 and 13.40d for females of *A. bambawalei* and *A. advena* respectively. In both the species, when fed with glucose 1 M and fructose 1 M (females); sucrose 1 M and glucose 1 M (males) the longevity was on par with each other. Longevity with distilled water and control were on par for both the sexes of *A. bambawalei* and *A. advena*. Waçkers (2001) identified that sucrose and its two monosaccharide components, glucose and fructose are key components of nectar and honeydew. So in the present study sugars like, sucrose, glucose and fructose were chosen as food sources.

Aenasius bambawalei and *A. advena* fed with 100 per cent honey survived 11-14 and 7-13 times longer than those maintained under starved (control) condition respectively (table 1). Distilled water and control were

on par and gave least longevity in both the species. Similar to the present study. Chong and Oetting (2006) also observed that when provided with a carbohydrate source in the form of honey solution, both sexes of *Anagyrus* sp. nov. nr. *sinope* lived 3-13 times longer than those provided with only distilled water or starved. Distilled water apparently did not provide any nutritional value to *Anagyrus* sp. nov. nr. *sinope* because the longevity of the hydrated parasitoids was identical to that of the starved parasitoids. Similar observations were also made by Ferreira de Almeida *et al.* (2002) who stated that provisions of honey solution increased the longevity of the encyrtid parasitoid *Tachinaephagus zealandicus* Ashmead by one to three times of those individuals fed with only water.

Longevity of *Coccidoxenoides perminutus* Girault (Hymenoptera : Encyrtidae) was maximum when the parasitoids were provided with honey solution compared with parasitoids provided no food, flowers, nectar, or mealybug honeydew (Davies *et al.*, 2004). Similarly, Jervis *et al.* (1992) also reported that most adult parasitoids require food, such as host haemolymph, host honeydew, nectar, pollen, or other carbohydrate- or protein-rich substitutes, to maintain somatic and reproductive fitness and to obtain energy for foraging and reproductive activities. Adult longevity often declines in the absence of food sources.

Among the sugars, sucrose extended the longevity of *A. bambawalei* (females, males) and *A. advena* (females, males) 4.6, 4.9 and 4.5, 2.7 times than control respectively. Glucose and fructose also increased the longevity of females and males of both the species by 2 - 4.7 times than control. This is in agreement with the results of Chen and Fadamiro (2006), who compared the longevity of phorid fly, *Pseudacteon tricuspis* Borgmeier (Diptera : Phoridae) provided with 1 M solutions of five naturally occurring sugars, fructose, glucose, sucrose, trehalose and melezitose. They stated that longevity is increased by 2.4 – 2.6 fold by the two monosaccharides, fructose and glucose and by 2.6 – 2.8 fold by the disaccharides, sucrose and trehalose.

Differences in longevity between *A. bambawalei* and *A. advena* might be because food sources may differ in their suitability to different species of natural enemies. Females of both *A. bambawalei* and *A. advena* lived longer than males. This is in accordance with the results of Oo *et al.* (2009), who reported that food positively influenced *Tetrastichus brontispae* longevity and females survived significantly longer than males. They also observed that when provided with 10 per cent honey

Table 1 : Effect of food resources on longevity of *Aenasius bambawalei* and *A. advena*.

Food source	Longevity (days)*			
	<i>A. bambawalei</i>		<i>A. advena</i>	
	Female	Male	Female	Male
100% Honey	30.00 ^a	19.40 ^a	25.00 ^a	14.80 ^a
50% Honey	15.00 ^b	5.40 ^c	13.40 ^b	11.00 ^b
Sucrose 1 M	12.80 ^c	6.80 ^b	9.80 ^c	5.40 ^c
Glucose 1 M	7.60 ^d	6.60 ^b	5.40 ^d	6.00 ^c
Fructose 1 M	7.80 ^d	5.00 ^c	6.00 ^d	4.00 ^d
Distilled water	2.20 ^e	1.40 ^d	1.60 ^e	1.40 ^e
No food (Control)	2.80 ^e	1.40 ^d	2.20 ^e	2.00 ^e
S.Ed.	0.76	0.52	0.60	0.65

*Mean of five replications.

In a column means followed by a common letter are not significantly different by DMRT (P = 0.05).

solution, the mean longevity was nine and 14.00 days for males and females, respectively.

Effect of different host densities on sex ratio and parasitism of *Aenasius bambawalei* and *A. advena*

The data on the effect of host density on sex ratio and per cent parasitism of *A. bambawalei* and *A. advena* are furnished in table 2. Number of female progeny produced ranged from 1.60 to 26.40 and 1.40 to 23.00 for *A. bambawalei* and *A. advena*, respectively. Number of male progeny produced was 0.40 to 7.80 and 0.40 to 7.60 for *A. bambawalei* and *A. advena*, respectively. Number of females and males of *A. bambawalei* and *A. advena* were significantly different from each other for each host density. Per cent female of *A. bambawalei* and *A. advena* was 83.33, 77.58 (5, 50 host densities) and 83.33, 75.50 (5, 50 host densities), respectively. Per cent parasitism of *A. bambawalei* and *A. advena* was 68.40 and 61.20 at highest host density. In *A. bambawalei* per cent parasitism of host densities with five and 15

Table 2 : Effect of different densities of *Phenacoccus solenopsis*[#] and *Ferrisia virgata*[#] on sex ratio and parasitism of *Aenasius bambawalei* and *A. advena*.

Host density	Mean number of progeny produced per female*				Sex ratio (per cent females)*		Per cent parasitism*	
	<i>A. bambawalei</i>		<i>A. advena</i>		<i>A. bambawalei</i>	<i>A. advena</i>	<i>A. bambawalei</i>	<i>A. advena</i>
	Females	Males	Females	Males				
5	1.60 ^d	0.40 ^c	1.40 ^d	0.40 ^d	83.33 (73.18) ^a	83.33 (73.18) ^a	40.00 (39.01) ^b	36.00 (36.47) ^b
15	4.80 ^c	2.40 ^c	4.60 ^c	2.40 ^c	65.14 (54.25) ^b	63.95 (53.28) ^b	48.00 (43.82) ^b	46.66 (43.05) ^{ab}
25	8.00 ^b	5.50 ^b	7.40 ^b	4.80 ^b	60.41 (51.05) ^b	61.91 (51.97) ^b	53.60 (47.09) ^{ab}	48.80 (44.23) ^{ab}
50	26.40 ^a	7.80 ^a	23.00 ^a	7.60 ^a	77.58 (61.81) ^{ab}	75.50 (60.40) ^{ab}	68.40 (55.94) ^a	61.20 (51.54) ^a
SEd	0.99	0.98	0.97	0.93	7.68	7.49	4.40	4.64

*Mean of five replications.

[#] *Aenasius bambawalei* on *Phenacoccus solenopsis*; *A. advena* on *Ferrisia virgata*.

In a column means followed by a common letter are not significantly different by DMRT (P = 0.05).

Data in parentheses are arc sine transformed values.

Table 3 : Influence of mating status on sex ratio and parasitism of *Aenasius bambawalei* and *Aenasius advena*

Host density	Mean number of progeny produced per female*				Sex ratio (per cent females)*		Per cent parasitism *	
	<i>A. bambawalei</i>		<i>A. advena</i>		<i>A. bambawalei</i>	<i>A. advena</i>	<i>A. bambawalei</i>	<i>A. advena</i>
	Females	Males	Females	Males				
Mated	5.00 ^a	1.17 ^b	3.80 ^a	1.33 ^b	30.72 (33.02) ^b	25.28 (30.04) ^b	61.67 (51.82) ^a	51.67 (45.96) ^a
Virgin	0.00 ^b	4.83 ^a	0.00 ^b	3.83 ^a	100.00 (88.83) ^a	100.0 (88.83) ^a	48.33 (44.00) ^b	38.33 (38.04) ^b
SEd	0.37	0.57	0.17	0.52	12.07	1.74	3.33	3.41

*Mean of six replications.

In a column means followed by a common letter are not significantly different by DMRT (P = 0.05).

Data in parentheses are arc sine transformed values.

were on par and in *A. advena* the same at host densities with 15 and 25 were on par.

Number of female and male progeny produced was in decreasing order between 50 hosts and 5 hosts in both the species (table 2). Female proportion was high and similar in *A. bambawalei* and *A. advena* at both host densities 5 and 50. Among the four host densities, the largest host density (50 hosts) induced the highest percent of parasitism in *A. bambawalei* and *A. advena*.

The results are in accordance with the result of Sagarra *et al.* (2000), who found that *A. kamali* emergence was low in *M. hirsutus* at the densities of two and five hosts (0.1 ± 0.32 and 0.7 ± 0.95 hosts emerging, respectively) and plateaued at a density of 50 hosts with an average progeny emergence of 8.15 ± 2.46 individuals.

Influence of mating status on sex ratio and parasitism of *Aenasius bambawalei* and *A. advena*

The data on influence of mating status on sex ratio and per cent parasitism of *A. bambawalei* and *A. advena* are furnished in table 3. Virgin females of *A. bambawalei* and *A. advena* produced only male progenies. Mated females produced significantly more number of female progenies in *A. bambawalei* (5.00) and *A. advena* (3.80). Proportion of male was significantly high (100.00%) with virgin in both *A. bambawalei* and *A. advena*. The per cent parasitism by mated females of *A. bambawalei* and *A. advena* was 61.67 and 51.67, respectively.

Mating status was studied as it influences reproductive longevity, fecundity and progeny sex ratio of parasitoids. In both *Aenasius bambawalei* and *Aenasius advena* mated females had more female progenies and higher per cent parasitism (table 3). Virgin females did not produce any female progeny in both the species. Chong and Oetting (2006) reported that many encyrtid parasitoids are arrhenotokous, meaning that virgin females produce only male progeny from unfertilized eggs, whereas mated females are capable of producing both male and female progenies. They also found that virgin parasitoids produced 100 per cent male broods at all temperatures. Mated parasitoids produced broods with 35-41 per cent males at 20-30°C. This is in accordance with the results of present study. Sagarra *et al.* (2002) concluded that mated *Anagyrus kamali* females had a lower tendency to superparasitize their hosts, thus mated females may parasitize more hosts and produce more progeny than virgin females. A similar result was also reported for *Anagyrus pseudococci* (Avidov *et al.*, 1967) and *Ageniaspis citricola* (Hymenoptera : Encyrtidae) (Edwards and Hoy, 1998).

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