

Reproductive Fitness of Mealybug Parasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae)

Zain ul Abdin, Fiaz Hussain, Muhammad Ahsan Khan,
Saqi Kosar Abbas, Atif Manzoor and Hoor Shaina

Department of Entomology, University of Agriculture, Faisalabad, 38040, Pakistan

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Abstract: *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) is a solitary endoparasitoid of cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has been recorded as an effective natural enemy of mealybugs in cotton fields of Pakistan. The current study was conducted to determine the developmental time, sex ratio and progeny fitness of this wasp on different developmental stages of its host. Our results show that maximum developmental time was recorded for 2nd instar host nymph as compared to 3rd instar and adult stage of the host while females of the wasp take longer time for their development (16.2 d) as compared to males (15.6 d) on 2nd instar host stage. Females developed rapidly with increasing number of the host stages while males developed faster than females in all host stages. The overall sex ratio of the resulting parasitoid male and female was 1:2 in all host stages. Maximum number of males were recorded at 2nd instar host stage (43%) followed by 29.4% and 32.2% in 3rd instar and adult host stages respectively. Maximum number of female wasps were developed at 3rd instar nymph (59.6%) followed by adult host stages (50.8%). The 3rd instar host nymph appeared to be the most suitable host stage for mass rearing and high progeny fitness of the wasp *A. bambawalei* for sustainable biological control programmes of mealybugs.

Key words: Wasp • Sex ratio • Developmental time • Host stages

INTRODUCTION

Cotton crop is not only a backbone of Pakistan's economy but it is also a main source of foreign exchange earnings [1]. The cotton improvement programme responded to the needs of the growers and industry and strived to combine high yield, early maturity fiber quality [2]. Mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) emerged as a highly invasive pest species on cotton crop and many other crop plants of economic importance and it has been reported to cause heavy loss to this crop in Pakistan [3]. It has become a devastating polyphagous pest spreading rapidly throughout cotton growing areas of the country and has become a serious pest of cotton [4, 5] with broad host range of plant species including, vegetables, weeds and ornamentals [5, 6]. Like other insect pests, mealybugs are also being controlled by the extensive use of synthetic insecticides which however, not only have negative impact on the environment/ecosystem but also

detrimental to non-target organisms [7]. Whereas insect natural enemies e.g predators and parasitoids are a diverse group of biological control agents [8]. The mealybug parasitoid *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) was recorded first time in Pakistan in 2008. It is an endophagous nymphal parasitoid of cotton mealybug which parasitizes mealybugs up to more than 80 percent on cotton [9]. This is one of the most successful examples of biological control of mealybug [10]. Many studies reported that the reproductive success of parasitoids mainly depends on the host stage parasitized, i.e., variation in their development time [11], body size [12, 13], progeny sex ratio [14, 15] and egg load [12, 16, 17]. Mostly the preference of any host stage occurs when it is found in excess as compared to the other stages. Egg laying by the wasps also depends on the host size which is considered as host quality index, i.e., large size hosts contain more resources as compared to the small ones [18, 12]. Adult parasitoids emerging out singly from the large size hosts

take more advantage of the resources available which are directly related to their fitness, such as fecundity and survival of the progeny is enhanced [11]. Endoparasitoids, however show acceptance of different host stages or sizes because they allow the younger host to develop after parasitization. So during the assessment of quality of host, they can use different signals i.e., future growth potential, nutritional status [19]. Although the range of the host stages for koinobiont parasitoids is wider but there are high costs invest in case of smaller hosts because development time is lengthened and their survival is also in at risk [14].

Discussion of the host stage selection resulted that the gregariously feeding parasitoids should adopt such oviposition behavior which can provide maximum profit in the form of their fitness return relative to the hosts of different sizes or ages [20]. Most of the parasitoids are able to select the proper host on the basis of its quality or size and preferably parasitize the host with specific size [21]. The egg loading capacity, searching ability and longevity depend on the size of female. Similarly the ability to find females, number of matings and longevity depend upon the male size [14].

The objective of the present research work is to determine the developmental time, sex ratio and reproductive fitness of mealybug parasitoid, on different life stages of its host.

MATERIALS AND METHODS

Preliminary studies on the developmental time, sex ratio and reproductive fitness of mealybug parasitoid, *Aenasius bambawalei* were conducted in the “Insect Molecular Biology Lab.” Department of Entomology, University of Agriculture Faisalabad, Pakistan.

Rearing and Handling of Host-parasitoid Culture:

Parasitized mealybugs/mummies were collected in plastic jars directly from the fields of cotton, vegetables, weeds and ornamental plant (*Hibiscus rosa-sinensis* L) located in the campus of University of Agriculture, Faisalabad. The parasitic wasps were reared on the colonies of their natural host, *Phenacoccus solenopsis* at 28 ± 1 , $70 \pm 5\%$ relative humidity (RH) and 18:6 (L:D) hour, photoperiod in the laboratory. The culture of unparasitized mealybugs was maintained in separate glass jars under the same environmental conditions.

Insect parasitoids to be used in the experiments were obtained directly from the mummies of mealybugs and were isolated singly in glass vials plugged with cotton swab and provided with honey and water as a food source upon emergence.

Progeny Fitness: The preliminary experiments were conducted to determine the progeny fitness of mealybug parasitoid, *A. bambawalei* on four different host stages including three nymphal instars and one adult host stage. The parasitoid adults emerged from mealybug mummies that were parasitized at the host stage of 3rd instar nymph. One mated female was released into each plastic jar containing a piece of pumpkin infested with 40 mealybugs (10 of each stage), the female parasitoid was allowed to parasitize mealybugs for 24 hours. The above experiment was repeated five times. After 24 hours of parasitization, the female parasitoid released again in the parasitoid rearing jars and data was recorded regarding developmental time, sex ratio and progeny fitness.

Statistical Analysis: Regression analysis was used to obtain the relationship between developmental time, sex ratio and progeny fitness on different host stages (Fig. 1, 2 and 3) (PROC REG, SAS, Institute, 1996). The parasitization percentage of newly emerged females increased with both host stage and sex ratio, so the estimated value of the response variable is given by the polynomial equation. The other data was analyzed statistically by software Statistix version 8.1 [22] (Analytical software, 2003) and subjected to the analysis of variance under complete randomized design. Means were separated by Least significant difference test (LSD-Test) and a significant level of ($P < 0.05$) was used to analyze the data.

RESULTS

Parasitoid Developmental Time: The results show that males developed within a shorter period of time as compared to females in all host stages. The developmental time for male wasp on 2nd instar host stage was (15.6d) followed by female wasp on the same host stage (16.2d). For both the sexes, developmental time decreased with the increase in host age. The maximum developmental time was recorded for 2nd instar host stage (15.6d) while minimum time was recorded in adult host stage (12.8d) in males (Table 1 and Fig. 1). Female parasitoid took longer time for its development than males.

Table 1: Mean ($\pm$ SE) developmental time (days) of *A. bambawalei* from egg to adult in different host stages and proportion of resulting female progeny

Host stage at Parasitization	(DT) Male	(DT) Female	Female progeny (%)
1 st Instar	0 $\pm$ 0.0 d	0 $\pm$ 0.0 d	0 $\pm$ 0.0 d
2 nd Instar	15.6 $\pm$ 2.0 a	16.2 $\pm$ 3.16 a	10 $\pm$ 3.16 c
3 rd Instar	14.8 $\pm$ 1.87 b	15.4 $\pm$ 3.53 b	59.6 $\pm$ 2.48 a
Adult	12.8 $\pm$ 1.73 c	13.2 $\pm$ 2.54 c	50.8 $\pm$ 1.90 b

Means with different letters at the same column are significantly different at $P < 0.05$.

DT= Developmental Time in days

Table 2: Mean ($\pm$ SE) percentage of sex ratio and reproductive fitness of mealybug parasitoid, *Aenasius bambawalei* in different host stages

Host stage at Parasitization	Male Sex ratio (%)	Female Sex ratio (%)	Total progeny (%)
1 st Instar	0 $\pm$ 0.0 c	0 $\pm$ 0.0 d	0 $\pm$ 0.0 c
2 nd Instar	43 $\pm$ 2.0 a	10 $\pm$ 3.16 c	53 $\pm$ 2.0 b
3 rd Instar	29.4 $\pm$ 1.07 b	59.6 $\pm$ 2.48 a	89 $\pm$ 2.46 a
Adult	32.2 $\pm$ 2.13 b	50.8 $\pm$ 1.90 b	83 $\pm$ 2.50 a

Means followed by same letter (s) within each column (denoted by lower-case letters) are not significantly different by at $P < 0.05$.

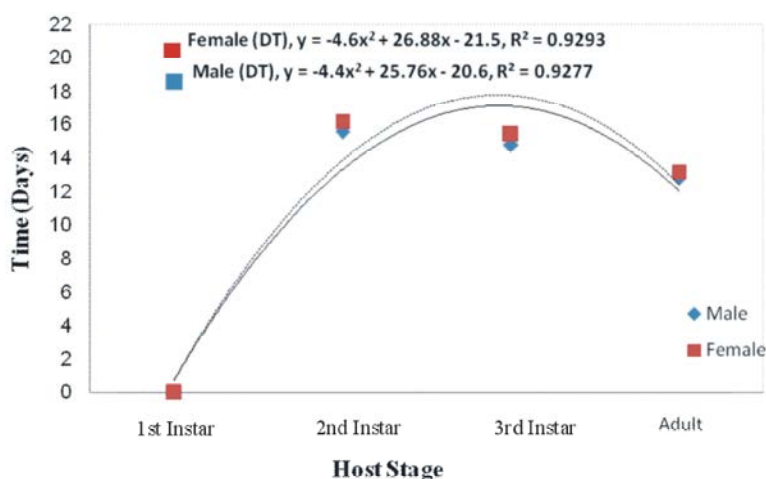


Fig. 1: Relationship between developmental time of *A. bambawalei* and the host stage at parasitization

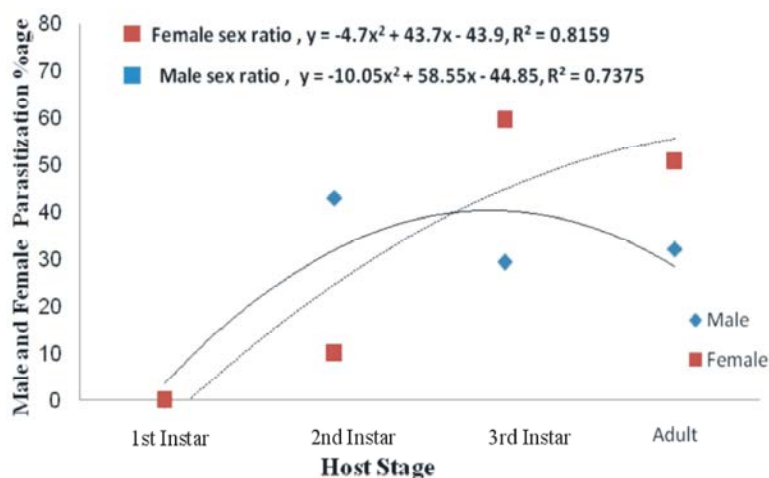


Fig. 2: Relationship between the sex Ratio (%) of *A. bambawalei* (Male and Female) and host stages at parasitization

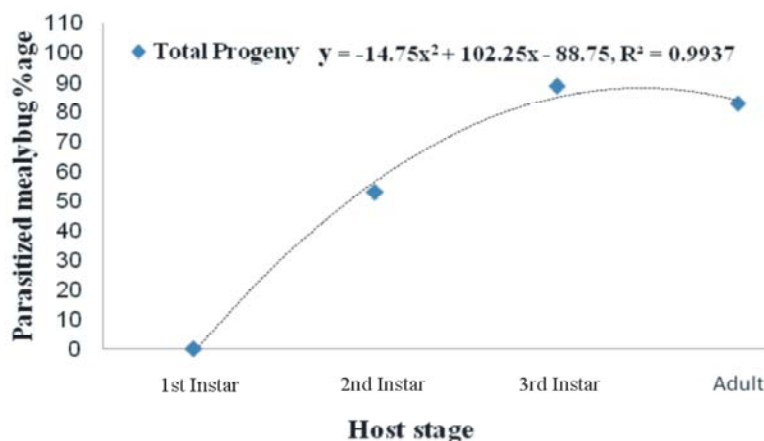


Fig. 3: Relationship between the total progeny fitness (%) of *A. bambawalei* (Female) and host stages at parasitizations

Sex Ratio and Progeny Fitness: More female parasitoids were emerged from the 3rd instars and adult host stages of the mealybugs whereas the 2nd instar host stage produced a significantly higher proportion of males (Table 2 and Fig. 2). Maximum number of female parasitoids were developed from 3rd instar host stage (59.6%) followed by 2nd instar and adult host stages (10% and 50.8%) respectively. The overall maximum parasitization (89%) occurred in 3rd instar host stages followed by the adult (83%) and 2nd instar host stages (53%) as shown in figure (Table 2 and Fig. 3).

DISCUSSION

The preliminary study was conducted to determine the sex ratio, development time and progeny fitness of mealybug parasitoid, *A. bambawalei* under control conditions. Our data shows that male parasitoids developed within shorter period of time than females in all host stages. In both sexes developmental time decreased with the increase in host stage (Table 1). This might be due to inadequate resources available at younger stages of the host [23-25]. A similar relationship between parasitoid developmental time and host age at oviposition has been reported [24, 26, 27]. *A. bambawalei* preferred 3rd instar host nymphs and adult host stages for parasitization. Similar findings were reported by Chong and Oetting [27], that *Anagyrus sp. nov.nr. sinope* preferred third instar immature and pre-reproductive adult female *P. madeirensis* for oviposition and progeny development. It has been investigated that in parasitoids, female fitness depends on the host size than for males [28, 29]. Highest parasitism was observed in third instar host nymphs than adult stages of mealybug (Table 2). It is due to their defensive behavior, which may result in increased

parasitoid handling time [26]. Besides these, parasitoids were unable to get resources that are consumed by adult mealybug for its own egg production, thus affecting fitness of the parasitoid progeny [30]. The parasitoids that developed in these preferred host stages had a shorter developmental time, a lower mortality rate, a higher proportion of females and larger brood and body sizes. Second instars nymph were considered the least preferred and of the least suitable for parasitoid growth and development. While, there was no parasitization at all on 1st instar host nymph. Mealybug parasitoid, *A. bambawalei* laid eggs in all host stages except 1st instar host nymph, but female parasitoid preferred later instar host nymphs and adult host stage for oviposition. Similar preference may also be attributed to highest fitness return [31].

It has been observed from various studies that the female's progeny fitness increased with the increase in host size and stage [29, 32]. Many parasitoids, such as *Anagyrus sp. nov.nr. sinope*, aggressively select hosts of a specific age or size that have sufficient resources to produce progeny of a higher fitness [14, 27, 33-35]. Host stage is positively correlated to fitness of the parasitoids, which is determined by the number of progeny that can be developed by each female offspring and the number of females that can be desired by each male offspring [36, 37]. But there is no direct relation of body size to fitness; it may be used as a primary proxy when its relationship with another primary proxy (for example fecundity, longevity or mating ability) is known [38]. The egg loading capacity, searching ability and longevity depend on the size of female similarly the ability to find females, number of matings and longevity depend upon the male size [14]. In general, the size of *Trichogramma* spp. seems to be positively related to performance [39].

It is also observed that 2nd instar host stage produced more number of male parasitoid while 3rd instars and adult host stages produced large number of female parasitoids and the same findings have also been reported by Karamaouna and Copland [40] that oviposition at 2nd instar nymph yielded primarily males.

CONCLUSION

The 3rd instar host stage of the mealybug found to be more suitable for mass rearing and high progeny fitness of the wasp *A.bambawalei* for sustainable biological control programmes of mealybugs.

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