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Parasitic Effects of Solitary Endoparasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) on Cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

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Abstract

Parasitization has an enormous impact on host physiology, development and reproduction. The effect of parasitism by endoparasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) on survival and reproduction of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on different host stages was studied. Our data reveals that host mealybugs parasitized by the wasp at the 2nd instar stage were died during the 3rd instar stage of their life. However, those parasitized at the 3rd instar stage could reach the adult stage and were able to produce their progeny. After 6 days of parasitization, all parasitized hosts of the 2nd, 3rd and adult stages were died except the 1st instar. Results showed that parasitized host mealybugs had significantly lower reproductive potential than the unparasitized ones. Maximum parasitoid emergence was recorded in the 3rd instar host stage. This basic research regarding survival and reproduction of the parasitized host mealybugs would be very helpful in devising sustainable biological control strategies for cotton mealybug.

Keywords

Parasitization, Cotton Mealybug, *A. bambawalei*, Instars, Reproduction

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1. Introduction

Monoculture farming in Pakistan resulted in a significant crop loss every year mainly due to the attack of different insect pests as well as diseases. A complex of chewing and sucking insect pests attacks on cotton crop [1]. An invasive insect species, mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) appeared first time in 2005, reported from District Vehari (Punajb) and Sanghar (Sindh) in Pakistan, The economic damage caused by this pest was 40% and reached up to 3.1 million bales of cotton during 2006 and 2007,which was very devastating and catastrophe [2] [3]. *P. solenopsis* is a polyphagous pest which damages vegetables (eggplant, pumpkin, okra and tomato), fruits, wheat, tobacco, fodders and also some ornamental plants [4]-[6]. Cotton plant is the most preferred host of this pest as it destroys both *Bt* and non *Bt* cultivars [7]. *P. solenopsis* feeds on leaves, branches, fruits, stems and roots by sucking sap from phloem and secretes honey dew, resulting in sooty mold growth, which hinders the process of photosynthesis [1].

No doubt the extensive use of chemicals has reduced the pest population but on the other hand it has so many negative impacts on the environment and human health [6]. To avoid such problems, biological management of mealybug by using of its predators and parasitoids is gaining importance [8]. Keeping in view the aftermaths of chemical management, Common Wealth Agricultural Bureaux International (CABI) has introduced its natural and biological control agents. The most effective natural enemy of cotton mealybug was first reported from Tando Jam (Sindh), Pakistan in August 2008 and identified as *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae). It is an efficient endoparasitoid of cotton mealybug, *P. solenopsis* and has good mortality factor for controlling this pest under natural conditions [9]. It has been documented that parasitism has an enormous impact on the development, fecundity and population growth of the host [10] [11]. The main objective of this study was to study the effects of parasitism on the survival and reproduction of host *P. solenopsis*.

2. Material and Methods

2.1. Rearing and Handling of Host-Parasitoid Culture

The encyrtid parasitic wasp *A. bambawalei* was reared on the colonies of its host *P. solenopsis* maintained on pumpkin (*Lagenaria sicerata* M.) that is locally known as bottle gourd. Parasitized mealybug/mummies were collected in plastic jars directly from the fields of cotton and vegetables (e.g. tomato, eggplant, okra, pumpkin etc.) located in the campus of University of Agriculture, Faisalabad, Pakistan. Breeding colony of *A. bambawalei* was established from individuals emerged from the dark brown mummies of parasitized mealybugs. Prior to the experiments, the colony of the wasp was reared on mealybug feeding on pumpkin for five generations. Host and parasitoid cultures were maintained in glass jars, in growth chambers, both at 28˚C ± 1˚C, 70% ± 5% relative humidity (RH) and 12 h light/12 h dark photoperiod by following a slightly modified approach as described by [12]. One day old after emergence male and female adult parasitoids were obtained from the mummies of parasitized mealybugs and allowed to mate them for 24 hours. A solution of 30% honey and 70% water was provided as a food source.

2.2. Survival and Reproduction

The effect of parasitism by *A. bambawalei* on the survival and reproduction of mealybugs at different host stages of development (1st instar to adult) were investigated. There were 4 treatments (1st, 2nd, 3rd instars and adult host stage) and each treatment consisted of 10 hosts (mealybugs) with 15 replications (total of 150 insects for each host stage). To obtain parasitized mealybugs in each treatment two days old (one day after emergence and one day for mating) single mated female wasp was released into 250 ml plastic box containing 10 mealybugs of each stage. After 24 hours of parasitization, female wasp was removed from all host stages except first instar and parasitized hosts were shifted on pumpkins (*Lagenaria sicerata* M.) and allowed to develop independently by taking one host per small jar placed at 28˚C ± 1˚C, 70% ± 5% relative humidity (RH) and 12 h light/12h dark photoperiod for the completion of their development. The 1st instar of the host was allowed for parasitization till its conversion to 2nd instar. Data regarding the survival and reproduction of the parasitized hosts were recorded after 24 h intervals till the emergence of 1st instar of mealybug also called as crawlers or parasitoid progeny. Host's offsprings and wasps emerged from the parasitized individuals were counted and checked for their survival and reproduction. All the four treatments were compared with the same host stages mealybug reared under same environmental conditions, acts as control treatments.

2.3. Statistical Analysis

Data of all the treatments was analyzed statistically by using software [13] Statistix 8.1 (Analytical software, 2003) and subjected to the analysis of variance under complete randomized design. Means were separated by using LSD test (Least significant difference test) at a significant level of $p \leq 0.05$.

3. Results

3.1. Mean Comparison of Host Mealybugs Survived after Parasitization among Different Host Stages

The effect of parasitization was studied on all life stages of the host mealybug and it survived generally only for a few days after parasitization. A comparison of the mean percentages of survived host mealybugs was made for each host stage in order to find the most resistant host stage to parasitism. **Figure 1** shows the mean percentage of survival rate at different host stages. Maximum survival rate of the host was observed at its 1st instar stage (94%), as none of the host was parasitized till its conversion to 2nd instar stage; which reveals that it is non-preferred host stage hence *A. bambawalei* does not accept this host stage for parasitization. Mealybugs parasitized at 2nd host stage turned to mummies in 3rd instar before reaching to adult stage. However host mealybugs parasitized during 3rd and adult stages continued their development. The longevity of parasitized host mealybugs for all of their life stages except 1st instar was significantly less as compared to unparasitized hosts.

Survival rates in case of 2nd instar hosts were zero (0) percent, as all of them were died before reaching to the adult stage and turned to mummies. Hosts parasitized during 3rd and adult stages were also died after 6 days of parasitization, but they laid eggs before their death and the progeny emerged from these eggs was survived.

3.2. Mean Comparison of Egg Laying Potential of Host after Parasitization

As discussed above 3rd instar and adult host stage offered resistance against parasitism, even they survived partially by completing their life cycle and the effect of parasitism on the egg laying potential of the host mealybug was studied. **Figure 2** shows none of the eggs/host mean number laid by 2nd instar parasitized host stage, because after parasitization they died before reaching the adult stage while data regarding parasitism effect on 1st instar host stage showed that wasp *A. bambawalei* did not parasitize this host stage, therefore, maximum number of 1st instar crawlers were observed. Mean number of eggs laid after parasitization was more at adult host stage (84.67 ± 1.45, LSD test at $P \leq 0.05$) than 3rd instar host stage (51.66 ± 1.85, LSD test at $P \leq 0.05$) followed by control treatment (233 ± 2.01, $P \leq 0.05$). The statistical analysis of data shows that egg laying potential of the wasp at adult host stage is significantly different than 3rd instar host stage as well as with the control treatment.

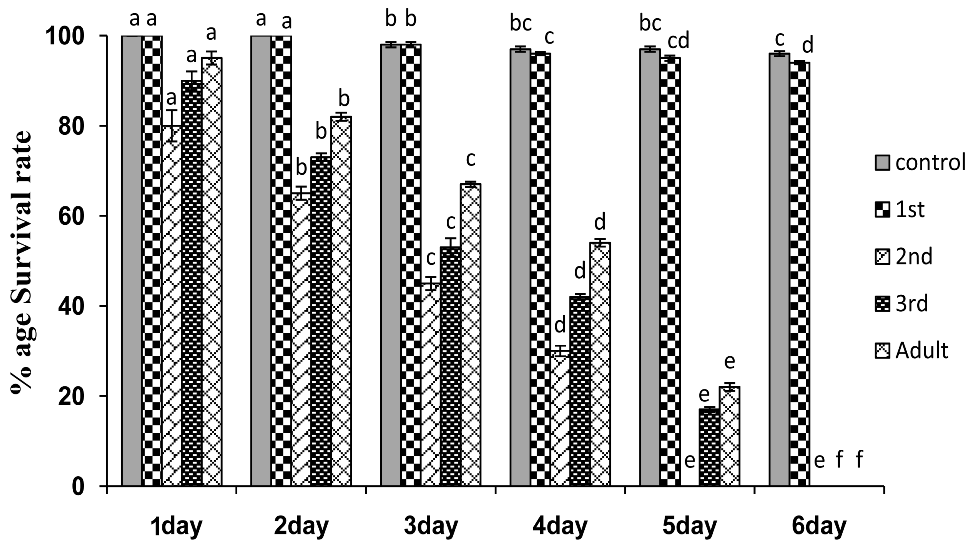

Figure 1. Mean percentage of survived rate of mealybugs after parasitization on different host stages, mean column bars denoted with different letters indicate statistically significant differences (LSD test, $P \leq 0.05$).

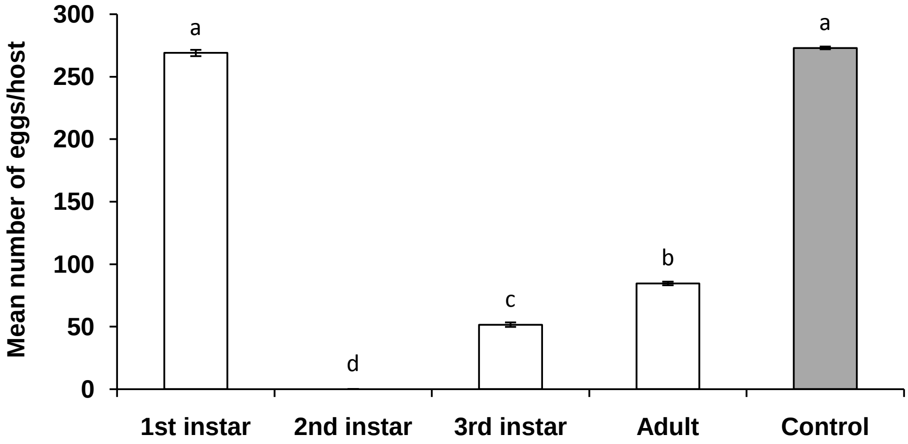

Figure 2. Comparison between mean numbers of eggs/host laid by parasitized host versus unparasitized (control). Mean column bars bearing different letters indicate statistically significant differences (LSD test, $P \le 0.05$).

3.3. Comparison between Mean Numbers of Crawlers Emerged from the Eggs Laid by Parasitized Host, Mealybug during Different Host Stages

The data given in **Figure 3** indicates the effect of parasitism by *A. bambawalei* on crawler's emergence from the eggs of parasitized host mealybugs at different host stages. Parasitization occurred in all life stages of the host except 1st instar, but eggs were laid by only 3rd instar and adult host stage which were hatched into crawlers while 2nd host stage was died before reaching to adult stage, so, there was no egg laying and no crawler emergence. The number of crawlers emerged from the eggs of parasitized host mealybugs were compared within different life stages and as well as with the control treatment (unparasitized hosts). The results showed that the treatments containing parasitized hosts, maximum mean number of crawlers emerged from the eggs laid by adult host stage was 41.95 ± 3.81 out of 84.67 ± 1.45 and from eggs of 3rd instar host stage was 12.33 ± 2.02 out of 51.66 ± 1.85 (LSD test at $P \le 0.05$, **Figure 3**). While in control treatment mean number of crawlers emerged i.e. 233 ± 1.52 out of 273 ± 1.14. The mean number of crawlers emerged from the eggs of 1st instar hosts were approximately equal to the control treatment; which further confirms that 1st instar was not parasitized at all. These results showed that parasitism has sharp negative effect on the reproduction and development of host mealybugs as the eggs produced by parasitized mealybugs were defective, most of them could not hatch which resulted very low number of crawler emergence as compared to unparasitized hosts (Control treatment). It means that venom which is injected by the parasitic female in the host body has bioactive components that affect the development as well as reproduction of the host mealybugs [12].

3.4. Mean Comparison of Parasitoids Emergence from Mummies of Mealybug at Different Host Stages

In the parasitization process, when a female wasp lays its egg in the host body, the host turns into a hard leathery structure called mummy. Larvae of parasitoid develop inside the host body and after a certain time they come out the host body by making a hole. **Figure 4** shows the mean number of parasitoids emerged from mummies of the parasitized host mealybugs. As the 1st instar crawlers of the host were not accepted/preferred by the wasp for parasitization so they remain alive and resultantly there was no emergence of parasitoid. The maximum number of mean parasitoid emergence was observed in 3rd instar, followed by adult and 2nd host stage respectively. Mean number of parasitoids emerged from mummies of different host stages clearly indicate the significant difference from each other.

4. Discussion

Parasitization by the wasp *A. bambawalei* induces significant reduction in the survival rate, fecundity and fertility of the host *P. solenopsis*. The survival rate of 1st instar hosts was recorded maximum as none of them was

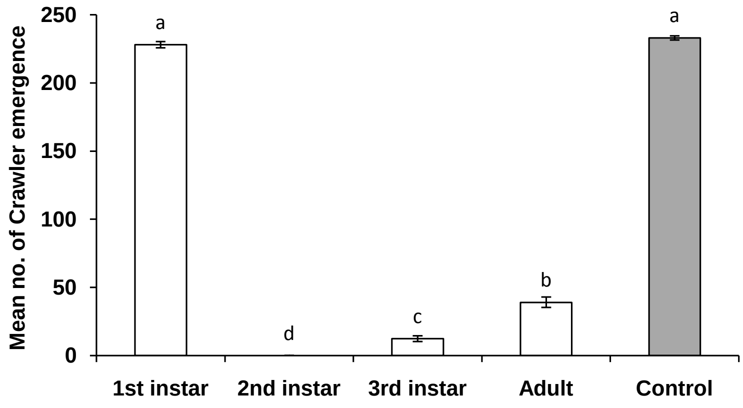

Figure 3. Comparison between mean numbers of crawlers/host emerged from parasitized versus unparasitized host (control) on different host stages. Column bars bearing similar letters for each host stage indicate statistically non-significant differences (LSD test, $P \leq 0.05$).

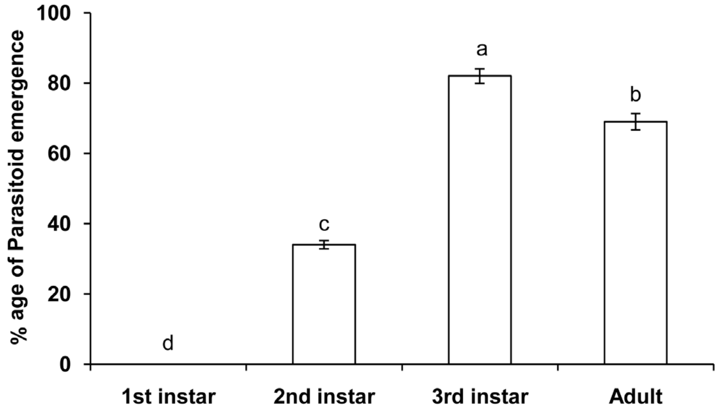

Figure 4. Comparison between mean percentages of parasitoids emergence from mummies of mealybug at different host stages. Different letters above each column bar for each host stage show statistically significant differences (LSD test, $P \leq 0.05$).

parasitized till it was shifted to 2nd instar life stage. It may be speculated that wasp did not accept/prefer 1st instar host stage for parasitization due to its small size and highly active behavior. Vijaya Ram [14] also reported the same that first instar of *P. solenopsis* was not parasitized at all. The survival rate of 2nd instar parasitized host stage was zero as their development continued till 3rd instar but all of them were ultimately died. The development of 3rd instar parasitized hosts was highly influenced by parasitization, as they reached to adult stage and produced progeny. Similarly parasitized adult host stage also produced progeny but both host stages were died very soon as compared to unparasitized one. Hence reproductive period of parasitized hosts were very short as compared to control treatment.

Our results are in line with the findings of [10] who reported during the investigation of the effect of parasitism on the development and reproduction of pea aphid by its parasitoid *Aphidius ervi* that development and survival of the host is the highest on the mature parasitized host stages and the aphids received strike of oviposition before 3 days of their life duration were died in early adult stages while those which got strike of oviposition after 4 days of their life span, successfully reached the adult stage and reproduced. Since our parasitoid is koinobiont, it exhibits a wider range of acceptable host sizes or host stages and in such parasitoids the small hosts may continue to develop after parasitism. Koinobiont parasitoids use alternative cues, such as future growth potential and nutritional status, as the basis of host quality assessment [15].

Although koinobiont parasitoids have a wider range of suitable host stages, there are costs involved in the parasitism of small hosts: developmental time is often lengthened and survival is often reduced [16]. Parasitism also affects the hatching ability of eggs as less number of crawlers were emerged or produced from the eggs laid by parasitized hosts as compared to unparasitized ones. Our findings are supported by [17] who reported the reduction in fecundity of *P. citri* parasitized by *Anagyrus pseudococci*, an encyrtid parasitoid which attacks all mealybug instars with preference for the third instar and adult female [17]. Parasitization induces cessation of fecundity of *P. citri* and the number of eggs produced by parasitized pre-ovopositing and reproductive mealybug adults were significantly different as compared to number of eggs produced by unparasitized adult females [17]. It was also revealed that the percentage of parasitoid's emergence from the mummies of parasitized hosts was different in different host stages. In our results maximum parasitoid progeny emerged from 3rd instar host stage of *P. solenopsis*. This may be speculated that 3rd instar is a richer source of nutrients for the development of wasp progeny and has a less efficient immune system as compared to adult while 2nd instar is deficient in nutrients. Mean parasitoids population on 2nd instar was recorded less as compared to 3rd instar host stage. Our results are similar to the findings of [18] who observed that 3rd instar of cotton mealybug, *P. solenopsis* is the most suitable host stage for mass-rearing of *A. bambawalei*. The emergence of parasitoids from adult host stage was less as compared to 3rd which further confirms that this host stage has more efficient immune system as compared tp 3rd. These findings are according to the [19] who reported less parasitism in adult mealybug than 3rd instar due to their defensive behavior. Similarly for other parasitoids of sucking insects, *A. bambawalei* could realize a strategy to provide enough and available nutritional substrate to the progeny development, reducing host reproductive activity [20]-[22]. This could be a common mechanism adapted by parasitoid of sucking insects and consisting in host metabolism redirection obtained with the injection of maternal secretions.

5. Conclusion

This preliminary information is very useful for developing sustainable pest management strategies for mealybug control in the field by releasing the mass reared population of the wasp at younger stages of the host. Further, it also provides information regarding which host stage is the best for the development and mass rearing of parasitoid for the farmers and other researchers. Further study will allow us to understand the specific function of parasitoid venom injected in the host body at the time of oviposition and to identify the host tissues targeted by these regulation factors responsible for the observed alterations of parasitized host reproduction.

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