

Bioecology and Efficacy of *Aenasius bambawalei* in Population Management of Invasive Mealybug, *Phenacoccus solenopsis* on Cotton Crop in Pakistan

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Abstract

Biology and parasitization efficiency of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) on mealybug, *Phenacoccus solenopsis* Tinsley were studied under laboratory condition. The rate of development of parasitoid varied on different life stages of mealybug. Total development time from oviposition to adult emergence was shortest on second instar followed by third instar and adult. Sex ratio of parasitoid also varied greatly on different life-stages.

Keywords: Cotton, *Phenacoccus solenopsis*, *Aenasius bambawalei*

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Introduction

Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) is a highly polyphagous and invasive mealybug species attacking more than 200 plants comprising field and horticultural crops, ornamentals, medicinal plants and weeds (Afzal et al. 2009, Wang et al. 2010, Fand and Suroshe 2015). It infests the leaves, fruits, branches, main stems, trunks and roots feeding on phloem sap (Wang et al. 2010). Attacked shoots and leaves are malformed due to production of large amounts of honeydew which is responsible for the development of sooty mold (Gullan and Kosztarab 1997). *Phenacoccus solenopsis* could complete about 8 -12 generations in a year (Fand and Suroshe 2015). The outbreak of *P. solenopsis* on cotton and other plants in Pakistan was first recorded in 2005 (Wang et al. 2010). Later, mealybug outbreak was also recorded from India (Yousif et al. 2007). This devastating pest has spread throughout the cotton growing areas of Pakistan and has become most serious pest of cotton and many other important crops (Arif et al. 2009). Reportedly outbreaks of *P. solenopsis* on cotton caused 30-60% yield losses in Pakistan and India during 2005-2009 (Fand and Suroshe 2015).

Phenacoccus solenopsis is difficult to control with insecticides because it is protected with a hydrophobic waxy layer which serves as a barrier to penetration of insecticides (Afzal et al. 2009). There are many mealybug species causing serious damage to agriculture and horticulture crops such as *Pseudococcus viburi*, citrus mealybug, *Planococcus citri* (Rissol) and cassava mealybug, *Phanacoccus herreni* Cox and Williams which are successfully managed by introduction and release of parasitoids in different parts of world. Among many parasitoids, the Encyrtids are documented as potent parasitoids of mealybug species and play important role in biological control of several species (Bertschy et al. 2000, Karamaouna and Copland 2000, Dorn et al. 2001).

Before introduction and release of any potential parasitoid for population management of a pest insect, basic studies are required on host-parasitoid relationships in terms of suitable host size and stage for optimal biology and population growth of parasitoid and influence of host size on sex ratio of parasitoid species. Development of a parasitoid on suitable stage is an important consideration and is generally positively correlated with progeny fitness (Hagvar and Hofsvang 1991, King 1993). Female biased sex ratio with large body size and fast development of parasitoid are required for successful biological control program (Karamaouna and Copland 2000, Chong and Oetting 2006). Female body size of parasitoids is positively correlated with fitness parameters and dispersal ability (Harvey et al. 2001, Chong and Oetting 2006). *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) is a new parasitoid species described in 2009 (Hayat 2009). This parasitoid was first time recorded in Indo-Pakistan in 2008 (Mahmood 2008). *Aenasius bambawalei* has been reported as an effective parasitoid of *P. solenopsis* in India (Gautam et al. 2009) and Pakistan (Mahmood et al. 2011, Solangi and Mahmood 2011). This is a newly discovered parasitoid species, information is lacking on its bioecology and parasitization efficiency under laboratory conditions (Ashfaque et al. 2010, Fand et al. 2011).

In the present investigation we studied the biology of *A. bambawalei* on different life stages of its host *P. solenopsis*. Influence of host stage on biological parameters of *A. bambawalei* such as days to parasitized mealybug mummy formation, adult emergence and their sex ratio.

Materials and Methods

Mealybug

The culture of mealybug, *P. solenopsis* was established in the laboratory from individuals collected from cotton fields and maintained on potato sprouts (cv. Aladin). Potato sprouts were grown in 15 cm plastic pots placed in wooden cage (30x30x45 cms). The cage has two sides made of glass panels and two sides of wire gauze. One side of cage had a circular hole (15 cm) covered with net for handling of insects. The laboratory temperature was maintained at $27\pm 1^{\circ}\text{C}$ with 50-65% RH and L16:D8 photoperiod. The parasitoid, *A. bambawalei* was reared on *P. solenopsis* feeding on potato sprouts in cages in the laboratory.

Biology of Aenasius bambawalei

Phenacoccus solenopsis were transferred from the mass culture on cotton (cv. NIAB-78) leaf. The leaf was kept with its lower surface upwards on a layer of potato dextrose agar in a plastic bottle (10 cm dia, 15 cm height) covered with muslin cloth tied with rubber band. Four stages of *P. solenopsis* were used in the study, that is, first, second and third instars and female mealybugs. Freshly moulted insects were used in the experiments.

Mummified mealybugs were collected from the mass culture and put into transparent gelatin capsules (5.0 mg size) and left in a ventilated plastic sandwich box (20x15x10 cm). They were checked twice per day at the same time for parasitoid emergence. Freshly emerged male and female parasitoids were paired together in a glass tube (10x2 cm) for 24 hours to mate. Next day, after mating, both male and female parasitoids were transferred to plastic bottles containing mealybugs on cotton leaf for parasitization. A moist cotton swabs and 50% honey solution drops on paper strips were provided for parasitoid feeding and for maintaining moist conditions inside the bottle.

The different host sizes were offered separately at 100 mealybugs / bottle to the experimental wasps in no choice experiments. Mealybugs were transferred to fresh cotton leaf with fine paintbrush daily in a clean bottle to avoid contamination. Cotton swabs and paper strip with honey drops were also changed daily. Parasitoids were transferred in a glass tube (8x2 cm) and released into the respective treatments for parasitization. The preliminary experiments on biology of *A. bambawalei* indicated that female wasps could lay eggs for 20-21 days. Therefore, fecundity and parasitization of females were observed up to 21 days. Data on parasitization were recorded on different days of parasitoid adult life and on cumulative basis. Each instar of *P. solenopsis* was exposed to *A. bambawalei* for 21 days in succeeding manner. As such, there were 21 pairs of *A. bambawalei* released for parasitization for a period ranging from one to twenty one days. Parasitoid pair one was released for one day, pair two for two days and so on up to 21 days. After stipulated period, parasitoids were discarded and mealybugs were retained for recording of parasitization. Mealybugs were then transferred from plastic bottle to Petri dish (15 cm dia, 1 cm height) lined with moist filter paper. Whatman[®] No. 1 filter paper was provided with a fresh cotton leaf daily and mummies were observed twice a day. When mummies appeared, they were recorded and transferred to a gelatin capsule till adult emergence. This experiment was replicated four times.

Statistical analysis

The *P. solenopsis* infestation was compared using analysis of variance (ANOVA). The treatment means were compared at the $P = 0.05$ significance level. Regression analyses were carried out between days of exposure of *P. solenopsis* and rate of parasitism of *A. bambawalei* (Zar, 2010). All the statistical analyses were carried out on a computer using statistical software (STATISTIX[®] VERSION-8.1, Analytical Software, Inc. Tallahassee, FL., USA).

Results

Biology of A. bambawalei

The biology of endoparasitoid, *Aenasius bambawalei* was greatly influenced by host development stage. Development time to mummy formation was shortest when second instar *P. solenopsis* was offered as a host for parasitization, followed by third instar. The longest time to mummy formation (Mean \pm SE, 11.09 \pm 09 days) was recorded on female adult *P. solenopsis* (Fig. 1).

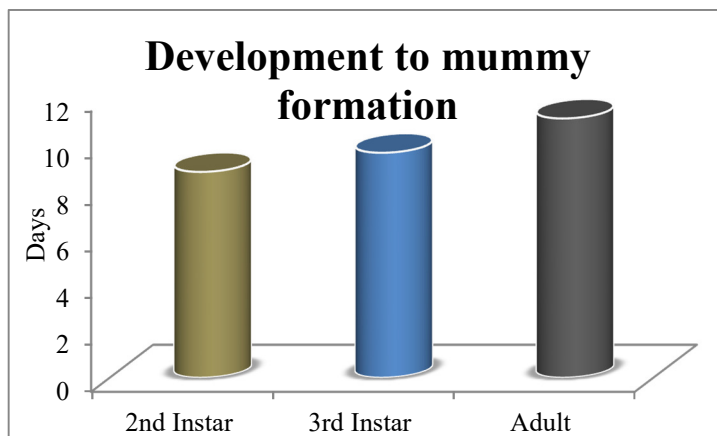


Fig.1. Development to mummy formation of *P. solenopsis* by the parasitism of *A. bambawalei* under laboratory conditions.

Majority of mummies were formed in 8 to 9 days, when second instar *P. solenopsis* was offered as a host. When third instar and female adult, *P. solenopsis* were offered as a host, duration of mummy formation was increased. More than 50% of mummies were formed in 9 and 10, and 10 and 11 days, respectively when third instar and female adult stages were given for parasitization (Fig. 2). Duration of mummy (pupal) stage of *A. bambawalei* did not vary greatly due to parasitization of different host stages. The length of pupal period of *A. bambawalei* depended on the development stage of *P. solenopsis*. Pupal stage lasted from 3 to 7 days on different host stages of *P. solenopsis* (Fig. 3).

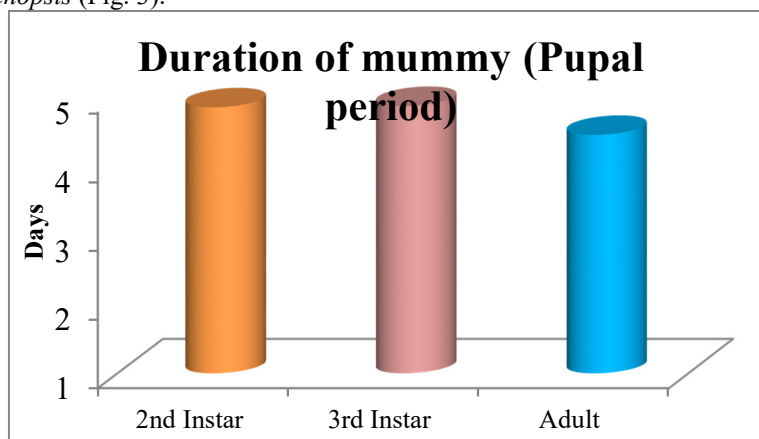


Fig.2. Duration of mummy of *P. solenopsis* by the parasitism of *A. bambawalei* under laboratory conditions.

Duration of mummy (pupal) stage of *A. bambawalei* was longest on third instar, *P. solenopsis* nymphs and the shortest on adult female. When second instar was offered as host 69.5% had completed their pupal period in 4 to 5 days. When *A. bambawalei* was released on third instar *P. solenopsis* to parasitize, 76.8% completed their pupal stage in 4 to 5 days. *A. bambawalei* when offered adults of *P. solenopsis* as a host, 93.3% had pupal period of 4 to 5 day. Total development time of *A. bambawalei* was also affected due to parasitizing on different life stages of *P. solenopsis*. Host development stage greatly affected the sex ratio of parasitoid. When feeding on second instar *P. solenopsis*, only male progeny was produced. When 3 instar and adult stages of host, *P. solenopsis* were offered for parasitization male and female biased sex ratios were observed, respectively. The highest range of parasitization was recorded on adult host stage compared with nymphal stages (Fig. 4). The life time cumulative and mean daily parasitization of *A. bambawalei* depended on the life stage of its host *P. solenopsis*. There was a significant correlation between days of exposure and rate of parasitism (Fig. 5). The average daily rate of parasitization of *A. bambawalei* on *P. solenopsis* was 2.27 ± 0.66 , 3.33 ± 0.74 and 3.66 ± 0.75 (mean \pm SE) when second, third and adult female, respectively were offered to parasitoids.

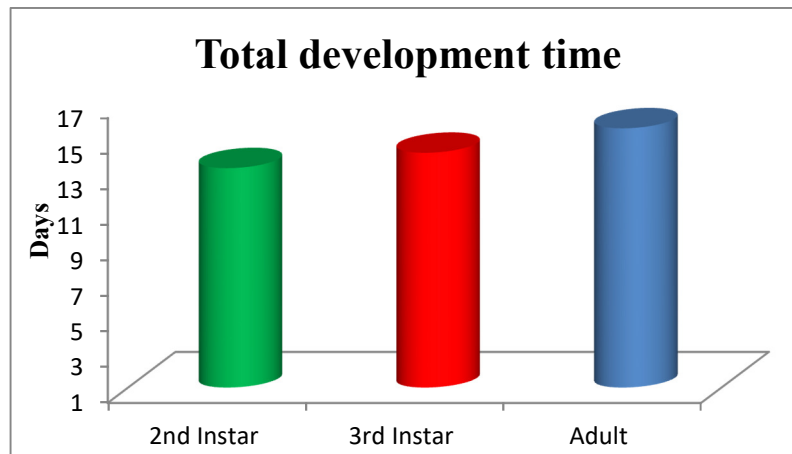


Fig.3. Total development time of *P. solenopsis* by the parasitism of *A. bambawalei* under laboratory conditions.

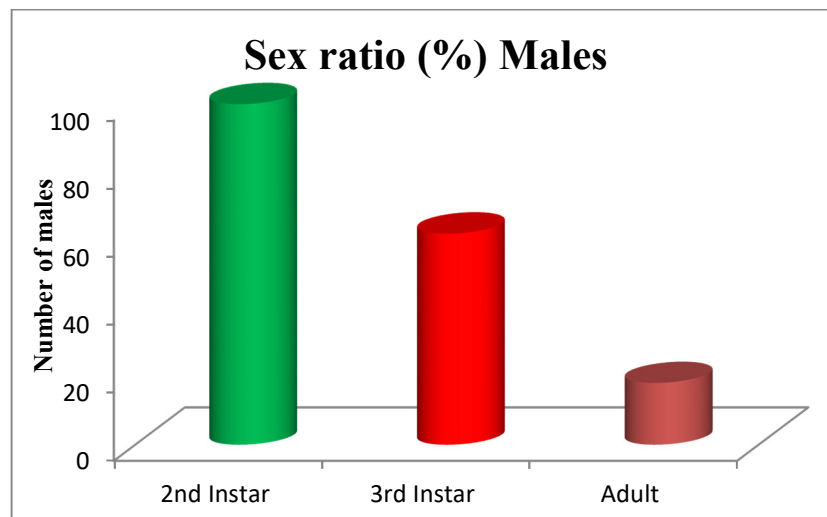


Fig.4. Sex ratio (%) males of *P. solenopsis* by the parasitism of *A. bambawalei* under laboratory conditions.

Discussion

Development time of parasitoid, *Aenasius bambawalei* from oviposition to adult emergence varied on different life stages of its host, *P. solenopsis* and it was observed that younger the host, faster the development of parasitoid. Moreover, in each host instar males developed faster than females. Depending upon host size, parasitoids started their emergence from 12 days and continued for 18 days after ovipositions. Ashfaque et al. (2010) recorded adult emergence of *Aenasius bambawalei* from 11 to 13 days after oviposition. The difference in adult emergence might be due to temperature and host stages. They carried out their studies in May and June when the laboratory temperature was 28 °C and our studies were conducted in October and November when laboratory temperature was 27±1 °C. When different host stages were offered to *A. bambawalei* for parasitization, it took longer time to develop on adult females compared with second and third instar nymphs. Ovipositing host females represent a depleting resource due to egg production, therefore, were less suitable for parasitoid development (de Jong and van Alphen 1989, Chong and Oetting 2006). No parasitization by *A. bambawalei* was observed on crawler stage (first instar nymphs) of *P. solenopsis* under no choice laboratory conditions. Under laboratory conditions, duration of second instar *P. solenopsis*, was 13 to 14 days and during first 10 days, of second nymphal instar, no parasitization was recorded. Parasitization was recorded in last four days of second nymphal instar; the emerging parasitoids were all male. Similar observations were recorded by Vijaya and Ram (2013). Many mealybug parasitoids of Encyrtidae showed preference for a range of host sizes and stages for parasitism (Chong and Oetting 2006).

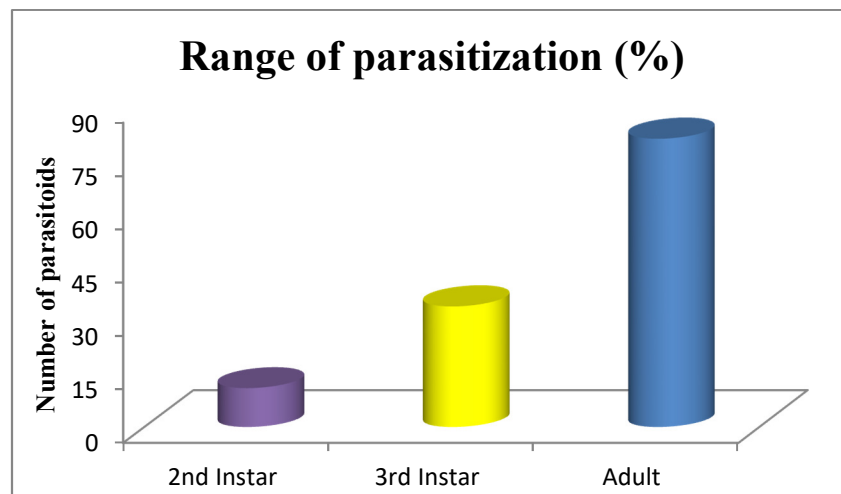


Fig.5. Range of parasitization (%) of *P. solenopsis* by the parasitism of *A. bambawalei* under laboratory conditions.

Anagyrus mangicole Noyes attacked all developmental host stages but preferred the larger host stages over smaller ones (Bokonon-Ganta et al. 1995). While citrus mealybug parasitoid, *Leptomastix dactylopii* Howard, preferred the preoviposting adults and completely rejected the first and second instar nymphs for parasitism (de Jong and van Alphen 1989). Sex ratio of *A. bambawalei* was different on various life stages of host. Adult and third instar mealybugs produced more female parasitoids, while second instar produced more males. Adult and third instar mealybugs may be most suitable host size for parasitoid female to lay preferentially female eggs and second instar the less suitable (King 1993, Bertschy et al. 2000, Abdin et al. 2013). Male biased sex ratio of *A. vexans* was found on second larval instar of cassava mealybug, *P. herreni* Cox and Williams (Bertschy et al. 2000), *Leptomastidea abnormis* parasitizing citrus mealybug, *Planococcus citri* (Cadee and van Alphen 1997), *Leptomastidea apona* (Walker) emerging from *P. viburni* (Signoret) (Karamaouna and Copland 2000) and *A. bambawalei* parasitizing *P. solenopsis* (Fand et al. 2011). Our studies showed that average female ovipositional period was 21 ± 2 days and it parasitized a mean of 81 ± 0.95 hosts in her life time. He et al. (2015) reported that female *A. bambawalei* ovipositional period was 21 days. Solongi and Mahmood (2011) reported that *A. bambawalei* females parasitized 80 ± 4.67 mealybugs in their life; Abdin et al (2012) reported a female *A. bambawalei* parasitoid parasitized 165 mealybugs in its entire life. Overall, results of our studies confirm the findings of other studies that *P. solenopsis* is an invasive and a very difficult pest to manage with a single strategy because of its biology and high reproductive potential. *Aenasius bambawalei*, although a very effective parasitoid of *P. solenopsis* and other predators active in cotton field could not bring pest population under control alone.

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