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BIOLOGICAL CHARACTERISTICS AND HOST STAGE PREFERENCE OF MEALYBUG PARASITOID *AENASIUS BAMBAWALEI* HAYAT (HYMENOPTERA: ENCYRTIDAE)

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ABSTRACT

Present study was conducted to investigate the biological characteristics and host stage preference of mealybug parasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) an endophagous, nymphal and solitary parasitoid of cotton mealybug , *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae). Preliminary experiments were conducted *in vitro* regarding its biological characteristics and host stage preference at controlled conditions (28 °C±1, 70 ± 5% % (RH) and 18 h L /6 h D). Our findings show that host swells, ceases its movement and turns in to hard leathery structure called "Mummy" after 4 and 8 days of parasitization respectively and 12-17 days are required to complete its development from first day of parasitization till emergence of adults from the mummies. Only single parasitoid adult was emerged from each mummy. Female lived for 15-32 days under laboratory conditions, whereas male died within one week. Female of the parasitoid parasitizes 165 mealybugs in its entire life and sex ratio of male and female was recorded as 1:2. Further, it is reported that 3rd instar host nymphal stage is the most preferred and suitable stage for parasitization by *A. bambawalei* as compared to other two host stages (1st and 2nd). This preliminary information regarding its biology and host stage preference will help us in its effective rearing and exploring its parasitization potential in biological control programmes of mealybugs.

Keywords: *Aenasius bambawalei, Phenacoccus solenopsis,* biology, host stage, preference

INTRODUCTION

The Mealybug, *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae), has recently emerged as a serious insect pest of cotton (Dhawan *et al.,* 2007), appeared first time in Pakistan in 2005, and became highly invasive on cotton and other plants of economic importance. It caused the serious loss of 0.2 million bales (a bale weighs 375 lbs or 170 kg each) in 2007 in Pakistan (Muhammad, 2007) and Sharma (2007) has also reported the same loss from Indian Punjab. This devastating polyphagous pest spread rapidly to all other cotton growing areas of the country and has become most damaging pest of cotton and many other economically important plants (Arif *et al*., 2009), causing heavy losses to this cash crop (Anonymous, 2005). It was found to attack a large number of plant species including crops, vegetables, ornamental plants and weeds (Arif *et al*., 2009; Abbas *et al*., 2010). It has many alternative hosts *viz*., sunflower, vegetables, weeds, ornamentals etc. (Saini *et al.* 2009). Use of chemical insecticides is an effective way for the control of this pest (Saeed and Ahmad, 2007). The use of chemicals for the control of this pest is not only expensive but also disturb the ecosystem of natural enemies (Meyerdrik *et al.,* 1982). The continuous use of chemical insecticides is the result of serious threats like development of resistance in insects, environmental pollution and side effects on human health (Pillmoor *et al*., 1993). According to sanitary and phytosanitary measures agreement on agriculture under WTO only those agriculture commodities can be exported or imported that are free from toxic pesticide residues. Keeping this in view, the native biocontrol agents predators and parasitoids were used for the control of mealybugs and an Encyrtid parasitoid of hymenoptera was identified as *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae). Parasitoids are important as diverse biological agents, which spend part of their life in the body or on the body surface of other invertebrates (Quicke, 1997; Renault *et al*, 2005). A very distinctive new mealybug parasitoids *Aenasius bambawalei* was first time reported by Mahmood (2008) in Tando jam in Pakistan in 2008. *A. bambawalei*

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(Hymenoptera: Encyrtidae) (Hayat, 2009) has been recently reported as solitary nymphal endoparasitoids *on Phenacoccus solenopsis* and as an effieient mortality factor for this mealybug under natural condition (Tanwar *et al*., 2008). Many studies have revealed that parasitisim can impact on the development, fecundity and population growth of the host (Lin and Ives, 2003; He *et al.,* 2005). The choice of host stage is an important factor in progeny fitness of parasitoids (Hagvar and Hofsvang, 1991). Usually, parasitoids fitness is positively correlated with host size especially for females (King, 1993).The present study was conducted to determine the development and host stage preference of mealy bug parasitoid *Aenasius bambawalei,* on cotton mealybug, *Phenacoccus solenopsis*.

MATERIALS AND METHODS

Preliminary experiments on the biology and host stage preference of mealybug parasitoid, *Aenasius bambawalei* were conducted in the "Insect Molecular biology lab.", Department of Agri. Entomology, University of Agriculture Faisalabad.

Rearing and handling of host-parasitoid culture in the lab

The endophagous Encyrtid parasitic wasps *Aenasius bambawalei* Hayat were reared on the colonies of its host *Phenacoccus solenopsis* Tinsley maintained on pumpkin. Parasitized mealybug/mummies were collected in plastic jars directly from the fields of cotton and vegetables (e.g. tomato, egg plant, okra, pumpkin etc.) located in the campus of University of Agriculture, Faisalabad. Mealy bug and parasitoid cultures were maintained in two separate glass jars, both at 28 °C±1, 70 ± 5% % relative humidity (RH) and 18 h light /6 h dark photoperiod. Naive/virgin parasitoid adults were obtained from mummies of mealybug kept singly in glass vials, plugged with cotton wool in the laboratory. Effective parasitization was obvious when the parasitized mealybug sheds its wax, swells and hardens into a leathery, brown colored structure called "mummy". Complete development of parasitoid larvae depends upon the temperature and other environmental factors. Non parasitized mealybugs were kept in a separate glass jars under the same environmental conditions. Insect parasitoids to be used in the experiments were obtained from the muumies of mealybugs and were isolated singly in glass vials plugged with cotton wool and provided with honey and water as a food source upon emergence. The adults of *A. bambawalei* were separated by sexes: morphologically the females are easily distinguished from the males by their pointed/conical abdomen (ovipositor).

Developmental and biological attributes of *Aenasius bambawalei*

Synchronized 10 mealybugs of 3rd instar nymph were taken from the rearing culture of the host in a petri dish (90×15mm) and one pair of newly emerged male & female pararsitoid was released in that petridish. Parasitoids were allowed to parasitize the hosts in the petridish for 24 hours and then parasitized mealy bugs were shifted on the pumpkins, kept in the separate jar in climatic chambter at 28 °C±1, 70 ± 5 % relative humidity (RH) and 18 h light /6 h dark photoperiod. The developmental period of the parasitoid was examined regularly till the emergence of adult parasitoids from mummies.

Host stage preference of the parasitoid

A preliminary study was conducted regarding the host stage preference of mealybug parasitoid whether *A. bambawalei* females have any preference for different host stages of *P. solenopsis* for oviposition. Four host stages including three nymphal and one adult were used in this experiment. The adult parasitoids emerged from mealybug mummies that were parasitized at the stage of 3rd instar nymphs were used in this experiment. 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 for parasitizing mealybugs for 24 hours. The above experiment was repeated five times, then 10 parasitized mealybugs were selected randomly and dissected for the observation of number of developing and diapausing parasitoid larvae. The dissection was done 4 days after parasitization in 70% alcohol under stereomicroscope. As a result a total of 200 mealybugs were dissected for different host stages. The data were analysed statistically.

Statistical analysis

The obtained data was analyzed statistically by using software Statistix version 8.1 (Analytical software, 2003), and subjected to analyse variance under completely randomized design. Means were separated by LSD- Test. A significance level of $P<0.05$ was used for all statistical tests.

RESULTS

Developmental and biological attributes of *Aenasius bambawalei*

After 2-4 days of parasitization, the swelling of the host mealybugs was observed which turned in to hard leathry structure " mummies" after 8-days of parasitization. The developmental period of the parasitoid (1st day of parasitization till emergence of adult wasp from mummies) was recorded 12-17 days at controlled conditions (28 °C±1, 70 ± 5% relative humidity (RH) and 18 h light /6 h dark photoperiod). Single parasitoid adult was emerged from each mummy of the host, evidenced that female of parasitoid lay single egg in its host. Male wasp was died within 7-10 days whereas female was survived for 15-32 days. Female parasitoid parasitizes 30-165 mealybugs in its whole life and sex ration for male and female of the parasitoid was observed 1:2.

Host stage preference

The results regarding the host stage preference showed that maximum mean number (9.2) of parasitoid larvae and adults were emerged from adult host stage followed by 3rd instar nymph (4.8), 2nd instar nymph (3.2) and 1st instar nymph (0)

of host stages respectively, indicating very clearly that there was no parasitoid larvae emerged from 1st instar nymph host stage as shown in Fig. 1. The obtained results revealed that maximum percentage of parasitization of host was occurred in adult host stage (92%) Followed by 3rd, 2nd and 1st instar nymph of host stages i.e. 48%, 32% and 0% respectively Fig. 2.

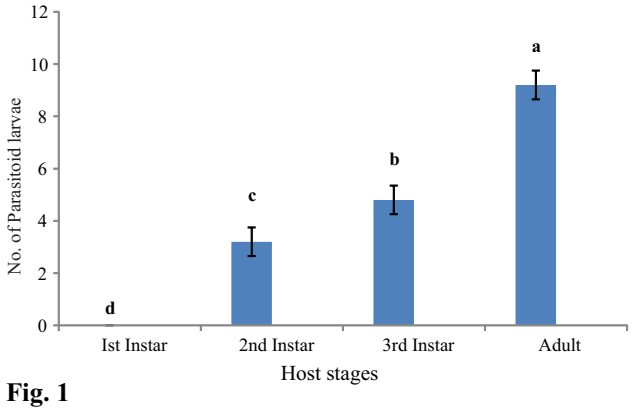

Fig. 1
Number of parasitoids larvae at different host stages.

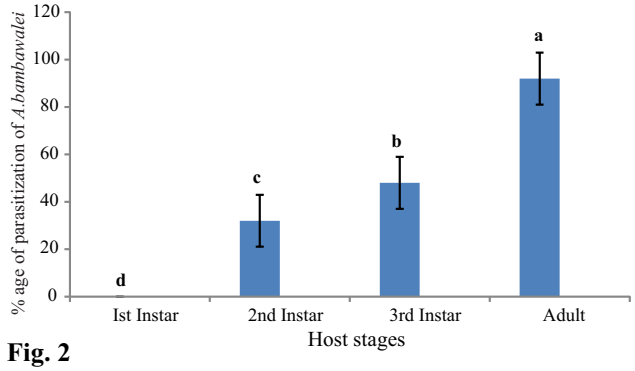

Fig. 2
Percentage of parasitization of different host stages by *A. bambawalei.*

DISCUSSION

The present study was conducted to determine the developmental period and host stage preference of mealybug parasitoid *Aenasius bambawalei*. The results indicate that the complete development of *Aenasius bambawalei* from the first day of parasitization till emergence of adult parasitoids take 12-17days. Mummification occur after 8 days of parasitization and only single parasitoid emerged from each mummy. The similar finding was also reported by (Solangi and Mahmood, 2010).

The female of *A. bambawalei* laid eggs on all host stages except first instar host stage during oviposition. The results showed that maximum mean (9.2) number of parasitoids larvae and adults were emerged from adult host stage whereas minimum mean number of larvae were emerged from 2nd instar nymph (3.2) and no wasp larvae and adults were emerged from 1st instar host stage at all. (Fig. 1). Therefore, the above results showed that maximum percentage of parasitization was reported from adult host stage (92%) while minimum percentage of parasitization was recorded in 2nd instar nymph (32%) as shown in (Fig.2). A relationship was established between the number of eggs laid and the host stage, i.e, *A. bambawalei* laid increasingly more eggs with the increase of host stages. Different studies also suggest that large hosts may generate superior parasitoid fittness (Charnov e*t al.,* 1981; Liu, 1985; Harvey *et al*., 1994). The parasitoid developmental period was longer in younger host stages. This might be due to inadequate resources available at younger stages of the host (Hu *et al*., 2002, 2003; Colinet *et al*., 2005). A similar relation between parasitoid developmental time and age of the host at time of oviposition has been reported by (Bertschy *et al*., 2000; Colinet *et al*., 2005). The conclusion of the study is that 3rd instar host stage is the most suitable and preferred host stage for mass-rearing of *A. bambawalei* in biocontrol programmes as it produces more female progeny of the best fitness as compared to the other two stages (1st and 2nd) of the mealybugs.

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