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The Role of Parasitoid Age on the Fecundity and Sex Ratio of the Parasitoid, *Aenasius bambawalei* (Hayat) (Hymenoptera: Encyrtidae)

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Authors' Contributions
ZA conceived the project and wrote the article with the help of MSI and MT. MSI executed the experimental work. SKA analyzed the data. AM helped in collection and rearing of insect parasitoid. MA was member of advisory committee. AJ collaborated in this project.

Key words
Parasitoid age; fecundity; sex ratio; *Phenacoccus solenopsis; Aeansius bambawale;* host stage.

A B S T R A C T

The effect of parasitoid age on the fecundity and sex ratio of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) was determined on different life stages of the host *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) by keeping both at 27±2°C and 65±5% relative humidity during parasitization. Fecundity of the female parasitoid did not change significantly during the first 5 days of its life span but afterwards it decreased significantly under laboratory conditions. While offspring sex ratio of 1 day old female wasp was found to be female biased on both 3rd and adult host stages but afterwards it changed to male biased sex ratio of 5 and 10 days old parasitoid female. The male emergence was more by 5 days old parasitoid female than 1 day old at 3rd instar host stage while at adult host stage it was more by 1 day old female than the 5 and 10 days old female wasp. Emergence of total progeny of the wasp (adult female and male) was significantly higher on adult host stage than 3rd instar host stage. The interaction of parasitoid age and host stage has significant effect on the emergence of male than on the female wasp. The fecundity and the parasitic potential of the female parasitoid reared on adult host stages were higher than those reared on 3rd instar host stages.

INTRODUCTION

Cotton is the world's most important fiber crop and cultivated on more than 30 million hectares in about 80 countries. The cotton having a share of 1.4 percent in Gross Domestic Product (GDP) and 6.7 percent in agriculture value addition is an important source of raw material to the textile industry (Anonymous, 2014). The land under cotton cultivation in Pakistan has increased overtime; however, the yield per hectare is much lower as compared to other cotton growing countries (Dhawan *et al.*, 2009). Insect pests and diseases are considered the primary reason of the low productivity under field conditions. The mealybug *Phenacoccus solenopsis* Tinsley is a new invasive pest of cotton crop in Pakistan. It was first reported in 2005 damaging cotton and other plants at Agriculture Research Station, Vehari (Punjab) and some places in Sanghar, Sindh. It caused huge economic loss of about 3.1 million bales in 2006-07. In 2006 about 12% of the crop and in 2007 about 40% of the crop in Punjab was damaged by this insect (Kakakhel, 2007; CAIR, 2007). Feeding of *P. solenopsis* causes growth abnormality characterized by curling and crinkling of leaves. Flowers, mature bolls and even leaves fall. Heavy infestations result in plant death. Infested flowers often drop and usually there is little or no fruit production. *P. solenopsis* also secretes large quantities of honeydew which encourages growth of black sooty mold on the leaves and twigs resulting in reduced photosynthetic activity (Mahmood *et al.*, 2011). Different management strategies like chemical, biological and cultural control have been used to combat this threat. Since pesticides lead to many serious problems like air and water pollution, health hazards, killing of beneficial organisms, pest resistance, pest resurgence, secondary pest outbreak, interruption in eco-cycles and biodiversity (Bellows, 2001), so we cannot rely on pesticide use for pest control. Biological control of insect pests utilizing parasitoids has been encouraged as one of the most recent techniques and unlike other control measures its effect is permanent, ecologically non-disruptive, self-sustaining and after the initial costs involving investigations and release, the recurrent costs are nominal (Chaturwedi *et al.*, 2013). According to above limitation native biocontrol agents predators and parasitoids were used for the control of mealybugs. A parasitic wasp *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) was identified as a successful biocontrol agent for this pest. It has been reported as a solitary nymphal endoparasitoid of *P. solenopsis* (Hayat, 2009). It is the only dominant and aggressive parasitoid reported so far, responsible for the

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suppression of mealybug population in field (Kumar *et al.,* 2009). Feng *et al*. (2014) reported that *A. bambawalei* has a high parasitism level on *P. solenopsis* under laboratory conditions and should be considered as a biological control agent. The parasitic wasp injects different host regulation factors in order to make favorable host environment at the time of parasitization, which is the requirement of the immature parasitoid for successful progeny development (Boivin *et al.,* 2004). Various studies have demonstrated that fecundity, progeny sex ratio and parasitism ability of parasitoid depend on parasitoid age (Melton and Browning, 1986; Drost and Carde, 1992; Morales-Ramos and Cate, 1993; Medeiros *et al.,* 2000), host species (Andow *et al.,* 1997), host instar (Sengonca and Peters, 1993), number of eggs laid by the female (Petitt and Wietlisbach, 1993), time passed till finding hosts (Orr *et al.,* 1986), host abundance (Hirashima *et al.,* 1990) and presence of hyper parasitoid species (Godfray, 1994). In classical biological control programs, the success rate is closely related to behavioral and physiological interactions between parasitoid and host (Andow *et al.,* 1997). This rate can be improved through greater knowledge about the physical, chemical and mechanical variables involved in these interactions, and the biological characteristics including fecundity, sex ratio and ecology of host and parasitoid species. Detailed investigations on these subjects will contribute to the success of biological control agent applications and mass production of parasitoids. Therefore, this study was proposed to investigate the effect of parasitoid age on the fecundity and sex ratio of the parasitoid *A. bambawalei* Hayat at various developmental host stages.

MATERIALS AND METHODS

Rearing and handling of host-parasitoid culture

The endophagous Encyrtid parasitic wasp *A. bambawalei* Hayat was reared on the colonies of its natural host *Phenacoccus solenopsis* Tinsley. Parasitized mealybugs/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 main campus of University of Agriculture, Faisalabad. A breeding colony of *A. bambawalei* were 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. Mealy bug and parasitoid cultures were maintained in glass jars, both at 27±2ºC, 65±5% relative humidity (RH) and 18 h light/6 h dark photoperiod by following a slightly modified approach as described by Zain-ul-Abdin *et al.* (2012). Naive/virgin parasitoid adults were obtained from mummies of mealybug and they were provided with honey and water as a food source.

Fecundity and sex ratio

Newly emerged wasps were used for the fecundity and sex ratio experiments on two different host stages (3rd and Adult). To investigate the age dependent fecundity and sex ratio of adult females of *A. bambawalei* these newly emerged females were divided into age classes at four day intervals, ranging from one, 5 and 10 days old. The females were kept in unmated conditions by keeping male and female in separate jars. As the parasitoid wasp is endo-parasitoid it essentially needs host for oviposition, its oviposition was inhibited by not providing mealybug host. One day old female parasitoid was kept for mating in a vial (2cm × 10cm), which was provided with a male and fine honey drops were sprinkled on the inner side of vial for feeding. In the 1st age group (One day old) a 24 h mated female was exposed to 20 hosts (mealybugs) maintained in the plastic jar on a piece of pumpkin for parasitization. The plastic jar was covered with muslin cloth. On alternate day, the female was removed. Similarly in other experiments (5 days old and 10 days old) a 24 h mated female was provided with 20 hosts in each plastic jar and from each jar female was removed after 24 h parasitism interval, respectively. Plastic jars were examined daily and the number of wasps emerging and sex ratio was recorded at various host stages. The experiment was replicated five times.

Statistical analysis

The effect of parasitoid age on offspring production and sex ratio was statistically analyzed by statistix 8.1 (Analytical software, 2003) and subjected to the analysis of variance under factorial design and means were separated by using the LSD test when the ANOVA was significant at $P < 0.05$.

RESULTS

Effect of parasitoid age and host stages on adult emergence

Table I shows comparison of total adult progeny, female and male emergence in different parasitoid age groups and different host stages. The total adult progeny emergence per female changed in relation to the different host stages and parasitoid age groups. The total adult progeny and female emergence of parasitoids were statistically significant on day 1 and 5 of parasitoid age on adult host stage (ANOVA, df=1, F=78.24, P<0.00, LSD, P>0.05) Compared to 3rd instar host. However, for 10 day old parasitoid female, the adult emergence was

Table I.- Comparison of means (Mean ± SE) for the total progeny emergence with interaction of different parasitoid age groups and different (3rd instar and adult) host stages.

Host stage	Parasitoid age (Day)	Total adult emergence	Male emergence	Female emergence
Adult	1	81.67±0.88a	38.33±1.07ab	43.33±6.27a
	5	78.00±0.57a	41.00±3.26a	37.00±6.01ab
	10	47.00±1.73c	26.67±0.81d	20.33±0.64d
3rd Instar	1	71.67±1.45b	33.67±1.33c	38.00±0.57bc
	5	69.00±2.08b	34.67±1.85bc	34.33±1.20c
	10	44.33±1.85c	23.00±0.69d	21.33±2.83d

Analysis of variance regarding the effect of parasitoid age on the male emergence at different (3rd and adult) host stages.

Source of variation	DF	SS	MS	F	P
Instar	1	234.72	234.72	78.24	0.0000
Age	2	3491.44	1745.72	581.91	0.0000
Host stage*Parasitoid age	2	47.44	23.72	7.91	0.0064
Error	12	36.00	3.00		
Total	17	3809.61			

Means followed by different letter (s) within each column (denoted by lower-case letters) are significantly different by LSD test at *P* <0.05.

significantly different from the other two parasitoid age groups on both host stages (ANOVA, df= 2, *F*=581.91, *P*<0.00, LSD, *P*<0.05 for parasitoid age). Male production was low by the 1 day old female both in 3rd instar and adult host, which then increased in 5 day old parasitoid in both host stages. On 3rd instar it was high on 1 day old female and decreased significantly with the increase in age. The analysis of variance shows that host stage, parasitoid age and interaction of host stage and parasitoid age have significant (P < 0.05) effect on the male emergence at different host stages.

Figure 1 shows the effect of parasitoid age on the sex ratio of adult and 3rd instar host, *P. solenopsis*. The parasitization effect showed that one day old female wasp produce female biased sex ratio, while with the increase in parasitoid age male biased sex ratio was recorded in both host stages (3rd and adult).

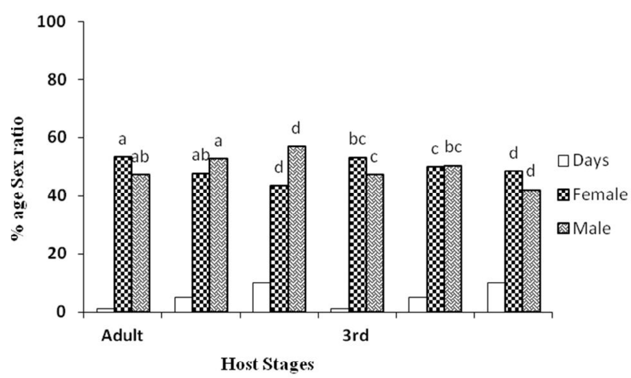

Fig. 1. Comparison of mean percentage for the sex ratio emerged from different parasitoid age groups and different (3rd and adult) host stages.

DISCUSSION

Present study was conducted to check the effect of parasitoid *A. bambawalei* age on its fecundity and sex ratio at different life stages of its host *P. solenopsis*. Our findings reveal that parasitoid age affect the fecundity and sex ratio of the parasitoid. As the age of the parasitoid increases its reproductive capability or fecundity decreases with the decrease in its sex ratio at its host cotton mealybug. It was found that offspring production per female decreased with the female age. The decrease in offspring production during the first 5 days of the female lifespan was not significant (P>0.05), whereas in the last age group it was significantly different (P<0.05) (Table I). Similar results have been reported for *Dibrachys boarmiae* (Walker), *Catolaccus grandis* (Burks), *Bracon hebetor* (Say) and *Apantales galleriae* (Wilkinson) (Gulel, 1982; Morales-Ramos and Cate, 1992; Gunduz and Gulel, 2005; Uckan and Gulel, 2002). As older females laid fewer eggs than younger ones (Gul and Gulel, 1995), the decrease in fecundity during the female's lifespan was physiologically age-dependent

(Ode *et al.*, 1997; Bressac and Chevrier, 1998). The period of declining fecundity starts when the females have oviposited approximately 60% of their total oviposition potential and ends with their death (Morales-Ramos and Cate, 1992; Medeiros *et al.*, 2000). We determined that the declining fecundity period started at the tenth day of age and ended with the death of females. This result was similar to those of Gul and Gulel (1995) who reported that age of the parasitoid is also crucial on successful parasitism. Hameed *et al.* (2010) studied the effect of age on reproduction and sex ratio of *Plodia interpunctella*, he concluded that all variables were decreased significantly with the increase in age. A younger parasitoid is more fecund than the older ones. The effect of age of the parasitoid on their ability to parasitize their host has been documented on some parasitoids (Hentz, 1998; Honda and Kainoh, 1998). For instance, the optimum age for *Cotesia marginiventris* (Cresson), to successfully parasitize larvae of *Spodoptera frugiperda* (Smith) ranges from 48 to 96 h (Rajapakse, 1992). *C. marginiventris* younger or older than the above age were not able to parasitize a host.

Present results showed that the offspring sex ratio of I day old female was female biased on both host stage but afterwards it changed to male biased sex ratio of 5 day and 10 day old parasitoid female (Fig. 1). Our results were supported by the work of Hameed *et al*. (2010), who investigated that when 3 days old virgin female were paired with newly emerged males, there was a male biased sex ratio (54-56%) as males were produced in all delayed mating combination. Zhao *et al.* (2013) reported that male progeny developed faster than females and older host pupae produced higher proportion of males. He also reported that initial host stages produced female biased while later stages produced male biased sex ratio. A similar increase in the proportion of male progeny with maternal age of *T. minutum* was reported by Houseweart *et al.* (1983), Smith and Hubbes (1986) and Li *et al.* (1993). Our findings are in accordance with Zipproah *et al*. (2013) who reported with the conclusion that highest progeny production, parasitism and sex ratio (females percentage) was obtained during the first day of host exposure and decreased with parasitoid age while investigating the reproductive fitness *i.e.*, parasitism, adult progeny production and sex ratio of a native egg parasitoid Trichogrammatoidea sp.nr. lutea Girault (Hymenoptera: Trichogrammatidae). The progeny sex ratio of *A. bambawalei* became male biased with the increase in age on both host stages. Fuester *et al*. (2003) reported similar results for the braconid *Glypapantales flavicoxis*. Increased female age also leads to a higher proportion of male offspring in *M. zaraptor* (Coats, 1976).

Parasitoid age and host stage interaction results were significant as given in Table I. Fecundity and sex allocation in insects are also age dependent, because egg supply and sperm reserves are depleted and the mother's investment in terms of clutch size, egg size and nutrient contents decrease with time (Giron and Casas, 2003). Among parasitoids, the existence of a positive relationship between maternal age and male production has been documented (Uçkan and Gülel, 2002; Gündüz and Gülel, 2005). Given that a single mating seems to be the rule in parasitoid wasps (Assem *et al.*, 1989), large and well-fed females that live longer should have higher sex ratios because they have exhausted their sperm supply (Godfray, 1994).

CONCLUSION

It may be concluded that age is a critical factor affecting the progeny and sex ratio in host parasitoid interaction. Sex ratio of *A. bambawalei* was found to be male biased with the increase in female parasitoid age under laboratory conditions. Further experimental studies are required to determine which other factors are responsible for sex allocation in *A. bambawalei*.

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