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Developmental biology of *Encarsiasophia* (Girault and Dodd) (Hymenoptera: Aphelinidae) on *Bemisiatabaci* (Gennadius) (Hemiptera: Aleyrodidae)

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Abstract

Encarsiasophia(Girault and Dodd) (Hymenoptera: Aphelinidae) is an abundant parasitoid of *Bemisiatabaci (*Gennadius) (Hemiptera: Aleyrodidae) in Pakistan. Biological studies of *E. sophia* were carried out on brinjal (*Solanummelongena*) in Insectary Biological Control Labs., NARC, Islamabad at 25±1°C, 60±5% RH and 14:10 L:D Photoperiod. Results demonstrated that mated *E. sophia* laid female eggs internally in whitefly nymphs (primary host) while male developed hyperparasitically by laying male eggs on the surface of 3rd instar larva (secondary host) by a virgin female. Both sexes have same developmental stages i.e. egg, 1st, 2nd, and 3rd instar larva, a pre-pupal and pupa stage. Time from egg to adult development till emergence was lower in female *E. sophia* (12.61±0.13 days) and was observed higher in male *E. sophia* (13.94±0.16 days). Super-parasitism was not observed in controlled experimental conditions but was common in glasshouse conditions. For developing mass-rearing protocols, our results will provide useful directions and contribute during field releases trials for controlling *B. tabaci*populations.

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Introduction

The whitefly*Bemisiatabaci*Gennadius (Hemiptera: Aleyrodidae) is an insect pest of economic importance in agronomic and horticultural crops in tropics and subtropics. It is a polyphagus insect causing direct yield losses by sucking cell sap and indirect losses by vectoring many viral diseases (Chu *et al.,* 1999). These losses aggravate when sooty molds growing on honey dews secreted by white fly cover the plant foliage and reduce their photosynthetic activities (Bethke*et al.,* 1991; Perring, 2001).

Despite commercially available insecticides, 'Biological Control' provides a useful substitute especially for the pests of greenhouses where climatic conditions are comparatively stable. Biological control of insect pests is an important component of integrated pest management system that can reduce insecticide applications load in different crops grown in diversified ecosystems (Ali and Rizvi, 2007). There are a variety of parasitoids and predators that can suppress whitefly population successfully. These days, about 125 species of beneficial organism are commercially available to control many insect pests in greenhouses (Van Lenteren, 2000).

Aphelinids in the genera *Encarsia*and *Eretmocerus*(Hymenoptera: Aphelinidae) are the most prevalent parasitoids with a successful record in biological control programmes to supress whitefly (Polaszek*et al.,*1992; Zang & Liu, 2008). *Encarsiasophia* (Girault and Dodd) (Hymenoptera: Aphelinidae) belongs to 'strenua group' is an autoparasitoid, firstly described by Timberlake in 1926. It is known as worldwide leading parasitoid against whitefly (Gerling, 1983; Giorgini and Baldanza, 2004; Otim*et al.,* 2005), which produces female offspring within the host by consuming the phytophagous host insect and male offspring develop inside the same phytophagous host insect and consume the immature female offspring (Walter, 1983), either of *E. sophia* or other parasitoid species (*Encarsia*and *Eretmocerus)* (Hunter and Kelly, 1998). Modern researches validates the effectiveness of *E. sophia*in the biological control, and it was concluded that this parasitoid suppresses more whiteflies through parasitism as well as by feeding its host contents as compare to other generally used species (Hunter and Kelly, 1998; Zang and Liu, 2009).

Studies revealed that there are a number of natural enemies that were likely to compete but the taxonomy and biology of most of these species especially parasitoids are not well-known. A number of research studies indicated that *E. sophia*from Pakistan as extremely effective biological solution against cotton pests (Roltsch and Goolsby, 1998). Efficient mass production and parasitoid field releases in *B. tabaci* control programs are totally depends upon the extensive information about biological relationships between host and parasitoid. To massrear *E. sophia*,understanding of the biological aspects particularly important to determine its exact developmental strategy about male and female wasps for its use in field. However, current study was designed to investigate the developmental behaviour of the *E. sophia* female in primary host and male in secondary host under laboratory conditions to fulfil requirements of its mass rearing.

Materials and methods

All studies were carried out in Insectary Biological Control Labs., NARC, Islamabad under 25±1°C and 60±5% RH and 14:10 L: D photoperiod.

Host Plants

Brinjal plants (*Solanummelongena*) were sown and grown in plastic pots (15cm×12cm) having potting mixture (compost: soil: sand). A number of 2-3 seedlings/pot were sown in each pot and placed the pots inside a greenhouse. After successful plantation, the plants reached to approximately 3-5 fully developed leaf stage were used to rear *B. tabaci.* Plants were seeded every two weeks interval to maintain a continuous supply of plants for colony maintenance. Healthy plants were grown in an isolated glasshouse to hold a pest free environment.

The plants were fertilized and watered well.

Hostand Parasitoid Culture

*B. tabaci*leaves withpupae and adults were collected originally from the fields of cotton areas from the Punjab province and released on brinjal plants inside the glasshouse. Emerged adults settled on the host plants placed in the glasshouse. The established whitefly colonies were maintained in the glasshouse at (27±2°C, RH 60±5% and 14:10 L: D photoperiod). Two weeks after infestation, the plants were monitored to ensure that *B. tabaci* 3rd and 4th instar nymphs had started their development on all host plant.*E. sophia*stock culture was established by getting melanised black pupae from the cotton plants in the field. They were brought in the Insectary Biological Control Labs., NARC and carefully removed from the leaf parts with the help of a camel hair brush. Pupae were placed in the glass petri dishes till emergence. The emerged adults of the parasitoid were released in the cage (48cm x 36cm x 36cm) containing brinjal plants infested with *B. tabaci*instars (mostly 3rd and 4th).

Preparation of B. tabaci Host Stage

Approximately 50 adult *B. tabai* containing an equal ratio (M:F) were collected with an electric aspirator and released into each clip cage (3cm×1.5cm) and placed lower surface of brinjal leaves and left for oviposition. After 24h, all the adults were removed by an electric aspirator and the leaf portion occupied by the clip cage encircled by an inedible ink pen. The plants were kept in air-conditioned Insect Holding Rooms under controlled conditions till the nymphs reached to 3rd instars. Choice of stage for *B. tabaci* based on earlier literature recommendations that unmated female *E. sophia* oviposit unfertilized eggs in *B. tabaci*under 'dry environment 'conditions.

Development of Female E. sophia

To understand the development of female immatures of *E. sophia*, pupae of *E. sophia* (50) were collected from the brinjal plant with the help of a camel hair brush and placed in the glass vials under light. Upon adult emergence male and female parasitoids were allowed for mating for a period of <24 h and provided with 10% sugar solution as their diet. Two mated*E. sophia*females were exposed in a clip cage with *B. tabaci*3rd instars (10). After 24h, the clip cages containing adult females were removed and plants were kept undisturbed inside the insect holding rooms with controlled conditions to see the development of female *E. sophia*. Parasitized *B. tabaci*instars were encircled and copied the same leaves on the A4 size paper to mark the locations of the instars. Instars were observed daily under binocular stereomicroscope (16SZX-Japan) equipped with a digital Olympus camera system to identify parasitized stage egg, 1st, 2nd, 3rd instars, pre-pupa and pupa stage of *E. sophia*was achieved. Development time was calculated from the 1st day a female was introduced. Egg period was recognized as 1st instar larva was observed. Developmental duration for 2nd and 3rd instars, pre-pupa and black melanised pupae were observed by the similar approach. Furthermore, morphological changes of nymphs were examined. A total of thirty parasitized *B. tabaci* instars were observed for parasitoid developmental stages.

Development of Male E. sophia

Male development was much complicated and was observed by a different method as *E. sophia*is an autoparasitoid and shows its male and female developmental behaviour differently. *E. sophia*female (virgin) desires to oviposit male eggs in already parasitized *B. tabaci*nymphs (secondary host) of later stages (dry environments) (Gerling, 1983; Hunter and Kelly, 1998;Zang and Liu, 2008, 2009). Newly emerged female *E. sophia*<24 h old was exposed in a clip cage arena on the leaf surface containing a number of 3rd instar of primary parasitoid larva (Zang*et al.,* 2011) of brinjal separated from the plant and placed in a plastic vial (50ml) containing water in it. The vial contains a hole from which the petiole is inserted in the water at the bottom and left there for oviposition.After 24h, female parasitoids were removed carefully without damaging any host instar

and the vials were kept undisturbed in insect holding room. Time for each stage was recorded as described for female development by observing instars under stereomicroscope until pupae formation. Pupae were isolated from the leaf parts and placed in (7cm×1.5cm) petri dishes under light and all of wasps that emerged were sexed and counted. A total number of 100 hosts comprising five instars in each arenawere used to observe the male development. Total 20 replicates were allocated for each treatment. Out of 100 secondary hosts, 23 successful males were obtained for development.

Statistical analysis:

The development time of *E. sophia* was analysed by Analysis of Variance (ANOVA), and means were compared by using the Least Significant Difference (LSD) at $P \leq 0.05$ with use of Statistics 8.1 software.

Results

Development Time

Mean development duration for *E. sophia*from egg deposition till adult emergence was significantly different 12.61±0.13 days for female and 13.94±0.16 days for male (F=37.4; df=1, 43; P=0.00). The developmental stages were observed as eggs, 1st, 2nd and 3rd larval instars, pre-pupa and black pupa (Table 1).

Table 1. Duration of developmental stages of *E. sophia* Male and Female (Mean±S.E).

Immature stages	Female	Male
Egg	1.60±0.08b	2.33±0.10a
1st instar	2.10±0.06a	2.09±0.07a
2nd instar	1.53±0.07b	2.00±0.08a
3rd instar	1.40±0.09b	1.76±0.10a
Total larval period	5.07±0.11b	5.90±0.12a
Pre-pupa	1.00±0.04a	1.11±0.04a
Pupa	5.03±0.11a	4.55±0.14b
Egg to Adult	12.61±0.13b	13.94±0.16a

Means with different letters are significantly different from each other at P≤0.05 LSD-test.

Development of Female E. sophia

Female *E. sophia* penetrates its ovipositor inside the whitefly (primary host) by standing over the host body and lays an egg (Fig. 1A). At this stage the egg changed apparently and migration of the cleavage nuclei started towards the margins away from each other (Fig. 1a). Development of egg to 1st instar occurred in average 1.60±0.08 days. The 1st instars were transparent with no clear segments on body. The larvae freely moved inside the body fluid of the host (*B. tabaci*). The development time from 1st to 2nd instar takes average 2.10±0.06 days (Table 1). In the 2nd instar, transparent cuticle was observed and most of the internal organs of the parasitized nymphs were visible through it. Averaged developmental days from 2nd to 3rd instar larva happened in 1.53±0.07 days (Table 1). The 3rd instar larva was observed clear and wide with an outer boundary and clearly distinguishable body segments (Fig. 1D). Illeolabial gland (Fig. 1b) and spiracles (Fig. 1c) and exuviae (Fig. 1d) were easily observed at this stage. Development of 3rd instar confirms a dry environment in host. The 3rd instar larvae completed its developmental duration in average 1.40±0.09 days (Table 1). Larvae were like sickle-shaped (Fig. 1e). Total larval period was observed as 5.07±0.11 days in female development (Table 1). The 3rd instar larva moved to the front side in host puparium and changed into pre-pupal stage. Pre-pupae were lie in central peripheral region with very light-yellow or sometimes milky white in colour and can easily be visible through the cuticle (Fig. 1F). The colour of meconium pallets of pre-pupa changed from yellow to light brown at the mesolateral position and was

visible easily through the nymph cuticle (Fig. 1f). Pre-pupa was converted to full black pupae in 1.00±0.04 days. Black pupae of females take 5.03±0.11 days to complete its development (Table 1). Later in pupal stage, distinct body parts can be easily distinguished from the host cuticle (Fig. 1H). Inner black pupal cuticle first removed by the female and the adult orange body comes outside which can be easily observed during emergence (Fig. 1I) and then female made an exit-hole in the host cuticle by chewing it antero-dorsum, which takes few min for *E. sophia* to come in environment with fully developed body parts. *E. sophia* female can easily be recognized with yellowish colour and dark colour band at the head and among mesosoma and metasoma (Fig. J).

Fig. 1. (A-I). Developmental biology of Female *E. sophia*; (A); Female *E. sophia* parasitizing whitefly nymph; (B) primary host with freshly laid egg; (C) a 1st instar larva (D) 3rd instar larva with exuviae, b: illeolabial glands, c: spiracles, d: exuviae (E) later stage 3rd instar larva e: sickle shape (F) pre-pupa at central peripheral region, f: meconium (G) Black pupa (I) adult *E. sophia* prior to emergence (H) Newly emerged *E. sophia* female

Development of Male E. sophia

Male eggs (unfertilized) were laid by virgin females outside the body of 3rd instar female parasitoid (secondary host) enclosed within hostpuparium (Fig. 2b) comprising dry environment (Fig. 2A). The externally laid male eggs were not easily distinguished in the day 1 but in later stages occasionally present with the body of host (Fig. 2b). Development of male egg extended for 2.33±0.10 days (Table 1). During the male development only a single egg was observed with host and no super-parasitism was seen in the whole study. 1st instars larva completed its development in 2.09±0.07 days (Table 1). At this stage, secondary host stopped working and growth ceased (Fig. 2d). Male larvae were found attached with the host (Fig. 2e) by

consuming its internal contents successfully to continue its further development (Fig. 2f). No clear segmentation was perceived during early developmental stages. Duration of male 2nd and 3rd instar (Fig. 2g) recorded as an average 2.00±0.08 days and 1.76±0.10 days respectively. Average larval period in male was observed 5.90±0.12 days (Table 1). Meconium pallets appeared on the pre-pupal stage as brown in colour (Fig. 2h) with an average 1.11±0.04 days. Remains of the consumed female *E. sophia*larvae were visible inside the primary host (Fig. 2i). Pupa completed its development in 4.55±0.14 days (Table 1). Additional meconium pallets were observed on together on the posterior peripheral region (Fig. 2j) during cocoon formation (Fig. 2F). Appearance of a distinctive body parts and adult colouration was observed during black pupal phase (Fig. 2G). During emergence time, black pupal case was first shed-off (Fig. 2k) and adult *E. sophia* prepared a circular hole by chewing the host dorsal cuticle and ready to escape (Fig. 2l). Fully developed body parts were easily distinguishable in the male *E. sophia* and was a little smaller in size than its female with a darker colouration than female with total brown metasoma (Fig. 2I).

Discussion

*Encarsiasophia*was considered as a major potential and dominant parasitoid against whitefly *B. tabaci*(Palaniswami*et al.,* 2001). *E. sophia* was recognized as a highly biological control agent on cotton against whitefly (Roltsch and Goolsby, 1998).

Fig. 2. (A-I). Development of Male *E. sophia*; (A) parasitized *E. sophia* 3rd instar, a: Male egg in later 3rd instar *E. sophia* b: secondary host (dry environment) (B) c: 1st instar larva attached with its host, d: paralyzed host. (C) 2nd instar larva of male *E. sophia*, e: larva consuming its host, f: partially consumed secondary host (D) male 3rd instar, g: fully developed 3rd instar after consuming its host (E) pre-pupa, h: meconium pallets, i: remains of secondary host (F) cocoon formation, j: additional meconium in male pupae (G) black pupa (H) Exuviae, k: remains of black pupal case, l: exit-hole (I) Newly emerged male *E. sophia*.

In the current study, *E. sophia*development duration from egg to adult emergence was higher in males and lower in females. These results was in agreement with Wylie (1983) who described that parasitoid larvae spend more time in development in super-parasitized hosts as compare to normal parasitized hosts and deny with those of Gerling (1983) where development takes 15 days to complete. Total time of immature development for *E. sophia* takes approximately 14 days and passes nearly half duration in the last stage (pupa) as confirmed by Hunter & Kelly(1998). The variation in development duration for both sexes of *E. sophia* might be because of different host characters or because of competition with larvae.

According to the observations male eggs cannot be distinguished from female eggs but only because of larval attachment with secondary host. Results showed that females could lay male eggs into a primary host in later stages. Antony *et al.,* 2003 found that *E. sophia* might lay male eggs on 3rd instar parasitoids of 6-7 days old after parasitization. Similarly, Zang*et al.*(2011) exposed nymphs with late 3rd instar *E. sophia* larva to unmated female wasp to obtain male offspring. Male and female *E. sophia* larvae were perceived nearly alike in external form but with some differences in the male larval behaviour because of its development in secondary host and also because of rivalry with another larva. Female larvae travelled freely inside the body fluid of host but male larvae were observed attached with body of the primary host. Female *E. sophia* larvae were larger in appearance as compare to male larvae, as in agreement with Hunter and Woolley (2001)and Heraty and Polaszek (2000) who described that *E. sophia* adult female was larger than male.

In current study, super-parasitism was observed rare in *E. sophia* under controlled laboratory conditionsand was only observed in mass rearing glasshouses with lavish host material. Commonly, one male egg was found attached with primary host. Hunter and Goldfray (1995) also described that males were seen in abundance because of surplus host. Hence, the ratio (M: F) depends upon female abundance. Wylie (1983)also described that parasitoid larvae took more time for development in super-parasitized hosts as compare to normally parasitized hosts which was according to the present results.

Whitefly *B. tabaci* has a very range of hosts and also its parasitoids possibly found on a diverse assemblage with host plants all over the year. Evidence obtained from this recent research will contributes to a better understanding of association between *E. sophia* and its host and also be valuable during establishment of mass production techniques.

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