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RESEARCH

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Parasitizing efficiency of *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) on *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) larva and pupa

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Abstract

Background This study evaluated the parasitizing efficiency of *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) on the *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) larvae and pupae. The sixth instar larvae and 48 h. old pupae were individually exposed for 24 h. to 15 newly emerged mated females of *T. howardi*. Following the emergence of parasitoids, observations were recorded on the percentage of parasitized and emergent pupae and larvae, life cycle duration, progeny, male and female emergence, sex ratio, longevity of male and female and adult per mg weight.

Results In both pupal and larval, parasitization and emergence were 100%. The pupa produced 438.56 progenies, with 394.76 females and 43.2 males and a sex ratio of 0.89. In larvae, 311.93 progenies emerged, with 259.83 females and 53.0 males and a sex ratio of 0.83. The *T. howardi* life cycle duration was 17.66 days in pupae and 20.13 days in larvae. Longevity of female and male progeny that emerged from pupae and larvae was 15.83, 13.40, and 10.40, 8.76 days respectively. The overall progeny production in pupae and larvae was 2.75 and 1.60 individuals per mg body weight, respectively.

Conclusions The highest parasitism as well as biological and reproductive development of *T. howardi* in *G. mellonella* pupae and larvae suggests that this host could be employed as an alternate host for mass multiplication.

Keywords Biological control, *Tetrastichus howardi*, *Galleria mellonella*, Larval and pupal parasitism

Background

Tetrastichus howardi (Olliff) (Hymenoptera: Eulophidae) is a gregarious and polyphagous larval and pupal parasitoid of lepidopteran pests, with significant potential for the biological control of sugarcane borers (La Salle and Polaszek 2007). Its potential as a biological control agent for several agriculturally important pests has been investigated in different countries (Rodrigues et al. 2021). This parasitoid parasitizes the different biological stages like larva, prepupa, and pupa of sugarcane borers *Diatraea saccharalis* F. sensu Guenee (Lepidoptera: Crambidae), *Chillo partellus* (Swinhoe) (Lepidoptera: Pyralidae), *Chillo auricilius* Dudgeon (Lepidoptera: Crambidae),

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Scirpophaga excerptalis (Walker) (Lepidoptera: Crambidae), and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Pereira et al. 2015), and it is active all year, especially during the warmer months in the tropical part of India (Jalali and Singh 2001). This is attributed to the longer life span of the adult stage of *T. howardi* (Vargas et al. 2011) than the widely used egg parasitoids *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (Oliveira et al. 2013) and the larval parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) (Simoes et al. 2012). Thus, this eulophid is more successful in regulating and suppressing the borer cycle in sugarcane fields.

The efficacy of biological control programs depends on mass rearing of parasitoids on their host (Pereira et al. 2009a) and the specific hosts used for rearing; therefore, it is essential to investigate their biological interaction (Nakajima et al. 2012). It is critical to study the parasitizing efficiency, including sex ratio, reproductive potential, life cycle duration (from egg to adult), host size, parasitization capacity, and parasitoid sensitivity to abiotic factors, particularly temperature, light, and humidity, in order to develop mass rearing techniques (Rodrigues et al. 2013). Extensive research on parasitoids parasitizing efficiency is also required to standardize mass-rearing methods and minimize the cost of parasitoid production (Favero et al. 2013).

Tetrastichus howardi exhibits a high reproductive potential when reared on a different alternate host, i.e., *Chilo parteullus* (Swinhoe) (Lepidoptera: Pyralidae), *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae), *Scirpophaga excerptalis* (Walker) (Lepidoptera: Crambidae), *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae) (Sankar and Rao 2016). There is limited information on the parasitism and biological and reproductive attributes of *T. howardi* on *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) larvae as well as pupae as it is difficult to rear a natural host of *T. howardi* in vitro conditions for large-scale mass multiplication. Therefore, this study aimed to assess *T. howardi* parasitizing ability on the *G. mellonella* larvae and pupae in order to develop mass production methods for the efficient management of sugarcane borers.

Methods

Mass multiplication of Galleria mellonella

The nucleus culture of *G. mellonella* in the last larval stage (and/or already pupae) was obtained from the ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. A total of 10 to 30 larvae/pupae were placed in plastic containers (28 cm height × 16 cm diameter) with holes in the covers. After the emergence of adult moths, paper folds were placed vertically inside the container for egg-laying and incubated at room temperature for 1 week. The egg mass containing paper was cut into 1—2 cm strips and placed within a plastic container (17.5 cm height × 14 cm diameter), with a thin layer of an artificial diet applied over the egg mass. The egg containers were incubated at 28 °C for about 15 days. When the larvae began to form galleries inside the diet, they were transferred to another plastic container (37 cm length × 25 cm breadth × 11.43 cm height) and incubated at 28 °C for another 15 days, until they reached a larva size of 1.0—2.0 cm. Newly hatched larvae were fed on an artificial diet composed of a semi-solid mixture of wheat bran, milk powder, yeast extract powder, honey and glycerol (Woodring and Kaya 1988). When the larvae reached a sufficient size to be handled (approximately 1—2 cm), they were separated and transferred to new containers supplied with the artificial diet until they reached the eight instar and pupal stage. An artificial diet of 10—20 gm was provided for every alternate day from hatching until the gallery formation stage, and a 35—40 gm diet was provided every day for the subsequent 15 days of incubation until sixth instar and pupae (Jorjao et al. 2018).

Mass multiplication of T. howardi

Rearing of *T. howardi* began with the adults from the stock colony maintained at the Biological Control Laboratory of the Division of Crop Protection, ICAR-Indian Institute of Sugarcane Research, Lucknow, India. In the initial study, *G. mellonella* third, fourth, fifth, and sixth larval instars and pupae were individually exposed for 24 h. parasitism by newly emerged and mated females of *T. howardi* at the different levels of densities of 1, 5, 10, 15, and 20 in a glass tubes sealed with cotton and containing honey water droplets. Following this, they were individualized and maintained at 25 ± 2 °C, 60 ± 5%, RH and a 12:12 h. (L: D) photoperiod in controlled climate chamber until the emergence of adults (Pereira et al. 2015). The stock colony were developed from 24 h. old *T. howardi* females that emerged from parasitized larvae and pupae in the preliminary study, which was individualized and given access to the *G. mellonella* pupa in a glass tube (8.5 × 2.5 cm) (height × diameter) closed with a cotton plug. The parasitoid was given a fine streak of honey-water solution (1:1v/v) as food inside the glass tube (Baitha and Maurya 2012). *T. howardi* parasitism and emergence were observed in all larval instars and pupal stage, as well as at all densities. As the highest adult emergence of *T. howardi* occurred when 15 females were used for parasitisation of fifth, sixth larval instars and pupae, this level of density and larval stages were selected for the experiment.

Experiment 1: Tetrastichus parasitism and development on G. mellonella pupae

Galleria mellonella pupae 48 h. old, weighing 0.1 to 0.25 g were individualized with fifteen 24 h. old *T. howardi* mated females in flat-bottomed glass tubes (8.5 × 2.5 cm) (height × diameter) sealed with cotton for 24 h. at 25 ± 2 °C, 60 ± 5%, RH and a 12:12 h. (L: D) photoperiod. The *T. howardi* females were removed at the end of the parasitism exposure period and *G. mellonella* pupae were kept in the tubes until the emergence of parasitoids offspring.

Experiment 2: Tetrastichus parasitism and development on G. mellonella sixth instar

Galleria mellonella sixth instar, weighing 0.1 to 0.3 g were individualized with fifteen 24 h. old *T. howardi* mated females in flat-bottomed glass tubes (8.5 × 2.5 cm) (height × diameter) sealed with cotton for 24 h. at 25 ± 2 °C, 60 ± 5%, RH and a 12:12 h. (L:D) photoperiod. After the removal of *T. howardi* females, *G. mellonella* larvae were kept in sterilized glass Petri plates with artificial diet until the emergence of parasitoids offspring. In order to minimize disruption to the parasitism process and the growth of the *T. howardi* progeny, the larvae were fed on a daily basis.

Evaluation of biological characteristics

The parasitism percentage [(number of pupae/larvae without *G. mellonella* adult emergence)/ (number pupae/larvae offered to parasitoids females) × 100]; emergence percentage [(number of *G. mellonella* pupae/larvae with emergence of parasitoids adults) / (number of pupae/larvae parasitized) × 100]; life cycle duration (from pupae/larvae exposure parasitism to parasitoids adult emergence), progeny (number of parasitoids emerged per pupae and larvae (males + females), and sex ratio (number of females/total number of progeny); male and female longevity (adult emergence period until death) were evaluated in experiment 1 and 2. The sex differentiation of adult *T. howardi* was based on the morphological characteristics of the antenna. The female antenna bears a dark brown pedicel, club, three funicular segments, and a pale-yellow scape lacking a sensory plate on the ventral margin. The male antenna contains pale yellow to light brown pedicel, four funicular segments, and a scape that carries a sensory plate on the ventral margin, with a black to dark brown club that often appears distinctly wider than funicles (La Salle and Polaszek 2007). *Tetrastichus howardi* adult longevity was estimated daily with 30 females and 30 males randomly selected from the offspring of each treatment, individualized in glass tubes, and fed with honey-water solution (1:1v/v) dispersed inside the glass tube.

Statistical analysis

The experiment was conducted using a completely randomized design, with two treatments (pupa and larva) and 30 replications. The data were analysed by one-way analysis of variance (ANOVA) through the online program OPSTAT (Sheoran et al. 1998) and the treatment means were compared with t-test at 5% probability.

Results

Tetrastichus howardi females were able to parasitize, develop, and emerge from *G. mellonella* pupae and larvae. The parasitized larvae were bloated, dry, and rigid, with brown and black coloration, whereas the parasitized pupa had brown and black coloration. The parasitization and adult emergence rates were 100% in both stages of the host insect (Table 1). Compared to larvae, pupa produced the highest number of progeny (Table 1).

Table 1 Biological characteristics of *Tetrastichus howardi* in pupae and larva of *Galleria mellonella*

Biological parameter	Host		T test	CV (%)
	Pupa	Larva		
Parasitism (%)	100	100		
Emergence (%)	100	100		
Progeny	438.56 ± 23.66[a]	311.93 ± 18.15[b]	18.53**	25.67
Life cycle duration (D)	17.66 ± 0.21[b]	20.13 ± 0.16[a]	81.71**	4.58
Total no. of male	43.2 ± 2.52[b]	53.0 ± 4.63[a]	17.12**	44.60
Total no. of female	394.76 ± 22.48[a]	259.83 ± 15.00[b]	17.55**	26.62
Sex ratio	0.89 ± 0.01[a]	0.83 ± 0.01[b]	137.05**	6.58
Longevity of females (days)	15.83 ± 0.26[a]	13.4 ± 0.35[b]	60.21**	10.45
Longevity of males (days)	10.26 ± 0.30[a]	8.76 ± 0.45[b]	33.85**	17.50
Adults /mg weight	2.75 ± 0.14[a]	1.60 ± 0.08[b]	19.18**	26.06

Mean ± standard error followed by a different lowercase letter in a row is significantly different by t test at 5% and 1% probability. ** Significant at 1% probability

The pupae and larvae stage produced 438.56 and 311.93 progenies, respectively ($p < 0.05$). The parasitoid life cycle duration differed significantly ($p < 0.05$) between the larval and pupal stages. The life cycle duration was 17.66 and 20.13 days in the pupae and larvae, respectively (Table 1). The emergence of female progeny was 394.76 and 259.83 in pupae and larvae ($p < 0.05$), respectively (Table 1). A positive correlation was observed between total progeny produced from the parasitized pupa ($R^2 = 0.99$) and larva ($R^2 = 0.94$) and female emergence (Fig. 1). The male progeny emergence was 43.2 and 53.0 in pupae and larvae ($p < 0.05$), respectively (Table 1). The parasitoid sex ratio had a significant impact on its population dynamics. *Tetrastichus howardi* emerged from the pupae and larvae had sex ratios of 0.89 and 0.83, respectively (Table 1). The females and males progeny that emerged from pupae exhibited longevity of 15.83 and 10.26 days respectively, whereas the female and male emerged from larvae had longevity of 13.40 and 8.76 days (Table 1). The longevity of the progenies emerged from larvae was significantly lower than that of pupae. The body weight of the host stage also had a significant impact on parasitoid production ($p < 0.05$). The progeny production in pupae and larvae was 2.75 and 1.60 per mg body weight, respectively (Table 1).

Discussion

Tetrastichus howardi parasitized and emerged from *Galleria mellonella* pupae and larvae demonstrating its potential for mass multiplication with that host. *Galleria mellonella* pupae exposed to a single female parasitoid demonstrated 40.0% parasitism and the emergence of 49.50 progenies (Baith et al. 2003). Pereira et al. (2015) observed that seven female parasitoids caused 2 and 56% parasitism and 14, 100% emergence in fifth-instar larvae and pupa of *D. sachharalis,* respectively, while Simonato et al. (2020) recorded parasitism in the fifth instar caterpillar, and parasitoids emergence during the pupal phase in *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). A single female parasitoid was enough to cause parasitization and mortality in *D. saccharalis* caterpillars from the second to fifth instars. However, the rate of parasitism was affected by caterpillar instars and increased with the density of *T. howardi* females used for parasitization (Vargas 2013). Present findings revealed that the high density of female parasitoids was responsible for the 100% parasitism and emergence in *G. mellonella* pupae and larvae. These results also indicated that the parasitoids immature stages efficiently utilized the host's nutritional resources for its growth and development. During co-evaluation with arthropod pests, female parasitoids developed defence mechanisms like chemical secretion from the ovaries which inhibit the host's defences, kill or paralyze the host, or shield the eggs from the host's immune system (Kaeslin et al. 2005). This enables female parasitoids to suppress the host's cellular defence and utilize their most nutritive resources, which is reflected in the efficient development of premature stages of the progeny (Andrade et al. 2010).

In the present study, pupae produced the highest progeny than larvae because *T. howardi* is a parasitoid of pupae and, thus, preferred the natural host. A similar finding was reported by Luccheta et al. (2022), who found out that the prepupal phase of *S. frugiperda* produced higher progenies than fifth and sixth instar caterpillars. Pupae of *H. armigera* and *D. saccharalis* parasitized by 15 female parasitoids, produced 689 and 358 progenies, respectively (Simonato et al. 2020). The significantly lower progeny production in the larval stage may be caused by changes in the structure and metabolism of various tissues, which affect the availability of nutrients and restrict the development of immature stages (Consoli & Vinson 2009). Higher progeny output per pupa, on the other hand, may be linked to the high density of female parasitoids for parasitism and the nutritional quality of the pupa. The high reproduction rate of parasitoid in *G.*

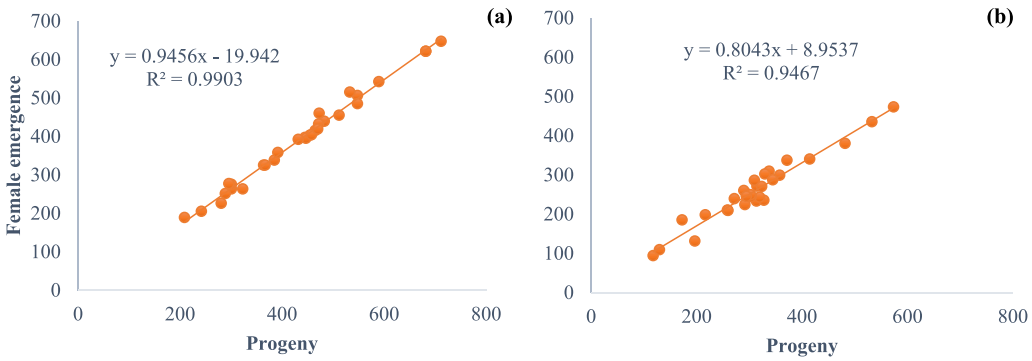

Fig. 1 Relationship between total progeny and female emergence **a** pupa and **b** larva exposed to 15 *Tetrastichus howardi* females for 24 h

mellonella pupae suggested that they could be beneficial as alternate hosts for mass multiplication. The highest progeny production contributed to the mass multiplication of parasitoids in laboratories, and the large quantity of release preserves parasitoids in the field condition following inundative releases.

Life cycle duration was significantly varied in *G. mellonella* pupal and larval stages exposed to 15 female parasitoids. The parasitoids life cycle duration was longer in larvae than pupae. *Tetrastichus howardi* had a short life cycle duration in pupae (20 days) than the fifth instar larvae (27 days), and adult (33 days) of *D. saccharalis* (Prereira et al. 2015). Luccheta et al. (2022) reported the longest life cycle of 31.0 days in fifth instar larvae and the shortest of 20.10 days in the prepupal stage of *S. frugiperda*. The life cycle duration (egg to adult) depends on several aspects and the host species (Zago et al. 2006). The nutritional status of pupae which supports the rapid growth and development of parasitoids than the larval stage may be the cause of the variation in the life cycle duration of *T. howardi* (Pascua and Pascua 2004). The shortened life cycle is an important characteristic for the mass multiplication point of view because it favours more generations of parasitoids in less time, which also speeds up the adult-rearing process in laboratories.

An increased proportion of females over males in *T. howardi* was also reported in different hosts (Baitha et al. 2011). The highest number of female progenies existed in both stages, which is considered an essential characteristic for field release and for increases in the number of offspring produced in the following generation (Pereira et al. 2009b). The higher number of female progenies promotes the persistence of parasitoids in the field crops, and 60% of females in the field releases are sufficient for effective biological control (Vacari et al. 2012). The *T. howardi* males are essential for mating in the first hours after emergence (González and Ravelo 2003). The low proportion of males in progeny was also reported in other parasitoids of the Eulophidae family, including *Trichospilus diatraeae* Cherian and Margabandhu and *Palmistichus elaeisis* Delvare and La Salle (Pastori et al. 2012).

The *T. howardi* sex ratio was significantly higher in pupae compared to larvae. A sex ratio index of 0.5 or above is considered ideal for parasitoid mass rearing (Dias et al. 2008). In pupae parasitized by a single female, Baitha et al. (2003) observed a sex ratio (Male: Female) of 1:13.75 in *G. mellonella* and 1:26.62 in *C. partellus*. However, the sex ratio of *T. howardi* emerged from the pupae parasitized by a high density of female parasitoid was 0.91 in *H. armigera* (Simonato et al. 2020), 0.93 in *D. saccharalis* (Costa et al. 2014), 0.94 in *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) (Oliveira 2013) and 0.96 in *Erinnyis ello* (Linnaeus) (Lepidoptera: Sphingidae) (Barbosa et al. 2015). Female biased sex ratio was seen in both stages, which is important for biological control because female parasitoids are the agents that regulate parasitism and pest control. It also helps in mass multiplication and its effectiveness in field conditions (Rodrigues et al. 2021).

There was a significant difference in the longevity of female and male progeny that emerged from pupae and larvae. In the present study, the female parasitoids had a longer longevity than male progeny in both stages. Pratissoli et al. (2005) reported that a longevity of more than 10 days was sufficient for female parasitoids to reproduce and find their hosts when they are released in the field. It indicates that *G. mellonella* is an excellent quality host that supports the longer longevity of *T. howardi*. The females and males of *T. howardi* had a longer life span than other parasitoids in the genus *Trichogramma* spp. and *Cotesia* sp. (Parra 2002) and therefore this natural enemy is most suitable for field conditions. The extended survival of female parasitoids, which are responsible for parasitism and maintaining the existence of species in the ecosystem, helps in evaluating and selecting the best quality host for progeny growth and performance (Pereira et al. 2010). It also supports increased parasitism under field conditions, which is adequate to control and prevent the pest cycle from continuing. Longevity enhancement is considered to be useful in the breeding of biological control agents because it ensures survival during operational procedures and encourages a longer stay of natural enemies in cultures (Vargas 2013).

In this investigation, pupae weighed between 0.1 to 0.2 g, produced more progeny than the larvae, which were weighed between 0.1 to 0.3 g. Pupae of *H. armigera* weighed between 0.328–0.418 g produced 669.3 individuals of *T. howardi*, with 609.9 females and 59.40 males (Oliveria et al. 2016). However, the pupae of *D. saccharalis* with a body weight between 0.187 g and 0.228 g produced 358 progeny (Simonato et al. 2020). It is important to note that in both studies, each pupa of *D. saccharalis* and *H. armigera* was exposed to 15 parasitoid females. Thus, the production of offspring can increase in proportion to the size of hosts. Similar findings have been reported by Lucchetta et al. (2022) in the 5th and 6th instars, and the prepupal phase of *S. frugiperda*. Obtained results suggested that the high progeny production in pupae may be related to the nutritional quality, which increases as it grows and develops from larvae to the pupal stage (Consoli and Vinson 2009). These findings showed that larvae and

pupae of *G. mellonella* fit perfectly as ideal hosts for the multiplication of *T. howardi.*

Conclusion

Obtained findings confirmed *T. howardi* parasitism and reproductive development in *G. mellonella* larvae and pupae under laboratory conditions. However, the high performance of these characters in pupae, indicates that this stage is an ideal alternative host for *T. howardi* mass multiplication under laboratory conditions.

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Author contributions
D. N. B. designed and conducted the experiment, analyzed data and prepared the original manuscript. Arun Baitha provided a nucleus culture of parasitoids, helped in designing the experiment, reviewed and edited the manuscript. Y. E. T. and B.E. K. assisted in experimental work and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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