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ORIGINAL RESEARCH ARTICLE

Biological characteristics of ectoparasitoid, *Fulgoraecia melanoleuca* (Fletcher) on various stages of its host, *Pyrilla perpusilla* (Walker)

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Abstract

Fulgoraecia melanoleuca (Fletcher) is a key parasitoid of *Pyrilla perpusilla* (Walker) nymphs and adults in the sugarcane ecosystem. The effect of different host stages, viz. five nymphal instars and adults (male and female) on the biological parameters, i.e. larval survival, larval period, pupal/chrysalis survival, pupal/chrysalis period and total development period of *F. melanoleuca* was examined. When 1st instar nymphs were exposed to the parasitoid, the host died completely without presenting any symptoms of parasitism.The larval survival, larval period, pupal/chrysalis survival, pupal/chrysalis period and total development period of the parasitoid was less on the younger nymphal instars as compared to later instars of the host. Likewise, adult longevity of parasitoid whose larvae had fed on younger instars lived for shorter period of time as compared to later instars. Between the sexes, the survival and development parameters of parasitoid was more when reared on female adult host as compared to male adults. The sex-ratio of *F. melanoleuca* adults was female-biased on all the host stages. However, more female progeny was observed in later nymphal instars and female adult host. The fecundity of *F. melanoleuca* increased with increase in host age and was comparatively more in the later nymphal instars. However, 4th instar pyrilla nymphs serving as host for *F. melanoleuca* favoured a significant increment in number of eggs laid by the parasitoid females as compared to 3rd and 5th instar nymphs. Among the host stages, 4th instar nymphs and adult females were more suitable for the parasitoid, *F. melanoleuca*.

Keywords *Pyrilla perpusilla* · *Fulgoraecia* (=*Epiricania*) *melanoleuca* · Parasitism · Biology · Sugarcane

Introduction

Pyrilla perpusilla Walker (Hemiptera: Lophopidae) is a key pest of sugarcane crop (family Poaceae) in India (Kumar et al. 2015), China (Liang 1997), Nepal (Neupane 1976), Pakistan (Rasul et al. 2014), Sri Lanka (Ganehiarachchi and Fernando 2006), Thailand (Fennah 1963), Cambodia (Liang 1997), and Vietnam (Emeljanov 2018). It has also been documented on different host plants from the families Gramineae, Leguminosae, Cucurbitaceae, and Moraceae. Both nymphs and adults of *P. perpusilla* suck the cell sap from the leaves near the mid-rib and produce symptoms with yellowish and whitish spots on the leaves (Pandey et al. 2008).The leaves become yellow due to phloem sap sucking by leafhopper which results in drying and withering of leaves. It excretes a sticky fluid in the form of honey dew on the surface of leaf which promotes the quick growth of fungus and sooty mould covers the leaves completely. As a result, photosynthetic activity is greatly reduced resulting in poor crop growth, yield and sugar recovery (Rasib et al. 2020). It also injects enzymes into leaves while sucking sap from the leaves that inhibits the formation of sucrose (Seneviratne and Kumarasinghe 2002) but glucose content increases considerably that leads to formation of soggy masses during *gur* making (Chaudhary and Ansari 1988). Though various practices like cultural, mechanical and chemical insecticides have been recommended for the management of *P. perpusilla* on sugarcane (Singh et al. 2001;Verma et al. 2002) but biological control through natural enemies has been the most successful approach for its effective management (Gangawar et al. 2008; Srikanth et al. 2016).

Several bioagents from different groups including predators, parasitoids and entomopathogens have been documented against *P. perpusilla* across India (Srikanth et al.

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2016). *Fulgoraecia melanoleuca* (Fletcher) (= *Epipyrops* or *Epiricania melanoleuca* Fletcher) is a key ectoparasitoid of *P. perpusilla* nymphs and adults. Female *F. melanoleuca* moths lay their eggs on the leaves. After hatching, the larvae catch hold the nearby pyrilla nymph or adult by its hooked claws with head directed posteriorly. Firstly, the parasitoid catches hold the tarsi of the host and gradually proceeds to orientate on the dorsal surface where it continues to feed until maturity. The larvae penetrate the host cuticle with its sharp mandibles and suck the body fluids. During the larval period, it secretes a large amount of wax over its body, making it conspicuous. The caterpillar leaves the host body after completion of larval period and migrates to the leaf surface where it spin white, oval-shaped cocoon inside which pupation occurs. As soon as the host is released by the parasitoid, it dies. Upon emergence, the female moth remains near the cocoon. However, the male flies to the cocoon for mating with the female. The female starts laying eggs soon after termination of copulation alongside the cocoon (Rajak et al. 2006). Under field conditions, the parasitoid produced up to 80% parasitization of *P. perpusilla* on sugarcane (Rajak et al. 2008).

The fitness of parasitoid progeny is dependent on the availability of suitable host stage or host quality (Hagvar and Hofsvang 1991; Kant et al. 2012; Shera and Karmakar 2018; Karmakar and Shera 2018). Previous studies revealed that the parasitoid *F. melanoleuca* has a negative impact on the fitness of its host, *P. perpusilla* (Sharma and Shera 2021). Information about whether and to what extent various host stages of *P. perpusilla* affect the detailed biological parameters of this ectoparasitoid are still lacking. Consequently, we investigated the biological characteristics of *F. melanoleuca* on various life stages of *P. perpusilla* (five nymphal stages) and adults (male and female) to know the parasitoid's preference among different host life stages. The identification of suitable host stage could be helpful in standardizing mass rearing methodology of this parasitoid and also implementing effective strategy for augmentative biological control program against *P. perpusilla* under field conditions.

Materials and methods

Host plant

Sugarcane seed setts (Co 238) were sown in the field at the Punjab Agricultural University's Entomological Research Farm in Ludhiana (India). The crop was raised without any plant protection measures. The fresh and young sugarcane leaves were used for the rearing of host insect and parasitoid cultures.

Rearing of *Pyrilla perpusilla*

Pyrilla perpusilla culture was reared on sugarcane leaves under controlled temperature (27 ± 2ºC) and relative humidity (70 ± 5%) conditions (Sharma and Shera 2021). Conical flasks (250 mL capacity) were filled with water and two fresh and healthy sugarcane leaves (size 40 cm) were placed in each flask with their one-third part inside and two-third part outside the flask. Cotton was plugged between the two leaves of each flask to avoid the falling of pyrilla nymphs and adults into the water. The flasks (2–4 in number) were kept in aluminum screen cages (30 cm x 30 cm x 45 cm). Pyrilla eggs, nymphs, and adults were collected from the sugarcane fields and released into aluminium screen cages for rearing on sugarcane leaves. Flasks containing fresh sugarcane leaves were changed on a regular basis for providing fresh food to nymphs and adults.

Rearing of *Fulgoraecia melanoleuca*

Egg masses and cocoons (pupae) of *F. melanoleuca* were collected from sugarcane fields. *Pyrilla perpusilla* culture maintained on sugarcane leaves were used for rearing of the parasitoid in the aluminium cages. Males and females were sexed morphologically (Kumar et al. 2015) and were placed in cages to allow for mating and egg laying on sugarcane leaves. As a food for the adults, a cotton swab was dipped in a 10% honey solution and hung in each cage. For parasitism, sugarcane leaves with newly deposited eggs were collected and placed in the cages with pyrilla population (nymphs and adults).The experiment was conducted with newly hatched eggs from a maintained culture.

Biological parameters on host nymphs and adults

Two hundred individuals from each of the five nymphal instars (1st, 2nd, 3rd, 4th, and 5th) and adults (male and female) of *P. perpusilla* were taken from the culture maintained under laboratory conditions to investigate the biological parameters. With the use of an aspirator, they were released in separate aluminium screen cages (size 30 × 30 × 45 cm) on sugarcane leaves held in water filled conical flasks. Sugarcane leaf bits having parasitoid egg masses were stapled on the underside of the sugarcane leaves in cages (4–7 days old egg masses) of *F. melanoleuca* for parasitism of pyrilla nymphs and adults. To record different parameters, twenty individuals of each stage (5 replicates; n = 100) showing symptoms of parasitism were extracted and preserved in separate plastic vials (35 mm diameter 100 mm height). Parasitized nymphs and adults of *P. perpusilla* were differentiated by presence of white cottony

cushion on back and on pleural part of abdominal region, respectively. Sugarcane leaves in small pieces were offered as food for the pyrilla nymphs in each vial. On one side, leaves were enfolded in water-soaked cotton to keep them turgid.Observations were recorded on various parameters like larval survival, larval period, pupal/chrysalis survival (adult emergence), pupal/chrysalis period, sex-ratio (% female progeny), adult longevity, total development period, fecundity, pre-oviposition, oviposition and post-oviposition periods to assess the preference of parasitoid among different *P. perpusilla* life stages.

Larval survival The larval survival was worked out by dividing the pupae formed from parasitized nymphs or adults of pyrilla with total number of parasitized nymphs or adults initially.

$$\text{Larval survival (\%)} = \frac{\text{No. of pupae formed from parasitized nymphs or adults of pyrilla}}{\text{Total No. of parasitized nymphs or adults}} \times 100$$

Larval period The duration from hatching of eggs to formation of pupa after death of parasitized nymphs or adults was considered as the larval period.

Pupal/chrysalis survival (adult emergence) The pupal/chrysalis survival was calculated out by dividing the number of pupae from which the adult emerged with the total number of pupae formed initially.

$$\text{Pupal/chrysalis survival (\%)} = \frac{\text{Number of pupae from which the adult emerged}}{\text{Total number of pupae formed}} \times 100$$

Pupal/chrysalis period The time duration between formation of pupa (cocoon) to adult emergence was considered as pupal/chrysalis period.

Sex-ratio The parasitoid adults emerging from cocoons were differentiated morphologically into males and females based on their wing colour and antennal structure (Kumar et al. 2015). The sex-ratio was calculated by dividing the total number of males with the total number of females.

Adult longevity (male and female) The time period starting from adult emergence from the cocoons till their death was considered as longevity of the adults for both the sexes.

Total development period The time duration right from hatching of eggs till death of the adult was considered as the total development period.

Pre-oviposition, oviposition and post-oviposition periods A pair of male and female adult was released in an aluminium screen cage having sugarcane leaves kept in conical flasks filled with water for egg laying. A cotton swab was hung in each cage after dipping in 10 per cent honey solution as food for the adults. The time period between newly emerged female adults until it started egg laying was recorded as pre-oviposition period. After mating, female immediately started egg laying and duration till it continued egg laying was considered as its oviposition period. After egg laying, the time interval for which the female survived was taken as post-oviposition period. The time period was recorded with the help of stopwatch. The experiment was replicated five times.

Fecundity The number of eggs laid by a female in an egg mass was counted using binocular stereo microscope (SZ40, Olympus, India) to determine the fecundity per female.

Data analyses

The mean and standard error of the mean are used to present all data (SE). Because the data on percent survival was not normally distributed, it had to be translated into a new set of scores with a bell-shaped distribution. Prior to analysis of variance, the % data on larval and pupal/chrysalis survival were arcsine transformed. The count data of eggs was normalised using the square root transformation. Before statistical analysis, the values of 0% and 100% were replaced by 1/4n and 100-1/4n, respectively. The data on the larval duration, pupal/chrysalis period, adult longevity, total developmental period, and reproductive parameters were normally distributed and analysed using ANOVA. Tukey's test was used to differentiate the means. The significance level was calculated by comparing the parasitoid's developmental and reproductive parameters on adult male and female hosts using the student's t-test. IBM SPSS 22.0 for Windows was used to conduct all statistical tests (IBM Corporation, Armonk, New York, USA).

Results

Biological parameters of *F. melanoleuca* on *P. perpusilla* nymphs

Larval period and survival

The larval survival rate and larval period of *F. melanoleuca* showed significant differences on various nymphal stages. Among nymphal stages, the larval survival was highest on 4th nymph and significantly lower on 2nd nymph (Fig. 1). Similarly, the larval period was also significantly higher on 4th nymph while, it was lowest on 2nd

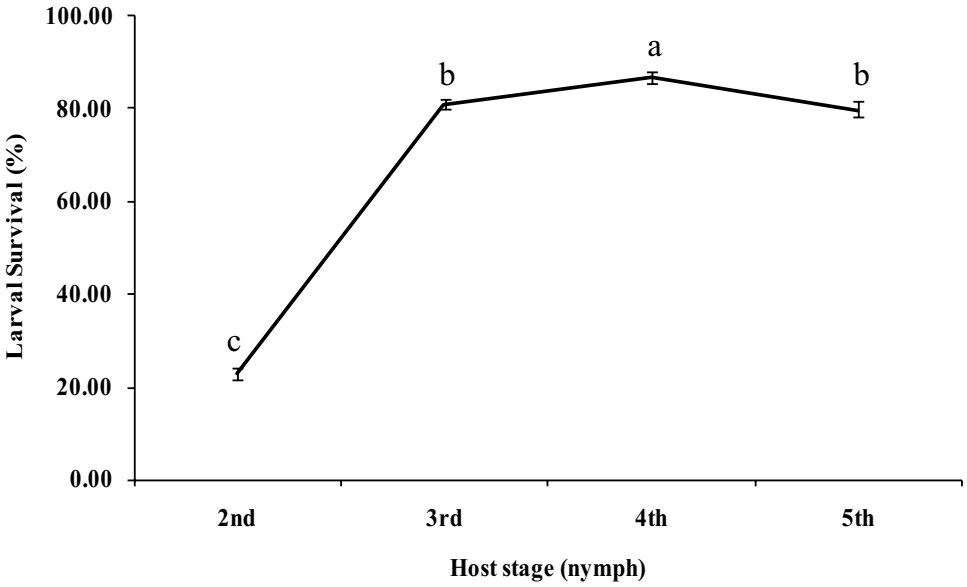

Fig. 1 Larval survival (%) of *F. melanoleuca* when various nymphal stages of *P. perpusilla* were offered to the parasitoid for parasitism; Treatments with different letters are significantly different according to a Tukey's test at $P < 0.05$. Error bars represent the standard error of the mean

nymph as compared to other nymphal stages (F = 127.08; df = 3, 16; $p < 0.0001$; Table 1). It is pertinent to mention that no successful parasitism was observed on 1st nymph.

Pupal/chrysalis period and survival

The survival rate and duration of *F. melanoleuca* pupal/chrysalis on various nymphal instars of *P. perpusilla* showed significant differences. Highest pupal/chrysalis survival was recorded on 4th instar nymphs and it did not differ significantly from survival on 5th instar nymphs. Significantly lower pupal/chrysalis survival was observed on 2nd nymphal instars (Fig. 2). The pupal/chrysalis period did not differ significantly on 3rd instar, 4th instar and 5th instar nymphs of *P. perpusilla*. However, lowest pupal/chrysalis period was recorded on 2nd instar nymphs (F = 30.71; df = 3, 16; $p < 0.0001$; Table 1).

Sex-ratio

The sex-ratio of *F. melanoleuca* adults was female-biased on all the nymphal instars of *P. perpusilla*. Among different nymphal stages, relatively more female adults (1:1.86) emerged from 4th instar nymphs followed by 5th instar (1:1.80) and 3rd instar (1:1.60) nymphs. However, the sex-ratio of male and females was 1: 1.25 on 2nd instar nymphal stage (Table 1).

Adult longevity (male and female)

Females lived longer than males in *F. melanoleuca* adults from distinct nymphal instars of *P. perpusilla* (Table 1). The longevity of adult female from 4th instar nymphs was significantly higher (5.73 ± 0.12 days) as compared to 4.02 ± 0.05days from 5th instar nymphs and 3.83 ± 0.09days

Table 1 Effect of *P. perpusilla* nymphal stages on the developmental parameters of *F. melanoleuca*

Host stage (Nymphs)	Mean duration (± SE_m) in days*						Sex ratio (♂ : ♀)
	Larval period	Pupal period	Adult longevity		Total development period		
			Male	Female	Male	Female	
1st	**	**	**	**	**	**	**
2nd	5.30 ± 0.24	3.10 ± 0.33	0.21 ± 0.03	0.25 ± 0.03	8.38 ± 0.15	8.30 ± 0.13	1:1.25
3rd	10.38 ± 0.33	6.45 ± 0.46	2.89 ± 0.11	3.83 ± 0.09	19.70 ± 0.26	20.60 ± 0.14	1:1.60
4th	12.10 ± 0.20	7.41 ± 0.30	3.54 ± 0.16	5.73 ± 0.12	23.00 ± 0.31	25.22 ± 0.31	1:1.86
5th	10.88 ± 0.27	6.43 ± 0.24	3.47 ± 0.13	4.02 ± 0.05	20.75 ± 0.30	21.30 ± 0.34	1:1.80
LSD (p = 0.05)	0.80	1.03	0.35	0.25	0.80	0.75	-
F value	127.08	30.71	180.84	792.26	615.52	864.69	-
p value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-

*Mean of 5 replications; **no successful parasitism in 1st stage *P. perpusilla* nymphs

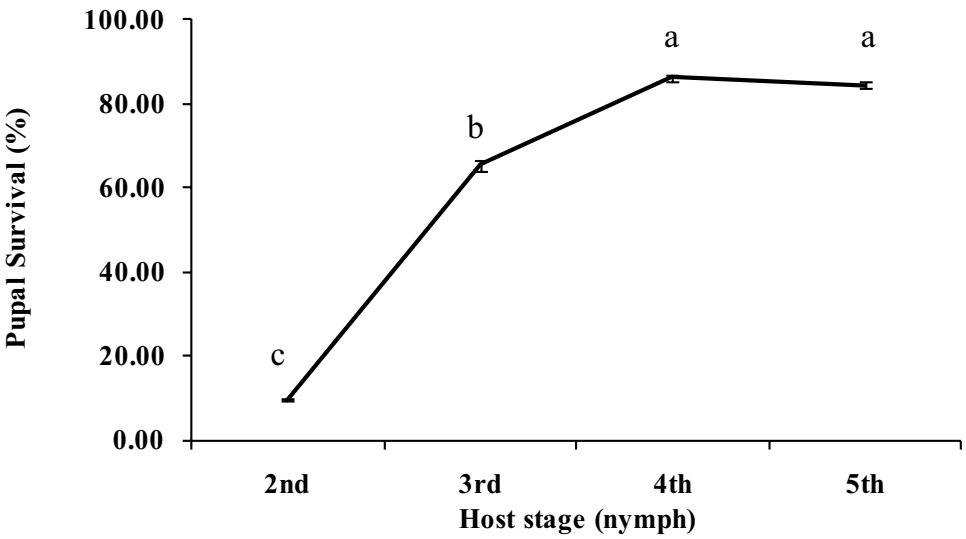

Fig. 2 Pupal survival (%) of *F. melanoleuca* when various nymphal stages of *P. perpusilla* were offered to the parasitoid for parasitism; Treatments with different letters are significantly different according to a Tukey's test at $P < 0.05$. Error bars represent the standard error of the mean

from 3rd instar nymphs (F = 792.26; df = 3, 16; $p < 0.0001$). Similarly, adult males from 4th instar nymphs (3.54 ± 0.16days) survived for longer duration and it did not differ significantly from 5th instar nymphs (3.47 ± 0.13 days) (F = 180.84; df = 3, 16; $p < 0.0001$). However, both male and female adults of parasitoid from 2nd instar nymphs of *P. perpusilla* survived only for 0.21 ± 0.03 and 0.25 ± 0.03 days, respectively.

Total development period

The total development period of *F. melanoleuca* showed significant differences among various host nymphal instars (Table 1). In comparison to females, males had a shorter developmental period. The total development period of *F. melanoleuca* females was longest (25.22 ± 0.31days) on 4th instar nymphs, whereas the duration on 5th instar nymphs (21.30 ± 0.34 days) and 3rd instar nymphs (20.60 ± 0.14 days) was not significantly different from each other (F = 864.69; df = 3, 16; $p < 0.0001$). However, it was minimum (8.38 ± 0.15 days) on 2nd instar host nymphs. Similar observations were recorded for adult males also. The maximum developmental time (23.00 ± 0.31days) was recorded on 4th instar nymphs, while it was minimum (8.38 ± 0.15days) on 2nd instar nymphal stage (F = 615.52; df = 3, 16; $p < 0.0001$).

Pre-oviposition, oviposition and post-oviposition periods

Pre-oviposition period of *F. melanoleuca* was highest (18.27 ± 0.25 min) in adults that were reared on 4th instar *P. perpusilla* nymphs as compared to 15.35 ± 0.23 and 13.58 ± 0.24 min in adults from 5th and 3rd instar nymphs, respectively(F = 95.87; df = 2, 12; $p < 0.0001$; Tables 2 and 3).The parasitoid females reared on 4th instar *P. perpusilla* nymphs showed significantly longer oviposition period (31.65 ± 0.44 min) which did not differ significantly from females reared on 5th instar nymphs (31.54 ± 0.24 min) (F = 333.68; df = 2, 12; $p < 0.0001$) While, significantly shorter oviposition period (22.16 ± 0.13 min) was observed in females from 3rd instar nymphs (Table 3). Significantly longer post-oviposition period (5.36 ± 0.24 days) was recorded in parasitoid females on 4th instar *P. perpusilla* nymphs as compared to 3.92 ± 0.08and 3.75 ± 0.07days in females reared on 5th and 3rd instar host nymphs, respectively (F = 33.25; df = 2, 12; $p < 0.0001$; Table 3).

Table 2 Effect of *P. perpusilla* adult stage (sex) on the developmental parameters of *F. melanoleuca*

Host stage (Adults)	Mean duration (± SE_m) in days*						Sex ratio (♂ : ♀)
	Larval period	Pupal period	Adult longevity		Total development period		
			Male	Female	Male	Female	
Adult male	10.52 ± 0.18	8.32 ± 0.10	3.95 ± 0.07	4.60 ± 0.11	22.74 ± 0.34	23.42 ± 0.21	1:1.66
Adult female	13.28 ± 0.15	9.16 ± 0.13	4.91 ± 0.13	5.87 ± 0.06	27.30 ± 0.32	28.24 ± 0.26	1:1.90
t- value	12.58	5.18	6.41	11.05	10.40	10.38	-
p value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-

*Mean of 5 replications

Table 3 Effect of *P. perpusilla* nymphal stages on the reproductive parameters of *F. melanoleuca*

Host stage (Nymphs)	Mean duration (±SE_m)*		
	Pre-oviposition period (min)	Oviposition period (min)	Post-oviposition period (days)
1st	**	**	**
2nd	***	***	***
3rd	13.58 ± 0.24	22.16 ± 0.13	3.75 ± 0.07
4th	18.27 ± 0.25	31.65 ± 0.44	5.36 ± 0.24
5th	15.35 ± 0.23	31.54 ± 0.24	3.92 ± 0.08
LSD (p = 0.05)	0.75	0.93	0.48
F value	95.87	333.68	33.25
p value	< 0.0001	< 0.0001	< 0.0001

*Mean of 5 replications; **no successful parasitism in 1st stage *P. perpusilla* nymphs; ***no successful mating occurred in *F. melanoleuca* adults from parasitized 2nd stage nymphs of *P. perpusilla*

Fecundity

The eggs laid by parasitoid females from 4th instar host nymphs were significantly higher (845.03 eggs/female) followed by females from 5th instar host nymphs (634.98 ± 1.89 eggs/female). However, lowest fecundity (318.75 ± 0.52 eggs/female) was recorded in females reared on 3rd instar nymphs of *P. perpusilla* (Fig. 3).

Biological parameters of *F. melanoleuca* on *P. perpusilla* adults

Larval period and survival

The larval period of *F. melanoleuca* showed significant differences when reared on male and female *P. perpusilla* adults (t = 12.58; $p < 0.0001$; Table 2). The larval survival and duration of the parasitoid was significantly longer on adult females as against adult males (Fig. 4).

Pupal/chrysalis period and survival

The pupal/chrysalis period was significantly longer (9.16 ± 0.13 days) on female adults as against that recorded on male adults (8.32 ± 0.10days) (t = 5.18; $p < 0.0001$; Table 2). Significantly higher pupal/chrysalis survival was recorded when the parasitoid was reared on adult female as compared to adult male (Fig. 5).

Sex-ratio

The sex-ratio of *F. melanoleuca* adults was female-biased on both the sexes of its host, *P. perpusilla* (Table 2). However, more female adults (1:1.90) emerged on parasitized female adults as compared to parasitized male adults (1:1.66).

Adult longevity (male and female)

The longevity of adult females that were reared on host female adults was significantly higher (5.87 days) as compared to females reared on host male adults (4.60 ± 0.11days) (t = 11.05; $p < 0.0001$; Table 2). Similar observations were recorded for adult males of the parasitoid also (t = 6.41; $p < 0.0001$).

Total development period (male and female)

Irrespective of host sex stage, the male adults developed within shorter period of time as compared to females (Table 2). The development time of female parasitoids

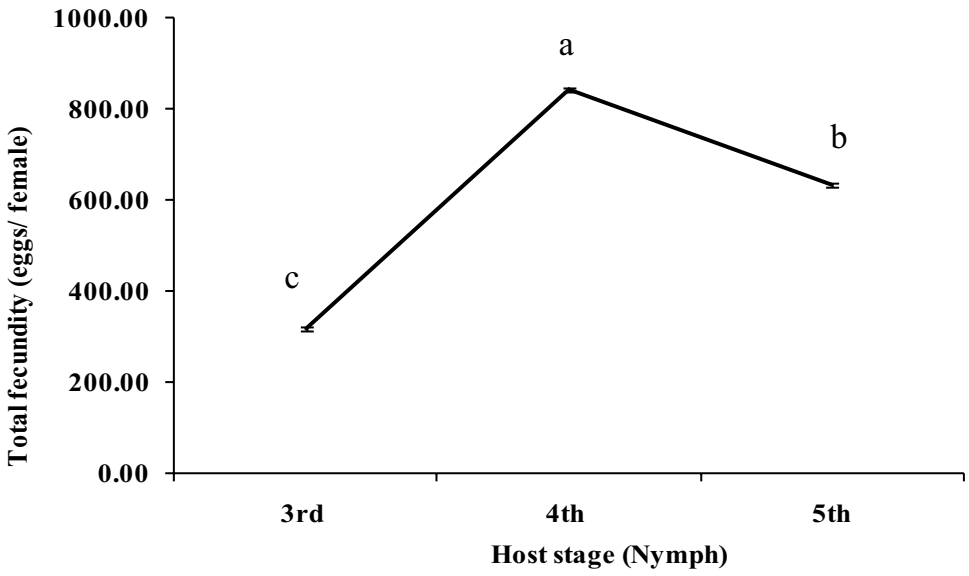

Fig. 3 Eggs laid by parasitoid females emerged from various various parasitized nymphs of *P. perpusilla*; Treatments with different letters are significantly different according to a Tukey's test at $P < 0.05$. Error bars represent the standard error of the mean

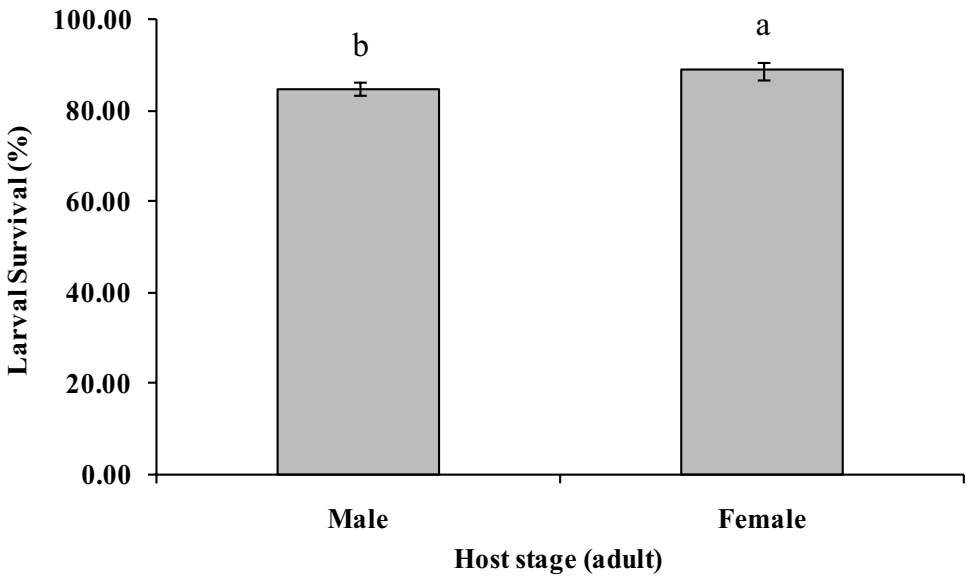

Fig. 4 Larval survival (%) of *F. melanoleuca* when male and female adults of *P. perpusilla* were offered to the parasitoid for parasitism; Treatments with different letters are significantly different according to a student's t-test at $P < 0.05$. Error bars represent the standard error of the mean

was significantly more (28.24 ± 0.26 days) on female host adult as compared to parasitoid reared on male host adult (23.42 ± 0.21days) (t = 10.38; $p < 0.0001$). Similarly, longer development period (27.30 ± 0.32 days) of male parasitoids was recorded on female adult stage as against male adult stage of its host (22.74 ± 0.34 days) (t = 10.40; $p < 0.0001$).

Pre-oviposition, oviposition and post-oviposition periods

The pre-oviposition period of *F. melanoleuca* female adults was less than one hour on both the sexes of host adult stage (Table 4). Significantly higher pre-oviposition period (25.07 ± 0.32 min) was recorded in parasitoid adults from host female adultsas compared to that from adult males (18.06 ± 0.40 min) (t = 13.61; $p < 0.0001$).The parasitoid females that emerged from parasitized *P. perpusilla* adult females showed significantly longer oviposition period (35.10 ± 0.43min) in comparison to the females that emerged from parasitized *P. perpusilla* adult males (29.47 ± 0.26 min) (t = 11.41; $p < 0.0001$; Table 4).Significantly longer post-oviposition period (5.73 ± 0.11days) was recorded in parasitoid females from *P. perpusilla* adult females as compared to 4.47 ± 0.12days in females reared host male adults(t = 6.76; $p < 0.0001$; Table 4).

Fecundity

The parasitoid females that emerged from parasitized female adults of *P. perpusilla* laid significantly more eggs (1024.65 ± 1.63 eggs/female) as compared to those which emerged from parasitized male adults (763.79 ± 0.72eggs/female) (Fig. 6).

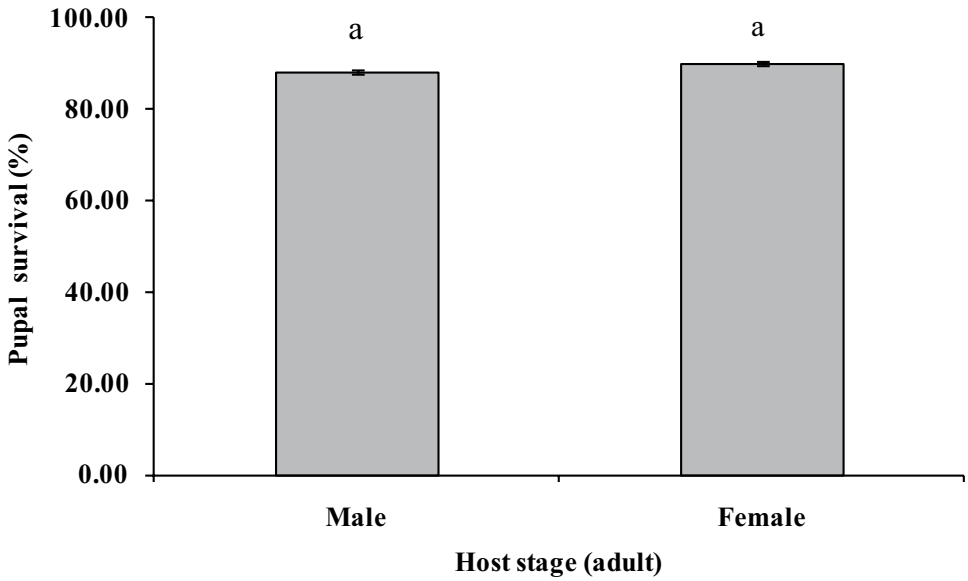

Fig. 5 Pupal survival (%) of *F. melanoleuca* when male and female adults of *P. perpusilla* were offered to the parasitoid for parasitism; Treatments with same letters are not significantly different according to a student's t-test at $P < 0.05$. Error bars represent the standard error of the mean

Table 4 Effect of *P. perpusilla* adult stage (sex) on the reproductive parameters of *F. melanoleuca*

Host stage (Adults)	Mean duration (± SE_m)*		
	Pre-oviposition period (min)	Oviposition period (min)	Post-oviposition period (days)
Adult male	18.06 ± 0.40	29.47 ± 0.26	4.47 ± 0.12
Adult female	25.07 ± 0.32	35.10 ± 0.43	5.73 ± 0.11
t- value	13.61	11.43	6.76
p value	< 0.0001	< 0.0001	< 0.0001

*Mean of 5 replications

Discussion

Our results showed that the exposure of 1st instar nymphs to the parasitoid resulted in cent per cent mortality of the host before showing any signs of parasitism. High mortality might be due to parasitoid inflicted injuries in the early nymphal instars which corroborates with findings of Bal et al. (1990). Therefore, the development and reproductive parameters were possible only for parasitoids reared on 2nd to 5th instar nymphal and adult (both male and female) stages of host.

The larval survival, larval period, pupal/chrysalis survival, pupal/chrysalis period and total development period of the parasitoid was less on the younger nymphal instars as compared to later instars of the host. This could be related to the small size of the parasitoid and/or insufficient resources for survival and development on younger nymphal instars. Furthermore, the parasitoid feeding may have been too much for the younger instar nymphs, causing them to perish early. As a result of early death in younger instars of host, there was early pupation in parasitoid and emergence of small moths. However, the later instars (4th and 5th) had sufficient vigour and tolerance to support adequate feeding of the parasitoid. Mukerji and Venktaraman (1948) also reported that in case of early death of the host, the parasitoid larvae were unable to transfer to new host but pupated and produced small moths. Likewise, adult longevity of parasitoid whose larvae had fed on younger instars lived for shorter period of time as compared to later instars. Shorter larval period, pupal/chrysalis period, small size cocoons and emergence of small moths might be possible reason for shorter adult longevity in younger nymphal instars. Jervis et al. (2008) and López et al. (2009) also observed that in parasitoids, the amount of dietary resources available during larval development is a key predictor of progeny fitness. Regardless of host stage, previous research (Rajak et al. 2006; Patel et al. 1993) on larval period, pupal/chrysalis period, adult longevity, and overall development period are in agreement with the current results, with minor differences that could be attributed to host stage and environmental variables. However, studies by Madan and Chaudhary (1991) on effect of host age revealed no significant differences in larval and pupal/chrysalis period of the parasitoid when reared on 5 to 30 days old nymphs are in variation with the present results. However, they also reported that pupal/chrysalis rate increased with increase in age of nymphs upto 15 days old nymphs and was minimum on 5 days old pyrilla nymphs are in agreement with the our findings.

The fitness of *F. melanoleuca* was comparatively more when reared on adults as compared to nymphs of *P. perpusilla*, which may be due to sufficient vigour and resources available for the parasitoid larvae from adults. However

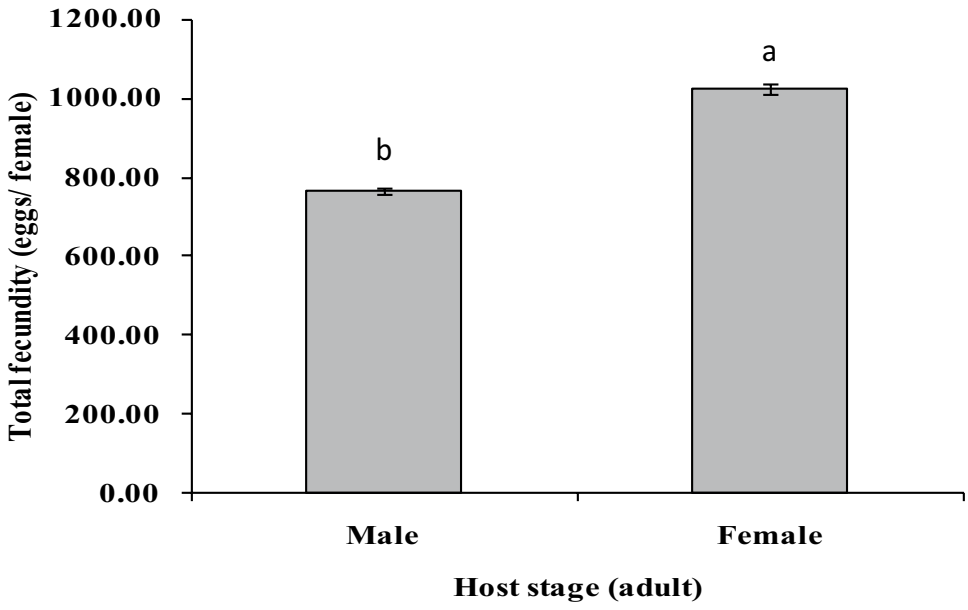

Fig. 6 Eggs laid by parasitoid females emerged from parasitized male and female adults of *P. perpusilla*; Treatments with different letters are significantly different according to a student's t-test at $P < 0.05$. Error bars represent the standard error of the mean

between the sexes, the survival and development parameters of parasitoid was more when reared on female adult host as compared to male adults. Female adults might have provided adequate and uninterrupted food resources as they do not disturb and dislodge the parasitoid larvae for their body by frequent jolts and during jumping as they are basically sluggish in nature unlike males (Misra and Krishna 1986; Madan and Chaudhary 1991) have reported no effect of host adult age (1 to 30 days old pyrilla) on the larval period, pupal/chrysalis period and adult emergence, however, pupation rate increased with increase in age of adults upto an age of 20 days.

The sex-ratio of *F. melanoleuca* adults was female-biased on all the host stages which are in conformity with the studies conducted by Bal et al. (1990). However, more female progeny was observed in later nymphal instars and female adult host. This is related to the host size-dependent sex allocation concept (Charnov et al. 1981), which suggested that bigger size hosts may generate a higher proportion of females due to greater dietary requirements and reproductive benefits for the female progeny. For successful biological control programmes, female-biased sex ratios are greatly desired because mature females are responsible for controlling insect pest populations (through host feeding or oviposition) (Berndt & Wratten, 2005; Chow & Heinz, 2006; Ode & Hardy, 2008). This is especially important for augmentative biological control, which aim to quickly suppress an insect pest population by mass-releasing enormous numbers of insects raised in the insectaries. Our results on the pre-oviposition period in conformity with studies conducted by Madan and Singh (1981);Patel et al. (1993) and Rajak et al. (2006) who reported it to be 1–31 min, 1–81 min and 1–35 min, respectively. The fecundity of *F. melanoleuca* increased with increase in host age and was comparatively more in the later nymphal instars. However, 4th instar pyrilla nymphs serving as host for *F. melanoleuca* favoured a significant increment in number of eggs laid by the parasitoid females as compared to 3rd and 5th instar nymphs. The lower fecundity of parasitoid female moths reared on younger nymphal instar may be due to their insufficient nourishment during immature period, shorter larval duration and /or formation of small cocoons owing to early death of the host. Early pupation and production of small moths in case of early death of parasitized host has also been reported by Mukerji and Venktaraman (1948). However, the larval period of parasitoid was longer on 4th instar pyrilla nymphs which could have provided sufficient opportunity to the parasitoid larvae for ingesting adequate food resources and ultimately yielded larger cocoons. A positive correlation between cocoon size and fecundity of *E. melanoleuca* has also been reported by Chandra and Tewari (1978). Moreover the oviposition period, i.e. the duration for which eggs were laid was significantly more in female moths from 4th instar nymphs as against other nymphal instars which might have also resulted in the enhanced egg yield. It is pertinent to mention that no successful mating occurred between male and female adults of *F. melanoleuca* whose caterpillars derived nourishment from parasitized 2nd instar *P. perpusilla* nymphs. Due to poor nourishment and early death of the host, there was early pupation and small sized cocoons. The adults from these small sized cocoons were weak and small in size. Further, they died prematurely or soon after emergence from the cocoons.

The oviposition period in female adults was also more when parasitoid was reared on female adult pyrilla as compared to male adult which further led to significantly higher egg deposition. The pyrilla females are basically sluggish in nature as compared to males. This might be possible reason that they do not disturb and dislodge the parasitoid larvae for their body by frequent jolts and during jumping unlike males. Due to favourable lodging on the body, females might have provided the parasitoid larvae an opportunity to ingest sufficient and uninterrupted quantity of food. Hence, more egg yield was recorded in female moths whose caterpillars fed on female pyrilla as compared to males. Similar findings on fecundity of females (1021.8 eggs/ female) reared on female host pyrilla have also been reported by Misra and Krishna (1986). Madan and Chaudhary (1991) reported that fecundity of *E. melanoleuca* varied from 794. 3 to 808.7 eggs per female which did not differ when reared on 1 to 30 days old pyrilla adults. The present results depicting the oviposition period, post-oviposition period and fecundity of *F. melanoleuca* corroborate with Madan et al. (1982), Patel et al. (1993) and Rajak et al. (2006).

Conclusion

With regard to higher larval survival, adult emergence, more proportion of females in the progeny, and higher fecundity, it can be inferred that 4th instar nymphs and adult females of the host, *P. perpusilla*, were more suited for the parasitoid, *F. melanoleuca*. These host stages can be gainfully exploited for the mass rearing of the parasitoid under laboratory conditions and subsequent use in the biological management of *P. perpusilla* under field conditions.

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Declarations

Conflicts of interest The authors reported no potential conflicts of interest.

References

Bal RS, Dhaliwal ZS, Bains SS (1990) Biological effects of parasitism by *Epiricania melanoleuca* (Fletcher) on *Pyrillaperpusilla* (Walker). J Biol Control 4:77–79

Berndt LA, Wratten SD (2005) Effects of alyssum flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid *Dolichogenidea tasmanica* Biol Control 32:65–69

Chandra J, Tewari NK (1978) Biology and fecundity of *Epipyrops melanoleuca* Fletcher, and ectoparasitoid of *Pyrilla perpusilla* Indian Sugar 28:333–335

Charnov EL, Los-den Hartogh RL, Jones WT, van den Assem J (1981) Sex ratio evolution in a variable environment. Nature 289:27–33

Chaudhary NA, Ansari MA (1988) Insect pests of sugarcane in Pakistan. Progressive Farming (Pakistan) 8:10–18

Chow A, Heinz KM (2006) Control of *Liriomyza langei* on chrysanthemum by *Diglyphus isaea* produced with a standard or modified parasitoid rearing technique. J Appl Entomol 130:113–121

Emeljanov AF (2018) A review of the fauna of the plant hopper family lophpopidae (Homoptera, Fulgoroidea) from Vietnam with descriptions of new genera and species and with taxonomic notes. Entomologicheskoe Obozrenie 97:293–316

Fennah RG (1963) The species of *Pyrilla* (Fulgoroidea:Lophopidae) in Ceylon and India. Bull Entomol Res 53:715–735

Ganehiarachchi GASM, Fernando IVS (2006) Biology of *Pyrill aperpusilla* Walker (Homoptera: Lophopidae), a pest of sugarcane in the wet zone of Sri Lanka. J Entomol 3:26–33

Gangwar SK, Srivastava DC, Tewari RK, Singh MR, Rajak DC (2008) Management of *Pyrilla perpusilla*Walker in sugarcane with ectoparasitoid *Epiricania melanoleuca* Fletcher during epidemics in sub-tropical India. Sugar Tech 10:162–165

Hagvar EB, Hofsvang T (1991) Aphid parasitoids (Hymenoptera: Aphidiidae): biology, host selection, and use in biological control. Biocontrol News Inf 12:3–41

Jervis MA, Ellers J, Harvey JA (2008) Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Ann Rev Ent 53:361–385

Kant R, Minor MA, Trewick SA (2012) Fitness gain in a koinobiont parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) by parasitising hosts of different ages. J Asia-Pacific Ent 15:83–87

Karmakar P, Shera PS (2018) Seasonal and biological interactions between *Aenasius arizonensis* and its host *Phenacoccus solenopsis*). Phytoparasitica 46:661–670

Kumar R, Mittal V, Chutia P, Ramamurthy VV (2015) Taxonomy of *Fulgoraeci amelanoleuca* (Fletcher, 1939), (Lepidoptera: Epipyropidae) in India, a biological control agent of *Pyrilla perpusilla* (Walker) (Hemiptera: Lophopidae). Zootaxa 3:431–439

Liang A (1997) Sugarcane and rice plant hoppers of the genus *pyrilla* stal in Southern China (Insecta: Homoptera: Auchenorrhyncha: Lophopidae). Reichenbachia 32:33–39

López OP, Hénaut Y, Cancino J, Lambin M, Cruz-López L, Rojas JC (2009) Is host size an indicator of quality in the mass-reared parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)? Fl Entomol 92:441–449

Madan YP, Chaudhary JP (1991) Effect of host (*Pyrilla perpusilla* Walker) age on the biology of *Epiricania melanoleuca* (Fletcher). J Insect Sci4:160–162

Madan YP, Singh RA (1981) Some observations on the *Epipyrops melanoleuca* Fletcher an ectoparasite of *Pyrilla perpusilla* Walker in laboratory. Indian Sugar Crops J 8:19–20

Madan YP, Sharma SK, Chaudhary JP (1982) Studies on the reproductive biology of *Epipyrops melanoleuca* Fletcher, a nymphal adult parasite of *Pyrilla perpusilla* Walker. Agric Sci Digest 2:11–13

Misra MP, Krishna SS (1986) Variation in the reproductive performance of *Epiricania melanoleuca* (Fletcher) (Lep., Epipyropidae) in relation to stage and sex of the host *Pyrilla perpusilla* (Walker) (Horn., Lophopidae) during rearing. Anzger Fur Schadlingskunde, Pflanzenschutz Umweltschutz, 59:20–21

Mukerji S, Venktaraman TV (1948) Studies on *Epipyrops melanoleuca* Fletcher (Lepidoptera: Epiloyropidae), and ectoparasite of sugarcane leafhopper, *Pyrilla* spp. (Homoptera: Fulgoridae). Proc Zool Soc Bengal 1:91–102

Neupane FP (1976) Studies on the effectiveness of different insecticides against the nymphs of sugarcane leaf hopper *pyrilla perpusilla* (Walk) Nepalese J Agric 6:131–137

Ode PJ, Hardy ICW (2008) Parasitoid sex ratios and biological control. In: Wajnberg E, Bernstein C, van Alphen JJM (eds) Behavioural ecology of insect parasitoids: from theoretical approaches to field applications. Blackwell, Oxford, pp 253–285

Pandey KP, Pandey MN, Mishra VK, Singh S, Singh DN, Singh SB (2008) Studies on the effect of eco-friendly bioagents *Epiricania melanoleuca* for the control of sugarcane pyrilla(*pyrilla perpusilla*) in eastern UP. Bhartiya Krishi Anusandhan Patrika 23:91–95

Patel DR, Patel MB, Patel CB (1993) Population dynamics of sugarcane leafhopper, *Pyrilla perpusilla* Walker and its ectoparasite *Epiricania melanoleuca* Fletcher in relation to climate. Gujarat Agric Univ Res J 19:56–63

Rajak DC, Singh RP, Prasad K, Chandra U, Saroj SR, Lal H (2008) Parasitism and hyper parasitism on *Pyrilla perpusilla*Walker in sugarcane ecosystem. Sugar Tech 10:150–153

Rajak DC, Singh RP, Tripathi RA, Kumar S (2006) Studies on the biology of *Epiricania melanoleuca* Fletcher a potent parasitoid for management of *Pyrilla perpusilla* Walker in sugarcane. Indian J Sugarcane Tech 21:49–53

Rasib KZ, Abru S, Malik A (2020) Comparative studies on quantity and quality of pests' incursion Sugarcane grown at Rahim Yar Khan Pakistan. Agriculture Research Technology: Open Access J 24:62–76

Rasul A, Mansoor-Ul-Hassan FM, Khan RR (2014) Sustainable management of sugarcane pyrilla, *pyrilla perpusilla* wlk. (Homoptera: Lophopidae) by using cultural, chemical and biological practices in Punjab, Pakistan. Pakist Entomol 36:45–49

Seneviratne JAUT, Kumarasinghe NC (2002) Biological control of the sugarcane plant hopper by the moth *Epiricania melanoleuca* (Fletcher) in Sri Lanka. Sugar Tech 4:26–32

Sharma T, Shera PS (2021) Biological anomalies in the sugarcane leafhopper, *pyrilla perpusilla* (walker) due to parasitism by *Fulgoraecia melanoleuca* (Fletcher) (Lepidoptera: Epipyropidae). Egypt J Biol Pest Control 31:137. https://doi.org/10.1186/s41938-021-00486-3

Shera PS, Karmakar P (2018) Effect of mating combinations on the host parasitization and sex allocation in solitary endoparasitoid, *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae). Biocontrol Sci Tech 28:46–61

Singh SP, Jagmohan S, Brar KS, Maninder S, Darshan S (2001) Demonstration of biological control based integrated pest management on sugarcane in Morinda mill area in Punjab. Indian J Sugarcane Tech 16:58–64

Srikanth J, Easwaramoorthy S, Jalali SK (2016) A 100 years of biological control of sugarcane pests in India: review and perspective. CAB Rev 11. https://doi.org/10.1079/PAVSNNR201611013)

Verma A, Chandra J, Tanwar RK (2002) Eco-friendly pest control in sugarcane. Indian Farming 51:40–43