

Larval Age-Dependent Parasitization Performance of *Cotesia flavipes* on *Sesamia inferens*

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Abbreviation: Nat. Env. & Poll. Technol.
Website: www.neptjournal.com

Received: 26-07-2024

Revised: 01-09-2024

Accepted: 21-09-2024

Key Words:

Cotesia flavipes
Sesamia inferens
 Parasitism
 Mass rearing
 Braconidae

ABSTRACT

Cotesia flavipes is an important hymenopteran larval parasitoid that belongs to the family Braconidae. Its usage in pest management strategies is promising due to its parasitic impact on the larval stage of lepidopteran pests. The current investigation aims to determine the optimal host age for the parasitoid's mass proliferation and augmentative releases. The experiments showed that the female *C. flavipes* parasitizes all larval age groups of *Sesamia inferens*. Among all the larval ages, *C. flavipes* preferred second to third instars for parasitism during the spring (up to 90%) and kharif (up to 80%) seasons. There was no substantial difference in the development period between stinging, cocoon production, and the adult emergence of parasitoids. The age of the host has a substantial impact on adult longevity, with females taking longer than males. Thus, larval instars (second and third) are also recommended for high-quality mass-rearing larval parasitoids, especially *C. flavipes*, due to their strong parasitism and high net reproductive rate. Therefore, the second and third instars of *S. inferens* will recommend the mass rearing of *C. flavipes* and the release of these parasitoids in the field as a successful bio-control program.

INTRODUCTION

Biological control with various living organisms such as predators, parasitoids, and entomopathogens has significant potential in integrated pest management (Sedaratian-Jahromi 2021). Adult parasitoids are free-living, whereas their egg and larval stages live in or on a single host to complete their life cycle to reach the adult stage (Dodiya & Pathan 2022, Fei et al. 2023, Holmes et al. 2023). Parasitoids eventually kill their hosts throughout the parasitism process by inducing paralysis (Quicke 2014, Butcher & Quicke 2023, Dai et al. 2024). It lays eggs in the hemocoel of the host insect (Jervis et al. 2023), complete larval development inside the host, and emerge just before the pupal formation in the silken cocoons (Van Noort & Broad 2024, Virla et al. 2023, Farahani et al. 2012). However, larval endoparasitoids like *Cotesia* are being used in biological pest control programs worldwide (Chepkemol et al. 2023, Parker & Kingsolver 2024) and parasitize various lepidopteran pests, including *Sesamia inferens*. Pink stem borer, *Sesamia inferens*, is a polyphagous pest reported on wheat, sorghum, oats, rice, pearl millet, barley, sugarcane, finger millet, and grasses (Jeer et al. 2021, Jadhao et al. 2020, Baladhiya et al. 2018). This pest caused damage of up to 78% in maize (Deole et al. 2017, Upadhyay et al. 2023).

Chemical communication is a very important factor in the host selection of parasitoids (Han et al. 2024). When the plant is infested by the insect pest, which emits volatile cues that are responsible for the identification of the host location for the parasitoid (Penaflor et al. 2011, Uefune et al. 2012, Furstenberg

Citation for the Paper:

Sonawane, V.K., Gharde, S.K., Ghodekar, K.S., Raut, A.M. and Assouguem, A., 2025. Larval age-dependent parasitization performance of *Cotesia flavipes* on *Sesamia inferens*. *Nature Environment and Pollution Technology*, 24(2), p. B4249. <https://doi.org/10.46488/NEPT.2025.v24i02.B4249>

Note: From year 2025, the journal uses Article ID instead of page numbers in citation of the published articles.



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et al. 2013, Bogka et al. 2023, Yi et al. 2023) or emitted by herbivores and their frass after feeding on plants (de Rijk et al. 2016, Liu et al. 2024), host preference could influence the host-parasitoid interaction (Furstenberg-Hagg et al. 2013), the development, success of reproduction, and the percentage of survival of the parasitoid on the hosts in field condition (Kher et al. 2024, Han et al. 2013, Ehteshami et al. 2023, Gomes et al. 2024). Consequently, numerous parasitoids exhibit host preferences based on particular stages of host or species and enhance the percentage of parasitism in invitro conditions (Stacconi et al. 2013, Yazdani et al. 2015, Hegazi et al. 2024, Kher et al. 2024).

The ability of parasitoids to search behavior is based on the parasitism rate against hosts (Hardy & Wajnberg 2023). After the parasitism of the host by the parasitoid, it goes through various phases such as host habitat location, host location, host acceptance, host suitability, and host regulation (Harris et al. 2012 Kuramitsu et al. 2019 Benrey 2023 Kathirvelu et al. 2024 Fei et al. 2023 Abram et al. 2023). The steps to be followed for effective colonization of a host by a parasitoid according to chemical cues are employed by the male wasps to find conspecific females in the field or an artificial cage (Malek et al. 2021). Certain parasitoids possess the capability to locate potential mates over a considerable distance through the emission of highly volatile pheromones, whereas, in certain instances, less volatile pheromones are employed for short-range (Ruther 2013). It is believed that male wasps may use cues other than female pheromones to locate females, other cues that help to find mates through host-associated volatiles or host-induced plant volatiles (Reddy & Guerrero 2004, Xu et al. 2017). Female parasitoids use plant volatiles to locate host habitats (McCormick et al. 2012, Wäschke et al. 2013, Turlings & Erb 2018). Some insects also take volatile stimuli from the habitat for mating (male dung flies are attracted to the odor of fresh cow manure) (Parker 1978, McAuslane et al. 1990, Webster & Cardé 2017).

The biocontrol program is successful when bio-agents are available at ground level, and natural enemies are mass culturing in the laboratory. The natural population available in the field will not survive for a longer period due to the application of pesticides to protect the crops. In agriculture, crops are protected through synthetic pesticides, causing several problems like the development of resistance and resurgence in pests, habitat loss, the decline of ecological service members, environmental pollution, and many more (Onaho et al. 2023, Ahmad & Akhtar 2016, Shimeng et al. 2001, Mruthunjayaswamy et al. 2016, Nwankwo et al. 2016). Therefore, the laboratory mass culture of braconid wasps requires lepidopteran larvae as a feeding

source. However, one issue encountered during laboratory production of *Cotesia flavipes* is parasitoid quality (Prezotti & Parra 2002). The parasitoid's inferior quality is the reason for its production failure and lack of demand (Ramalho et al. 2012). Hence, the knowledge of the parasitoid is most important for the suitable host age used for augmentative parasitoid releases (Li et al. 2006). Therefore, the present study mainly focuses on the performance of *Cotesia flavipes* on parasitization and net reproductive rate on different larval instars of the pink stem borer, *Sesamia inferens*, to enhance the mass-rearing process of parasitoids.

MATERIALS AND METHODS

The experiment entitled Parasitization performance of *Cotesia flavipes* on different larval instars of pink stem borer, *Sesamia inferens*, was conducted under laboratory conditions at the School of Agriculture, Lovely Professional University, Phagwara (Punjab). The experiments were conducted during the spring and kharif seasons of 2023.

Mother Culture of *Sesamia inferens*

A fourth and fifth instar of the pink stem borer, *Sesamia inferens* Walker, were collected from a maize field and reared under laboratory conditions. The insects were kept in in-vivo conditions at $26 \pm 2^\circ\text{C}$, RH $65 \pm 5\%$, and a 12L:12D photoperiod. The emerged adult from this culture was reared on a maize plant in the ovipositional chamber (Kumar et al. 2011) by providing 10% honey solution to emerging adults. After five to six days, remove the potted plant from the ovipositional chamber, separate the leaf sheath from the plant where the egg mass was, and rear in the incubator up to the hatching of neonate larvae. These larvae were reared on a maize stem (7×2.5 cm) in tubes, with this method, culture was maintained for future study (Sharma et al. 2017). The old stems were replaced by fresh stems every day. Two generations were reared under laboratory conditions before starting the experiments.

Mother Culture of *Cotesia flavipes*

Parasitized cadavers were collected from the maize field and brought into the laboratory for further mass culturing. The cocoons were kept at $26 \pm 2^\circ\text{C}$, RH $65 \pm 5\%$, with a photometer for 12 hours of equal day and night length. The cocoons of parasitoids were reared in the rearing chamber by covering them with brown paper (22×15×5 cm). The observations were recorded daily until adults emerged. Emerged adults were then shifted to the rearing cage by providing a 20% honey solution in cotton swabs as food for adult parasitoids. After 24 hours, keep the old larvae separate and the fresh larvae with the parasitoids,

and continue this process till the death of the parasitoid. All parasitized larvae of *S. inferens* were reared at $26 \pm 2^\circ\text{C}$, RH $65 \pm 5\%$, with a photoperiod of 12 hours equal to the length of day and night. The honey solution was provided daily to the adult parasitoid, and three generations of *Cotesia flavipes* were reared in laboratory conditions with the same procedure.

Preference of *Cotesia flavipes* on Different Larval Instars

To study the preference of female parasitoid *Cotesia flavipes* on different larval instars of pink stem borer, a pair of adult parasitoids were released for 24 hours in a glass tube for mating (Saini et al. 2019) with a 20% honey solution in a cotton swab as food. For mating, a no-choice experiment was carried out, with 10 larvae of each instar in a rearing box ($22 \times 15 \times 5$ cm). The separation of the larvae was done (instar-wise) according to their size and color (Sharma et al. 2017, Nagarjuna et al. 2015). The parasitized host larvae of different instars of pink stem borer were then reared individually and observed until the pupation and emergence of the adult parasitoid.

Impact on the Developmental Biology of *Cotesia flavipes*

The experiment on the developmental biology of the parasitoid *Cotesia flavipes* on the larval instars of pink stem borer was investigated through a series of experiments involving the parasitization of various larval instars of stem borer. The eclosion of adults was sorted by sex, and a pair of parasitoids were exposed separately to batches of ten larvae of host II to VI instars in a no-choice

experimental design. After 24 hours, the old larvae were replaced with new ones.

The old larvae were reared according to the prescribed procedure to facilitate the emergence of parasitoids (Saini et al. 2019). Since immature stages (egg and larvae) of the parasitoid were present in the body of the host, various parameters like duration of egg and larval stages (stinging to cocoon formation stage), pre-pupa and pupal stage (cocoon stage), longevity of adults, and sex ratio were recorded throughout the experimental period.

Statistical Analysis

Different parameters recorded during the study were subjected to a one-way analysis of variance (ANOVA) using SPSS 22.0 software. We investigated the interactions of season and instar on the parasitization and developmental period of *Cotesia flavipes*, considering season and instar as fixed effects. The means that showed significant differences were distinguished using the least significant difference (LSD) method.

RESULTS

Parasitization Rate of *Cotesia flavipes* on Different Larval Instars

The *C. flavipes* parasitization rate significantly differs between the different larval instars of *S. inferens*. The most preferred stage and the highest level of parasitism (80% and 90%) were observed in third-instar larvae in the spring ($F_{(4,9)} = 3.68$ $p = 0.011$) and kharif ($F_{(4,9)} = 5.07$

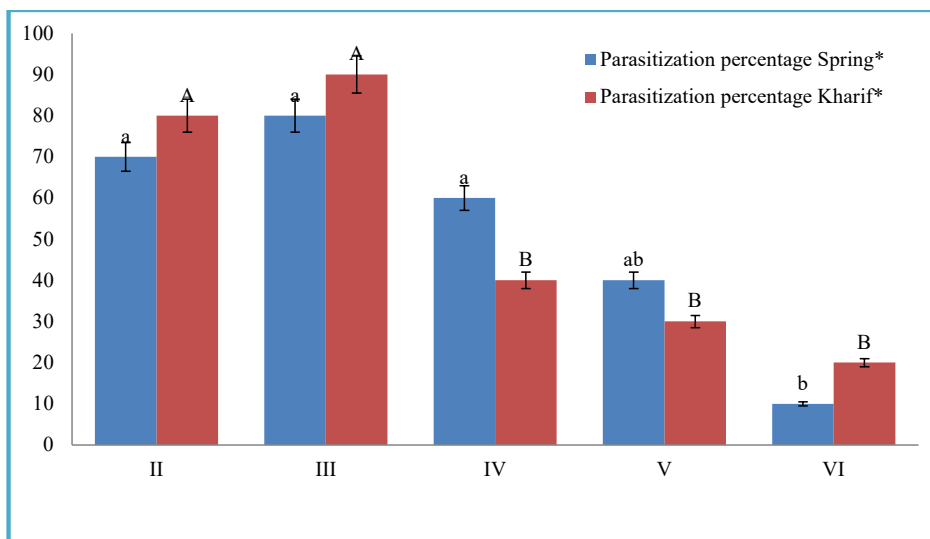


Fig. 1: Parasitization capability of *Cotesia flavipes* on different larval instars. ^a, ^A values with the same letters in a column are non-significant, as per DMRT. ^{*} significant at 5% level of significance.

$p=0.002$) seasons, respectively (Fig 1). The parasitization rate gradually decreased after the third instar until the sixth instar during both seasons. However, there was no statistically significant impact of season on the parasitization of *C. flavipes* with respect to larvae host age.

Impact on the Developmental Biology of *Cotesia flavipes*

The development period of immature parasitoids on host age was not significantly different ($p > 0.05$) in both seasons (Table 1). The age of the parasitized host showed a substantial effect on adult longevity ($p < 0.05$), with females living longer in both seasons. In *spring* 2023, the third instars had the longest development period, followed by the second, fourth, fifth, and sixth instars, and a similar pattern was seen in *Kharif* 2023 (Table 1).

Net Reproductive Rate

The maximum oviposition period of *C. flavipes* was up to 96 hours observed in the second and third instars and then gradually decreased with host age in the *spring* season, and the same trend was observed in the *Kharif* season (Fig. 2). However, the female adult who emerged from the cocoon was twice as old as the male adult in all the time periods and

the age of the host in both seasons. Overall, there was no significant variation in the cocoon-forming population and adult emergence between seasons (Fig. 2). The sex ratio was 0.40:1 and 0.37:1 (male: female) found in the *spring* and *kharif* seasons, respectively.

DISCUSSION

Bio-agents have the potential to check pest populations in the available ecosystem and therefore They are an important tool in the integrated pest management (IPM) system (Sampaio et al. 2009). The biological control includes predators, parasitoids, and entomopathogens successfully accommodated in the environment by the availability of suitable habitat, the application of eco-friendly pesticides, etc. Among the natural enemies, parasitoids are usually the best option for pest management due to their high host specificity and free-living behaviors (Colmenarez et al. 2018, Trejo & Contreras 2024). Therefore, mass culturing of parasitoids is an important step, and a temperature of 25-30°C indicates a faster development rate (Adamo et al. 2012, Huey et al. 2012, Moore et al. 2020). However, the mass production of *Cotesia* species in the laboratory is very cost-effective for pest management (Zang et al. 2021). Our research study showed that the *Cotesia flavipes* preferred

Table 1: Development time and adult longevity of parasitoids at different ages of the host.

Spring 2023					
Host instars	Development time (days) \pm SD			Adult longevity (days) \pm SD	
	Stinging to cocoon formation	Cocoon stage	Stinging to Adult emergence	Male	Female
II	8.2 \pm 4.42 ^a	3.3 \pm 1.83 ^{ab}	11.5 \pm 6.13 ^a	4.9 \pm 3.48 ^a	5.1 \pm 3.57 ^a
III	8.5 \pm 7.40 ^a	4.0 \pm 3.5 ^a	12.5 \pm 10.8 ^a	5.2 \pm 2.86 ^a	5.5 \pm 3.10 ^a
IV	7.1 \pm 5.02 ^{ab}	3.0 \pm 3.89 ^{ab}	10 \pm 7.04 ^{ab}	4.5 \pm 4.00 ^a	4.7 \pm 4.1 ^a
V	6.2 \pm 8.01 ^{ab}	2.9 \pm 2.08 ^{ab}	9.2 \pm 11.89 ^{ab}	2.9 \pm 3.78 ^b	3.2 \pm 4.21 ^{ab}
VI	1.7 \pm 5.38 ^b	0.8 \pm 2.53 ^b	2.5 \pm 7.91 ^b	0.7 \pm 2.21 ^b	0.9 \pm 2.85 ^b
F _{value}	2.25	2.00	2.17	3.72	3.19
P _{value}	0.116 ^{NS}	0.159 ^{NS}	0.129 ^{NS}	0.022*	0.041*
Kharif 2023					
Host instars	Development time (days) \pm SD			Adult longevity (days) \pm SD	
	Stinging to cocoon formation	Cocoon stage	Stinging to Adult emergence	Male	Female
II	8.0 \pm 4.37 ^{a#}	3.5 \pm 1.90 ^a	11.5 \pm 6.15 ^a	4.7 \pm 2.71 ^a	5.6 \pm 3.17 ^a
III	8.8 \pm 7.60 ^a	4.0 \pm 3.50 ^a	12.8 \pm 11.08 ^a	5.1 \pm 3.03 ^a	5.3 \pm 2.95 ^a
IV	6.0 \pm 3.27 ^a	3.2 \pm 4.16 ^a	9.2 \pm 11.93 ^a	4.0 \pm 3.53 ^{ab}	4.3 \pm 3.74 ^{ab}
V	6.0 \pm 7.79 ^a	2.9 \pm 1.66 ^a	8.9 \pm 4.91 ^a	2.5 \pm 3.37 ^{ab}	2.8 \pm 3.65 ^{ab}
VI	3.1 \pm 6.54 ^a	1.7 \pm 3.59 ^a	4.8 \pm 10.13 ^a	1.2 \pm 2.57 ^b	1.4 \pm 2.95 ^b
F _{value}	1.27	0.761	1.088	2.803	2.44
P _{value}	0.293 ^{NS}	0.556 ^{NS}	0.374 ^{NS}	0.037*	0.035*

The values with the same letters in a column are non-significant, as per DMRT.

‘*’ significant at 5% level of significance (P<0.05), NS - non-significant

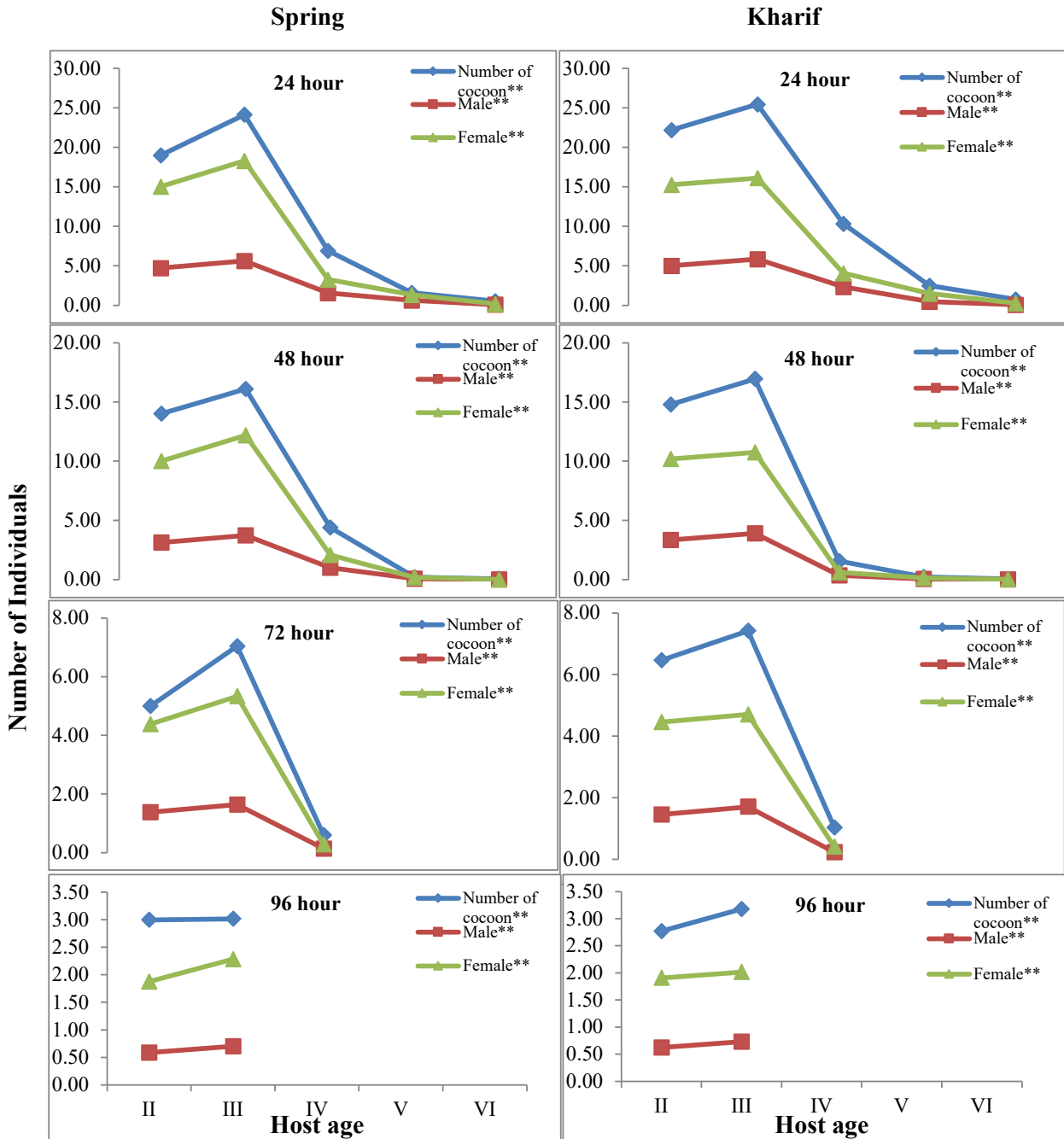


Fig. 2: Variation in the larval Parasitoid Reproductive Rate Across Different Host Ages
*** Significant level at $P \leq 0.01$

the second and third instars of the host for parasitism at a temperature of 25°C due to the availability of high protein, sugar, and lipid content in early instars larvae (Shekharappa & Kulkarni 2003, Jervis et al. 2008, Silva-Torres et al. 2009, Sokame et al. 2021).

However, the old age instars were not preferred by the

female *C. flavipes* for parasitization because the old larval instars developed defense mechanisms themselves and were aggressive, which led to the death of parasitoids (Potting et al. 1997).

Abiotic factors, especially temperature, play a crucial role in parasitoid growth and development (Adamo et

al. 2012, Huey et al. 2012). However, parasitoid wasp emergence and survival rate were maximum at 20°C and 25°C, but when it exceeded 30°C exhibited the rate of wasp emergence and survival rate (Moore et al. 2020). The longer development process, cocoon formation, and duration from stung to the emergence of adult parasitoids were observed at 25°C in the present investigation. A similar result was observed in *Cotesia* species; development periods were longer in the second instar of *Chilo partellus* (Sarkar et al. 2020 Khan et al. 2017), and the formation of the highest number of parasitoid cocoons was recorded in the third instar of *Spodoptera frugiperda* (Obala et al. 2023).

Augmentation is the release of natural enemies or environmental manipulation to increase the effectiveness of naturally occurring natural enemies (Hoy 2008). Environmental manipulation can include supplying alternate hosts or prey, food or nesting locations, or changing agricultural methods to benefit bioagents (Hoy 2008). The periodic release of natural enemies is evaluated based on fitness factors such as offspring sex ratio (Mohamad et al. 2015, Kruitwagen et al. 2018). Our research reported that males emerged early as compared to females, and the population of female adults was almost double the of male adults. This result may be due to mated female parasitoids being able to manage progeny sex ratios by regulating fertilization during oviposition. The present result, supported by (Heimpel & de Boer 2008), revealed that females develop from fertilized eggs and are diploid, while males develop from unfertilized eggs and are haploid. However, the previous research showed that the female adult emerged more than the male adult of *Cotesia* sp. when the host was *Spodoptera frugiperda* (Obala et al. 2023) and *S. littoralis* (Agbodzavu et al. 2018).

CONCLUSIONS

The present investigation concludes that *Cotesia flavipes* preferred the second or third instars of *Sesamia inferens* for parasitization. These larval stages are used for mass rearing of *C. flavipes* in laboratory conditions and are periodically relived in the field to manage the lepidopteron pest. In laboratory conditions, a temperature of 25–30°C will enhance the fecundity, cocoon formation, and emergence rate of *C. flavipes*. However, for the conservation of parasitoids, we will provide alternate hosts and food by planting nectarine plants and avoiding the application of non-selective pesticides. The conservation of the larval parasitoid *C. flavipes* enhances the efficacy of biological pest management strategies aimed at mitigating the impact of lepidopteran pest infestations in field conditions.

ACKNOWLEDGMENT

All the authors are thankful to Lovely Professional University for providing the facility for conducting the experiments.

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