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Research Article

Effect of temperature on the development of two pupal parasitoid species of *Xanthopimpla* Saussure (Hymenoptera: Ichneumonidae) on *Sesamia inferens* Walker

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ABSTRACT: The biology of two pimplini parasitoids *viz., Xanthopimpla flavolineata* and *X. stemmator* were investigated in the laboratory by using the pupae of *Sesamia inferens* Walker. The host insect pupae were collected from the field and maintained in the laboratory for rearing the pimplini parasitoids. The results showed that the developmental period of *X. flavolineata* was 33.87 ± 0.18 and 18.54 ± 0.15 days, and *X. stemmator* was 36.08 ± 0.16 and 18.95 ± 0.19 days under 20°C and 28°C, respectively. The morphometric studies of two pimplini parasitoids showed that *X. stemmator* was larger than *X. flavolineata* in all the life stages. The longevity of *X. flavolineata* males and females was 21.09 ± 0.14 days for males and 34.53 ± 0.26 days for females when fed with 50 per cent honey at 20°C., while *X. stemmator* males and females survived for 26.94 ± 0.22 days and 38.90 ± 0.25 days. At 28°C, the lifespan of *X. flavolineata* male and female was 16.17 ± 0.09 and 26.55 ± 0.13 days, respectively, while in *X. stemmator,* it was 17.47 ± 0.16 and 27.08 ± 0.17 days in male and female, respectively, when fed with 50 per cent honey. Thus, females lived longer than males when fed with honey (50%) solution as a food source followed by sucrose (50%). From the results, it is concluded that the temperature and developmental period are inversely proportional and these details can be integrated into the development of a standardized mass-production technique for both parasitoids.

KEYWORDS: Biology, parasitoid, *Sesamia inferens*, temperature, *Xanthopimpla*

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INTRODUCTION

Ichneumonidae is the most biologically diverse group of the Order Hymenoptera order (Gauld *et al.,* 2002). The subfamily, Pimplinae is a moderately large group but includes several big, strikingly coloured parasitoid species of Ichneumonidae (Gómez *et al.,* 2014). Its classification has undergone many changes by various authors and presently the subfamily is divided into four tribes *viz.,* Ephialtini, Pimplini, Delomeristini and Perithoini (Gauld *et al.,* 2002). All species of Pimplini are generalist parasitoids (rather than specialists) that can feed on more than one species of host. The most primitive pimplines are idiobionts of the endopterygote insects. *Xanthopimpla* Saussure, 1892 is a large genus belonging to the subfamily Pimplinae and containing 265 species from all zoogeographical regions (Gadad *et al.,* 2023). The detailed review of studies on the developmental stages of endophagous parasitoids including those of Ichneumonidae was summarised by Hagen (1964). Arthur (1963) described the life history and developmental periods of immature stages of four ichneumonid parasitoids of the European pine shoot moth, *Rhyacionia buoliana* Schiff which included, *Itoplectis conquisitor* Say. The biology, morphology, development and behaviour of immature stages of many related endoparasitic ichneumonid species were studied by many workers, of which *Nemeritis canescens* Grav. (Corbet and Rotheram, 1965), *Temelucha* sp. (Oatman and Planter, 1974), *Bathyplectes anurus* Thomp. (Bartell and Pass, 1980) and *Eriborus trochanteratus* Morley (Swamiappan, 1984) were noteworthy.

Pillai and Nair (1983) reported the biology, longevity, courtship behaviour, life cycle, fecundity, sex ratio and oviposition behaviour of *X. nana nana* on concealed and naked hosts of *O. arenosella*. Ueno and Ueno (2007) observed the reproductive biology of six Pimplinae ichneumonid parasitoids and their developmental period, longevity, number of ovarioles, mature eggs per female, emergence rate

and sex ratio on different lepidopteran hosts. Additionally, Kathirvelu *et al.* (2023) recorded the courtship and mating behaviour of the pupal parasitoid, *X. flavolineata.*

Keeping the above in consideration, the present study was carried out to investigate the developmental period of parasitoids, *X. flavolineata* and *X. stemmator,* and the effect of adult diet and temperature on adult longevity of their adults. This knowledge can be utilized to improve the mass-rearing techniques of these parasitic species and employ these parasitoids as a candidate for biological control programmes to manage lepidopteran borer pests like *Scirpophaga incertulas* Walker and *S. inferens* in rice and other economically important agricultural pests.

MATERIALS AND METHODS

The experiment was conducted at the Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India and the following methods were followed. One of the host insects of *Xanthopimpla* is the rice pink stem borer, *Sesamia inferens* Walker. The larvae and pupae of *S. inferens* and adults of *X. flavolineata* and *X. stemmator* were collected from rice fields of Annamalai University experimental farm premises of Cuddalore District, Tamil Nadu. The host insect was reared as described by Lingappa (1978), *X. flavolineata* and *X. stemmator* parasitoids were reared using the obtained pupae of *S. inferens* under laboratory conditions at 27±2ºC and 65±5% RH. Newly emerged adults were fed with a small drop of honey and kept individually in vials (Diameter: 30 mm, Height: 15 cm) closed with nylon cloth at the top. All *X. flavolineata* and *X. stemmator* parasitoids were used just once in the experiments (Avila *et al.,* 2017; Chen *et al.,* 2023).

The host pupae were exposed to *X. flavolineata* and *X. stemmator* by individual exposure method and kept in plastic rearing containers (14 x 12 cm) in a BOD incubator at 20 and 28ºC. The parasitized pupae of *S. inferens* were dissected out in series one by one, two times a day (0900 h and 1800 h) till the 15th day to know the developmental period of egg, larval instars and pupa under Carl Zeiss stemi DV4 stereomicroscope. The life stages were photographed using a Canon 12 MP, 6x optical zoom camera and measured using the micrometry technique. The total developmental period from egg to adult for the parasitoid was observed and recorded. The host pupa was exposed to oviposition by respective parasitoids. Once oviposited the host pupa was removed and dissected out to locate the parasitoid egg (Pourian *et al.,* 2015). The shape, size, colour and incubation period were observed. The number of larval instars of the parasitoid was fixed based on the measurement of the mandible as described by Swamiappan (1984). The period of each instar was also noted. The prepupal and pupal stages of the parasitoid were also dissected from the parasitized *S. inferens* pupae. The *Xanthopimpla* parasitized pupa of *S. inferens* was kept individually in the glass tubes (7.5 x 2.5 cm) and observed daily for the emergence of *Xanthopimpla* (Li *et al.,* 2019; Wang *et al.,* 2022). The emergence behaviour of the parasitoids was observed and photographed.

The effect of different adult diets, *viz.* 50 per cent sucrose and honey on the male and female parasitoid's longevity was studied in the laboratory by keeping them individually in cages (15 x 15 x 15 cm) and compared with control (Nawaz *et al.,* 2021). The sucrose and honey solutions were prepared by diluting them with distilled water. Fifteen female and male adults each were used in this study. Wherever a single observation was made using a single parasitized pupa, the standard error of the mean (SE ±) was calculated (Panse and Sukhatme, 1961).

RESULTS AND DISCUSSION

Biology and developmental period of *X. flavolineata* and *X. stemmator*

The data on the biology and developmental period of *X. flavolineata* and *X. stemmator* are furnished in Tables 1, 2 and 3, and their life stages are shown in Figures 1 and 2.

Egg

White, elongate-oval, translucent and shiny eggs were laid, and their chorion was smooth and unsculptured. It became pale yellow and slightly increased in size before hatching. *X. flavolineata* and *X. stemmator* egg were measured at 1.20 ± 0.04 mm and 1.40 ± 0.05 mm in length, and 0.24 ± 0.01 mm and 0.26 ± 0.02 mm in width (Figure 1A and 2A). The egg period was observed to be 4.87 ± 0.09 and 5.00 ± 0.07 days at 20ºC and 2.87 ± 0.07 days and 3.03 ± 0.08 at 28ºC, respectively. The morphology of the egg stage of both the species of *Xanthopimpla* reported here is in accordance with Moutia and Courtois (1952). The colour changes from white to pale yellow before hatching, and a marginal increase in size (Table 1 and 2, Figure 1A) might be due to osmosis or by the absorption of haemolymph and ions from the host as opined by Machekano *et al.* (2018), Cusumano *et al.* (2021). An increase in the size of the egg after oviposition was also observed in other endoparasitic species *viz., Itoplectis naranyae* (Ueno and Ueno, 2007), *Therophilus javanus* (Aboubakar *et al.,* 2021), *Apocrypta* sp. (Chou *et al.,* 2023).

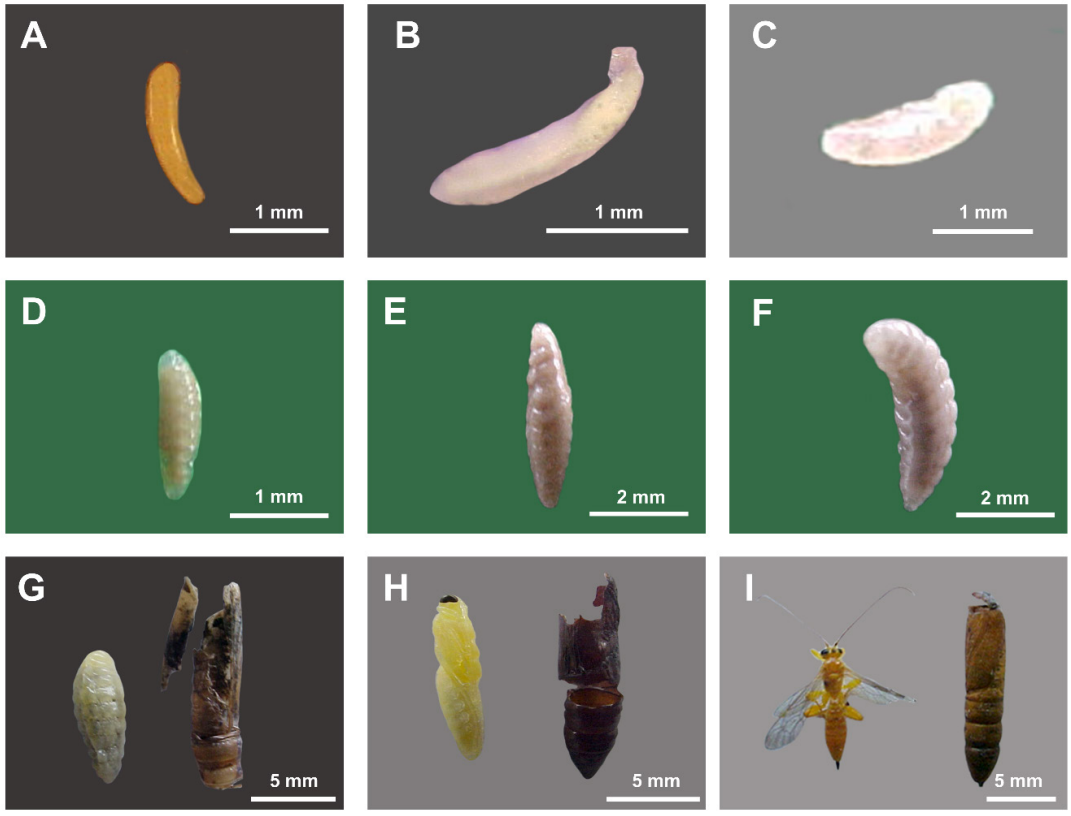

Figure 1. Biology of *X. flavolineata on S. inferens* pupa. A) egg, B) neonate larva, C) 1st instar, D) 2nd instar, E) 3rd instar, F) 4th instar, G) prepupa, H) pupa, I) *X. flavolineata adult* emerged from host pupa.

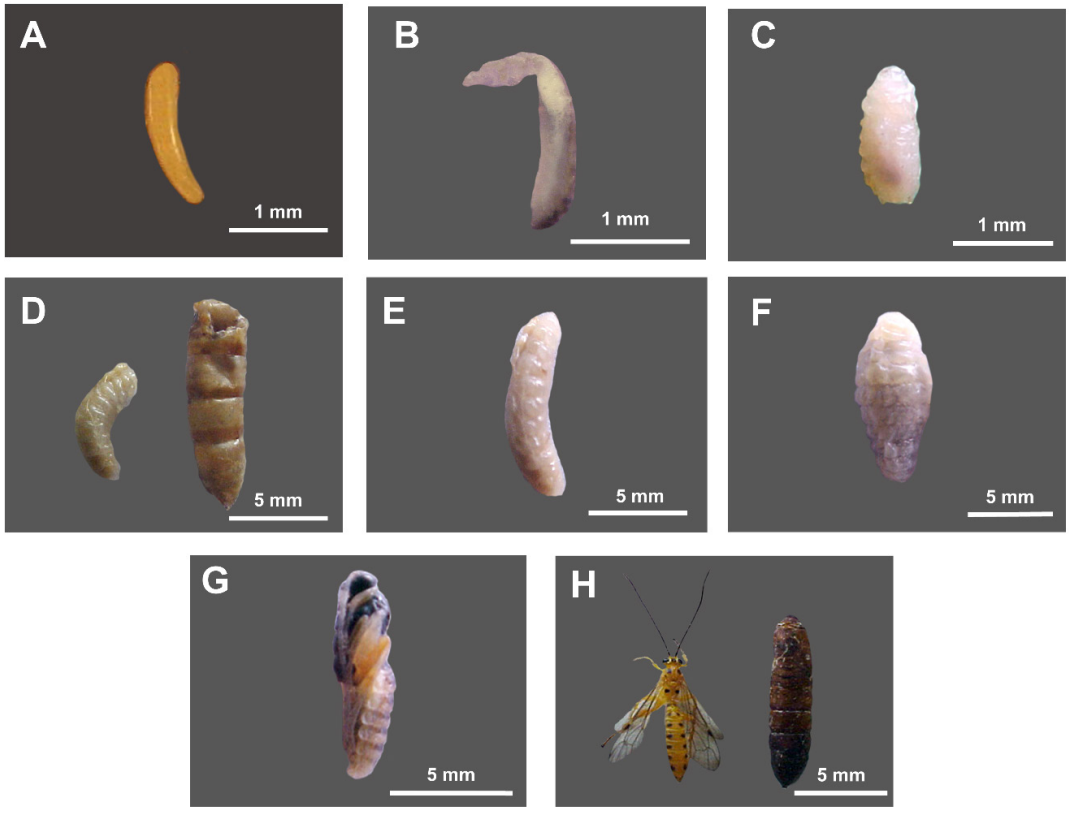

Figure 2. Biology of *X. stemmator* on *S. inferens* pupa. A) egg, B) neonate larva, C) 2nd instar, D) 3rd instar, E) 4th instar, F) prepupa, G) pupa, H) *X. stemmator* adult emerged from host pupa.

Table 1. Morphometry of *X. flavolineata* and *X. stemmator* life stages

Sl. No.	Stage	Measurement (mm)*			
		X. flavolineata		*X. stemmator*	
		Length	Width	Length	Width
1.	Egg	1.20 ± 0.04	0.24 ± 0.01	1.40 ± 0.05	0.26 ± 0.02
2.	1st larval instar	2.77 ± 0.31	0.57 ± 0.02	3.03 ± 0.13	0.69 ± 0.09
3.	2nd larval instar	5.00 ± 0.17	1.01 ± 0.04	5.82 ± 0.16	1.25 ± 0.04
4.	3rd larval instar	6.38 ± 0.12	2.35 ± 0.03	8.48 ± 0.16	2.47 ± 0.03
5.	4th larval instar	12.30 ± 0.25	3.02 ± 0.06	13.80 ± 0.28	3.65 ± 0.09
6.	Prepupa	13.34 ± 0.10	2.92 ± 0.09	14.19 ± 0.16	3.24 ± 0.12
7.	Pupa	13.80 ± 0.12	3.00 ± 0.08	14.25 ± 0.22	3.41 ± 0.11
8.	Adult	13.92 ± 0.14	3.02 ± 0.07	14.37 ± 0.15	3.45 ± 0.09

* Mean of 10 observations, Mean values followed by Standard Error (SE), Single parasitized pupa used per observation

Table 2. Developmental period of *X. flavolineata* and *X. stemmator* on *S. inferens* pupae at 20 and 28ºC

Sl. No.	Stage	Developmental Period (in days) at*			
		20ºC		28ºC	
		X. flavolineata	*X. stemmator*	*X. flavolineata*	*X. stemmator*
1.	Egg	04.87 ± 0.09	05.00 ± 0.07	02.87 ± 0.07	03.03 ± 0.08
2.	Larva	16.93 ± 0.13	18.05 ± 0.14	08.77 ± 0.15	08.95 ± 0.11
3.	Pupa	12.07 ± 0.11	13.03 ± 0.11	06.90 ± 0.09	06.97 ± 0.09
4.	Total life cycle	33.87 ± 0.18	36.08 ± 0.16	18.54 ± 0.15	18.95 ± 0.19

* Mean of 10 observations, Mean values followed by Standard Error (SE), Single parasitized pupa used per observation

Larva

First instar

White, clear segmentation was visible at the time of hatching (Figure 1B and 2B). The head capsule was white, transparent, unsclerotised, and the mandibles (0.15 mm) were also unsclerotised. The cuticle of the 1st instar larva hardened during the first day and became amber-coloured.

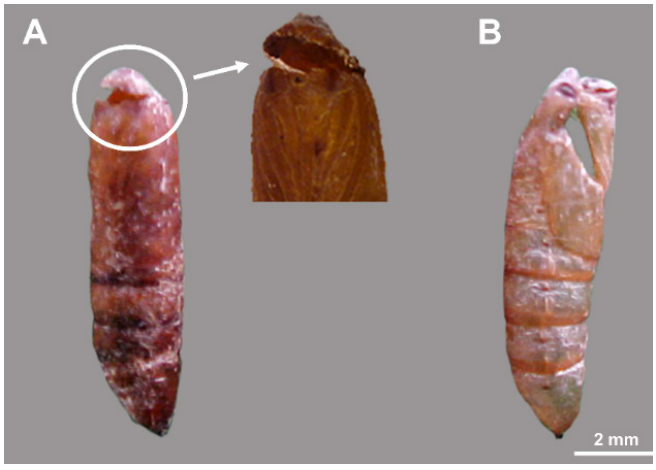

Figure 3. Comparison of *Xanthopimpla* and *S. inferens* pupal case. A) *Xanthopimpla* emerged pupal case (insert: closeup view of circular opening), B) *S. inferens* pupal case.

The antennae were vestigial, and the cuticle of the body was shiny and had numerous conical setae all over each segment. Spiracles were absent, and the larva of *X. flavolineata* and *X. stemmator* measured 2.77 ± 0.31 mm and 3.03 ± 0.13 mm in length, and 0.57 ± 0.02 mm and 0.69 ± 0.09 mm in width, respectively (Figure 1C).

Second instar

It was white and transparent. The head capsule was not sclerotised, and the mouth parts had slightly sclerotised mandibles (0.19 mm), which were simple, smooth and brown at the tip. The cuticle was very shiny and had very short spines, which are more so at the posterior end of the body. Spiracles were absent, and the larva of *X. flavolineata* and *X. stemmator* measured 5.00 ± 0.17 mm, 5.82 ± 0.16 mm and 1.01 ± 0.04 mm, 1.25 ± 0.04 mm in length and width, respectively (Figure 1D and 2C).

Third instar

The third instar larva was cylindrical, creamy white and opaque. the head remained unsclerotized, but the mandibles (0.20 mm) were more sclerotised than in the second instar and developed spiracles, which differed from the previous stage. The length and width of *X. flavolineata* and *X. stemmator*

larva were 6.38 ± 0.12 mm, 8.48 ± 0.16 mm, and 2.35 ± 0.03 mm, 2.47 ± 0.03, respectively (Figure 1E and 2D).

Fourth instar

The larva was cigar-shaped and buff to light brown in colour. It had a hemispherical head, which was slightly inclined ventrally and unsclerotised. The mouth parts were sclerotised, and the mandible was pointed and smooth with dark brown tips (0.25 mm). The humps were more pronounced in segments 6 to 11. The cuticle was shiny, translucent and had small conical papillae, which were distributed on all body segments and were stout on lateral lobes.

The length of the *X. flavolineata* and *X. stemmator* larvae measured 12.30 ± 0.25 mm, 13.80 ± 0.28 mm, and the width was 3.02 ± 0.06 mm, 3.65 ± 0.09 mm, respectively (Figure 1I and 2H). The total larval period of *X. flavolineata* and *X. stemmator* were 16.93 ± 0.13 and 18.05 ± 0.14 days at 20ºC and 8.77 ± 0.15 and 8.95 ± 0.11 days at 28ºC, respectively. The larval instars were slightly brownish (Figure 2B to F). The egg, larval instars of *X. stemmator* were similar to *X. flavolineata* whereas the size of the above-mentioned stages of *X. stemmator* was found to be a little bigger than the former (Table 1 and 2, Figure 2A to E). The results are in confirmation with the earlier research from Majumdar *et al.* (2021) and Gadad *et al.* (2023), who worked on *Xanthopimpla* spp. Gathalkar *et al.* (2017) reported the *Xanthopimpla* larva was white and turned to cream, then subsequently to yellow in the later instars. The head capsule was unsclerotized in early instars (I to III) but hardened in the last instar. The cuticle was shiny, translucent and soft at birth, and the final instar had conical papillae all over the body. The mandibles gradually became more sclerotized from early to late instars and were long, sickle-shaped without serrations and light brown in colour. The anterior end was pointed and smaller than the posterior end. Accumulation of fat globules was found towards the middle region (Table 1 and 2, Figure 1B to F).

Prepupa

The *X. flavolineata* prepupa was pale yellowish white (Figure 1G), while *X. stemmator* was pale whitish (Figure 2F). The length and width were 13.34 ± 0.10 mm, 14.19 ± 0.16 mm in length and 2.92 ± 0.09 mm, 3.24 ± 0.12 mm in width, respectively.

Pupa

The *X. flavolineata* pupa was pale yellowish-white and darkens with age to the colour of the adult, while the *X. stemmator* pupa was amber to orange coloured. The eyes were light reddish brown. A pair of antennae and three pairs of legs could easily be seen from the exarate pupa. The pupa measured 13.80 ± 0.12 mm, 14.25 ± 0.22 mm in length and 3.00 ± 0.08 mm, 3.41 ± 0.11 mm in width. The pupal period was 12.07 ± 0.11 days and 13.03 ± 0.11 days at 20ºC, and 6.90 ± 0.09 days and 6.97 ± 0.09 days at 28ºC, respectively (Figure 1H and 2G). As the adult developed inside, the colour became darker (Tables 1 and 2). Moutia and Courtois (1952) reported that the exarate pupa was of typical hymenopteran form and became completely yellowish orange just before adult emergence.

Adult

The active adults were flying to and fro and seldom remained motionless. They were attracted to light and were yellowish orange in colour. Both male and female adult insects were good fliers. The adults, both males and females were medium-sized and measured 13.92 ± 0.14 mm in length and 3.02 ± 0.07 mm in width (Figure 1I). The total life cycle of the *X. flavolineata* was completed in 33.87 ± 0.18 days at 20ºC and 18.54 ± 0.15 days at 28ºC. While *X. stemmator* was orange-coloured and had black spots on the dorsal side of the pronotum, propodeum and gaster segments (Figure 2H). The length and width of the adults measured 14.37 ± 0.15 and 3.45 ± 0.09 mm, respectively. It required 36.08 ± 0.16 days to complete its life cycle at 20ºC and 18.95 ± 0.19 days at 28ºC.

Pillai and Nair (1983) recorded similar observations for *X. flavolineata*, including its yellowish-orange body colour, adult length, ovipositor length, and wing expanse. Also, found that these parasitoids were attracted to light and copulate readily after a brief premating period, and they were active and seldom motionless (Table 1 and 2, Figure 1I). Similar reports were observed by Moutia and Courtois (1952) and Sokame *et al.* (2021).

The total life cycle observed here as 33.9 and 18.5 days at 20 and 28ºC in *X. flavolineata* is contrary to the earlier reports of Hailemichael and Smith (1994), who found that the developmental time was 42 days at 20ºC and 15 days at 28ºC. The difference in the development time might be due to host variation. Gitau *et al.* (2005) and Yi *et al.* (2020) also explained that the life cycle might be varied depending on the host insect. In addition, the longevity of females was reduced, if the host insects were readily available upon emergence. Picciau *et al.* (2019) and many others proved that female parasitoids can trade off longevity with fecundity because the eggs of the parasitoids could be resorbed for additional survival if the host is absent. They further reported that the developmental time of *X. stemmator* and their size were purely dependent on the size of the host pupa and temperature. At near room temperature (28ºC), both had the same developmental period, but at lower temperature (20ºC), *X. stemmator* took two more days than *X. flavolineata*. Though many researchers have reported that the developmental period is inversely proportional to temperature within a certain

Table 3. Effect of adult diet and temperature on adult longevity of *X. flavolineata* and *X. stemmator*

Sl.No.	Adult diet	Mean longevity (in days) at*							
		20ºC				28ºC			
		X. flavolineata		*X. stemmator*		*X. flavolineata*		*X. stemmator*	
		♂	♀	♂	♀	♂	♀	♂	♀
1.	honey (50%)	21.09 ± 0.14	34.53 ± 0.26	26.94 ± 0.22	38.90 ± 0.25	16.17 ± 0.09	26.55 ± 0.13	17.47 ± 0.16	27.08 ± 0.17
2.	Sucrose (50%)	20.49 ± 0.11	33.27 ± 0.23	24.82 ± 0.16	37.41 ± 0.13	14.88 ± 0.14	24.57 ± 0.22	13.93 ± 0.14	23.94 ± 0.22
3.	Water alone (control)	12.40 ± 0.10	14.90 ± 0.11	12.51 ± 0.11	15.88 ± 0.09	7.96 ± 0.09	10.55 ± 0.13	7.73 ± 0.16	10.57 ± 0.20

* Mean of 15 observations, Mean values followed by Standard Error (SE), Single parasitoid used per observation

range, the reasons for the extended developmental period could not be explained. Moutia and Courtois (1952) observed that the *Xanthopimpla citrina* female laid a total of 22 eggs during its life of 25 days. Maximum longevity was 45 days, but without food, it was eight to ten days only at 23.5ºC. The sex ratio of adults breed in the laboratory was three males to two females, in 75 individuals. The life cycle in summer at 24.5ºC was found to be 17 days for *X. stemmator* and 14 days for *X. citrina*. In winter, at a mean temperature of 18.5ºC, the difference was more apparent, the complete life cycle of *X. stemmator* was 31 days, and that of *X. citrina* was 25 days (Moutia and Courtois, 1952).

Effect of adult diet and temperature on adult longevity of *X. flavolineata* and *X. stemmator*

The effect of diet and temperature on the longevity of *X. flavolineata* and *X. stemmator* are shown in Table 3. The data showed that *X. flavolineata* males and females lived for 21.09 ± 0.14 and 34.53 ± 0.26 days when they were fed with 50 per cent honey followed by 50 per cent sucrose with 20.49 ± 0.11 and 33.27 ± 0.23 days, respectively at 20ºC. At the same temperature, *X. stemmator* male and female lived for 26.94 ± 0.22 and 38.90 ± 0.25 days, respectively in 50 per cent honey. The same trend was noticed at 28ºC also, whereas the longevity of male and female *X. flavolineata* was 16.17 ± 0.09 and 26.55 ± 0.13 days, respectively and in *X. stemmator,* it was 17.47 ± 0.16 and 27.08 ± 0.17 days in male and female respectively, when fed with 50 per cent honey.

Provision of 50 per cent honey increased the longevity of adult parasitoids as compared to 50 per cent sucrose and water (Table 3). This might be because honey not only served as a carbohydrate source but also provided other needed vitamins and minerals. carbohydrates (sucrose) helped in additional fecundity and longevity of adults in *Scambus buolianae* (Leius, 1963). Similar findings were reported by prabhu (2006), kanagarajan (2008), Picciau *et al.* (2019), who found that the provision of honey enhanced the life of adult Dryinidae, Chalcididae and Torymidae, respectively. The maximum longevity of 38.9 days reported here is much less compared to the findings of Moore and Kfir (1996), who reported the *X. stemmator* longevity as 140 and 87 days for females and males respectively. The possible reason for this reduced longevity might be the host ooze out feeding after oviposition and the local variation in the species used for the study. Moutia and Courtois (1952) reported that longevity was 30 days for females and 20 days for males. when the insects were kept in breeding jars and amply supplied with food at 23.5ºC, the maximum longevity was 45 days. The adult lived for eight to ten days without food.

Emergence behaviour of *X. flavolineata* and *X. stemmator*

In the present study, it was noted that *Xanthopimpla* egressed during early morning hours by making a circular opening at the anterior top of *S. inferens* pupae and slowly pushing the cap upward with its head and coming out (Figure 3A). Similar adult emergence was earlier reported by Kallekkattil *et al.* (2019) and Bredlau *et al.* (2020). *Xanthopimpla* egressed host pupal case was dark and hard because of the presence of deposition of excreta of the parasitoid (may be combined with some host tissue) almost to ¼ of pupal height at the posterior part of the inner region. In contrast, the normal host moth emerged pupal case was transparent and brittle because the entire pupal content was completely utilized for the development of the moth. The host moth emerged pupal case was transparent and with a 'T' shaped tearing through which the adult escaped (Figure 3B). Pupal parasitoids, *Brachymeria lasus* and *Tetrastichus* sp. were also recovered during host rearing, the former emerged by making an exit hole at the first segment of the host pupa while the latter emerged from different places of the host pupal case.

CONCLUSION

The developmental period of *X. flavolineata* and *X. stemmator* on *S. inferens* pupae varied at 20 and 28ºC, when the temperature is increased, the total life cycle is reduced

and vice versa. The study on the effect of adult diet and temperature on adult longevity of *X. flavolineata* and *X. stemmator* revealed that females lived longer than males when fed with honey (50%) solution as a food source followed by sucrose (50%) solution. Therefore, the obtained results of the study may be incorporated in devising a standard mass production protocols of these parasitoids.

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