

## Comparative biology of *Coccinella septempunctata* and *Cheilomenes sexmaculata* on mustard aphid, *Lipaphis erysimi* (Kalt.)

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### Abstract

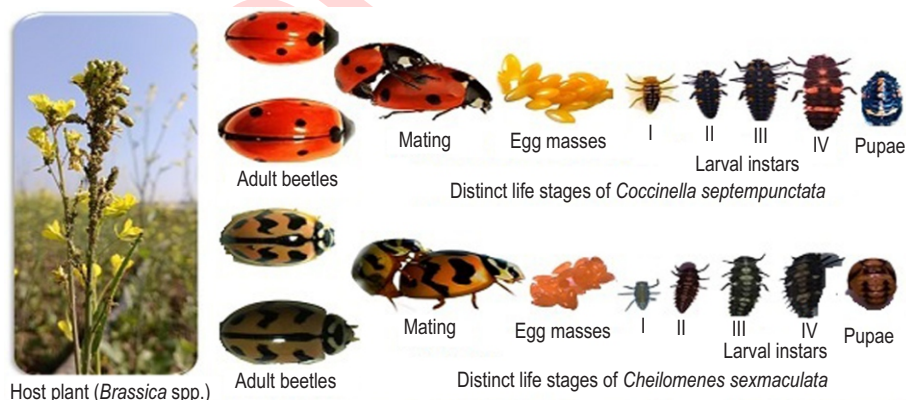
**Aim:** To compare the biology of *Coccinella septempunctata* (L.) and *Cheilomenes sexmaculata* (Fab.) on mustard aphid *Lipaphis erysimi* (Kalt.) for assessing their biocontrol potential.

**Methodology:** A laboratory study was conducted at the Department of Entomology, College of Agriculture, Banda University of Agriculture & Technology during 2023 to evaluate the biology of *C. septempunctata* and *C. sexmaculata* reared on mustard aphid, *Lipaphis erysimi*. Ten pairs of adults of each species were observed for developmental stages, reproductive traits, and longevity.

**Results:** *C. septempunctata* showed longer life cycle ( $22.10 \pm 0.84$  days), combined grub to pupal period averaged  $17.95 \pm 0.65$  days, total developmental period (egg to adult emergence) spanned  $22.10 \pm 0.84$  days and higher fecundity ( $324.50 \pm 23.43$  eggs) as compared to *C. sexmaculata*, which developed more rapidly, where total developmental period (egg to adult) averaged  $16.68 \pm 0.17$  days and the fecundity was averaged  $198.8 \pm 13.70$  eggs.

**Interpretation:** The study indicates that *C. septempunctata* had higher fecundity and longer development, making it suitable for sustained aphid control, while *C. sexmaculata* developed faster, favoring rapid biocontrol response. Both species showed longer female longevity and strong potential as biocontrol agents in eco-friendly farming systems.

**Key words:** Comparative biology, *Coccinella septempunctata*, *Cheilomenes sexmaculata*, Mustard aphid



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## Introduction

Coccinellids (Coleoptera: Coccinellidae), commonly known as ladybirds or ladybird beetles, have been identified as potential predators to be used as biocontrol agents of aphids and other insect pests because of their high predation efficiency, adaptability, and wide distribution (Hodek and Honek, 1996; Michaud, 2012; Rosagro et al., 2019). Around 6,000 species of coccinellids are known worldwide (Hodek et al., 2012), with 261 species from 57 genera documented in India (Omkar and Pervez, 2004). *Coccinella septempunctata* and *Cheilomenes sexmaculata* have been found to be particularly effective in suppressing aphid populations and identified as the most abundant species in the rapeseed and mustard ecosystem (Kumar et al., 2024). *C. septempunctata* is known to be abundant during the initial phase of mustard aphids (*Lipaphis erysimi*) infestation, with eggs and grubs being most abundant in the early stages and adults appearing during the downturn phase of aphid populations (Kumar, 2015). Similarly, *C. sexmaculata* is known for its voracious feeding behaviour, high searching efficiency and adaptability to both laboratory and field conditions making it a promising candidate for biological control (Venkatesan et al., 2006; Kumar, 2015). A comparative study showed that *C. sexmaculata* has distinct advantages over other coexisting species, such as *C. transversalis*, in terms of predation efficiency and easy handling (Omkar et al., 2005; Rakshith et al., 2018).

Early detection of resident coccinellids in mustard ecosystems plays a crucial role in suppressing aphid populations in the early stages. The results should provide insights into the developmental stages of these two coccinellid species and contribute to the development of population models, improved sampling techniques, and innovative pest control strategies (Papadopoulos et al., 2002) by reducing dependence on chemical insecticides, promoting the use of natural predators, and supporting sustainable agriculture and environmental protection. Many region-specific reports state that *C. sexmaculata* emerged earlier than *C. septempunctata*, but its population was later displaced by the dominance of *C. septempunctata*, which exhibits optimal development and fecundity at temperatures around 30°C, with a maximum fecundity of 250.67 eggs per female and high hatching rates under controlled conditions (Aman et al., 2023). Conversely, *C. sexmaculata* shows increased attraction to aphid-infested plants through semiochemical cues, which promotes recruitment and retention in the field (Jagdish et al., 2013).

Conventional control methods have proven to be inefficient and harmful to the environment. They lead to the development of resistance, contamination of soil and water and negative impacts on non-target species. These challenges highlight the urgent need for sustainable pest management strategies with an assurance of environmental sustainability, with biological control being considered the most appropriate approach (Jalali et al., 2009; Skouras et al., 2017). Despite comparative studies on the developmental stages and predation

efficiency of two most abundant coccinellids viz., *C. septempunctata* and *C. sexmaculata* on mustard aphid remain scarce. To address this gap, the utilization of these predators into sustainable pest management programme is a practical and ecologically sound strategy to regulate aphid population (Varshney et al., 2016; Gurung et al., 2025). Therefore, the present study aimed to systematically evaluate the biology, developmental stages and reproductive traits of *C. septempunctata* and *C. sexmaculata* to provide essential insights for optimising their mass-rearing and release strategies in mustard ecosystems.

## Materials and Methods

The study was conducted in 2023 under ambient laboratory in the Department of Entomology, College of Agriculture, Banda University of Agriculture and Technology, Banda, India to evaluate the biological parameters of two most common coccinellid predators, *C. septempunctata* (L.) and *C. sexmaculata* (Fab.). Nucleus cultures were collected from untreated mustard plots and maintained until adult emergence. Ten pairs of newly emerged adults of each species were reared in insect cages (20 × 10 cm) and fed ad libitum with fresh mustard leaves infested with *L. erysimi* (Kalt.), with filter paper provided for oviposition. A pronounced sexual dimorphism was observed and species-specific morphological characteristics were documented. The laid eggs were collected daily with a camel hairbrush and incubated separately. The duration of incubation, hatchability and developmental stages, including larval (grub) instars, prepupal and pupal periods and total development time were recorded. Pre-oviposition, oviposition and post-oviposition periods were recorded according to the standard methods (Varshney et al., 2016; Venkanna et al., 2022), as well as adult emergence, sex ratio, longevity, fecundity and life cycle duration. Observations were replicated ten times in a Completely Randomized Design (CRD) and data were analyzed using mean and standard deviation in Microsoft Excel.

## Results and Discussion

Comparative biological observations, reproductive parameters, and life cycle stages of *C. septempunctata* and *C. sexmaculata* are presented and discussed separately under the following headings:

**Biology of *C. septempunctata*:** The results on the biology of *C. septempunctata* on the mustard aphid (*L. erysimi*) showed promiscuous mating behaviour in which females showed their selectivity for males of specific sizes or colour, a trait that could improve genetic diversity and fitness of offspring (Majerus, 2003). Females laid bright yellow eggs in clusters near food sources to ensure immediate access to food for the emerging larvae, a strategy maximizes offspring survival in predatory coccinellids (Hodek and Honek, 1996). The mean pre-oviposition, oviposition, post-oviposition and incubation periods were  $6.80 \pm 1.40$ ,  $9.10 \pm 2.18$ ,  $7.50 \pm 1.43$  and  $4.15 \pm 0.21$  days with a range of 4–9, 5–10

and 5–10 and 2–5 days, respectively, while the mean fecundity was  $324.50 \pm 23.43$  eggs with a range of 280–356 eggs (Fig. 1). The freshly laid eggs were small, spindle shaped and bright yellow in colour, deposited in clusters near food sources, gradually turned into light grey in colour and 76.66–86.56% hatched at a temperature of  $25 \pm 2^\circ\text{C}$  and relative humidity of  $75.0 \pm 5.0$  per cent. Yadav *et al.* (2016) recorded comparatively longer periods before egg laying ( $7.92 \pm 2.45$  days), egg laying ( $21.60 \pm 3.39$  days), after egg laying ( $7.16 \pm 1.77$  days) and incubation ( $4.40 \pm 0.77$  days) and lower egg hatching rate ( $73.30 \pm 5.27\%$ ) at a temperature of  $23 \pm 2.47^\circ\text{C}$  and relative humidity of  $62.67 \pm 5.84\%$ . However, Varshney *et al.* (2016) recorded slightly shorter pre-oviposition and oviposition periods. Singh *et al.* (2009) and Singh and Singh (2014) also recorded a slightly longer incubation period; however, higher fecundity (586 and 657.7 eggs) was also recorded by Skouras *et al.* (2015) and Mishra and Kanwat (2017). This variability can be attributed to different environmental conditions and food availability. This agreement between the studies highlights the stability of the reproductive behavior for *C. septempunctata* under controlled conditions and suggests that the ecological acceptability and favourability in the present study.

The neonate grubs initially feed on egg contents and passed through four larval instars. The mean developmental duration of the first, second, third and fourth larval instars and the total grub period were  $3.23 \pm 0.24$ ,  $2.73 \pm 0.10$ ,  $2.65 \pm 0.17$ ,  $3.55 \pm 0.26$  and  $12.15 \pm 0.61$  days, respectively, with a range of 2–4 days for the first to third grub instars, 2–5 days for the grub instar and 9–15 days for the total grub period (Fig. 2). Rauf *et al.* (2013) recorded a slightly longer duration for the 1<sup>st</sup> ( $3.44 \pm 0.07$  days) and 2<sup>nd</sup> ( $2.78 \pm 0.10$  days) grub instars, while Kumar *et al.* (2019) recorded a longer duration for the 3<sup>rd</sup> grub instar ( $3.67 \pm 0.33$  days). On the other hand, Varshney *et al.* (2016) recorded shorter developmental duration for each grub instar and total grub period at two different temperature regimes. Singh *et al.* (2009) also recorded a shorter total grub period ( $10.95 \pm 0.35$  days), while Khursheed *et al.* (2006) observed a longer duration ( $13.5 \pm 0.87$  days). These slight differences in the results of previous and current studies maybe due to variations in host species, temperature and other microclimatic variables during the experiments. The fourth grub instar ceases feeding and becomes sluggish before pupation, attaching its abdominal segment to a suitable surface and becoming immobile. This marks the transition to the prepupal stage, which is characterized by a curved shape and lasts 1–2 days, with a mean of  $1.55 \pm 0.14$  days and pupation lasts  $5.80 \pm 0.12$  days with a range of 4–8 days. Khursheed *et al.* (2006) also reported a similar prepupal period ( $1.5 \pm 0.29$  days) as in the present study. Sahito *et al.* (2019) recorded a longer pupal period (6.40 days), while Gurung *et al.* (2025) documented a comparatively shorter pupal period ( $5.2 \pm 1.03$  days) than in the present study on *L. erysimi*. Pupation involves the splitting of the larval skin longitudinally along the mid-dorsal line, which extends from the lateral arms of the epicranial suture to the anterior edge of the sixth abdominal tergite and marks a critical developmental transition from the grub to the pupal stage. The mean duration of the grub and pupal period was

$17.95 \pm 0.65$  days and ranged from 13–22 days, while the mean total developmental time from egg to adult emergence was  $22.10 \pm 0.84$  days and ranged from 16–27 days (Fig. 2). Saini *et al.* (2024) recorded similar observations for grub and pupal period ( $18.90 \pm 1.16$  ranging from 17–21 days) and the time from egg to adult emergence ( $23.45 \pm 1.20$  ranging from 21.25 - 25.75 days) on *L. erysimi* at a  $25^\circ\text{C}$  and 65% relative humidity.

The newly emerged adults of *C. septempunctata* showed typical morphological features, including an oval and convex body with red elytra with seven black spots and a black pronotum with white lateral margins (Fig. 3). The sex ratio of male to female was 1:1.11, indicating a female-biased population. The result is in partial agreement with the findings of Yadav *et al.* (2016), who also reported a female-biased population with a sex ratio of 1:1.39 on *L. erysimi*. The adults mated 2–3 days after emergence and the females exhibited longer longevity ( $34.25 \pm 1.04$  days and a range of 29–38 days) than the males ( $26.58 \pm 1.46$  days and a range of 23–33 days). The result on the longevity of females is in conformity with the finding of Kumar *et al.* (2019) who observed that females lived longer ( $36.67 \pm 0.33$  days) than males, and a similar result was also observed by Paliwal and Singh (2024) on *L. erysimi*. On the other hand, Rauf *et al.* (2013) recorded a comparatively longer longevity of male on *Schizaphis graminum* and the differences in adult life expectancy may be attributed to differences in prey quality, environmental conditions, experimental methods and a higher proportion of females, and longer longevity is a common feature in coccinellids reflecting reproductive roles and energy allocation strategies with increased population growth potential (Aman *et al.*, 2023).

### Biology of *C. sexmaculata*

A quiescent mating behaviour of *C. sexmaculata* involves males detecting females using pheromones and initiating courtship using tactile and visual cues, coupled for several minutes to hours and facilitate sperm transfer for egg fertilization (Omkar *et al.*, 2010). Females start egg laying 2–3 days after mating in clusters of 5–26 near aphid colonies, on twigs and on the edge of petri dishes to ensure immediate availability of food for hatching larvae. The mean pre-oviposition, oviposition, post-oviposition and incubation periods were  $5.1 \pm 0.74$ ,  $12.9 \pm 1.20$ ,  $7.9 \pm 1.73$  and  $3.68 \pm 0.17$  days with a range of 4 to 6, 11–15 and 6–11 and 2–6 days, respectively (Fig. 1), while the mean fecundity was  $198.8 \pm 13.70$  and ranged from 186 to 220 eggs. The results are in partial agreement with those of Abbas *et al.* (2020) who documented  $5.67 \pm 1.45$ ,  $10.33 \pm 6.90$ ,  $9.70 \pm 4.33$  and  $3.60 \pm 0.12$  days for pre-egg laying, egg laying, post-egg laying and incubation period, respectively, with a mean fecundity of  $199.10 \pm 17.10$  eggs. On the other hand, Bhadauria *et al.* (2001) and Tank and Korat (2007) documented higher fecundity of 277 and 195–839 eggs, respectively. The freshly laid eggs were bright yellowish, cigar-shaped, smooth, turned into black colour and 82.21% hatched. Eggs from the same batch usually hatched within an hour, although differential hatching occasionally led to cannibalism, which consistent with other results, where egg

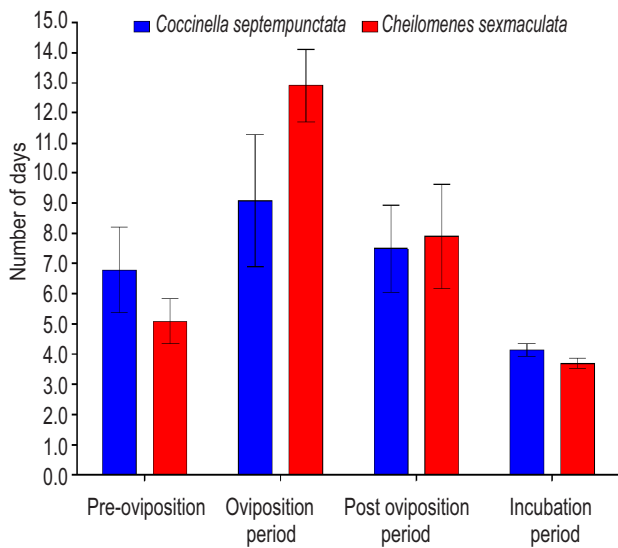


Fig. 1: Comparative reproductive parameters of *Coccinella septempunctata* (L.) and *Cheilomenes sexmaculata* (Fab.).

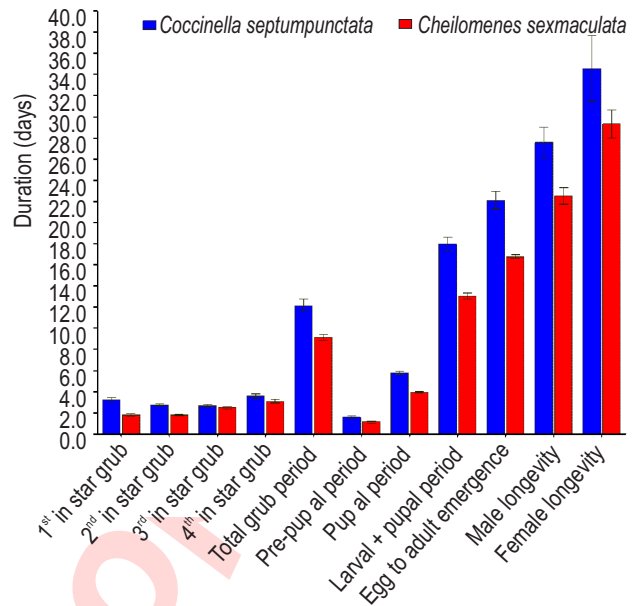


Fig. 2: Comparative biological observations of *Coccinella septempunctata* (L.) and *Cheilomenes sexmaculata* (Fab.).

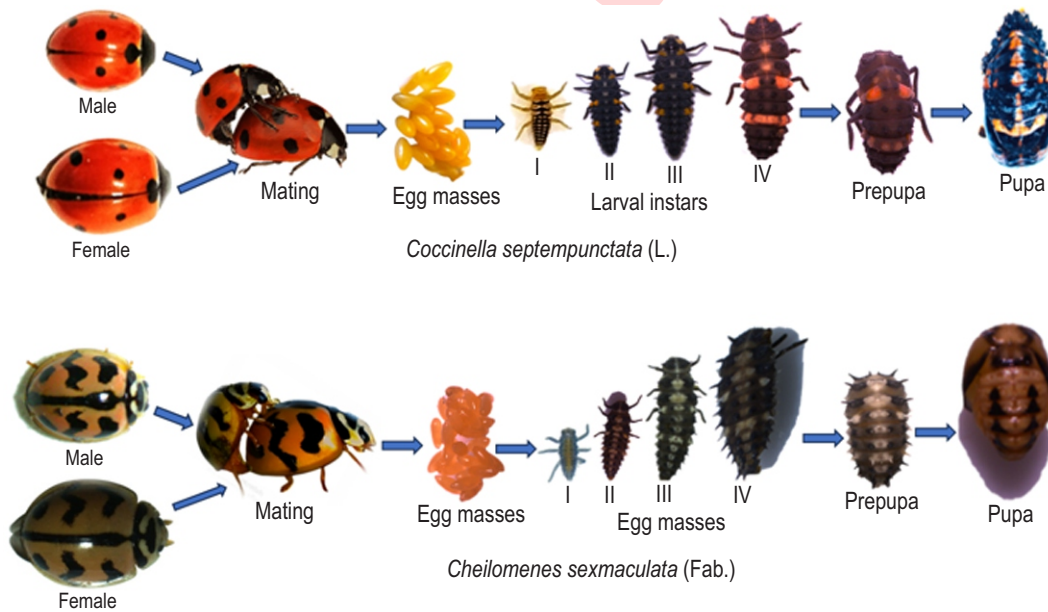


Fig. 3: Distinct life stages of *Coccinella septempunctata* (L.) and *Cheilomenes sexmaculata* (Fab.).

cannibalism greatly enhanced the survival of adult females and allowed them to expand their search for aphid food (Agarwala, 1991). The differences in the same findings of many researchers may be due to differences in prey species, host plants, and experimental conditions.

The neonate grubs had a campodeiform and porcupine-like appearance and underwent four successive instars that differed morphologically and stage-wise development. The mean

developmental duration of the first, second, third and fourth grub instars and the total grub period were  $1.70 \pm 0.14$ ,  $1.80 \pm 0.08$ ,  $2.48 \pm 0.10$ ,  $3.10 \pm 0.16$  and  $9.08 \pm 0.30$  days, respectively, with a range of 1–3 days for first and second instars, 2–4 days for the third and fourth instars and 6–13 days for the total grub period (Fig. 2). The result of the first grub instar was longer than that of Abbas *et al.* (2020), who recorded  $1.45 \pm 0.18$  days on *L. erysimi*, and aligned with that of Rai *et al.* (2003), who recorded  $1.60 \pm 0.15$  days on *Aphis craccivora*, while it was lower than that of Tank

and Korat (2007), who documented  $1.80 \pm 0.50$  days on *A. gossypii*. The result of the second instar was comparatively longer than that of Pandi et al. (2012), who recorded  $1.60 \pm 0.10$  days. The third grub instar was characterized by two white mottled areas on the dorsum of the thoracic segments and the duration of the present study is partially in agreement with Routray et al. (2016), who recorded a duration of  $2.25 \pm 0.86$  days. The result of the developmental duration of fourth instar was shorter than Singh et al. (2008) and longer than the records of Abbas et al. (2020), who documented  $3.85 \pm 0.08$  days and  $2.47 \pm 0.12$  days, respectively. The result of total grub period was comparatively shorter than those of Singh (1994) and Bhadauria et al. (2001), who documented that *C. sexmaculata* developed in 10.83 and 11.80 days, when reared on *L. erysimi*. The differences in the results of many researchers could be due to the different quality of hosts and the timing of the experiment, as climatic conditions play an important role in the larval development, which may vary from month to month (2.5–2.9 days) (Mishra et al., 2022).

The pre-pupal, pupal, grub and pupal developmental time and total developmental period from egg to adult emergence were  $1.13 \pm 0.10$ ,  $3.93 \pm 0.10$ ,  $13.00 \pm 0.28$  and  $16.68 \pm 0.17$  days, respectively, with a range of 1–2, 3–5, 10–15 and 11–21 days. The result of pupation period is partial agreement with the records of Reddy et al. (2001) and Rai et al. (2003) who observed 3.60 days and  $2.68 \pm 0.14$  day on the variable host, respectively. However, the result of the duration from egg to adult emergence agrees with Singh et al. (2008) who documented that *C. sexmaculata* takes  $18.24 \pm 0.49$  days to develop from egg to adult emergence.

The newly emerged adults were soft-bodied, translucent, and yellowish in colour and later developed into beetles with yellowish-orange-red elytra, two zigzag black lines, a posterior black spot, and a pronotum with a T-shaped black band (Fig. 3). The sex ratio of males to females was 1:1.22, indicating a female-biased population and the results are in partial agreement with the earlier records 1:1.30 to 1:1.45 (Tank and Korat, 2007 and Shanmugapriya et al., 2017). The adults mated 2–3 days after emergence and the females exhibited longer longevity ( $29.33 \pm 1.33$  days and a range of 23–37 days) than the males ( $22.50 \pm 0.78$  days and a range of 18–26 days). The result on longevity of male and females was comparatively longer than those reported by Shanmugapriya et al. (2017) who observed that females lived longer ( $22.04 \pm 2.42$  days) than males ( $16.44 \pm 1.19$  days) when reared on *L. erysimi*. Based on the results obtained, the study concluded that both *C. septempunctata* and *C. sexmaculata* exhibited efficient reproductive and developmental traits when reared on *L. erysimi*. *C. septempunctata* showed higher fecundity and longer developmental periods, while *C. sexmaculata* had a shorter life cycle with adaptive behaviors like cannibalism. Female-biased sex ratios in both species favor population growth. These traits confirm their potential as effective biological control agents against aphids.

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**Authors' contribution:** M.K. Mishra and R. Pandey: Conceived, designed research and edited the manuscript. S.K. Mishra and B.S. Tiwari: Conducted experiments and wrote the manuscript. M.K. Mishra: Analyzed data and all authors read, revised, and approved of the manuscript.

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**Data availability:** All data generated or analyzed during this study are included in this article.

**Consent to publish:** All authors agree to publish the paper in *Journal of Environmental Biology*.

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