



# Assessment of Biology and Fertility Life Table of *Coccinella septempunctata* Linnaeus Predating on Mustard Aphid, *Lipaphis erysimi* Kalténbach

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

*Lipaphis erysimi* (Kalt.), commonly known as the mustard aphid (Homoptera: Aphididae), is the most notorious, globally distributed, and obligatory ectoparasite of many field crop including rapeseed and mustard. An investigation was carried out to assess the biology and fertility life table of *Coccinella septempunctata* in Bio-control lab, Department of Entomology at CCS Haryana Agricultural University, Hisar following the standard protocol at the laboratory condition of temperature (T)  $25\pm 2^{\circ}\text{C}$ , relative humidity (RH)  $65\pm 5\%$  RH, and 16:8 light:dark (L:D). Biological studies revealed that *Coccinella septempunctata* (L.), predatory female laying eggs either singly or in masses (546.90 eggs/female) were vibrant yellow and spindle shape with an incubation period of 3.50 days. Hatchability was  $86.00\pm 5.62$  per cent. There were four grub instars followed by pre-pupal stage, mean grub duration of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar was  $1.68\pm 0.47$ ,  $3.18\pm 0.60$ ,  $3.08\pm 0.65$  and  $5.02\pm 0.73$  days. Total grub duration extended for  $12.97\pm 0.61$  days. Larval survival was 74.44 per cent, followed by a pre-pupal period of  $1.05\pm 0.18$  days and pupal duration of  $5.93\pm 0.86$  days. Total grubs and pupal period were computed to be  $18.90 \pm 1.16$  days with adult emergence 77.22 per cent. Total developmental period, from egg to adult was  $23.45\pm 1.20$  days. Adult became darker in colour and have seven spots, three on each wing and one in the middle, near the head and act as predator to control mustard aphid. Females of beetle survived longer than the males with ovipositional period of 23.10 days and preponderance of female has been noticed with female: male ratio of 1.62: 1 in the study.

**Keywords:** Biology; *Coccinella septempunctata*; *Lipaphis erysimi*; rapeseed-mustard.

## 1. INTRODUCTION

Mustard crop genus, *Brassica* belongs to family *Brassicaceae* are grown in 53 countries across the globe and are cultivated in India during the Rabi season in a variety of agro-climatic conditions ranging from the North-Eastern/North-Western highlands to the low lands, under irrigated/rainfed, timely/late sown and mixed cropping. Total area of rapeseed-mustard in India is about 6.7 million ha with a total production of 10.21 million tonnes (Ahlawat, 2008, Anonymous, 2021). Rajasthan is the largest producer of mustard with an area of 3.07 million ha and production 4.20 million tones, which accounts for 49 per cent of the total mustard produced in India (Darekar and Reddy 2018).

Significant biotic factor that poses a serious constraint to mustard crop from germination to harvest, albeit of among other production related restrictions, are insect-pests and over 50 insect species have been known to infest rapeseed-mustard in India. Mustard aphids, *Lipaphis erysimi*; cabbage aphid, *Brevicoryne brassicae* and green peach aphid, *Myzus persicae* are important and assumed to be of regional and sporadic importance (Sharma and Singh, 2010). Mustard aphid, *Lipaphis erysimi* (Homoptera: Aphididae) often known as turnip aphid, is of great importance and one of the most serious destructive global pests having a detrimental

effect on rapeseed-mustard (*Brassica spp*) crops. *L. erysimi* (Kalt.) is found worldwide (Pradhan and Moorthy, 1995) and is a key cruciferous pest (Atwal et al., 1969) of cauliflower, turnip, kohlrabi, radish, Chinese cabbage, brussels sprout, broccoli, kale and a minor pest of bean, beet spinach, pea, celery, onion, stock, cucumber and potato. It feeds by sucking sap from its host and causes crop damage ranging from 9 to 96 per cent in various agro-climatic conditions in India (Singh and Sachan, 1995; Parmar et al., 2007). Huge aphid populations may cause the plant to become distorted due to leaf curling and shrivelling (Dhillon et al., 2018). *Lipaphis erysimi* prefers to feed on mustard blossoms as well as on leaves (Singh et al., 1965). In severe infestations, both sides of the leaves are attacked (Yadav et al., 1988). It reduces oil content up to 66.9 per cent and causes significant seed yield losses of up to 73.3 % (Sekhon, 1989).

Mustard aphid, *L. erysimi* have been successfully managed by bio-control agents like coccinellids and chrysopids (Shukla et al., 1990; Singh and Singh, 2013). The aphidophagous lady bird beetle, *Coccinella septempunctata* (Linn.) is the most substantial group of entomophagous predators feeding on soft bodied insect-pests particularly mustard aphids among other predators and parasitoids. *C. septempunctata* could be a potential predator in the formulating eco-friendly strategy using the

bio-agents for the management of mustard aphids in the present context and found in a variety of environments, such as cities, mountains, sea coasts, gardens, and fields (Ali and Rizvi, 2009).

Keeping this in the mind, comprehensive study of biology of predaceous coccinellids' supposed to be essential in Trans-Gangetic Plain Region for their sustained usage in the integrated pest management programme, experiment was planned get compact outcome.

## 2. MATERIALS AND METHODS

Biology parameters of *Coccinella septempunctata* were studied as per standard protocol in Bio-control lab, Department of Entomology at CCS Haryana Agricultural University, Hisar. Geographically, Hisar is located at 29°08'49"N latitude; 75°42'28"E longitude; 215.2 m above mean sea level (MSL) altitude having a semi-arid and subtropical climate.

Mustard aphid, *Lipaphiserysimi* fed to the field collected ladybeetles in glass battery jars (size 15x10 cm) covered with muslin cloth maintained as stock culture "A". Jars lined with corrugated pieces of blotter paper at the bottom to enhance surface area of activity and provide a good medium for gravid female beetle. Wet camel hair brush used to remove the eggs that the female laid on the surface of the blotter paper, eggs laid on the surface of leaves or twigs moved as a whole in battery jar. Eggs grown upto adult, males and females, emerged were kept in separate glass battery jars and provided mustard aphid for feeding and maintained as Stock culture "B" to eliminate any experimental errors brought on by heterogeneity in the population collected from the field. Stock culture "B" was employed for further distinct investigation that required eggs, larvae, pupae, or adults.

Stock culture "B" further used to collect eggs laid by females in petridishes with a diameter of 5cm that held the eggs bottomslined with circular blotting papers. In a BOD incubator, the petri dishes having the eggs were kept at a constant temperature of 25°C, 65% RH, and 16:8 L:D. Hourly observations on incubation duration and percentage of hatching were made. With the help of camel hair brush, each neonate grub emerged from the egg collected to put in petri dishes covered with circular blotting paper to avoid larval cannibalism (Michaud, 2000;

Michaud, 2005). Information on the length of the various larval instars, the pre-pupal phase, percentage of pupation, and mortality, sixty larvae (sample size) were recorded daily. Growing grubs were fed with fresh field mustard aphid every morning at about 9 o'clock. Exuvia examined at hourly interval in order to monitor the grubs' moulting. Pre-pupal stage *i.e.*, fourth instar larvae stopped feeding and attached their abdomen to blotting paper or rapeseed mustard twigs or leaves until the pupae turned a light-yellow colour. All larval instar durations were continually tracked from the first instar to pupation. Sixty pupae were observed to record information on the pupal time period, male and female emergence (%), and sex ratio (M:F).

Stock Culture "B" served the *C. septempunctata* pupae. A day after emergence, adults male coupled with sexually mature adults of the opposite sex and housed in separate glass petri dishes (9 cm diameter and 2.5 cm height) in ten replication with aphid, *Lipaphis erysimi* as feed, on host plant twigs until maturation under laboratory conditions and observed every day at 10:00 AM. Using the same pairs of adults, observations on pre-oviposition, oviposition, post-oviposition periods, fecundity, adult longevity and total life span were also made. If they were the same age, adult females were larger than adult males, and females had straight abdomens while males had curled and hairy ones.

## 3. RESULTS AND DISCUSSION

Coccinellidae beetles are recognized as a beneficial and an effective biological control agent, they eat insects like aphids, mealybugs, citrus psyllids, mites and many others for a wide range of pests of field crops due to their ravenous appetites. Female of *C. septempunctata* laid her eggs in groups (Plate 1). Eggs placed in the glass jar surface laid on the twigs and leaves of the mustard plant. Colour of eggs was vibrant yellow, tiny rice grains shape and spindle shape. Eggs near to hatch changed from yellow to brownish or dark brownish in colour (Plate 2). Incubation period of *C. septempunctata* ranged from 3 to 4 days with an average of  $3.50 \pm 0.53$  days (Table 1). Aforesaid results reported by various authors, namely Sipioet al., 2017, Kumar et al., 2019, Mishra et al., 2017 and Manpoonget al., 2016 alike about incubation period of  $3.00 \pm 0.58$  days,  $3.33 \pm 0.33$  days, 3.00 days and  $3.5 \pm 0.5$  days respectively and contradicted by Singh et al., 2009, Singh and Singh, 2013,

Yadav et al., 2016 and Mari et al., 2016 in their studies. Average hatchability ranged from 76.7 to 96.7%, with an overall average of  $86.00 \pm 5.62$  per cent. Yadav et al., (2016), Sipioet al., (2017) and Mishra et al., (2017) supported the present findings that hatching percentage ranged between 66.03 to 100.00 per cent, an aggregate average of  $73.30 \pm 5.27$  per cent,  $85.21 \pm 5.77$  and 71.69 to 100 per cent, respectively in their studies. On the other hand, Mari et al., (2016) notified that hatching percentage as 66.03%. Environmental condition in the laboratory may have fluctuated results and aphid species fed to *C. septempunctata* may be different or gustatory appeal may be of different.

Grubs of *C. septempunctata* developed through four instars before entering the pre pupal stage that eventually emerged as a pupa. Newly hatched grubs were uniformly pale brown in colour (Plate 3), with no discernible markings on it. First instar grubs spend 1 to 2 days to moult to develop into second instar with an average of  $1.68 \pm 0.47$  days (Table 1). Second instar grubs larger in size compared to first instar (Plate 4). Second instar grubs were more active and took 2 to 4 days to become third instar with an average of  $3.18 \pm 0.60$  days. Third stage grubs were easily distinguished by presence of pair of yellow thoracic pigments (Plate 4). Third instar grubs took 2 to 4 days to become fourth instar with an average of  $3.08 \pm 0.65$  days. Fourth instar grubs possessed morphologically notable yellow coloured pair of pigmentation on the thoracic and abdominal segments as well as a noticeable yellow coloured head capsule (Plate 4). Fourth instar grubs spent 4 to 6 days to complete fourth instar period with an average of  $5.02 \pm 0.73$  days. Total grub period of *C. septempunctata* ranged from 12 to 14 days with an average of  $12.97 \pm 0.61$  days (Table 1). Yadav et al., (2016) also reported the larval duration of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> larval instar and total larval period of *C. septempunctata* was  $3.73 \pm 0.78$ ,  $2.80 \pm 0.76$ ,  $3.50 \pm 0.78$ ,  $3.63 \pm 0.85$  days and  $13.67 \pm 1.77$  days, respectively. Findings on larval duration by authors Kumar et al., 2019, Singh and Singh (2013), Mishra et al., (2017) also supported these finding of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar and total grub period of *C. septempunctata*. Report of Mari et al., (2016) about the duration of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar and total grub period of *C. septempunctata* was 5.23, 5.66, 5.91 and 8.50 days and  $26 \pm 3$  days respectively and contradicted the present study. Differences in present findings in comparison to findings of earlier workers may be owing to the differences

in micro and macro environment prevailing in the laboratory and host species i.e., aphid species fed to *C. septempunctata* may be different or gustatory appeal may be of different.

Grubs ceased to eat and became inactive just before the beginning of the pre-pupal period (Plate 5). Prior to entering its pupal stage, the larvae lost its vibrant colours, acquiring a dismal grey colour. Recently developed pupa start out pale yellow and evolved into a dark brown colour (Plate 5). Average pre-pupal period ranged from 0.8 to 1.25 days with an overall average of  $1.05 \pm 0.18$  days (Table 1). Ozderet al. (2003) reported pre-pupal period of 1.00, 1.29, 1.00 and 1.33, while feeding on *Rhopalosiphumpadi*, *Sitobionavenae*, *Metopolophiumdirhodumand* *Myzuscerasi*, respectively, gave support to outcome of existing findings.

Survival of grubs of lady bird beetle found to be average 74.44 per cent. In initially stages of development, mortality rate was higher in the first and second instars that decreased gradually over the subsequent instars. Mean pupal period ranged from 5 to 7 days with an average of  $5.93 \pm 0.86$  days (Table 1). According to Singh et al., (2009), also mean pupal period was  $5.35 \pm 0.15$  days in their studies, give support to present findings. Singh and Singh (2013) also supported present investigations as they reported pupal period of  $5.60 \pm 0.18$  days. Kumar et al., (2019) and Varshney et al., (2016) also communicated pupal period of *C. septempunctata* to be  $5.67 \pm 0.33$  days and  $5.8 \pm 0.91$  days, respectively. Total grubs and pupal period of *C. septempunctata* ranged from 17 to 21 days with an average of  $18.90 \pm 1.16$  days (Table 1). Minor differences in results of different workers and present investigations may be due to different aphid species as host, differences in temperature and other micro-climatic variables under laboratory conditions at time of experimentation. Total developmental period, from egg to adult of lady bird beetle ranged from 21.25 to 24.75 days with an average of  $23.45 \pm 1.20$  days.

Newly formed adults were spotless, brilliant yellow with black coloured head. Female transcribed noticeably larger with length of 7.34 mm and breadth 6.18 mm compared to male in general, males were smaller than their female counterparts with 6.75 mm length and 5.21 mm breadth. Four white spots present, two on the pronotum and two near the compound eyes (Plate 6 and 7). Over time, the adult became

darker in colour and popular name "seven-spotted ladybird beetle" comes from the fact that mature coccinellids have seven spots, three on each wing and one in the middle, near the head. Male possessed a curve rounded, hairy ventral region, while females were flat and grooved. Size and abdominal features helped to differentiate between the sexes. During the *Rabi* season of 2022-23, it was found that the seven-spotted ladybird beetle *C. septempunctata* (Coleoptera: Coccinellidae) exhibits polymorphism, with five distinct morphs

observed in rapeseed-mustard experimental field (Plate 8). Female longevity ranged from 35 to 43 days (average  $38.90 \pm 2.28$  days) while; male longevity ranged from 26 to 36 days (average  $30.10 \pm 3.98$  days). Males were short lived (Table 1) compared to females. Similar results were obtained by Kumar et al. (2019) who reported adult male and female longevity of 30.67 days and 36.67 days feeding on *L. erysimi*. Also, similar findings were reported by Yadav et al. (2016) about male and female adult longevity  $31.33 \pm 3.28$  and  $37.30 \pm 3.44$  days, respectively.

**Table 1. Biology of lady bird beetle, *Coccinellaseptempunctata* (Coleoptera: Coccinellidae) on mustard aphid, *Lipaphiserysimi***

Biological parameter/Stage	Number Observed/sample size	Mean ± SE	Range
<b>Incubation period (in days)</b>	300	$3.50 \pm 0.53$	3-4
Hatchability (%)	300	$86.00 \pm 5.62$	76.7-96.7
<b>Larval period</b>			
First instar period (days)	60	$1.68 \pm 0.47$	1-2
Second instar period (days)	60	$3.18 \pm 0.60$	2-4
Third instar period (days)	60	$3.08 \pm 0.65$	2-4
Fourth instar period (days)	60	$5.02 \pm 0.73$	4-6
Total grub period (days)	60	$12.97 \pm 0.61$	12-14
<b>Pre-pupal period (days)</b>			
Grub survival (%)	90	74.44%	---
<b>Pupal period</b>			
Pupal period (days)	60	$5.93 \pm 0.86$	5-7
<b>Total grubs + pupal period (days)</b>	60	$18.90 \pm 1.16$	17-21
<b>Total developmental period, egg to adult (days)</b>	60	$23.45 \pm 1.20$	21.25-25.75
<b>Adult period</b>			
Adult male longevity (days)	10(P)*	$30.10 \pm 3.98$	26-36
Adult female longevity (days)	10(P)*	$38.90 \pm 2.28$	35-43
<b>Pre-oviposition period(days)</b>	10(P)*	$8.40 \pm 1.96$	6-10
<b>Oviposition period(days)</b>	10(P)*	$23.10 \pm 2.69$	19-27
<b>Post-oviposition period (days)</b>	10(P)*	$7.30 \pm 1.95$	4-10
Sex ratio (Female: Male)	120**	1.62: 1	---
Adult emergence (%)	90	77.22%	---
Fecundity (Eggs/female)	10(P)*	$546.90 \pm 95.51$	384-672

\* (Ten pairs of male and female beetle)

\*\* (3 replication with 120 insects per replication)



**Plate 1. Newly laid eggs of *Coccinella septempunctata***



**Plate 2. Eggs near to hatching of *Coccinella septempunctata***



**Plate 3. First instar grub of *Coccinella septempunctata***



**Plate 5. Pre pupal stage of *Coccinella septempunctata***



**Plate 7. Ventral view of male and female adult beetles of *Coccinella septempunctata***



**Plate 4. Second, third, fourth instar grubs of *Coccinella septempunctata***



**Plate 6. Pupal stage of *Coccinella septempunctata***



**Plate 8. Polymorphs-elytral patterns in *Coccinella septempunctata* (1 to 5)**

Pre-oviposition period of *C. septempunctata* varied from 6 to 10 days with a mean of  $8.40 \pm 1.96$  days (Table 1). Oviposition period of lady beetle ranged from 19 to 27 days with an average of  $23.10 \pm 2.69$  days. With an average of  $7.30 \pm 1.95$  days, post oviposition period of *C. septempunctata* varied from 4 to 10 days. These results are in agreement with findings of Yadav et al., (2016), who reported that the pre-oviposition, oviposition and post-oviposition period of female were  $7.92 \pm 2.45$  days,  $21.60 \pm 3.39$  days and  $7.16 \pm 1.77$  days, respectively. Similar results were communicated by Kumar et al., (2019) that the pre-oviposition period of female found to be as  $6.00 \pm 1.16$  days. Further support to the present results was protracted by Mari et al. (2016) that the ovipositional period of female was 23.62 days. Sex ratio (Table 1) of *C. septempunctata*

was 1.62:1 female: male (F: M) in the present study. Similar results were obtained by Sattar et al. (2000) and Mishra et al., (2017), they reported sex ratio (M: F) of 1:1.5 and 1:1.6, respectively. Per cent adult emergence (Table 1) of *C. septempunctata* during the experimentation concluded as 77.22 per cent. Tomar et al. (2009) also reported 78.20 per cent emergence of *C. septempunctata*. Perusal of fecundity data presented in (Table 1) indicated that a single female has capacity to lay approximately 384-672 eggs (average  $546.90 \pm 95.51$  eggs) during its life span. Singh et al., (2009), Singh and Singh (2013), and Kumar et al. (2019), Yadav et al. (2016), Mari et al. (2016) and Mishra et al. (2017), all of them supported present findings by reporting, fecundity of *C. septempunctata* to be  $357.45 \pm 22.41$ ,  $378.00 \pm 26.51$ ,  $582.44 \pm 93.15$ , 601 eggs and 657.7 eggs, respectively.

#### 4. CONCLUSION

Biology of *C. septempunctata* studied on mustard aphid, *L. erysimi* in lab at ambient laboratory condition of 25±1°C, 65±5% RH, 16:8 L:D period. Eggs laid in masses or singly ranged from 546.90±95.51 eggs/female with incubation period of 3.50±0.53 days with hatchability 86.00±5.62 per cent. Four grub instars followed by pre-pupal stage, mean grub duration of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar recorded as 1.68±0.47, 3.18±0.60, 3.08±0.65 and 5.02±0.73 days. Duration of total grub period averaged 12.97±0.61 days. Survival of larva counted 74.44 per cent, larval period followed by a pre-pupal period of 1.05±0.18 days and pupal duration of 5.93±0.86 days. Total grubs and pupal period computed to be 18.90 ± 1.16 days with adult emergence 77.22 per cent. Total developmental period, from egg to adult, lasted upto 23.45±1.20 days. Females survived longer than the males i.e., 38.90 ± 2.28 days and 30.10±3.98 days, respectively. Pre-oviposition, oviposition and post-oviposition period of *C. septempunctata* established as 8.40±1.96, 23.10±2.69 and 7.30±1.95 days, respectively. Preponderance of female has been noticed with female: male ratio of 1.62:1.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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