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Biology of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) in lower temperate region of Tehri Garhwal Himalaya, India

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Abstract
Study on biology of *Z. bicolorata* carried out on *P. hysterophorus* plant at Department of Zoology. The *Z. bicolorata* reared on the *Parthenium* potted plant and insect rearing cages. The study show that the Mexican beetle oviposit small, oblong, elongated, light yellow or orange eggs mostly on upper and lower surface on leaf occasionally on flowers rarely on stems. The mean value of fecundity 581.25±61.16, incubation period in days 4.6±0.68, hatching percent (%) 69.55±4.33, larval period 1st, 2nd, 3rd and 4th instar respectively 4.25±0.72, 3.40±0.50, 3.95±0.83, 4.85±0.88, pupal period 1.4±0.50, Pupal period, Pre-oviposition period 9.25±0.72, Oviposition period 35.45±4.30, Post-oviposition period 16.90±2.36, Total life cycle days were recorded 32.50±5.39, adult longevity male 32.85±8.56, and female was 40.75±6.50 and total survival of days of adult beetle were 73.60±9.06.

Keywords: *Zygogramma bicolorata*, Biology, *P. hysterophorus*, Tehri Garhwal Himalayas

Introduction
Zygogramma bicolorata Pallister is the prominent and significant bio-control agent, feeds on leaves and flowers of the harmful, invading weed plant *P. hysterophorus* L. The beetle was first introduced to Australia from Mexico in 1980 [16] (McFadyen and McClay, 1981) [17] and subsequently to India in 1983 (Jayanth, 1987). Indian Institute of Horticultural Research (IIHR), Bangalore first introduced this particular beetle for the bio-control of *Parthenium* weed (Jayanth and Nagarkatti, 1987) [15] and *Zygogramma* beetle reduced the noteworthy density of *Parthenium* in neighboring region within only three years after getting introduced and it achieved a large quantity and gets in abundance (Jayanth and Bali, 1994; Jayanth and Visalakshy, 1996) [14, 5]. In the year 1989, biocontrol programs were initiated in Jammu and Kashmir, and a huge number was released of this beetle (Gupta *et al*., 2002) [7]. Since then, beetle was scattered over an area of more than 9000 km2 (Gupta, 2008) [9] and weed suppression was quite evident in numerous regions of the state (Gupta *et al*., 2004) [6]. The geographical allocation of *Z. bicolorata* was determined by Dhileepan and Senaratne (2009) [4] through GIS (Geographical Information System)-based map that emphasizes the information on *P. hysterophorus* incidence in different states and territories in India, as well as in neighboring countries like Bangladesh, Bhutan, Nepal and Pakistan. Occurrence of *Z. bicolorata* was also recorded as three distinct clusters, covering several regions of India (Dhileepan and Senaratne, 2009) [4]. Shushil Kumar and Ray (2011) [23] determined the physical dimension of male and female *Z. bicolorata* and stated that it may have a mean body length and weight of 5.6-6.5 mm and 30-40 mg respectively with undulating dark brown lines running longitudinally over off-white background on elytra. Sex can be distinguished effortlessly by the mere shape of last abdominal sternite. Posterior margin of the last abdominal sternite in the female is entire while in males, it is slightly serrated at the tip with a faint depression in the center (Mc Clay, 1980) [16]. The present study documented on the biology of Mexican beetle in laboratory study and its cultural maintenance.

Materials and methods
1. Study area
The experiment was conducted during weed growing season in the laboratory at the room temperature and humidity at Hemvati Nandan Bahuguna Garhwal University (A Central University), SRT Campus Badshahithaul Tehri, Tehri Garhwal Himalaya (Indian Himalayan Region, IHR), Uttarakhand (India) in 2021. District Tehri Garhwal is situated at latitude 30.1˚N to 30.9˚N and longitude 77.9˚E to 79.1˚E. Garhwal region is situated between North latitude 29˚26' 15" and 30˚5' 31" and between East longitude 78˚18' 45" and 80˚8' 0" in North West Himalayas (Atkinson, 1884). Geographical location of experimental site is 30˚23`02.837"N 78˚25`25.633"E and elevation is 1750 m asl surrounded by evergreen mountains and situated near Tehri dam at New Tehri city. The climate is lower temperate, snow falls at least in every winter season. All experiments carried out at room temperature at the laboratory.

2. Collection, Rearing and biology of *Zygogramma*
The work on the biology of *Z. bicolorata* on *P. hysterophorus* was executed in the laboratory at temperature (maximum) 30.37±2.63°C, temperature (minimum) 15.94±1.63°C, relative humidity 70.50±8.59% and rainfall 5.78±2.04 mm during June to October in 2019. 20 eggs were used to and biology aspects. *Z. bicolorata* beetles have been collected from field and reared on the *Parthenium* potted plant, insect cages at the laboratory. 20 matting mating pair released on the potted plant and cages. Female beetles started after matting. All methods followed in the present study as reported by various workers, viz., (Jayanth & Geetha, 1993), Sushil Kumar and Ray, (2011) [23]; Singh *et al*., (2017) and Jaiswal & Jayalaxmi Ganguli, (2020) [10].

Result and discussion

The life cycle of *Z. bicolorata* was studied from egg to adult. Some parameters like fecundity, incubation period of eggs, larval period, pre-pupal, pupal period, hatching percentage, pre-oviposition period, oviposition period, post-oviposition period, male and female longevity were studied.

Basically, adult beetle found more active during day period. Male and female beetle mainly recorded as moving on *Parthenium* plant both in lab and field conditions. Generally male beetle can be seen sitting on the back above of the female beetle. This shown their meeting behavior. After mating, female beetle start to lay eggs. Life cycle of *Z. bicolorata* undergoes four larval (grubs) instars, pre- pupa, pupa, and adult beetle. All larval instars, and adult male and female feed on *Parthenium* leaves. Larvae feed more greedily than adult beetles.

1. Egg

Female beetle generally prefers to lay eggs singly or in clusters of about 4-11 eggs on the ventral surface of the leaves. Sometimes oviposited on the surface of leaves, stems, and flowers also. Mucilaginous secretion of the eggs was glued tightly to the substratum. Twenty eggs were used for the morphometric study of *Z. bicolorata.* Incubation was observed between the dates of egg-laying and egg hatching. The specific colour and shape of the eggs and hatched larval instar and duration were observed.

The eggs were yellowish-orange with sub-shining, occasionally orange in colour. The shape was oblong- oval, smooth, slightly tapper toward the ends, end bluntly rounded. The colour ranged from light yellow to yellowish orange, and just before hatching, they turned slightly reddish (Fig. 1). The incubation period and hatching percentage were observed 4.6±0.68 (5- 6) days and 69.65±4.33 or 60-75% respectively. Similar finding of incubation period was reported by Saruk (2001) [24], Pawar and Korat (2013a). However, the hatching percentage showing some variations as reported by some authors. The egg hatching percentage was reported 30-52% by Jayanth and Bali (1992),63.41- 70.45% by Parise *et al*., (2010) [21], 66.82 ± 2.17%by Siddhapara *et al*., (2012) [25], 30 to 93% by Chaudhari *et al.*, (2012) [3], 90- 95% by Singh (2017), 67-82% by Mehta and Rahuraman, (2019), 84.11% by Jaiswal and Ganguli (2020) [10].

2. First instar larva

The newly hatched first instar larvae are fleshy and fusiform and ellipsoidal. The colour of larvae was yellowish and turn creamy or curdy white as they grow. The colour of the head of the grubs was yellowish but darker than the thorax and abdomen. Mid-dorsal portion of the abdomen bears a faded longitudinal yellowish line get extends till the abdominal end which persist in consecutive larval instars. The duration of the grub was noted 3-5 (4.25±0.72) days.

The 1st instar larva was 1.52 ± 0.11 mm in length and 0.63 ± 0.08 in breadth. Foreleg, middle leg, and hind leg were smaller than successive instars. The duration of first instar larva was 4.49±0.93 days reported by Mehta and Raghuraman (2019) [18] which is slightly varying with present result. our measurement of regarding first instar larva shown the greater body length with Mehta and Rahuraman (2019).

3. Second instar larva

The first instar grub is mounted into the second instar grub. Body segments (thoracic and abdominal) were clearly distinguished in the second instar grub. The colour of the grub was pale- yellow. The duration of the second instar grub was 3-4 (3.4±0.50) days. The first instar and second instar grubs were almost similar in colour and shape but the second instar was larger than the first instar. The prolongs of the second instar grubs were yellow, shiny, and almost similar in colour. Mehta and Raghuraman (2019) [18] observed the length, width, and duration of second larval instar as 2.50 ± 0.21 mm, 0.73 ± 0.11 mm and 2.82±0.78 days respectively.

4. Third instar larva

The second instar larvae moult into third instar grubs and their spiracles easily distinguish them from the first and second instar larvae.

On the thoracic and abdominal segments of third instar larvae, nine pairs of spiracles were noticed. As the larvae reach or assume a more convex shape in the fourth instar, they develop more curves. Later instar larvae have been observed their posterior body segments fused together, which makes them look narrower and pointed than the middle part of their bodies. This is because the fused final abdominal segments are smaller than the rest of the body parts. The duration of the third instar larva was 3-5 (3.95±0.83) days.

5. Fourth instar larva

Third instar larva moult into fourth instar larva. The fourth instar larva appears like that of the third instar larva excluding in size, with a mean6.38 ± 0.20 mm in length2.48±0.18 mm in width and 1.45±0.08 mm in head capsule with was observed. The circulatory system was visible just below the thin larval skin, which appeared darker, and ran parallel to the faint middorsal yellow longitudinal line (fig. 1). Jayanth & Bali (1993), as well as Parise *et al*., (2010) [21] had previously made similar statements.

Setae were sparsely distributed on the larvae. Form psychosomatic, i.e., sides narrowed anteriorly from the first abdominal segment and posteriorly from the sixth abdominal segment, and head hypognathous type fourth or last instar larvae become sluggish and reduce or stop feeding, and enter the pre-pupal stage before entering the soil or pupation. The larva pupates in earthen pots or chambers dug into the ground. The pupa was light yellow in colour and exarate in type. The duration of the fourth instar larva was 4.85±0.88 range 4-6 days.

Grubs are yellowish in colour and gradually turn creamy white with age. The 3rd and 4th instars larvae of the beetle are easily distinguished from the earlier instars by their spiracles. Spiracles are a distinguishing characteristic of third and fourth instar larvae (Pawar and Korat, 2013b). Whereas, studying the biology of *Z. bicolorata*, Siddhapara *et al.*, (2012) [25]; Mehta and Raghuraman (2019) [18], Mohapatra (2021) [19] made similar observations.

6. Pre-pupal period

The fourth instar larva goes through a pre- pupal stage that lasts 1.40.50 (1-2) days. Sluggish behaviour could be noted at the start of this stage, as it stopped feeding and gradually moved toward the bottom of the plant, eventually reaching the soil.

7. Pupa

Before entering the pupal stage, the larvae remained pre-pupae for nearly a one to two days. When they were prepared to pupate, they changed colour from clear creamy white to light yellow (fig. 1) and buried themselves 1 to 3 cm deep into the soil for pupation after constructing a round-shaped earthen cocoon around them. They spent roughly 8 to 12 days in the soil before emerging as fully developed adults. The pupa has a length of 6.35 0.38 mm and a width of 3.23 0.16 mm.

8. Adult

The adult emerged adults the soil after pupation. The adult has an oval shape with a convex and seriated dorsal surface and is completely white with a deep yellow notum; the colour of the adult changes over time. The colour of the head changes to black, the Pronotum to ground colour, and the fore pair of wings, i.e., elytra creamy yellow with a pinkish tinge or luteous yellow to buff. A median hat-shaped black marking on the pronotum that does not cover the lateral and anterolateral corners.

In addition to a black stripe on the anterior half of the sutural line, the Elytra have the following markings: two elongate spots arranged longitudinally, a hook-like elongate marking, and two smaller, posterolateral spots. An undulating dark brown or blackish line on the elytra runs longitudinally over an off-white background in the insects' attractive appearance.

Elytra had a non-uniform longitudinal marking pattern. The base of each elytron was found to have a blackish elongated spot (fig. 1). Within and between populations, the elytral pattern is frequently highly variable. Both serpentine and bifurcated forms of the black marking were found on the elytra's costal margin. There are dark brown longitudinal lines on the Elytra.

It appeared that the hind pair of wings were transparent and in a folded state beneath the elytra. The costal and subcostal veins of the hind wings were a reddish-brown colour, similar to that of the forewings. In the female, the posterior margin of the last visible abdominal ventrite is completely smooth, whereas in the male, it is slightly serrated at the tip. Males also had a depression in the centre of the last abdominal ventrite, which was more prominent than in the females.

Males are typically smaller than females as adults. The female's posterior margin was entire, while the males was slightly serrated at the tip.

Males were smaller (6.44±0.22 mm in length and 3.32±0.24 in width and head capsule width 1.62±0.07) mm than females (7.85±0.25 mm in length and 3.36±0.32 mm in width and head capsule width 1.80±0.05). The male beetle longevity period was observed 32.85±8.56 (25-50) and the female beetle could live 40.75±6.50 (35-55) at the lower temperate climatic condition in laboratory (Table 1, Figure 1).

The shape of the last abdominal sternite can be used to identify sexes of *Z. bicolorata*. Both male and female sternites have an entire posterior margin, while the males have a serrated tip with a small depression in the centre reported McClay (1980) [16].

Other parameters regarding biology of *Z. bicolorata* like fecundity 5.81±61.16 (400-680), incubation period of eggs 4.6±0.68 (5-6) days, hatching percentage 69.55±4.33 (60-75) %, Total larval period 16.45±2.93 (13-20) days, pre-pupal period 1.4±0.50 (1-2) days, pupal period 9.95±1.28 (8-12) days, pre-oviposition period 9.25±0.72 (8-10) days, oviposition period 35.45±4.30 (30-45) days, post-oviposition period 16.90±2.36 (15-20), male longevity 32.85±8.56 (25-50) and female longevity 40.75± 6.50 (35-55) days were recorded (Table 1, Fig. 1).

Many workers have been worked on biology of *Z. bicolorata* (viz., McClay, 1980b [16]; Jayanth and Bali 1993 [13]; Pandey *et al.,* 2001; Viraktamath *et al.,* 2004; Dhiman and Bhargava, 2005; Parise et al., 2010 [21]; Singh., *et al.,* 2017; Chandravanshi *et al.,* 2018; Mehta and Raghuraman, 2019 [18]; Jaiswal and Ganguli., 2020 [10]; Mohapatra *et al.,* 2021) [19]. Mohapatra *et al.,* (2021) [19] observed the biology of *Z. bicolorata* at the temperature 25°C, 30°C and 35°C and reported almost similar results at all three different temperatures. Here only those results have been discussed which observed at 25°C as reported by Mohapatra *et al.,* (2021) [19]. Our results showed similarity with researchers with some differences, these differences may be due to different climatic conditions and altitudinal variations.

The fecundity (egg laying/female) reported 1695-3360 by Jayanth and Bali (1992), 684 Kulkarni *et al.,* (1997), 550-960 by Sushil kumar (1998a), 551 by Aherkar *et al.,* (1992), 2500 by Panday *et al.,* (2001), average 40 egg by Dhiman and Bhargawa (2005), 1019.08 ± 18.37 days by Siddhapara *et al.,* (2012) [25], 663 days Mehta *et al.,* (2019), 683.22±14.18 by Jaiswal *et al.,* (2020) [10], 621-690 by Mohapatra *et al.,* (2021) [19].

Egg group size (Number of eggs/group) 6 by Jayanth and Bali 1992; 3-5 by Sushilkumar (1998a), 4-5 by Aherkar *et al.,* (1992) [1], 1-4 by Gautam (2002), 4-6 by Panday *et al.,* (2001),

The egg incubation period was recorded 5 days by Jayanth and Bali (1992), 4-5 days Kulkarni *et al.,* (1997), 3-5 days by Sushilkumar (1998a), 2-6 days by Aherkar *et al.,* (1992) [1], 4-6 days by Gautam (2002), 3-4 days by Panday *et al.,* (2001), 11.78 ± 1.02 days by Siddhapara *et al.,* 2012 [25] 4.53±1.04 days by Mehta *et al.,* 2019, 3-5 days by Jaiswal *et al.,* (2020) [10], 4-5 days by Mohapatra *et al.,* (2021) [19].

The hatching percentage recorded 30-53 by Jayanth and Bali (1992), 85-91 Kulkarni *et al.,* (1997), 45-93 by Sushilkumar (1998a), 51-100 by Gautam (2002), 66.82 ± 2.17 by Siddhapara *et al.,* (2012) [25], 78.72 ± 0.50 by Mehta *et al.,* (2019), 84.11 by Jaiswal *et al.,* (2020) [10], and 79-80 days by Mohapatra *et al.,* (2021) [19].

Larval period for first, second, third and fourth instar 3-5 days, 3-3.5 days, 2-3 days, and 4-8 days by Dhiman and Bharagawa (2005), 3.02 ± 0.51 days, 2.88 ± 0.33 days, 2.40 ± 0.49 days, 3.48 ± 0.73 days by Siddhapara *et al.,* 2012 [25]; 4.49±0.93, 2.82±0.78, 3.40±0.63, 4.60±0.74 by Mehta *et al.,* 2019 [18], Jaiswal *et al.,* (2020) [10], 2-3 days, 3-4 days 3-5 days, 4-6 days by Jaiswal *et al.,* (2020) [10], by Mohapatra *et al.,*(2021) [19], 4-6 days, 2-4 days,3-4 days, 4-6 days by Mohapatra *et al.,*(2021) [19] respectively.

Total larval period reported 11-13 days by Jayanth and Bali 1992; 19-23 days Kulkarni *et al.,* (1997), 12-21 days by Sushilkumar (1998a), 15-20 days by Aherkar *et al.,* (1992) [1], 13-16 days by Gautam (2002), 10-12 days by Panday *et al.,* (2001), 11.78 ± 1.02 days by Siddhapara *et al.,* 2012 [25], Mehta *et al.,* 2019 [18], 12-18 days by Jaiswal *et al.,* (2020) [10], and 13-20 days by Mohapatra *et al.,* (2021) [19].

The pupal period reported 10-12 days by Jayanth and Bali (1992), 7-10 days Kulkarni *et al.,* (1997), 5-11 days by Sushilkumar (1998a), 10-12 days by Aherkar *et al.,* (1992)

[1], 5-6 days by Gautam (2002), 8-10 days by Panday *et al.*, (2001), 10-12 days by Dhiman and Bharagawa (2005), pre-pupal 1.32 ± 0.47 Days, Pupal period 6.56 ± 0.50 Days by Siddhapara *et al.*, 2012, 13.80±1.36 days Mehta *et al.*, 2019 [18], 8-10 by Jaiswal *et al.*, (2020) [10], and 9-11 days by Mohapatra *et al.*,(2021) [19].

The egg to adult emergence period reported 27-29 days by Jayanth and Bali 1992; 30-38 days Kulkarni *et al.*, (1997), 20-37 days by Sushilkumar (1998a), 27-38 days by Aherkar *et al.*, (1992) [1], 23-27 days by Gautam (2002), 23-27 days by Panday *et al*., (2001), Mehta *et al.*, 2019 [18], and 24-35 days by Jaiswal *et al.*, (2020) [10].

The pre-oviposition period reported 10-70 days by Jayanth and Bali (1992), 6-14 days by Sushilkumar (1998a), 3-8 days by Gautam (2002), 7-10 days by Panday *et al.*, (2001), 8.68 ± 0.65 days by Siddhapara *et al.*, 2012 [25], 8.59±0.72 days Mehta *et al.*, 2019 [18], Jaiswal *et al.*, (2020) [10], and 8-10 days by Mohapatra *et al.*, (2021) [19].

The oviposition period recorded 89-138 days by Jayanth and Bali (1992), 50-120 days by Sushilkumar (1998a), 75-102 days by Panday *et al.*, (2001), 53.30 ± 1.53 days by Siddhapara *et al.*, (2012) [25], and 44-47 days by Mohapatra *et al.*, (2021) [19].

The post oviposition period reported 1-21 days by Jayanth and Bali (1992), 1-8 days by Sushilkumar (1998a), 18.58 ± 0.81 days by Siddhapara *et al.*, (2012) [25], 44.26±1.53 days, 18.35±0.81 days Mehta *et al.*, (2019) [18], and 17-20 days by Mohapatra *et al.*, (2021) [19].

Male longevity reported 122-271 days by Jayanth and Bali (1992), 31.97 days by Kulkarni *et al.*, (1997), 62-110 days by Sushilkumar (1998a), 30 days by Aherkar *et al.*, (1992) [1], 35-90 days by Gautam (2002), 120-240 days by Panday *et al.*, (2001), 85.48 ± 2.17 days by Siddhapara *et al.*, 2012, 61.42 ± 7.31 days by Mehta *et al.*, 2019 [18], 31- 36 days Jaiswal *et al.*, (2020) [10], and 33-45 days by Mohapatra *et al.*, (2021) [19].

Female longevity reported 109-198 days by Jayanth and Bali (1992), 35.05 days Kulkarni *et al.*, (1997), 60-115 days by Sushilkumar (1998a), 38 days by Aherkar *et al.*, (1992) [1], 49-105 days by Gautam (2002), 90-180 days by Panday *et al.*, (2001), 80.56 ± 1.47 days by Siddhapara *et al.*, (2012), Mehta *et al.*, (2019) [18], 35-48 days by Jaiswal *et al.*, (2020) [10], and 46-56 days by Mohapatra *et al.*, (2021) [19]. The present study revealed similar results, with some differences from the previous studies. The causes of these differences may be due to altitudinal and climatic variation in the study area.

Table 1: Duration of different life stages of *Z. bicolorata* in laboratory

S. N.	Life stages	Period(days)(Mean±S.D.)	Range
1	Fecundity	581.25±61.16	400-680
2	Incubation period (Days)	4.6±0.68	5-6
3	Hatching percent (%)	69.55±4.33	60-75
Larval period (days)			
4	1st instar grub	4.25±0.72	3-5
5	2nd instar grub	3.40±0.50	3-4
6	3rd instar grub	3.95±0.83	3-5
7	4th instar grub	4.85±0.88	4-6
Total larval/ grub period (days)		16.45±2.93	13-20
8	Pre-pupal period	1.4±0.50	1-2
9	Pupal period	9.95±1.28	8-12
Total life cycle(days)		32.50±5.39	27-40
10	Pre-oviposition period	9.25±0.72	8-10
11	Oviposition period	35.45±4.30	30-45
12	Post-oviposition period	16.90±2.36	15-20
Adult longevity(days)			
13	Male	32.85±8.56	25-50
14	Female	40.75±6.50	35-55
Total periods(days)		73.60±9.06	53-75

Source: Primary Data collected in 2019-20

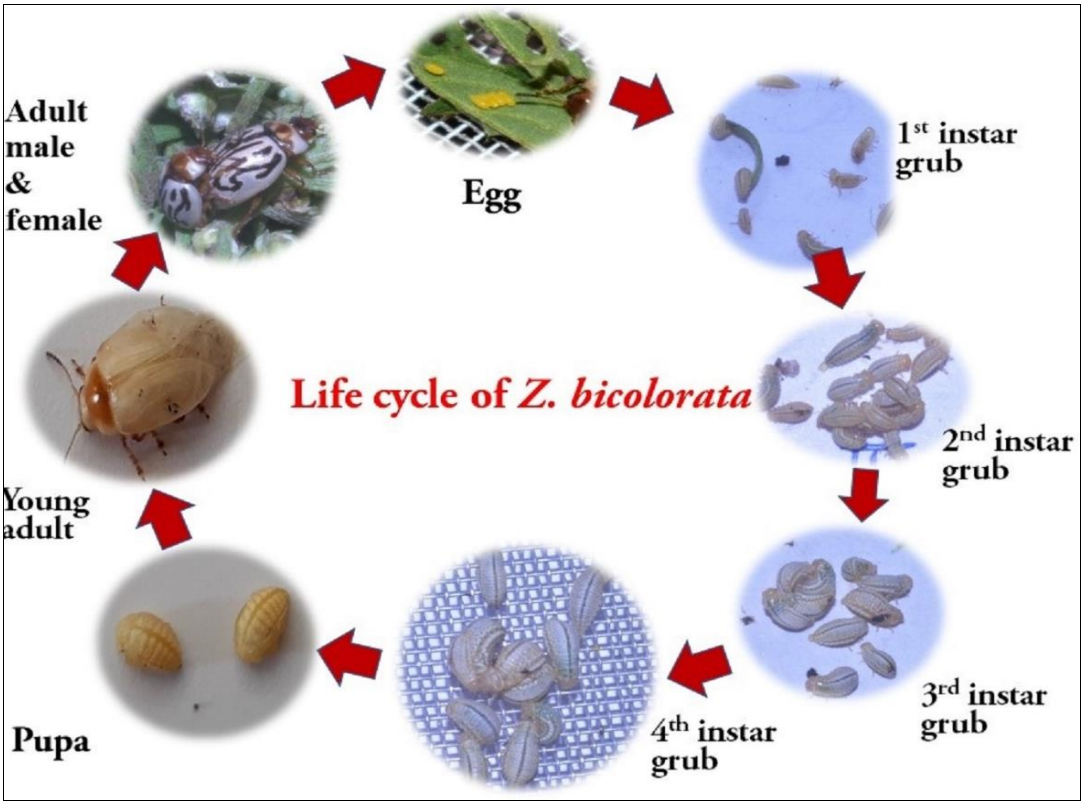

Fig 1: Life cycle of *Z. bicolorata* showing different life stages (Photographs by Nikon 5600D in 2019-20 at laboratory)

Conclusion

Zygogramma bicolorata is widely used a classical bioagent for management of *Parthenium hysterophorus* L. weed. For any biocontrol program is necessary to maintain the appropriate or large number of beetles in laboratory. So, the rearing and culture of beetle is most important for further study. *Z. bicolorata* undergoes four larval instars i.e., 1st instar, 2nd instar, 3rd and 4th instar grubs. They all are voraciously feeder on parthenium leaves. Grubs as well as adult beetle feed on *Parthenium* plant significantly managing spread of this noxious weed classical biocontrol agent.

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