

Biology of *Zygogramma bicolorata* Pallister on *Parthenium hysterophorus* Linn. under Laboratory Conditions

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ABSTRACT

Parthenium hysterophorus Linn. is one of the most aggressive, invasive weeds threatening natural and agricultural ecosystems in the world including Nepal. Augmentative release of host-specific, leaf feeding beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) is one of the sustainable management approaches of parthenium weed. Successful mass rearing program requires knowledge on biology of *Z. bicolorata* for augmentative release. Thus, life cycle and biology of *Z. bicolorata* were studied in lab at 26±2 °C temperature and 70±10% relative humidity at NARC, Lalitpur, Nepal during March to September, 2016. The average incubation, larval and pupal periods of the insect were 4.33, 12.20, and 11.00 days, respectively. A single female could lay 1,837 eggs in 58.80 days oviposition period with 73.01% egg hatchability. The insect completed its life cycle in 108.40 and 105.50 days with adult longevity of 91.90 and 75.00 days for females and males, respectively. The average length of egg, pupa, adult female and male was 1.19 mm, 5.90 mm, 6.58 mm and 5.32 mm and breadth 0.51 mm, 3.74 mm, 3.94 mm and 3.20 mm, respectively. The average lengths of the first, second, third and fourth instar larvae were found 1.30 mm, 3.02 mm, 4.96 mm and 8.08 mm and breadth of 0.44 mm, 1.13 mm, 2.02 mm and 3.16 mm, respectively. These findings will help in mass multiplication of *Z. bicolorata* for biological control of parthenium weed.

Keywords: Mexican beetle, parthenium, life cycle, head capsule, instars and oviposition period.

1. INTRODUCTION

Parthenium hysterophorus L. (Asteraceae; Heliantheae), commonly known as parthenium, is an annual or short-lived perennial, herbaceous plant. Parthenium is a noxious, invasive weed of both agricultural and natural ecosystems (Shrestha *et al.* 2019). It is a weed of global significance, which causes losses in agriculture, adverse effects on human and animal health and degrades natural ecosystems (Kaur *et al.* 2014). This weed has characteristics of prolific nature producing up to 25,000 viable seeds per plant (Shrestha *et al.* 2019). The weed is native to tropical America and is problematic in tropical and subtropical regions of the world. It has been spread into 96 countries of Africa, Asia, Europe, North

America, Oceania and South America (CABI 2020a). The weed entered India from the United States of America along with wheat imported into Pune during 1956 (Vertak 1968). It is suspected that parthenium entered Nepal from India along with vehicles or contaminated seeds, in 1960s (Mishra 1991). The significant expansion of parthenium occurred in Nepal during 1990 (Shrestha *et al.* 2015). Presently, this weed has been found in terai, mid hill and high hill regions reaching up to the altitude of 2,000 m asl (Shrestha *et al.* 2019).

Parthenium can be managed through physical, chemical and biological approaches. Biological control with insects is one of the most important approaches used in the sustainable management of the weed (Adkins & Shabbir 2014). Nine host-specific insect species were introduced in Australia, including *Z. bicolorata* (Adkins & Shabbir 2014). Learning from successful biological control of parthenium weed in Australia, *Z. bicolorata* was released in Bangalore, India in 1984 (Jayant 1987). There is no record of the deliberate introduction and release of *Z. bicolorata* in Nepal, but it fortuitously arrived from India and spread into various parts of Nepal (Shrestha *et al.* 2010; Shrestha *et al.* 2011).

The beetle, *Z. bicolorata*, is commonly known as the Mexican beetle, but also called as parthenium beetle (CABI 2020b). Larval and adult feeding on parthenium results in

skeletonization, defoliation and reduction in flowers and seed production. *Z. bicolorata* can cause 100% defoliation of parthenium, resulting in reduced weed density, plant height, plant biomass, flower production and soil seed bank (Dhileepan *et al.* 2000). Augmentative release of *Z. bicolorata* is one of the sustainable management approaches of pernicious weed *P. hysterophorus*. However, several augmentative releases may be required to ensure the establishment and effective control of parthenium (Sushilkumar & Ray 2011). Successful mass rearing technology is needed for an effective augmentative release program of *Z. bicolorata* against parthenium weed. Knowledge of the life cycle and biology of *Z. bicolorata* is indispensable to initiate any mass rearing and augmentative release program. Hence, the present study was undertaken to understand the life cycle and biology of *Z. bicolorata* under lab conditions.

2. MATERIALS AND METHODS

Studies were conducted in the laboratory of National Entomology Research Center, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal. A temperature of $26\pm 2^\circ\text{C}$ and relative humidity of $70\pm 10\%$, were maintained in the laboratory with the help of air conditioner and humidifier, respectively. The average monthly temperature and relative humidity during the study period are presented in Fig. 1.

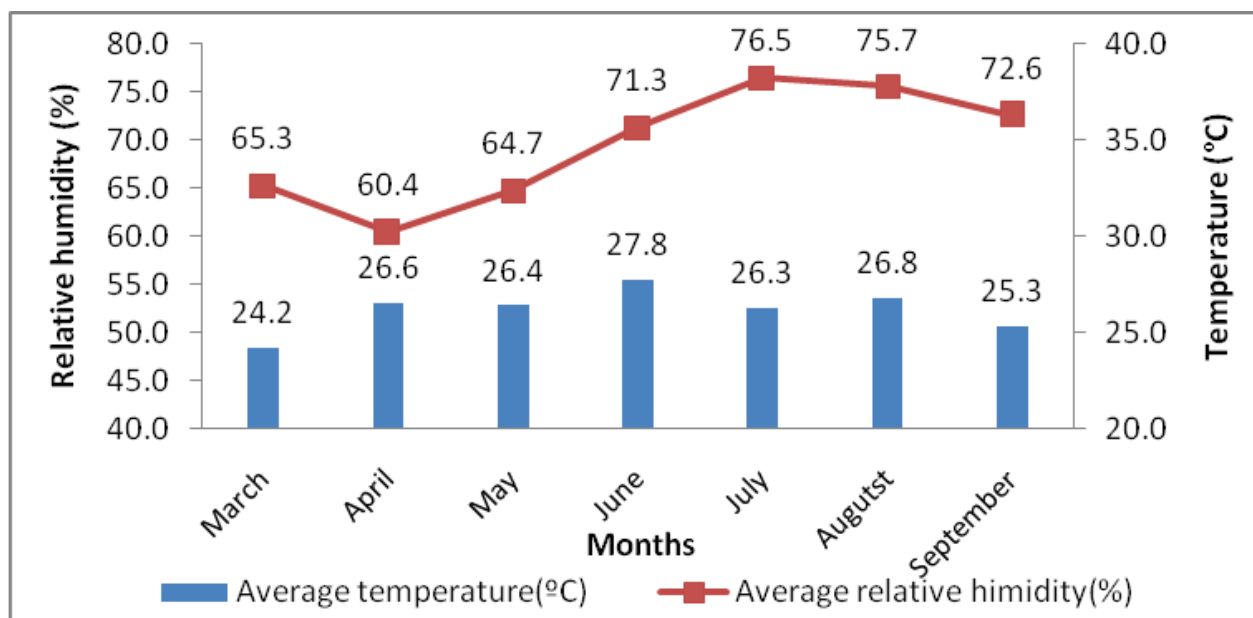


Fig. 1. Average monthly temperature and relative humidity in the laboratory during the study period

2.1 Culture of *Z. bicolorata*

Adult males and females of *Z. bicolorata* were collected from Khageri irrigation dam site (N 027°34.110', E 084°43.792') of Chitwan district. The insects were reared in rectangular plastic boxes of 22.7 cm X 16.3 cm X 9.0 cm length, breadth, and height, respectively. (Fig. 2a). Ventilation was provided in boxes by cutting holes of 10 cm X 5 cm dimensions on lid, which was made insect proof with a black muslin cloth. Bouquets of fresh parthenium leaves with few flowers (Fig. 2b) were provided as food for adults and larvae of *Z. bicolorata*. Cut ends of leaves were covered with moistened cotton and wrapped with aluminium foil to delay drying. The bouquets of leaves were changed daily in rearing cages after cleaning, and *Z. bicolorata* larvae and adults attached to old leaves were transferred back with the help of a camel hair brush. Eggs laid on leaves and black muslin cloth of window in rearing

boxes were collected with a camel hair brush and further used to multiply the insect. The eggs were kept in a 9 cm Petri plate and first instar larvae immediately after hatching were transferred into plastic, rearing cages along with bouquets of parthenium leaves. Pupation cages were made of plastic rearing boxes with 5 cm depth of soil as pupation media. Pupation media were prepared from field-collected soil and sand mixed in a 1:1 ratio. Medium was sterilized in a hot air oven at 80 °C for 30 minutes, and distilled water was sprinkled to maintain soil moisture. When the larvae stopped feeding and became pre-pupa, fully grown larvae, were transferred into pupation cages, and moisture in soil was maintained by regularly sprinkling distilled water on the soil surface. Newly emerged adults of *Z. bicolorata* were again transferred into plastic rearing cages with parthenium leaves for mating and further multiplication.



Fig. 2. (a) Experimental set up in plastic cages, (b) Bouquets of fresh Parthenium leaves with flowers

2.2 Life cycle and biology

The life cycle and biology of *Z. bicolorata* were studied in laboratory conditions at 26±2°C average temperature and 70±10% average relative humidity during March to September, 2016. Newly laid eggs were used for the study of developmental periods of immature stages. Eggs were kept in a 9 cm diameter Petri plate to determine the incubation period (duration between egg laying and hatching). Newly hatched, first instar larvae were transferred individually into 9 cm Petri plates, and fresh parthenium leaves were provided as food. Completely randomized design (CRD) was followed and 30 replications were maintained. Moulting and duration of larval instars were recorded daily, and parthenium leaves were also changed daily. Pre-pupa were transferred individually into pupation cages

made of plastic boxes of 18.7 cm X 12.6 cm X 7.8 cm length, breadth, and height, respectively. The pupation period was recorded as the time between dates of pupation to adult emergence.

Thirty pairs of newly emerged adults were used to determine pre-oviposition, oviposition and post-oviposition periods, longevity of adult females and males and egg-laying capacity of *Z. bicolorata*. Pair of adults were individually kept in plastic rearing cages of size 18.7 cm X 12.6 cm X 7.8 cm length, breadth, and height, respectively, with the provision of ventilation. Parthenium leaf bouquets were provided as adult food. The daily observation on initiation of egg laying, number of eggs laid by each pair and cessation of egg laying, and live and dead adults was recorded on the number of eggs laid. The pre-oviposition

period of an adult female was determined as the time between the date of adult emergence and initiation of egg-laying. Similarly, the oviposition period was recorded as the time between the initiation and cessation of egg-laying. Post-oviposition period was recorded as the duration of adult female survival after cessation of egg-laying. The date of male and female adults' death was recorded to calculate adult longevity.

Twenty different clusters of eggs were kept separately in 9 cm Petri-plates to determine the hatching percentage. On an average, each cluster consisted of 57 eggs (ranging from 42 – 80 eggs). Number of eggs hatched each day were counted and the average hatching percentage was calculated. Similarly, pupae were collected daily from *Z. bicolorata* rearing and were kept in separate pupation boxes for 20 days during June, 2016. Emergence of adult males and females was recorded daily. Percent adult emergence, percent male and female adults' emergence were calculated.

Length and breadth of immature and mature life stages (egg, larva, pupa & adults) of *Z. bicolorata* were measured with the help of an ocular micrometer in a stereomicroscope

(Bestscope BS-3040T). The ocular micrometer was calibrated with the stage micrometer of one-millimetre size (ERMA INC. Japan) at the observed level of magnification. Length and breadth of egg, larvae, pupae and adult moths were measured. Head capsule widths from larval exuviae of all larval instars were also measured. Similarly, the weight of all immature and mature stages of *Z. bicolorata* was measured with digital balance (Sartorius ENTRIS2241-1S). Weight of five first instar larvae were measured at a time due to the small size, and weight of one larva was calculated. Similarly, two larvae were taken for second instar larvae. Data obtained from observations were entered into Microsoft excel, and the mean values, ranges and standard error of the mean were calculated using statistical program Genstat Discovery Edition 4.

3. RESULTS AND DISCUSSION

3.1 Life cycle of *Z. bicolorata*

The beetle *Z. bicolorata* consisted of four life stages viz., egg, larva, pupa and adult with complete metamorphosis (Fig. 3a-d). The duration of various life stages of the insect is presented in Table 1.

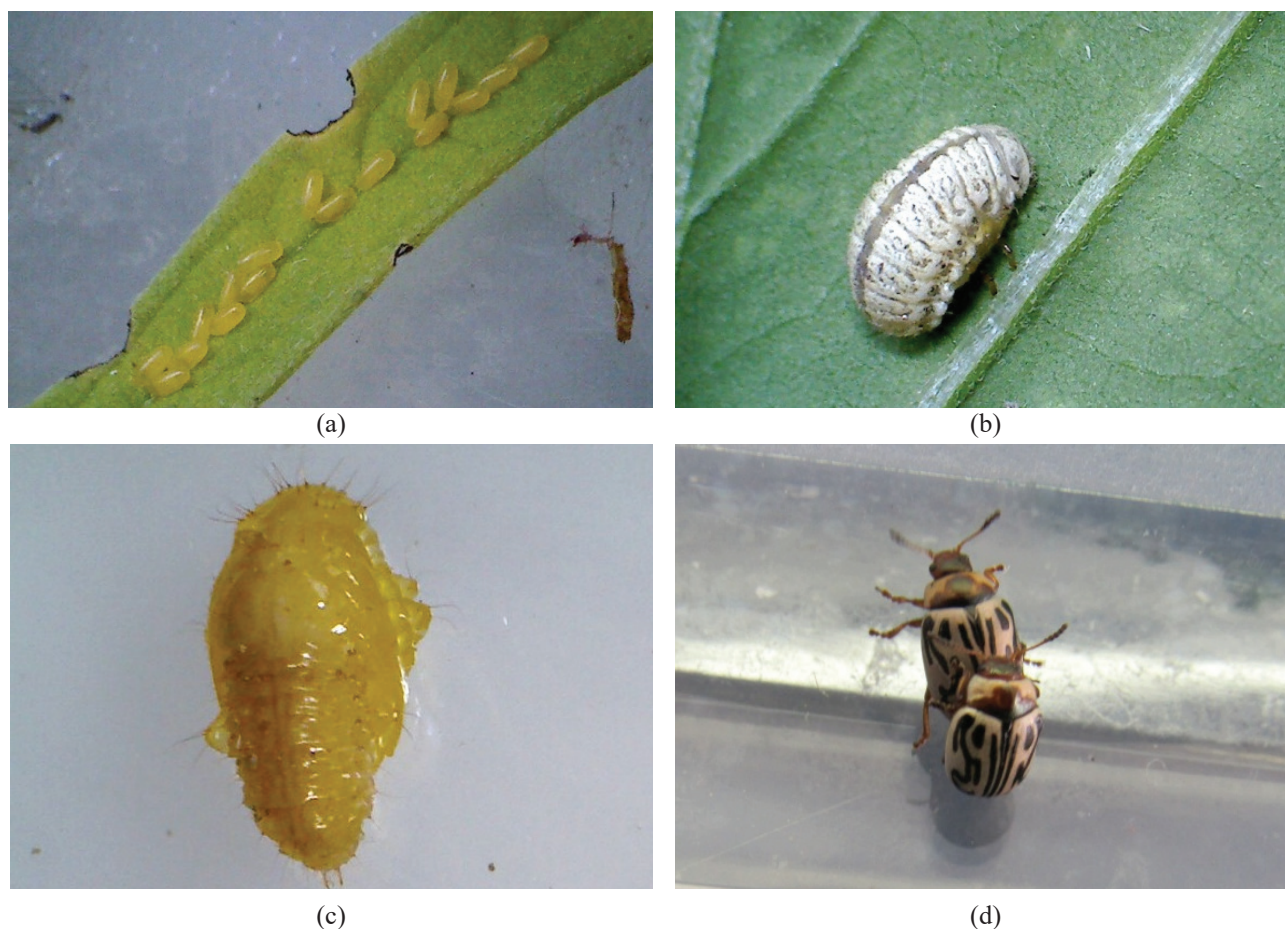


Fig. 3. Different developmental stages of *Z. bicolorata*, (a) Egg, (b) Larva, (c) Pupa and (d) Adults

Eggs were generally laid singly or in a cluster of 2-5 on the leaf surface or muslin cloth on the lid of rearing boxes. Eggs were oblong in shape and light yellow with a smooth surface that turned into dark yellow before hatching. The average incubation period of an egg was found 4.33 days ranging from 4 - 5 days. The larva of the insect consisted of four instars and moulted three times before pupation. The newly hatched larva was pale yellow which turned into creamy white as it grew. The first and second instar larvae were similar in appearance except in size, whereas, third and fourth instars were similar in appearance, and black coloured spiracles were visible on the abdomen and on lateral sides of thorax. The larvae were slightly curved with a protrusible proleg-like structure at the posterior end for locomotion. Larval duration of first, second, third and fourth instars was 3.33, 2.33, 2.13, and 4.40 days, respectively. The mean larval period was 12.20 days ranging from 11-14 days.

Pupation took in the soil about 1-2 cm deep in the pupal cage in the laboratory. The pupae were of exarate type and off-white to pale yellow in color. The pupal period was 11 days ranging from 10-12 days. The total developmental period of the immature stages was found 27.53 days ranging from 25-30 days. Various previous workers also reported similar duration of developmental period of egg, larva and pupa (Jayanth & Bali 1993; Pandey *et al.* 2001; Dhiman & Bhargawa 2005; Sidhpara *et al.* 2012; Rathod *et al.* 2012; Mehata & Raghuraman 2019).

Table 1. Duration of various developmental stages of *Z. bicolorata* under lab conditions.

Stages	Mean (days)	Range (days)
Egg (incubation period)	4.33±0.088*	4-5
First larval instar	3.33±0.088	3-4
Second larval instar	2.33±0.088	2-3
Third larval instar	2.13±0.063	2-3
Fourth larval instar	4.40±0.123	4-6
Total larval period	12.20±0.147	11-14
Pupa	11.00±0.127	10-12
Total developmental period	27.53±0.243	25-30
Pre-oviposition period	10.50±0.150	9-12
Oviposition period	58.80±2.630	39-96
Post-oviposition period	22.60±1.873	2-49
Adult female longevity	91.90±2.092	57-107
Adult male longevity	75.00±3.571	46-124
Total life cycle of female	108.40±2.062	84-134
Total life cycle of male	105.50±3.584	73-151
* Standard Error of Mean		

Adults emerged from the soil after completion of pupation. Adults were oval, convex dorsally and flat ventrally. Elytra were creamy yellow with black, elongated elytral markings, and the head was black. Average adult female longevity was 91.90 days, ranging from 57-107 days, and adult male longevity was recorded 75 days, ranging from 46-124 days. Adult females were found to survive longer than adult males, such longer female longevity was also reported by previous researchers (Jayanth & Bali 1993; Mehta & Raghuraman 2012). Jayanth and Bali (1993) reported adult female and male longevity as 210.00 and 129.30 days, respectively. Siddhapra *et al.* (2012) found 80.56 days and 85.48 days of adult female and male longevity, respectively, which is quite similar to the present study, though they found adult males survived longer than adult females.

In the present study, the total life cycle was recorded 108.40 days for an adult female, ranging from 84-134 days, and 105.50 days for an adult male, ranging from 73-151 days. Sidhpara *et al.* (2012) reported a similar total life cycle of *Z. bicolorata*, i.e., 104.08 days and 109.54 days of adult females and males, respectively. The average oviposition period was found 58.80 days, ranging from 39-96 days. The oviposition period was recorded 53.30 days by Sidhpara *et al.* (2012), which was close to the present study. The pre-oviposition and post-oviposition periods in this study were recorded 10.50 days and 22.60 days, respectively. The duration of pre- and post-oviposition periods were similar to the findings of Sidhpara *et al.* (2012) (8.68 days & 18.58 days) and Mehta and Raghuraman (2019) (8.59 days & 18.35 days, respectively).

3.2 Hatching and adult emergence

The average number of eggs laid by a pair of *Z. bicolorata*, hatching percentage, adult emergence and sex ratio of emerged adults are presented in Table 2. A female laid 1,837 eggs on an average during its life, which ranged from 1,229-2,489 eggs. Previous workers had reported different average fecundity in female *Z. bicolorata* ranging from 663.5 eggs per female (Mehta & Raghuraman 2019) to 2,520.9 eggs per female (Jayanth & Bali 1993) during its lifetime. The hatching percentage of the egg was found 73.01% ranging from 62.50-83.78%. A similar hatching percentage of 66.82 to 78.72 in laboratory was reported by previous workers (Rathod *et al.* 2012; Sidhpara *et*

al. 2012; Mehta & Raghuraman 2019). Adults' emergence from pupa in the present experiment was 75.50%. Among the emerged adults, 60.53% were female, and 39.47% male with an average sex ratio of 1:1.55 (male:female). Previous workers also reported higher adult female emergence with male to the female sex ratio of 1:1.29 (Siddhapara *et al.* 2012) and 1:1.35 (Mehta & Raghuraman 2019).

Table 2. Egg laying, hatching and adult emergence of *Z. bicolorata* in laboratory

Stages	Mean (days)	Range (days)
Total eggs laid, no.	1837±106.9*	1229-2489
Egg hatching, %	73.01±4.628	62.50-83.78
Adult emergence, %	75.50±1.754	57.78-89.09
Adult fe-male emergence, %	60.53±0.711	53.85-68.89
Adult male emergence, %	39.47±0.711	31.11-46.15
Average sex ratio	1:1.55 (Male: Female)	
* Standard Error of Mean		

3.3 Egg-laying capacity of *Z. bicolorata*

The average number of eggs laid per week by a single adult female of *Z. bicolorata* during the oviposition period is presented in Fig. 4. The number of eggs laid per week increased up to the third week and decreased from the fourth week onward. The highest number (346 eggs) was laid during the third week of the oviposition period. Eggs laid per week remained above 122 till the 8th week, and scanty after the 10th week of the oviposition period. Pandey *et al.* (2001) reported that a single female could lay 65 eggs per day during peak production of the oviposition period.

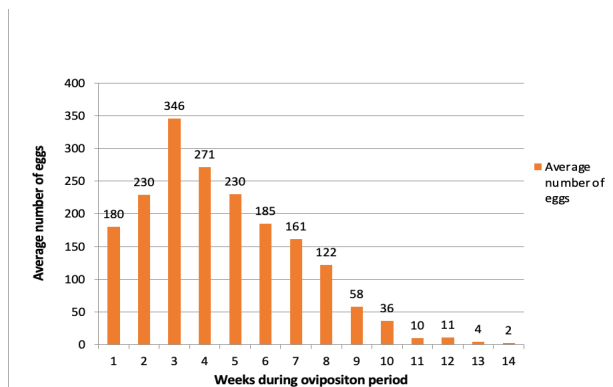


Fig. 4. Egg laying pattern of *Z. bicolorata* females in laboratory

3.4 Morphometrics of *Z. bicolorata*

Length and breadth of all life stages of live *Z. bicolorata*, including different instars, were measured with the help of an ocular micrometer (ERMA Inc. Japan) in stereomicroscope (Bestscope BS-3040T). Data obtained are presented in Table 3. The eggs of *Z. bicolorata* were measured 1.19 mm long and 0.51 mm wide, and a similar finding was reported by Rathod *et al.* (2012) (1.22 mm length & 0.56 mm width) and Mehta and Raghuraman (2019) (1.33 mm length & 0.53 mm width). Similarly, Siddhapra *et al.* (2012) found *Z. bicolorata* egg 1.51 mm long and 0.59 wide, which was a little bit larger than the present finding. Length of the first, second, third and fourth instars larvae was found 1.30 mm, 3.02 mm, 4.96 mm, and 8.08 mm, respectively. Similarly, breadth was recorded 0.44 mm, 1.13 mm, 2.02 mm and 3.16 mm, respectively. Similar measurements were also reported by previous workers (Siddhapara *et al.* 2012: Dhiman & Bhargawa, 2005; Mehta & Raghuraman 2019). Bhusal *et al.* (2020) reported body size pattern of offsprings of *Z. bicolorata* depended on body size of parents; off springs from larger parents were larger in size.

Table 3. Morphometrics of different life stages of *Z. bicolorata* reared in laboratory.

Life stages	Length (mm)	Range (mm)	Breadth (mm)	Range (mm)
Egg	1.19±0.006*	1.13-1.22	0.51±0.004	0.47-0.53
First instar larva	1.30±0.019	1.10-1.50	0.44±0.004	1.05±1.29
Second instar larva	3.02±0.028	2.76-3.33	1.13±0.011	1.05-1.29
Third instar larva	4.96±0.047	4.38-5.50	2.02±0.023	1.75-2.25
Fourth instar larva	8.08±0.047	7.50-8.63	3.16±0.010	3.13-3.25
Pupa	5.90±0.233	5.75-6.25	3.74±0.011	3.50-3.88
Adult female	6.58±0.039	6.38-7.00	3.94±0.018	3.75-4.13
Adult male	5.32±0.055	5.00-5.88	3.20±0.020	3.13-3.50
* Standard Error of Mean				

The length of the pupa was recorded as 5.90 mm and breadth 3.74 mm. Previous workers reported pupal length of 6.18 mm and width of 3.34 mm (Mehta & Raghuraman 2019), whereas 5.65 mm length and 2.28 mm breadth were reported by Siddhapara *et al.* (2012). The length of adult female was 6.58 mm and width 3.94 mm. Similarly, adult male had length and breadth of 5.32 mm and 3.20 mm, respectively. Thus, the adult female of *Z. bicolorata* was larger than the male. The larger size of adult females compared to adult males of *Z. bicolorata* was also reported by previous workers (Jayanth & Bali 1993; Dhiman & Bhargawa 2005; Mehta & Raghuraman 2019). A similar size of adult female (6.21 mm length & 3.85 width) and male (5.74 mm length & 3.61 mm width) was reported by Jayanth and Bali (1993).

The head capsule was measured from larval exuviae of the first, second and third instars and are presented in Table 4. The widths of head capsule were measured 0.62 mm, 0.90 mm and 1.34 mm of the first, second, and third instar larval exuviae. Jayanth and Bali (1993) reported the head capsule size of 0.52 mm, 0.80 mm, and 1.08 mm for the first, second, and third instar larvae, respectively. Similarly, the head capsule was measured 0.56 mm, 0.71 mm, and 1.07 mm for the first, second, and third instars, respectively, by Mehta and Raghuraman (2019).

Table 4. Head capsule width of different instars of *Z. bicolorata* measured from exuviae in laboratory.

Larval moults	Width (mm)	Range (mm)
First instar	0.62±0.004*	0.58-0.67
Second instar	0.90±0.003	0.85-0.94
Third instar	1.34±0.008	1.21-1.51
* Standard Error of Mean		

The average weight of various life stages of *Z. bicolorata* is presented in Table 5. Average larval weight of the first, second, third and fourth instars was recorded 0.24 mg, 2.55 mg, 10.06 mg and 21.00 mg, respectively. The weight of the pupa was 29.75 mg. Adult female and male weights, immediately after emergence from soil, were 36.17 mg and 25.82 mg, which, after one week at the mating stage increased to 43.43 mg and 30.00 mg, respectively. The weight of adult females increased to 46.29 mg at the egg laying stage, i.e., 15 days after emergence.

Table 5. Weight of various life stages of *Z. bicolorata* reared in laboratory.

Life stages	Weight (mg)	Range (mg)
First instar	0.24±0.005*	0.20-0.28
Second instar	2.55±0.087	1.80-3.60
Third instar	10.06±0.45	7.20-16.30
Fourth instar	21.00±0.74	15.00-29.80
Pupa	29.75±0.602	24.10-36.80
Newly emerged female	36.17±0.54	30.10-41.70
Female at the mating stage	43.43±1.12	32.50-53.20
Female at egg-laying stage	46.29±1.25	33.60-58.10
Newly emerged male	25.82±0.63	20.20-29.90
Male at the mating stage	30.00±0.57	23.20-36.30
* Standard Error of Mean		

4. CONCLUSION

The life cycle and biology of *Z. bicolorata* were studied in the National Entomology Research Center, Khumaltar laboratory Lalitpur, Nepal. Easy mass multiplication of *Z. bicolorata* feeding with parthenium appeared possible under lab conditions at NARC, Khumaltar. Its average egg incubation, larval and pupal periods appeared to be 4.33, 12.20, and 11.00 days, respectively, whereas, its life cycle was found 108.40 and 105.50 days for females and males, respectively. Present findings on biological parameters of *Z. bicolorata* would help in mass multiplication and release of *Z. bicolorata* for the biological control program of *P. hysterophorus* (parthenium) in Nepal.

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