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## Life cycle details and biometrics of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) on *Parthenium hysterophorus* in Raipur, Chhattisgarh

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### Abstract

*Parthenium hysterophorus* (Linnaeus), well known worldwide as 'carrot grass' or 'white top' or 'congress grass' and 'gajar ghans' or 'chatak chandni' in hindi, belongs to family Asteraceae, It can only be managed effectively by developing an integrated approach involving many options in combination. The biological control of *Parthenium* was explored through a leaf beetle, *Zygogramma bicolorata* Pallister, at Biocontrol laboratory, Raipur, Chhattisgarh. *Z. bicolorata* is considered as a safe biocontrol agent but till now it is not well established and spread in Chhattisgarh. Therefore, the present study was carried out to investigate the detailed life cycle, duration and site of different life stages being spent. The study revealed that the female beetle laid about  $683.22 \pm 14.18$  eggs and grub hatched after 3 to 5 days. There were four larval stages which fed on *Parthenium* for 12 to 18 days. After 4<sup>th</sup> instar, it entered into soil for pupation at mean depth of  $1.93 \pm 0.31$ . The beetles emerged after 8-10 days and completed their life cycle in about 55 to 83 days. Both the adults and grub (grubs) were capable of feeding on *Parthenium* leaves.

**Keywords:** *Parthenium hysterophorus*, Mexican beetle, *Zygogramma bicolorata*

### Introduction

*Parthenium hysterophorus* (Linnaeus), well known in India and throughout the world as 'carrot grass' or 'white top' or 'congress grass' and 'gajar ghans' or 'chatak chandni' in hindi, belongs to family Asteraceae. It is an invasive weed species in Africa, Australia and Asia, being native of tropical and sub-tropical South and North America. It is an herb of geotropical origin which has now spread in too many parts of the world [1], and has become one of the most noxious weeds in almost all types of agricultural lands besides infesting wasteland, community land, road and sides of railway track including forests [2]. In India, *Parthenium* was first reported as a weed from Pune in 1955 [3], and now occurs across the country. The infestation of the weed in cultivated land causes yield losses up to 40% in several crops and reduces forage production up to 90%. At present *Parthenium* has spread to 35 million hectares in India. The Mexican beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) was imported into India in 1983 for the biological control of the problematic *Parthenium* weed, *P. hysterophorus* L. grubs and adult beetles feed voraciously on the foliage and inflorescence and remains confined to the weed [4]. The bio-ecology [5] and *in vitro* rearing of the beetle on *Parthenium* has been well documented [6]. Although the insect established readily, population build-up was noticed only in 1988 and by 1994, it had spread over 2,00,000 sq km area in and around Bangalore from the epicenter [7]. The beetle is known to occur throughout the year but the insect diapauses over an extended period of time in nature [8] resulting in extensive proliferation of the weed in its absence. The life cycle of *Z. bicolorata* P., revealed that the female beetle laid about 650-700 eggs and grub hatched after 36 to 72 hrs, which fed on the *Parthenium* for 12 to 16 days. After 4<sup>th</sup> instar, it entered the soil and pupated below. The beetles emerged after 8-10 days and completed their life cycle in 25 to 28 days. Both the adults and grubs were capable of feeding on the *Parthenium* leaves but only 15-20% on the flowers [9].

### Materials and Methods

**Location of study:** The bionomics of the Mexican beetle, *Z. bicolorata* P. was studied under

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laboratory conditions in the Bio-control laboratory, Department of Entomology, IGKV, Raipur, Chhattisgarh, India.

#### Collection and Rearing of *Zygogramma bicolorata* P.:

Adult beetles of *Z. bicolorata* were collected from *Parthenium* plants at Raipur in the range of 2.0 km around Indira Gandhi Krishi Vishwavidyalaya, University campus, during 2018 from June - July, as the beetles emerged after diapause during these months. The collected beetles were reared under net house as well as in the Biocontrol laboratory. Beetles were placed in plastic basins. The basins were filled with soil up to 10 cms. Three fresh *Parthenium* plants of an average height of 24.60cms having flowers was planted in the basins for feeding of beetles. Plastic net covers were used for covering basins. (Plate 1) Egg laying, both on plastic net and leaves (both upper and lower side) were observed. Mated pairs of beetles were selected and transferred to new plastic basins of the same size with *Parthenium* plant. The basins were replenished with fresh *Parthenium* plant till the female beetle survived. Temperature was maintained at an average of  $25\pm 5^{\circ}\text{C}$  during the experimental period.



**Fig 1A:** Mass multiplication of Mexican beetles, *Z. bicolorata* in plastic basins covered with plastic net under laboratory condition



**Fig 1B:** Grubs and adults of Mexican beetles, *Z. bicolorata*

**Plate 1:** Mass multiplication of Mexican beetles, *Z. bicolorata* Pallister

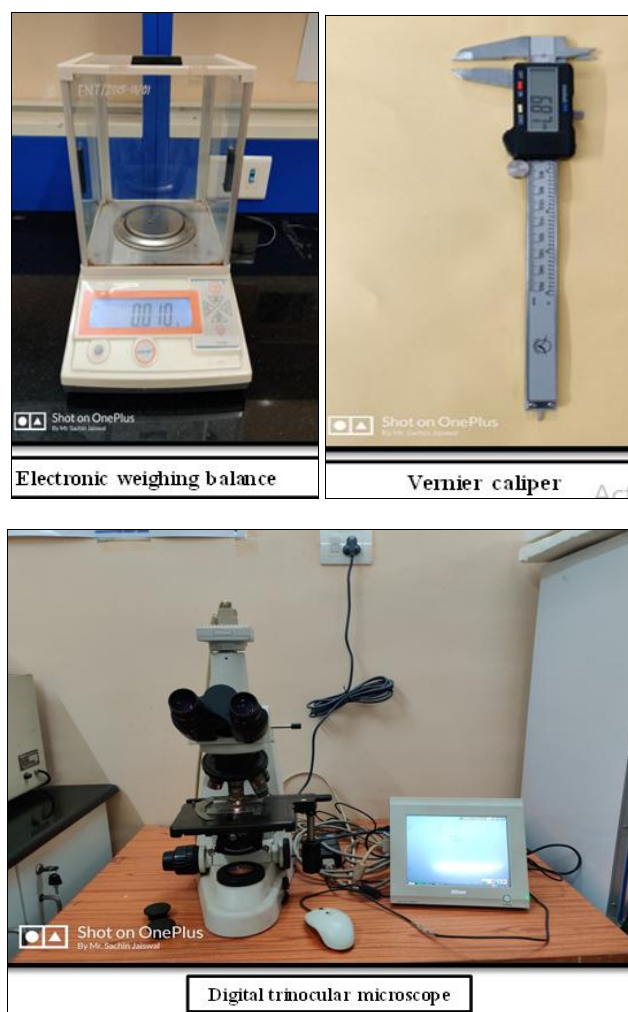
**Details of study:** The observations were recorded on fecundity, incubation period and duration, length and weight of different grub/larval instars, pupal period and longevity of male and female beetles using digital trinocular microscope and vernier caliper.

#### Observations recorded

**a. Incubation period (in days)-** Eggs were observed under microscope for confirming hatching. Incubation period

was calculated from the date of egg-laying to the date of hatching.

**b. Biometrics of the grub/larval neonate and different larval instars [length (in mm) and weight (in mg)] -** Newly hatched first instar grubs from stock culture were kept in transparent glass petridishes (10 cm. diameter) and reared on *Parthenium* leaves at constant laboratory temperature. Length of the neonate grubs and successive instars were recorded using an electronic Vernier caliper and weight was recorded using an electronic weighing balance. (Plate 2)



**Plate 2:** Instruments used for biometric measurements

**c. Biometrics (length) of head capsule (in mm) –** For measuring the length of head capsule, the shed head capsules of different instars were collected from representative samples after each moult and measured under digital trinocular microscope.

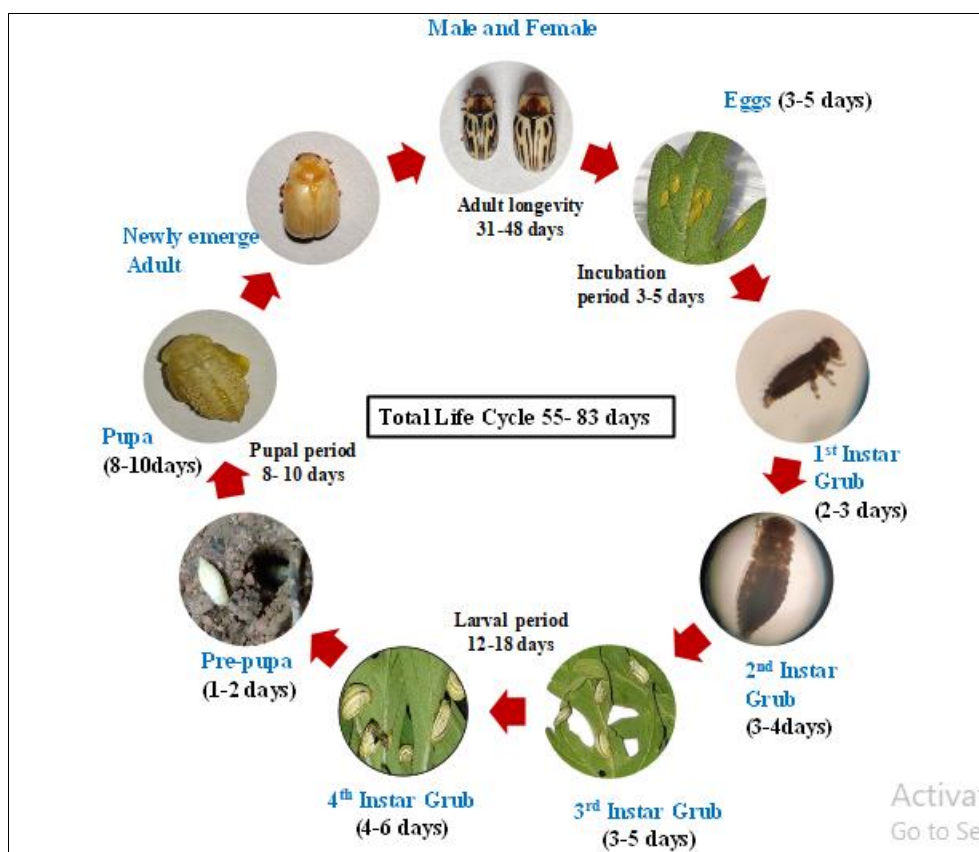
**d. Grub/larval period (in days) –** Grub/larval period was calculated by summing of the number of days from the date of egg hatching till the date of pupation including the time required by grub to undergo different instars. The instar wise confirmation was done by looking at the head capsule shed by the grubs during moulting

**e. Pupal period (in days) -** Pupal period was recorded by calculating the number of days between the date of formation of pupa and adult emergence.

- f. Biometrics of pupa [length, width (in mm) and weight (in mg)]** - Pupa of *Z. bicolorata* were collected from soil. Length and width of pupae were measured using an electronic Vernier caliper (or graph paper taking scale of 1 block equals to 1 mm). Weight of pupae were recorded using electronic weighing balance.
- g. Biometrics of adult male and female beetles [length, wing-span (in mm) and weight (in mg)]** – Adult male and female beetles were collected, and their morphometric characters such as body length and wing span were measured. Length and wing-span of beetles were measured using an electronic Vernier caliper, whereas weight was recorded using an electronic weighing balance.
- h. Fecundity of adult females** - Emerging adults were paired by observing morphometric characters of male and female. One pair beetle were reared in petridish provided with fresh leaves and replicated thrice. Egg laying of beetles were recorded daily. Fecundity was worked out by counting the total number of eggs laid by individual females during its life span.
- i. Biometrics of eggs laid [length, width (in mm) and weight (in mg)]** - Length and width of eggs laid by females reared in petridishes were measured under digital trinocular microscope. Weight of 100 eggs was recorded at a time using electronic weighing balance and weight of single egg was calculated from it.
- j. Adult longevity of male and female beetles (in days)** - Longevity of male and female beetle was computed from the date of emergence till their death.
- k. Developmental Period or Total Life cycle (in days)** - The period from eggs to adult emergence was considered as the total developmental period which was calculated by summation of incubation period, grub period, pupal period and adult longevity i.e., duration from egg to adult stage.

### Results and Discussion

The morphometric parameters of different stages of *Z. bicolorata* as revealed in the present studies is mentioned below: (Plate 3)



**Plate 3:** Life cycle of *Zygogramma bicolorata* Pallister

- 1. Eggs:** Eggs were laid singly or in clusters of 4 to 5 on the under surface of *Parthenium* leaf and occasionally on the stem and flowers in general. This is in total agreement with <sup>[12]</sup> who also reported egg laying singly or in clusters, mostly on the underside of the leaves. Eggs were glued securely to the substrate by a clear mucilaginous secretion. But in laboratory condition, the eggs were laid on both lower/upper surface of leaf, stems and flowers of *Parthenium*. Eggs were shiny, yellowish-orange, oblong-

oval, slightly tapering toward the ends, with ends bluntly rounded. The mean length and width of eggs were about  $1.21 \pm 0.07$  mm and  $0.55 \pm 0.03$  mm, respectively and the mean weight of eggs was recorded to be  $0.16 \pm 0.01$  mg (Table 2). This result slightly varies with <sup>[12]</sup> who reported the average length of egg to be  $1.51 \pm 0.05$  mm. The mean fecundity, incubation period and hatching percentage were  $683.22 \pm 14.18$ , 3-5 days, 84.11 per cent respectively. (Table 1)

## 2. Grub/larvae

**1. First instar grub:** The egg hatched to neonate larvae/grub. The grub was fleshy and fusiform or ellipsoidal in shape. Thorax and abdomen of early instar grub was yellowish in colour which gradually changed to curdy white and the head of the grub was yellowish but darker than the thorax and abdomen. The head and thorax of grub were covered with fine, minute hairs at the lateral sides of the abdomen with light yellow colored tubercle. Mid-dorsal portion of the abdomen of the grub possessed a faint longitudinal yellowish line extending till the abdominal end which became more prominent in successive instars. The mean length and width of the 1<sup>st</sup> instar grub was  $1.45 \pm 0.08$  mm and  $0.62 \pm 0.01$  mm respectively. The mean breadth of head capsule was  $0.56 \pm 0.04$  mm (Table 2). Fore leg, mid leg and hind leg were smaller than successive instars. The duration of the first instar grub was 2-3 days (Table 1) and average weight of the 1<sup>st</sup> instar grub was recorded to be  $0.26 \pm 0.03$  mg.

**2. Second instar grub:** The 1<sup>st</sup> instar moulted to 2<sup>nd</sup> instar. The segments (thoracic and abdominal) were clearly distinguishable in the 2<sup>nd</sup> instar grub and color of the grub was pale-yellow. The prolegs were yellow, shiny and translucent. 1<sup>st</sup> and the 2<sup>nd</sup> instar grubs were almost similar in colour, and form, except that it was larger in size having mean length of  $2.31 \pm 0.09$  mm, mean width of  $0.74 \pm 0.03$  mm and the mean breadth of head capsule  $0.65 \pm 0.16$  mm (Table 2), with longer fore legs, mid legs, and hind legs. The duration of 2<sup>nd</sup> instar grub was 3-4 days (Table 1) and the average weight was recorded as  $4.90 \pm 1.92$  mg.

**3. Third instar grub:** The 2<sup>nd</sup> instar moulted to form 3<sup>rd</sup> instar, which could be easily distinguished from early instars by their spiracles. Nine pair of spiracles was found on the thoracic and abdominal portion of 3<sup>rd</sup> instar grub. The body was covered by setae which were also visible on the femur, tibia and tarsi of all three pair of thoracic legs, dorsal surface of the body exhibited mosaic pattern with creamy white colour which gave a curdy appearance. Paired six black spots were seen like beads on the lateral side of the head, just below the base of the antennae. These six black spots or beads were arranged in two separate rows having three spots in each row. Out of six spots, four spots were located at equal distance in a rectangular fashion on the upper part of grub's head, whereas remaining two spots were located on lower part of head slightly away from the rectangular spots. As the grub reached maturity, the body got curved and assumed more convex shape in the 4<sup>th</sup> instar grub stage. Last 2 to 3 abdominal segments reduced in size than other segments most probably as posterior segments fused and looked narrow and pointed than the middle portion. The 3<sup>rd</sup> instar grub measured  $4.64 \pm 0.18$  mm in length and  $2.25 \pm 0.06$  mm in breadth with an average breadth of head capsule  $1.34 \pm 0.04$  mm (Table 2). The duration of the 3<sup>rd</sup> instar grub was 3-5 days (Table 1) and its mean weight was recorded to be  $14.50 \pm 1.78$  mg.

**4. Fourth instar grub:** 3<sup>rd</sup> instar grub looked alike 4<sup>th</sup> instar except in size, having mean length  $6.27 \pm 0.16$  mm and breadth  $3.35 \pm 0.12$  mm with a mean head capsule breadth

of  $1.49 \pm 0.05$  mm (Table 2). In continuous with the faint mid-dorsal yellow longitudinal line, circulatory system was visible just below the thin grub cuticle. The grub was sparsely clothed with setae. Head was exerted and, hypognathous in position. 4<sup>th</sup> instar grub became sluggish prior to entering into the soil, for pupation. The duration of 4<sup>th</sup> instar grub was 4-6 days (Table 1) and its average weight was recorded as  $31.90 \pm 1.85$  mg.

**3. Pre-pupa:** The grub remained as pre-pupae for about 1-2 day before entering into the soil for pupation.

**4. Pupa:** When the pre-pupa was about to pupate, it turned transparent creamish white to light yellow in colour and buried itself 1.0 to 3.0 cm deep in the soil for pupation after forming a spherical earthen cocoon around. It remained within soil for 8 to 10 days at an average depth of  $1.93 \pm 0.31$  cms before emerging as fully formed adult. This contradicts with the earlier report of [13] who mentioned the pupation depth of 10.0 cms. On an average pupae measured  $5.65 \pm 0.37$  mm in length, and  $4.10 \pm 0.41$  mm in breadth (Table 2) with a mean weight of  $37.8 \pm 5.75$  mg.

**5. Adult:** Adults which emerged from pupa out of the soil were elongate and oblong or oval in shape with strongly convex and glabrous dorsal surface and completely white in colour with dark yellow notum. Later the colour of the adult changed. Head turned to black in color and pronotum changed to ground color and elytra creamy yellow with a pinkish tinge or luteous yellow to buff. Pronotum possessed a median hat-shaped black marking not covering the lateral and antero-lateral corners. Elytra included the following markings: a stripe adjacent to sutural line, two elongate spots arranged longitudinally, a hook-like elongate marking, and two smaller, postero-lateral spots; sutural line with a black stripe, broader in the anterior half. The attractive insects bear an undulating dark brown or blackish line on elytra that run longitudinally over an off-white background. The pattern of longitudinal marking on elytra was non-uniform. Prominent blackish elongated spot was observed at the base of each elytron. The black marking originated at the costal margin of elytra was found either as serpentine fashion or somewhat bifurcate type. Elytra marked with dark brown longitudinal lines. Hind wings were transparent and folded beneath the elytra. Costal and sub-costal veins of hind wings were reddish-brown in colour. The posterior margin of the last visible abdominal ventrite was whole in the female and slightly serrated at the tip in the male, and the males also had a faint depression at the center of the last abdominal ventrite. Adult males were generally smaller than the adult females. Males were smaller ( $6.10 \pm 0.38$  mm in length and  $4.04 \pm 0.26$  mm in breadth) and females ( $6.65 \pm 0.25$  mm in length and  $4.33 \pm 0.21$  mm in breadth) (Table 2). The average weight of male and female adults were recorded as  $30.60 \pm 5.79$  mg and  $42.0 \pm 5.79$  mg, respectively. Similar findings with the present studies were reported by [9] and [10] who mentioned that the average egg, larval, pupal and adult period was found out to be  $4.53 \pm 1.04$  days,  $13.80 \pm 1.36$  days,  $9.95 \pm 0.85$  days,  $34.20 \pm 8.05$  days (male) and  $42.68 \pm 7.33$  days (female). However the present studies slightly differs with the studies of [11] who reported lesser hatching percentage ( $78.72 \pm 0.50$ ) than the present studies.

## Conclusion

Thus, from the present studies on the detailed biology and

biometrics of *Z. bicolorata* P. conducted at Raipur, Chhattisgarh revealed that the duration of the total life cycle was of about 55-83 days and consisted of four stages i.e. eggs, larva (grub), pupa and adults with duration of about 24-35 days. The pre-pupal stage was observed to be of 1-2 days there after it remained in the pupal stage up to 8-10 days in soil.

**Table 1:** Duration of different developmental stages of *Z. bicolorata* under laboratory conditions during 2018-19

S. No.	Life stages	Period (Days)
		Range (in days)
1	Fecundity	683.22±14.18
2	Incubation period	3-5
3	Hatching percentage	84.11
4	<b>Larval/grub period</b>	
	I Instar	2-3
	II Instar	3-4
	III Instar	3-5
	IV Instar	4-6
5	<b>Total larval/grub period</b>	<b>12-18</b>
6	Pre-pupal period	1-2
7	Pupal period	8-10
8	Total life cycle (Days)	24-35
9	<b>Adult longevity</b>	
	Male	31-36
	Female	35-48
	<b>Total Period</b>	<b>55-83</b>

**Table 2:** Morphometrics of different life stage of *Z. bicolorata* under laboratory conditions during 2018-19

S. No.	Life stages	Length (mm)	Width (mm)	Head width (mm)	Weight (mg)
1	Egg	1.21±0.07	0.55±0.03		0.16±0.01
<b>Grub/ larval stages</b>					
2	I Instar	1.45±0.08	0.62±0.01	0.56±0.04	0.26±0.03
3	II Instar	2.31±0.09	0.74±0.03	0.65±0.16	4.90±1.92
4	III Instar	4.64±0.18	2.25±0.06	1.34±0.04	14.5±1.78
5	IV Instar	6.27±0.16	3.35±0.12	1.49±0.05	31.9±1.85
6	Pupa	5.65±0.37	4.10±0.41		37.8±5.75
<b>Adults</b>					
7	Male	6.10±0.38	4.04±0.26		30.6±2.95
8	Female	6.65±0.25	4.33±0.21		42.0±5.79

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