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Study on biology and morphometric aspects of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) on parthenium in Varanasi region, India

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Abstract
The work on biology of *Zygogramma bicolorata* reared on *Parthenium hysterophorus* was done in laboratory at department of entomology and agricultural zoology. The study show that the Mexican beetle oviposit small, oblong, elongated, light yellow or orange eggs on leaf surface and lid of Petri plate under laboratory condition. The average egg, larval, pre-pupal, pupal and adult period was found out to be 4.53±1.04 days, 13.80±1.36 days, 1.65±0.58 days, 9.95±0.85 days, 34.20±8.05 days (male) and 42.68±7.33 days (female). Hatching percentage and adult emergence percentage was 78.72±0.50 %, 71.08±2.81 % respectively. The larvae molted four times and the larval periods are 4.49±0.93 days (1st instar), 2.82±0.78 days (2nd instar), 3.40±0.63 days (3rd instar), 4.60±0.74 days (4th instar) respectively. Entire life cycle escalates to 61.42±7.31 days for male and 69.33±8.26 days for female. The average fecundity, pre-oviposition, oviposition, post oviposition periods and sex ratio were 663.5 eggs, 8.59±0.72 days, 44.26±1.53 days, 18.35±0.81 days and 1:1.35 respectively. Total average developmental period lasted for 26.82 days.

Keywords: Biology, morphometry, life cycle, *Zygogramma bicolorata*, *Parthenium hysterophorus*

Introduction
Parthenium hysterophorus L. commonly called as carrot grass, or white top or congress grass in India and it belongs to family Asteraceae. This weed is very common along the road sides, around the agricultural fields and on waste lands. *P. hysterophorus* is considered as a noxious weed due to its prolific seed production, it produces up to 25,000 seeds per plant (Naïve *et al.*, 1996) [6] and 200,000 seeds/m^2 in abandoned fields in India (Joshi, 1991) [3] and fast spreading ability, allelopathic effect on other plants, strong competitiveness with crops and health hazard to human as well as animals. Parthenium has ill effects such as dermatitis and respiratory malfunction. The allelo-chemicals produced by parthenium plant suppress growth of native vegetation posing a threat to bio-diversity (Mausam *et al*., 2013) [7]. In India parthenium was first reported as weed from Pune in 1955 (Singh, 1997) [5], and now occurs throughout the country (Bhanu *et al.*, 2014). The infestation of the weed causes yield losses up to 40% in several crops and reduces forage production by up to 90%. At present parthenium has spread to 35 million hectares in India (kumar & Ray, 2007). The Mexican beetle was found to be effective bio-control agent of *P. hysterophorus*, was introduced from Mexico to Bangalore in 1983. There are many other bio-control agents like stem-galling moth *Epiblema sternuana* Walker (lepidoptera: Torticidae), stem boring weevil *Listronotus setosipennis* Hustache (Coleoptera: Curculionidae), the seed feeding weevil *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidae), *Bucculatrix parthenica* Bradley (Lepidoptera: Bucculatricidae), *Contrachelus albocinereus* Fiedler (Coleoptera: Curculionidae), and *Puccinia babruptvar. Partheniicola* (Jackson) Parmel that attacks parthenium but among them *Zygogramma bicolorata* proved to be most effective.

Materials and Methods
The experiment was carried out under laboratory conditions at constant temperature (25±1 ^{0}C) and relative humidity (70±5%). The wild Mexican beetle was collected from the agricultural field, roadsides and wastelands. The beetles were reared for two generations in laboratory, tender leaves of parthenium was changed daily and the third generation was used for studying the biology of the Zygogramma beetle in BOD incubator. The mating pair was kept in the petriplates of dimension (1.5 x 8 cm). The adults were fed with mixture of tender leaves, old leaves, and inflorescence. Rest of the beetles were kept in a transparent plastic jar (10 cm in

height and 5 cm in diameter). Forty freshly eggs laid that were laid on the lid surface or under the surface of parthenium leaves were removed with the help of fine camel hair brush and placed separately in petridishes (1.5 cm depth x 8 cm height). Proper attention must be given for any mechanical injury during transfer of eggs. On hatching early instar larva was also fed with tender leaves of parthenium as a food. Continuous supply of parthenium leaves was given to the larva for preserving the culture *Z. bicolorata*. The larvae molted three times and the instars were judged with the help of exuvia of the larva that was observed daily in the morning hours. Along with recording the incubation period and period between the instars, the measurements of the eggs and the larva were studied under ocular and stage micrometres. Morphometric observation was done for length, breadth, head width, fore leg, middle leg and hind leg of the first to fourth instars larva, pupa and adults (male and female). The size of adult male is smaller than the adult female. Many a times size is taken as a parameter to distinguish male and female adult. The data on size was collected and statistically analyzed and the growth index was calculated by using Howe's (1953) formula.

Growth index = percent larva pupated/Mean larval duration (days)

This research paper also seeks to provide information on description of the life stages of the Mexican beetle, their morphology and distinction of one life stage from another.

Biology of *Zygogramma bicolorata* on *Parthenium hysterophorus*

The work on biology of *Zygogramma bicolorata* on *Parthenium hysterophorus* was executed in laboratory during October 2017 to November 2017 at (25±1 ^{0}C) and relative humidity (70±5%). 40 eggs were used to study the morphometric and biology aspect of the *Zygogramma bicolorata*. Incubation period was enquired as the time between the dates of laying eggs and date of egg hatching. The specific colour and the shape of the egg were observed. Keeping in view the objective of study, the number and duration of larval instars was necessary to be taken into note. The hatched larvae from the eggs from each petridish were keenly observed for knowing the larval instars and duration of each larval instar. The morphometric measurement of the eggs and the larva is done using microscope under ocular and stage micrometres. Duration of first instar was calculated by noting the period between date of hatching and date of first moulting. Duration of second instar is calculated by noting the period between date of first moulting to date of second moulting. Duration of third instar is calculated by noting the period between date of second moulting and the date of third moulting. Duration of fourth instar is calculated by noting the period between Date of third moulting to date of fourth moulting. Pre-pupal period stage is characterized by lack of appetite, the full-grown larvae rest without feeding for a few hours to one day on the undersurface of leaves before burrowing into the soil for pupation (Jayanth & Geetha, 1993) [10]. The period of pupal stage is noted from the time the larva forms pupa, enters the soil to adult emergence. Pre-oviposition period was estimated by the duration between adult female emergence to becoming capable of laying eggs, and the interval between the inception of egg laying to termination of egg laying is estimated as oviposition period, and interval between termination of egg laying to the mortality of female was considered as post-oviposition period. For the purpose of determining the pre-oviposition, oviposition and post-oviposition period the mating pair were separated, forty numbered and kept in a plastic container (10 cm in height and 5 cm in diameter). The dimension viz. length and breadth of pupa was estimated with the help of a scale. Colour, shape, and size of the adult was also noted with the help of a scale. The adult mating pairs were regularly fed with fresh tender parthenium leaves, so that the female adult can realize maximum fecundity. The eggs laid by female in each plastic container was separated and counted every morning (9:00 am-10.00 am), then the mating pair were again transferred to their original plastic container after cleaning the debris to commence mating/oviposition. The total number of eggs laid in entire life span of adult female was called fecundity. Along with estimating and maintaining all the observation, the longevity of the male and female adults was also observed and noted, from date of emergence to date of death of the beetle. Total life cycle was estimated from the period between the date of egg laying to the death of the beetles. In order to estimate the sex-ratio (Male: Female) under controlled laboratory environment, the number of male and females that emerged were counted. The life cycle of *Zygogramma bicolorata* was drawn and depicted in figure 1.

Results

Biology of Mexican beetle, *Z. bicolorata* on congress grass, *P. hysterophorus*

Egg

Generally, in nature eggs were laid singly or in clusters of 4 to 5 on the, under surface of parthenium leaf, or occasionally on stem and flowers. Eggs were glued tightly to the substrate by a clear mucilaginous secretion. But in laboratory condition, the eggs were laid on surface of the lid of the container along with laying on leaf surface and stems and flowers of parthenium. Eggs were about 1.33±0.20 mm in length and 0.53± 0.02 mm in breadth (table 1). The incubation period was 4.53±1.04 days (table 2). Eggs were sub-shining, yellowish-orange (fig. 2). Oblong-oval, slightly tapered toward the ends, ends bluntly rounded. Chorion was sculptured with numerous hexagonal cells and aseptate. Micropyle was located terminally. The hatching percentage varied from 67 – 82 per cent.

First instar larva

The egg hatches to young larvae. The larva is fleshy and fusiform or ellipsoidal in outline (fig. 3). Thorax and abdomen of early instar larva was yellowish in colour which gradually changes to curdy white in colour and the head of the larva is also yellowish but darker than the thorax and abdomen. The head and thorax of larva is covered with fine, minute hairs and present at the lateral sides of the abdomen bore on light yellow colored tubercle. Mid dorsal portion of the abdomen of larva bear a faint longitudinal yellowish line extending till the abdominal end which gets prominent in successive instars of larva. than first instar. The 1st instar larva was 1.52 ± 0.11 mm in length and 0.63 ± 0.08 in breadth (table 1). Foreleg, middle leg and hind leg were smaller than successive instars. The duration of first instar larva was 4.49±0.93 days (table 2).

Second instar larva

The segments get distinguishable in second instar larva. The prolegs of the larvae are yellow, shiny and translucent. First and the second instar larva were almost similar in colour, and form, except that second instar larva is larger in size having

mean length of 2.50 ± 0.21 mm, and mean breadth of 0.73 ± 0.11 mm (table 1), with longer forelegs, middle legs, and hind legs. Color of the second instar larvae was pale-yellow (fig. 4). The duration of second instar larva was 2.82±0.78 days (table 2).

Third instar larva

Third instar larva can be easily distinguished from early instar larvae by their spiracles (fig. 5). Nine pair of spiracles are found on the thoracic and abdominal portion of third instar larvae. The body is covered by setae and setae were also visible on the femur, tibia and tarsi of all three pair of thoracic larva, but the setae are smaller than the first instar larvae. Dorsal surface of the body exhibits mosaic pattern with creamy white in colour which gives a curdy appearance. A pair of six, black spots appears like beads were located at each 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 equal distance in a rectangular fashion on the upper part of larval head, whereas remaining two spots were located on lower part of head slightly away from the rectangular spots. As the larva reaches later instars the body of the larva gets curved and assumes more convex shape in fourth instar larva. Last 2 to 3 abdominal segment was having reduced size than other segments most probably because posterior segments are fused and looks narrowed and pointed than the middle portion. The larva was 4.47 ± 0.39 mm in length and 2.23±0.14 mm in breadth on an average (table 1). The duration of third instar larva was 3.40±0.63 days (table 2).

Fourth instar larva

Fourth instar larva appears alike to third instar larva except in size, with mean length (5.72 ± 0.49 mm), and mean breadth (3.44±0.56 mm) (table 1). In continuous with the faint mid-dorsal yellow longitudinal line, circulatory system was visible just below the thin larval skin, which appeared darker (fig. 6). In past, similar description has been made by Jayanth & Bali (1993) [10] as well as Parise *et al.* (2010) [4]. The larva was sparsely clothed with setae. Form cyphosomatic i.e. sides curved, narrowed anteriorly from first abdominal segment and posteriorly from sixth abdominal segment. Head exerted, hypognathous Fourth instar larva then become sluggish and reduce or stops feeding and undergo pre-pupal stage, prior to entering into the soil, to undergo pupation. The larva pupates in earthen chambers that they make inside the soil. The pupa was light yellow in colour and exarate type. The duration of fourth instar larva was 4.60±0.74 days (table 2).

Pupa

The larvae remained as pre-pupae for about a day before entering the pupal stage. When they were about to pupate, they turned transparent creamish white to light yellow colour (fig. 1(7)) and buried 1 to 3 cm deep in the soil for pupation after forming a spherical earthen cocoon around. They remained within soil for 8 to 12 days before emerging as fully formed adult. The pupae measured 6.05 ± 0.37 mm in length, and 3.68 ± 0.83 mm in breadth (table 1).

Adult

Adults that 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, as time passes the colour of the adult changes. Head turns to black in color and Pronotum changes to ground color and elytra creamy yellow with a pinkish tinge or luteous yellow to buff. Pronotum with a median hat-shaped black marking not covering the lateral and anterolateral corners. Elytra with the following markings: a stripe adjacent to sutural line, two elongate spots arranged longitudinally, a hook-like elongate marking, and two smaller, posterolateral 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 (fig. 7).

The elytral pattern often shows considerable variation, both within and between populations. The black marking originated at the costal margin of elytra was found either as serpentine fashion or somewhat bifurcate type. Elytra is 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 is 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 are generally smaller than the adult female. The posterior margin of the female was entire it was slightly serrated at the tip in male. Males were smaller (6.05±0.19 mm in length and 3.15±0.16 in breadth) mm than females (6.21±0.34 mm in length and 3.25±0.39 mm in breadth) (table 2).

Table 1: morphometric particulars of different life stages of *Zygogramma bicolorata*

Stage	Length (mm)	Breadth (mm)	Head width (mm)
Egg	1.33 ± 0.20	0.53± 0.02	
Larval stages-			
1st instar	1.52 ± 0.11	0.63 ± 0.08	0.56 ± 0.02
2nd instar	2.50 ± 0.21	0.73 ± 0.11	0.71 ± 0.04
3rd instar	4.47 ± 0.39	2.23 ±0.14	1.07 ± 0.05
4rth instar	5.72 ± 0.49	3.44 ± 0.56	1.42 ± 0.09
Pupa	6.18 ± 0.15	3.34 ± 0.09	-
Adult			
Male	6.05±0.19	3.15±0.16	-
Female	6.21±0.34	3.25±0.39	

Table 2: parameter of Developmental stages of *Z. bicolorata* on *P. hysterophorus* under laboratory condition.

Particulars	Mean + S.D.
Incubation period (Days)	4.53±1.04
Hatching percentage (%)	78.72 ± 0.50
Larval period (Days)-	
1st instar	4.49±0.93
2nd instar	2.82±0.78
3rd instar	3.40±0.63
4rth instar	4.60±0.74
Total larval period (Days)	13.80±1.36
Pre-pupal (Days)	1.65 ± 0.58
Pupal period (Days)	9.95 ± 0.85
Pre-oviposition period (Days)	8.59±0.72
Oviposition period (Days)	44.26±1.53
Post-oviposition period (Days)	18.35±0.81
Adult emergence (%)	71.08 ± 2.81
Sex-ratio (Male: Female)	1:1.35
Adult longevity (Days)	
Male	34.20 ± 8.05
Female	48.42 ± 7.33
Total life cycle (Days)	
Male	61.42 ± 7.31
Female	69.32 ± 8.26
Fecundity	663.5

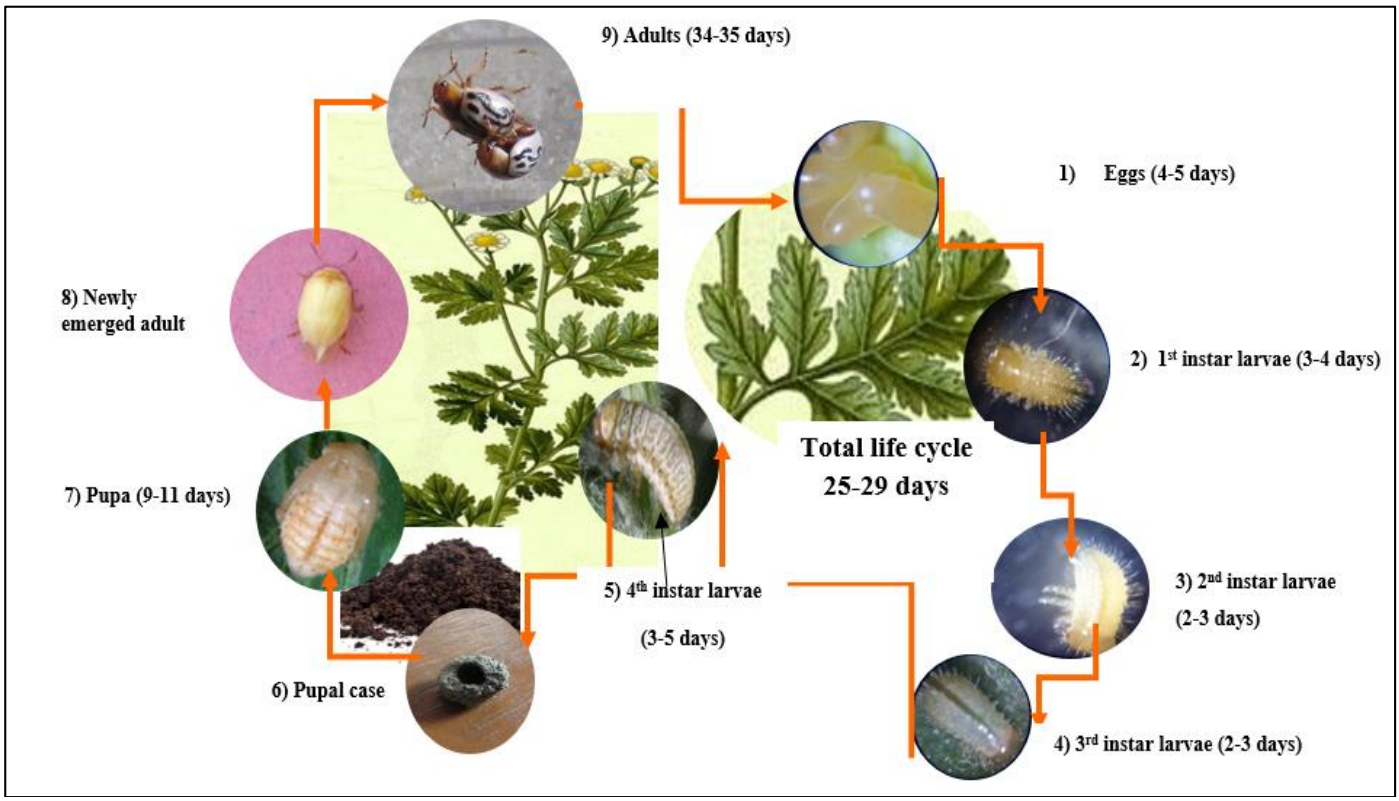

Fig 1: Life cycle of *Zygogramma bicolorata*

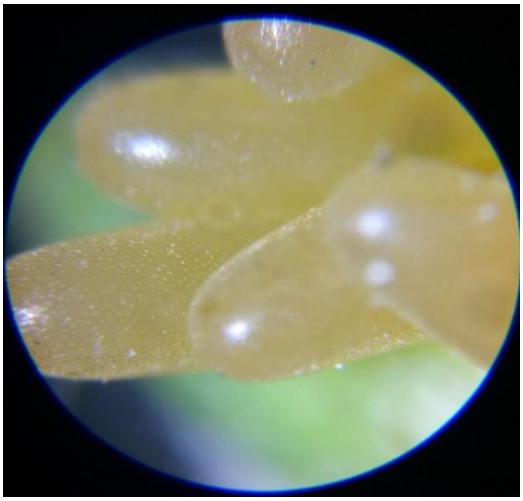

Fig 2: *Eggs glued in mass*

Fig 4: *Second instar larva.*

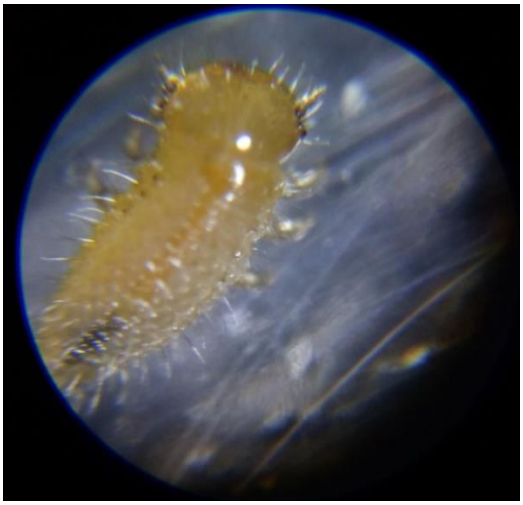

Fig 3: *First instar larva.*

Fig 5: *Third instar larva.*

Fig 6: *Fourth instar larva.*

Fig 7: *Adult Zygogramma beetle*.

Discussion and conclusions

Biology of Mexican beetle, *Z. bicolorata* on congress grass, *P. hysterophorus*

Egg

My present research concluded the average incubation period as 4.53±1.04 days (table 2). which was very similar to the result of Pawar *et al.,* (2015) [13] and the hatching percentage of my research came out to be 78.72 ± 0.50 similar with the result of Bhoopathi, (2006) [2], which was 84.40±4.81. the mean length of the egg of my present research was 1.33±0.20 mm (table 1), which was indeed very close to 0.59 ± 0.03 mm (Siddhapara *et al.,* 2012) [12]. The mean breadth of egg was 0.53± 0.02 mm that showed almost similar result with that of Siddhapara *et al.,* (2012) [12].

First instar larva

The mean duration of first instar larva was 4.49±0.93 days (table 2) according to the present research and it showed similarity with the value 4.40±0.49 days produced by Siddhapara *et al.,* (2012) [12]. The 1st instar larva was 1.52 ± 0.11 mm in length and 0.63 ± 0.08 mm in breadth (table 1), which was near to the value of 0.55 ± 0.02 mm and 1.61 ± 0.14 mm respectively (Pawar *et al.,* 2015) [13].

Second instar larva

The mean duration of the second instar larva was 2.82±0.78 days (table 2) and exhibited a close relation with 2.88 ± 0.33 (Siddhapara *et al.,* 2012) [12]. The morphometric mean data of my present research turn out to be 2.50 ± 0.21 mm in length (table 1) that was in between the value of 2.38 ± 0.26 mm (Siddhapara *et al.,* 2012) [12] and 2.84±0.12 mm (Pawar *et al.,* 2015) [13]. The mean breadth of the first instar larva was 0.73 ± 0.11 mm (table 1) that showed close value with 0.71 ± 0.03 mm (Siddhapara *et al.,* 2012) [12].

Third instar larva

The average duration of the third instar larva according to my present research was 3.40±0.63 days (table 2) and it had resembled a in between value with 2.40 ± 0.49 days (Siddhapara *et al.,* 2012) [12] and 3.93±0.81 days (Pawar *et al.,* 2015) [13]. In the present research the larva had a length and breadth of 4.47 ± 0.39 mm and 2.23±0.14 mm respectively (table 1) that showed a closer value with 4.11 ± 0.06 mm and 2.03 ± 0.06 mm respectively (Bhoopathi, 2006) [2].

Fourth instar larva

The fourth instar larva had a mean duration of 4.60±0.74 days (table 2) which was far from the value of 3.48 ± 0.73 days (Siddhapara *et al.,* 2012) [12] and 3.80±0.94 days (Pawar *et al.,* 2015) [13] and was lesser than 8.05 ± 0.76 days (Bhoopathi, 2006) [2]. This may be due to the seasonal difference that prevail over Varanasi region, having been impelled with hot and humid climate. The morphometric data of the 4th instar larva with mean length (5.72 ± 0.49 mm) (table 1) which was close to 5.63 ± 0.53 mm (Siddhapara *et al.,* 2012) [12], and mean breadth (3.44±0.56 mm) (table 1) fell in between 3.05 ± 0.07 mm (Bhoopathi, 2006) [2] and 4.05±0.13 mm (Pawar *et al.,* 2015) [13].

Pupa

The pupa remained in the soil for 8 -12 days according to my present research which was similar to the findings of Jayanth & Geetha, (1993) [10]. The pupa measured 6.05 ± 0.37 mm in length that lied in between the value of 5.65 ±0.11 mm (Siddhapara *et al.,* 2012) [12], and 6.55±0.11 mm (Pawar *et al.,* 2015) [13], (Pawar *et al.,* 2015) [13]. And the breadth of the pupa was 3.68 ± 0.83 mm in my research that had close relation with the mean value of 3.71 ± 0.07 mm (Bhoopathi, 2006) [2].

Adult

The characteristics and morphological feature of the adult Zygogramma beetle was explained by many scientist and those explanations matched with the findings of my present research like the elytra of the adult Elytra is marked with dark brown longitudinal lines that was also observed by Aherkar *et al.* (1992) [1], Jayanth (1996), Pandey *et al.* (2001) [3], Saruk (2001) [14] and Parise *et al.* (2010) [4]. The posterior margin of the last visible abdominal ventrite is 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 (McClay, 1985). In my present research the dimension of the male beetle was 6.05±0.19 mm in length and 3.15±0.16 mm in breadth which was in accordance with the values such as 5.91 ± 0.10 mm in length and 2.97 ± 0.13 mm in breadth respectively (Siddhapara *et al.,* 2012) [12]. The dimension of the female beetle 6.21±0.34 mm in length (table 1), in my research was in between the value of 5.66 ± 0.15 mm (Siddhapara *et al.,* 2012) [12] and 6.64±0.08 mm (Pawar *et al.,* 2015) [13] in length and the breadth of 3.25±0.39 mm (table 1) lie in between the value of 2.50 ± 0.10 mm (Siddhapara *et al.,* 2012) [12] and 3.71±0.09 mm (Pawar *et al.,* 2015) [13]. The adult male longevity in my research was 34.20 ± 8.05 days and that of female longevity was 48.42 ± 7.33 days that was in close relation with the result of Siddhapara *et al.,* (2012) [12] (32.40±8.05 days and 44.53±7.33 days for male and female respectively).

So, the total life cycle of the *Zygogramma bicolorata* stretched to a mean duration of 25 – 29 days in the context of my present research.

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