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Biology and morphometric measurement of cowpea weevil, *Callosobruchus maculatus* fabr. (Coleoptera: Chrysomelidae) in green gram

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ABSTRACT

A study was conducted in the Laboratory of Entomology, D.M. College of Science, Imphal during May to June, 2013 at 28.5 ± 2.0 °C and $78.5 \pm 3.0\%$ relative humidity to study the biology and morphometry of *Callosobruchus maculatus*. The life cycle consisted of egg, four larval instars (L₁, L₂, L₃ and L₄), pupa and adult. The eggs hatched in 6-7 days. The larval duration varied from 18-22 days. The morphometric measurements revealed that the average length and breadth of egg were 0.47 ± 0.08 mm and 0.12 ± 0.21 mm respectively. The mean length and breadth of L₁, L₂, L₃ and L₄ were 0.60 ± 0.03 mm, 1.19 ± 0.05 mm, 2.43 ± 0.15 mm, 3.64 ± 0.18 mm and 0.22 ± 0.03 mm, 0.77 ± 0.02 mm, 1.22 ± 0.08 mm, 2.00 ± 0.11 mm respectively. The average length of male pupa and female pupa was 4.07 ± 0.00 , 4.57 ± 0.07 mm and breadth was 2.35 ± 0.07 mm, 2.60 ± 0.06 mm respectively. The average length and breadth of male and female adult was 3.21 ± 0.06 mm, 3.70 ± 0.12 mm and 1.91 ± 0.05 mm, 2.17 ± 0.05 mm respectively. The life cycle took 45-48 days for completion.

Keywords: biology, instar, relative humidity, morphometric measurement, *Callosobruchus maculatus*.

1. Introduction

The cowpea weevil, *Callosobruchus maculatus* Fab. (Coleoptera: Chrysomelidae), is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram, and black gram [1, 2, 3, 4]. It is a holometabolic insect with the egg and adult stage found on the grain and the larval and pupal stages living inside the grain. The larvae bore into the pulse grains and eat up the endosperms which become unsuitable for human consumption with reduced viability for replanting or for the production of sprouts. They are important pests of pulse crops in Asia and Africa under storage conditions [1, 2, 3, 5, 6]. In India the insect breeds freely from March to November and hibernates in the larval stage during winter. The pest causes maximum damage during February to August when all its developmental stages exist simultaneously [7]. It was used as a model organism in many biological laboratories due to its quick generation time and ease of maintenance. Such type of work has not been studied adequately in Manipur and review in this context was also very limited. So, the present study was carried out on green gram under laboratory conditions to reveal its biology by morphometric measurement of egg, larva, pupa and adult for finding out the suitable control measures.

2. Materials and Methods

2.1. Test insects and maintenance

A study on the morphological measurement of *C. maculatus* was conducted in the Laboratory of Entomology, D.M. College of Science, Imphal during the period of May to June, 2013 at 28.5 ± 2.0 °C and $78.5 \pm 3.0\%$ relative humidity.

C. maculatus was collected from grocery shop of Imphal West. The culture was maintained on green gram with 12% moisture content kept in plastic jar of 3 kg capacity. The equal sex ratio of 1-2 day old adults was released into a petri dish of 9 cm size for egg laying. The bruchid beetle, *C. maculatus* was cultured on green gram in petri dish. The freshly emerged adults were allowed to lay eggs on green gram for 24 hours. A pill cutter was used to make an initial cut adjacent to the bean and pin was used as leverage at the areas where the eggs were laid. A scalpel was also used for the initial incision creating a two sided cross section into the bean. The dissected portion

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was then examined to find the larvae. Remaining grain was deposited for observing the developmental stages of the beetle. Then the grain was bound by cello tape as the earlier one for observing the next stages of development. The observation was made in 3 days interval to confirm the stage of development. The observations on development, temperature and relative humidity were recorded every day and carefully observed for the size of egg, larva, pupa and adult. For the measurement of length and breadth of the different stages, a digital calliper was used. The experiment

was conducted using standard mean deviation with five replications. The data was analysed statistically.

3. Results and Discussion

3.1. Biology of *C. chinensis*

The details of the developing stages are presented in Table 1. The different stages of *C. maculatus* from egg to adult are shown in Plate 1. From the laboratory data, the life cycle of *C. maculatus* was studied.

Table 1: Morphological measurement of *C. maculatus* stages

Life stage	Duration (Days)	Length (Mean ± SE) (mm)	Breadth (Mean ± SE) (mm)	Average Temperature		Average Relative Humidity (%)
				Min (°C)	Max (°C)	
Egg	6-7	0.47±0.08	0.12±0.21	18.1	27.14	79.5
1 st instar larva	8-9	0.60±0.03	0.22±0.03	20.7	29.4	76.22
2 nd instar larva	3-4	1.19±0.05	0.77±0.02	22	31.5	75.5
3 rd instar larva	3-4	2.43±0.15	1.22±0.08	22	30	79.5
4 th instar larva	4-5	3.64±0.18	2.00±0.11	25	32	71.42
Pupa male	6-7	4.07±0.00	2.35±0.07	22.6	32.33	76.5
Pupa female	5-6	4.57±0.07	2.60±0.06	22.6	32.33	76.5
Adult male	9-12	3.21±0.06	1.91±0.05	13.7	27.1	77.9
Adult female	10-14	3.70±0.12	2.17±0.05	12.1	27.9	71.7



Fig 1a. Young eggs laid on the surface of the seed.



Fig1b. Development of young larva head



Fig 1c. Young larva are formed within the egg.



Fig 1d. Egg fills with frass from the larva.



Fig 2a. Newly emerge young larva.



Fig 2b. Larva penetrating the seed coat.



Fig 2c. Larva feeding inside the seed endosperm

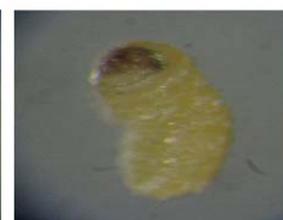


Fig 2d. First instar larva.



Fig 3a. 2nd Instar larva.



Fig 3b. 3rd Instar larva.



Fig3c. 4th Instar larva.



Fig 3d. Different larval stages.



Plate 1: Life cycle of *Callosobruchus maculatus*

Egg: The egg was small, glued on the grain singly but many eggs could be seen on a single grain. The eggs when freshly laid, were translucent smooth and shining, which later became yellowish white and very prominent against the contrasting background of the grain surface. The duration of egg ranged from 6 – 7 days. It was measured 0.47 ± 0.08 mm in length and 0.12 ± 0.21 mm in breadth [8]. Reported that egg was 0.75 mm in length. The average minimum, maximum temperature and humidity was 18.14 °C, 27.14 °C and 79.5% respectively.

First instar larva: Upon hatching, they bite through the base of the eggs and bored into the seeds. The first instar larva was formed within the eggs prior to penetrating the seed coat with a convenient visual marker signalling that embryonic development was nearly complete. After the formation of the pigmented larval head capsule they borrowed from the egg through the seed coat and entered into the bean endosperm. The remaining egg shell became opaque white or mottled as it was filled with frass from the larva. The larva was curve, white, and had a small head. The larval period ranged from 8-9 days [9]. Reported that the larvae hatched out of egg and burrowed straight into the bean about 4-8 days after oviposition. The length of larva measured from 0.52 to 0.69 mm with an average of 0.47 ± 0.08 mm and the breadth varied from 0.20 to 0.26 mm with a mean of 0.22 ± 0.03 mm respectively. The average minimum, maximum temperature and humidity was 20.7 °C, 29.4 °C and 76.22% respectively.

Second instar larva: The 2nd instar larva burrowed and fed on the bean endosperm. The larval duration ranged from 3-4 days. The length of 2nd instar larva was 1.00 to 1.3 mm with an average 1.19 ± 0.05 mm and the breadth was 0.68 to 0.8 mm with an average

0.77 ± 0.02 mm respectively. The average minimum, maximum temperature and humidity were 22 °C, 31.5 °C and 75.5% respectively.

Third instar larva: The 3rd instar larvae were most active and fed on the entire endosperm voraciously. The larval period ranged from 3-4 days. The length of the 3rd instar larva was 2.10 to 2.99 mm with an average of 2.43 ± 0.1 mm and the breadth was 0.95 to 1.50 mm with a mean of 1.22 ± 0.08 mm respectively. The average minimum, maximum temperature and humidity were 22 °C, 30 °C and 79.5% respectively.

Fourth instar larva: The larva was white, yellowish and somewhat C-shaped with a small head. The 4th instar larvae were similar to 3rd instar but differed in size and shape. In this instar the larva seemed to become larger and fed on the entire endosperm voraciously. It burrowed into a position just underneath the seed coat prior to pupation. The duration of the larva ranged from 4-5 days. The body length of the 4th instar larva was 3.9 to 4.06 mm with an average of 3.64 ± 0.18 mm and the breadth was 2.00 to 0.11 mm with an average of 2.00 ± 0.11 mm respectively. The average minimum, maximum temperature and humidity were 25 °C, 32 °C and 71.72% respectively.

Pupa: During the time of pupation larval structures were broken down and adult structures developed; the rudiments of the wings appeared at the first day, at second day appendages such as legs, antenna and proboscis developed freely and at 3rd day eyes, mouth part, forewing, hind wing and legs with cuticular hair developed. At 4th day almost all the parts developed but intersegmental region of the abdomen remained colourless and forewing was light green

in colour. At 5th day forewings changed into dark brown with black patches. The male and female pupal period ranged from 6-7 days and 5-6 days respectively. The length of male pupa was 4.07±0.00 mm and breadth was 2.23±0.07 mm. The length and breadth of female pupa was 4.57±0.00 mm and 2.60±0.07 mm respectively. The average minimum, maximum temperature and humidity was 22.6 °C, 32.33 °C and 76.5% respectively.

Adult: After pupation, the adult remained in the cell for several days before pushing or biting out the window with its mandibles. The adult emerged by chewing and removing a circular piece of the seed coat to form a round hole. The adult elytra were shorter in comparison to the rest of its body leaving the last segment of the abdomen exposed. Adults were oval in shape, chocolate or reddish brown in colour, with long and erected antennae. The adult male was smaller and possessed a more round shape than the female whereas female adults had dark stripes on each side of dorsal abdomen. The adult male and female period ranged from 9-12 day and 10-14 days respectively^[10]. Reported that adults have a mean lifespan 7 days in lab environment, but some have been able to live up to 14 days. The length of male moth was 3.21±0.06 mm and breadth was 1.91±0.05 mm whereas the length and breadth of female moth was 3.70±0.1 mm and 2.17±0.05 mm respectively^[11]. Reported that the average body length of adult beetle was 4-6 mm. The average minimum, maximum temperature and humidity was 13.7 °C, 27.9 °C and 77.9% respectively. The results of the study show that the developmental period of the egg to adult was 45-48 days. It took one and half month to complete its life cycle, which could take longer under unfavourable conditions depending on the food supply, temperature and humidity. This beetle could breed throughout the year.

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