

Study of the embryonic development of the pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) eggs laid on cowpea (*Vigna unguiculata*) seeds

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Abstract

A short-term experimental study was undertaken for a week in the laboratory to study the development of the pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) during the incubation period of its eggs laid on cowpea (*Vigna unguiculata*) seeds. The eggs laid on the pulse seeds were observed through the stereo zoom microscope and its pictures taken at the same resolution, daily for a period of one week with the help of smartphone camera. The compiled photomicrographs have been documented in this research paper. This small-scale study was undertaken by an undergraduate student, and minimal resources were used. This information can be used by researchers for designing experimental bioassays aimed to inhibit the growth and development of this economically important insect pest of pulses, and contribute towards its management in the initial stages of its development itself so as to prevent further damage. Experiments are in progress to study the efficacy of botanicals as ovicidal compounds, which can be used in the management of *C. chinensis*.

Keywords: Pulse beetle, *Callosobruchus chinensis*, embryonic development, cowpea, lobia seeds, *Vigna unguiculata*

Introduction

Globally, food legumes primarily consisting of chickpea, lentil, cowpea, field pea, green gram, black gram, French bean, faba bean, and grass pea, constitute a major component of the nutritional diet. Pulses are an essential nutritional source for millions of people worldwide, especially those in developing and least developed nations (Guru *et al.*, 2022) [2]. Pulses, being the rich source of protein, several amino acids, minerals and certain vitamins, constitute a key component of vegetarian diet of common people of Asian countries including India. The year 2016 was declared as the 'International Year of Pulses' by the Food and Agriculture Organization (FAO), United Nations to increase awareness of the nutritional benefits of pulses for sustainable production for food security and world nutrition. Pulses are also known as "Poor man's meat" as they are a rich source of protein, and reasonably priced (Sharma, 1984) [8]. On an average, an adult person needs to consume only about 56 g of pulses (Das, 2016) [1]. Unfortunately, the pulses are very much vulnerable to attack and consequent damage by insect pests. Pulse beetle (Coleoptera: Chrysomelidae) is a notable insect pest of green grams, garden peas, chickpeas, cowpeas, lentils, black grams, and pigeon peas. The pulse beetle, *Callosobruchus chinensis* L. is one of the most serious insect pests of stored pulses as it can cause huge quantitative and qualitative losses and also decrease the nutritional values of stored grains/pulses. It being a rapid and prolific breeder, causes serious qualitative and quantitative damage by reducing the nutritive value of stored grains, and renders it unfit for human consumption. This insect is cosmopolitan in distribution but is more widely spread throughout the tropical and sub-tropical regions (Mensah, 1986) [6]. The routine measures used to protect the pulses from stored grain pests include the use of synthetic chemical insecticides and fumigants, which are the sources of toxic residues in food, cause potential health hazards, and environmental pollution (Kim *et al.*, 2017) [3]. The present study was undertaken to comprehend the

embryonic development of *C. chinensis* before conducting some experiments towards developing some safe, economical and effective ovicidal compounds so as to prevent the population build-up of this serious pest of pulses, and the consequent economic damage.

Materials and Method

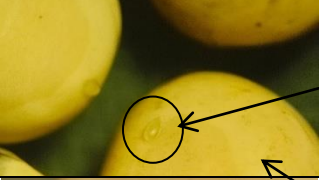


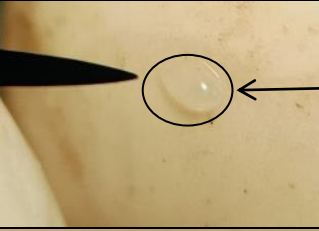

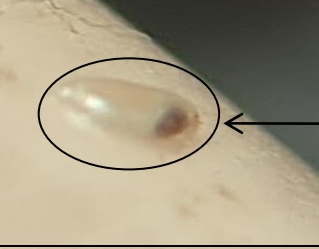
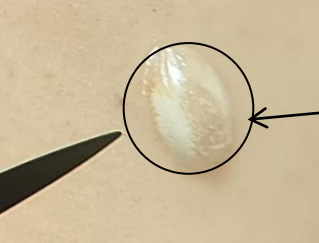
The culture of pulse beetle was maintained on the cowpea (*lobia*), *Vigna unguiculata* seeds procured from the grocery store in the local market in the University of Delhi area. The insects were reared in transparent plastic containers normally used for storing pulses. Freshly emerged adult beetles were removed from the stock culture and used for the experiment. For the experiment, 3 plastic dibbis/ petri dishes (dia. 4 cm) with tight-fitting lid were taken, which were sterilized by wiping its inner surface (bottom and lid) clean with a piece of absorbent cotton moistened with 70% EtOH. It was allowed to dry and then fresh lobia seeds were spread in a single layer at the bottom of the plastic dibbis, which were properly labelled. A pair of freshly emerged adults were transferred to these containers with the lids properly fastened to the dibbis, before putting them in the B.O.D. incubator maintained at $30\pm 2^{\circ}\text{C}$, 60% R.H. and 16hL:8hD conditions. After noting the oviposition on lobia seeds, the adults were removed and used for culture. Observations on the embryonic development of the pulse beetle were taken daily (for a period of 7 days) after focussing the experimental plastic dibbis under the stereo zoom microscope, and photographs captured with the help of the smartphone attached to the microscope's eyepiece with the help of a mobile holder. Pictures of eggs laid on lobia seeds were taken daily for all the 3 replicates set up for the experiments. The recorded pictures were transferred to the computer and then compiled. The details are depicted in Table 1 below. The observations could only be taken for a period of 7 days only since the larvae penetrate the seed for further development in the protective environment of seeds which also provide all the nutrients required for the larval

growth and development for about 15 days (nearly a week before pupation during which there is no consumption of food). Normally, the adults emerge out of the seeds after completing the developmental process of 27-30 days (as observed in the insect's culture), although this experiment

was confined to taking the observations of development of embryonic development only during the incubation period of eggs for 7 days.

Conclusion

Table 1: Observations of the embryonic development of pulse beetle, *Callosobruchus chinensis*, during the incubation period of its eggs laid on cowpea (*lobia*) seeds.

Observation Day	Photographic record of embryonic stage of pulse beetle on cowpea (<i>lobia</i>) seeds	General description
1		Egg encased in the protective shell called 'chorion' attached to the seed coat of cowpea seed.
2		The bottom most seed out of the 3 seeds in the above picture was followed for studying development by zooming on that particular seed daily.
3		An oval patch on the seed surface beneath the egg was observed.
4		Significant growth and development of larva within.
5		Blackhead stage: Head capsule of first-instar larva is visible
6		Increase in size of the first-instar larva.
7		Larva almost hatched and partially penetrated the seed as its anterior end (head capsule) is not visible.

As per the details in Table I, the freshly laid eggs of the pulse beetle on the surface of cowpea seeds, and the progress of embryonic development could be monitored daily and captured through the mobile phone (camera) installed on the stereo zoom microscope. Normally, only a single egg was laid on each seed. Various morphological changes in the growing embryo (larva) were visible through the transparent chorion, and observed through the stereo zoom microscope, during the incubation period of 6-7 days, as stated below.

- Briefly after the oviposition, the eggs appeared to be oval, translucent and stuck firmly to the seed surface.
- Shortly after oviposition, the cleavage and mitosis began inside the egg, forming a mass of cells referred to as Blastoderm.
- The blastoderm later developed into a germ band, which is the early embryonic structure. Segmentation also became visible on the germ band.
- The 'blackhead stage' of the larva with the prominent head capsule was clearly visible. This stage indicates that hatching process would be complete in the next 24 hours.

The larvae successfully hatched and penetrated the lobia seeds, leaving the hollow egg shell behind on the seed surface, after 6 days of oviposition.

It has been reported in scientific literature that the incubation period, larval and pupal period and total developmental period of *C. chinensis* varies from 3 to 6 days, 21-26 days and 31 to 42 days, respectively (Limma *et al.*, 2022) [5]. Latha and Tayde (2022) [4] conducted studies on biology and morphometrics of pulse beetle, *C. chinensis* (L.) on stored cowpea under laboratory conditions and reported that that eggs incubation period ranged from 3-5 days, the grub stage consisted of four instars and its development ranged from 15-26 days, pupation lasted for 7-10 days, whereas, female adult longevity ranged from 8-12 days, male adult longevity ranged from 7-11 days.

The short-term experimental study conducted by us in the laboratory for about 8-9 days since the setting up of the experiment resulted in key insights about the embryonic development of the pulse beetle, *C. chinensis* in a non-invasive and non-destructive manner with the help of simple instruments like stereo zoom microscope, and the smartphone available with everyone these days. And, it can be easily performed by even any undergraduate student of biological sciences, under proper guidance and supervision. The incubation period of *C. chinensis*, which varies with the pulses used and the environmental conditions for rearing, ranged from 5-7 days which is in consonance with earlier reports (Sekar *et al.*, 2021) [7]. The results of this study can be utilized to evaluate the efficacy of botanicals as potent ovicidal compounds or in inhibiting the embryonic growth and development of *C. chinensis*, and prevent the build-up of its population up to the Economic threshold/ Economic injury level. This experiment can be easily replicated with any other pulses or with any other stored grain pest, and extrapolated to study the developmental process in greater detail by using advanced instruments, and contribute

towards the management of this economically important insect pest of pulses.

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