Doi: HTTPS://DOI.ORG/10.23910/2/2023.4937a

Biology and Morphometrics of Pulse Beetle, *Callosobruchus chinensis* L. on Chickpea

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Article History

Article ID: IJEP4937a
Received on 29th September, 2023
Received in revised form on 08th November, 2023
Accepted in final form on 23rd November, 2023

Abstract

A study was conducted during year 2022–23 for a period of six months for several generations in controlled laboratory environment at the College of Agriculture, Iroisemba, Imphal, Manipur India on the biology and morphometrics of the pulse beetle, *Callosobruchus chinensis*, on GNG 2207 chickpea cultivar. The experiments were conducted within a BOD incubator, maintaining a constant temperature of 30±1°C and relative humidity ranging from 70±1 %. Life cycle of *C. chinensis* encompassed four distinct stages: egg, grub, pupa, and adult. The collected data unveiled essential insights such as the egg incubation period spanned 5–8 days, while grub stage consisted of four instars, taking 16–21 days for complete development. Pupation occurred over a period of 8–9 days. Female adult displayed a longevity range of 8–10 days. In terms of morphometric measurements, the average dimensions for eggs were found to be 0.60±0.05mm in length and 0.35±0.02 mm in breadth. The respective lengths and breadths (mm) for the four grub instars, G1, G2, G3, and G4, were as follows: 0.55±0.05 and 0.30±0.02, 1.56±0.09 and 0.91±0.06, 2.68±0.07 and 1.45±0.08, 3.62±0.16 and 1.93±0.08. Pupa measurements yielded an average length and breadth of 3.76±0.22 mm and 2.21±0.05 mm, respectively. Moreover, the dimensions (mm) of adult male and female bruchids were found to be 3.88±0.07, 2.31±0.07, and 4.19±0.10, 2.12±0.11, respectively. The comprehensive life cycle of *C. chinensis* spanned from 38 to 42 days, encapsulating the various developmental stages.

Keywords: Pulse beetle, chickpea, GNG 2207, larva, pupation, morphometric

1. Introduction

Pulses, often referred to as the "Wonderful gift of nature," hold a significant position in both the Indian economy and diet and known as 'poor man's meat' Hossain et al. (2014). Grain legumes are popularly known as pulses Bharathi et al., (2017). They form a crucial component of Indian agriculture, following cereals and oilseeds. Chickpea (*Cicer arietinum* L.) is a good source of energy, protein, minerals, vitamins, fibre, and also contains beneficial phyto-chemicals (Wood and Grusak, 2007). Serving as a primary source of dietary protein and essential vitamins, pulses belong to the Leguminosae family, which encompasses various vital agricultural and food crops. Chickpea stand as a primary source of vegetable protein in the human diet, boasting a protein content of 21% along with carbohydrates ranging from 38% to 59%. The Indian Council of Medical Research recommends 65 g/day/person, the projected availability of pulses has decreased from 70.10 g day^{-1} $person^{-1}$ in 1951 to 54.70 g day^{-1} $person^{-1}$ in 2016–17 Ready et al. (2012).

Chickpea (*Cicer arietinum* L.) is a noteworthy leguminous crop cultivated widely in dry and rainfed areas worldwide, especially in regions like India, South Asia, West Asia, and Southern Europe, catering to vegetarian dietary protein needs. During 2021–22, India saw 13.75 mt of chickpea production from 10.91 mha, with productivity of 12.6 q ha^{-1} Anonymous (2023). Pulse beetle, *Callosobruchus* spp. is an important primary pest and major constraint Mounika et al. (2021), Duan et al. (2014). India reports an 8.5% loss due to the beetle, causing 10–95% seed weight loss and 45.5%–66.3% protein loss during storage Paikaray et al. (2022*)*. The pulse beetle *C. chinensis*, a Coleoptera from the Bruchidae family, leads to 40–50% pulse losses during storage. Gosh and Durbey (2003). Rapidly damaging seeds up to 30%, *C. chinensis* caused a 15–17% loss in Indian chickpea storage. Parameshwarappa et al. (2007).

Worldwide, the *Callosobruchus* genus poses a threat to grain legumes both before and after harvesting. The most destructive bruchid species to chickpea are *C. chinensis* and *C. maculatus* Mishra et al. (2015). These grain insects spend their entire immature life within individual legume seeds, leading to weight loss, reduced germination potential, and a decrease in the commodity's market and nutritional value. Particularly, *C. chinensis* Linnaeus, commonly known as the pulse beetle, inflicts significant losses on various pulse varieties during storage. The grain characters, which also interfere the normal physiology or feeding of the insect, affects the biology of the pest adversely and these make a variety resistant to insect

attack Jat et al. (2013)

The extent of damage varies depending on the type of legumes, the duration of exposure, storage facilities, and other seed-related factors, Srinivasan and Durairaj (2007). They multiply rapidly in suitable environmental conditions such as high humidity and optimum temperature (Appleby and Credland, 2004).

The duration of various life stages also varies among the different types of pulses studied by Mehta and Negi (2020) as well as different varieties describing the varietal preference of the pulse beetle studied by Singh et al. (2016). Jaiswal et al. (2019) and Kamble et al. (2016).

Over 150 insect pests afflict pulse crops, significantly affecting seed weight (55–60%) and protein content (45.5–66.3%) in pulses like pea, cowpea, pigeon pea, adzuki bean, and lentil Hosamani et al. (2018). Post-harvest and storage losses in Indian pulses are estimated at 8.5% Rahman et al. (2010) despite their role in enriching soil with atmospheric nitrogen. Pulse beetle infestations peak in storage during July-August. In order to direct the specific management practices as well as feeding behaviour this study was conducted to know the biology and morphometrics of pulse beetle.

2. Materials and Methods

To study biology of *Callosobruchus chinensis* L. in storage, a laboratory experiment was carried out in the Department of Entomology, College of Agriculture Iroisemba, CAU Imphal, Manipur, India on chickpea variety GNG 2207 during 2022–2023. The experiments were conducted at a temperature of 30±1°C and relative humidity of 70±5%. The data obtained are then analysed statistically by Completely Randomized Design.

2.1. Identification of test insect

The adult beetles were identified following taxonomic keys Hackston (2016). Notable features included chocolate reddish-brown coloration, distinct antennae differences between males (pectinate) and females (serrate), ivory-like spots on the body, and characteristic bands on the elytra (Figure 1).

2.2. Collection and maintenance of test insect

The pulse beetle adults were collected from infested chickpea seeds in the storage section of the Department of Seed Science at the College of Agriculture, CAU Imphal. These adults were identified and placed in plastic containers with sterilized chickpea grains. The containers were covered with muslin cloth and kept in a controlled B.O.D. chamber, with fresh grains periodically provided. Regular observation prevented infections, and dead adult beetles were removed periodically.

Female adult beetle Male adult beetle

Figure 1: Antennae of male and female pulse beetle

2.3. Procurement and preparation of seed

For the research purpose Chickpea were provided from AICRP chickpea with the recommendation of Department of GPB, COA, CAU Imphal. The grains were then cleaned made for sun drying for three days to get healthy grain for experiment purpose. The grains were properly inspected for any other insect infestation or any other types of secondary infestation. Later the grains were subjected to fumigation with help of phosphine after a period of 72 hrs and they were kept in open for another 24 hrs to get rid of any residual effects.

2.4. Biology of Callosobruchus chinensis L. in chickpea

To investigate the biology of the test insect, *Callosobruchus chinensis*, in relation to chickpea, we employed six plastic containers, each with a capacity of 200 grams and containing 100 grams of seeds. The lids of these containers were covered with muslin cloth and tied with a rubber band to check insect escape and also to ensure adequate aeration for the insects. On the subsequent day, ten pairs of male and female *C. chinensis* were selected from the uniparental culture and introduced into each of the six containers containing fresh chickpea seeds to facilitate egg laying. Proper labelling was applied to the containers, and they were maintained under standard laboratory conditions. The following morning, grains for pulse beetle oviposition were examined. Eggs laid daily on grains from the initial three containers were isolated in Petri dishes, and observations on various biological parameters were documented. For the remaining three containers, left undisturbed throughout the study to avoid potential disruption or damage to eggs, we recorded the multiplication rate. This precaution was taken to prevent any interference during the observation and handling of eggs from the first three containers, as it could potentially disturb adult emergence and compromise the integrity of the data.

Observations were made on various biological aspects including fecundity, incubation period, larval and pupal periods, oviposition and post-oviposition periods, sex ratio, adult emergence, and adult longevity. Measurements of egg, larval, pupal, and adult dimensions were taken using Stereoscopic zoom Leica Microscope

2.4.1. Fecundity

A pair of beetles was observed daily, counting eggs laid by each female on chickpea grains until death

2.4.2. Egg incubation period

The time taken for the transition from egg laying to hatching was determined by the change in color of the egg, specifically the shift to an opaque or creamish white hue. This

transformation was attributed to the accumulation of bored material inside the egg.

2.4.3. Larval and pupal period

Following hatching, the larva burrowed into the seed, rendering the eggshell empty, and underwent its larval and pupal stages within the seeds. The pupal stage was indicated by the formation of a net or circular translucent exit hole on the seed's surface. However, documenting the precise larval and pupal periods within the seed for the respective season proved challenging. As a result, the data were recorded as the duration of time taken from egg hatching to adult emergence. Meanwhile the observations for each instar were taken by checking on the seed by immersing them in the water for few hours which was later broken and the larvae was exposed. On the basis of previous researches, the chickpea was subjected for observation at different intervals of time.

2.4.4. Oviposition period

The duration from start to end of egg laying by the female beetle was observed. The interval from the emergence of the female to the initiation of egg laying was defined as the pre-ovipositional period. The duration from the commencement of egg laying to its cessation was recorded as the ovipositional period, whereas the timeframe from the cessation of egg laying to the death of the female was categorized as the post-ovipositional period.

2.4.5. Sex ratio and adult emergence

Oviposited grains were examined for emerged adults to determine sex ratios based on size and antennae differences.

2.4.6. Adult longevity

Freshly emerged male and female beetles were observed for longevity in separate containers with chickpea grains.

2.4.7. Hatchability percentage

A total of hundred numbers of eggs on seed were observed separately in a Petri dish and hatchability percentage was recorded using the formula:

=(Number of hatched eggs/Total number of eggs observed) ×100

2.4.8. Developmental stages measurement

Grubs hatched from soaked chickpea seeds were examined under a microscope to measure the length and width of different developmental stages of *C. chinensis*.

This comprehensive study investigated various aspects of *Callosobruchus chinensis* behaviour and biology on chickpea, providing valuable insights into its interactions and development.

3. Results and Discussion

3.1. Biology of pulse betle

3.1.1. Fecundity

Females typically laid one to three eggs per seed which were white in colour with oval in shape, smooth and translucent in appearance. sometimes depositing additional eggs on a single seed. Eggs were occasionally found scattered, even on container edges. Fecundity ranged from 84 to 93 eggs per female, with an average of 88.80±4.08 eggs which is in accordance with the findings of Kumari et al. (2020) found fecundity in the range of 71–87 with a mean of 78.93±4.83. The highest number of eggs was laid on the second day after adult release, with the first day following closely. Subsequently, the number of eggs laid gradually decreased until the conclusion of oviposition, with no eggs laid on the sixth day. The female retained the ability to lay eggs until the male bruchid's death, even though it remained alive until the tenth day (Table 1). Following the male's demise, the female's oviposition gradually diminished and eventually ceased. The observed increase in egg laying also indicated that chickpea is the preferred host for depositing a larger number of eggs.

3.1.2. Incubation period

Eggs were firmly attached to seed surfaces. Initially transparent and cream-colored, eggs turned grayish-white before hatching. The average incubation period was 4.18±0.23 days, ranging from 3 to 4 days which is similar to reported by Augustine and Balikai (2019) 4–6 with an average of 4.60± 0.70 days. Varma and Anandhi (2010) reported a mean egg period of 4.0±1.0 in the range of 3–5 days. This variation is may be due to the climatologically differences especially temperature and relative humidity in the laboratory, geographical

location and host seed. The female of *C. chinensis* typically laid eggs individually, often depositing several eggs on a single grain, reaching a maximum of 6–10 eggs on a single grain. The eggs exhibited a cigar-shaped, oval form, were white in color, and possessed a smooth appearance. When freshly laid, the eggs were transparent and white, firmly adhering to the seed surface. As they approached hatching, the eggs transformed into a milky white color and became opaque, a result of the accumulated bored material inside the egg (Figure 2).

Figure 2: Microscopic image of unhatched egg and Hatched egg of pulse beetle on chickpea

3.1.3. Larval period

The emerging grub entered the seed, showing a curvy, creamy white appearance. The larva progressed through four instar stages, with an average stage length of 18.57±1.95 days, spanning from 16 to 21 days. This collaborated with the

findings of Sharma et al. (2018) who reported a larval period of 21.30 days. Augustine and Balikai (2019) reported a total grub period in the range of 14–23 with a mean of 18.20±3.58. The grub looks short, creamy color, apodous, with brown color head and 'C' shaped scarabeiform larvae. As the day advances it increases in length and width of its body by every change in instar. The observation are in accordance with Varma and Anandhi, (2010).

3.1.4. Pupal period

Pupation occurred beneath the seed coat after a circular hole was made by the fully developed larva. The pupal stage lasted 8 to 9 days on average (8.51±0.17 days). This is in accordance with the findings of Hosamani et al. (2018) who reported a pupal period of 7–8 days with an average of 6±0.39 days in chickpea. Singal and Borah (2001) reported pupal periods of 7–9 days on black gram and 7.2±0.18 days on pigeon pea, respectively. Pupa is the physically inactive stage; it is white to creamy color and obtect type. The indication of pupal stage was formation of circular translucent exit hole or circular window on the surface of the seed by the last instar grub. The observation are in accordance with Varma and Anandhi, (2010).

3.1.5. Egg-laying period

The egg-laying period spanned from 5 to 8 days, averaging 6.4±1.14 days. The highest egg deposition occurred on the second day of oviposition, gradually declining towards the end. It was in accordance with the result by Gopi and Singh, (2020) who reported the egg-laying period to be 5–7 days with an average of 5.80 days.

3.1.6. Hatchability percentage

The hatching of eggs was determined by monitoring the shift in the egg's color from translucent to white. This change to an opaque or creamish white hue resulted from the accumulation of bored material inside the egg. Not all eggs laid by the insect would hatch, primarily due to the competition arising from population density, where multiple eggs were deposited on each seed. Even if hatching occurred, it could lead to developmental malformations. To accurately record the hatchability of eggs, one egg per seed was selected, removing all other seeds with the assistance of forceps to eliminate competition. In this study, a total of 100 seeds were observed, each with one egg, Mature eggs exhibited an average hatching rate of 83.8±1.92, with a range of 81–86 eggs hatching. This was in accordance with Varma and Anandhi (2010) who reported 85.6% of hatchability.

3.1.7. Adult emergence

The final instar grub, just before pupation, created a distinct circular window through which the adult later emerged. During the emergence of the adult, the head protruded first, and the circular window was chewed and removed using its mandible. Adult beetles displayed a brownish coloration, an oval shape, and featured black, grey, and white patches. The elytra exhibited a pale brown hue with small dark patches in the middle. In females, the elytra were shorter compared to the rest of the body, allowing the tip of the abdomen to extend beyond the hard wing cover. The visible portion of the abdomen was white, marked with two black oval spots. Adults of this species were capable of flight. Sexual dimorphism was evident, with males having a pectinate type of antenna, while females exhibited a serrate type. The adult female appeared larger and heavier than the male. This observation with respect to adult beetle is in confirmation with Hosamani et al. (2018), Varma and Anandhi (2010), and Kumari et al. (2020) (Figure 3).

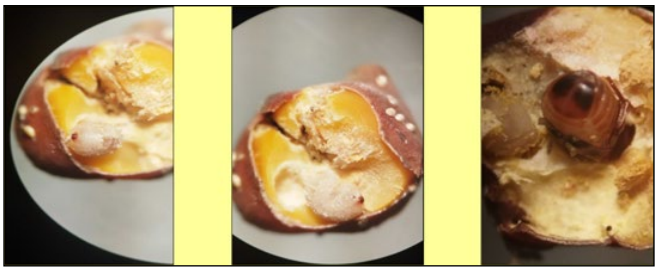

Figure 3: Feeding grub inside chickpea and emerging adult

3.1.8. Adult longevity

The average lifespan of female and male *C. chinensis* was 9.10±0.58 and 7.20±0.59 days, respectively. Ranging from 8–10 days (female) to 6–8 days (male), adult longevity varied. It was also in close accordance with the Udagi et al. (2021) findings who reported average life span of females in pigeon pea was 9.95±0.83 days. Augustine and Balikai (2019) reported adult longevity in Males to be in the range of 7–11 with a mean value of 8.30±1.25 whereas in females in the range of 8–12 days and a mean of 9.50±1.58 days. Patel et al. (2005) also reported adult longevity of 8 to 14 days having an average 11.75 days on greengram.

3.1.9. Total developmental period

The complete pulse beetle development lasted an average of 31.26±3.25 days, with a range of 29 to 33 days. This result is supported by the findings of Sharma et al. (2018) who reported a total developmental period of 33.30 days. Augustine and Balikai (2019) also reported the total developmental period in days to be 26–40 with a mean of 30.90±4.28. This difference in adult longevity may be due to difference in environment condition or difference in temperature and relative humidity in different geographical location and laboratory.

3.1.10. Total life cycle

Female and male *C. chinensis* exhibited average life cycle lengths of 40.36±3.00 and 38.46±2.94 days, respectively, varying from 38–42 (female) to 37–40 (male) days. Obtained data is in accordance with the one reported by Udagi et al., (2021) who stated that *C. chinensis* completed its life cycle in 38.50 to 49.00 days with a mean longevity of 48.53±1.98 days. Dalal et al. (2020) reported total life cycle of male and

female pulse beetle were completed in 39.03 and 42.97 days, respectively (Figure 4 and 5).

Figure 4: Pupa of pulse beetle

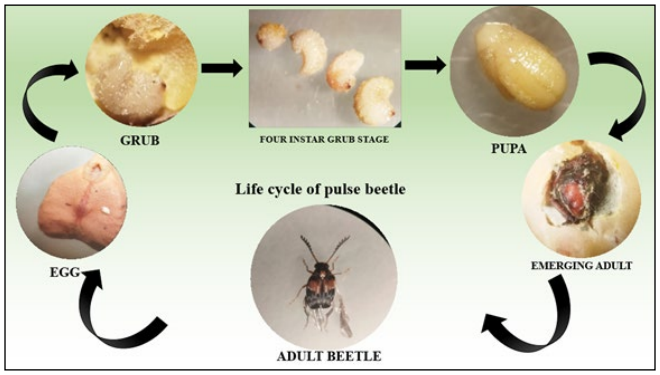

Figure 5: Life cycle of pulse beetle (*Callosobruchus chinensis*)

3.1.11. Sex ratio

The sex ratio (Female: Male) of *C. chinensis* was found to be 0.83:1.21, indicating the dominance of males over females in the beetle population. which is in accordance with the data reported by Kumari et al. (2020) on sex ratio with an average of 0.83:1.21 observed in mungbean.

These results provide a comprehensive understanding of the various developmental stages and behaviours of *Callosobruchus chinensis* on chickpeas, shedding light on its lifecycle and characteristics.

3.2. Morphometrics of pulse beetle

Morphometrics of pulse beetle was carried out on the GNG 2207 chickpea variety (Table 2).

3.2.1. Egg

The current study showed that the egg had a length and breadth of 0.60 mm±0.05 mm and 0.35 mm±0.02 mm respectively. This is in accordance with the result reported by Udagi et al. (2021) who reported that the mean length and breadth of the egg were 0.55 mm±0.02 mm and 0.33 mm±0.01 mm respectively.

Table 1: Biological parameters of pulse beetle on GNG 2207 variety of chickpea

Sl. No.	Parameters	Range	Mean±SD
1.	Egg Laying (days)	5.00–8.00	6.4±1.14
2.	Incubation Period (days)	3–4	4.18±0.23
3.	Larval Period (days	16–21	18.57±1.95
4.	Pupal Period (days	8–9	8.51±0.17
5.	Hatchability percentage	81–86	83.80±1.92
6.	Adult longevity female (days)	8–10	9.1±0.58
7.	Adult longevity male (days)	6–8	7.20±0.59
8.	Fecundity	84–93	88.80±4.08
9.	Total developmental period (days)	29–33	31.26±2.35
10.	Sex-ratio (F:M)	–	0.83:1.21
11.	Total life cycle (Female)	38–42	40.36±3.00
12.	Total life cycle (Male)	37–40	38.46±2.94

Table 2: Morphometrics of pulse beetle

Sl. No.	Parameters	Length (mm)±SD*	Breadth (mm)±SD*
1.	Egg	0.60±0.05	0.35±0.02
2.	1st Instar	0.55± 0.05	0.30±0.02
3.	2nd Instar	1.56±0.0.09	0.91±0.06
4.	3rd Instar	2.68±0.0.07	1.45±0.08
5.	4th Instar	3.62±0.16	1.93±0.08
6.	Pupa	3.76±0.22	2.21±0.05
7.	Female Adult	4.19±0.10	2.31±0.07
8.	Male Adult	3.88±0.07	2.12±0.11

*SD: Standard deviation

3.2.2. Grub

3.2.2.1. First instar grub

The size of the grub had a mean length and breadth of 0.55mm±0.05mm and 0.30 mm±0.02 mm respectively which is in tune with the data obtained by Augustine and Balikai, (2019) who reported that 1st instar measured 0.50±0.03 mm in length and 0.31 ±0.01 mm in breadth.

3.2.2.2. Second instar grub

The second instar grub was found to be 1.56±0.0.09 mm in length with a breadth of 0.91±0.06 mm. Data obtained are in strong accordance with Kota et al. (2022) who reported a length of 1.45 mm±0.06 mm and a breadth of 0.81±0.02 mm.

3.2.2.3. Third instar grub

Length of 2.68 mm ±0.0.07 mm and 1.45 mm±0.08 mm as its

breadth was observed during the present investigation. It is in accordance with Kota et al. (2022) who reported a length of 2.58 mm±0.10 mm and a breadth of 1.21 mm±0.03 mm when studied on the ICPL 161 variety of pigeon pea.

3.2.2.4. Fourth instar grub

The data obtained about the length and breadth of the last grub stage during the current investigation was 3.62 mm±0.16 mm and 1.93 mm±0.08 mm respectively. It is close proximity to Kota et al. (2022) who reported length and breadth to be 3.47±0.08 mm and 1.82 mm±0.08 mm respectively while studying on ICPL 161 variety of pigeon pea. It is somewhat close to Udagi et al. (2021) who reported a mean length of 2.58 mm±0.45 mm and breadth of 1.76 mm±0.29 mm

3.2.3. Pupa

During the current study carried out length and breadth of pupa were found to be 3.76 mm±0.22 mm and 2.21 mm±0.05 mm respectively. The pupal size recorded is in conformity with the Udagi et al. (2021) as well as Kota et al. (2022) where they reported length and breadth of 3.27 mm±0.34 mm, 1.97 mm±0.13 mm and 3.6 mm±0.11 mm, 2.1 mm±0.06 mm respectively (Figure 6).

Figure 6: Four instars grub stage of pulse beetle and pupa

3.2.4. Adult

The female adult had a length of 4.19 mm±0.10 mm and a breadth of 2.31 mm ±0.07mm whereas the male adult was found to be smaller than the female with a length and breadth of 3.88 mm±0.07mm and 2.12 mm respectively. This tunes with data reported by Augustine and Balikai (2019) who obtained the length and breadth of adult males 3.49 mm±0.25 mm, 1.81 mm±0.12 mm and females to be 4.00 mm±0.42 mm, 2.01 mm±0.13 mm respectively.

4. Conclusion

GNG 2207 variety of chickpea to know the various developmental stages. A total life cycle of 38–42 days in female and 37–40 in male which signifies those female lives slightly longer than male. Fecundity was found to be in the range of 80–95. The incubation period larval period pupal period was found to be 3–4 days,16–21 days and 8–9 days respectively. The sex ratio was found to be 0.83:1.21 which means female population is lesser than the male.

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