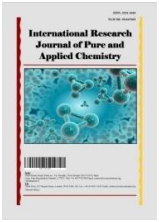

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Biology and Morphometrics of Pulse Beetle, *Callosobruchus chinensis* (L.) on Chickpea

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KS and JJ designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SPM and JJ revised the first draft. Authors SPM and AV managed the analyses of the study and performed the analysis. Authors BPK and DKR helped in finalizing protocol. Author KS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The biology and morphometrics of pulse beetle, *Callosobruchus chinensis* were studied on chickpea cultivar ICCV 2 under laboratory conditions at ICRISAT, Patancheru, Telangana, India during 2018-19 and 2019-2020. The experiments were carried out in the BOD incubator at temperature of 28 ± 2°C and relative humidity of 65 to 70%. The life cycle of *C. chinensis* included four stages egg, grub, pupae and adult. The data revealed that eggs incubation period in 4-5 days, the grub stage consisted of four instars and its development ranged from 20-25 days, pupation lasted for 6-7 days, whereas, female adult longevity ranged from 9-12 days. The morphometric measurements revealed that the average length and breadth of eggs were 0.62 ± 0.03mm and 0.34 ± 0.02 mm respectively. The length and breadth (mm) of four grub instars G1, G2, G3 and G4 were 0.58 ± 0.02 and 0.30 ± 0.01, 1.53 ± 0.06 and 0.92 ± 0.04, 2.71 ± 0.10 and 1.47 ± 0.06, 3.59 ± 0.14 and 1.96 ± 0.08 respectively. The average length and breadth of pupa were 3.72 ± 0.12 mm,

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2.15 ± 0.09 mm. Whereas, the length and breadth (mm) of adult male and female bruchids were 3.87 ± 0.08, 2.07 ± 0.05 and 4.23 ± 0.14, 2.31 ± 0.07 respectively. The total life cycle of *C. chinensis* ranged from 33-42 days.

Keywords: Fecundity; growth and development; longevity; Azuki bean weevil; Bruchids and Cicer arietinum (L.).

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important leguminous crops and is extensively cultivated in dry and rain fed areas of the world. Chickpea ensures high quality dietary protein for vegetarian population in many countries like, India, South Asia, West Asia and Southern European countries [1]. Chickpea contains proteins (18.22%), carbohydrates (52-70%) and fats (4-10%). In addition, chickpea contains minerals calcium (6%), iron (54%), copper (73%), zinc (25%), and phosphorus (36%) of DV (daily values) potassium (15%) and sodium (1.6%).

The protein content in chickpea significantly varies as percentage of the total dry seed mass before (17-22%) and after (25.3-28.9%) dehulling [2,3].

There are two distinct types of cultivated chickpea, *Desi* and *Kabuli*. *Desi* (microsperma) types have pink flowers, anthocyanin pigmentation on stems, and a colored and thick seed coat. The *kabuli* (macrosperma) types have white flowers, lack anthocyanin pigmentation on stem, white or beige-colored seeds with a ram's head shape, thin seed coat and smooth seed surface [4].

The area and production of chickpea in India are 8.9 million ha, and 83.65 million tonnes with a productivity of 937 kg per hectare [5]. The pulse beetle, *C. chinensis* (L.) (Coleoptera: Bruchidae) is one of the three main species that causes significant damage to the stored legumes causing up to 55.7 per cent of damage in severe infestation [6]. It not only infests the cultivated host plant and stored chickpea but also a few other legumes [7]. Feeding of larvae on the cotyledons leads to significant loss in seed weight and viability. It reduces the biochemical characters for seed quality affecting seed storability. The larvae of bruchid feed on the pulse seeds making them unfit for planting and human consumption [8]. The present studies on the biology, larval development, and fecundity of *Callosobruchus chinensis* will help in maintaining continuous culture of this pest for experiments on host plant resistance, toxicological bioassays, evaluation of transgenics and pheromones development. This study can be used for developing integrated pest management of pulse beetle *C. chinensis*.

2. MATERIALS AND METHODS

The nucleus culture of pulse beetles were collected from infested seeds of chickpea from the ICRISAT, storehouses. The stock culture was maintained on chickpea by releasing 10 pairs of freshly emerged beetles separately in plastic jars covered with muslin cloth and fastened by rubber band. Fresh grains were provided periodically for the development of beetles. The pure culture was maintained by infesting insect-free chickpea grains (500 grams) with freshly emerged pair of beetles in plastic jars. The culture was maintained in the laboratory throughout the experimental period.

Mated female adult beetle (less than 24 hours after emergence) was released singly in a glass jar containing 300 grams of ICCV 2 grains with five replications. The jars were covered with muslin cloth on the top and tied with rubber bands. The experiments were carried out in the BOD incubator under controlled temperature of 28 ± 2ºC and relative humidity of 65 to 70 per cent. Oviposition was recorded daily and grains containing eggs were separated out by examining under magnifying lens and from each replication 30 grains were used to record data with respect to various parameters like eggs incubation period, grubs period, pupae period and adult longevity of male and female. The morphometrics of length and breadth were measured using a digital caliper

3. RESULTS AND DISCUSSION

3.1 Egg

The incubation period of *C. chinensis* eggs laid on chickpea genotype, ICCV 2 under lab conditions ranged from 4-5 days. The eggs were laid singly which were oval, white and smooth in

appearance. The mean length and breadth of the egg were 0.62 ± 0.03 mm and 0.34 ± 0.02 mm respectively (Table 1). The freshly laid eggs appeared transparent and sticked to the surface of seed by a secretion from the mother. The hatching of eggs were determined by the change in colour of the eggs. The hatched eggs turned to creamish white colour. The results were in tune with Neenu Augustine and RA Balikai [9] who reported that incubation period of the eggs under laboratory conditions ranged from 4 to 6 days.

3.2 Grub

The grubs of *C. chinensis* had four instars and were identified based on size of grub and head capsule casting. The first instar grub had brown coloured head and body annulations were clearly visible. It was also characterized by a pair of pro-thoracic plates and the thoracic legs were represented by conical stumps. The duration of grub ranged from 3-4 days. After the formation of the pigmented larval head capsule, the grubs borrowed from the egg through the seed coat and entered into the bean endosperm. The remaining eggshell became opaque white or mottled as it was filled with frass from the larva. It measured 0.58 ± 0.02 mm in length and 0.3 ± 0.01 mm in breadth.

The second instar grub was similar to first instar except its size and absence of prothoracic plates, whereas it had a length of 1.53 ± 0.06 mm and a breadth of 0.92 ± 0.04 mm and the grub duration ranged from 4-5 days.

The third instar grub was identified based on size and presence of three castings of head capsule, which was sandwich between the faecal pellets of the preceding instars. The third instar larvae were most active and fed on the entire endosperm voraciously. It took 3-4 four days for its development and mean larval length and breadth were 2.71 ± 0.10 mm and 1.47 ± 0.06 mm respectively.

The final grub stage was white, yellowish and C-shaped with a small head and three castings of head capsules were visible between the faecal pellets of the preceding instar. It took 4-5 days for its development with a mean length of 3.59 ± 0.14 mm and breadth of 1.96 ± 0.08 mm (Table 1). An end of the larval period, the fourth instar grub constructed a pupal chamber which was oval in shape and was prepared by compacting faecal matter against the walls of the tunnel. Finally, it stopped feeding and became inactive.

The results were in corroboration with M. B. Devi and N. V. Devi [10] who reported that the larval duration of *C. chinensis* on cowpea varied from 18-22 days and 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; 0.22 ± 0.03 mm, 0.77± 0.02 mm and 1.22 ± 0.08 mm, 2.00 ± 0.11 mm, respectively.

3.3 Pupa

During the time of pupation larval structures were broken down and adult structures developed, the rudiments of the wings appeared on the first day, on second day appendages such as legs, antenna and proboscis developed freely and on third day eyes, mouth parts, forewings, hind wings and legs with cuticular hair developed. On fourth day almost all the parts developed but intersegmental region of the abdomen remained colourless. On fifth day forewings changed to dark brown with black patches. The pupal duration lasted for 6-7 with a mean length and breadth of 3.72 ± 0.12 mm, 2.15 ± 0.09 mm respectively. The results were in suppoerted with Neenu Augustine and RA Balikai [9] who reported pupal development of *C. chinensis* on cowpea lasted for 6.80 ± 0.63 days with a mean length and breadth of 3.24 ± 0.31 mm and 1.95 ± 0.13 mm respectively.

3.4 Adult

The adults emerged by chewing and removing a circular piece of the seed coat to form a round hole. Adults were oval in shape, with long and erected antennae. The adult male was smaller and possessed a more round shape than the female. The female adults had dark stripes on each side of dorsal abdomen. The male antennae were pectinate, whereas the female antennae were serrate and pygidium of female was covered with white coloured setae. The longevity of male and female ranged from 9-10 days and 10-12 days respectively. The length of adult male was 3.87 ± 0.08 mm and breadth was 2.07 ± 0.05 mm. Whereas, the length and breadth of adult female were 4.23 ± 0.14 mm and 2.31 ± 0.07 mm respectively. Similar findings were reported by Singh et al. [11] who observed that adult longevity of *C. chinensis* male and female on chickpea were, 7.07 and 8.8 days respectively. Neenu Augustine and RA Balikai [9] also reported that adult longevity of male and

Table 1. Morphometrics of *C. chinensis* on chickpea genotype, ICCV 2

Different stages of bruchids	Length (Mean ± SE) (mm)	Breadth (Mean ± SE) (mm)	Duration (Days)
Egg	0.62 ± 0.03	0.34 ± 0.02	4-5
I Instar	0.58 ± 0.02	0.3 ± 0.01	3-4
II Instar	1.53 ± 0.06	0.92 ± 0.04	4-5
III Instar	2.71 ± 0.10	1.47 ± 0.06	3-4
IV Instar	3.59 ± 0.14	1.96 ± 0.08	4-5
Pupae	3.72 ± 0.12	2.15 ± 0.09	6-7
Female	4.23 ± 0.14	2.31 ± 0.07	10-12
Male	3.87 ± 0.08	2.07 ± 0.05	9-10

female were 8.30 ± 1.25 days and 9.50 ± 1.58 days respectively. While, the length and breadth of adult male and female were 3.49 ± 0.25 mm 1.81 ± 0.12 mm; and 4.00 ± 0.42 mm, 2.01 ± 0.13 mm respectively.

4. CONCLUSION

The study on biology of pulse beetle, *C. chinensis* on chickpea variety, ICCV 2 is a pioneer work. The beetles reared on ICCV 2 exhibited excellent larval growth, healthy adults and high fecundity. The findings of the current studies clearly indicated that ICCV 2 is highly conducive for large scale rearing this insect under laboratory conditions. The findings of this study have significant contribution towards development of effective control strategy to be a part of integrated pest management of pulse beetle *C. chinensis* for shorter and longer storages of chickpea grains.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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