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Morphological and morphometrical analysis of *Callosobruchus maculatus* (Coleoptera: Bruchidae) reared on green gram (*Vigna radiata* L.), and black gram (*Vigna mungo* L.)

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Abstract
A research studies on morphology and morphometric analysis of cowpea beetle was supervised in Entomology lab, Department of Zoology, BSNV PG College, University of Lucknow, Lucknow during September to October 2023, when temperature is about 27±1 ºC and 65±5% RH with a photoperiod of 12 h light: 12 h darkness is available. The developmental characters *viz*. body size, colour, abdomen, antennae, mobility, first egg lay time, fecundity, oviposition, incubation period, larval and pupal development, and longevity of cowpea weevil on green gram (*Vigna radiata*) and black gram (*Vigna mungo*) were studied in this experiment. The present study result revealed that the adult males were smaller in size, brownish in colour, with obtuse abdomen, pectinate antennae and hypermobility while females are larger in size, blackish in colour, pointed abdomen, serrate antenna and hypomobility. Mating took time within 40-60 minutes of adult emergence where male had a stimulant role in copulation and its duration was 30-40 seconds. The breeding period was 4-5 days duration in mung bean while 8-9 days in urad bean seeds. The larval (L_1, L_2, L_3, L_4) time span assorted from 4-5, 3-4, 3-4, 3-4 in *Vigna radiata* and in *Vigna mungo* was about 5-6, 4-5, 4-5, 4-5 days. Morphometric measurement revealed that the eggs length and breadth were measured in SE 0.48±0.08 mm and 0.13±0.22 mm reared on *Vigna radiata* whereas in *Vigna mungo* the length and breadth of eggs were 0.54±0.01 mm and 0.14±0.01 mm. The larval mean length and breadth of cowpea pulse beetle reared on *Vigna radiata* (Greengram) (L_1, L_2, L_3, L_4,) were 0.61±0.04 mm, 1.21±0.04 mm, 2.30±0.05 mm, 3.58±0.13 mm, and breadth were 0.24±0.02 mm, 0.79±0.03 mm, 1.24±0.08 mm, 1.96±0.07 mm whereas on *Vigna mungo* length were 0.69 ±0.03 mm, 1.26±0.04 mm, 2.35±0.10 mm, 3.62±0.15 mm, and breadth were 0.31 ±0.02 mm, 0.82±0.04 mm, 1.28±0.04 mm, 2.06±0.08 mm. Pupa of Male and female was about 4.08±0.01mm, 4.31±0.02 mm, and 2.36±0.08 mm, 2.62± 0.05 mm reared on *Vigna radiata* and in *Vigna mungo* had 4.11± 0.03 mm, 4.38±0.07 mm and 2.39±0.05 mm, 2.65±0.03 mm. The average length and breadth of male and female adult was about 3.22±0.06 mm, 3.58±0.24 mm and 1.93±0.07 mm, 2.10±0.08 mm reared on green gram seeds in 10 to 14 days whereas on black gram was 3.25±0.06 mm, 3.61±0.12 mm and 2.01±0.04 mm, 2.13±0.06 mm in 12-16 days duration respectively.

Keywords: Morphology, instar, relative humidity, morphometric analysis *C. maculatus*, *Vigna radiata*, *Vigna mungo* seeds

Introduction
Pulses are the members of family Leguminosae are main source of dietary proteins and mineral in humans across the world (Mahfuz I and Khalequzzaman M, 2007, Oladipupo JA and Sanni AY 2019,) [20, 25]. In underdeveloped nations pulses are main protein source and play a pivotal role in the diet of common people (Rahman MA *et al.* 2020) [30]. Pulses are nutrient rich legume crops and good source of protein and micronutrients, in which fat content is low and dietary fibre is high. Among the grown pulses, green gram (*Vigna radiata*) and black gram (*Vigna mungo*) is the main pulse crop grown in India after chickpea and pigeon pea. In globally production India is the biggest consumer and producer of *Vigna radiata* (green gram), which alone show worldwide production is about 65% and globally production is 54% (Pratap A *et al.* 2012) [28]. Green gram is highly nutritious containing 24.6% protein, 1.0% fat, 56.6% carbohydrates, calcium 0.08 g/100 g, phosphorus 0.045/100 gm, and iron 5.7 mg/100 gm and provides 234 cal energy / 100 gm (Srivastav and Ali, 2004) [37].

The black gram (*Vigna mungo*) a major leguminous crop, is commonly cultivated in different area of Bangladesh and used as important protein supplement of diet as food item. In worldwide pulse beetles overspread in stock grain legumes which was included in Bruchidas family (Dobie P 1974) [14]. Major stored grains pests are generally three type among of these five familiar species of *Bruchidae* these three are specifically *Callosobruchus maculatus*, *C. chinensis* and *C. analis* (Bressani R). Crucial and poorly valuable pulse beetle cowpea weevil, *C. maculatus* generally encounter in legume stored grain such as cowpea, black gram and green gram (Okonkwo EU 1996, Mulatu B 2000, Raja N 2000, Park C 2003, *et al.*) [24, 22, 33, 26]. Cowpea weevil is a notorious pest of economically important leguminous stored grains (Okonkwo and Okoye, 1996, Raja *et al.* 2000, Sarwar 2012, Beck and Blumer 2014, Tahmasebi *et al.*, 2022) [24, 33, 35, 6, 40]. *C. maculatus* infest grain and cause severe damage in the field as well as in storage (Prevett, 1961; Ashamo *et al.*, 2022) [29, 2]. *C. maculatus* prefer to attack the stored grain legumes of the leguminous genus *Vigna* (Cope and Fox 2003) [11], especially cowpea *Vigna unguiculata*, causing significant losses in grain weight, germination ability and nutritional quality (Caswell 1981, Babu *et al.* 2021, Hamzavi *et al.* 2022) [10, 3, 15]. All stored grains can be lost in few months due to their short lifecycle, high reproductive capacity, rapid development and continual generations of *C. maculatus* (Turaki JM 2012) [42]. During winter *C. maculatus* hibernates and breeds freely from March and November. From February to August cowpea weevil cause maximum damage during their developmental stage (Tapondjou *et al.*, 2002) [41]. Cowpea weevil is a holometabolic insect with ovipositional and developmental stage was seen on upper side of the grain and larva and pupa stages found inside the grain. In pulses grain grub makes cavity and endosperm eat by cowpea weevil that not singly reduce the viability of seeds for germinating and sowing yet as well as produce it unwilling for human being consumption (Ajayi FA 2001, Nair RM *et al.* 2019, Somta P *et al.* 2007, War AR *et al.* 2017) [1, 23, 36, 43]. During their storage condition nourishment and development of larva found inside the legumes whereas pupa and adult showing aphagous (Kergoat GJ *et al.* 2007, Stearns SC 1992) [17, 38]. Throughout the world cowpea weevil decrease the stored grain legumes quality and quantity due to this it is a major problem to prevalent (Messina FJ *et al.* 1985) [21]. Due to its fast stage of development period pests increase exponentially and losses overcome uncontrolled 100% during their storage condition (Lal D *et al.* 2012, Somta P *et al.* 2007) [19, 36]. Due to their severe infestation by *C. maculatus* seeds become hollow completely and also showing unmarketable (Khalil Y *et al.* 1999) [18]. Female lay eggs on the seeds and cement with its coat after mating with male. During oviposition the egg are oval and translucent. Beneath the egg larvae hatched and cut the coat of seed by their chewing mouthparts for their feeding, developing and sheltering them start to bore the endosperm. Eggs show white colour due to their frass which is formed by larvae and thrown to the egg. After pupation the larvae undergo the process of moulting and make burrow exactly below the testa. Larva metamorphosed into pupa before the adult emergence, where imaginal disc developed another necessary organs to this got different appearance (Barnes *et al.* 2001) [5]. Adult beetles cut the seed coat as round hole and come out from seed. In worldwide pulses play major role for the upliftment of agriculture economy so presently research studies on control methods of pests in pulses are focal demand due to their severe damage (Sarwar M *et al.* 2003) [34]. In their storage conditions insecticides (Rahman MA *et al.* 2018) [31] botanicals (War AR *et al.* 2017, Swami and Singh 2023) [43, 39], gamma radiations (Islam *et al.* 2001, Rahman MA *et al.* 2005) [16, 32], fumigants (Pieper *et al.* 2014) [27] and nanoparticles (Rahman MA *et al.* 2020) [30] are used as controlling measure of pest population in pulses.

For both research and education due to its quick generation time, sexual dimorphism, and its maintenance *C. maculatus* used as model organism in many biological laboratories (Beck *et al.* 2006, Devi MB *et al.* 2014) [8]. The longevity of cowpea weevil consist egg, four grub instar (L_1, L_2, L_3, L_4), pupa and adult in *Vigna radiata* and *Vigna mungo* (Devi MB *et al.* 2014) [13]. Day by day storage technologies are developing and without these technologies insect pest damages of pulses are not controlling. Complete information on morphology and morphometric analysis of cowpea weevil including adults, mating egg laying, hatching, development period of larva, pupa and adult emergence, adult longevity was seen in mungbean and uradbean. Therefore, present lab work was managed to investigate morphology, morphometric characters of *C. maculatus* on *Vigna radiata* and *Vigna mungo* seed to find out its biology by morphometric analysis of various developmental period including egg, larva, pupa, and adult on green gram and black gram seeds under laboratory condition.

2. Materials and methods

The study was carried on morphology and morphometric measurement of *C. maculatus* was conducted in the laboratory of entomology, Department of Zoology, BSNV PG College, Lucknow. During the research study *Vigna radiata* and *Vigna mungo* was collected for their culturing and sample preparation. Under laboratory condition when temperature is about 27±1 ºC and 65±5% RH with a photoperiod of 12 h light: 12 h darkness is available. The adult pulse beetles were ready to mate, oviposit, and grow their progeny, and observe adult emergence in daily basis, (0-1 day old) adults, which might be helpful for developing control measure and their biology and morphometric analysis.

2.1 Preservation of cowpea weevil under laboratory condition

In the present experiment cowpea weevil was used. The culture of *C. maculatus* strain was collected from the overspread green gram and black gram, which were purchased from different grocery shop of Lucknow market. In this study, firstly seeds were pick out and all debris and dust particles were removed. Only mature and healthy intact seeds were used for the experiment. Before experiment the seed of pulses were kept in freezer for a week to kill any life phases of the pest if they are previously found on them and dry for 24 h at laboratory conditions. Due to their distinctive abdominal characters, male and female beetles, cowpea weevil were identified (Bandara and Saxena, 1995) [4]. The cultured of cowpea weevils were introduced in approximately 250 g of screened mungbean and uradbean put in jars (5 lit. capacity) and wrapped with muslin cloth sealed with rubber bands with 27±1 ºC and 65±5% RH with a photoperiod of 12 h light:12 h darkness in laboratory conditions inside a growth chamber. When growth chamber was sealed with muslin clothes then the cowpea weevil were ready for breeding and egg laying. One week later the adults were shift to some other jar and black gram, green gram which was infested by beetles transferred to another breeding jar that covered with muslin

cloth and seize on with elastic loop to stop the pollutant and escape of beetles.

2.2 Preparation of test insect and sample

Cowpea weevil was stocking on *Vigna radiata* and *Vigna mungo* seeds in (9 cm) size glass jars, which contained 90 seeds of mungbean and uradbean. Freshly emerged adults (1-2 days) which was taken in equal sex ratio was cultured in to glass jars for oviposition on seeds of green and black gram for 24 hrs. The sample were put in to incubator at their mentioned degree of temperature and constant humidity. In this experimental study scalpel was taken to clear an initial incision by larva on the seed coat beneath the egg. Damage seed coat and endosperm was removed by forceps which was blunt and sharp type and also taking needle in this experiment. The seeds of *Vigna* species were dissected under a simple microscope to find out the stage of larva and pupa, they are collected from the ovipositional stage to their developmental days. At least 10-20 seeds were broken to get larva and pupa for each days of oviposition. The result on development, temperature and humidity are recorded every day and observed the size of egg, larva, pupa, and adult. A spring caliper and IMAGE J software was used to find out the length and breadth of different developmental stages of cowpea weevil. The research was done by using standard mean deviation with three generation and data was analyzed statistically.

3. Result and Discussion

Egg

The shape of egg was tiny ovoid and spindle, which was sticked to the *Vigna radiata* and *Vigna mungo* seed individually but also more than single egg may be seen in a isolated seed. Eggs which was freshly laid were seen clear, smooth, shining and translucent colour which convert in yellowish colour at their later on stage. The time period of egg at 35 ^{o}C temperature and constant humidity was fluctuate from 4-5 days in mungbean and 8-9 days in uradbean. The average length and breadth of egg was about 0.48±0.08 mm and 0.13±0.22 mm on green gram whereas in black gram the length and breadth was 0.54±0.01 mm and 0.14±0.01 mm. Devi *et al.* (2014) [13], reported the egg size, was 0.47 mm in length and 0.12 mm breadth.

Larva

(L_1)

On fourth day after oviposition hatching start, the first instar larval period begin and larva cut seed coat and a hole is made. It is known as grub which was small in size and caraboid with well develop pigmented head which help entry in seed, it made burrow in endosperm and take nutrition up to pupation stage. The body behind the head was broad and tapering at ends. After oviposition larva hatch out of egg and burrowed directly seed about 4-5 days in green gram seeds and 5-6 days in black gram seeds. The mean length of first instar larva was about 0.61±0.04 mm and breadth was 0.24±0.02 mm reared on *Vigna radiata* seeds whereas on *Vigna mungo* was about 0.69±0.03 mm and 0.31±0.02 mm. According to Devi *et al.*, the larval period (L_1) average length is about 0.60 mm and 0.22 mm breadth was found respectively.

(L_2)

From the 10th day of development segmentation of larva was made. Behind the head, the anatomy of grub expanded and tapering end became humpback appearance. The larval size larger in size by feeding seed endosperm with developed tooth plates in mouth and started to be curve from posterior portion. This time duration ranged from 3-4 days in green gram and in black gram 4-5 days next to first instar stage. The length of (L_2) was 1.21±0.06 mm and breadth was about 0.79±0.03 mm on *Vigna radiata* seeds while on *Vigna mungo* was about 1.26±0.04 mm and 0.82±0.04 mm.

(L_3)

These were most active and increased their shape, size by feeding on endosperm of mung seed and found 'C' shape. The body segmentation was clearly visible with unaided eyes. The leg buds were appeared and this stage ranged 3-4 in green gram and in black gram was about 4-5 days. The length and breadth of third instar on green gram was 2.30±0.05 mm and 1.24±0.08 mm whereas on black gram was 2.35±0.10 mm and 1.28±0.08 mm respectively.

(L_4)

The Fourth instar larva were similar to third instar larva but they are different in their shape and size. The colour of larva is white, yellowish and C shaped with a small and dusky head. The leg buds were prominent and segmentation ridges of body were seen clearly. The time duration of the larva ranged from 3-4 days in green gram and 4-5 days in black gram. The length of (L_4) was 3.58±0.13 mm, breadth was 1.96±0.07 mm reared on green gram whereas in black gram was 3.62±0.15 mm and 2.06±0.07 mm.

Pupa

From the last phase of larval period (L_4) the pupation period was developed. Pupal period started where adult formation gradually developed day by day (12-14 days) and ended to adult emergence at 27 day of development. During this period stage adult structure and rudiments of wings appeared at first, and then legs, antenna, and proboscis developed freely followed by eyes, mouth part, forewing, hindwing and legs with cuticular hair but intersegmental region of the abdomen and remained colourless. Forewings changed into dark brown with black patches at the end of this stage. The pupal period ranged from 5-6 to 6-7 in green gram while in black gram it took 6-7 to 7-8 days at 35 ^{o}C respectively. Length and breadth of male and female pupa was measured in *Vigna radiata* was about 4.08±0.01 mm, 4.31±0.02 mm, and 2.36±0.08 mm, 2.62±0.05 mm, whereas in *Vigna mungo* the length and breadth of pupa was 4.11±0.03 mm, 4.38±0.07 mm, and 2.39±0.05 mm, 2.65±0.03 mm respectively.

Adult

The cowpea weevil was sexually dimorphic, females were larger in size than the male and had antennae which is serrate in shape while male has pectinate antennae. The female cowpea beetles overall darker in colour while males were brown. The female antennae were shorter than that of male. The female abdomen was pointed and modified to assist egg laying whereas male was obtuse. Two set of wings, first set was convert into elytra sheathing which is only a part of abdomen was dark coloured in female than male. Hindwings were membranous and longer than forewings and shield by the elytra. Male was hyperactive for breeding as well as female was comparatively hypoactive. Adult emerged out from the *Vigna radiata* and *Vigna mungo* seeds by grinding and form a round hole. Adults were small in size than pupa

and brown in colour. Throughout the larval period, head of cowpea weevil was hypognathous and biting type of mouthpart was found. According to AK Raina the mean lifespan of adult have 6-7 days under laboratory conditions, but few have been capable to alive upward to 15 days. The average length of male and female was about 3.22±0.06 mm, 3.58±0.24 mm while breadth of both was1.93±0.07 mm, 2.10±0.08 mm reared on *Vigna radiata* seeds whereas on *Vigna mungo* the length and breadth of cowpea beetle 3.25±0.06 mm, 3.61±0.12 mm and 2.01±0.04 mm, 2.13±0.06 mm in 10 to14 and 12 to 16 days duration. For rapid development of *C. maculatus* optimum temperature is about 35 ºC at a constant humidity. According to Devi *et al.*, the growth period of oviposition to adult emergence was about 45-48 days.

Table 1: Morphological characters of *Callosobruchus maculates*

S. No.	Morphological characters	Male *Callosobruchus maculatus* characters	Female *Callosobruchus maculatus* characters
1.	Body size	Smaller	Larger
2.	Body colour	Brownish	Blackish
3.	Abdomen	Obtuse	Pointed
4.	Antenna	Pectinate	Serrate
5.	Antenna size	Larger	Shorter
6.	Elytral pattern	Two prominent brown spot present on elytra	Two prominent black spot present on elytra
7.	Pygidium	Pygidium with two brown spot	Pygidium with two black spot
8.	Mobility	Hypermobile	Hypomobile

Table 2: Morphometric analysis of *C. maculatus* cultured on *Vigna radiata* and *Vigna mungo*

S. No	Morphometric traits (mm)	*Vigna radiata* (Green gram)		*Vigna mungo* (Black gram)	
		Length (mm) Mean ±SE	Breadth (mm) Mean ±SE	Length (mm) Mean ±SE	Breadth (mm) Mean± SE
1.	Male body size	3.22±0.06	1.93±0.05	3.25±0.06	2.05±0.04
2.	Female body size	3.58±0.24	2.10±0.07	3.72±0.15	2.19±0.05
3.	Male antenna	1.81±0.07	-	1.85±0.07	-
4.	Female antenna	1.49±0.07	-	1.53±0.07	-
5.	Male head	1.10±0.06	0.63±0.03	1.13±0.05	0.66±0.03
6.	Female head	1.19±0.15	0.73±0.05	1.21±0.18	0.75±0.06
7.	Male pronotum	0.81±0.07	1.12±0.07	0.84±0.05	1.15±0.08
8.	Female pronotum	0.87±0.04	1.18±0.08	0.89±0.06	1.22±0.05
9.	Male elytra	2.12±0.02	0.99±0.04	2.14±0.03	1.02±0.03
10.	Female elytra	2.20±0.14	1.10±0.08	2.22±0.12	1.12±0.07
11.	Male pygidium	0.94±0.08	0.77±0.07	0.96±0.06	0.79±0.05
12.	Female pygidium	1.19±0.10	0.93±0.09	1.22±0.08	0.95±0.08

Table 3: Morphometrical analysis of *C. maculatus* reared on *Vigna radiata* and *Vigna mungo*

S. No.	Life Stages	*Vigna radiata* (Green gram)			*Vigna mungo* (Black gram)		
		Duration (days)	Length (mm) Mean ±SE	Breadth (mm) Mean ±SE	Duration (days)	Length (mm) Mean ±SE	Breadth (mm) Mean ±SE
1.	Egg	4-5	0.48±0.08	0.13±0.22	8-9	0.54±0.01	0.14±0.01
2.	1st instar larva	4-5	0.61±0.04	0.24±0.02	5-6	0.69±0.03	0.31±0.02
3.	2nd instar larva	3-4	1.21±0.06	0.79±0.03	4-5	1.26±0.04	0.82±0.04
4.	3rd instar larva	3-4	2.30±0.05	1.24±0.08	4-5	2.35±0.10	1.28±0.08
5.	4th instar larva	3-4	3.58±0.13	1.96±0.07	4-5	3.62±0.15	2.06±0.07
6.	Pupa male	5-6	4.08±0.01	2.36±0.08	6-7	4.11±0.03	2.39±0.05
7.	Pupa female	6-7	4.31±0.02	2.62±0.05	7-8	4.38±0.07	2.65±0.03
8.	Adult male	10-12	3.22±0.06	1.93±0.07	12-14	3.25±0.06	2.01±0.04
9.	Adult female	12-14	3.58±0.24	2.10±0.08	14-16	3.61±0.12	2.13±0.06

4. Conclusion

The results of the current study revealed the morphology and morphometric analysis of bean beetles cultured on *Vigna radiata* and *Vigna mungo* is a pioneer work during September to October 2023, when temperature is about 27±1ºC and 65% RH with a photoperiod of 12h light:12h darkness is available. The cowpea weevil characters *viz*. body size, colour, abdomen, antennae, mobility, first egg lay time, fecundity, oviposition, incubation period, larval and pupal development, and longevity of *C. maculatus* on mungbean and uradbean were studied in this research article. The present study result revealed that the adult males were smaller in size, brownish in colour, with obtuse abdomen, pectinate antennae and hypermobility while females are larger in size, blackish in colour, pointed abdomen, serrate antenna and hypomobile. Morphometric measurement revealed that the mean length and breadth of cowpea weevil eggs larva pupa and adult were seen in this research study with a particular time duration which was reared on both *Vigna radiata* and *Vigna mungo* seeds respectively.

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