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Studies on the life history traits of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) reared in black gram (*Vigna mungo* L.)

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Abstract
The life history traits *viz.* body size, colour, abdomen, antennae, mobility, mating time, mating duration, mating behaviour, first egg lay time, fecundity, oviposition, incubation period, larval and pupal development, and longevity of *Callosobruchus maculatus* (F.) on black gram (*Vigna mungo* L.) were studied in this experiment. The present results revealed that the adult males were smaller, brownish, with obtuse abdomen, pectinate antenna (larger) and hypermobility whereas the females were larger, blackish, pointed abdomen, serrate antenna (shorter) and hypomobility. Mating took place within 40 to 60 minutes of adult emergence where male had a stimulant role in copulation and its duration was 30-40 seconds. Sometimes male and female showed courtship behaviour before mating and the male always ran after the female. During mating the male gripped the female's abdomen behind by its leg tightly and penetrated intromittent organ with single effort and sometimes took several efforts. The mated female showed no tendency to mate more than single time and started to lay eggs within 45-60 minutes after mating. The fecundity of females was gradually decreased with the increase of time and single egg was laid on single seed wherever seeds were adequate. The eggs were translucent in colour, glued with seed coats just after oviposition and became whitish in the next day. The incubation period of the egg was 3 days and in 4th day the developing larva cut the seed coat as a round structure. The duration of first, second, third and fourth larval instars were 6, 3, 4 and 4 days whereas pre-pupal and pupal were 1 and 5 days followed by 1 day of emergence. The first instar larvae were small and second and third were apparently similar except the growth and development. However, before pupation, the fourth instar larva started to change its morphology completely and ongoing development turned it to adult individual. The average longevity of virgin male and female was estimated as 6.5±1.5 days and 10.5±3.5 days. The findings of morphological, reproductive and developmental traits can be used as basic data which might keep a pivotal role to design the control measures of this insect pest to save the pulses from their infestation and damage.

Keywords: traits, mating, oviposition, fecundity, larva, longevity

Introduction
Callosobruchus maculatus (Fabricius) (Coleoptera: Bruchidae), is an agricultural insect pest ranges throughout the tropical and subtropical world [7, 54]. It is the most destructive on cowpeas, *Vigna unguiculata* (L.), causing over 90% yield reduction [12]. It infests stored legumes, particularly of the genus *Vigna* [14]. The black gram (*Vigna mungo* L.) an important leguminous crop, is commonly cultivated in different parts of Bangladesh and used as important protein supplement of diet as food item. The beetles infest it in the field condition and often make severe damage in storage condition [44] which is one of the major limitations for increasing pulses production [54].
In storage condition the larvae feed and develop inside the legumes' (Fabaceae) seed whereas the pupae and adults are aphagous [24, 60]. Thus, the insects reduce the quality and quantity of stored grains and by this way it is a major problem prevalent throughout the world [29]. Bruchids, the most notorious insect pests are causing 40% damage of pulses [36], in chick pea it is 50% during storage [12] and in cowpea up to 60% [3]. The insect multiplies very fast in storage due to its quick generation time and if storage condition is uncontrolled losses become about 100% [26, 59]. The seeds in case of severe infestation by *C. maculatus* become completely hollow and are unmarketable [25].
After mating females lay eggs on the seed and cement with its coat [30]. The eggs are oval and translucent at the time they are laid. Upon hatching the larvae cut the coat by their chewing type mouthparts beneath the egg and started to bore the endosperm for their feeding, developing and secured sheltering. The larvae create holes and eat up the seed endosperms which not only decrease the seed viability for sprouting and replanting but also make them reluctant for human consumption [35, 67, 59, 2]. The frass is formed by the larva and thrown to the egg and thus eggs got white colour. The larvae undergo a series of molts and make burrow to a position just underneath the seed coat prior to pupation. Before the adult emergence larva metamorphosed into pupa where necessary organs

are developed from imaginal discs [6] and thus got quite different appearance. Before emergence adult beetles cut the seed coat as round hole and finally emerged from the seed.

Pulses are one of the main protein sources and play a pivotal role in the diet of common people in underdeveloped nations [48]. Animal protein meat is beyond the capability of poor and that's why they are called as "poor man's meat" which contains 20-40 percent plant protein content [9, 56]. Black gram is one of them and used as common food item in Bangladesh and heavily damaged by *Callosobruchus maculatus* [48]. Presently studies on pest control methods in pulses are focal demand which is increasing day by day in the world because pulses play an important role for the upliftment of agricultural economy worldwide [15, 53]. Insecticides [49], botanicals [67], gamma radiation [50, 21], fumigants [43], nanoparticles [48] are used as controlling measures to suppress the pest population in pulses in storage condition. To develop economically feasible, ecologically sound, and socially acceptable pest management strategies detailed information about the pest complex, the status and the sequence of appearance of the pest, the losses and type of damages are of great importance [10].

Callosobruchus maculatus used as a model organism in many biological laboratories for both research and education due to its quick generation time, sexual dimorphism and ease of maintenance [16, 8]. The population of *Callosobruchus maculatus* can grow exponentially, leading to significant loss in seed weight, germination viability, and the market value of the crop [13, 58, 61, 7]. The life cycle consisted of egg, four larval instars (L1, L2, L3 and L4), pupa and adult [16] in mung bean (*Vigna radiata* L.). Nowadays, the storage technologies are developing day by day and without these developments insects damage stored grains and pulses. Detailed information on life history traits including adult, mating, egg laying, egg, hatching, boring, the day-wise series of larval development, pupal series, adult emergence, adult longevity in black gram are still wanting. The insect pests of stored product can effectively be controlled only by analyzing the life history traits of different life stages in their life cycle and such type of work has not yet been studied in broad scale. Therefore, the present study was designed to investigate the life history traits of *Callosobruchus maculatus* (F.) on black gram seeds under laboratory condition might be helpful for developing control measures through proper management.

Materials and Methods

A study on the life history traits including morphological, reproductive and developmental stages of *C. maculatus* was conducted at 28.0±2.0oC and 80.0± 2.0% RH in the Laboratory of Genetics and Molecular Biology, Department of Zoology, University of Rajshahi, Bangladesh.

Collection of fresh seeds

Fresh mung bean (*Vigna mungo* L.) seeds were collected from local market under Rajshahi City Corporation Area. Large particles in them were cleaned by hand picking and by sieve for small particles. They were dried in the sunlight for 2-3 hour per day for three days regimen.

Collection of test insects and culture maintenance

C. maculatus was collected from grocery shop of local market under Rajshahi City Corporation Area. The culture was maintained on black gram kept in glass petridishes (9 cm). Seeds with egg were separated individually in glass vials so that virgin insects would be collected easily.

Mating of insects and releasing them on seeds

Just after emergence the virgin male and female insects were got together in glass vial for their reproduction. After confirmation of mating, 50-60 fresh seeds were supplied for egg laying in the same vial for 24 hours. After copulation males were returned into their original vials because females were disturbed by them. Here individual seed was with a single egg. A total of 30 pairs emerged adults both male and female (30:30) were used in this experiment.

Seed coat removal and endosperm breaking

A scalpel was used to find out an initial incision by larva on the seed coat beneath the egg. Both sharp and blunt forceps and needle were used to remove the seed coat and gradual breaking of endosperm of seeds.

Collection of larva and pupa

The seeds were dissected under a simple microscope to find out the larvae and pupae. They were collected from the oviposited seeds from consecutive developmental days. The observation was continued to confirm the stage of development. At least 10-15 seeds were broken to get the larva and pupa for each day of oviposition.

Photographs

All photographs were taken by a digital Camera (Canon, IXUS, A3400; 10 megapixels) and better one represented as figure for each.

Results

Life cycle traits of *C. maculatus* were studied in Genetics and Molecular Biology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh and the details of morphological, reproductive and developmental traits are presented in Table 1, 2 and 3.

Table 1: Morphological traits of *Callosobruchus maculatus* (F.).

Stage	Sex	Size	Colour	Abdomen	Antenna	Antenna size	Mobility
Adults	Male	Smaller	Brownish	Obtuse	Pectinate	Larger	Hyper
	Female	Larger	Blackish	Pointed	Serrate	Shorter	Hypo

Table 2: Reproductive traits of *Callosobruchus maculatus* (F.).

Mating after emergence	Mating duration	Oviposition after mating	Egg laying potential	Choice of egg laying
40-60 min	30-40 sec	45-60 min	24h>48h>72h	1 egg in 1 seed and more than 1 when seeds are inadequate.

Table 3: Developmental (egg-adult) traits of *Callosobruchus maculatus* (F.).

Incubation (Days)	Larval stage (Days)				Pupal stage (Days)		1st Emergence (Day/s)	Longevity (Days)
Egg	Instar				Pre-pupa	Pupa	Adult (♂ & ♀)	♂ 6.5±1.5 ♀ 10.5±3.5
	1st	2nd	3rd	4th				
1-3	4-9	10-12	13-16	17-20	21	22-26	27	
3	6	3	4	4	1	5	1	

Adult

The beetle was sexually dimorphic and males were easily distinguished from females (Table 1) because the females were larger than males and had serrate shape of antennae while the males had pectinate antennae (Fig. 2e-f). The females were darker overall (Fig. 1b), while males were brown (Fig. 1a) in colour. The antennae of female were shorter than that of male. The abdomen of female was pointed might be modified to assist egg laying whereas it was obtuse in male. The male was always hyperactive and followed female for mating and the female was comparatively hypoactive (Table 1). Adult emerged at 27th day of development where the average male longevity was 6.5±1.5 days and female 10.5±3.5 days, respectively (Table 3).

Mating

Just after emergence the beetles were remained inert and after 40-60 minutes visible movements of male and female were found in the glass vials. Mating was induced by putting male into the glass vial containing female or vice versa. It was found that male beetle always ran after the female and stimulated her by his legs, antennae and head. Thus, after 1-2 minutes of first initiative male became successful and mating duration lasted about 30-40 seconds (Table 2). During mating a long intromittent organ was used by male to penetrate the opening of oviduct of female. It was observed that male tried second time mating about one hour later from the first one but the inseminated female strongly opposed and ran away to lay egg on the seed.

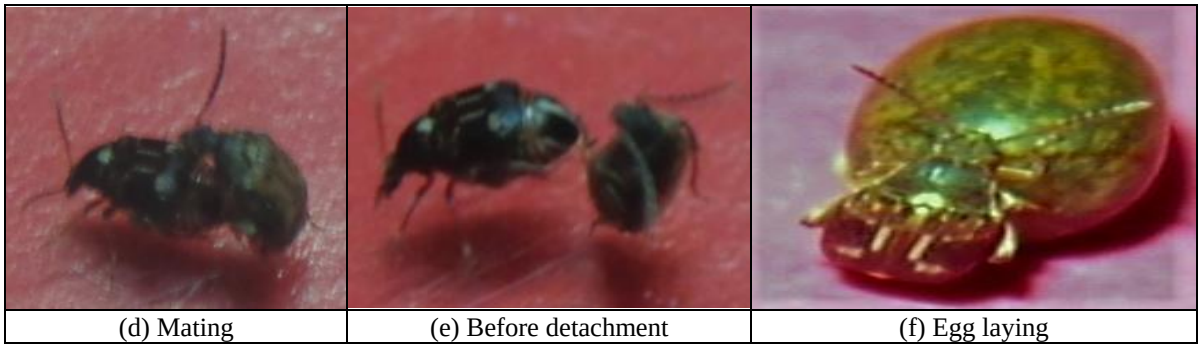
Egg laying

About an hour interval (45-60 minutes, Table 2) of mating, female gripped seed (Fig. 1f) tightly and laid egg on seed coat (Fig. 2a). Without seed coat female did not lay eggs on endosperm (data not shown) and did not loss her attention to any cues. Some glue like substances secreted by female during egg lay which helped to adhere the egg with the seed coat. The egg laying potential was high in 1st day (first 24h) then decreased gradually (Table 2). The tendency of single egg laying on single seed (Fig. 2a) was observed and laid multiple eggs on a single seed whenever fresh seeds were wanting (Table 2). Females understood that single egg was laid on a single seed for their own oviposition markers (Wijeratne and Smith, 1998).

Egg shape and colour

The oviposited egg looked like oval shaped and transparent in colour (Fig. 2a). The females laid fertilized eggs and glued on the seed coats singly one after another. The translucent eggs later became white in appearance (Fig. 2a).

(a) Adult (♂) (b) Adult (♀) (c) Courtship



(d) Mating	(e) Before detachment	(f) Egg laying

Fig 1: The adult morphology, courtship, mating behavior and egg laying of *C. maculatus* (F.).

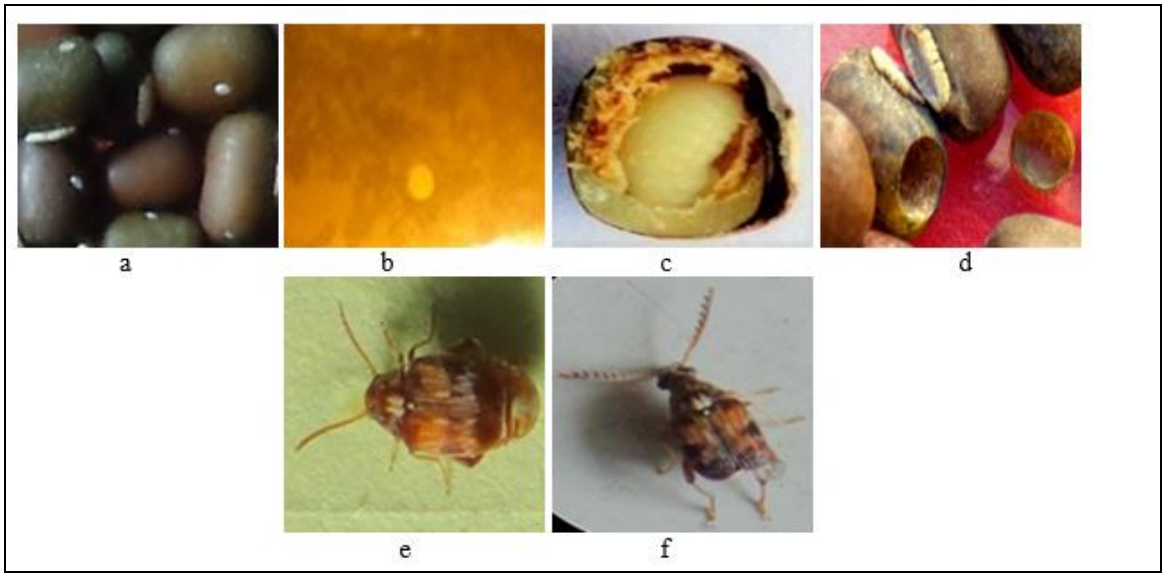
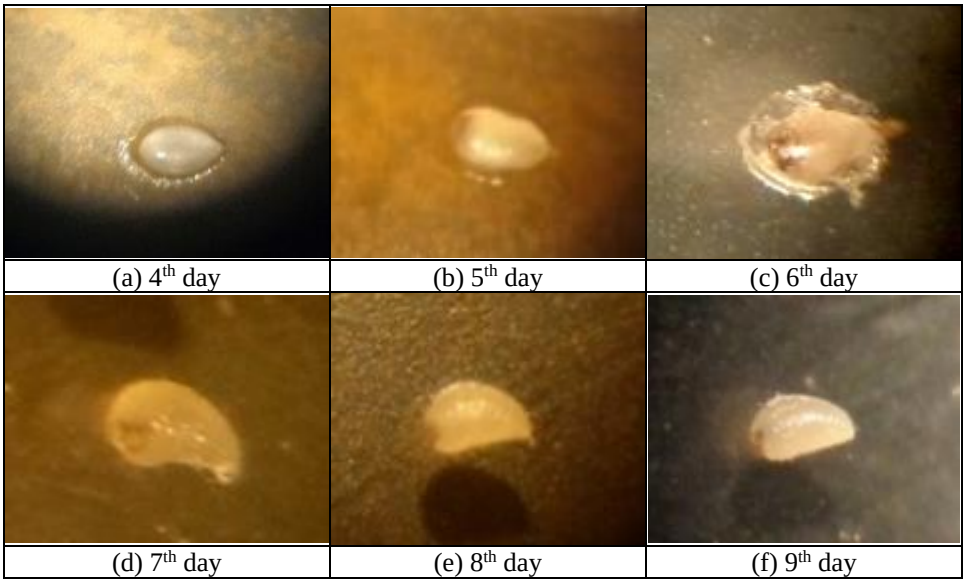


Fig 2: (a) Egg (arrow) on seed coat b), Hole (arrow) by 1st instar larva (c), Larva (arrow) in seed (d), Round hole (arrow) and cut coat (arrow), (e) ♀, (arrow- serrate antenna) (f) ♂, (arrow- pectinate antenna), Egg to adult developmental phenomena of C. maculatus (F.).

Larva

The development of embryos started in the eggs and the larva underwent metamorphosis. The larva made a hole by its mouthparts on the seed coat as a round structure (Fig. 2b) and after that burrow inside the seed endosperm (Fig. 2c) and got abundant food for spending larval stages with secured shelter where it grew and developed. The frass to its posterior side provided secured optimum environment. There were four instars (first, second, third and fourth) in larval stage.



(a) 4th day	(b) 5th day	(c) 6th day
(d) 7th day	(e) 8th day	(f) 9th day

Fig 3: 1st instar larval day-wise development of *Callosobruchus maculatus* (F.).

First instar

Upon hatching the first instar larval stage started and larva cut seed coat as round structured hole (Fig. 2b) at 4th day after oviposition. It is called grub which was small, caraboid with well-developed pigmented head (Fig. 3b) to help entry into the seed (Fig. 3c). Then it started to made burrow in endosperm and ate as food and continued until pupation. The transparent egg became opaque white in colour (Fig. 2a, 3a) because air touch hardened the egg chorion (van der Meer, 1979) and frass deposited backwardly by larva. The body behind the head was broad and gradually tapering to the end (Fig. 3, 4, 5 and 6). The body segmentation was observed from 7th day of incubation (Fig. 3d). The growth and development of larva occurred continuously as the developmental time increased (Fig. 3a-f) (Table 3). This stage ranged till 9th day of development.

(a) 10th day | (b) 11th day | (c) 12th day

Fig 4: 2nd instar larval day-wise development of *Callosobruchus maculatus* (F.).

Second instar

The 2nd instar larvae developed from first instar larvae where they burrowed and fed on the mung endosperm. The segmentation of larva was conspicuous from the 10th day (Fig. 4a) of development. The body of larva was expanded behind the head and tapering end became humpback appearance (Fig. 4a-c). The larva got large in size by eating seed endosperm with developed tooth plates in mouth and started to be curve from posterior portion. This period ranged 3 days (Fig. 4a-c) (Table 3) next to first instar stage.

Third instar

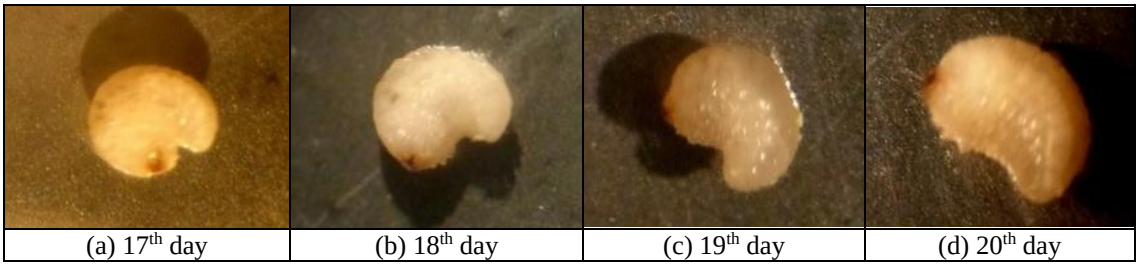
The third instar larvae were most active and increased in shape and size by eating endosperm of mung seed voraciously and got "C" shape. The body segmentation was clearly visible with unaided eyes. The leg buds were appeared and this stage ranged 4 days (Fig. 5a-d) (Table 3).

(a) 13th day | (b) 14th day | (c) 15th day | (d) 16th day

Fig 5: 3rd instar larval day-wise development of *Callosobruchus maculatus* (F.).

Fourth instar

The 4th instar larvae were similar to 3rd instar but differed in size and shape. They were white, yellowish in colour and C-shaped with a small and pigmented head (Fig. 6a-d). The leg buds were prominent and segmentation ridges of body were clearly visible. The larger body size was resulted from the voracious feeding of seed endosperm. They burrowed the endosperm and resided to a position just underneath the seed coat prior to pupation. The duration of the larva ranged 4 days (Fig. 6a-d) (Table 3).



(a) 17th day | (b) 18th day | (c) 19th day | (d) 20th day

Fig 6: 4th instar larval day-wise development of *Callosobruchus maculatus* (F.).

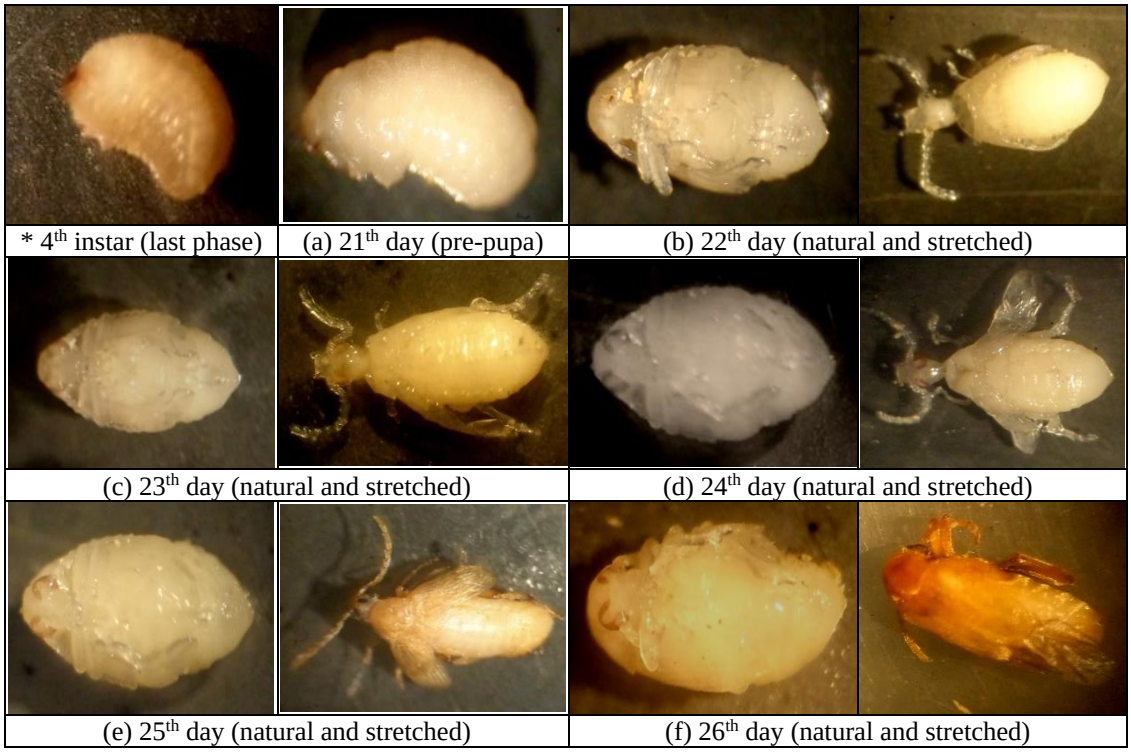


Fig 7: Pupal development of *Callosobruchus maculatus* (F.). * indicates last phase of fourth instar larva and (a) for pre-pupal stage. (b-f) first one of each is natural and second one is stretched condition.

Pupa

Pre-pupa and pupa constituted pupal stage. The pupation stage developed from the last phase of 4th instar larva (Fig. 7.*). There was an intermediate pre-pupal stage (Fig. 7.a), an inactive stage in between the grub and pupal stages. Unlike the larval stages, the body divisions of pre-pupa were distinct and the abdominal portion was markedly broader than the thorax region (Fig. 7a). This period was for one day (21st day) (Table 3). Then pupal stages started (Fig. 7b-f) (Table 3) where adult formation gradually developed day by day (22-26th day) and ended prior to adult emergence and adult emerged at 27 day of development (Table 3; Fig. 1. a,b; Fig. 2e,f).

Discussion

Females are larger and darker (blackish) than males which were also reported by previous worker [7]. The antennae of both sexes are slightly serrate [28] but in this experiment male possessed larger pectinate antennae whereas female was with shorter serrate antennae. Pygidium becomes much greater due to the eggs [22] and the pointed abdomen of female might provide mechanical support for reproduction with male which is in agreement with the reports of workers [18]. The adult male was with agile (hyper) mobility for its excitement and always ran after the female because of the female-produced sex pheromone [42, 45]. Adults have a life span of 12-14 days [8], male with average 15.6 days and female 13.50 days [48], male 9.50 and female 18.75 days [49]. In this experiment the average male and female longevity do not support the aforesaid findings and differences might be resulted from seasonal temperature variations.

A single pair of newly emerged adults immediately after releasing on host seed, moved around the host and then go for mating [23]. Females are usually mated with males within minutes of emergence [63, 32, 62, 51, 11, 20] soon after emerging [41] or within a short time after emergence [54]. Whereas it was reported that the adults of *Callosobruchus maculatus* were found to mate between 50-60 minutes after their emergence [46]. Similarly, it was observed that the adults mated within one hour after emergence [51]. We are in agreement with the reports of reporters [46,51] because in this experiment the adult beetles for their copulation took 40-60 minutes after their emergence. Copulation usually lasts for about 3-8 minutes [45, 51], on an average 4½ minutes for first mating [23], 4-8 minutes [46]. None of these results were similar with our findings because in the present experiment copulation duration was found to be 30-40 seconds.

Unmated females do not lay eggs [38] and if the beetles were deprived of a laying substrate, the eggs did not start embryonic development while still in the oviduct [64]. Female beetles took oviposition decisions and attach their eggs individually to the testa of seeds [54, 32] and this ovipositional behaviour of *C. maculatus* is closely related to the size of the seed, mating, thickness of seed coat, photoperiod etc. [23]. Females preferred to lay eggs uniformly (1egg/seed) [23, 7] on healthy seeds when hosts (seeds) are superabundant [19, 31] and more than one egg wherever necessary seeds were wanting. The less preference of egg laying on rough coated seeds was due to thicker testa or poor nutritive quality of seed cotyledon or due to presence of more linoleic acid which affect oviposition and larval feeding [54] or might be due to the chemical cues associated with seed coat [23]. Oviposition may be reduced in the presence of previously infested seeds [39, 40] and adult females may have the ability to distinguish their own

oviposition markers which led them to lay eggs singly on the seeds [68]. In our study as there were many seeds to lay eggs it was evident that females laid single egg in each seed and fecundity gradually decreased with the increase of oviposition day which confirmed the reports of previous workers [47, 49]. In the presence of a suitable host females normally begin ovipositing within 1 hour [54], 8-10 hours after mating [46], 1 hour and 35 minutes interval [23]. In the present study it was seen that egg laying on the seed started within 45-60 minutes after mating which did not support the abovementioned reports by previous workers. The females copulating with multiple males laid more eggs than those copulating repeatedly with the same male [23,17] but in this study we found that the single female did not show any tendency to mate more than one time by the same male let alone multiple.

The entire development took four instars plus pupal stadium in the seed [14,20]. The larva fed on the contents of grain and moults five times inside the seed [27] whereas it was found that moulting took 4 times inside the seed [46, 20]. On the other hand, it was reported that the grub moulted three times and thus has four instars [66]. In the present study we have found four times moulting in larval stage after egg hatch (first instar; first moulting, second instar; second moulting, third instar; third moulting, fourth instar; fourth moulting). Thus, we are in agreement with the findings of previous workers [46, 20].

The first instar larvae burrow seeds 4-5 days later of egg laying [55], the larvae hatch in around 5 days [54], in 4-7 days [37, 20], 8-10 days after oviposition [8] or 3 days [46]. In black gram seed we found that no seed with egg has a round cut mark on the surface of seed coat before 4 days of incubation. Thus, the present findings support several earlier reports [55, 37, 20] mentioned here.

The larva had three body portions *i.e.* head, thorax and abdomen with segmentations where it was reported that the first instar larva consisted of 19 segments: 5 cephalic, 3 thoracic and 11 abdominal segments [64]. The mean duration of *C. maculatus* first instar larva was 9.25 days for February-March and 8 days were for April-May in soybean [65], 5 to 8 days (6.32±1.14 days), 8-10 days (8.76±0.72 days) and 3-5 days (4.08±0.90 days) during July-August, November-December, 2012 and April-May 2013, respectively [66]. The duration of first instar larva was 8-9 days [16] and similarly it ranged 4-5 days at 30˚C [1] whereas in *C. chinensis* it was 3-5 days [5]. All were similar with the present report where first instar larval duration was 4-9 days (6.32 days).

2nd instar larva took 6-9 days for development [66], 2-3 days [1], 3-4 days [16], in *C. chinensis* 3-6 days [5]. In case of 3rd instar larva it took 3-4 days [16], 2-3 days [1] and in *C. chinensis* it was 4-6 days [5]. In the present study we found that the second instar larval duration was 3 days (10th-12th day) and for 3rd instar it was 4 days (13th -16th day). The fourth instar grub before pupation chewed a circular hole near the seed coat until only a thin layer of testa was left intact which gave an appearance of window or spot on moong seeds which was an indication of pupation and the round hole's diameter was 1-2 mm [54, 27, 51]. In the present study a sign of circular hole was found before adult emergence and afterwards cut round seed coat looked like an operculum. The 4th instar larva took 4-5 days [16], 2-3 days [1] and in *C. chinensis* 4-7 days [5] for its development. In the present study 4th instar larval duration was 4 days (17th-20th day) and thus we are in agreement with previous workers. The pupal period of *C. maculatus* on cowpea was 7.40 days [57], on soybean 9.92 days and 8.15 days during winter and summer [65] and 8.68 days [52]. The pre-pupal and pupal period ranged from 3.41 and 3.67 days, respectively [33]. Pupa male 6-7 days and female 5-6 days [16], 4-5 day [1]. In *C. chinensis* the pre-pupal duration was 2-3 days whereas pupal period was 6-8 days [5]. In this experiment pre-pupal and pupal duration was 1 day and 5 days respectively (total 6 days) which supported the findings with the previous reports on *C. maculatus* except this report [33]. The adult that resulted from pupation chews through the seed coat and emerges from the seed and can complete one generation within 3-4 weeks [7], 25-30 days after oviposition [54], 25-40 days [55]. The total life cycle was completed in black gram in 3 days for incubation and 23 days for larval and pupal stages with a total of 26 days [46], and 17-23 days [1]. It is reported that generation time depends on the species of legume [7]. These reports are similar with the present study. Beetles emerged from seeds are reproductively mature and well adapted to storage conditions, requiring neither food nor water to reproduce [14] whereas the adults become sexually mature after 24-48 hours of emergence [7]. The damage is caused by this insect pest during larval stages and the reduction of the amounts of carbohydrates and proteins of the grain, leading to the degradation of the nutritional quality and forcing the growers to sell their commodity with low economic value after harvest [34,4]. Thus, it is important to control the black gram from the pulse beetles' infestation and the strategies should be taken on the basis of life history different stages. The life history traits of this species discussed above might vary depending on the seed coat texture, endosperm, seasonal temperature and humidity, photoperiod, availability of necessary seeds, seeds genome, freshness of seeds, age, sex and genetic constitution of experimental beetle etc. The findings of present experiment could provide basic data on several life history traits which might be needed to take measures to control the pulses from *Callosobruchus maculatus* infestation and damage.

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