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Effect of temperature on the biology and morphometric measurement of cowpea beetle, *Callosobruchus maculatus* fabr. (Coleoptera: Chrysomelidae) in cowpea seed

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Abstract
The pulse beetle, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae), is a worldwide insect pest that infests pulses in the fields and storages. Laboratory experiment was conducted to study the biology and morphometry of *C. maculatus* under the effect of three different temperature degrees (25, 30, 35℃) and 65 % related humidity. The life cycle consisted of egg, four larval instars (L1, L2, L3 and L4), pupa and adult. The eggs hatched in 6-7, 2-4 and 4-5 days, and the larval duration varied from 15-19, 10-14 and 10-14 days at 25, 30 and 35℃ respectively. The morphometric measurements showed that the average length and breadth of egg were 0.54 mm and 0.13 mm respectively. The mean length and breadth of L1, L2, L3 and L4 were 0.68, 1.26, 2.30, 3.58 mm and 0.30, 0.80, 1.29, 1.95 mm respectively. The average length of pupa was 4.30 mm and breadth was 2.27 mm respectively. The average length and breadth of adult was 3.57 mm and 2.10 mm respectively. The life cycle took 30-37, 19-26 and 20-27 days for completion at 25, 30 and 35℃ and constant relative humidity. Which concluded that the standard optimum temperature for *C. maculatus* fitness lie between the range of 30-35^{0}C and a relatively high humidity of 65 percent.

Keywords: temperature degree, biology, morphometric measurement, *callosobruchus maculatus.* Cowpea seed

1. Introduction
The insect pests included in the family of *Bruchidas* are infesting stored products, which are found in the tropical and sub-tropical area of the world [1]. Among the five known species of *Bruchidae* three are the most important pests of the stored products namely *Callosobruchus maculatus*, *Callosobruchus chinenesis* and *Callosobruchus analis* [2]. The cowpea beetle, *C maculatus* commonly met in leguminous grain such as cowpea, lentils, black gram and green gram being their major and economically important pest [3, 4, 5, 6]. It is one of the cosmopolitan pests of cowpea seeds, *Vigna unguiculata* which is causes serious damage during storage condition [7]. The insect multiplies very fast in storage due to its quick generation time and the cowpea seed are significantly affected by the beetle infestation causing weight losses of up to 60 % [8]. The larval and pupal stages of this holometabolic insect reside inside the grain, although the egg and adult stage is found on the grain. The larva creates holes in the pulse grains and eats up the endosperms which not only decreases the seed viability for sprouting and replanting but also make it reluctant for human consumption [9, 3, 4, 5, 10]. The use of synthetic insecticide has been employed to control pest infestation in storage, which had positive effect on the pest, but associated with harmful health hazards to warm-blooded animals and environment [11]. The insect pests infesting stored product can effectively controlled only by analyzing the life cycle, temperature degree and moisture preference for the insect oviposition and development of different life stages. Such type work has not been studied adequately in Jeddah, Saudi Arabia and review in this context was also very limited. So, the present study was conducted on cowpea seed under laboratory experimental condition to reveal its biology by morphometric measurement of different developmental stage such as egg, larva, pupa and adult for finding out the suitable control measures.

2. Materials and Methods
A study on the biology and morphological measurement of *C. maculatus* was conducted in the laboratory of plant protection, Faculty of Meteorology, Environment and Arid Land Agriculture, Department of Arid Land agriculture, King Abdulaziz University, Jeddah, Saudi Arabia during March to April 2016 under the effect of three different temperature degrees (25, 30 and 35 ˚C) and 65% relative humidity. Cowpea seed commonly grown in Saudi Arabia was selected for the culture and sample preparation during the experimental study. Culture of insects was prepared and grown in the laboratory at a temperature of 30 ± 2℃, 12: 12 L: D and 70% RH kept in plastic jar of 1kg capacity.

2.1 Laboratory Maintenance of the Experimental Insects
The bruchid beetle, *C. maculatus* (Fabricus) was used in the present experiment. Cleaned and sterilized (heating at 70℃ for 1hr) cowpea seeds (*Vigna unguiculata*) were placed in glass jar to reabsorb moisture. Then, transferred amount 200 gm cowpea seeds to depth of 4 cm to separately sterilized culture jar. A small population of pulse beetle with equal sexual ratio was released from previous culture under laboratory conditions on cowpea seeds inside a growth chamber at 30 ± 2℃, 12:12 L: D and with 70 ± 5.0% RH. The growth chambers were sealed with muslin and the beetles were allowed for mating and oviposition. After one week the parental insects were discarded or transferred to another jar and infested cowpea seed containing eggs was transferred to fresh cowpea seeds in the

breeding jars that were covered with pieces of cloth fastened with rubber band to prevent the contamination and escape of beetles.

2.2 Test insects and Sample preparation

The cowpea beetle *C. maculatus* was cultured on cowpea seeds in petri dish of 9 cm size; contained 80 seeds of cowpea. The equal sex ratio of freshly emerged adults (1-2 day old) was reared into a petri dish for egg laying, and allowed for 24 hours to lay eggs on cowpea seeds. The samples were placed in the incubator at three mentioned degrees of temperature and constant RH (65%) separately. A pin was used as leverage at the areas where the eggs were laid and pill cutter was used to make an initial cut adjacent to the cowpea seed. The initial incision creating a two sided cross section in to the cowpea seed was done by using scalpel. The dissected portion was examined under the plate Microscope (Nikon SMZ-2B/japan) to see and find the larvae. Remaining seeds were deposited for observing the other developmental stages of the insect. Then the petri dish contained cowpea seeds was closed and kept in incubator as the earlier one for testing and observing the next stages of development. The observation was conducted in 2 days interval to confirm the different stages of development. The observation was recorded every day on development, and carefully observed for the size of different stages (egg, larva, pupa and adult). A digital caliper was used for the measurement of breadth and length of the different developmental stages. The experiment was conducted using standard mean deviation with four replications. The data was analyzed statistically.

2.3 Development-Hatching

The eggs was tested from the day of culturing infested cowpea seeds in petri dish and exposing to incubator for hatching and the time taken by the eggs to hatch was calculated and recorded.

2.4 Larval and Pupal Development

After recording and calculating the hatching time of eggs they were allowed to continue their development. The larva of insect bores its way directly from the egg into the seed of cowpea and develops inside the seed, since the larva moults within the seed. The interval between the various larval instar was recorded and calculated separately. At the end of larval period, the larva drills a minute of circular hole near the seed the seed coat till only a thin layer of seed coat left intact and the larva change to pupa. This was an indication of pupation then the incision creating a two sided cross section in to the seed was done by using scalpel to observe the pupation stage.

2.5 Adult emergence

The adult was also indicated as a pupation stage using scalpel for the incision creating a two sided cross section in to the seed and observing the adult stage of the insect. The emergence of adult insect was determined by emergence hole on the seed which was taken at the final stage of the growth phase.

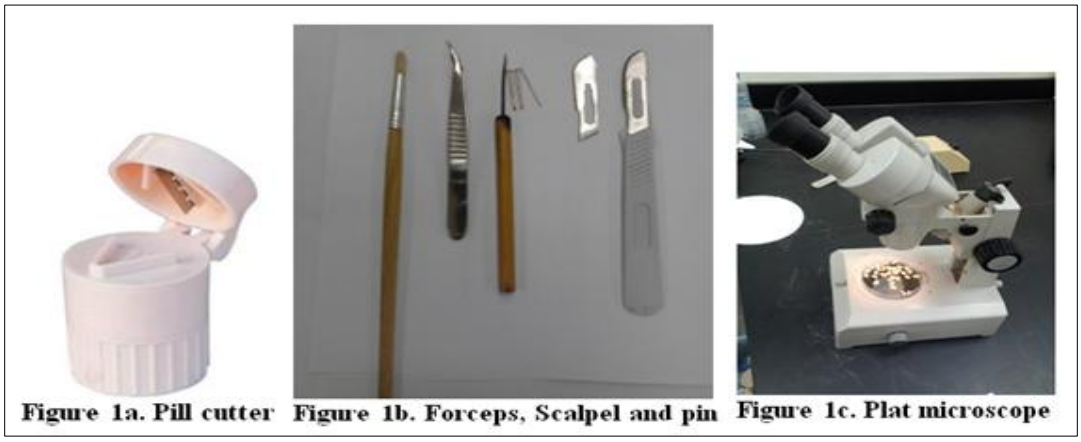

Plate 1: Equipment used during experimental work.

3. Results and Discussion

The biological parameters and details of the development stages of *C. maculatus* fed on cowpea seed under the effect of different temperature degrees are presented in table 1. The different stages of development from egg to adult of *C. maculatus* are shown in plate 2. From the laboratory experimental data, the life cycle of *C. maculatus* was studied.

Table 1: Biological and morphological measurement of *C maculatus* development stages

Life stages	Duration under the effect of different temperature degree			Length	Breadth
	Duration (days) at 25 ˚C	Duration (days) at 30 ˚C	Duration (days) at 35 ˚C	(mean ± SE) (mm)	(mean ± SE) (mm)
Egg	6-7	3-4	4-5	0.54 ± 0.01	0.13±0.01
1st instar larva	5-6	4-5	4-5	0.68±0.03	0.30±0.02
2nd instar larva	3-4	2-3	2-3	1.26±0.04	0.80±0.04
3rd instar larva	3-4	2-3	2-3	2.30±0.05	1.29±0,05
4th instar larva	4-5	2-3	2-3	3.58±0.13	1.95±0.08
Pupa	5-6	4-5	3-4	4.30±0.02	2.27±0.09
Adult	4-5	2-3	3-4	3.57±0.23	2.10±0.07
Total life spam (days)	30-37	19-26	20-27		

Fig 2a: Young eggs laid on the surface of the seeds.

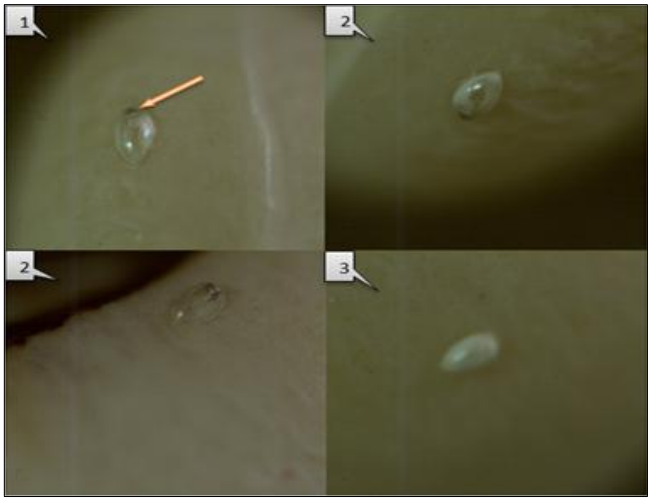

Fig 2b: 1-Development of young larva head. 2- Young larva is formed within the egg. 3- Egg fills with frass from the larva.

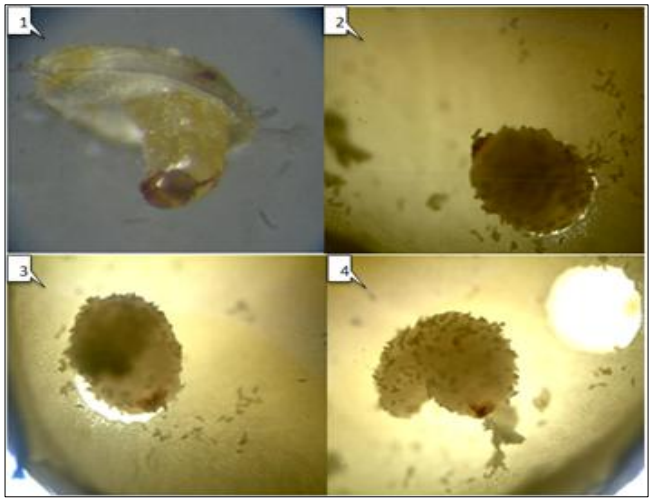

Fig 3a: 1-Newly emerged young larva, 2- Larva penetrated into the seed. 3- Larva feeding inside the seed endosperm. 4- Circular hole made by the larva in the seed.

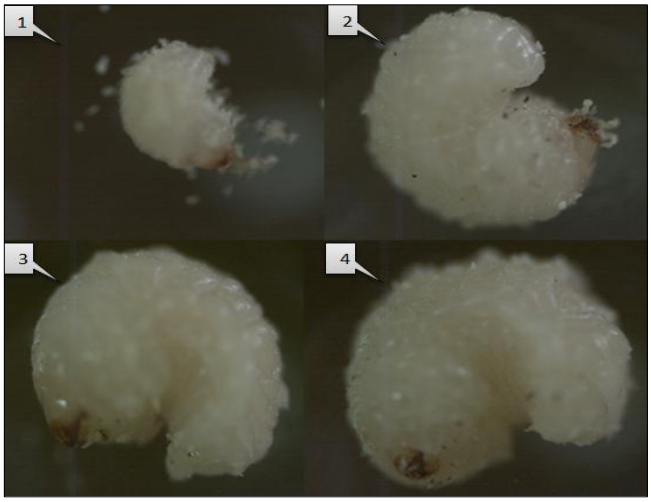

Fig 3b: 1-1st instar larva, 2- 2nd instar larva, 3- 3rd instar larva, 4- 4th instar larva.

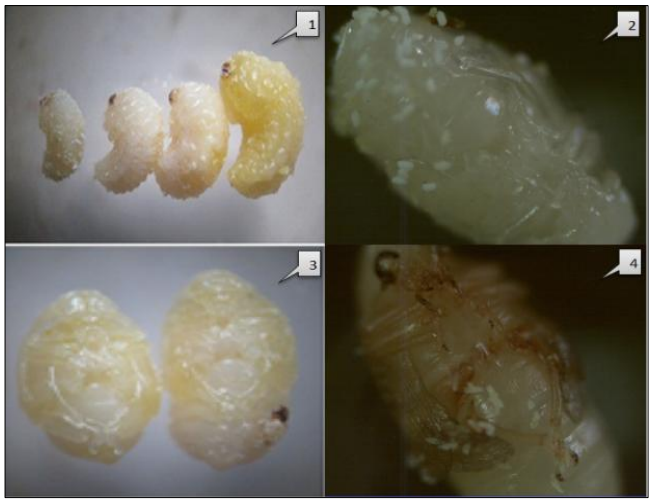

Fig 4a: 1- Different larval stages. 2-1st pupal stage. 3- 3rd pupal stage of male and female.

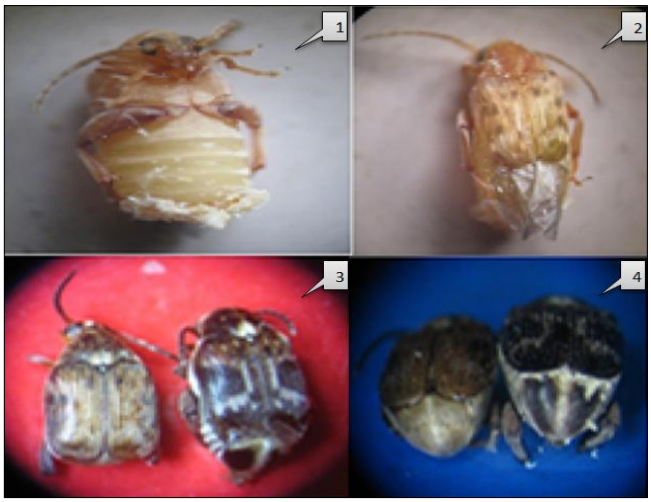

Fig 4b: 1- 4th pupal stage. 2- Young adult formed inside the seed. 3- Adult of male and female. 4- Male has no dark strips and female have dark strips on the dorsal sides of abdomen.

Plate 2: Life cycle of *Callosobruchus maculatus*

Egg

The egg was small oval or spindle shaped, and firmly glued to the cowpea seed individually but many eggs could be seen on a single seed. The freshly laid eggs were clear, translucent smooth and shining, which later become yellowish white. The duration of egg at different temperature degrees (25, 30 and 35 ˚C) and constant 70% RH was ranged from 6-7, 3-4 and 4-5 days respectively. The average length of egg was 0.54± 0.01 mm and breadth was 0.13± 0.01 mm [12]. Reported that egg was 0.75 mm in length. The size of egg was reported by [13]. That it was 0.47 mm in length and 0.12 mm in breadth. The differences here could be attributed to characteristic genetic differences of the strains themselves and age of insect which laid eggs.

First instar larva

1st instar larva was formed inside the eggs out of cowpea seed coat and after the formation of pigmented larval head capsule they bored from the bases of the egg by biting through the seed coat and entered into the cowpea seed endosperm without moving outside the protection of the egg. The remaining egg shell become opaque white or mottled as it was filled with frass from the larva. The larva was curve, white in color and had a small head. The larval period ranged from 5-6, 4-5 and 4-5 days at 25, 30 and 35 ˚C respectively [14]. Reported that the larvae hatched out of egg and borrowed directly into the been about 4-8 days after oviposition. The 1st instar larva measured 0.68± 0.03 mm in length and 0.30± 0.02mm in breadth [13]. Reported that the average length of larva was o.60 mm and breadth was 0.22 mm.

Second instar larva

The 2nd instar larva developed from first instar larva, they burrowed and fed on the cowpea endosperm. The duration of larval period at 25, 30 and 35 ˚C, ranged from 3-4, 2-3 and 2-3 days respectively. The length of larva was ranged from 1.17 to 1.35 mm with an average 1.26± 0.04 mm and the breadth was 0.70 to 0.90 mm with an average 0.8± 0.04 mm respectively.

Third instar larva

The duration of the larva at mentioned three incubated degree of temperature was similar as a 2nd instar larva. The average body length of the 3rd instar larva was 2.30± 0.05 mm and the breadth was 1.29± 0.05 mm. The 3rd instar larvae were most active and fed on the entire endosperm of cowpea seed.

Fourth instar larva

The 4th instar larvae were similar to 3rd instar larvae but they were different in size and shape. It was white, yellowish in colour and somewhat C-shaped with small and pigmented head. This instar larva seemed to become larger and fed on the entire cowpea endosperm voraciously and burrowed into a position just underneath the seed coat prior to pupation. The larva period ranged from 4-5, 2-3 and 2-3 days at 25, 30 and 35 ˚C incubation periods respectively. The length of the 4th instar larva was 3.30 to 3.86 mm with in average of 3.58± 0.13 mm and the breadth was 1.75 to 2.15 mm with in average of 1.95± 0.08 mm respectively. The present study show that the total larva period long as 17 days in a 25 ˚C which was short as 12 days in a 30 and 35 ˚C.

Pupa

The pupation stage developed from 4th instar larva. During this stage adult structures developed; the rudiments of the wings appeared at first, than appendages such as legs, antenna and proboscis developed freely followed by eyes, mouth part, forewing, hind wing and legs with cuticular hair developed but intersegmental region of the abdomen and remained colourless. Forewing was looked as light green in colour. At the end of this stage forewings changed into dark brown with black patches. The pupal period ranged from 5-6, 4-5 and 3-4 days at 25, 30 and 35 ˚C respectively. The length and breadth of pupa was measured 4.30± 0.02 mm and 2.27± 0.09 mm respectively.

Adult

The adult of insect results from pupation remained in the cowpea seed for several days before pushing or biting out the window with its mandibles. The adult emerged out from the cowpea seed by chewing and removing a circular piece of the seed coat to form a round hole. Adult were small in size than pupa with typical rounded appearance and reddish brown in colour. The head of this insect was hypognathous in type with biting type of mouthparts, which are best used during the larval stages. Head of adult was provided with a pair of segmented antennae, which were serrate in type comparatively longer in males than in females. Among the two pair of wings; first pair was modified into elytra covering only a part of the abdomen which was dark colored in female than in male. The hind wings ware membranous and longer than forewings and protected by the elytra. The adult male was smaller in size and possessed a more round shape than the female whereas female adults beetle had dark stripes on each side of dorsal abdomen. The adult period of insect inside cowpea seed up to emerge out of the seed was 4-5, 2-3 and 3-4 days at 25, 30 and 35 ˚C respectively. The lifespan of adult after emerged from the seed was 7-10 days [14]. Reported that adult have a mean lifespam 7 days in lab environment, but some have been able to live up to 14 days. The average male and female length and breadth was 3.57± 0.23 and 2.10± 0.07 mm respectively [16]. Reported that the average body length of adult was 4-6 mm. the average length of male was 3.21± 0.06 mm and breadth was 1.91± 0.05 mm whereas the average length and breadth of female beetle was 3.70± 0.1 mm and 2.17± 0.05 mm respectively [13]. The results of the present study show that the developmental period of the egg to emerged adult from the seed was longer (30-37 days) at 25 ˚C and it was short as 19-26 days in a 30 ˚C followed by 35 ˚C (20-27 days). The optimum temperature for rapid growth of *C. maculatus* lies between 30 and 35^{0}C at a relative high humidity [13]. reported that the developmental period of the egg to adult was 45-48 days. It took one and half month to complete its life cycle, which could take longer time under unfavorable conditions depend on the different strain of insects, food supply, temperature and humidity.

4. Conclusion

The results of the present study revealed that the standard optimum temperature for *C. maculatus* fitness lie between the range of 30-35^{0}C and a relatively high humidity of 65 percent. This standard is valid only to the *Callosobruchus* reared on a standard feed. The period of life cycle stage of the insect could be increase at low temperature <25 and high >35 ^{0}C and a low

humidity combination. The pupa incidentally is not affected by high temperature for its period of pupation but increase in temperature greater than 30^0C may reduce the pupal period.

5. Acknowledgment
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6. References

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