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Studies on the biology of *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) with stereomicroscopic images of its life stages

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ABSTRACT: The life cycle and season's studies of *Tribolium castaneum* indicated that the most favourable season as monsoon (27±5<" C & 80±05 % RH) where they have completed their life cycle within 22 days. The most unfavourable season was the winter (15±3<" C & 35±05 % RH) where it extended till 45 days. Results showed the presence of seven larval instars in the life cycle of the beetle. Stereomicroscope was used to study the microscopic stages like eggs, sexual dimorphism of pupae and adults, morphometric of the beetle. This is the first compiled stereomicroscopic photographs of the life cycle of *T. castaneum*. © 2021 Association for Advancement of Entomology

KEYWORDS: Morphometric, stereomicroscopy, life stages, seasons

Tribolium castaneum (Coleoptera: Tenebrionidae) is one of the highly resistant pests and known to damage a wide range of stored grains (White and Lambkin, 1988; Hagstrum, 2017). Abiotic and biotic factors, which greatly influence the life cycle of the pest, are vital to the mass-rearing (Santos et al., 2018). However, standardisation claims for a major share of time. Hence, the collective knowledge of basic biology is of great importance to validate the efficacy of the new formulations (Arthur and Hoernemann, 2004). Interestingly, it has emerged as a better model for developmental studies than *Drosophila* (Richards *et al.*, 2008). Additionally, they work as an efficient early warning system in transgenerational epigenetic side effects caused by different pharmaceuticals (Bingsohn *et al*., 2016). A few studies on the biology of the pest have been recorded from different parts of India (Devi and Devi, 2015; Sreeramoju *et al*., 2016). However, distinctive photography explaining microscopic characteristics are lacking in the literature. As the number of larval instars varies with temperature, humidity (Karuppaiah and Sujayanad, 2012), detailed biology of *Tribolium castaneum* with unique characteristics of each stage, photographs and morphometric data were undertaken.

T. castaneum culture was collected from the Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India and reared in the defined culture media of wheat flour, wheat grains, and Baker's yeast in the ratio of 6:3:1. The jars were covered with the muslin cloth for the ease of aeration. Cultures were divided into two sets where one was maintained in the laboratory to understand the growth curve in the warehouses. And other set was maintained in the humidity

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chamber at 27±2°C, 70±5 RH to be used for bioassay studies. Here, the biology study was conducted with the culture set maintained in the laboratory. This experiment continued throughout the year (April 2018- March 2019) to analyse the most favourable and unfavourable season for the growth of the beetle. The cultures were observed regularly for oviposition. A 250 µm sieve of 60 mesh size was used to separate the microscopic eggs from the media. Eggs were then isolated and kept in separate glass petriplate to study their incubation period. Newly emerged larvae were immediately transferred in the plastic container containing the media. The pupae were separated with the help of a paintbrush and transferred to a glass petriplate for the ease of study. On adult emergence, they were again transferred to the vials containing media. The time required to complete their life cycle was recorded. Morphological characteristics, morphometric analyses and photography were done using stereomicroscope (Computer assisted 13.1 mega pixel catcam stereomicroscope; Model no: LEICA MZ 16 A) at 200x magnification.

Biology:

Flour beetles reared in the natural conditions indicated that the most suitable season was monsoon i.e. July- August (27±5 °C & 80±05 % RH) where they have completed their life cycle within 22 days. The most unfavourable season was the winter (December - January) where it extended till 45 days. The temperature and humidity range were 15±3 °C and 35±05 per cent respectively. Each stage viz. eggs, larvae, pupae and adults is identified by unique characteristics and is explained below (Fig. 1) (Table 1).

Egg: Eggs are microscopic, oval in shape and pale white in colour. Flour particles often stick to their surface as they are sticky when laid. This makes it more difficult to identify even under the microscope. However, when observed carefully, they appear pale compared to flour particles. They are measured by about 0.088±0.004 mm in length and 0.056±0.005 mm in breadth.

Larva: A total of seven instars were recorded. They are campodeiform, slender in shape. Their dorsal surface is covered with fine bristles and the last abdominal segment is demarcated by the presence of anal cerci. Each larval stage is smaller in size from its succeeding stage. The 1st instar larva, emerged post incubation, is very tiny and hence very difficult to see it with the naked eye. They are ivory white in colour and measures about 0.87±0.04 mm in length and 0.096±0.005 mm in breadth. 2nd instar is mobile, linen white in colour, and measured about 1.78±0.04 mm in length and 0.28±0.02 mm in breadth. The 3rd instar is thread like, light yellowish in colour. They measure about 2.06±0.05 mm in length and 0.376±0.03 mm in breadth. The 4th instar is light brownish in colour & measured about 2.852±0.06 mm in length and 0.45±0.05 mm in breadth. The next one is tortilla colored, 5th instar, immature, and measured about 3.84±0.2 mm in length and 0.67±0.03 mm in breadth. The 6th instar is large and bulgy in appearance. They are light brown and measured about 4.97±0.04 mm in length and 0.79±0.02 mm in breadth. The 7th instar is highly mobile, heavy, and tawny in color and measured by about 5.95±0.05 mm in length and 0.972±0.03 mm in breadth.

Pupa: The pupal phase can be differentiated into three stages i.e. Pre pupal, pupal & post pupal stage based on their progressive development. The pre pupal stage is light yellowish, smaller in size. The dorsal side possesses fine bristles. Their head region is broad and curved whereas the tail region always bears shedding exuvia. The pupal stage is recognised by the dark coloration and shorter size. Hind limbs developed on the ventral side and small eyespots are seen. The post pupal stages are demarcated by the presence of fully developed eyes, hind limbs, and dark brownish coloration. The pupal stage shows sexual dimorphism where females and males have forked and stubby genital papillae respectively. Moreover, papillae in the case of females are longer and reach the length of the urogomphi whereas in males it is small and restricted to the last abdominal segment. Pupa measured about 3.88±0.04 mm in length and 0.96±0.05 mm in breadth.

Adult: Adults are dark brownish in colour. They have capitate type of antennae which is very prominent. They measure about 3.96±0.05 mm in

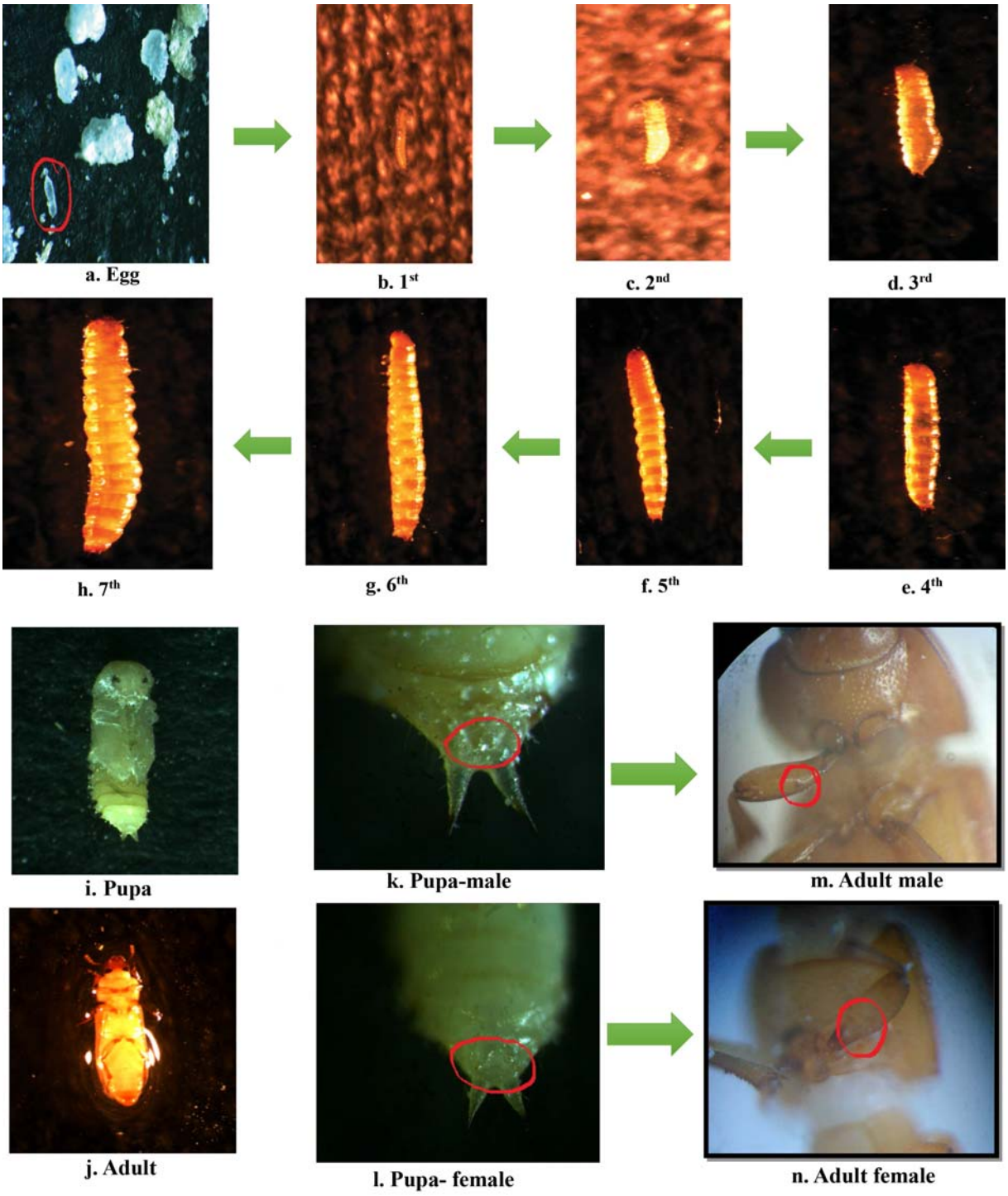

Fig.1 Different stages of *Tribolium casteneum* captured using the computer assisted 13.1 mega pixel catcam stereomicroscope with fine details.

a: egg, b: 1st instar, c: 2nd instar, d: 3rd instar, e: 4th instar, f: 5th instar, g: 6th instar, h: 7th instar, i: ventral view of pupal stage, j: ventral view of adult insect, k: male pupa where genital papilla is stubby, l: female pupa where genital papilla is forked reaching the urogomphi, m: male adult marked by the presence of setiferous patch on the forefemur, n: absence of setiferous patch in female adult

Table 1. Morphometric data of different stages of *Tribolium castaneum* of F1 generation (Mean of 10 individuals)

F1 Gen.	Size	Morphometric data(mm)			
		Mean	Range	±SD	SE
Egg	L	0.088	0.08- 0.09	0.004	0.001
	W	0.056	0.05-0.06	0.005	0.001
1st instar	L	0.87	0.8-0.9	0.04	0.01
	W	0.096	0.09-0.1	0.005	0.001
2nd instar	L	1.78	1.7-1.8	0.04	0.01
	W	0.28	0.25-.3	0.02	0.008
3rd instar	L	2.06	2-2.1	0.05	0.01
	W	0.376	.32-.4	0.03	0.01
4th instar	L	2.852	2.78-2.9	0.06	0.01
	W	0.45	0.4-0.5	0.05	0.01
5th instar	L	3.84	3.6-4	0.2	0.06
	W	0.67	0.64-.7	0.03	0.01
6th instar	L	4.97	4.9-5	0.04	0.01
	W	0.79	0.75-.0.8	0.02	0.006
7th instar	L	5.95	5.9-6	0.05	0.01
	W	0.972	0.93-1	0.03	0.01
Pupa	L	3.88	3.8-3.9	0.04	0.01
	W	0.96	0.9-1	0.05	0.01
Adults (Both male & female)	L	3.96	3.9-4	0.05	0.01
	W	1.08	1-1.1	0.04	0.01

L= Length; W = Width; SD= sample standard deviation SE= Standard error of the mean

length and 1.08±0.04 mm in breadth. They show sexual dimorphism where males possess a setiferous patch in the forefemur which is absent in the females. However, the character is microscopic and cannot be seen with the naked eyes.

Different temperature and humidity in different geographical locations has a profound effect on the life cycle of insect (Good, 1933; Karuppaiah and Sujayanad, 2012). The larval stage has a varying number of instars depending on the abiotic factors like temperature, humidity, and food availability (Good, 1933; Mukerji and Sinha, 1953). The present study has recorded seven larval instars. However, very minute differences were seen between the instars. So morphometric and associated morphological characteristics were taken into account to establish a complete comparative exposition. Microscopic egg stage and distinguishing characteristics of pupae and adults are fine enough to be observed by the naked eyes hence stereomicroscope was used in the study. In a similar study, biology of the beetle was documented from the Imphal, Manipur in the laboratory conditions from January to July (Devi and Devi, 2015). Result shows that the beetle took 164-194 days to complete its life cycle. Singh and Prakash (2015) documented the effect of temperature and humidity on the life cycle of *T. castaneum* collected from two different areas, Dayalbagh and Cantonment area of the Agra

city. Leelaja *et al*. (2007) have confirmed the presence of microscopic eggs of flour beetles using staining techniques. In a study, abnormal larva and adult of *T. castaneum* was photographed along with their normal counterpart using stereomicroscope (Santos *et al.*, 2011). A compiled micro photographic report on the life cycle of red flour beetle is the first study.

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