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BIOLOGY OF RUST RED FLOUR BEETLE *TRIBOLIUM CASTANEUM* (HERBST) ON STORED WHEAT

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ABSTRACT

The laboratory studies on the biology of the rust red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera) conducted on stored wheat revealed that adult females deposited the scattered eggs within the food material preferably on the upper layer with an average fecundity of 54.3±5.63 eggs / month while the incubation period was 3.45±0.68 days with 80.2±7.59 per cent hatching. The average larval period of the first, second, third, fourth, fifth, and sixth larval instar was 4.85±0.74, 3.60±0.75, 4.40±0.99, 5.40±1.39, 7.70 ±1.52 and 5.95±1.09 days, respectively. After completion of the last larval instar, it formed the pre-pupal stage before transforming into a complete pupa. The pre-pupal period lasted for 2.90±1.07 days and the duration of the pupal period was 6.45±1.05 days. Newly emerged adults were yellowish brown in colour, but later itturned into reddish brown. The total life cycle of males with and without food was 106.15±28.19 days and 64.7±12.48 days, respectively whereas in the case of adult females, it was 119.2±22.04 days with food and without food was 68.35 ±15.49 days.

Key words : *Tribolium castaneum*, Morphology, Morphometrics, Insect rearing, Stored wheat.

Introduction

To meet the increasing global need for food in the form of micronutrients, protein and energy, cereals are necessary. The three main cereal crops are wheat, maize and rice. India's staple food is wheat, which ranks next to rice. Wheat, *Triticum aestivum* L. is rich in carbohydrates, proteins and vitamins; it gives millions of people a balanced diet every day. In Gujarat, 4132.74 thousand tonnes of wheat were produced in 2022-23 on an area of 59.1 thousand hectares (Anonymous, 2023). About 95 species of the Tenebrionidae family are found to be associated with stored products. Among these species, *T. castaneum* is one of the major and most destructive secondary pests of stored grains (Prakash *et al*., 1987). *T. castaneum* may be present in large numbers in infested grains. The adults are attracted to light but go towards cover when disturbed. Normally, *T. castaneum* has been found inside infested grain products, but sometimes also found in cracks and crevices where grain may have spilt (Howe, 1965). They are attracted to grain with high moisture content and can cause a grey tint to the grain they are infesting (Ahmed *et al*., 2019). In addition to causing harm, they contaminate food and make it unfit for human consumption. The quantity and nutritional value of the stored wheat were both decreased by them (Singh *et al*., 2022). Hence, to find out the activity of various life stages and the nature of damage, the present investigation on the biology of *T. castaneum* infesting stored wheat was carried out under laboratory conditions.

Materials and Methods

Under sterile laboratory conditions, the stock culture was mass-multiplied on wheat. For four hours, the wheat grains were sterilized at 55°C in a hot air oven to get rid of the insect infestation. Every time the food was changed, which happened once a month, the culture in the previous rearing jar was agitated and pieces of white paper strip were placed on top of it. After a short time, the insects on the paper strip were collected and

meticulously separated using a camel hair brush into a fresh food-filled glass jar. Adult males and females were paired and confined into glass jars. Freshly laid 50 eggs of *T. castaneum* were taken from the stock culture and kept individually in a glass Petridish with the help of a moist camel hair brush and observed twice a day (morning and evening) daily till hatching. Eggs were considered hatched when tiny larvae came out of them and the percent egg hatchability was calculated by using the following formula:

$$\text{Egg hatchability (\%)} = \frac{\text{The number of neonate larvae hatched out}}{\text{Total number of eggs kept under observation}} \times 100$$

While, the incubation period was calculated from the date of egg laying to the date of egg hatching. Moreover, twenty hatched larvae were kept in another glass Petri dish individually to avoid cannibalism along with wheat grains as a portion of food and reared till they underwent pupation. Petridishes were checked regularly for the presence of exuviate, to calculate the duration of each larval instar. The total larval period was recorded from the date of egg hatching to the date of the formation of the pre-pupa. When a fully grown larva ceases feeding and becomes inactive, it is considered a pre-pupa. The pre-pupal period was recorded from the date of inactivation of the fully grown larva to the date of pupa formation. The pupal period was calculated from the date of formation of the pupa to the date of emergence of adult beetles. Newly emerged adults were separated as male and female based on their body size and morphological characteristics.Twenty pairs of adult male (♂) and female (♀) beetles were confined in glass jars individually, provided with crushed grains of wheat and reared to study pre-oviposition period, fecundity, longevity of male and female with and without food, sex ratio (male: female) and total life cycle. Pre-oviposition period was calculated from the date of emergence to the date of starting egg laying. To determine the fecundity, the total number of eggs laid during the entire life span of the adult female was collected and counted daily in the morning till the death of the female. The longevity of males and females was calculated from the date of emergence to the date of death of adults. The sex ratio was determined by counting the number of newly emerged adult male and female from pupae based on their morphological characteristics. The period from eggs laid to death of an adult was considered a total life cycle.

The morphological parameters like colour and shape of twenty replicates of each stage *viz*., eggs, larval instars, pre-pupa, pupa and adult (male and female) were observed using a binocular microscope Olympus SZ-61 while, the trinocular microscope (SZ-61; make: Olympus) attached to the software scope photo (Version 3.1) was used for measuring the morphometric parameters like length and breadth of eggs, larval instars, pre-pupa, pupa and adult (male and female). The data on temperature and relative humidity were recorded daily during the entire investigation period by using a digital thermo-hygrometer. The data on different parameters were statically analyzed.

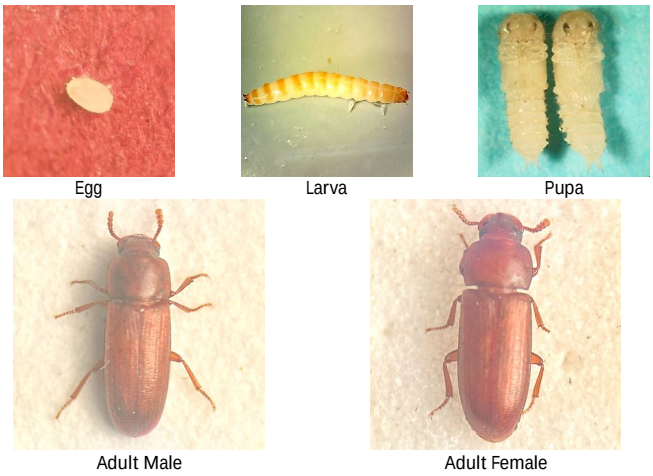

Fig. 1 : Life stages of rust red flour beetle, *Tribolium castaneum* (Herbst).

Results and Discussion

The findings of the present study are presented and discussed here. Eggs laid by mated gravid female beetlewere microscopic, whitish, cylindrical and rounded at both ends. While, the oviposition pattern was singly, loosely and scattered within the food material preferably on the upper surface. Moreover, the eggs were covered with a sticky secretion so that the flour particles and other debris stuck up making the eggs difficult to locate. Morphometric studies revealed that thelength of the eggs ranged from 0.44 to 0.58 mm while the breadth ranged from 0.23 to 0.36 mm (Table 2). The duration of development between egg laying and hatching was very short and it varied from 3 to 5 days. Moreover, the average hatching percentage was 80 percent (Table 1). Similar observations were reported by Gentry *et al*. (1991), Adil (2002), Alabi *et al*. (2008), Shrikant (2013) and Sundar *et al*. (2021).

Larva was the damaging stage which passed through six distinct instars and five moults. Moulting was confirmed by the presence of a casted-off head capsule and attended size. The newly emerged first instar larva of *T. castaneum* was slender andcylindrical, milky white in colour and later on changed into brownish white in colour. The black colour eyes were visible. It possessed

Table 1 : Details of the biology of *T. castaneum* reared on stored wheat.

S. no.	Life stage	Particulars		Min.	Max.	Mean± SD
1	Egg	Incubation period (days)		3.00	5.00	3.45± 0.68
		Hatching percentage (%)		70.00	92.00	80.2± 7.59
2	Larva	1st instar period (days)		4.00	6.00	4.85± 0.74
		2nd instar period (days)		3.00	5.00	3.60± 0.75
		3rd instar period (days)		3.00	6.00	4.40± 0.99
		4th instar period (days)		4.00	8.00	5.40± 1.39
		5th instar period (days)		6.00	10.00	7.70± 1.52
		6th instar period (days)		5.00	8.00	5.95± 1.09
		Total larval period (days)		27.00	38.00	31.9± 3.05
3	Pre-pupa	Total period (days)		2.00	5.00	2.90± 1.07
4	Pupa	Total period (days)		5.00	8.00	6.45± 1.05
5	Adult male	Longevity (days)	With food	32.00	90.00	63.25± 18.74
			Without food	6.00	32.00	19.55± 8.88
		Total life cycle (days)	With food	65.00	155.00	106.15± 28.19
			Without food	44.00	85.00	64.7± 12.48
6	Adult female	Pre-oviposition period (days)		3.00	7.00	4.45± 1.27
		Fecundity (No. of eggs/female)	Perday	0.00	5.00	2.60± 1.66
			Permonth	46.00	65.00	54.3± 5.63
			Entire life span	196.00	256.00	214.6± 17.5
		Longevity (days)	With food	60.00	125.00	88.70± 18.33
			Without food	10.00	34.00	21.25± 7.57
		Total life cycle (days)	With food	96.00	174.00	119.2± 22.04
			Without food	42.00	94.00	68.35± 15.49
7	Sex ratio (male: female)			1:1	1:1.67	1.33± 0.26

a dark brown head, three thoracic and eight abdominal segments. Three segmented antennae were visible undera microscope. Each thoracic segment had a pair of true legs, each leg with four visible segments and had a claw at the terminal segment. The larva also possessed finehairs on its whole-body segment. Moreover, the length of the first instar larva ranged from 0.84 to 1.01mm while the breadth varied from 0.22 to 0.29 mm. However, the duration of the first instar larva ranged from 4 to 6 days. The second instar larva *T. castaneum* looks like the first instar though it waspale brownish in colour with a well-developed light brown head capsule with three pairs of thoracic legs. The segments of the body were distinctly visible as they were separated by less sclerotized and light-colored inter-segmental. The length of the second instar larva of *T. castaneum* ranged from1.82 to 2.04 mm while the breadth ranged from 0.26 to 0.43 mm. The duration of the second instar larvawas varied from 3 to 5 days. The third instar larva was slender and cylindrical, pale brownish in colour with a dark brownish head and two distinctive, dark, and forked projections visible on the posterior side of the larva. It resembled the second instar larva except for the size of the body. The length of the third instar larva was found to vary from 2.34 to 2.87 mm while the breadth ranged from 0.41 to 0.55 mm. Moreover, the duration of the third instar larva ranged from 3 to 6 days.The fourth instar larva of *T. castaneum* resembled the previous instar larva except in size. The colour was slightly changed in to dark brownish and small hairs were observed on the body. The length of the fourth instar larva ranged from 3.21 to 3.67 mm and the breadth ranged from 0.51 to 0.67 mm whereas, the duration ranged from 4 to 8days. The fifth instar larva of *T. castaneum* resembled the previous instar and it was well developed having a stout body with a dark brown head and the colour changed slightly from pale brownish to

Table 2 : Details of the morphometrics of *T. castaneum* reared on stored wheat.

S. no.	Life stage		Length (mm)			Breadth (mm)		
			Min.	Max.	Mean± SD	Min.	Max.	Mean± SD
1	Egg		0.44	0.58	0.49± 0.04	0.23	0.36	0.28± 0.03
2	Larva	1st instar	0.84	1.01	0.89± 0.04	0.22	0.29	0.25± 0.02
		2nd instar	1.82	2.04	1.92± 0.06	0.26	0.43	0.33± 0.05
		3rd instar	2.34	2.87	2.61± 0.16	0.41	0.55	0.47± 0.04
		4th instar	3.21	3.67	3.43± 0.13	0.51	0.67	0.58± 0.05
		5th instar	3.26	4.25	3.73± 0.36	0.62	0.70	0.65± 0.02
		6th instar	4.44	4.71	4.58± 0.09	0.74	0.85	0.78± 0.03
3	Pre-pupa		4.19	4.55	4.40± 0.10	0.79	1.03	0.91± 0.07
4	Pupa	Male	3.58	4.96	4.50± 0.44	1.08	1.35	1.23± 0.07
		Female	3.54	4.56	4.32± 0.28	1.11	1.32	1.21± 0.06
5	Adult	Male	3.21	4.01	3.65± 0.22	1.06	1.30	1.19± 0.07
		Female	3.26	4.05	3.74± 0.25	1.06	1.41	1.27± 0.10

dark brown dorsally on anterior and posterior abdominal segments. The length of the fifth instar larva ranged from 3.21 to 4.25 mm while, the bread thronged from 0.62 to 0.70 mm whereas, the duration varied from 6 to 10 days.The sixth instar larva was almost similar to the fifth instar except in size. It was bigger in size, fully brownish in colour and three pairs of legs on the thoracic region and having small hairs visible on the body. The length and the breadth ranged from 4.44 to 4.71mm and 0.74to0.85mm, respectively while, the duration ranged from 5 to 8 days. The total larval period ranged from 27 to 38 days. Present findings are in line with previous studies by Mason (2003), Singh *et al*. (2006), Sundar *et al*. (2021) and Ushasri *et al*. (2023).

Beginning of the pre-pupal stage, the last instar larva stopped feeding became sluggish and slightly contracted in length. The body was shrunken and assumed a curved shape during the formation of the pre-pupa. It was pale brownish and dirty white in colour. The length of the pre-pupa ranged from 4.19 to 4.55 mm while, the breadth ranged from 0.79 to 1.03 mm whereas, the pre-pupal period lasted for 2 to 5 days. Pupation generally took place on the upper surface of the wheat grains or in between the grain particles. Freshly formed pupae were dirty white in colour and gradually changed in to yellowish, the pupae were naked, exarate type and visible compound eyes on the head. The body appendages on the thorax region were also clearly visible. The abdomen was five to six segmented with two spines - like processes present on the terminal segments. On the lateral side of the abdomen, it possessed two dark brown spiny processes on the 2nd, 3rd and 4th segments and a single spiny process on the 1st and 5th abdominal segments. The female pupae possessed prominent genital lobes which would be used for sex differentiation. The data on measurement of pupae revealed that the length of male and female pupae ranged from 3.58 to 4. 96 mm and 3.54 to 4.56 mm, respectively while the breadth of male and female ranged from 1.08 to 1.35 mm and 1.11 to 1.32 mm, respectively. The duration of the pupal period ranged from 5 to 8 days. Previous research by Sattigi *et al*. (1989), Adil (2002), Singh *et al*. (2006), Evaldo (2019), Sundar *et al*. (2021) and Ushasri *et al*. (2023) supported the above findings.

The freshly emerged adults were yellowish brown in colour, but they turned to reddish brown after one day. Adults were oblong with reddish brown for wings in the form of elytra and the hind wing was white and semi-transparent. The elytra possessed parallel in esofas lightly darker colour on the dorsal surface. Male and female can be easily distinguished based on their body size; the male was smaller than the female. Antennae were eleven segmented with distinct three segmented clubs. The data on measurement revealed that the length of male and female beetles varied from 3.21 to 4.01mm and 3.26 to 4.05 mm, respectively while, the breadth varied from 1.06 to 1.3 mm and 1.06 to1.41 mm, respectively. However, the female was found slightly bigger and broader than the male.These results are consistent with what has been found in previous studies by Sundar *et al*. (2021), Awadalla *et al*. (2023) and Ushasri *et al*. (2023).

The pre-oviposition period ranged from 3 to 7 days. Moreover, the female beetle laid 5 eggs per day and 46

to 65 eggs per month. The fecundity during the entire life span was also recorded as196 to 256 eggs. The longevity of adult males with food varied from 32 to 90 days and 6 to32 days without food while the longevity of adult females with food ranged from 60 to 125 days and 10 to 34 days without food. The sex ratio (male: female) was female biased *i.e.*, 1:1.67. The total life cycle of males with and without food ranged from 65 to 155 days and 45 to 85 days, respectively whereas, in the case of adult females it varied from 96 to 174 days with food and 42 to 94 days without food. The findings of the present investigation are in close confirmation with the findings of Evaldo (2019), Sundar *et al*. (2021), Awadalla *et al*. (2023) and Ushasri *et al*. (2023).

Conclusion

From this study, it could be concluded that the biological and morphological description of *T. castaneum* will be useful for the identification of various life stages *viz*., egg, different larval instars, pre-pupa, pupa, adult male and adult female. Overall, it will be useful to distinguish *T. castaneum* from other species of genus *Tribolium*. Furthermore, the larval and adult life span, nature of damage and damaging stages, and weak link/ target stage of *T. castaneum* will be useful for the planning of various management strategies in stored wheat.

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Conflict of interest

The authors declare that the research work was carried out without any financial or commercial dealings that could be raised as a probable conflict of interest.

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