Effect of Temperature and Humidity on the Culture of *Tribolium castaneum*, Herbst (Coleoptera: Tenebrionidae) in the laboratory

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Abstract- In the present study for the establishment of culture in the laboratory adults of *Tribolium castaneum* were collected from two different areas, Dayalbagh and Cantonment area of the Agra city and pooled together to have a heterogeneous population with large variability. The progeny thus reared in the laboratory was designated as parental strain (P1). Effect of temperature and humidity were studied on the strains of this laboratory culture.

Key words: culture, heterogeneous, variability, temperature, humidity

INTRODUCTION

Tribolium belongs to the order Coleoptera, which is the largest order of insects and contains the most common and important stored product pests. The name "Coleoptera" was given by Aristotle for the hardened shield- like forewings (coleo=shield + ptera= wing). The Coleoptera contains more described species than in any other order in the animal kingdom, constituting about 25% of all known life-forms. Forty percent of all described insect species are beetles (about 350,000 species), and new species are frequently discovered.

This is a large and varied group of insects that contains more than 15,000 species of which about 600 are associated with stored products. Most of the Tenebrionids are black or dark brown in colour and mainly phytophagous. Infestation by these beetles results in an unappealing smell due to the secretion of benzoquinones from abdominal glands. Adult beetles may be distinguished by their hardened protective forewings, the elytra. They usually have membranous hind wings which are folded beneath the elytra. Like other insects with a complete metamorphosis, beetles have four distinct stages in their life cycle: egg, larva, pupa and adult. The larvae of the beetles are found associated with stored food products.

Tribolium, being most cosmopolitan and abundant among the stored grain pests, has always attracted researchers for experimenting for their control. The Red flour beetle was identified and classified as early as 1797 by Herbst. In the late eighties, the process of adaptation to new environment and genetic aspect of cannibalism in flour beetles were discussed (Bergerson and Wool *et al.*, 1988; Stevens and Lori, 1989) but no systematic effort was made on its study throughout the 19^{th} century. The possible reason for it may be the lack of micro instruments to visualize such small beetles.

Among the genus *Tribolium* two species have been reported in India *T. confusum.* and *T. castaneum. T. confusum* is now reported to be absent from major part of the India and restricted to only few pockets of colder zones of Kashmir and parts of Himachal Pradesh **(**Sarup, personal communication**).** We have selected *T. castaneum* for our studies as it is suggested that this group has now advanced to a critical point from where they are now ready to break into newer environment (attacking previously uninfested grains), developing new mutations and increasing cross resistance day by day to various control measures. Reproductive performance of *Tribolium* exposed to various conditions like temperature, food availability and structural complexity, genetic variation and the ability to colonize new niches (food media) on the capture of *Tribolium castaneum* in simulated warehouses were studied. (Langer and Young, 1976; Bergerson, and Wool, 1986; Arnaud et al., 2005; Toews, 2005).

Review of literature available on red flour beetle reveals that work on *Tribolium* in India is extremely scanty in comparison to the work done in the rest of the world. In the present study culture of *Tribolium castaneum* was established in the laboratory and maintained. For maintaining a culture in the laboratory it is necessary to study the effects of environmental factors on the development or growth of culture. Organisms adapted to live in a given area are subjected to fluctuation in temperature and humidity because of seasonal changes. Temperature and humidity are two physical variables seemingly easy to control and modify in experimental research with laboratory organisms, upon which future applied studies, can be based. The present study was undertaken to determine the effect of temperature and humidity on the development of *T. castaneum*.

MATERIAL AND METHODS

Sampling- Adults of *Tribolium castaneum* were collected from two different areas of the city of Agra, Dayalbagh and Cantonment area and pooled together to have a heterogeneous population with large variability (Fig.1). These adults were identified according to the identification proposed by British Museum of Natural History (Freeman, 1990).

Media- The laboratory culture of *Tribolium* was maintained under standard food medium of wheat flour supplemented with 5% brewer's yeast.

Rearing of strains- The standard routine procedure (prescribed by WHO) for the strain isolation and culture were utilized. Adults of *Tribolium* from two different sampling sites were mixed and allowed to oviposit for 5days in the food medium and then they were removed. The larvae obtained were counted and allowed to pupate. It is technically impossible to separate eggs from the media therefore larval selection ensured the number of viable eggs.

The adults obtained from this laboratory maintained culture were introduced for oviposition in a glass rearing jar containing sterilized food media. Replicates of such jars were initially prepared and kept in a B.O.D Incubator where the temperature was maintained at 30 ± 1ºC. After allowing five days for oviposition the adults were removed by passing the wheat flour through a 20 mesh sieve. The sieved wheat flour containing the eggs was replaced in the same jar and kept in the incubator for the completion of the life cycle. The insect start emerging 30ºC ± 2 days after oviposition. To maintain uniformity in age, the rearing jars were regularly examined. Beginning with the first emergence of adult, the wheat flour was sieved every third day to obtain 1-3 days old adults. Such adults are later transferred in the glass rearing jars having fresh sterilized wheat flour supplemented with 5% brewer's yeast. On emergence of appreciable number of adults, insects were sieved out and used either for experiments or rearing the next generation. Subsequent generations of experimental strain were maintained in similar manner. The progeny thus reared in the laboratory was designated as parental strain (P1) which can be used for other studies. Effects of temperature and humidity on the development of *Tribolium* were observed.

RESULTS

Effect on the larval stage

Larvae of this species are more sensitive than eggs to extreme conditions. No larvae pupated at 40^{o}C and 90, 30 and 10 percent humidity, but a few are pupated at this temperature and 70, 50 percent relative humidity. At 28^{o}C no pupae formed at 10 percent relative humidity, but high proportions of larvae became adults at higher relative humidity. At 20^{o}C no larvae reached the adult stage at any humidity. The optimum temperature for larval development to wheat feed is 35^{0}C (Table-1). At every temperature the larvae develop most quickly at the highest relative humidity except the temperature 20^{0}C or below it.

Table: 1 Effect of temperature and humidity on the larval stage

Temperature (ºC)	Humidity (%)	Effect
40	90, 30, 10	No larvae pupated
40	70, 50	Few pupated
28	10	No pupation
28-35	70, 75	High proportion of larvae pupated
20	Any	No pupation

Effect on the pupal stage

The length of the pupal period is not affected by humidity, but it is affected by temperature. The shortest period for the pupa stage was 4 days at 40^{0}C. The period is slightly increased with decreasing temperature : 4 days at 40^{0}C, 5 days at 35^{0}C, 8 days at 30^{0}C, 12 days at 25^{0}C, and 24 days at 20^{0}C. Below 20^{0}C all pupae died at emergence (Table- 2).

Table- 2 Effect of temperature on the pupal stage

Temperature (ºC)	Length of pupal period
40	4 days
35	5 days
30	8 days
25	12 days
20	24 days
<20	All pupa died at emergence

Effect on the total developmental time

Table-3 and Fig.1 shows temperature conditions for development of *T. castaneum*. The optimum temperature for rapid growth of *T. castaneum* lie between 30 and 37^{0}C at a relative high humidity, *T. castaneum* larvae at 35^{0}C develop in about 13 days (Howe, 1960), while at 34^{0}C they develop in 15.5 days (Park, 1948). In our results larvae at 35^{0}C and 70 % relative humidity develop in about 27 days. The differences here could be attributed to characteristic genetic differences of the strains themselves. Beetle may complete their life cycle in 32-40 day, at optimum conditions.

Table-3 Temperature conditions for *Tribolium csataneum* development

TEMPERATURE LIFE STAGES	25^{0}C	30^{0}C	35^{0}C
Egg	**4-5 Days**	**3-4 Days**	**11-12 Days**
Larva	**40-41 Days**	**27-28 Days**	**25-27 Days**
Pupa	**12-14 Days**	**6-8 Days**	**5-6 Days**
Adult	**2-3 Days**	**3-4 Days**	**5-6 Days**

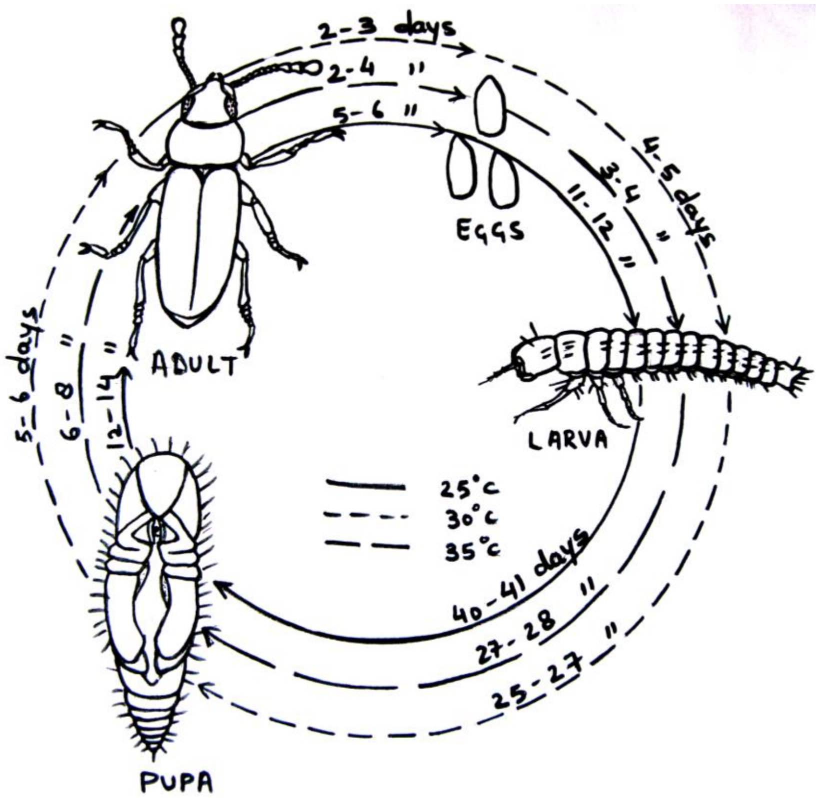

Fig.1 Life cycle of *T. castaneum* at different Temperature

CONCLUSION

The results of the present study revealed that the standard optimum temperature for *T. castaneum* fitness lie between the range of 30-35^{0}C and a relatively high humidity of 70 percent. This standard is valid only to the *Tribolium* reared on a standard feed. Larval mortality has been found to be due to the combination of temperature and humidity rather than any other. Low temperature

(<20^{0}C) and a low humidity (< or = 30%) combination has been found to be the critical point from where mortality begins. The pupa incidentally is not affected by humidity for its period of pupation but increase in temperature greater than 30^{0}C may reduce the pupal period.

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