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Effect of temperature on the biology of flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) in the laboratory

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Abstract
The flour beetles (*Tribolium* spp) are the most extensive pest in the world. *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) is associated with human stored products and cause damage resulting quantitative and qualitative losses. Laboratory experiment was conducted to study the biology of *T. confusum* under the effect of three different temperature degrees (25, 30, 35℃) and 70 % related humidity. The life cycle consisted of egg, larval, pupa and adult. The eggs hatched in 4-5, 2-3 and 3-4 days, the larval duration varied from 32-33, 19-20 and 18-19 days, pupal period of insect ranged 9-11, 6-8 and 5-6 days and adult period from new emerged up to able to produce (oviposition) ranged 5-6, 3-4 and 4-5 days at 25, 30 and 35℃ respectively. The average total life spam durations from eggs to adult's oviposition were 53, 33 and 32 days at 25, 30 and 35 ˚C and constant relative humidity. Which concluded that the standard optimum temperature for *T. confusum* fitness lie between the range of 30-35^{0}C and a relatively high humidity of 70 percent.

Keywords: biology, temperature degree, humidity, *tribolium confusum*, wheat flour

1. Introduction
Trib*olium* belong to the order of Coleoptera, which contains high number of described species than in any other order in animal kingdoms, constituting about 25 percent of all known life forms. The name of Coleoptera was given by Aristotle for the hardened shield, like forewings (coleo=ahield + ptera = wing). This is the largest order of insects, which contains about 370, 000 named species [1] and contains the most common and important stored product pests. About 40 beetle's families are associated with stored food products, however only the members of 7 families (Bostrichidae, Bruchidae, Curculionidae, Dermestidae, Laemopholidae, Silvanidae and Tenebionidae) are considered economically important.

Tenebrionidae is a large, varied and cosmopolitan group of insects, which commonly called darkling beetles, like other insects with complete metamorphosis. The number of species in the family of Tenebrionidae is estimated at more than 20, 000 of which about 600 are associated with stored food products. Infestation by *Tribolium* beetles results in an unappealing smell due to the secretion of benzoquinones from abdominal glands.

Tribolium, being most cosmopolitan and abundant among the Tenebrionidae stored product pests has always attracted researcher for investigating of their control. Among the genus *Tribolium* two species have been reported with economically important in different place of the world *T. confusum* and *T. castaneum. T. confusum* is now reported to be absent from major part of the world and restricted to only few place of colder zones. We have selected *T. confusum* 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 stored 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, humidity, food availability, and structural complexity.

Variation and the ability to colonize new niches (food media) on the capture of *T. castaneum* and *T. confusum* in simulated warehouses were studied by Langer and Young [2], Bergerson and Wool, [3], Arnaud *et al*. [4], Toews [5].

Review of literature available on confused flour beetle reveals that work on *Tribolium* in Saudi Arabia is extremely scanty in comparison to the work done on the rest of the world. In the present study culture of *Tribolium confusum* was established in the laboratory and maintained. For maintaining a culture in the laboratory it is necessary to study the effect of environmental factors such as temperature and humidity 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 conducted to find out the effect of temperature and humidity on the development of *T. confusum.*

2. Materials and Methods
A study on the biology of *T. confusum* 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 70% relative humidity.

2.1 Sampling
Adults of *Tribolium confusum* were collected from previous culture available in the laboratory of plant protection. The collected samples of insects were identified by morphological characters according to the taxonomic keys provided by Shomar [6].

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

2.3 Test insects and Sample preparation
The red flour beetle, *T. confusum* was cultured in petri dish of 9 cm size; contained 10 gr of wheat flour. Ten pair of freshly emerged adults (1-2 weeks old) from laboratory maintained culture were introduced into a petri dish containing sterilized food media for egg laying, and allowed to oviposit for 24 hours in the food medium. Replicates of such petri dish were initially prepared on the laboratory table and kept in an incubator where the temperature was maintained at 30℃. After allowing 24 hours for oviposition the parental insects removed by passing the wheat flour through a 20 mesh sieve. The sieved wheat flour contained the eggs of insect was replaced in the same petri dish and kept in the incubators at three different mentioned degree of temperature and constant RH (70%) separately (Plate 1a, 2b) for development and completion the life cycle of insects. The each replicate was examined under the plate Microscope (Nikon SMZ-2B/japan) to find out the first instar larvae. Then the petri dish contained infested food media was closed and kept in incubator as the earlier one for observing the next stage of development. The observation was made in 2 days interval to confirm the stage of development.

2.4 Development-Hatching
The eggs were tested from the day of culturing infested wheat flour in petri dish and exposing to incubator for hatching. The time taken by the eggs to hatch was calculated and the larvae obtained were counted. Technically impossible to separate eggs from the media, therefore larval selection ensured the number of viable eggs.

2.5 Larval and Pupal Development
After calculating the time of hatching they were allowed to continue their development. The larva fed and develops inside the food media. Since the larva moults several times in the wheat flour and allowed to pupate. The duration time of larval period was calculated and recorded. At the end of the larval period, the pupa was indicated according to the morphological characters. The plate was shocked by hand to smooth the flour and pin was used to observe the pupation stage. The time taken by larval period was recorded.

2.6 Adult emergence
The adult was also indicated as a pupation stage according to the morphological characters of adult beetle by shocking plat and using pin for observing the adult stage of the insect. The emergence of the adult insect was taken at the final stage of the growth phase. Effects of temperature on the last developmental stage of insect were observed. To maintain uniformity in age, the rearing plates 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 next experiments or rearing the next culture.

Fig 1a: Incubators used for the biological study of insects under the effect of three different temperature degrees.

Fig 1b: Incubator with exposed sample of insects.

3. Results
The data pertaining to the effect of three different temperature degrees (25, 30 and 35℃) on the biology and details of the development stages of *T. confusum* fed on wheat flour is given in Table 1. The different stages of development from egg to adult of *T. confusum* are shown in Plate 2. From the laboratory experimental data, the life cycle of *T. confusum* was studied.

Table 1: Temperature conditions for *T. confusum* development

Life stages	Duration under the effect of different temperature degrees		
	Duration (days) at 25 ˚C	Duration (days) at 30 ˚C	Duration (days) at 35˚C
Eggs	4-5	2-3	3-4
Larva	32-33	19-20	18-19
Pupa	9-11	6-8	5-6
Adult	5-6	3-4	4-5
Total life spam (days)	53	33	32

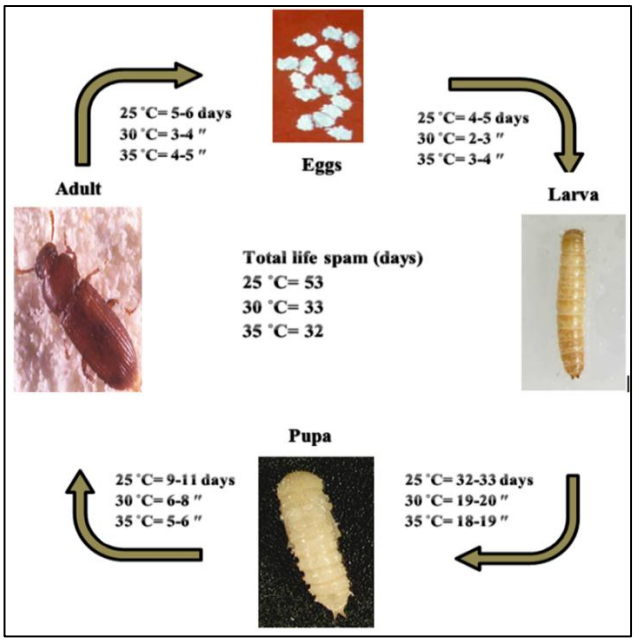

Fig 2: Life cycle of *T. confusum* at different temperature degrees

Eggs

The eggs of *T. confusum* were very small, white in color, oblong in shape, typically coated with flour (Plate 2) and were almost impossible to find without the use of a dissecting microscope. Wheat flour particles were confused with the eggs and were approximately the same size as the eggs. Under the wheat flour, the eggs were always white in color while the wheat particles were light brown or tan. The duration of egg at different temperature degrees (25, 30 and 35 ˚C) and constant 70% RH was ranged from 4-5, 2-3 and 3-4 days respectively. The optimum temperature for rapid develop of *T. confusum* eggs was 30 ˚C followed by 35 ˚C at relative humidity (70%). The longer period for the eggs stage was 4-5 days at 25 ˚C.

Larva

The larvae immediately after hatching were so small that they were difficult to find, even with a microscope, but they were easy to distinguish from their surroundings, provided. The larvae were creamy yellow to light brown in color, slender in shape and typically go through different instars (moltings) before maturing to pupa. They had two dark pointed projections on the last body segment. The larval period ranged from 32-33, 19-20 and 18-19 days at 25, 30 and 35 ˚C respectively. The period is slightly increasing with decreasing temperature.

Pupa

This was the easiest stage to deal with. Since they were pupating, they did not move unless they were prodded gently with insect forceps. The pupa was pale yellow to tan in color and appear to be crusted over. The pupal period of insect ranged from 9-11, 6-8 and 5-6 days at 25, 30 and 35℃ incubation periods. In this results 35 ˚C was the optimum temperature followed by 30 ˚C for the growth of *T. confusum* pupa. The longer period for pupa stage was 9-11 days at 25 ˚C.

Adults

The easiest ways to handle the adults were with insect forceps or by letting them crawl on to a small piece of paper. Immediately after emerging from the pupal stage, adults were pale yellow colors and after a few days, they were darken in color to a dark reddish-brown. The adult periods from new emerged up to able to produce (oviposition) were ranged 5-6, 3-4 and 4-5 days at 25, 30 and 35℃ respectively. 30 ˚C was optimum temperature for development of adult stage followed by 35 ˚C at 70% related constant humidity.

Total life spam durations from eggs to adult's oviposition of *T. confusum* were 53, 33 and 32 days at 25, 30 and 35 ˚C respectively. The total life spam duration period is slightly increasing with the decreasing temperature. The optimum temperature for rapid growth of *T. confusum* lies between 30 and 35 ˚C at a relative humidity (70%).

4. Discussion

Beetle life cycles include four stages: egg, larva, pupa, and adult [7]. In the present study we observed that, *Tribolium confusum* eggs were very small, white in color, oblong in shape and typically coated with wheat flour. Wheat particles in the flour were confused with the eggs. The larvae were creamy yellow to light brown in color, slender in shape. They have two dark pointed projections on the last body segment. The pupae were pale yellow to tan in color and appear to be crusted over. Immediately after emerging from the pupal stage adults were pale yellow colors and after a few days, they were darken in color to a dark reddish-brown. This was conformity with finding of Bousquet [8], Lale and Vidal [9], Pai [7].

In the present investigation we observed that the optimum temperature for rapid develop of *T. confusum* eggs was 30 ˚C that gave shorter duration period (2-3 days) followed by 35 ˚C (2-4 days). The longer period for the eggs stage was 4-5 days at 25 ˚C. Shweta Singh and Sant Prakas [10]; reported that at 25, 30 and 35 ˚C, the duration of *T. castaneum* egg ranged from 4-5, 3-4 and 11-12 days and the larval period ranged from 40-41, 27-28 and 25-27 days, respectively. In our study the larval period ranged from 32-33, 19-20 and 18-19 days at 25, 30 and 35 ˚C respectively. The period is slightly increasing with decreasing temperature. 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 [11], while at 34^{0}C they develop in 15.5 days [12]**.** Larvae at 35^{0}C and 70 % relative humidity develop in about 27 days [10]**.** In the present results 35 ˚C was the optimum temperature followed by 30 ˚C for the growth of *T. confusum* pupa. The adult periods from new emerged up to able to produce (oviposition) were ranged 5-6, 3-

4 and 4-5 days at 25, 30 and 35℃ respectively. Shweta Singh and Sant Prakas [10]; reported that the optimum temperature for the rapid growth of *T. castaneum* is 25 ˚C followed by 30 ˚C and adult period was 2-3, 3-4 and 5-6 days at 25, 30 and 35 ˚C. The life span of virgin adults in laboratory conditions of *T. castaneum* and *T. confusum* is 7–11 months [7]. The differences here could be attributed to characteristic genetic differences of the strains themselves, insect species, food types and availability, environmental condition and structural complexity. Various investigators [13, 14, 15, 16, 17, 18, 19, 20, 21] have studied the onset of sexual maturity in *Tribolium. T. castaneum* was found to mature earlier then *T. confusum* which was earliier then *T. destructor*. The onset of sexual maturity was earlier and production of eggs greater at higher then at lower temperatures. Males mature earlier then females. It has also been found that yeast-supplemented diet resulted in earlier sexual maturity and genotype by diet interaction for this trait [22]. In our results the optimum temperature for rapid growth of *T. confusum* lies between 30 and 35 ˚C at a relative humidity (70%).

5. Conclusion

The results of the present study showed that the standard optimum temperature for *C. maculatus* fitness lie between the range of 30-35^{0}C and a relatively high humidity of 70%. This standard is valid only to the *Tribolium* 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.

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7. References

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