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Impact of Temperature and Nutrient Medium on Confused Flour Beetle Development

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ABSTRACT

Tribolium confusum, commonly referred to as the confused flour beetle, is a widespread pest insect that commonly attacks and infests stored grain and flour. The present study aims to determine the growth of the larvae of the confused flour beetle, *T. confusum*, following feeding on wheat, barley, and yellow corn flour. The shortest duration and highest survival of the larvae were obtained after feeding them wheat flour. In contrast, the longest duration and least survival of the larvae were obtained after feeding on yellow corn flour. There was no definite pattern to the effect of the type of flour on the duration of pupae at the different temperatures (15, 27, 35°C). The highest survival of pupae was obtained after feeding larvae on wheat flour. However, the least survival was obtained after feeding larvae yellow corn flour. The larval and pulpal duration decreased with the increase in temperature from 15 to 35 °C. Contrariwise, the survival of larvae and pupae increased with the increase in temperature. It is clear that the maximum number that can be obtained for the developmental stages (larvae, pupae, and adult insects) from a similar clan of flour terns (*T.confusum*) under practical conditions is that the larvae are raised on wheat flour and kept at a temperature of 35°C until the adult stages of the insect emerge.

INTRODUCTION

Insects that infest stored grains and their products not only consume the grains but also make them unsuitable for human consumption. These insects contaminate the stored grains and their products with their feces and shed skins; it is their salivary secretions that cause the entanglement of threads in the grains or their products. At the global level, it has been estimated that about 5–10% of global grain production is lost during storage. In tropical countries where temperatures and humidity are high, grain losses during storage may reach 50% (De Lim & Saqib, 1985; Wilson *et al.*, 1969). Beetles can survive for more than a year as adults. Female-deposited eggs hatch in five to twelve days. Larvae are cylindrical, thin, white, and have a hint of yellow. They grow to about 3/16 inch long in as little as 30 days after going through 5 to 12 stages of development, or instars. In ideal circumstances, a female's life span can span many years, during which she can lay up to 1,000 eggs (Merchant *et al.*, 2018). The confused flour beetle, *Tribolium confusum*, is a worldwide pest that is dangerous to stored grains and their products, such as flour. Each insect species' rate of population expansion is greatly influenced by the type and quantity of food available. Population growth is the final result of the influence of many factors, including fertility, survival (especially the survival of immature stages), and rate of development. Each of these factors may be affected either directly or indirectly by the quality of food, its quantity, or both. The life history strategy adapted by a particular species results in complex relationships between these factors (Longstaff, 1995). The study aims to investigate the effect of three types of flour (wheat, barley, and yellow corn) and three types of temperatures (15, 27, and 35°C) on the development and survival of the immature stages of *Tribolium confusum* by determining the larval development period, percentage of larval survival, period of parthenogenesis, percentage of virgin survival, evidence of evolution, survival guide, and percentage of development per day.

The larval stage of stored product pests is affected by changes in external environmental conditions such as temperature and type of food. Chittenden stated in 1896 that beetles of the genus Tribolium can be raised on any type of ground grain. Although the beetle has the ability to survive and reproduce on whole grains, it prefers to grow on ground grains. (Lhaloui *et al.,* 1988; Daniels, 1956; Birch, 1947; Miller, 1944) compared six types of food in terms of their effect on the growth of *T. castaneum* larvae and concluded that wheat was the best of all. Magis (1954) also found that the fastest growth of *T. castaneum* larvae occurred when they were fed on wheat, barley, and corn, compared to rice and millet. The results of Khalifa and Badawy (1955) showed that wheat bran gave slightly faster growth to *T. castaneum* larvae compared to brown flour. Mickel and Standish (1947) concluded that a temperature of 35% is the ideal temperature for the growth of *T. castaneum* larvae. Howe (1956) confirmed this result when he stated that this temperature is the ideal temperature and added that the larvae fail to grow into adults at temperatures of 20°C and 40°C when the relative humidity is 30.90%. The growth rate of larvae was fastest at 35°C, and the percentage of larval mortality at this temperature was less than 20%. The results of Khalifa and Badawy (1955) indicated that the majority of *T. castaneum* larvae that were raised on brown wheat flour produced six molts, and a few of them produced 5 to 7 molts. As for the larvae that were fed on white wheat flour or corn flour, they had 6–8 molts. He also added that the growth of wheat flour was faster than the growth of corn flour. Likewise, yeast has been used by many researchers to feed *T. castaneum* (Park & Frank, 1948; Mickel & Standish, 1947). The results of the statistical analysis showed that there were significant differences between the growth period of the larval stage when fed on wheat, corn, and soybeans compared to that period when fed on rice, which is considered the least preferred food type because it contains a low percentage of fat and protein, while soybeans were the most preferred type due to their high percentage of components. On the other hand, Wagiman *et al.* (1999) found that *T. castaneum* larvae benefit from broken rice due to the activity of rice weevil larvae, Sitophilus oryzae. White (1987) reported that temperature has a significant effect on the growth period of the larval stage of the red flour beetle *T. castaneum*. At temperatures of 22.5, 25, 30, 35, and 37.5°C and a relative humidity of 65%, the duration of larval stage growth was 49.1, 29.9, 18.4, 15.5, and 17.6 days, respectively. At a relative humidity of 55% and the same previous temperatures, the growth period of the larval stage was 58.3, 37, 22.9, 18.6, and 21 days, respectively. These results indicate that the growth rate was high at temperatures higher than 30°C, and that rate decreased clearly at temperatures of 22.5 and 25°C. When the relative humidity decreased to 40%, temperatures had a different effect, as the larvae failed to complete their growth at temperatures of 22.5, 25, 30, and 37°C, while the growth period increased to 35.5 days at 30°C. The average larval growth period of the *T. castaneum* beetle that was reared on brown rice at temperatures of 25, 28, 34, and 36 °C was 53, 33.4, 30.6, and 31 days, respectively (Chon et al. 1991).

The biological activity of the parthenogenetic stage of stored product pests, like the rest of the life stages of other insect species, is affected by changes in the surrounding environmental conditions, including temperatures. Good (1936) noted that the development of the pupal stage of the mealybug beetle, *T. castaneum*, usually takes a relatively long time at a temperature of 25°C. In another study by Miller (1944a & b), it was noted that the pupal growth period of the red flour beetle *T. castaneum* ranged between 5.3 and 6.1 days at a temperature of 30 °C and a relative humidity of 75%. While Mickel (1947) and Standish found that the period of development of the pupal stage in the red flour beetle *T. castaneum* was 4-5 days at a temperature of 35°C, and it was 9–10 days at a temperature of 25°C, In a study conducted by Howe (1956), it was noted that the period of development of the pupal stage was in The red flour beetle *T. castaneum* decreases with the increase in temperature, as that period was 4.4, 3.9, 4.5, 4.6, 5.5, 7.2, 10.2, 13.4, and 24.4 days at temperatures of 40, 37.5, 35, 32.5, 30, 2, 7, 25, 22.5, and 20°C, respectively. These results indicate that a temperature of 37.5°C is the ideal temperature for the growth of the red flour beetle *T. castaneum.* King (1918) registered that the shortest growth period for the pupa in the *Sitotroga crealella* was 5.9 days at a temperature of 30 °C and a relative humidity of 80%, while the length of the period was 14.10 days at a temperature of 20 °C and a relative humidity of 40%. Cox (1973) also noted that the temperature of 30°C is the ideal temperature for the development of the parthenogenesis of the dried fruit moth *E. figulilla*, where the average parthenogenesis growth was 27, 18, 12, 10, 9, 7, and 8 days at temperatures of 17.5., 22.5, 25.5, 27.5, 30, and 35°C, respectively, and a relative humidity of 70%. Also, the growth rate of pupae in the lesser grain borer *R. dominica* was directly proportional to the temperatures that ranged between 27 and 33°C, and the pupae failed to grow at high temperatures (36°C) and relative humidity (Bains, 1971). As for the effect of the type of food that the larvae feed on the period of pupal development, Mukerji and Sinha (1953) found that the larval food has no significant effect. On the duration of parthenogenesis in the red flour beetle *T. castaneum.*

MATERIALS AND METHODS

Adults of the confused flour beetle *Tribolium confusum* were collected from a flour store in the city of Misrata and identified using taxonomic keys such as the presence of Caduceus antennae (Fig. 1) (Borror & Delong, 1964). In 1980, Freeman used three types of flour as a nutritional medium in raising the insect as well as in conducting experiments: wheat, barley, and yellow corn. The three previous nutritional media were then placed separately in a laboratory (300 ml) in an oven for an hour at a temperature of 60 °C to obtain maximum disinfection of the flour and get rid of any infection that may be present in it (Saleem *et al.*, 2001). After leaving the tester containing each type of flour to cool at room temperature, it was filled to about a quarter only with flour, and 100 adult insects were added to it to lay eggs. White paper strips were placed in the tester for the insects to climb on, and the mouth of the tester was closed with a piece of gauze cloth held in place with a rubber band. Place the laboratory, including the adults, in the incubator (2735 Kottermann) at a temperature of 27 °C and a relative humidity of 60% (Bray & Davis, 1985). To obtain this level of relative humidity inside the incubator, 30 g of potassium hydroxide (KOH) (manufactured by the British company BDH) was dissolved in 100 ml of distilled water (Solomon, 1951). After preparing the solution, place it inside a glass desiccator where the test containing the adults is placed, and keep the desiccator at a temperature of 27°C. The flour was sifted through a sieve (sixty holes per inch) every two days to collect the eggs (Bray and Davis, 1985).

Fig. 1: The complete insect of the confused flour beetle, *Tribolium confusum*

Experimental Methods:

1.The Effect of Flour Type and Temperature on The Development and Survival of Immature Stages:

In this part, three temperatures were chosen (15, 27, and 35 °C) in addition to confusum, separately, in a glass tube (1 x 3 cm) filled with only one gram of flour and marked with the date of hatching and the type of flour. The nozzle of the tubes was closed with a piece of gauze cloth secured with a rubber band and placed in the incubator at a temperature of 15, 27, 35°C and a relative humidity of 60%. The larvae were monitored until they reached the pupal stage, and the transition period from larvae to pupa was calculated at each type of flour and previous temperatures in order to calculate the length of the larval stage's lifespan under the previous conditions. The experiment was repeated three times. Also, in this experiment, the percentage of pupation was calculated as a measure of the percentage of larval survival. The virgins were collected by sifting the flour using a sieve (60 holes per inch) in a glass tube (1 x 3 cm) marked with the date of separation and the type of flour. The nozzle of the tubes was closed with a piece of gauze cloth using a rubber band and placed in the incubator at the previous temperatures (15, 27, and 35 °C) and 60% relative humidity. The pupae were monitored until the emergence of adults, and the date of emergence was recorded for each pupa in order to calculate the length of the pupal lifespan. In this experiment, the percentage of adult emergence was also calculated as a measure of the percentage of pupae survival. The experiment was repeated three times.

2.Evidence of Development and Survival:

To calculate the indicators of development and survival of the larvae (from hatching until the completion of larval development) at a temperature of 27 °C and a relative humidity of 60%, and for the three types of flour (wheat, barley, and yellow corn), the following equations were applied:

-Percentage of evolution per day =. 100 x 1/larval period (days) (Power and Oatman, 1984).

-Development index = percentage of survival / larval stage period (days). (Moonis, 1979).

-Survival index = percentage of survival/maximum survival in all treatments. (Moonis, 1979).

RESULTS AND DISCUSSION

The Effect of the Type of Nutrient Medium (wheat, barley, and yellow corn) on the Development and Survival of Immature Stages:

1. The Larval Stage:

The results shown in Table 1 and Figure 2, show that, in general, the shortest growth period for the larvae of the confused flour beetle *T. confusum* was obtained when the larvae were raised on wheat grain flour, followed by that period when the larvae were raised on flour. Barley grains. The longest growth period for larvae was when they were raised on yellow cornmeal during the temperature range of 15-35°C. The difference between the average larval growth period when the larvae were reared on wheat flour and when they were reared on barley flour was not significant ($P > 0.05$). However, this difference was significant ($P < 0.05$) between the average growth period of the larval stage when fed wheat flour and yellow corn flour at a temperature of 15° C. Likewise, this difference was significant ($P < 0.05$) between the larvae fed on barley flour and yellow corn flour at the same temperature. The same results were statistically obtained at a temperature of 35°C. However, at a temperature of 27°C, there was no significant difference between the average growth period of the larval stage of the confused flour beetle *T. confusum* when fed wheat, barley, or yellow corn flour. The reason for the decrease in the growth period of the larval stage of the confused flour beetle *T. confusum*, when raised on wheat flour compared to that period when raising larvae on barley and yellow corn flour, is that wheat flour contains a greater amount of protein compared to barley or yellow corn flour, and the current results are similar to Mickel's. Standish (1947 found that brown wheat flour (the same flour used in the current study) was more important than white wheat flour or barley flour in the growth of the larvae of the red flour beetle *T. castaneum*. After that, it became clear that brown wheat flour contains a higher percentage of protein and vitamin B1 compared with white wheat flour or barley flour (Mukerji and Sinha, 1953). Similarly, wheat was better than brown rice in rearing the larvae of the red flour beetle *T. castaneum* (Chon *et al.*, 1991). When analyzing the seeds of cowpeas, chickpeas, and peas, it was found that the seeds of the cowpea contained a higher percentage of protein than the seeds of chickpeas and peas (Hulse and Davis *et al.*, 1991; Duke, 1994; Huisman and Van der *et al.*, 1981; Poel, 1994). These results indicate that protein is the determining nutritional component for larval growth.

Table 1: The larval development period of the confused flour beetle *T. confusum* when raised on three types of flour (wheat, barley, and yellow corn) and preserved at three temperatures (15, 27, 35° C).

Temperature (°C)	Type of flour	Larval development period ± standard error (Days)	Larval stage survival ± measurement error (%)
15	Wheat	79.00 ±1.00 a	46.67± 0.33 a
	Barley	81.67 ± 0.67 a	30.00 ±0.77 b
	Yellow corn	100.00 ± 2.51b	26.67± 0.68 b
27	Wheat	18.00 ± 0.58 a	70.00 ± 1.00 a
	Barley	19.00 ± 0.62 a	46.67 ± 0.82 b
	Yellow corn	18.67 ± 0.53 a	30.00 ± 0.15 c
35	Wheat	10.33 ± 0.33 a	80.00 ± 1.16 a
	Barley	10.00 ± 0.30 a	65.00 ± 1.13 b
	Yellow corn	15.67 ± 0.67 b	45.00 ± 1.77 c

• Numbers followed by unlike letters are significantly different ($P < 0.05$) for the three types of flour at each temperature and variable separately.

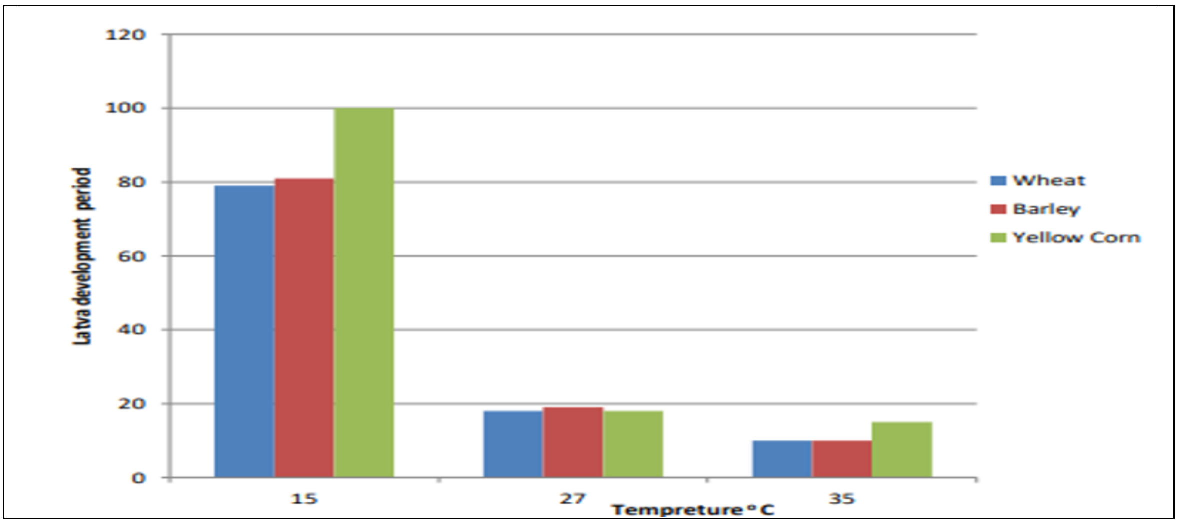

Fig. 2: The larval development period of the confused flour beetle *T. confusum* when raised on three types of flour (wheat, barley, and yellow corn) and preserved at three temperatures (15, 27, and 35° C).

Miller (1944a) compared six types of food in terms of their effect on the growth of larvae of the red flour beetle *T. castaneum*. He concluded that wheat was the best of all. The same results were reached by Khalifa and Badawy (1955) when they compared the effects of wheat and corn flour on the same species. On the other hand, Table 1 and Figure 2, showed that the growth period of the larval stage of the confused flour beetle *T. confusum* decreased with the increase in temperature in all three-nutrient media (wheat flour, barley, and yellow corn). It was the longest period when the larvae were raised at a temperature of 15°C, while the shortest period was at a temperature of 35°C. These results indicate that a temperature of 35°C is the ideal temperature for the growth of confused flour beetle larvae. The current results are similar to those reached by many of the previous researchers on the larvae of the red flour beetle, *T. castaneum*. The growth period of the larval stage of this type of stored product pest decreased with the gradual rise in temperature, and a temperature of 35°C was the ideal temperature for the growth of larvae, regardless of the type of nutrient medium (Chon, 1991; White, 1987; Howe, 1956; Mickel and Standish, 1947). The current study indicated that the larvae of the confused flour beetle *T. confusum* were raised on wheat flour, followed by barley flour. While the lowest survival of larvae was when they were raised on yellow cornmeal (Table 1, Fig. 3), the difference between the average survival rate of larvae raised on wheat flour and those raised on barley or yellow corn flour was significant ($P < 0.05$) at a temperature of 15°C. However, the difference between the average survival of both barley and yellow corn was not significant ($P > 0.05$) at the same temperature (15°C). On the other hand, the difference between the average survival of the three types of flour (wheat, barley, and yellow corn) was significant ($P < 0.05$) at the two temperatures of 27 and 35°C. The present results are similar to those obtained by Howe (1956), where the survival rates of the larvae of the red flour beetle *T. castaneum* were greatest when they were reared on wheat flour, while the lowest survival rates were when the larvae were reared on peanuts. Likewise, wheat flour was the best food medium for the survival of red flour beetle larvae compared to brown rice (Chon *et al.*, 1991).

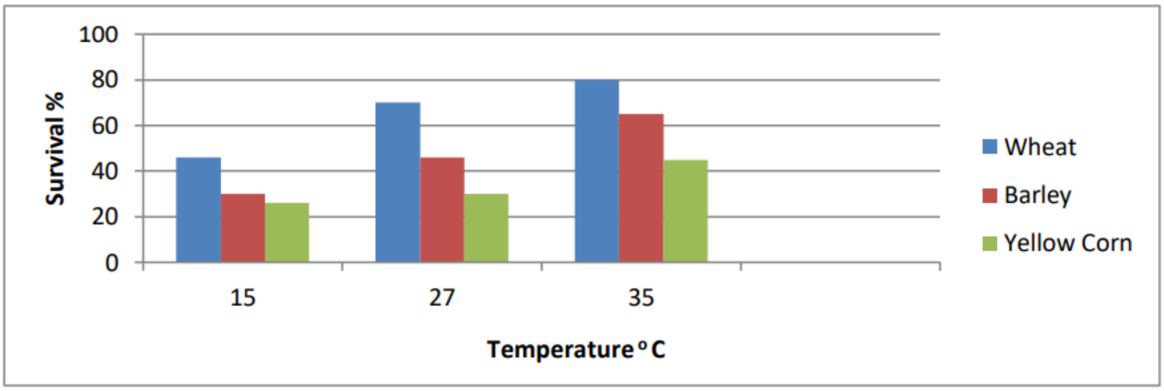

Fig. 3: Survival of the larval stage of the confused flour beetle *T. confusum* when raised on three types of flour (wheat, barley, and yellow corn) and kept at three temperatures (15, 27, and 35 °C).

On the other hand, the percentage of survival of the larvae of the confused flour beetle *T. confusum* increased with the gradual increase in temperature from 15 to 35°C, regardless of the type of food medium. The highest percentage of larval survival was at a temperature of 35°C in all three nutritional media in which the larvae were raised (Table 1, Fig. 3). Likewise, Howe (1956) noted that the percentage of larval stage survival of the red flour beetle *T. castaneum* was zero, 87, 94, and 87 at a temperature of 40, which is the longest period. Statistically, the difference between the average period of parthenogenesis of barley and yellow corn flour and that of barley and wheat was significant ($P < 0.05$).

Table 2: Development and survival of pupae of the confused flour beetle *T. confusum*.1 resulting from larvae raised on three types of flour (wheat, barley, and yellow corn) and preserved at three temperatures (15, 27, and 35°C).

Temperature (°C)	Type of flour	Parthenogenetic development period ± standard error (The days)	Parthenogenetic survival ± standard error (%)
15	Wheat	15.33 ± 0.20 a	65.00± 1.15 a
	Barley	16.67 ± 0.67 a	52.33 ± 0.88 b
	Yellow corn	14.00 ± 0.58 a	44.67± 1.45 c
27	Wheat	9.33 ± 0.67 a	93.00 ± 1.16 a
	Barley	6.67 ± 0.68 b	90.00± 1.14 a
	Yellow corn	8.33 ± 0.66 a	82.00 ± 1.15 b
35	Wheat	6.67 ± 0.31 a	97.00 ± 1.13 a
	Barley	5.67 ± 0.33 a	92.00 ± 1.11 a
	Yellow corn	4.00 ± 0.58 b	87.00 ± 1.19 b

• Numbers followed by unlike letters are significantly different ($P<0.05$) for the three types of flour at each temperature and variable separately.

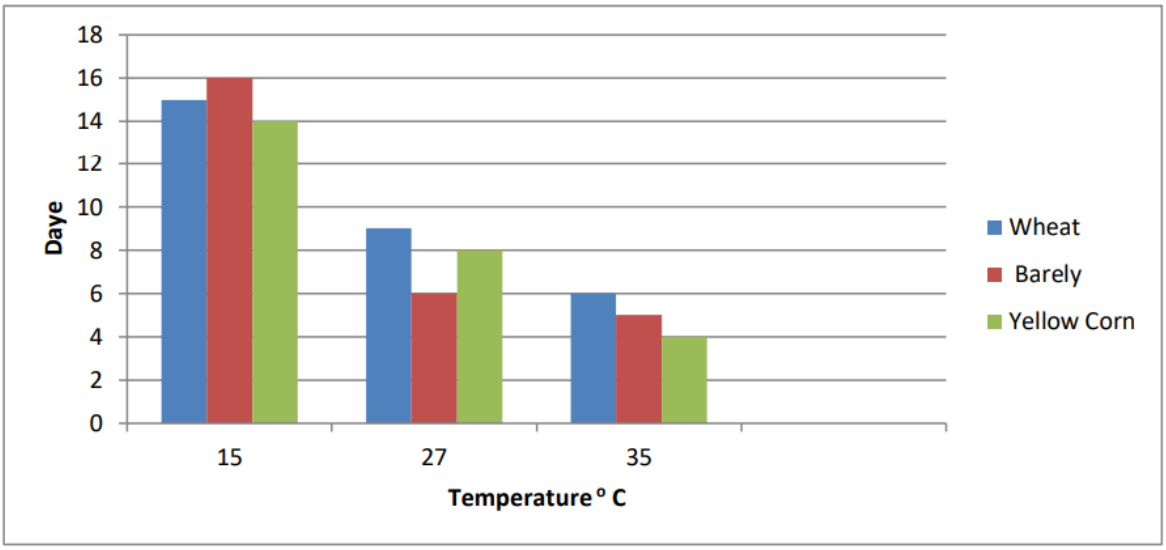

Fig. 4: The period of development of the pupal stage of the confused flour beetle *T. confusum* resulting from larvae raised on three types of flour (wheat, barley, and yellow corn) and kept at three temperatures (15, 27, and 35° C).

Whereas, when the larvae were kept at a temperature of 35°C, the growth period of the pupae resulting from the larvae that were raised on yellow corn flour was the shortest period, followed by the growth period of the pupae resulting from the larvae that were raised on barley flour, and the period of growth of the pupae was the longest. It was obtained from larvae raised on wheat flour. The difference between the average period of parthenogenesis of wheat flour and yellow corn, as well as the difference between the average period of parthenogenesis of barley flour and yellow corn, was significant ($P < 0.05$). On the contrary, the difference between the average parthenogenesis period of wheat and barley flour was not significant ($P > 0.05$). In general, based on the results obtained (Table 2, Fig. 3), the shortest growth period for pupae of the confused flour beetle *T. confusum* was obtained when raising larvae on yellow cornmeal. In contrast, the longest growth period of these pupae was obtained from larvae that had been raised on wheat flour. It was found that the diet of the larvae of the red flour beetle *T. castaneum* has no significant effect on the growth period of the resulting parthenium. (Howe, 1956; Mukerji and Sinha, 1953). This result is completely similar to the three types of flour used in current studies of the larvae of the confused flour beetle *T. confusum* at a temperature of only 15°C. However, this similarity was partial later, as it differed according to the temperature of the surrounding environment. The difference between the average periods of development of the pupal stage of the confused flour beetle *T. confusum* was not significant at a temperature of 27°C except between the pupae resulting from larvae that were fed on wheat and yellow corn flour. Likewise, this difference was not significant at a temperature of 35°C except when the larvae were fed wheat and barley flour (Table 2, Fig. 3**).** This is similar to the results reached in the current study on the same type of insect, only among the pupae resulting from the larvae fed on barley and yellow corn flour at two temperatures of 27°C and 35 °C, as well as among the virgins resulting from Table 2 and Figure 4. Larvae are fed on wheat and yellow corn flour at a temperature of 35°C. The period of development of the pupal stage of the confused flour beetle, *T. confusum* decreased with the gradual increase in temperature from 15°C to 35°C in all three-nutrient media (wheat, barley, and yellow corn) on which the larvae were fed before, as it was the longest development period. At a temperature of 15°C, while the shortest development period was at a temperature of 35°C. These results indicate that 35°C is the ideal temperature for the development of pupae of the confused flour beetle, *T. confusum*, regardless of the type of nutrient medium (Table 2, Fig. 4). This result is similar to that reached by many researchers on the red flour beetle, *T. castaneum*, where the period of pupal development decreased with the increase in temperature, and the temperature of 35°C was the ideal temperature for pupal development (Miller, 1944a; Good, 1936; Mickel & Standish, 1947, Howe, 1956). The current results (Table 2, Fig. 5) showed that the percentage of survival of the gnats of the confused flour beetle *T. confusum* was affected depending on the type of flour on which the larvae were previously seen, and wheat flour was the best type of flour. Compared to barley and yellow corn flour, it had the highest survival rate of larvae, and on the contrary, the survival rate of hydatid was obtained when the larvae were fed on yellow sorghum flour. Statistically, the difference between the percentages of hydatid survival of the confused beetle, *T. confusum* was significant ($P < 0.05$). When the larvae were fed on wheat flour, barley, and yellow corn at a temperature of 15 °C. When the larvae were kept at two temperatures of 27 °C and 35 °C, the difference between the average survival of the larvae was significant ($P < 0.05$) only between wheat and yellow corn and between barley and yellow corn. The percentage of survival of pupae of the confused flour beetle *T confusum* increased with the gradual increase in temperature from 15 to 35°C at the three types of flour on which the larvae had previously been raised (Table 2, Fig. 5**).** Likewise, CON (1973) noted that the survival rate of pupae of the fruit moth *E figulilla* was 85.96.84.98.97, or 31%, at temperatures of 17.5, 20, 22.5, 30, 32.5, and 35 °C and relative humidity of 70%, respectively. On the other hand, the survival of pupae of the small grain borer *R. dominica* was not affected by the change in temperature from 27 to 33 °C (Bains, 1971).

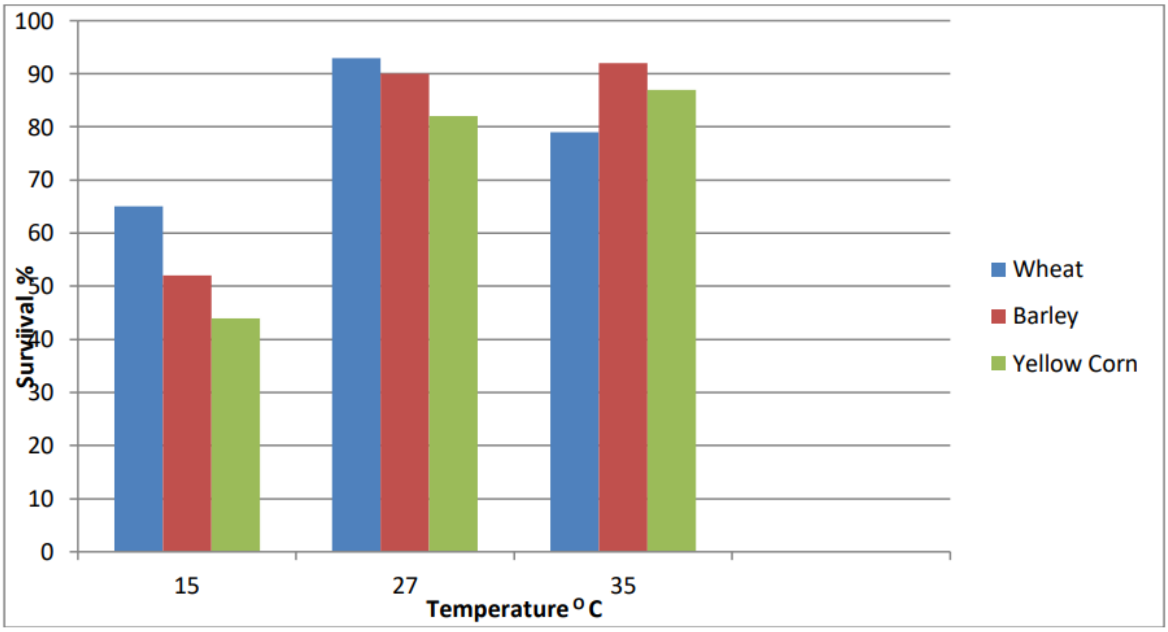

Fig. 5: Survival of the pupal stage of the confused flour beetle *T. confusum* resulting from larvae raised on three types of flour (wheat, barley, and yellow corn) and kept at three temperatures (15, 27, and 35°C).

Based on the results obtained (Tables 1 and 2, Figs. 2–5), it is clear that the maximum number that can be obtained for developmental stages (larvae, pupae, and adult insects from the confused flour clan, *T. confusum*.), under practical conditions, is that the larvae are reared on wheat flour and stored at a temperature of 35° C until the adult stages of the insect emerge.

2-The limitation of the Study:

One limitation of our study is that we only focused on the confused flour beetle, *T. confusum*, without considering how it interacts with different types of flour.

Conclusion

The larvae of the confused flour beetle *T. confusum* that were fed on wheat flour had the shortest lifespan and the highest rate of survival. By contrast, the larvae that were fed on yellow corn flour had the greatest duration and the lowest survival rate. The duration of pupa at each of the three temperatures (15, 27, and 35°C) was not significantly impacted by the type of flour. Feeding larvae wheat flour resulted in the highest pupa survival rate. However, feeding larvae yellow corn flour resulted in the lowest survival rate. A temperature rise from 15 to 35°C resulted in a decrease in the larval and pulpal duration. However, when the temperature rose, the larval and pupal survival rates increased. This study indicated that feeding the larvae wheat flour at a temperature of 35°C provided the optimum environment for their growth and survival. It not only advances scientific understanding but also offers practical solutions to real-world challenges faced by grain storage facilities and agricultural industries.

Declarations:

Ethical Approval and Consent to Participate: Not applicable.

Consent For Publication:All authors declared that there are no issues related to journal policies.

Competing Interests: The authors declare that they have no conflict of interest.

Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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