**Salahaddin University-Erbil**

**College of Agricultural Engineering Sciences**

**Department of Plant Protection**

**Instar Determination for** ***Tribolium*** ***confusum* (Hbst.) (Coleoptera: Tenebrionidae) Using Head Capsule Widths and Lengths**

Research Project

Submitted to the Department of (plant protection) in partial fulfillment.

of the requirements for the degree of **BSc.** in **plant protection**

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**Abstract**

The confused flour beetle, *Tribolium confusum*, is a widespread pest that infests stored grains globally, and it is essential to understand its life cycle and physical characteristics, including morphometric parameters, to manage the pest effectively. This study aimed to examine the morphometric parameters of *T. confusum* larvae raised on white and whole wheat flour. The results revealed no significant differences in morphometric parameters of larvae reared on different types of flour. However, there were noteworthy differences in morphometric parameters, such as head capsule width, head capsule length, and body length, among various larval instars. As the larval instar advanced from first to seventh, the average values of these morphometric parameters also increased significantly.

**Keywords:** *Tribolium confusum*, confused flour beetle, physical characteristics, morphometrics, white flour, whole wheat flour.

# **1.Introduction**

The flour beetle, also known as the confused flour beetle (*Tribolium confusum*), is a type of darkling beetle that infests stored flour and grain, causing significant damage to these products. They are a common and destructive pest in silos, warehouses, grocery stores, and homes (Alanko *et al*. 2000). In Iraq, wheat is a staple food and makes up 75% of the grain consumed. Unfortunately, cereal grain losses during storage can be as high as 50% of the total harvest in some countries, with insects being a major cause of this loss in quality (Fornal *et al*., 2007).

Understanding the biology of insects is crucial for developing effective pest management strategies. To study the development of insects, it is important to be able to accurately determine the instar of the larvae. However, previous research on *T. confusum* has not reached a consensus on the number of instars of this beetle, and it is possible that differences in geography and other factors may affect this. Many insects have been observed to have a variable number of instars due to various factors, such as temperature and host plant (Aguilon and Velasco, 2015).

The study aimed to determine the number of instars in *T. confusum* and compare it to past research. They used head capsule width to measure the instar of individual larvae. Morphometric parameters of *T. confusum* larvae were measured on both white and whole wheat flour during the larval stage.

# **2.Literature Review and Background**

## **2.1-Taxonomic classification**

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Hexapoda

Class: Insecta

Order: Coleoptera

Superfamily: Tenebrionoidea

Family: Tenebrionidae

Subfamily: Tenebrioninae

Genus: *Tribolium*

Species: *confusum*

(Haines, 1991; Bolev, 2014; Myers *et al*., 2016).

## **2.2-Origin and Distribution**

The confused flour beetle, which is believed to have originated in Africa, has a wide distribution and is found in cooler climates all around the world. In the United States, it tends to be more abundant in the northern states (Smith and Whitman, 1992).

## **2.3-Morphological description of different larva instars of *T. confusum***

### **2.3.1-** **Larvae**

The larvae of *T. confusum* exhibit distinct morphological features. Unlike adult beetles, the maxilla of last larval instars have only three palp segments, and two endites are present in each of the two maxillae. The labium of larvae has two segmented palps. The antennae of the larvae have three segments and are inserted in a membranous structure called anta corium that connects them to the head capsule. The basal segment (antennomere I) is subcylindrical, while the antennomere II has a lobe and the antennomere III is tiny and elongated, internally situated on the apex of antennomere II, and bears a long seta.

Most last larval instars range from 5.75 to 6.9 mm in length and 0.75 to 0.95 mm in width, with the head, thorax, and abdomen covered in minute spines. The first larval instars are 5.0– 5.1 mm long and 0.5–0.6 mm wide, with a shortened antenna and a reduced number of antennomeres. They also possess well-developed and moderately long legs. The general body shape of the larvae is elongate and cylindrical, mostly white, weakly sclerotized surface with sparse vestiture of whitish setae, with segment IX dorsally forming divided sclerite (pygidium). Appendages of tergum IX, called urogomphi, are present in the first and last larval instars. Segment X is not visible in dorsal view, inserted on the ventral side posterior to sternum IX, and may be represented by two lobes, which is probably developed as a pygopod. The larvae have three pairs of legs, each consisting of five segments (coax, trochanter, femur, tibia, and tarsus), and a single claw is present in the first and last instar larvae (Zohry, 2017).

## **2.4-Biology and life cycle**

When disturbed, adult confused flour beetles are highly active and move quickly. On average, they live for about one year. Female beetles lay approximately 450 small, clear white eggs on fine materials and broken kernels where they reside. These eggs are loosely laid and covered in a sticky secretion, which helps to adhere them to the surrounding material. If previous grain residue is not removed, fresh material placed in a grain bin can quickly become infested. Larvae, which are small brownish-white worms, hatch within five to twelve days and mature in one to four months. When fully grown, the larvae are about three-sixteenths of an inch long and have a yellow tinge. They feed on fine materials and broken grain kernels.

After this stage, the larvae transform into small, naked pupae. At first, these pupae are white but gradually change color to yellow and then to brown. Shortly afterwards, they develop into reddish-brown adult beetles. Under favorable weather conditions, the egg-to-adult cycle of the confused flour beetle typically takes about six weeks, although this can be prolonged by cold weather, which is the case for all grain pests. In general, the life cycle of the confused flour beetle is shorter than that of the red flour beetle.(Sreeramojo. et al.,2016)

Diagram

Description automatically generated

Fig. Life cycle of a flour beetle, *Tribolium* sp.

## **2.5-Host range and damage**

*Tribolium confusum* is a widely distributed pest of stored products, known for its polyphagous feeding habits and ability to attack a variety of stored goods and their by-products (Fedina and Lewis, 2007; Bachrouch et al., 2010; Obeng-Ofori and Reichmuth, 1999). This pest is often found infesting cocoa beans in both farm and commercial warehouses (Bateman, 2015), and is a common culprit in the damage of wheat and wheat flour, as well as prepared cereal-based foods like biscuits, nuts, beans, pasta, cornflakes, and even dried fruits (Kheradpir, 2014; Devi and Devi, 2015). Wheat flour is considered its most preferred substrate (Lu et al., 2010; Kheradpir, 2014), although *T. confusum* has also been reported toattack groundnuts/peanuts, maize, rice, sorghum, and their flours (Ajayi and Rahman, 2006; Ranga Rao *et al*., 2010). Other food items that have been reported as hosts of *T. confusum* include beans, dried flours, pasta, spices, cocoa beans and chocolate, dried pet food, dried specimens in museums, yam, cassava, pearl millet, wheat grain, soya bean meal, and other stored grain and grain products (Ajayi and Rahman, 2006; Tettey *et al.*, 2014; Ahmed *et al*., 2010; White and Lambkin, 1988; Cox and Simms, 1978; Nadeem *et al*., 2012).

*T. confusum* causes both quantitative and qualitative damage to its host products, resulting in a loss of overall value (Ridner and Dias, 2007; Bachrouch*, et al.,* 2010). Infested products are characterized by the presence of frass, carcasses, and exuviae, and have a pungent odor due to the release of benzoquinones, a defensive chemical compound produced by the pest's prothoracic and abdominal glands (Devi and Devi, 2015). Both larvae and adults feed externally on grains and processed foods, causing significant damage, with larval damage typically affecting the germ of wheat grains (White and Lambkin, 1988).

This damage leads to a reduction in the overall value of the product, as well as additional costs associated with the use of pesticides and pest-proof packaging (Lale and Yusuf, 2000). Therefore, there is a need to develop cost-effective and safer methods of controlling this pest that can be applied to all its host products. Such measures will help enhance food security, particularly for subsistence farmers who may have limited resources to invest in expensive insecticides and protective measures.

# **3. Materials and methods**

The research was carried out during the period of 2022-2023 in the laboratories of the Agriculture College at Salahaddin University-Erbil.

## **3.1-Test insect**

*T. confusum*, the confused flour beetle, was sustained on a standardized diet containing wheat flour and 5% dry yeast powder supplement (Singh and Prakash, 2015). The diet containing insects was stored in sizeable plastic containers with a capacity of 500 ml, and fine mesh cloth covers were placed to allow ventilation and restrict insects from escaping. The cultures were maintained in an incubator set to a temperature of (30±2℃) and relative humidity of (60±2) for ideal insect growth and survival.

## **3.2- products**

For each treatment, we used two types of flour commonly available in local markets: white flour and whole wheat flour, with 250 g of each flour in every replicate. Three replicates were performed for each treatment. Before starting the experiment, wheat samples were stored in a freezer for two weeks and then conditioned for several days at room temperature, following the method described by (Almaši and Poslončec ,2014).

## **3.3- Experimental Procedures (Measurements).**

To collect morphological data on each instar of *T. confusum* larvae, approximately 42 samples were used. The measurements involved using a binocular microscope equipped with a micrometer to determine the width and length of the head capsule at its widest and longest points, respectively, for each discovered larva. This methodology was described in (Blomefield and Giliomee's, 2009 )study.

## **3.4-Statistical analysis**

The statistical analyses for this study were performed using version 25 of the SPSS software. To compare the mean of treatment, the Duncan test was used at a significance level of 0.05

# **4.Results and discussion**

The life cycle of *Tribolium confusum*, also known as the confused flour beetle, consists of four stages: egg, larva, pupa, and adult. During the larval stage, which is composed of seven instars, morphometric parameters such as head capsule width, head capsule length, and body length were measured on both white and whole wheat flour.

Based on the data presented in Table 1, the study did not find a significant relationship between the physical characteristics of different larval instars and the two types of flour used. This suggests that the morphometric parameters of the larvae were not significantly affected by the type of flour they were raised on. These findings are consistent with the previous work of (Mohammad et al.,2012), which reported that the presence of confused flour beetles can lead to a decline in flour quality.

Table 1 also reveals that there were significant differences in the morphometric parameters of the confused flour beetle between different larval instars, including head capsule width, head capsule length, and body length. As the instar progressed from the first to the seventh, the mean values for each of these measurements increased. For example, in the first instar, the mean values for head capsule width, length, and body length were 0.163 ± 0.003, 0.165 ± 0.005 and 1.627 ± 0.046, respectively, while in the seventh instar, these measurements increased to 0.658 ± 0.005, 0.507 ± 0.006, and 6.136 ± 0.056, respectively. These differences in mean values between the first and seventh instars were statistically significant, indicating that the larval instar had a significant effect on the physical characteristics of the insect.

According to Table 2, the interaction effect revealed that there was no significant difference in the morphometric parameters of the larvae, including head capsule width, length, and body length, between white and whole wheat flour. However, the data indicated that instar seven had the largest mean values for these parameters, while instar one had the lowest. Both types of flour exhibited a geometric progression in growth rate, with mean values that conformed to (Dyar, 1890). Additionally, the head capsule width measurements recorded in this study were similar to those reported by Frank in 2014.

Table 4.1 Average of head capsule widths, lengths, and body lengths of larval instars 1-7 of T. *confusum*



**The data represent mean ±S.E. The different letters indicate significant difference larval instars according to Duncan multiple range test , p < 0.05**

Table 4.2: Average impact of the interplay between larval instar morphometrics of *T. confusum* and different varieties of white and whole wheat flour**.**



**The data represents mean** **±S.E. The different letters indicate significant difference larval instars according to Duncan multiple range test ,** **p < 0.05.**

# **5.****Conclusions and recommendation**

## **5.1 -Conclusions**

In conclusion, the present study investigated the morphometric parameters of the confused flour beetle during the larval stage, which consists of seven instars, on both white and whole wheat flour. The results indicate that the type of flour did not have a significant effect on the physical characteristics of the larvae. However, the larval instar had a significant effect on the morphometric parameters, with increasing mean values for head capsule width, head capsule length, and body length observed from the first to the seventh instar. These findings are consistent with previous research and suggest that the physical characteristics of the larvae are primarily determined by their developmental stage. The study provides useful information on the life cycle and development of the confused flour beetle, which is an important pest in the food industry. Further research is needed to explore the factors that affect the growth and development of the insect in different environments.

## **5.2-Recommendation**

It is recommended that flour manufacturers implement pest control measures to prevent *T. confusum* infestations, which can have a detrimental effect on flour quality. Additionally, regular monitoring and inspection of flour storage areas can help detect and prevent infestations. As the larvae's physical characteristics were not affected by their diet, it may not be necessary to use specific types of flour to deter *T. confusum* infestations.

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