

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

By

Hataw Himdad Ahmed

Supervised by

Dr. Gona Sirwan Sharif

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1.Introduction

The confused flour beetle (*Tribolium confusum*) (Order: Coleoptera, Family: Tenebrionidae), a type of darkling beetle known as a flour beetle, is a common pest insect known for attacking and infesting stored flour and grain. They are one of the most common and most destructive insect pests for grain and other food products stored in silos, warehouses, grocery stores, and homes (Alanko *et al.* 2000; Baldwin and Fasulo, 2010). In Iraq the wheat is one of the most foods used in meals and constitute 75% of the grain consumed, cereal grain losses during storage can reach 50% of total harvest in some countries; a worldwide loss quality of grain is caused by insects (Fornal *et al.*, 2007).

To study the biology of insects, it is very important to be able to determine the instar of the larvae, Taking into account that these previous works on *T. confusum* are not in agreement to each other about the number of instars of this beetle, the aim of my work was to determine the number of instars of *T. confusum* in the area of study and to compare my data with those reported in these works, considering also that geographical differences could occur. In fact, a variable number of instars has been recorded for many insects in relation to various factors, such as temperature and host plant(Aguilon. and Velasco, 2015). The measurement of head capsule width is the most frequently used method to determine the number of larval stages and to classify larvae on the basis of their instar(Dyar 1980).

The aim of this research was to assess the larval instar number of *T. confusum* (Hbst.) and to facilitate the study of its biology by identifying a reliable method to determine the instar of individual larvae.

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, originally of African origin, has a different distribution in that it occurs worldwide in cooler climates. In the United States it is more abundant in the northern states (Smith and Whitman, 1992).

2.3-Morphological description of different stages of *T. confusum*

2.3.1-Adults

Adults of *T. confusum* are reddish brown elongate beetles varying from 4.0 to 4.5 mm in body length and from 1.0 to 1.2 mm in width. The head of adult *T. confusum* is visible from above, it has no beak, and the thorax is slightly parallel on its sides. Legs consist of coxa, trochanter, femur, tibia, and five tarsi in the fore and middle legs and four tarsi in the hind legs ending with two claws which are simple, curved apically, and are equipped with sensillae. The antenna of adult *T. confusum* is composed of 11 annuli, the scape, the pedicel, and a flagellum of nine annuli (Zohry, 2017).

The adult mandible is short, and the posterior edge have many spines. The base of the maxilla consists of two segments, the cardo contains a single sclerite which is semicircular in shape in adults, the main sclerite of the stipes is triangular and articulates with the cardo along its diameter. The galea has a brush of spines at its apex. The lacinia is short, not extending beyond the galea with bidentate apical. The maxillary palp consists of four segments. The distal palp segment is elongated and terminates in a field of sensory receptors (Zohry, 2017).

The adult labium is a midventral structure, its base consists of the mentum, which contains a single sclerite. Distal to the mentum is the prementum to which the lateral labial palps are tied. Between the labial palps is the ligula, a structure fringed with long distal setae. The adult labial palps consist of three segments and, like the maxillary palps, terminate in a field of sensillae (Zohry, 2017).

2.3.2-Larvae

The structure of last larval maxilla differs from the adult maxilla in having only three palp segments. Two larval endites are present in the two maxillae. In larval labium two segmented labial palps are present. The antenna of the larval stage of *T. confusum* consists of three segments inserted in a membranous structure called anta corium which connects it to the head capsule. The antenna consists of a basal subcylindrical segment (antennomere I), a segment equipped with a lobe (antennomere II) and an apical segment (antennomere III) which is both tiny and elongated internally situated on the apex of antennomere II and bearing a long seta. Most last larval instars are varying from 5.75 to 6.9 mm long and 0.75 to 0.95 mm wide. The head, thorax, and abdomen are covered with minute spines. The first larval instars are 5.0– 5.1 mm long and 0.5–0.6 mm wide. A specific feature of the first instar larvae is the extreme shortened antenna with a reduced number of antennomeres (Zohry, 2017).

Another feature is the presence of well-developed and moderately long legs. General body shape of the larvae is as follows elongate, cylindrical, body mostly white, weakly sclerotized surface with sparse vestiture of whitish setae, segment IX dorsally forming divided sclerite (pygidium). Urogomphi are appendages of tergum IX of beetle larvae of *T. confusum* which are reported for the first and last larval instars. Segment X is not visible in dorsal view; it is inserted on the ventral side posterior to sternum IX and may be represented by two lobes which is probably developed as a pygopod. In the larval stage, there were three pairs of legs each of them consists of five segments; these are coxa, trochanter, femur, tibia, and tarsus, and a single claw is present in the first and last instar larvae (Zohry, 2017).

2.3.3-Pupa

The mature formed pupa is white to light yellow, and the head is depressed beneath the pronotum. The male pupa measured about 3.25–4.15 mm in length (from the anterior edge of pronotum until before the urogomphi) and 0.95–1.25 mm in width and the female pupa measured about 3.6–4.0 mm in length and 1.0– 0.25 mm in width. The pupal abdomen was conical and the last segment has two pointed structures, these are the urogomphi. The female genital papillae, which are much larger than those of the male, are two finger-like structures just anterior to the pointed urogomphi. The male papillae are small enough that they look like just fingertips. Pupae of beetle *T. confusum* have jaws called gin traps on the lateral margin of their jointed abdominal segments from segment 1 to 6 (Zohry, 2017).

2.4-Biology and life cycle

The adult beetles are very active and move about rapidly when disturbed. The average life of adults is about one year. Females lay an average of about 450 eggs, which are small and clear white. The eggs are laid loosely on fine materials and broken kernels where the adults reside. The eggs are covered with a sticky secretion which the fine material adheres to. Fresh material placed in a grain bin will become rapidly infested if previous grain residue is not removed. Larvae (small brownish-white worms) hatch in five to twelve days and are full-grown in one to four months. Full grown larva are about three-sixteenths inch long and tinged with yellow. These larvae feed on fine materials and broken grain kernels. The larvae transform into small naked pupae, which are white at first and then gradually change to yellow and then to brown and shortly afterwards into the reddish-brown adult beetle. The period from egg to adult averages about six weeks under favorable weather conditions, but is greatly prolonged by cold weather, as is true of all grain pests. The life cycle of the red flour beetle is usually shorter than the confused flour beetle.

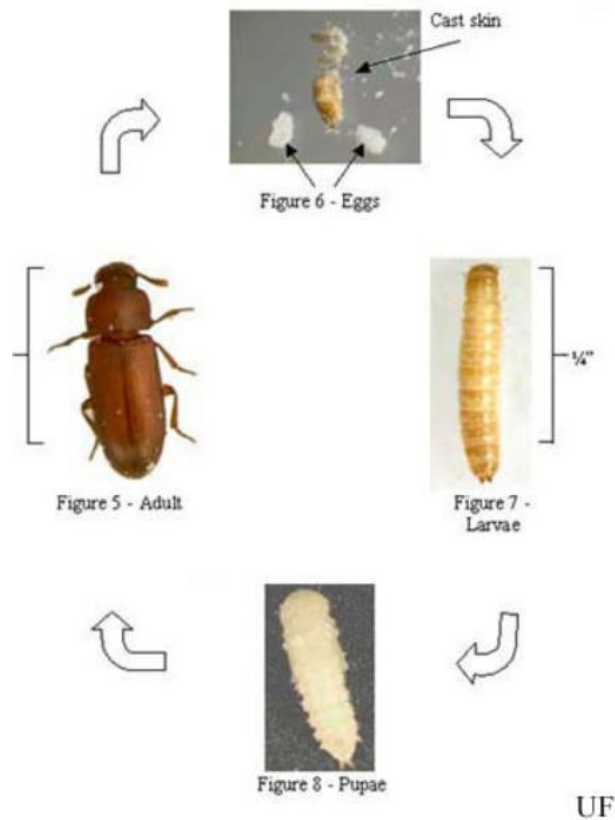


Fig. Life cycle of a flour beetle, *Tribolium* sp.

2.5-Host range and damage

Tribolium confusum is one of the most cosmopolitan pests of stored products (Fedina and Lewis, 2007; Bachrouh *et al.*, 2010), with a polyphagous feeding habit (Bachrouh, *et al.*, 2010), and attacking widespread variety of stored products and their by-products (Obeng-Ofori, and Reichmuth, 1999). It is one of the communal pests of cocoa beans at both farm and commercial warehouses (Naravvo *et al.*, 2007; Bateman, 2015). It has been consistently reported on wheat and wheat flour, and often causing serious damage in prepared cereal based foodstuffs such as biscuit, nuts, beans, pasta, cornflakes, and even dried fruits (Kheradpir, 2014; Devi and Devi, 2015). Wheat flour is, however, regarded as its most preferred

substrate (Lu *et al.*, 2010; Kheradpir, 2014). It has also been reported attacking groundnut/Peanuts (Ranga Rao *et al.*, 2010) and other cereal like Maize, rice, sorghum etc. and their flours (Ajayi and Rahman, 2006). Other researchers reported beans, dried flours, pasta, spices, cocoa beans and chocolate, dried pet food, dried specimens in museum, yam and cassava (Ajayi and Rahman, 2006; Tettey *et al.*, 2014), Pearl millet (Ahmed *et al.*, 2010), wheat grain (White and Lambkin, 1988), Soya bean meal (Cox and Simms, 1978), and other stored grain and grain product (Nadeem *et al.*, 2012) as being host to *T.confusum* worldwide.

Damage by this pest is usually manifested by a loss in both quantity and quality (Ridner and Dias, 2007; Bachrouch, *et al.*, 2010). Product attacked by *T. confusum* usually contains frass, carcasses, and exuviae. It also turns grey in colour with a pungent unacceptable odour due the elicitation of benzoquinones, a defensive chemical material from their prothoracic and abdominal glands; making the product unsuitable for humans (Devi and Devi, 2015). Both larvae and adults feed externally on both grains and processed foodstuff causing devastating damage, but the damage by larvae are mostly confined to the germ of the grains in wheat (White and Lambkin, 1988).

All the above manifestation of damage normally lead to the loss in overall gain as a result of the loss of price and/or the extra cost needed for control in form of pesticides and expensive pest proof packaging (Lale and Yusuf, 2000). There is then, the need to find cheaper and safer methods of control for this pest that can be applicable to all its host range. This will go a long way in enhancing food security especially in subsistent families with little resources to afford the prohibitive costs of insecticides and other protective measures.

2.6-Control of *Tribolium confusum* Infestation in Stored Product Environment

The global pest-harvest grain losses by insect damage and other bioagents range from 10% to 40%. Methods used to control stored grain insect pest included physical, chemical, and biological treatments (Isman,2006).

2.6.1-Chemical Control Methods

Chemical control is the most commonly adopted techniques of insect pest management (Golshan *et al.*, 2014). Fumigation with synthetic chemicals has also for long been established to be one of the most economical means of controlling insect pests of stored products (Wang *et al.*, 2009)

For decades, organophosphorus compounds, such as malathion, pirimiphos-methyl and carbaryl, along with methyl-bromide have been applied against stored product pests in many countries. At present, application of pirimiphos-methyl and carbaryl has been banned, and methyl-bromide will be banned in near future, which may result in increase of malathion application. It is best to not apply chemical insecticides in stored-product silos; however, sometimes it is necessary to control the insect pests in empty silos before filling them up. (Javadzadeh,*et al.*, 2017)

However, the control of stored product pest using synthetic pesticides and fumigants had some limitations such as pest resurgence, environmental disturbances, and pest resistance to pesticides, increasing cost of application, lethal effects on non-target species, and poisoning of farm workers and consumer (Obeng-Ofori *et al.*, 1998; Lale, 2002; Isman, 2006; Obeng-Ofori, 2010). This necessitated the exploration and adoption of other suitable alternatives to these synthetic insecticides for the management of insect pest infestation on stored products over the last few decades.

2.6.2-Biological Control of *T. confusum*

Entomopathogenic fungi, as both biological control agents and sources of bioactive compounds active against the insect pests, could provide an alternative to chemical pesticides (Isaka *et al.*, 2005; Monlar *et al.*, 2010), since they have low mammalian toxicity, high effectiveness and a natural origin (Moore *et al.*, 2000). Entomopathogenic fungi as natural enemies of insect pests in different ecosystems have high potential to control pests in agroecosystems (Fiedler & Sosnowska, 2007; Jaronski, 2010; Jaber, 2015). There are approximately 90 genera and 700 species of entomopathogenic fungi known (Roberts & Humber, 1981) and the common species of *Beauveria*, *Metarhizium*, *Lecanicillium* and *Isaria* are quite amenable for mass production. Previous studies have mostly focused on *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Akbar *et al.*, 2004; Batta, 2004, 2005; Kavallieratos *et al.*, 2006; Michalaki *et al.*, 2006; Vassilakos *et al.*, 2006). The anamorphic entomopathogenic fungi such as *B. bassiana*, *M. anisopliae*, *Lecanicillium muscarium* (Petch) Zare & W. Gams, *Isaria farinosa* (Holmsk.) Fr. (formerly *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm.), *Isaria fumosorosea* Wize (formerly *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.), and *Lecanicillium muscarium* (Zimm.) Zare & W. Gams from the order Hypocreales (Ascomycota) are natural enemies of wide range of insect pests, and these fungi may produce enormous numbers of conidia over many asexual life cycles in a single cultivation season (Roberts & St. Leger, 2004; Rehner, 2005; Gurulingappa *et al.*, 2010).

Species of entomopathogenic fungi such as *Beauveria bassiana*, *Paecilomyces farinosus*, *Isaria fumosorosea*, *Isaria farinosa*, *Lecanicillium muscarium* using as biological control agent against *Tribolium confusum*. (Komaki *et al.*, 2017).

Press *et al.*, (1975) reported a successful use of the predaceous warehouse bug *Xylocoris flavipes* (Reuter) for the control of *T. confusum* on groundnut kernel stored in plywood bins in a warehouse in Georgia.

2.6.3-Use of high Temperature for the Control of *T. Confusum*

The use elevated temperature for the control of *T. confusum* on stored product as a standalone method or in combination with other methods has been demonstrated. High mortality of adult *T. confusum* at a temperature of ≥ 380 C coupled with the controlled atmosphere was reported under laboratory condition within 24 to 48 h of treatment (Soderstrom *et al.*, 1992). Donahaye *et al.*, (1996) reported 99% mortality of all life stages at 35 0C under the modified atmosphere, 44 h after treatment. Successful use of elevated temperature (>50 C) for achieving high mortality (99%) in *T. confusum* life stages was also reported by Mahroof *et al.*, (2003a; b). Similarly, Arthur and Dowdy (2003) also found high mortality in adults in a laboratory experiment involving heating of the concrete for 4, 8 or 16 hours at 45 or 550C.

Earlier, Dowdy (1999) studied the mortality of *T. castaneum* exposed to high temperature and diatomaceous earth combinations and reported a mortality increase from 8-100% at 500C. Similarly, Mahroof *et al.*, (2003b) studied the effect of constant temperature at 42, 46, 58 and 600C on all life stages of *T. castaneum* and discovered a linear dose-dependent response in all stages to an elevated temperature as exposure time increases. Very high mortality (99%) was achieved at temperature regime of ≥ 500 C in larvae. In an later study, Mahroof *et al.*, (2001) exposed adults, eggs, younger instar larvae, oldest instar larvae, and pupae of *T. castaneum* to chains of constant temperature in the range of 40-600C and found that mortality in all tested stages increased proportionately along

higher temperature gradient and treatment time; until a peak mortality of 95% was attained at $\geq 50^{\circ}\text{C}$ and 120 minutes of exposure.

Brijwani *et al.*, (2012) measured the effect of elevated temperature and sanitations on *T. castaneum* at several floors in new flour mills and recorded a significant mortality of $93.2 \pm 6.7 - 100\%$ and $55.5 \pm 12.9 - 98.6 \pm 0.8\%$ on others floors and first floor respectively. These mortality intensities were attained gradually at $\geq 50^{\circ}\text{C}$ over series of time differential which ranged from 8-12 hours and the temperature regimes were also maintained for 10-14 hours. A similar study involving the use of high temperature on *T. confusum* recorded 100% mortality for all its life stages at most of the sampling locations with $\geq 50^{\circ}\text{C}$ temperature levels. Lower mortalities were only recorded in locations where such high-temperature regime could not be sustained for a reasonable time frame (Mahroof *et al.*, 2003a).

Additionally, Arthur (2006) in a trial exposed all life stages of *T. castaneum* and *T. confusum* to 51°C and 54°C of elevated temperatures in a laboratory oven for 2 and 1 hours respectively and recorded a 100% mortality in all the life stage for both species. Generally, the use of temperature extremes below or at 15°C and above or at 50°C for the management of stored product pests through produce and/or structural disinfestation is termed advantageous and eco-friendly stored product protection method (Fields, 2006) and can be utilized to manage insect pests of stored product on all durables (Downes *et al.*, 2008). These recorded magnitudes of mortalities above consolidate the potential of high temperature regime as a very suitable alternative to phosphine, methyl bromide and other chemical in use on store product to control *T. castaneum*. This, therefore, need to be further

investigated to expand our knowledge base on its definitive potentials in this regard by exploring the use of different sources of heat, especially solar energy.

Time Mortality responses of all *T. Confusum* life stages increased with an increase in exposure time and temperature. Both time mortality and Axed time responses showed eggs and young larvae to be most susceptible at elevated temperatures and old larvae to be least susceptible. (Boina and Subramanyam, 2004)

3. Materials and methods

The study is going to be conducted in 2022- 2023 in in high education laboratories of Agriculture College at Salahaddin University-Erbil.

3.1-Test insect

confused flour beetle, *T. confusum* was maintained under stander food medium of wheat flour supplemented with 5% dry yeast powder (singh and Prakash, 2015)

The diet with insects were placed in large plastic (500 ml) capacity, covered with fine mesh cloth which simultaneous allow ventilation and prevent insects from escaping. All cultures were maintained in incubator at (30±2°C) temperature and (60±2) relative humidity.

3.2- products

The flour available in local markets of the kind Turkish (called Zer) was used after sterilization in the oven for 3 hours and by 250 g for each replicate and three replicates of each treatment experience

3.3- Experimental work (Measurements).

Approximately 42 samples were used to morphological measurements for each larva instar of the tribolum *confusum* (Blomefield & Giliomee 2009). The head capsule of each larva was measured with a binocular microscope equipped with a micrometer. For each larva founded, head capsule width and length were measured, respectively, at the widest and the longest point.

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