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Study on hemogram and the effect of thermal stress on hemocytes and development in *Dacus ciliatus* (Diptera: Tephritidae)

Maryam Ajamhassani[1*], Mohamed El Aalaoui[2], and Bita Valizadeh[3]

Abstract

This study investigated the impact of temperature stress on the immune system of *Dacus ciliatus* Loew (Diptera: Tephritidae) by examining the morphology and density of hemocytes, which are crucial components of insect immunity. Various factors, such as temperature stress, dietary changes, and the entry of contaminants and infections into the hemolymph, are known to affect insect immune responses by altering hemocyte profiles. The research focused on the hemocyte profile, hemogram across all biological stages, and the morphological and frequency changes of hemocytes in third instar larvae exposed to temperature stress. Cucumber fruits infected with insect larvae were collected and brought to the laboratory, where third instar larvae were extracted from the fruit tissue. The hemolymph was then collected, and after staining with Giemsa solution, hemocytes were identified under a light microscope. The hemogram analysis included measurements of DHC, THC, blood volume, and AHC across all biological stages. In third instar larvae, plasmatocytes and granulocytes were the most abundant, comprising about 56% of the hemocyte population. In contrast, prohemocytes were most frequent in the first instar larvae, accounting for approximately 37%. THC was highest in third instar larvae, indicating a direct correlation between hemolymph volume and total hemocyte count. Temperature stress had a significant impact on hemocyte numbers. Heat stress, with temperatures up to 30 and 35°C, led to a notable increase in total cell count, granulocytes, and plasmatocytes. Conversely, cold temperatures resulted in a decrease in prohemocytes, plasmatocytes, granulocytes, and the total cell count compared to the control group. Additionally, temperature stress induced hemocyte deformation, with plasmatocytes and granulocytes showing the most pronounced changes under heat stress, including torn cell walls and loss of cell contents at 35°C. Cold stress had a greater effect on the shrinkage of prohemocytes than on other cell types. Temperature stress also significantly affected the developmental characteristics of the fruit fly. Heat stress reduced the pupation period length and emergence rates, while cold stress more prominently impacted birth rates. This study

[1] Department of Plant Protection, Faculty of Agriculture, Shahrood University of Techonology, Shahrood, Islamic Republic of Iran.
[2] National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat principal, 10090 Rabat, Morocco.
[3] Delta Research and Extension Center, Mississippi State University, Stoneville, MS 38776. USA.
*Corresponding author, e-mail: shahroodm@gmail.com

represents the first identification of hemocytes and analysis of the immune response of *D. ciliatus* to temperature changes, providing a foundation for further research into the physiological defense mechanisms of this pest.

Keywords: hemocyte identification, DHC, THC, temperature, developmental stage, *Dacus ciliatus*.

Introduction

The Tephritidae family, commonly known as fruit flies, includes over 4,000 species worldwide. The larvae of this family feed on the seeds, fruits, and stems of various agricultural and horticultural crops, with approximately 30% of these species targeting the fruit tissue of truck crops (Norrbom, 2011). Among them, the Ethiopian fruit fly, *Dacus ciliatus* (Loew) (Diptera: Tephritidae), is an oligophagous pest primarily affecting the Cucurbitaceae family. This pest is active in tropical and subtropical regions of Asia and Africa (Vayssières *et al*., 2008, Abdallah *et al*., 2012, EPPO, 2018) and is particularly destructive in Iran's fields and greenhouses (Barzkar *et al*., 2017). The larvae of *D. ciliatus* cause significant damage to various cucurbit crops, including spring cucumbers, autumn cucumbers, Armenian cucumbers, pumpkins, melons, honeydew melons, and zucchini, leading to fruit spoilage and reduced market value. The damage begins when larvae penetrate the fruit tissue, leaving a visible entrance hole, and as they feed, they create tunnels that deform the fruit. Under severe infestation, a single fruit may contain multiple larvae at different developmental stages. Notably, *D. ciliatus* does not undergo obligatory diapause, allowing it to remain active year-round under favorable conditions, particularly in cucumber greenhouses, where it can devastate up to 90% of the crop yield if left unmanaged (Arghand, 1983, Paydar *et al*., 2020, Mohammad, 2022).

Understanding the physiological characteristics, particularly the immunological aspects of insects, is crucial for developing effective strategies to combat pests using chemical and microbial agents. The immune response of insects serves as a key indicator of hemolymph stress or contamination. Sensitivity and resistance to pathogenic agents vary across different insect species and developmental stages. A strong immune system in insects can prevent the development of infections during microbial challenges, with the outcome largely depending on the robustness of the insect's immune defenses (Washburn *et al*., 2000, Kanost *et al*., 2007). The first step in this field involves identifying hemocytes and their frequency across the insect's developmental stages (Valizadeh *et al*., 2017, Go *et al*., 2022).

The immune reactions of insects are influenced by various environmental and non-environmental factors, such as temperature changes, diapause, feeding, molting, starvation, and

the entry of contaminants or infections into the hemolymph. These factors underscore the sensitivity of the circulatory system to stress and osmolality changes (Siva-jothy and Thompson 2002; Lee *et al*., 2008). Osmolality, which refers to the concentration of solute particles in a solution, is a critical characteristic of hemolymph, playing an essential role in blood circulation, gas exchange, metamorphosis, adult emergence, and wing expansion (Jiang *et al*., 2023; Salcedo *et al*., 2023). Upon identifying a foreign agent, hemocytes such as plasmatocytes and granulocytes react by altering their shape, type, and density, followed by processes like phagocytosis and nodulation, which are vital for the insect's innate immune response (Pech and Strand, 2000, Black *et al*., 2022).

Temperature is a significant environmental factor that influences insect growth, body size, molting, reproduction, abundance, survival, generation time, and immunity (Vogel *et al*., 2022; Mutamisva and Mbande, 2023). Insects typically have an optimal temperature range for growth and development, with deviations from this range negatively affecting their survival (Foray *et al*., 2014, Cui *et al*., 2018). Exposure to high or low temperatures can significantly alter hemocyte density, depending on the insect's growth stage and species, thereby affecting the insect's resistance to control measures (Browne *et al*., 2014; Vogel *et al*., 2022). Various studies have documented the effects of temperature on hemocyte morphology and numbers in different insects, including *Phthorimaea operculella* Zeller (Lep: Gelechiidae), *Gromphadorhina coquereliana*, and *Megastigmus pistaciae* (Hym: Torymidae) (Pourali and Ajamhassani, 2018; Lubawy and Stocinska, 2020, Ajamhassani *et al*., 2023).

Dacus ciliatus is a serious pest in fields and greenhouses, remaining active throughout the year. Environmental temperature fluctuations or temperature stresses in greenhouses can affect the growth and physiological activities of this insect. These environmental changes may also alter the insect's sensitivity to various pesticides or natural enemies (Zhu *et al*., 2012). By studying how environmental changes affect the immune system of this fly, more effective control methods can be developed and implemented. Therefore, the purpose of this research was to identify the hemocytes, assess the hemogram, and evaluate the effects of thermal stress on the hemocyte profile and some biological characteristics of *D. ciliatus*.

Materials and Methods

Insect Rearing

Cucumbers infected with *D. ciliatus* larvae were collected from infested cucumber greenhouses of Semnan (35.5767° North, 53.3949° East), Semnan province, Iran, during the 2022 growing

season. They were transferred to the laboratory under controlled conditions in growth chamber (temperature 24±1 °C, relative humidity 60%, and light-dark ratio 14:10 h). Growth chamber condition was checked daily. Contaminated cucumbers were placed in plastic containers (40 cm length×40 cm width×40 cm height). First, second, and third instar larvae were distinguished based on body length and head capsule width (Dyar, 1980) [Figure 1 (a, b, and c)]. The characteristics of larval instars are shown in Table 1. Feeding third instar larvae were utilized to identify hemocytes and determine parameters related to hemogram. Subsequently, infested cucumbers, consumed by larvae and nearing spoilage, were substituted with healthy cucumbers, and the larvae were carefully transferred to the healthy cucumbers using a brush.

Table 1. Morphometric size (mean±se) of different larval development of *Dacus ciliatus* (n= 20).

	Larval stages		
	First instar larvae	Second instar larvae	Third instar larvae
Body length (mm)	2.9±0.16	4.7±0.22	7.1±0.26
Head width (mm)	0.32±0.02	0.67±0.03	1.1±0.45

Hemocyte Identification

Hemocytes were identified by using Gupta keys and staining cells by Giemsa. Cells were observed using an Olympus BH2 light microscope at 40× magnification and identified based on size and morphological characteristics (Gupta, 1985; Jones, 1962).

Hemogram

Differential Hemocyte Count (DHC) in larvae, pupae, and adults of *Dacus ciliatus*

The larvae fed on greenhouse cucumber were used for DHC calculations. Differential hemocyte counts of larvae, pupae, and adults were calculated. Following hemolymph collection using a sterile needle from the area between abdominal segments 3 and 4, the samples were placed on a slide, and a smear was prepared using another slide. A staining solution composed of Giemsa (Merck KGaA, Germany) and distilled water in a 9:1 ratio was added to the slide and allowed to stand for 5 minutes. Subsequently, the slide was washed in distilled water and briefly immersed for 5 seconds in a saturated lithium carbonate solution to fix the cell staining (Yeager,1945). After another rinse, the underside of the slide was dried using filter paper. One hundred hemocytes were randomly selected at 40× magnification and differentially counted using an Olympus BH2 microscope. Twenty-five hemocytes from each biological stage were examined.

Total hemocyte count (THC) in larvae, pupae, and adults of *Dacus ciliatus*

For THC, approximately 1 µL of hemolymph from two larvae was collected using a capillary tube and mixed with 10 µL of Tyson buffer as an anticoagulant solution (NaCl2 72 Mm, Na2SO4 9 Mm, glycerol 43 Mm, methyl violet 0.06 Mm, distilled water) (Mahmood and Yusaf, 1985). Hemolymph and Tyson solutions were placed on Neubauer slides (HBG, Germany), and hemocytes were counted using the Jones formula and light microscopy at 40× magnification (Jones *et al*., 1962).

$$\frac{\text{Hemocyte in} \times 1\ \text{mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}}$$

Dilution= 10 times, Depth factor of the chamber= 10, No. of squares counted= 5

Hemolymph volume (HV)

Hemolymph volume was directly determined by extracting hemolymphs from various developmental stages using micropipettes (Terra *et al*., 1975, Ghasemi *et al*., 2013). The weight of a piece of filter paper was measured when dry. Subsequently, a proleg was cut from the larval abdomen, and all the hemolymph was collected by using Hamilton syringe (10 µl, Switzerland), placed on filter paper and weighed. Sampling was also conducted for pupae and adults. The difference between the weights of wet and dry filter paper sheets was recorded and considered as hemolymph volume; 15 insects from each developmental stage were included.

The total hemocyte count, plasmatocyte, granulocyte, oenocytoid, prohemocyte count, and morphological changes of cells in late larvae of *D. ciliatus* affected by thermal stress

The effect of temperature stress on the number of hemocytes comprised four treatments (5, 24±1, 30 and 35°C) and four repetitions. Based on previous observations, infected fruits with larval entrance holes and deformation due to larval activity were found to contain various larval instars. These fruits were divided into four groups and exposed to different conditions: controlled (24±1°C), cold stress (5°C), and heat stress (30°C and 35°C). Hemocyte counts in third instar larvae of *D. ciliatus* were assessed after 24 hours. The control group comprised larvae kept under growth chamber conditions (24±1°C). In each replicate, the hemolymph of three larvae (approximately 3 µL) was collected via a capillary tube and mixed with 20 µL of Tyson (anticoagulant solution). Cells in 3 µL of hemolymph were counted using a hemacytometer. To observe morphological changes in hemocytes under heat and cold stress, infected fruits were exposed to thermal stress (Pourali and Ajamhassani, 2018, Ajamhassani *et al*., 2023). After 24 hours, hemocytes from third instar larvae were stained with Giemsa and

examined using a light microscope at 40× magnification. Hemocyte deformation was then recorded.

Effect of thermal stress on pupal weight, pupal duration, percentage of adult emergence, adult longevity

Fruits infected with larvae were divided into four groups within rearing containers and were subjected to test temperatures (5, 24±1, 30, and 35°C). After 24 h, the fruits were transferred to growth chamber conditions (for the control treatment, the fruits were kept under growth chamber conditions (temperature 24±1°C, relative humidity 60%, and light-dark ratio 14:10 h) for 24 h), The dead larvae were removed from the fruits and the alive third instar larvae were transferred to fresh fruits to complete their life cycle and become pupae and adults. These fruits were checked daily. New puparium usually has a light brown color and body length is 4.8±0.17 mm [Figure 1 (d)]. New pupae were separated daily and 2-old-days pupa were weighed. The other characteristics such as the pupal period, percentage of adult emergence, and adult longevity were examined (40 third instar larvae were examined for each treatment). After emerging, adult flies were gently transferred to the falcon tubes and supplied with a solution of water and honey to determine their longevity.

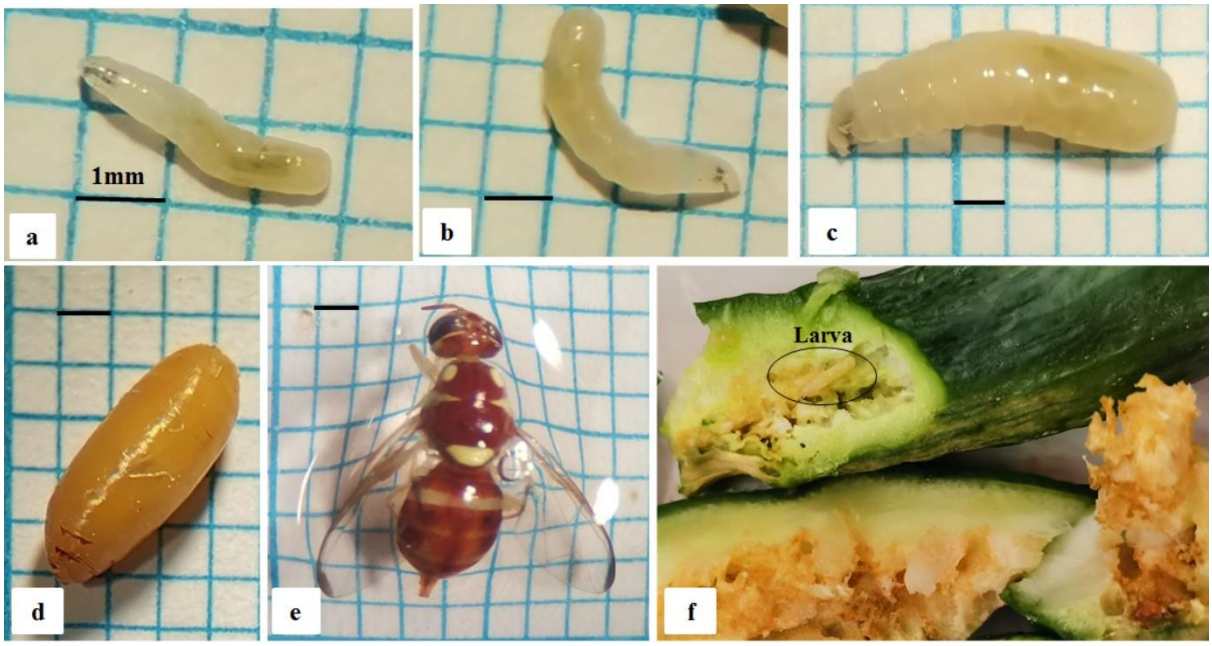


Figure 1. Developmental stages of *Dacus ciliates*, (a) First instar larva, (b) Second instar larva, (c) Third instar larva, (d) Pupa, (e) Adult (female), and (f) Damage of larvae on cucumber (original photo).

Statistical analysis

All data obtained from a complete randomized design were compared by one-way analysis of variance (ANOVA) followed by Tukey's test when significant differences were found at $p \leq 0.05$ (SAS, 9.4). Differences between samplings (n= 3) were considered statistically significant at a probability less than 5% and marked in figures and tables.

Results

Identification of hemocytes in *D. ciliatus* larvae and determination of their abundance percentage in biological stages

Five types of hemocytes were observed in the hemolymphs of *D. ciliatus* larvae, namely prohemocytes, granulocytes, plasmatocytes, oenocytoids, and spherulocytes.

Prohemocytes are round and the smallest cells in terms of size (Table 2). They feature a large, central nucleus that occupies majority of the cytoplasmic volume (Figure 2). The highest abundance of prohemocytes was observed in first instar larvae (37±2.2%), whereas their number decreased in subsequent stages, with the lowest abundant observed in third instar larvae and pupae (Table 3) ($F= 56.3$, $df_{t,e}= 4,14$, $P\leq 0.0001$).

Granulocytes with central or semi-central nuclei varied in sizes and were sometimes the largest cells (Figure 2). The cytoplasm surface contained numerous granules, which were visualized with Giemsa blue. The frequency of these cells was higher in third instar larvae (29±1.5%) compared to other stages and lowest in first instar larvae (16.5±2.3%) (Table 3) ($F= 84$, $df_{t,e}= 4,14$, $P\leq 0.0001$). Plasmatocytes exhibited a spindle-shaped or eye-shaped morphology with varying sizes (Figure 2). The abundance of plasmatocytes was highest in third instar larvae (26.4±2%) and lowest in first instar larvae (21±1.6%) (Table 2), ($F= 55.4$, $df_{t,e}= 4,14$, $P\leq 0.004$). Oenocytoids were egg-shaped with lateral nuclei and were similar but slightly larger in size compared to prohemocytes (Figure 2, Table 2). The frequency of these cells was lower than the previous cells (Table 3) ($F=107$, $df_{t,e}=4,14$, $P\leq 0.0001$). Spherulocytes of medium to large sizes were observed in larval hemolymph (Table 2) (Figure 2). Small spherules around the nucleus occupied the cytoplasm surface and were the least frequency of cells (Table 3) ($F= 92.6$, $df_{t,e}= 4,14$, $P\leq 0.0001$).

Table 2. Morphometric measurements of hemocytes in larvae of *Dacus ciliatus* (n= 20).

Hemocyte type	Size (µm)	
	Length (mean±se)	Width (mean±SE)
Prohemocyte	3.1±2.4b	3±2.5b
Plasmatocyte	6.2±2.6ab	2.4±2.8bc
Granulocyte	8.2±3.3a	6.2±2.6a
Oenocytoid	3.2±1.5b	2.9±0.8b
Spherulocyte	6.5±3.1ab	5.3±2.8ab

Different letters in each column show significance using Tukey's test at P< 0.05).

Table 3. Frequency of hemocytes in developmental stages of *Dacus ciliatus* (n= 25).

Developmental stage	Frequency of hemocytes (%)				
	Prohemocyte	Plasmatocyte	Granulocyte	Oenocytoid	Spherulocyte
1th instar larva	37±2.2a	21±1.6bc	16.5±2.3c	11±0.5b	6±1.1a
2nd instar larva	30.3±1.6b	23±0.8b	20±2.4b	14±0.7a	8±1.3a
3rd instar larva	24.1±1.4cd	26.4±2a	29±1.5a	12±0.5b	7±1a
Pupa	24.2±2.2cd	25.4±1.6a	27±0.8a	11.5±1.1b	6.4±0.2a
Adult	27±1.3c	25.4±1.4a	26±1.3ab	8±0.6c	6a

Different letters in each column show statistical differences among biological stages (Tukey's test, P≤ 0.05).

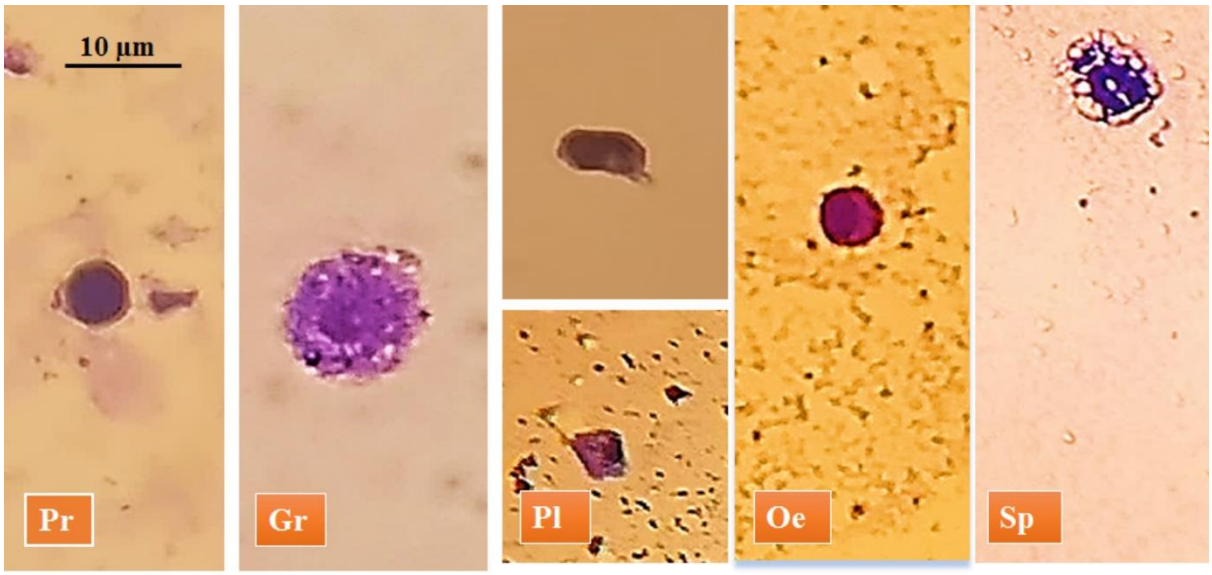


Figure 2. Light microscopy pictures of *Dacus ciliatus* hemocytes stained with Giemsa. PR (Prohemocyte), PL (Plasmatocyte), OE (Oneocytoid), GR (Granulocyte), SP (Spherulocyte), Scale bar= 10 µm.

Hemogram

According to Table 3, the weight of the first and second instar larvae was lower than that of other stages (F= 44.4, $df_{t,e}$= 4,14, P≤ 0.0001). Due to the higher feeding of the third instar larvae, the weight of these larvae was higher significantly than that of the younger larvae. On the other hand, the amount of nutrition is also effective on the hemolymph volume; so the hemolymph volume is higher in the third instar larvae, pupae, and adults than in the early larval stages (F= 87.7, $df_{t,e}$= 4,14, P≤ 0.0001). Hemocyte number of adults (230.2±21.4) cells/mm^3) decreased

compared to third instar larvae (314.4±22.4) cells/mm^3) (Table 4), (F= 35.5, $df_{t,e}$= 4,14, P≤ 0.0001).

Table 4. Body weight, hemolymph volum (HV), Total Hemocyte Count (THC), in developmental stages of *Dacus ciliatus*

Developmental stage	Weight (mg)	HV (µl)	THC (cell/mm^3)
1th instar larva	0.08±0.01d	1±0.33cd	85.5±10d
2nd instar larva	3±0.2c	1.5±0.2c	210±34.3c
3rd instar larva	17±0.4a	3.1±0.3a	314.4±22.4a
Pupa	14±0.4b	2.9±0.3a	256±16.4b
Adult	18±1 a	2.2±0.21b	230.2±21.4bc

Different letters in each column show statistical differences among biological stages (Tukey's test, P≤ 0.05).

Total hemocyte count, plasmatocytes, granulocytes, oenocytoids, and prohemocytes count in larvae of *D. ciliatus* affected by thermal stress

Significant changes were observed in the number of hemocytes of *D. ciliatus* larvae affected by cold and heat. The results showed that the total hemocyte count (F= 84.2, $df_{t,e}$= 3.10, P≤ 0.0001), granulocytes (F= 102.5, $df_{t,e}$= 3,10, P≤ 0.0001), plasmatocytes (F= 109.35, $df_{t,e}$= 3,10, P≤ 0.0001), and oenocytoids (F= 104, $df_{t,e}$= 3,10, P≤ 0.0001) of larvae subjected to heat (30 and 35°C) were significantly higher than those of control larvae. In all the aforementioned cases except for oenocytoids, a significantly lower number of hemocytes in larvae experienced cold stress compared to the control group. Prohemocyte number decreased under cold too stress. The total hemocyte count in the larvae exposed to 35°C (421±25 cells/mm^3) and 30°C (377±28.1 cells/mm^3) was higher than that of control larvae (340±11.5 cells/mm^3). Moreover, cold stress at 5°C significantly decreased the number hemocytes in larvae, reducing it to 262±15 cell/mm^3 hemolymph (Figure 3).

Furthermore, the granulocyte count was higher in larvae placed at 35°C (177±14 cells/mm³) cell in mm^3 and 30°C (145±15.5 cells/mm³) than in control larvae (107±11.3 cells/mm³). Similar to the previous case, the number of granulocytes significantly decreased under cold stress, being reduced to about half the number of hemocytes in the control larvae (45±6.5 cells/mm³ hemolymph) (Figure 4). The changes observed in plasmatocytes under the influence of high and low temperatures were similar to those in granulocytes. In other words, the increase of these cells in heat stress and the decrease of plasmatocytes in cold were significant compared to the control. At 30 and 35°C, the rate of increase of plasmatocytes, like granulocytes, fell into a statistical group (Figure 5).

Increases in oenocytoids differed at high temperatures, with 35°C caused a greater increase in the number of these cells. On the other hand, cold stress did not have a significant effect on the

reduction of oenocytoids. Larvae that experienced cold for 24 h showed no significant differences in the number of oenocytoids compared to the control group (Figure 6).

Under the influence of cold stress, prohemocytes exhibited a significant decrease compared to the control group, with their numbers reduced to about half. Based on the observations, the number of prohemocytes increased in higher temperatures but showed no significant difference with the control (Figure 7).

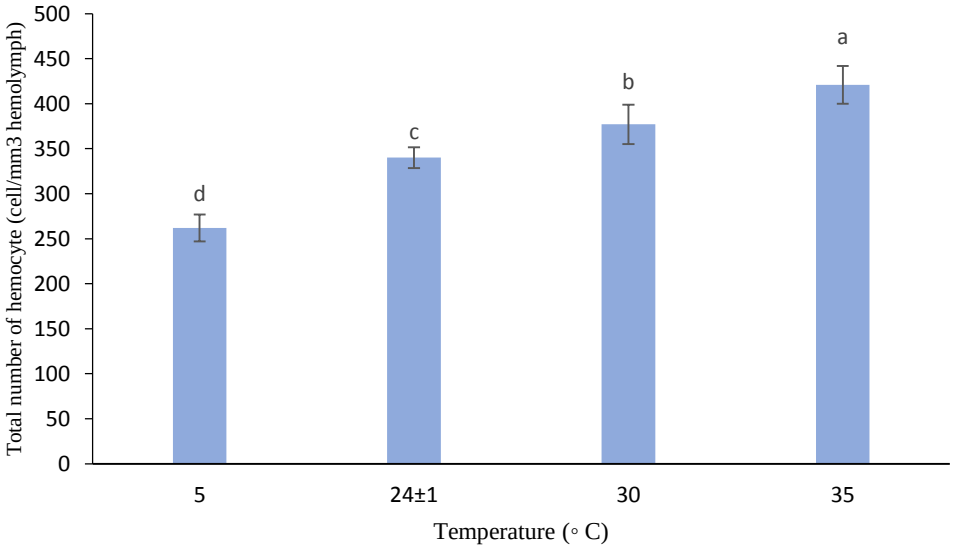


Figure 3. Effect of thermal stress on total hemocyte count in third instar larvae of *Dacus ciliates*.

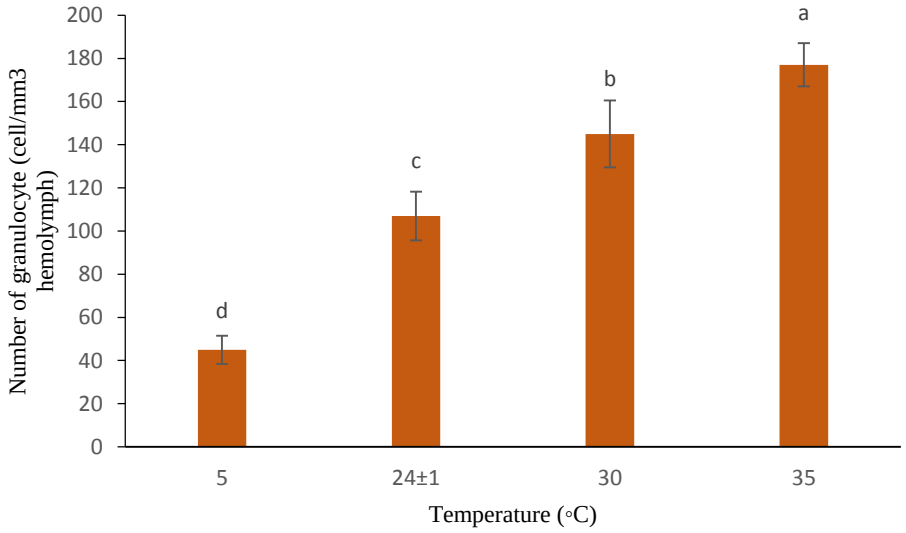
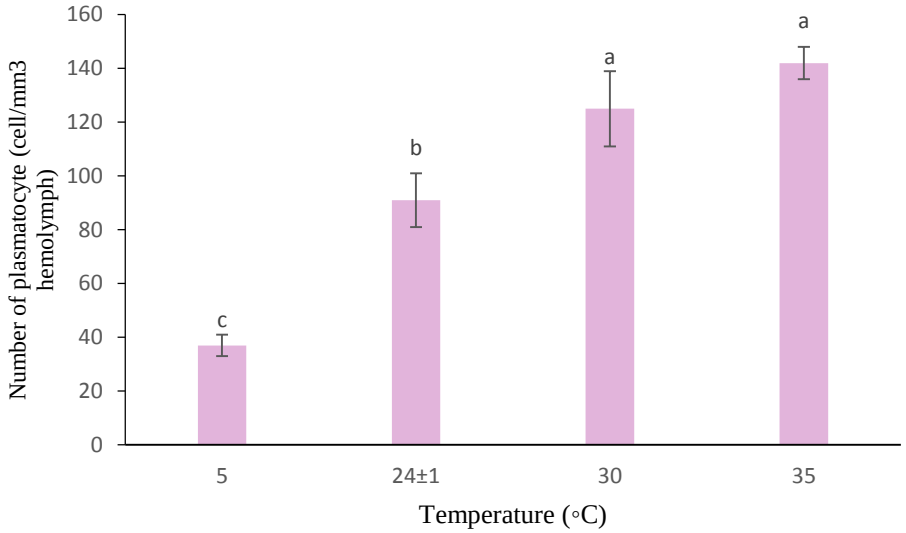


Figure 4. Effect of thermal stress on granulocyte number in third instar larvae of *Dacus ciliates*.



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285 **Figure 5**. Effect of thermal stress on plasmatocyte number in third instar larvae of *Dacus*
286 *ciliates*.
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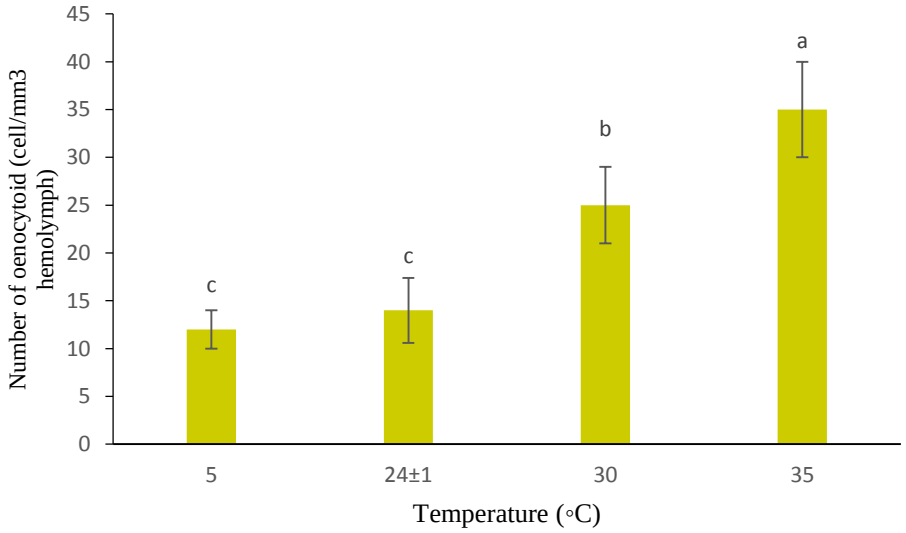

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291 **Figure 6**. Effect of thermal stress on oenocytoid number in third instar larvae of *Dacus ciliates*.
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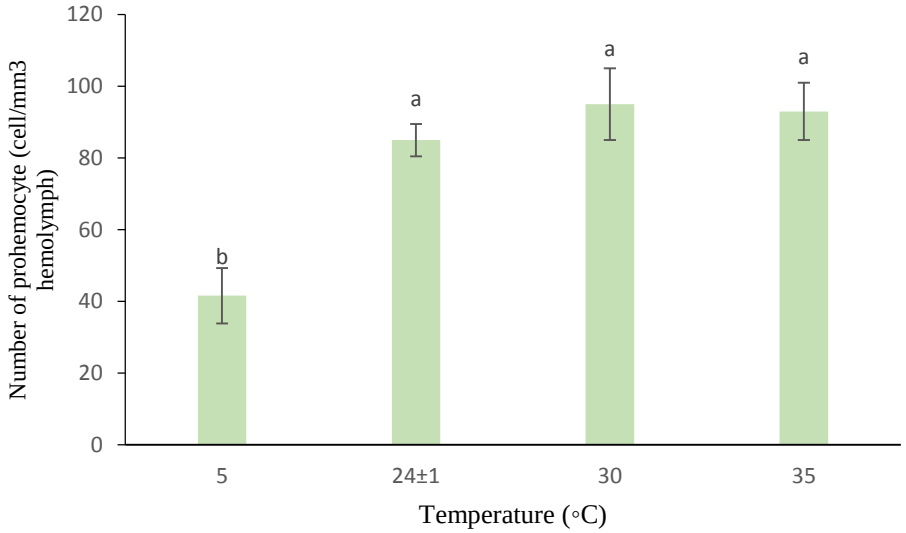

Figure 7. Effect of thermal stress on prohemocyte number in third instar larvae of *Dacus ciliates*.

Morphological changes of hemocytes in *D. ciliatus* affected by thermal stress

Heat and cold stress significantly affected the shape of hemocytes. Nonetheless, cells underwent greater changes in appearance under heat stress compared to cold. Granulocytes (55±2.3%) and plasmatocytes (42±7%) were deformed more than other cells by temperatures of 30 ℃ and 35℃ (Figure 9). At 30℃, the walls of the granulocytes were wrinkled; at the temperature of 35℃, after the cell wall was torn, the cell contents gradually came out of the cell (Figure 8). In some cases, cells were seen to disintegrate under the influence of 35℃. The cell walls of plasmatocytes and oenocytoids were also wrinkled by heat stress. Cold had the greatest effect on the morphology of prohemocytes (Figure 9). These cells were severely shrunk at 5 ℃, and the nuclei were compressed.

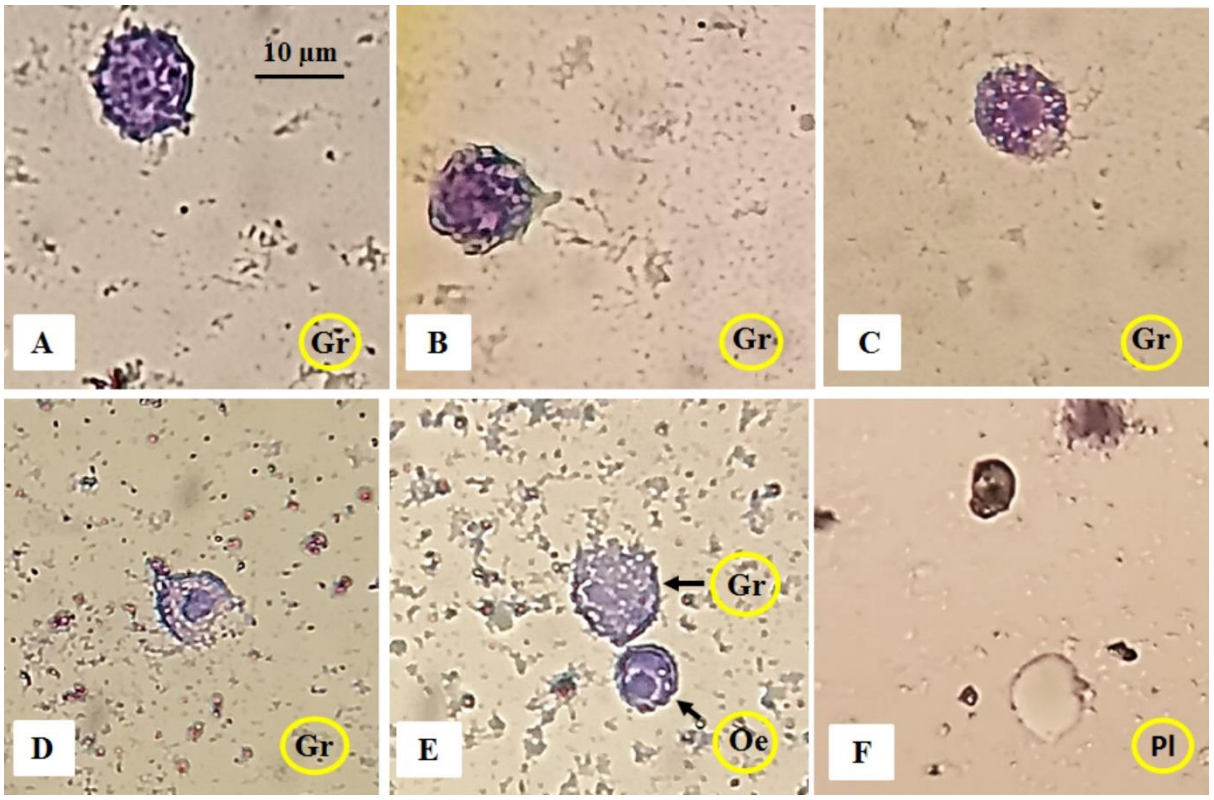

Figure 8. A, and B show the deformation of granulocytes; C shows the tearing of the cell wall in granulocytes affected by thermal stress at 30°C; D shows the exit of cellular contents from the granulocyte at 35°C; E shows the complete removal of cellular contents of the granulocyte and destruction of the nucleus as well as deformation of the oenocytoid, and F shows the shrinkage of the cell wall in plasmatocytes affected by cold stress

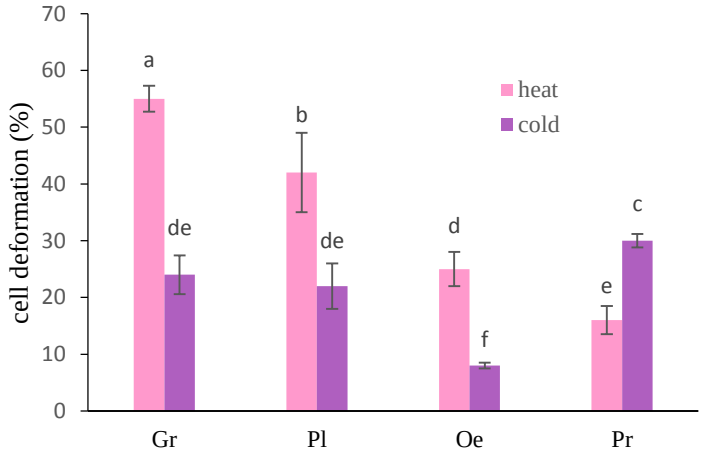

Figure 9. Cell deformation percentage of *Dacus ciliatus* affected by heat and cold stress.

Effect of thermal stress on pupal weight, pupal period, percentage of adult emergence, and adult longevity

The pupal period was observed to be shorter in larvae that experienced heat stress than in the control group ($F=76.5$, $df_{t,e}= 3, 10$, $P\leq 0.002$). The temperature of 35℃ decreased the pupal period (6.4±0.5 days) more than the temperature of 30℃ (8.5±0.2 days). The percentage of

adult emergence in larvae subjected to both heat and cold stress was lower than in the control, although cold stress had a greater effect on this parameter ($F= 98.3$, $df_{t,e}= 3, 10$, $P\leq 0.0001$). The percentage of adult emergence in the larvae exposed to cold (58±1.5%) was reduced by half compared to the control (98±5.5%) (Table 5).

Table 5. Survival status of *Dacus ciliatus* affected by thermal stress

Temperature (◦C)	Days until pupation	Pupal weight (mg)	Pupal period (day)	Percentage of adult emergence (%)	Adult longevity (day)
24±1	2±0.3a	14±0.4a	10±1a	98±5.5a	18.5±1.7a
5	2±0.6a	13.5±0.6a	11.7±0.6a	58±1.5c	20±2a
30	1±0.2a	12±0.2a	8.5±0.2b	84±5b	16.5±1.8a
35	0.5±0.1b	11.2±0.3a	6.4±0.5c	80±2.4b	17±2.2a

Different letters in each column show statistical differences among biological stages (Tukey's test, $P\leq 0.05$.

Discussion

Hemocytes play a crucial role in the cellular immunity of insects, responding to various stresses and infections by altering their number, type, size, and shape (Lavine and Strand, 2002, Ebrahimi and Ajamhassani, 2020, Duarte *et al*, 2020). In *Dacus ciliatus*, five types of hemocytes were identified in the hemolymph: prohemocytes, plasmatocytes, granulocytes, oenocytoids, and spherulocytes. It is important to note that in some insects, these cell types can be reduced or transformed into other forms. For instance, different types of hemocytes have been observed in flies and mosquitoes. In *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae), prohemocytes, granulocytes, plasmatocytes, adipohaemocytes, oenocytoids, and spherulocytes were identified in the hemolymph (Silva et al., 2002). Similarly, in *Musca domestica* Linnaeus, *Chrysomya megacephala* (Fabricius), and *Chironomus ramosus* (Fabricius), prohemocytes, plasmatocytes, granulocytes, oenocytoids, and spherulocytes were observed (Pal and Kumar, 2014; Gaikwad et al., 2024). This variability suggests that there is no universal hemocyte pattern within this order (Bruno *et al*., 2022). The types and functions of hemocytes can vary not only between different insect orders but also among families, genera, and species within the same order (Gábor *et al*., 2020).

The differential hemocyte count (DHC) in *D. ciliatus* revealed significant variations in the population of different hemocyte types across its life stages. Prohemocytes were the most abundant hemocytes in the hemolymph of first instar larvae, but their density decreased as the larvae aged. Prohemocytes differentiate into plasmatocytes before being released from hematopoietic organs, undergoing mitosis to become plasmatocytes and granulocytes during cellular defense or wound healing processes (Yamashita and Iwabuki, 2001). Plasmatocytes and granulocytes were the most abundant hemocytes in the third instar larvae and pupae, with a higher concentration in the third instar larvae. These cells are critical for cellular defense

processes. Previous research has shown that in Lepidoptera, plasmatocytes and granulocytes account for approximately 80-90% of the total hemocyte population (Strand, 2008). It has been demonstrated that older larvae exhibit greater resistance to foreign factors compared to younger larvae, likely due to the higher abundance of key immune cells like plasmatocytes and granulocytes (Valadez-Lyra, 2011). In *D. ciliatus*, the number of plasmatocytes and granulocytes peaked in the third instar larvae but decreased in the pupal and adult stages. In contrast, the numbers of oenocytoids and spherulocytes were low across all developmental stages, comprising approximately 6-11% of the total hemocyte population.

The hemogram of *D. ciliatus* revealed a direct correlation between the insect's weight and both the hemolymph volume and total hemocyte count (THC). The third instar larvae, which are larger and consume more food, cause more damage to crops and have greater body size and weight compared to other stages. Consequently, their blood volume and THC were significantly higher. In contrast, first instar larvae, which are smaller with less feeding activity, exhibited lower hemocyte counts and hemolymph volume. It has been established that nutrition and food type significantly influence hemolymph volume and hemocyte density (Manjula et al., 2020). Furthermore, the increased nutritional demands of older larvae and the heightened concentration of antimicrobial protein compounds may contribute to changes in the hemocyte population (Gupta, 1985; Mason et al., 2014). In adult flies, the reduced nutritional intake is associated with a significant decrease in circulating hemocytes compared to third instar larvae. Many insects are ectotherms, meaning their primary source of heat comes from the environment. Consequently, drastic temperature changes can significantly affect their homeostasis and survival. The ability to tolerate environmental stress is therefore crucial for their fitness, activity, reproduction, survival, and immunological potential (Boher *et al*., 2016; Herren *et al*., 2023). In our study, we observed physiological changes in *Dacus ciliatus* hemocytes under short-term temperature stress (24 hours), specifically in their number and morphology.

The effects of heat and cold stress on hemocyte count and morphology were evident. As the temperature increased to 35 °C, there was a significant rise in the number of hemocytes. This increase is attributed to cell division, particularly among prohemocytes, which differentiate into immunocytes in response to stress or the presence of foreign agents (Pandey et al., 2010). Additionally, granulocytes and plasmatocytes were observed to undergo cell division as a result of elevated temperatures (Amaral et al., 2010). However, at these higher temperatures, the cell walls of granulocytes and plasmatocytes were compromised, leading to the rupture of cell

membranes and the release of cell contents (Ghasemi et al., 2013). Similar observations were made in *Scrobipalpa ocellatella* (Boyd) (Lep: Gelechiidae), where granulocytes were severely deformed, and oenocytoids, despite their thick cell walls, were torn under high-temperature stress. Prolonged heat stress in larvae led to the complete disintegration of cells as their contents were fully expelled (Ajamhassani, 2021).

Conversely, cold stress resulted in a decrease in hemocyte numbers and caused compression and shrinkage of prohemocytes. Under unfavorable weather conditions or temperature drops, insects tend to reduce their vital activities, such as feeding and mobility (Ajamhassani *et al*., 2023). As a result, some hemocytes are removed from circulation and attach to the body walls (Rowley and Ratcliffe, 1978). In cold-exposed cockroaches, the hemocyte area was significantly smaller compared to the control larvae. Specifically, at 4 °C, the hemocyte size in *Gromphadorhina coquereliana* was markedly reduced (Lubawy and Stocinska, 2020). These cells were no longer part of the circulating hemocyte population. Similar effects have been reported in other insect species, such as *Nicrophorus vespilloides* Herbst (Coleoptera: Silphidae) (Urbanski *et al*., 2017), *Antheraea myllita* (Drury) (Pandey *et al*., 2010), and *Yponomeuta mallinellus* Zeller (Ajamhassani and Mahmoodzadeh, 2020). In our study, *D. ciliatus* exhibited comparable response to the thermal stress, where hemocyte size and morphology were notably altered by thermal stress. Granulocytes and plasmatocytes showed significant deformation under heat stress, while cold stress led to a marked reduction in prohemocyte size. These findings further support the sensitivity of hemocyte morphology to temperature extremes, consistent with observations in other insect species.

We observed a shortened pupal period in *Dacus ciliatus* individuals subjected to short-term heat stress. Additionally, the percentage of adult emergence was significantly lower in those exposed to both heat and cold, with cold stress having a more pronounced impact compared to the control group. Our findings suggest that the duration of heat stress plays a crucial role in influencing survival and the population dynamics of subsequent generations, which can ultimately affect damage levels, as highlighted by Herren *et al*. (2023). In their study, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) larvae were subjected to either short (2 hours) or long (14 hours) heat stress at 38°C, and the effects on larval survival and immune response were assessed. They found that brief exposure improved survival rates and enhanced antibacterial activity, whereas prolonged or delayed heat stress had less favorable outcomes, underscoring the importance of stress duration and timing.

In contrast, Zheng *et al*. (2017) investigated the effects of a 2-hour heat stress at 35°C on *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) pupae. Their results indicated a significant increase in adult longevity and heat resistance, though fecundity was negatively impacted. This study demonstrates how even short-term, mild heat stress can enhance certain aspects of fitness, such as longevity, which contrasts with the lack of significant changes in pupal weight and adult longevity observed in our study. Ouda *et al*. (2022) examined *D. ciliatus* at constant temperatures of 15°C, 20°C, 25°C, and 30°C, maintaining infested squash fruits at these temperatures to measure developmental rates from egg to adult. Their findings showed that higher temperatures accelerated the development of immature stages, consistent with our observation of accelerated development at elevated temperatures. Similarly, Mahmoud (2016) reported a reduction in the larval period of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) with increasing temperatures from 15°C to 30°C. This observation aligns with our findings on the accelerated development of *D. ciliatus*, highlighting the broader impact of temperature on developmental rates across different species.

Furthermore, high temperatures have been documented to reduce the developmental stages of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (de Campos *et al*., 2021), and *Athetis lepigone* (Möschler) (Lepidoptera: Noctuidae) when temperatures were increased to 30°C during their development from egg to adult. (Li *et al*., 2013). Despite insects having behavioral, morphological, and physiological adaptations to tolerate adverse environmental conditions, even slight deviations from the optimal temperature range for their growth can affect their survival and development (Mutamisva and Mbande, 2023).

In our study, we found that temperature treatments did not significantly affect pupal weight or adult lifespan. This may be due to the larvae inside the fruits experiencing only a brief 24-hour temperature stress after nearly completing their feeding, suggesting that under these conditions, pupal weight and adult longevity remained largely unchanged. However, it is crucial to consider the physiological characteristics and sensitivity of each species to environmental changes.

Our findings revealed a positive effect of thermal stress on certain biological and physiological aspects of *D. ciliatus*. While our study primarily focuses on the effects of thermal stress, understanding the broader implications, including the insect's immune response, can provide additional context. Specifically, future research should explore how thermal stress affects the insect's immune system and its ability to cope with other stressors, such as microbial agents and toxic substances. Investigating the interaction between thermal stress and immune parameters like antimicrobial peptides and detoxifying enzymes could offer valuable insights into the pest's

overall resilience. Such studies will be essential for developing comprehensive pest management strategies that account for both environmental stress and biological factors.

Conclusions

In the present study, we investigated the impact of thermal stress on certain developmental characteristics and the hemocyte density of *D. ciliatus*. Our findings suggest that this insect exhibits sensitivity to thermal stress, which affects its development and physiological parameters. Given these results, it is crucial to conduct further research on the effects of thermal stress in greenhouse and field settings over extended periods. Understanding how thermal stress influences the survival and immune system of the insect in both short and long terms could provide valuable insights for managing its population and mitigating the damage it causes.

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References

Abdallah, A.A., El-Saiedy, E.M.A., El-Fatih, M.M., and Shoula, M.E. 2012. Effect of some biological and biochemical control agents against certain squash pests. *Arch. Phytopathol*., **45 (1)**: 73-82.

Ajamhassani, M. and Mahmoodzadeh, M. 2020. Cellular defense responses of 5th instar larvae of the Apple Ermine Moth, *Yponomeuta malinellus* (Lepidoptera: Yponomeutidae) against starvation, thermal stresses and entomopathogenic bacteria *Bacillus thuringiensis*. *J. Anim. Res*., **4(2)**: 59–68. (In Persian with English summary).

Ajamhassani, M. 2021. Hemocyte changes of larvae of the beet moth, *Scrobipalpa ocellatella* (Lepidoptera: Gelechiidae) affected by thermal stress. *J. Entomol. Soc. Iran*., **41(1)**:101–103. (In Persian with English summary).

Ajamhassani, M., Ebrahimizadeh, Z., Abdos, F. and Ahangi rashti, B. 2023. Different pistachio cultivars impair hemocyte frequencies in diapausing and nondiapausing larvae of pistachio seed chalcid, *Megastigmus pistaciae* (Hymenoptera: Torymidae). *J. Entomol. Soc. Iran*., **43(4)**: 347-360.

Amaral, I.M.R., Neto, J.F.M., Pereira, G.B., Franco, M.B,. Beletti, M.E., Kerr, W.E., Bonetti, A.M., Ueira-Vieira, C. 2010. Circulating hemocytes from larvae of *Melipona scutellaris* (Hymenoptera, Apidae, Meliponini): Cell types and their role in phagocytosis. *Micron*. **41**: 123-129.

Arghand, B. 1983. Introduction flies Dacus sp. and study it in the province Hormozgan. Journal of Plant Pests and Diseases., **51(1)**: 9-3. (in Persian).

Barzkar, M., Goldasteh, SH., Eslamizadeh, R. and Usefi, B. 2017. Study on the population dynamics and spatial distribution of the cucurbit Fly; *Dacus ciliatus* Loew (Dip., Tephritidae). *J. Entomol. Res*., **9(2)**: 131-142.

Black, J. L.; Clark, M. K.; Sword, G. A. 2022. Physiological and transcriptional immune responses of a non-model arthropod to infection with different entomopathogenic groups. *PLoS ONE*., **17**: e0263620.

Boher, F., Trefault, N., Estay, S. E., Bozinovic, F., 2016. Ectotherms in variable thermal 521 landscapes: A physiological evaluation of the invasive potential of fruit flies species. *Front. Physiol*., **7**: 302.

Browne, N., Surlis, C. and Kavanagh, K., 2014. Thermal and physical stresses induce a short-term immune priming effect in *Galleria mellonella* larvae. *J. Insect Physiol*., **63**: 21-26.

Bruno, D., Montali, A., Gariboldi, M., Wronska, A., Kaczmarek, A., Mohamed, A., Tian, L., Casartelli, M. and Tettamanti, G. 2022. Morphofunctional characterization of hemocytes in black soldier fly larvae. *Insect Sci*., 1-21.

De Campos, M. R., Béarez, P., Amiens-Desneux, E., Ponti, L., Gutierrez, A. P., Biondi, A., Adiga, A. and Desneux, N. 2020. Thermal biology of *Tuta absoluta*: demographic parameters and facultative diapause. *J Pest Sci*., **94**: 829–842.

Dyar, H. C., 1890. The number of molts of lepidopterous larvae. *Psyche*. **5**: 420-422.

EPPO, 2018. EPPO Global Database (available online). https://gd.eppo.int [accessed on 23 May 2018] EPPO/CABI (1997) Quarantine Pests for Europe, 2nd edn. (Eds Smith IM, McNamara.

Foray, V., Desouhant, E. and Gibert, P. 2014. The impact of thermal fuctuations on reaction norms in specialist and generalist parasitic wasps. *Funct. Ecol*., **28**: 411–423.

Gábor, E., Cinege, G., Csordás, G., Rusvai, M., Honti, V. and Kolics, B. 2020. Identification of reference markers for characterizing honey bee (*Apis mellifera*) hemocyte classes. *DCI*., **109**: 103701.

Gaikwad, P., Gupta, A., Waghamare, N., Mukhopadhyaya, R. and Nath, B. B. 2024. Hemocytes of a tropical midge *Chironomus ramosus* (Diptera: Chironomidae). *Int. J. Trop. Insect Sci*., **44**: 265-271.

Ghasemi, V., Moharramipour, S., and Jalali Sendi, J., 2013. Circulating hemocytes of Mediterranean flour moth, *Ephestia kuehniella* Zell (Lep: Pyralidae) and their response to thermal stress. *ISJ*., **10**:128-140.

Go, M. S., Cho, Y., Park, K., Kim, M., Park, S., Park, J., 2022. Classification and characterization of immune haemocytes in the larvae of the Indian fritillary, *Papilio hyperbius* (Lepidopetra: Nymphalidae). *Eur. J. Entomol*. **119**: 430-438.

Hassan, G. M., El Aassar, M. R., and Khorchid, A. M. 2023. Implement of Some Biocontrol Tactics as an Innovative Management Against Cucurbit Fly, *Dacus ciliatus* and Western Flower Thrips, *Frankliniella occidentalis* on Squash Crop. *A J A S*., **54 (2)**: 202-219.
Jones, J. C. 1962. Current concepts concerning insect hemocytes. *Am. Zool.,* **2**: 209-246.

Herren, P., Hesketh, H., Dunn, A. M. and Meyling, N. V. 2023. Heat stress has immediate and persistent effects on immunity and development of *Tenebrio molitor*. *J. Insects Food Feed*., 1-19.

Lee K. P., Simpson, S. J. and Wilson, K. 2008. Dietary protein-quality influences melanization and immune function in an insect. *Funct. Ecol*., **22**: 1052-1061.

Li, L. T., Wang, Y. Q., Ma, J. F., Liu, L., Hao, Y. T., Dong, C., Gan, Y. J., Dong, Z. P. and Wang, Q. Y. 2013. The efects of temperature on the development of the moth *Athetis lepigone*, and a prediction of feld occurrence. *J. Insect Sci*., **13**: 103.

Lubawy, J. and Sticinska, M. 2020. Characterization of *Gromphadorhina coquereliana* hemolymph under cold stress. *Sci. Rep*., **10**: 12076.

Mahmood, A., and Yousaf, M. 1985. Effect of some insecticides on the haemocytes of *Gryllus bimaculatus*. de Geer. *Pak. J. Zool*., **17**: 71-84.

Mahmoud, A. A., 2016. Effect of temperature on the development and survival of immature stages of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Plant Protection Department, Faculty of Agriculture, South Valley University Qena, Egypt. African Journal of Agricultural Research. **11(36):** 3375-3381.

Manjula, P., Lalitha, K. and Shivakumar, M. S., 2020. Diet composition has a differential effect on immune tolerance in insect larvae exposed to *Mesorhabditis belari*, *Enterobacter hormaechei* and its metabolites. *Exp. Parasit*. **208**: 1-7.

Mason, A. P., Smilanich, A. M. and Singer, M. S., 2014. Reduced consumption of protein-rich foods follows immune challenge in a polyphagous caterpillar. *J. Exp. Biol*. **217**: 2250-2260.

Mohammad, A.K.H. 2022. Biological and Control Study of the Cucurbit Fruit Fly, *Dacus ciliatus* (Leow) (Diptera: Tephritidae). *Biochem. Cell. Arch*. **22 (1)** (Part II): 2923-2926.
tamiasva, R., Mbande, A., Nyamukondiwa, C. and Chidawanyika, F. 2023. Thermal adaptation in Lepidoptera under shifting environments: mechanisms, patterns, and consequences. *Phytoparasitica*., **51**: 929-955.

Norrbom, A. L. and Uchoa, M. A. 2011. New species and records of Anastrepha (Diptera: Tephritidae) from Brazil. *Zootaxa*., **2835**: 61-67.

Ouda, M. I., Mousa, E. A., M. and Fatina, b. 2022. Biological Study of Cucurbit Fruit Fly, *Dacus ciliatus* (Loew) on Constant Temperatures. *Egypt. Acad. J. Biolog. Sci*. **15(4)**:121-128
Pal, R. and Kumar, K. 2014. A comparative study of haemocytes in three cyclorrhaphous dipteran flies. *Int. J. Trop. Insect Sci*., **34**: 207–216.

Pandey, J. P., Mishra, P. K., Kumar, D., Singh, B. M. K., and Prasad B. C. 2010. Effect of temperature on hemocytic immune responses of tropical tasar silkworm, *Antheraea mylitta*. *RJI*., **3**: 169-177.

Paydar, M., Moeini-Naghadeh, N., Jalilian, F. and Zamani, A.A. 2020. Comparative field study of various attractants of the pumpkin fruit fly, *Dacus ciliatus* (Diptera: Tephritidae) in Kermanshah. *Iran J Plant Prot Sci***. 51(2)**: 171-179.

Pech, L. L., and Strand, M. R. 2000. Plasmatocytes from the moth *Pseudoplusia includens* induce apoptosis of granular cells. *J Insect Physiol*., **46**: 1565–1573.

Pourali, Z., and Ajamhassani, M. 2018. The effect of thermal stresses on the immune system of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *J. Entomol. Soc. Iran*., **37**: 515-525. (In Persian with English Summary).

Rowley, A. F., and Ratclife, N. A. A. 1978. histological study of wound healing and hemocyte function in the wax-moth *Galleria mellonella*. *J. Morphol*., **157**: 181–199.

Silva, J. E. B., Boleli, I. C. and Simoes, Z. L. P. 2002. Hemocyte types and total and differential counts in unparasitized and parasitized *Anastrepha obliqua* (Diptera, Tephritidae) larvae. *Braz. J. Biol*., **62(4A)**: 689-699.

Siva-Jothy, M., and Thompson, J. 2002. Short-term nutrient deprivation affects immune function. *Physiol. Entomol*., **27(3)**: 206-212.

Shapiro, M. 1979. Changes in hemocyte populations. In: Gupta A.P. (ed.), Insect hemocytes, Cambridge University Press, Cambridge, pp. 475-524.

Strand, M. R. 2008. The insect cellular immune response. *Insect Sci*., **15**: 1- 14

Terra, W. R., Bianchi, A. G., and Lara, F. J. S. 1975. Physical properties and chemical composition of the haemolymph of *Rhynchosciara americana* (Diptera) larvae. *CBP*., **47**: 117-129.

Urbanski, A., Czarniewska, E., Baraniak, E. and Rosinski, G. 2017. Impact of cold on the immune system of burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Insect Sci*., 24: 443-454.

Valadez-Lira, J. A., Gonzalez, J. M., Damas, G., Meja, G., Oppert, B., Padilla, C., and Guerra, P. 2011. Comparative evaluation of phenoloxidase activity in different larval stages of four lepidopteran after exposure to *Bacillus thuringiensis*. *J. Insect Sci*., **12(80)**: 1-11.

Valizadeh, B., Sendi, J., Khosravi, R. and Salehi, R. 2017. Establishment and characterizations of a new cell line from larval hemocytes of rose sawfly *Arge ochropus* (Hymenoptera: Argidae). *J. Entomol. Soc. Iran*., **38(2)**: 173–186. (In Persian with English Summary).

Vayssières, J. F., Carel, Y., Coubes, M. and Duyck, P. F. 2008. Development of immature stages and comparative demography of two cucurbit-attacking fruit flies in Reunion Island:

Bactrocera cucurbitae and *Dacus ciliatus* (Diptera: Tephritidae). *Environ. Entomol*. **37**: 307-314.

Vogel, M., Shah, P. N., Voulgari-Kokota, A., Maistrou, S., Aartsma, Y., Beukeboom, L.W., Salles, J. F., Van Loon, J. J. A., Dicke, M. and Wertheim, B., 2022. Health of the black soldier fly and house fly under mass-rearing conditions: innate immunity and the role of the microbiome. *J. Insects Food Feed*., **8**: 857-878.

Yamashita, M., and Iwabuchi, K. 2001. *Bombix mori* prohemocytes division and differentiation in individual microcultures. *J. Insect Physiol*., **47**: 325- 331.

Yeager, J. F. 1945. The blood picture of the Southern armyworm (*Prodenia eridamin*). *J. Agric. Res.,* **71**: 1–40.

Zheng, J., Cheng, X., Hofmann, A. A., Zhang, B. O., and Ma, C. S. 2017. Are adult life history traits in oriental fruit moth affected by a mild pupal heat stress? *J. Insect Physiol*., **102**: 36–41.

Zhu, Q., He, Y., Yao, J., Liu, Y., Tao, L. and Huang, Q. 2012. Effects of sublethal concentrations of the chitin synthesis inhibitor, hexaflumuron, on the development and hemolymph physiology of the cutworm, *Spodoptera litura*. *J. Insect Sci*., **12(27)**: 1-13.

بررسی هموگرام و تأثیر استرس حرارتی بر هموسیت ها و رشد در Dacus ciliatus (Diptera: Tephritidae)

مریم عجم حسنی، محمد العلوی، و بیتا ولی زاده

چکیده

این مطالعه، تاثیر تنشهای دمایی را بر سامانه ایمنی مگس جالیز *Dacus ciliatus* Loew (Diptera: Tephritidae) با بررسی مرفولوژی و تراکم سلولهای خونی به عنوان اجزای اصلی ایمنی حشره نشان می دهد. فاکتورهای مختلفی مانند تنش دما، تغییرات رژیم غذایی و ورود عفونتها و آلودگیها به همولنف، با تغییر پروفایل سلولهای خونی سبب پاسخ ایمنی حشره می شوند. این تحقیق متمرکز بر پروفایل هموسیتها، هموگرام همه مراحل زیستی و تغییرات مرفولوژیکی و فراوانی هموسیتهای لاروهای سن سوم مگس جالیز در مقابل استرسهای دمایی بود. میوه های خیار آلوده به لارو، جمع آوری و به آزمایشگاه منتقل شدند. لاروهای سن سوم از بافتهای میوه خارج شدند. پس از استخراج همولنف از لاروها و رنگ آمیزی با محلول گیمسا، هموسیتها با استفاده از میکروسکوپ نوری، شناسایی شدند. در مطالعه هموگرام، پارامترهای DHC، THC، حجم همولنف و AHC در همه مراحل زیستی اندازه گیری شد. در لاروهای سن سوم، گرانولوسیتها و پلاسماتوسیتها در مجموع شامل 56 درصد فراوانی، بیشترین جمعیت را بین هموسیتها داشتند. در مقابل، پروهموسیتها در حدود 37%، بیشترین فراوانی را در لاروهای سن اول به خود اختصاص دادند. بالاترین THC در لاروهای سن سوم مشاهده شد که نشان دهنده ارتباط مستقیم بین حجم همولنف و تعداد کل سلولها بود. تنشهای دما تاثیر معنی داری بر تعداد هموسیتها نشان داد. در تنش گرما، افزایش دما تا 30 و 35 درجه سلسیوس، منجر به افزایش بارز تعداد کل سلولها، گرانولوسیتها و پلاسماتوسیتها شد. در مقابل، تنش سرما، سبب کاهش پروهموسیتها، گرانولوسیتها، پلاسماتوسیتها و تعداد کل سلولها در مقایسه با شاهد شد. به علاوه، تنش دما سبب تغییر شکل هموسیتها شد. پلاسماتوسیت ها و گرانولوسیت ها بارزترین تغییرات را تحت تنش گرمایی نشان می دهند، از جمله پارگی دیواره سلولی و از بین رفتن محتویات سلولی در دمای 35 درجه سلسیوس. استرس سرما، تأثیر بیشتری بر انقباض پروهموسیت ها در مقایسه با سایر سلولها داشت. تنش دما همچنین به طور قابل توجهی بر ویژگی های رشدی مگس میوه تأثیر گذاشت. استرس گرمایی طول دوره شفیره و نرخ ظهور حشرات کامل را کاهش داد، در حالی که استرس سرما به طور برجسته‌تری بر نرخ تولد تأثیر داشت. این مطالعه نشان دهنده شناسایی اولیه هموسیت ها و تجزیه و تحلیل پاسخ ایمنی *D. ciliatus* به تغییرات دما است که پایه ای برای تحقیقات بیشتر در مورد مکانیسم های دفاع فیزیولوژیکی این آفت فراهم می کند.