

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

3  
4        Maryam Ajamhassani<sup>1\*</sup>, Mohamed El Aalaoui<sup>2</sup>, and Bita Valizadeh<sup>3</sup>

5        **Abstract**

6        This study investigated the impact of temperature stress on the immune system of *Dacus ciliatus*  
7        Loew (Diptera: Tephritidae) by examining the morphology and density of hemocytes, which  
8        are crucial components of insect immunity. Various factors, such as temperature stress, dietary  
9        changes, and the entry of contaminants and infections into the hemolymph, are known to affect  
10        insect immune responses by altering hemocyte profiles. The research focused on the hemocyte  
11        profile, hemogram across all biological stages, and the morphological and frequency changes  
12        of hemocytes in third instar larvae exposed to temperature stress. Cucumber fruits infected with  
13        insect larvae were collected and brought to the laboratory, where third instar larvae were  
14        extracted from the fruit tissue. The hemolymph was then collected, and after staining with  
15        Giemsa solution, hemocytes were identified under a light microscope. The hemogram analysis  
16        included measurements of DHC, THC, blood volume, and AHC across all biological stages. In  
17        third instar larvae, plasmatocytes and granulocytes were the most abundant, comprising about  
18        56% of the hemocyte population. In contrast, prohemocytes were most frequent in the first  
19        instar larvae, accounting for approximately 37%. THC was highest in third instar larvae,  
20        indicating a direct correlation between hemolymph volume and total hemocyte count.  
21        Temperature stress had a significant impact on hemocyte numbers. Heat stress, with  
22        temperatures up to 30 and 35°C, led to a notable increase in total cell count, granulocytes, and  
23        plasmatocytes. Conversely, cold temperatures resulted in a decrease in prohemocytes,  
24        plasmatocytes, granulocytes, and the total cell count compared to the control group.  
25        Additionally, temperature stress induced hemocyte deformation, with plasmatocytes and  
26        granulocytes showing the most pronounced changes under heat stress, including torn cell walls  
27        and loss of cell contents at 35°C. Cold stress had a greater effect on the shrinkage of  
28        prohemocytes than on other cell types. Temperature stress also significantly affected the  
29        developmental characteristics of the fruit fly. Heat stress reduced the pupation period length  
30        and emergence rates, while cold stress more prominently impacted birth rates. This study

---

<sup>1</sup> Department of Plant Protection, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Islamic Republic of Iran.

<sup>2</sup> National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat principal, 10090 Rabat, Morocco.

<sup>3</sup> Delta Research and Extension Center, Mississippi State University, Stoneville, MS 38776. USA.

\*Corresponding author, e-mail: shahroodm@gmail.com

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

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

### 37 Introduction

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

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

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

65 the entry of contaminants or infections into the hemolymph. These factors underscore the  
66 sensitivity of the circulatory system to stress and osmolality changes (Siva-jothy and Thompson  
67 2002; Lee *et al.*, 2008). **Osmolality, which** refers to the concentration of solute particles in a  
68 solution, is a critical characteristic of hemolymph, playing an essential role in blood circulation,  
69 gas exchange, metamorphosis, adult emergence, and wing **expansion** (Jiang *et al.*, 2023;  
70 Salcedo *et al.*, 2023). Upon identifying a foreign agent, hemocytes such as plasmatocytes and  
71 granulocytes react by altering their shape, type, and density, followed by processes like  
72 phagocytosis and nodulation, which are vital for the insect's innate immune response (Pech and  
73 Strand, 2000, Black *et al.*, 2022).

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

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

## 93 94 **Materials and Methods**

### 95 **Insect Rearing**

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

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

107 **Table 1.** Morphometric size (mean±se) of different larval development of *Dacus ciliatus* (n=  
 108 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

109

## 110 Hemocyte Identification

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

114

## 115 Hemogram

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

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

128

129

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

131 For THC, approximately 1  $\mu\text{L}$  of hemolymph from two larvae was collected using a capillary  
 132 tube and mixed with 10  $\mu\text{L}$  of Tyson buffer as an anticoagulant solution (NaCl 72 Mm,  
 133 Na<sub>2</sub>SO<sub>4</sub> 9 Mm, glycerol 43 Mm, methyl violet 0.06 Mm, distilled water) (Mahmood and Yusaf,  
 134 1985). Hemolymph and Tyson solutions were placed on Neubauer slides (HBG, Germany), and  
 135 hemocytes were counted using the Jones formula and light microscopy at 40 $\times$  magnification  
 136 (Jones *et al.*, 1962).

$$\frac{\text{Hemocyte in } \times 1 \text{ mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}} \quad \begin{matrix} 137 \\ 138 \end{matrix}$$

No. of squares counted

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

140

141 **Hemolymph volume (HV)**

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

149

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

152 The effect of temperature stress on the number of hemocytes comprised four treatments (5,  
 153 24 $\pm$ 1, 30 and 35 $^{\circ}\text{C}$ ) and four repetitions. Based on previous observations, infected fruits with  
 154 larval entrance holes and deformation due to larval activity were found to contain various larval  
 155 instars. These fruits were divided into four groups and exposed to different conditions:  
 156 controlled (24 $\pm$ 1 $^{\circ}\text{C}$ ), cold stress (5 $^{\circ}\text{C}$ ), and heat stress (30 $^{\circ}\text{C}$  and 35 $^{\circ}\text{C}$ ). Hemocyte counts in  
 157 third instar larvae of *D. ciliatus* were assessed after 24 hours. The control group comprised  
 158 larvae kept under growth chamber conditions (24 $\pm$ 1 $^{\circ}\text{C}$ ). In each replicate, the hemolymph of  
 159 three larvae (approximately 3  $\mu\text{L}$ ) was collected via a capillary tube and mixed with 20  $\mu\text{L}$  of  
 160 Tyson (anticoagulant solution). Cells in 3  $\mu\text{L}$  of hemolymph were counted using a  
 161 hemacytometer. To observe morphological changes in hemocytes under heat and cold stress,  
 162 infected fruits were exposed to thermal stress (Pourali and Ajamhassani, 2018, Ajamhassani *et*  
 163 *al.*, 2023). After 24 hours, hemocytes from third instar larvae were stained with Giemsa and



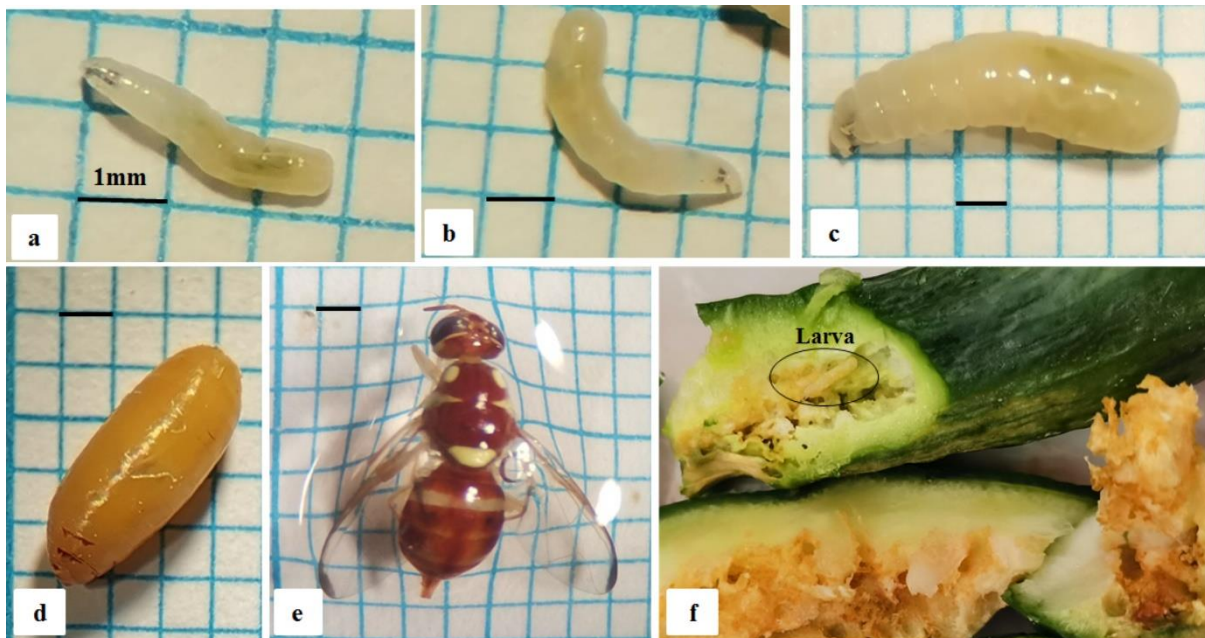
164 examined using a light microscope at 40× magnification. Hemocyte deformation was then  
165 recorded.

166

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

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

181



182

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

186

187

188 **Statistical analysis**

189 All data obtained from a complete randomized design were compared by one-way analysis of  
190 variance (ANOVA) followed by Tukey's test when significant differences were found at  $p \leq$   
191 0.05 (SAS, 9.4). Differences between samplings ( $n=3$ ) were considered statistically significant  
192 at a probability less than 5% and marked in figures and tables.

193  
194 **Results**195 **Identification of hemocytes in *D. ciliatus* larvae and determination of their abundance**  
196 **percentage in biological stages**

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

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

204 Granulocytes with central or semi-central nuclei varied in sizes and were sometimes the largest  
205 cells (Figure 2). The cytoplasm surface contained numerous granules, which were visualized  
206 with Giemsa blue. The frequency of these cells was higher in third instar larvae ( $29 \pm 1.5\%$ )  
207 compared to other stages and lowest in first instar larvae ( $16.5 \pm 2.3\%$ ) (Table 3) ( $F=84$ ,  $df_{t,e}=  
208 4,14$ ,  $P \leq 0.0001$ ). Plasmatocytes exhibited a spindle-shaped or eye-shaped morphology with  
209 varying sizes (Figure 2). The abundance of plasmatocytes was highest in third instar larvae  
210 ( $26.4 \pm 2\%$ ) and lowest in first instar larvae ( $21 \pm 1.6\%$ ) (Table 2), ( $F=55.4$ ,  $df_{t,e}=4,14$ ,  $P \leq 0.004$ ).

211 Oenocytoids were egg-shaped with lateral nuclei and were similar but slightly larger in size  
212 compared to prohemocytes (Figure 2, Table 2). The frequency of these cells was lower than the  
213 previous cells (Table 3) ( $F=107$ ,  $df_{t,e}=4,14$ ,  $P \leq 0.0001$ ). Spherulocytes of medium to large sizes  
214 were observed in larval hemolymph (Table 2) (Figure 2). Small spherules around the nucleus  
215 occupied the cytoplasm surface and were the least frequency of cells (Table 3) ( $F=92.6$ ,  $df_{t,e}=  
216 4,14$ ,  $P \leq 0.0001$ ).

217

218

219

220

221 **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

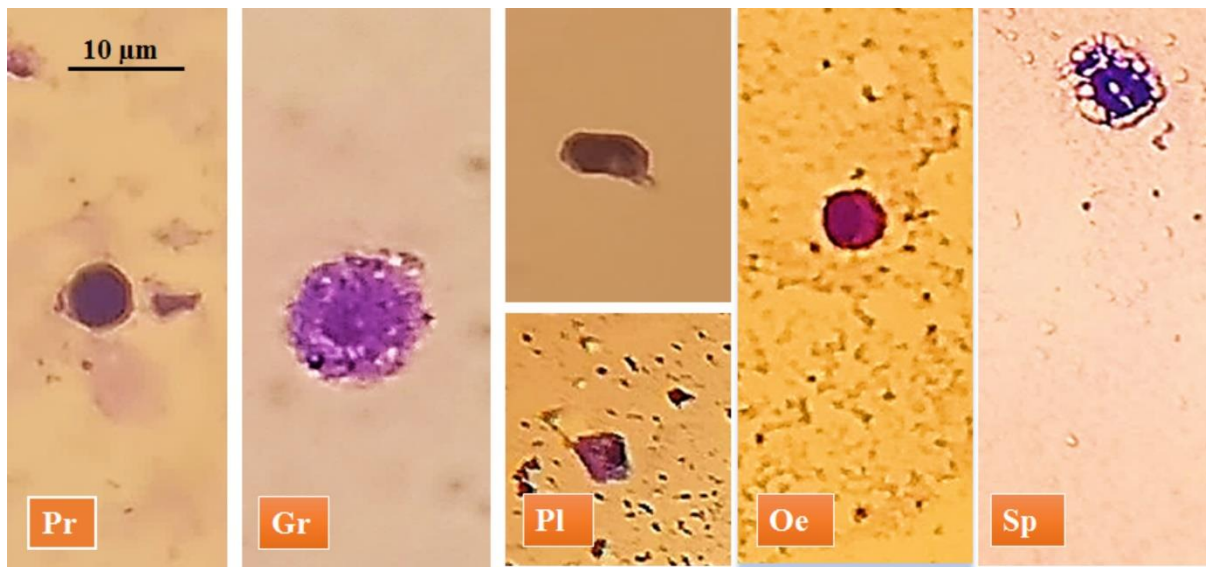
222 Different letters in each column show significance using Tukey’s test at P< 0.05).

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

Developmental stage	Frequency of hemocytes (%)				
	Prohemocyte	Plasmatocyte	Granulocyte	Oenocytoid	Spherulocyte
1 <sup>th</sup> instar larva	37±2.2a	21±1.6bc	16.5±2.3c	11±0.5b	6±1.1a
2 <sup>nd</sup> instar larva	30.3±1.6b	23±0.8b	20±2.4b	14±0.7a	8±1.3a
3 <sup>rd</sup> 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

225 Different letters in each column show statistical differences among biological stages (Tukey’s test, P≤  
226 0.05).

227



228 **Figure 2.** Light microscopy pictures of *Dacus ciliatus* hemocytes stained with Giemsa. PR  
229 (Prohemocyte), PL (Plasmatocyte), OE (Oneocytoid), GR (Granulocyte), SP (Spherulocyte),  
230 Scale bar= 10 µm.  
231

232

233 **Hemogram**

234 According to Table 3, the weight of the first and second instar larvae was lower than that of  
235 other stages (F= 44.4, df<sub>t,e</sub>= 4,14, P≤ 0.0001). Due to the higher feeding of the third instar larvae,  
236 the weight of these larvae was higher significantly than that of the younger larvae. On the other  
237 hand, the amount of nutrition is also effective on the hemolymph volume; so the hemolymph  
238 volume is higher in the third instar larvae, pupae, and adults than in the early larval stages (F=  
239 87.7, df<sub>t,e</sub>= 4,14, P≤ 0.0001). Hemocyte number of adults (230.2±21.4) cells/mm<sup>3</sup>) decreased



240 compared to third instar larvae ( $314.4 \pm 22.4$ ) cells/mm<sup>3</sup>) (Table 4), ( $F = 35.5$ ,  $df_{t,e} = 4, 14$ ,  $P \leq$   
 241  $0.0001$ ).

242

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

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

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

246

### 247 **Total hemocyte count, plasmatocytes, granulocytes, oenocytoids, and prohemocytes count** 248 **in larvae of *D. ciliatus* affected by thermal stress**

249 Significant changes were observed in the number of hemocytes of *D. ciliatus* larvae affected by  
 250 cold and heat. The results showed that the total hemocyte count ( $F = 84.2$ ,  $df_{t,e} = 3, 10$ ,  $P \leq$   
 251  $0.0001$ ), granulocytes ( $F = 102.5$ ,  $df_{t,e} = 3, 10$ ,  $P \leq 0.0001$ ), plasmatocytes ( $F = 109.35$ ,  $df_{t,e} = 3, 10$ ,  
 252  $P \leq 0.0001$ ), and oenocytoids ( $F = 104$ ,  $df_{t,e} = 3, 10$ ,  $P \leq 0.0001$ ) of larvae subjected to heat (30  
 253 and 35°C) were significantly higher than those of control larvae. In all the aforementioned cases  
 254 except for oenocytoids, a significantly lower number of hemocytes in larvae experienced cold  
 255 stress compared to the control group. Prohemocyte number decreased under cold too stress. The  
 256 total hemocyte count in the larvae exposed to 35°C ( $421 \pm 25$  cells/mm<sup>3</sup>) and 30°C ( $377 \pm 28.1$   
 257 cells/mm<sup>3</sup>) was higher than that of control larvae ( $340 \pm 11.5$  cells/mm<sup>3</sup>). Moreover, cold stress  
 258 at 5°C significantly decreased the number hemocytes in larvae, reducing it to  $262 \pm 15$  cell/mm<sup>3</sup>  
 259 hemolymph (Figure 3).

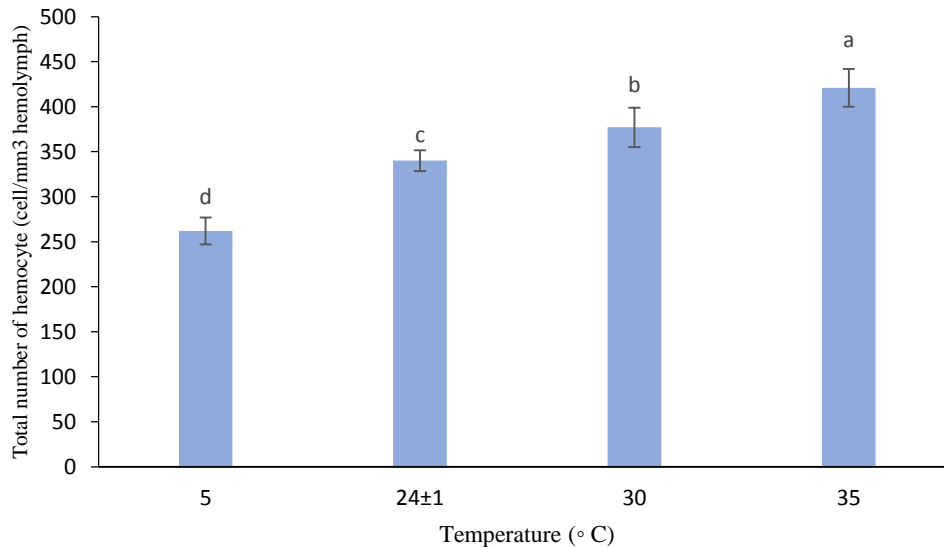
260 Furthermore, the granulocyte count was higher in larvae placed at 35°C ( $177 \pm 14$  cells/mm<sup>3</sup>) cell  
 261 in mm<sup>3</sup> and 30°C ( $145 \pm 15.5$  cells/mm<sup>3</sup>) than in control larvae ( $107 \pm 11.3$  cells/mm<sup>3</sup>). Similar to  
 262 the previous case, the number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

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

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

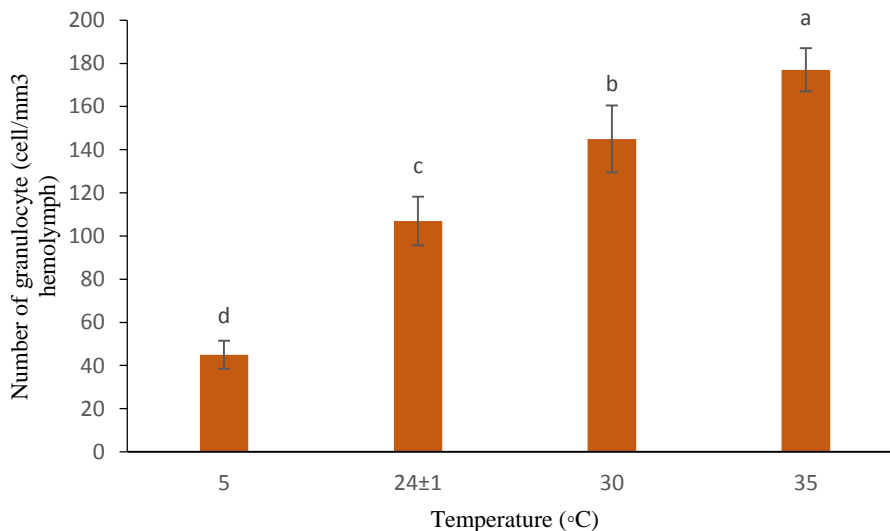
273 Under the influence of cold stress, prohemocytes exhibited a significant decrease compared to  
274 the control group, with their numbers reduced to about half. Based on the observations, the  
275 number of prohemocytes increased in higher temperatures but showed no significant difference  
276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

277



278

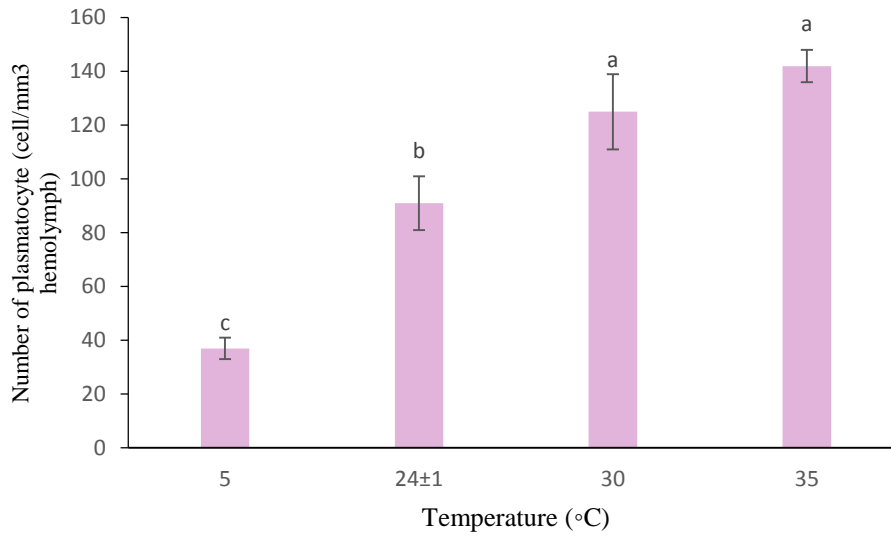
279 **Figure 3.** Effect of thermal stress on total hemocyte count in third instar larvae of *Dacus*  
280 *ciliates*.



281

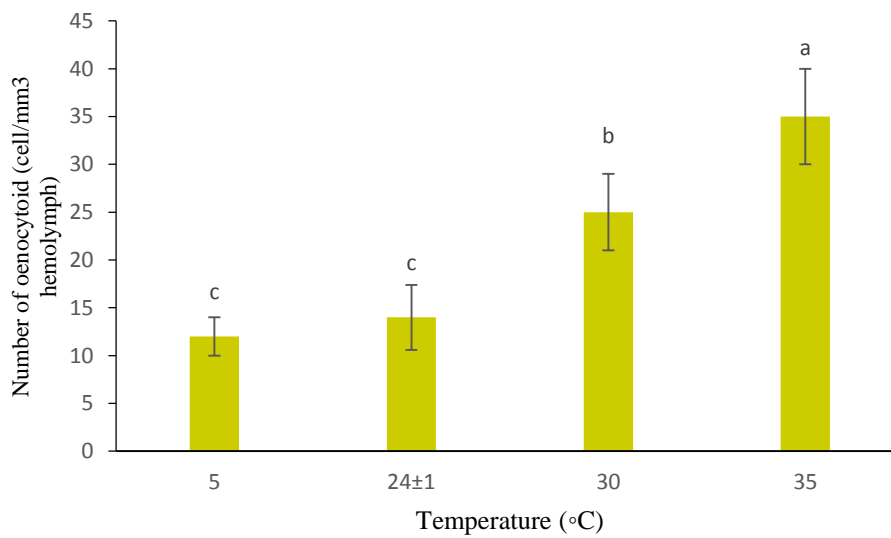
282 **Figure 4.** Effect of thermal stress on granulocyte number in third instar larvae of *Dacus ciliates*.

283



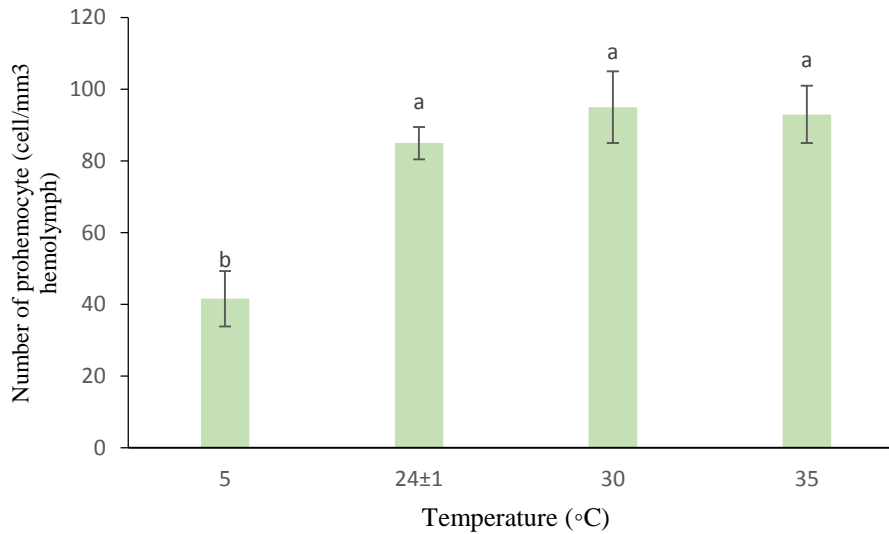
284 **Figure 5.** Effect of thermal stress on plasmatocyte number in third instar larvae of *Dacus*  
 285 *ciliates*.  
 286

287  
 288  
 289



290 **Figure 6.** Effect of thermal stress on oenocytoid number in third instar larvae of *Dacus*  
 291 *ciliates*.  
 292

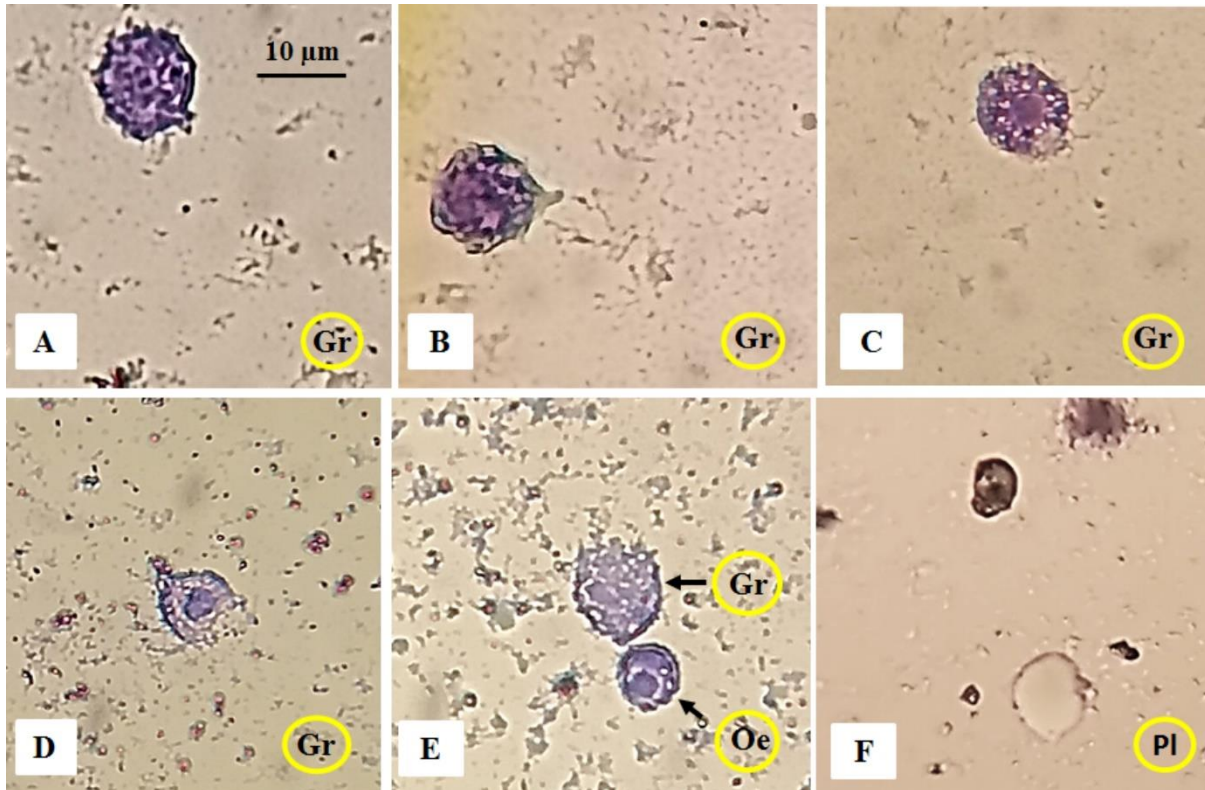
292



293 **Figure 7.** Effect of thermal stress on prohemocyte number in third instar larvae of *Dacus*  
 294 *ciliates*.  
 295

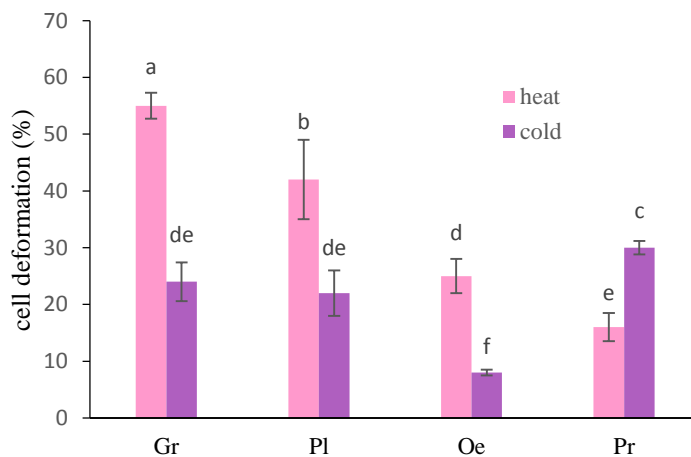
296  
 297 **Morphological changes of hemocytes in *D. ciliatus* affected by thermal stress**

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



307  
308  
309  
310  
311  
312  
313

**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



314  
315

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

316  
317  
318

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

319  
320  
321

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°C decreased the pupal period ( $6.4 \pm 0.5$  days) more than the temperature of 30°C ( $8.5 \pm 0.2$  days). The percentage of



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

326

327 **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

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

329

330 **Discussion**

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

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

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

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

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

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

392 Conversely, cold stress resulted in a decrease in hemocyte numbers and caused compression  
393 and shrinkage of prohemocytes. Under unfavorable weather conditions or temperature drops,  
394 insects tend to reduce their vital activities, such as feeding and mobility (Ajamhassani *et al.*,  
395 2023). As a result, some hemocytes are removed from circulation and attach to the body walls  
396 (Rowley and Ratcliffe, 1978). In cold-exposed cockroaches, the hemocyte area was  
397 significantly smaller compared to the control larvae. Specifically, at 4 °C, the hemocyte size in  
398 *Gromphadorhina coquereliana* was markedly reduced (Lubawy and Stocinska, 2020). These  
399 cells were no longer part of the circulating hemocyte population. Similar effects have been  
400 reported in other insect species, such as *Nicrophorus vespilloides* Herbst (Coleoptera:  
401 Silphidae) (Urbanski *et al.*, 2017), *Antheraea myllita* (Drury) (Pandey *et al.*, 2010), and  
402 *Yponomeuta mallinellus* Zeller (Ajamhassani and Mahmoodzadeh, 2020). In our study, *D.*  
403 *ciliatus* exhibited comparable response to the thermal stress, where hemocyte size and  
404 morphology were notably altered by thermal stress. Granulocytes and plasmatocytes showed  
405 significant deformation under heat stress, while cold stress led to a marked reduction in  
406 prohemocyte size. These findings further support the sensitivity of hemocyte morphology to  
407 temperature extremes, consistent with observations in other insect species.

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

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

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

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

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

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

#### 454 **Conclusions**

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

#### 462 463 **Acknowledgment**

464 This research was done with the financial assistance of Shahrood University of Technology,  
465 which is hereby acknowledged.

#### 466 467 **References**

468 Abdallah, A.A., El-Saiedy, E.M.A., El-Fatih, M.M., and Shoula, M.E. 2012. Effect of some  
469 biological and biochemical control agents against certain squash pests. *Arch. Phytopathol.*, **45**  
470 **(1)**: 73-82.

471  
472 Ajamhassani, M. and Mahmoodzadeh, M. 2020. Cellular defense responses of 5th instar larvae  
473 of the Apple Ermine Moth, *Yponomeuta malinellus* (Lepidoptera: Yponomeutidae) against  
474 starvation, thermal stresses and entomopathogenic bacteria *Bacillus thuringiensis*. *J. Anim.*  
475 *Res.*, **4(2)**: 59–68. (In Persian with English summary).

476  
477 Ajamhassani, M. 2021. Hemocyte changes of larvae of the beet moth, *Scrobipalpa ocellatella*  
478 (Lepidoptera: Gelechiidae) affected by thermal stress. *J. Entomol. Soc. Iran.*, **41(1)**:101–103.  
479 (In Persian with English summary).

480  
481 Ajamhassani, M., Ebrahimizadeh, Z., Abdos, F. and Ahangi rashti, B. 2023. Different pistachio  
482 cultivars impair hemocyte frequencies in diapausing and nondiapausing larvae of pistachio seed  
483 chalcid, *Megastigmus pistaciae* (Hymenoptera: Torymidae). *J. Entomol. Soc. Iran.*, **43(4)**: 347-  
484 360.

485  
486 Amaral, I.M.R., Neto, J.F.M., Pereira, G.B., Franco, M.B., Beletti, M.E., Kerr, W.E., Bonetti,  
487 A.M., Ueira-Vieira, C. 2010. Circulating hemocytes from larvae of *Melipona scutellaris*  
488 (Hymenoptera, Apidae, Meliponini): Cell types and their role in phagocytosis. *Micron*. **41**: 123-  
489 129.

490  
491 Arghand, B. 1983. Introduction flies *Dacus* sp. and study it in the province Hormozgan. *Journal*  
492 *of Plant Pests and Diseases.*, **51(1)**: 9-3. (in Persian).

493



- 494 Barzkar, M., Goldasteh, SH., Eslamizadeh, R. and Usefi, B. 2017. Study on the population  
495 dynamics and spatial distribution of the cucurbit Fly; *Dacus ciliatus* Loew (Dip., Tephritidae).  
496 *J. Entomol. Res.*, **9(2)**: 131-142.
- 497  
498 Black, J. L.; Clark, M. K.; Sword, G. A. 2022. Physiological and transcriptional immune  
499 responses of a non-model arthropod to infection with different entomopathogenic groups. *PLoS*  
500 *ONE.*, **17**: e0263620.
- 501  
502 Boher, F., Trefault, N., Estay, S. E., Bozinovic, F., 2016. Ectotherms in variable thermal 521  
503 landscapes: A physiological evaluation of the invasive potential of fruit flies species. *Front.*  
504 *Physiol.*, **7**: 302.
- 505  
506 Browne, N., Surlis, C. and Kavanagh, K., 2014. Thermal and physical stresses induce a short-  
507 term immune priming effect in *Galleria mellonella* larvae. *J. Insect Physiol.*, **63**: 21-26.
- 508  
509 Bruno, D., Montali, A., Gariboldi, M., Wronska, A., Kaczmarek, A., Mohamed, A., Tian, L.,  
510 Casartelli, M. and Tettamanti, G. 2022. Morphofunctional characterization of hemocytes in  
511 black soldier fly larvae. *Insect Sci.*, 1-21.
- 512  
513 De Campos, M. R., Béarez, P., Amiens-Desneux, E., Ponti, L., Gutierrez, A. P., Biondi, A.,  
514 Adiga, A. and Desneux, N. 2020. Thermal biology of *Tuta absoluta*: demographic parameters  
515 and facultative diapause. *J Pest Sci.*, **94**: 829–842.
- 516  
517 Dyar, H. C., 1890. The number of molts of lepidopterous larvae. *Psyche*. **5**: 420-422.
- 518  
519 EPPO, 2018. EPPO Global Database (available online). <https://gd.eppo.int> [accessed on 23  
520 May 2018] EPPO/CABI (1997) Quarantine Pests for Europe, 2nd edn. (Eds Smith IM,  
521 McNamara.
- 522  
523 Foray, V., Desouhant, E. and Gibert, P. 2014. The impact of thermal fluctuations on reaction  
524 norms in specialist and generalist parasitic wasps. *Funct. Ecol.*, **28**: 411–423.
- 525  
526 Gábor, E., Cinege, G., Csordás, G., Rusvai, M., Honti, V. and Kolics, B. 2020. Identification of  
527 reference markers for characterizing honey bee (*Apis mellifera*) hemocyte classes. *DCI.*, **109**:  
528 103701.
- 529  
530 Gaikwad, P., Gupta, A., Waghmare, N., Mukhopadhyaya, R. and Nath, B. B. 2024. Hemocytes  
531 of a tropical midge *Chironomus ramosus* (Diptera: Chironomidae). *Int. J. Trop. Insect Sci.*, **44**:  
532 265-271.
- 533  
534 Ghasemi, V., Moharramipour, S., and Jalali Sendi, J., 2013. Circulating hemocytes of  
535 Mediterranean flour moth, *Ephestia kuehniella* Zell (Lep: Pyralidae) and their response to  
536 thermal stress. *ISJ.*, **10**:128-140.
- 537  
538 Go, M. S., Cho, Y., Park, K., Kim, M., Park, S., Park, J., 2022. Classification and  
539 characterization of immune haemocytes in the larvae of the Indian fritillary, *Papilio hyperbius*  
540 (Lepidoptera: Nymphalidae). *Eur. J. Entomol.* **119**: 430-438.
- 541

- 542 Hassan, G. M., El Aassar, M. R., and Khorchid, A. M. 2023. Implement of Some Biocontrol  
543 Tactics as an Innovative Management Against Cucurbit Fly, *Dacus ciliatus* and Western Flower  
544 Thrips, *Frankliniella occidentalis* on Squash Crop. *A J A S.*, **54 (2)**: 202-219.
- 545 Jones, J. C. 1962. Current concepts concerning insect hemocytes. *Am. Zool.*, **2**: 209-246.  
546
- 547 Herren, P., Hesketh, H., Dunn, A. M. and Meyling, N. V. 2023. Heat stress has immediate and  
548 persistent effects on immunity and development of *Tenebrio molitor*. *J. Insects Food Feed.*, 1-  
549 19.
- 550
- 551 Lee K. P., Simpson, S. J. and Wilson, K. 2008. Dietary protein-quality influences melanization  
552 and immune function in an insect. *Funct. Ecol.*, **22**: 1052-1061.
- 553
- 554 Li, L. T., Wang, Y. Q., Ma, J. F., Liu, L., Hao, Y. T., Dong, C., Gan, Y. J., Dong, Z. P. and Wang,  
555 Q. Y. 2013. The effects of temperature on the development of the moth *Athetis lepigone*, and a  
556 prediction of field occurrence. *J. Insect Sci.*, **13**: 103.
- 557
- 558 Lubawy, J. and Sticinska, M. 2020. Characterization of *Gromphadorhina coquereliana*  
559 hemolymph under cold stress. *Sci. Rep.*, **10**: 12076.
- 560
- 561 Mahmood, A., and Yousaf, M. 1985. Effect of some insecticides on the haemocytes of *Gryllus*  
562 *bimaculatus*. de Geer. *Pak. J. Zool.*, **17**: 71-84.
- 563
- 564 Mahmoud, A. A., 2016. Effect of temperature on the development and survival of immature  
565 stages of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Plant  
566 Protection Department, Faculty of Agriculture, South Valley University Qena, Egypt. African  
567 Journal of Agricultural Research. **11(36)**: 3375-3381.
- 568
- 569 Manjula, P., Lalitha, K. and Shivakumar, M. S., 2020. Diet composition has a differential effect  
570 on immune tolerance in insect larvae exposed to *Mesorhabditis belari*, *Enterobacter*  
571 *hormaechei* and its metabolites. *Exp. Parasit.* **208**: 1-7.
- 572
- 573 Mason, A. P., Smilanich, A. M. and Singer, M. S., 2014. Reduced consumption of protein-rich  
574 foods follows immune challenge in a polyphagous caterpillar. *J. Exp. Biol.* **217**: 2250-2260.  
575
- 576 Mohammad, A.K.H. 2022. Biological and Control Study of the Cucurbit Fruit Fly, *Dacus*  
577 *ciliatus* (Loew) (Diptera: Tephritidae). *Biochem. Cell. Arch.* **22 (1)** (Part II): 2923-2926.
- 578 tamiasva, R., Mbande, A., Nyamukondiwa, C. and Chidawanyika, F. 2023. Thermal adaptation  
579 in Lepidoptera under shifting environments: mechanisms, patterns, and consequences.  
580 *Phytoparasitica.*, **51**: 929-955.
- 581
- 582 Norrbom, A. L. and Uchoa, M. A. 2011. New species and records of *Anastrepha* (Diptera:  
583 Tephritidae) from Brazil. *Zootaxa.*, **2835**: 61-67.
- 584
- 585 Ouda, M. I., Mousa, E. A., M. and Fatina, b. 2022. Biological Study of Cucurbit Fruit Fly,  
586 *Dacus ciliatus* (Loew) on Constant Temperatures. *Egypt. Acad. J. Biolog. Sci.* **15(4)**:121-128
- 587 Pal, R. and Kumar, K. 2014. A comparative study of haemocytes in three cyclorrhaphous  
588 dipteran flies. *Int. J. Trop. Insect Sci.*, **34**: 207-216.
- 589

- 590 Pandey, J. P., Mishra, P. K., Kumar, D., Singh, B. M. K., and Prasad B. C. 2010. Effect of  
591 temperature on hemocytic immune responses of tropical tasar silkworm, *Antheraea mylitta*.  
592 *RJI.*, **3**: 169-177.  
593
- 594 Paydar, M., Moeini-Naghadeh, N., Jalilian, F. and Zamani, A.A. 2020. Comparative field study  
595 of various attractants of the pumpkin fruit fly, *Dacus ciliatus* (Diptera: Tephritidae) in  
596 Kermanshah. *Iran J Plant Prot Sci.* **51(2)**: 171-179.  
597
- 598 Pech, L. L., and Strand, M. R. 2000. Plasmacytes from the moth *Pseudoplusia includens*  
599 induce apoptosis of granular cells. *J Insect Physiol.*, **46**: 1565–1573.  
600
- 601 Pourali, Z., and Ajamhassani, M. 2018. The effect of thermal stresses on the immune system of  
602 the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *J. Entomol. Soc.*  
603 *Iran.*, **37**: 515-525. (In Persian with English Summary).  
604
- 605 Rowley, A. F., and Ratcliffe, N. A. A. 1978. histological study of wound healing and hemocyte  
606 function in the wax-moth *Galleria mellonella*. *J. Morphol.*, **157**: 181–199.  
607
- 608 Silva, J. E. B., Boleli, I. C. and Simoes, Z. L. P. 2002. Hemocyte types and total and differential  
609 counts in unparasitized and parasitized *Anastrepha obliqua* (Diptera, Tephritidae) larvae. *Braz.*  
610 *J. Biol.*, **62(4A)**: 689-699.  
611
- 612 Siva-Jothy, M., and Thompson, J. 2002. Short-term nutrient deprivation affects immune  
613 function. *Physiol. Entomol.*, **27(3)**: 206-212.  
614
- 615 Shapiro, M. 1979. Changes in hemocyte populations. In: Gupta A.P. (ed.), *Insect hemocytes*,  
616 Cambridge University Press, Cambridge, pp. 475-524.  
617
- 618 Strand, M. R. 2008. The insect cellular immune response. *Insect Sci.*, **15**: 1- 14  
619
- 620 Terra, W. R., Bianchi, A. G., and Lara, F. J. S. 1975. Physical properties and chemical  
621 composition of the haemolymph of *Rhynchosciara americana* (Diptera) larvae. *CBP.*, **47**: 117-  
622 129.  
623
- 624 Urbanski, A., Czarniewska, E., Baraniak, E. and Rosinski, G. 2017. Impact of cold on the  
625 immune system of burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Insect Sci.*,  
626 **24**: 443-454.  
627
- 628 Valadez-Lira, J. A., Gonzalez, J. M., Damas, G., Meja, G., Oppert, B., Padilla, C., and Guerra,  
629 P. 2011. Comparative evaluation of phenoloxidase activity in different larval stages of four  
630 lepidopteran after exposure to *Bacillus thuringiensis*. *J. Insect Sci.*, **12(80)**: 1-11.  
631
- 632 Valizadeh, B., Sendi, J., Khosravi, R. and Salehi, R. 2017. Establishment and characterizations  
633 of a new cell line from larval hemocytes of rose sawfly *Arge ochropus* (Hymenoptera: Argidae).  
634 *J. Entomol. Soc. Iran.*, **38(2)**: 173–186. (In Persian with English Summary).  
635
- 636 Vayssières, J. F., Carel, Y., Coubes, M. and Duyck, P. F. 2008. Development of immature stages  
637 and comparative demography of two cucurbit-attacking fruit flies in Reunion Island:

- 638 *Bactrocera cucurbitae* and *Dacus ciliatus* (Diptera: Tephritidae). *Environ. Entomol.* **37**: 307-  
639 314.
- 640
- 641 Vogel, M., Shah, P. N., Voulgari-Kokota, A., Maistrou, S., Aartsma, Y., Beukeboom, L.W.,  
642 Salles, J. F., Van Loon, J. J. A., Dicke, M. and Wertheim, B., 2022. Health of the black soldier  
643 fly and house fly under mass-rearing conditions: innate immunity and the role of the  
644 microbiome. *J. Insects Food Feed.*, **8**: 857-878.
- 645
- 646 Yamashita, M., and Iwabuchi, K. 2001. *Bombix mori* prohemocytes division and differentiation  
647 in individual microcultures. *J. Insect Physiol.*, **47**: 325- 331.
- 648
- 649 Yeager, J. F. 1945. The blood picture of the Southern armyworm (*Prodenia eridamin*). *J. Agric.*  
650 *Res.*, **71**: 1-40.
- 651
- 652 Zheng, J., Cheng, X., Hofmann, A. A., Zhang, B. O., and Ma, C. S. 2017. Are adult life history  
653 traits in oriental fruit moth affected by a mild pupal heat stress? *J. Insect Physiol.*, **102**: 36-41.
- 654
- 655 Zhu, Q., He, Y., Yao, J., Liu, Y., Tao, L. and Huang, Q. 2012. Effects of sublethal concentrations  
656 of the chitin synthesis inhibitor, hexaflumuron, on the development and hemolymph physiology  
657 of the cutworm, *Spodoptera litura*. *J. Insect Sci.*, **12(27)**: 1-13.

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

661 مریم عجم حسنی، محمد العلوی، و بیتا ولی زاده

662 چکیده

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

683