Journal of Insect Physiology 167 (2025) 104907

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Influence of thermal stress on the cellular immunity of *Galleria mellonella* F. (Lepidoptera: Pyralidae), and the biological traits of *Bracon hebetor* (Hymenoptera: Braconidae) in a host–parasitoid interaction: implications under climate change

Erinç Çelik Biçer *, Olga Sak, Aylin Er

Department of Biology, Faculty of Science-Literature, Balıkesir University, Balıkesir, Turkiye

ARTICLE INFO

Keywords:
Heat stress
The host-parasitoid relationship
Cellular immunity
Egg fecundity
Hatching time
Life-history traits

ABSTRACT

Extreme temperatures, the most evident indicator of climate change, threaten the delicate balance among parasitoids, key components of the ecosystem, and their agricultural pest hosts. Understanding the effects of thermal stress on parasitoids is essential for improving the mass production of *Bracon hebetor* (Hymenoptera: Braconidae), and predicting how the climate change will affect host-parasitoid relationship. The immune system of the host *Galleria mellonella* (Lepidoptera: Pyralidae) varied with both temperature and duration of exposure. Total hemocyte count peaked at 40 °C after 24 h due to elevated granulocyte, plasmatocyte, spherulocyte, and other cell types. The mitotic index peaked at 38 °C (24 h) before dropping sharply at 40 °C. Strong encapsulation responses rose significantly at 40 °C compared to 38 °C. Re-exposing adult females of *B. hebetor* to thermal stress, along with its previously heat-stressed hosts, altered biological traits in both adult females and F_1 offspring, especially the reduced egg fecundity. Rising temperatures initially reduced the female sex ratio, but ultimately it nearly balanced out. At 36 °C, 95 % of the 24 eggs per female developed into adults, and prolonged adult longevity increased parasitoid numbers and extended their active period, a key finding. Overall, *B. hebetor* displayed high thermal tolerance, yet temperature-driven changes in host immunity and parasitoid traits may reshape their interactions under future climates.

1. Introduction

Climate change, with its rapidly escalating impacts, has become an undeniable reality in today's world (IPCC, 2022). Among its most evident consequences are the rise in average global temperatures and the heightened frequency of extreme weather events (Cracknell and Varotsos, 2021; Seneviratne et al., 2021). Temperature as one of the most critical ecological factors for ectothermic organisms, with the ability to withstand temperature fluctuations being vital for insect survival (Overgaard and Sørensen, 2008; Colinet et al., 2015). Rising temperatures, driven by climate change and global warming, pose a serious threat to the survival of parasitoids, which are among nature's key biological control agents (Jeffs and Lewis, 2013; Moiroux et al., 2014; Zhang et al., 2016). Parasitoids are known to be more vulnerable to climate change and other environmental challenges compared to most insects, largely due to their specific position within the food chain (Hance et al., 2007; Jeffs and Lewis, 2013). High temperatures, as a significant stressor, can impact parasitoid species not only directly but also indirectly through their hosts (Hance et al., 2007). Moreover, the effects of global warming on the host-parasitoid interaction become increasingly intricate when coupled with other environmental shifts, such as elevated levels of carbon dioxide and nitrogen, as well as the presence of competitive, mutualistic, or antagonistic species in the ecosystem (Jeffs and Lewis, 2013; Hance et al., 2007).

It is impossible for all individuals within a community to respond to climate change in the same way. Parasitoids are generally expected to react to climate warming in three main ways: (1) shifting their distribution to cooler environments, (2) altering their phenology, and (3) staying in place through phenotypic plasticity or evolutionary adaptation (Jeffs and Lewis, 2013). These varied responses inevitably lead to the reorganization of existing communities (Hance et al., 2007). To predict future species distributions, climate change scenarios often

---

* Corresponding author.
E-mail addresses: erinc.celik@baun.edu.tr (E. Çelik Biçer), altun@balikesir.edu.tr (O. Sak), asahin@balikesir.edu.tr (A. Er).

https://doi.org/10.1016/j.jinsphys.2025.104907
Received 7 September 2025; Received in revised form 24 October 2025; Accepted 12 November 2025

consider the dispersal capabilities of species within their physiological limits (Klok et al., 2003; Hance et al., 2007; Jeffs and Lewis, 2013). The phenological synchrony between hosts and parasitoids is critically important for maintaining ecological balance (Jeffs and Lewis, 2013). In examining the adaptation of host-parasitoid relationships to rising temperatures, the physiological impacts of high temperatures must be assessed holistically within the ecosystem. Ultimately, any change in a member of a co-evolved group of organisms will inevitably influence all other members. If host-parasitoid systems fail to adapt to global climate change, they will inevitably face extinction over time.

Insects have developed both behavioral responses and physiological adaptation strategies to cope with adverse environmental conditions such as high temperatures. Among these physiological responses, developmental arrest mechanisms like dormancy (diapause or quiescence) may also occur in certain species (Danks, 1987; Hance et al., 2007). For parasitoid insects, diapause is highly influenced by the conditions of the host, as it typically occurs within the host or its cocoon (Hance et al., 2007). Furthermore, temperature impacts the functional response of parasitoid species during their host-seeking process, altering parasitism rates and ultimately disrupting the host-parasitoid relationship (Hance et al., 2007; Jeffs and Lewis, 2013; Joodaki et al., 2018). Parasitism rates are also influenced by the parasitoids' ability to locate hosts successfully, select suitable hosts, lay eggs, and ensure that the deposited eggs or larvae can overcome or evade the host's immune defenses (Jeffs and Lewis, 2013). Parasitoid wasps must successfully suppress their host's immune system in order to complete their development and survive (Gupta and Ferkovich, 1998). The primary line of defense in host insects against parasitoids and other foreign invaders is the cellular immune system (Schmidt et al., 2001; Lavine and Strand, 2002). Since both low and high temperatures act as stressors for insects, hemocyte counts may vary directly in response to temperature changes (Ghasemi et al., 2013). Several studies have shown that thermal stress can affect the total hemocyte count (Nittono, 1960; Kiuchi et al., 2008), the distribution of different hemocyte types (Pandey et al., 2010; Ghasemi et al., 2013), and the number of mitotic cells (Bloemkolk et al., 1992; Ghasemi et al., 2013). Some studies have shown that the encapsulation response, a cellular defense mechanism, can be affected by thermal stress (Lynn and Vinson, 1977; Seehausen et al., 2017; 2018). However, these effects on cellular immunity may vary, showing either increases or decreases depending on the insect species, temperature intensity, and duration of exposure. Therefore, considering that different species may have different adaptive capacities, how insect immune systems respond to climate change remains a topic that requires detailed investigation.

Tritrophic interactions exist among ecosystem components such as plants, herbivorous insects, and their natural enemies (predators, parasitoids, and pathogens). Temperature variations driven by climate change can affect the biology of each species within the ecosystem differently, potentially leading to the extinction of certain species and causing unexpected changes in these tritrophic interactions. In this context, parasitoid species are organisms for which significant impacts are expected, as they represent the third and fourth trophic levels (Hance et al., 2007). Rising temperatures associated with climate change are expected to disrupt the natural balance between parasitoids, organisms of critical ecological importance, and their hosts. This disruption will, in turn, negatively impact the success of biological control and Integrated Pest Management (IPM) programs (Collier, 1995; Uçkan and Gülel, 2002; Alam et al., 2014). In our study, *Bracon hebetor* (Hymenoptera: Braconidae), a larval ectoparasitoid, was used along with the last (final) instar larvae (7th instar, according to Pietrykowska-Tudruj et al., 2025) of *Galleria mellonella* (Lepidoptera: Pyralidae) as the host within the framework of the host-parasitoid relationship. *B. hebetor* is a prominent biological control agent employed against pests in the order Lepidoptera due to its rapid life cycle, efficient biological development, and ease of mass rearing (Keever et al., 1985; Prozell and Schöller, 1998; Alam et al., 2014). *G. mellonella* is one of the most common pests of stored bee products and honeycombs (Ellis et al., 2013). Although it mainly damages stored combs by tunneling through the wax, it can also infest weak or poorly maintained colonies, posing a potential risk under certain conditions (Arifie et al., 2024). Effective management of this pest is crucial to prevent economic losses in apiculture, and biological control using parasitoids such as *B. hebetor* provides an environmentally friendly alternative to chemical insecticides. There are studies in the literature examining how the host-parasitoid relationship changes in response to thermal stress (Hance et al., 2007; Jeffs and Lewis, 2013; Joodaki et al., 2018). Additionally, although some studies have examined the relationship between various species of the order Lepidoptera and *B. hebetor* under high-temperature conditions (Payne, 1934; Singh et al., 2014; Abbes et al., 2015; Noor-ul-Ane et al., 2018; Wu et al., 2022), *G. mellonella* has been used as a host in only a few of these studies (Forouzan et al., 2008; Montazeri et al., 2019). Although the effects of climate change on insect immunity have been examined in various studies, the fact that each insect species possesses distinct physiological and immune responses highlights the importance of investigating a broader range of species before drawing general conclusions. Therefore, in this study, the potential effects of thermal stress on both host cellular immunity and the host-parasitoid relationship were thoroughly investigated. Total and differential hemocyte counts (THC and FHC), mitotic index (MI), and encapsulation response were assessed as the immunity parameters of the host species, *G. mellonella*. Subsequently, we determined both the parasitoid's response to its host exposed to specific temperatures and the potential changes in some biological traits (egg fecundity and hatching time, larval spinning time, pupal time, pupal period, adult emergence time, adult sex ratio, and adult longevity) of the first-generation parasitoids when exposed to heat along with their host. The findings obtained in this study are expected to contribute to the existing literature on the potential effects of climate change on the immune system of agricultural pest species. Furthermore, the study may help anticipate how similar host–parasitoid relationships could be altered under changing climatic conditions. Overall, our results are anticipated to provide valuable insights for research on insect physiology, mass-rearing practices, and biological control strategies in the context of climate change.

2. Materials and methods

2.1. Insects

Laboratory cultures of the host species, *G. mellonella* were established from individuals collected from several beehives near Balıkesir, while the source of the parasitoid, *B. hebetor* was Trimail Biological Agriculture of Ankara University's Teknokent. Host stock cultures were maintained at 30 ± 1 °C and 60 ± 5 % relative humidity, with a photoperiod of 12:12 h (L:D) in Animal Physiology Laboratory at Balıkesir University, Balıkesir, Turkey. Host insects were kept in 250 mL jars and fed with natural blackened honeycomb. *B. hebetor* were reared on the last (final) instar (7th instar) larvae of *G. mellonella* at 25 ± 2 °C, 50 ± 5 % RH and a photoperiod of 16:8 h (L:D). Adult parasitoids were fed with cotton balls soaked in 50 % (v/v) honey solution.

2.2. Treatment and Bioassays

2.2.1. Heat exposure

The effects of thermal stress on the cellular immunity of the host *G. mellonella* and on certain biological traits of the parasitoid *B. hebetor* within the host-parasitoid relationship were investigated in two different experimental designs. For the immunity and host-parasitoid interaction experiments, the 7th instar larvae of *G. mellonella* were collected manually from the stock culture and weighed to an accuracy of 1×10^{-4} g to ensure that all weighed 260 ± 25 mg. The species identification of *G. mellonella* was confirmed based on the morphological characteristics of larvae, pupae, and adults, including head capsule width, pronotum color and the presence of an ecdysial line, body shape, the presence of club-shaped setae, the arrangement of crochets on the prolegs, and the color of dorsal sclerites, following the diagnostic keys provided by Smith (1965) and Pietrykowska-Tudruj et al. (2025). The larvae were then individually placed in 60 × 15 mm plastic Petri dishes and prepared for thermal exposure. Different temperature points were selected for the immunity (30, 38, 40, and 42 °C) and the host-parasitoid interaction (30, 36, 38, and 40 °C) experiments, which were determined based on variable heat shock exposures (30, 32, 34, 36, 38, 40, 42, and 44 °C) in our previous study (Çelik Biçer et al., 2025). These differences were necessary because the thermal tolerance limits of *G. mellonella* larvae and *B. hebetor* females differ. For the immune assays, higher temperatures (30, 38, 40, and 42 °C) were chosen based on larval heat tolerance and developmental thresholds determined in our previous study (Çelik Biçer et al., 2025), whereas lower temperature points (30, 36, 38, and 40 °C) were used for the host-parasitoid experiments to ensure parasitoid survival and reliable interaction assessments. For immune parameters (THC, DHC and MI, and encapsulation response), 192 larvae (4 larvae × 4 replicates × 4 temperatures × 3 exposure times) were used, resulting in a total of 576 larvae exposed to heat treatments. In the host-parasitoid relationship assays, 192 host larvae and 192 mated female parasitoids (4 hosts or parasitoids × 4 replicates × 4 temperatures × 3 exposure times) were similarly exposed to heat before behavioral and biological trait evaluations.

2.2.2. *Immune assay*

The temperature selection was guided by the thermal stress responses of 7th instars, LTemp values, and temperature-dependent changes in biological traits (Çelik Biçer et al., 2025). Accordingly, the control temperature of 30 °C was included as the initial temperature, from which higher temperatures were subsequently applied. The other selected temperatures were the highest temperature at which larvae could successfully pupate under constant heat shock (38 °C), the first temperature at which pupation failed (40 °C), and the temperature closest to the LTemp_{50} value (42 °C). As a result, 7th instar larvae of *G. mellonella* were exposed to thermal stress for 2, 24, and 48 h at four different temperatures (30, 38, 40, and 42 °C). Each immune parameter (THC, DHC, MI, and encapsulation response) was tested in four replicates with a total of 16 larvae (four per replicate).

2.2.2.1. Total and differential hemocyte counts and mitotic index. Hemolymph samples were taken from 7th instar larvae of *G. mellonella* after 2, 24, and 48 h to examine the effects of thermal stress on THC in circulation, a measure of immunity. For this purpose, the larvae were punctured with a sterile 19-gaugle needle above their first right hind legs, and 4 µL of hemolymph was collected from each insect using a microcapillary tube (Sigma). The obtained hemolymph sample was transferred to 0.5 mL Eppendorf tubes containing 36 µL of anticoagulant buffer (0.098 M NaOH, 0.186 M NaCl, 0.017 M Na_2EDTA, and 0.041 M citric acid, pH = 4.5), which were kept in an ice-cold environment, and gently mixed with an automatic pipette. In this way, 10 µL was taken from the 1:10 diluted cell suspension with the buffer using an Eppendorf brand automatic pipette (0.5–10 µL) and loaded onto a Neubauer hemocytometer (Improved Neubauer Hemocytometer; Superior, Germany) with a depth of 0.100 mm, and the cells were counted under an Olympus BX51 (Japan) brand microscope.

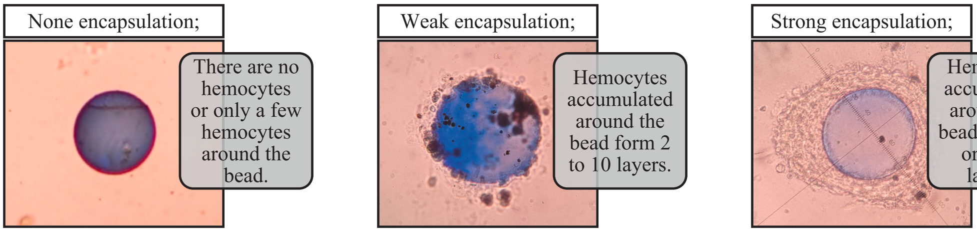
In order to investigate the effects of thermal stress on DHC (another measure of immunity), 7th instar larvae were punctured at the same site as in THC experiment and 5 µL of hemolymph was collected from each insect after 2, 24, and 48 h. The collected hemolymph was immediately spread on sterile slides and left to dry at room temperature to allow the hemocytes to adhere to the glass. After ensuring that the hemocytes were dry, 0.2 mL of methanol (Sigma) was added onto the dried slides, which were placed in a horizontal position, to fix the cells, and the preparations were left at room temperature for another 10 min. At the end of this period, the excess methanol was drained from the slides and the preparations were left to dry again. The fixed hemocytes were stained by immersing the slides freshly prepared Giemsa dye solution (57 mL PBS + 3 mL Giemsa dye (Merck, Giemsa's Azure Eosine Methylene Blue Solution, pH = 7.4)) for 15 min during each staining procedure. The stained slides were then removed from the dye solution one by one, first rinsed with distilled water and then with PBS, and left to dry in a vertical position for 24 h. To determine DHC, the dried preparations were examined under an Olympus BX51 microscope, and cell differences were determined according to the study by Er et al. (2010). While determining DHC, the number of hemocytes in the mitotic phase was also assessed. DHC and the MI data in the preparations made using 5 µL of hemolymph from each 7th instar larva were evaluated as percentage.

2.2.2.2. Encapsulation. The effect of thermal stress on the encapsulation behavior of hemocytes, an immune parameter formed by hemocytes, was investigated in *G. mellonella*. Sephadex DEAE A-25 (Sigma) beads (40–120 µm in diameter) were used to assess encapsulation levels. In addition, a 50 µL microsyringe with a fixed-tip and a 25-gauge needle was used during the injection of the bead solution into the 7th instars. Before injection, beads were stained blue in a 1 % Coomassie blue solution (Brilliant Blue G, Sigma) prepared in PBS (Richards and Edwards, 2002). To ensure proper staining, beads were incubated for one hour. After staining, the supernatant containing the dye-PBS mixture was removed from the stained bead sediment, and PBS was added to wash the beads (Richards and Edwards, 2002). Then, 10 µL of Sephadex A-25 stock bead solution (containing 7–9 beads) was withdrawn with a microsyringe and injected into the 7th instar larvae. After injection, the larvae were immediately placed in pre-heated incubators (30, 38, 40, and 42 °C) and exposed to these temperatures for 2, 24, and 48 h. At the end of these periods, the larvae were removed from the incubators and immediately dissected under an Olympus SZ61 stereomicroscope at 40x magnification. The recovered beads were transferred onto separate slides with a drop of PBS and then covered with a coverslip. These preparations were subsequently examined under an Olympus BX51 microscope to determine the encapsulation levels of hemocytes. The encapsulation response was categorized into three levels based on the hemocyte layers surrounding the beads: absent (no or minimal hemocyte accumulation around the bead), weak (formation of 2–10 hemocyte layers), and strong (formation of 10 or more hemocyte layers) (Fig. 1).

2.2.3. *The host-parasitoid relationship assay*

The thermal stress was applied first to the host alone and then to the host-parasitoid pairs in two successive phases to investigate its potential effects on *G. mellonella-B. hebetor* relationship. When selecting four different temperatures, priority was given to the survival of *B. hebetor* females at high temperatures, starting with the highest value of 44 °C, and then gradually lowering the temperatures to determine the threshold at which survival was no longer possible. Consequently, the three highest temperatures at which *B. hebetor* survived (36, 38, and 40 °C) were selected for use in experiments investigating the possible effects of thermal stress on the *G. mellonella-B. hebetor* relationship. A control temperature of 30 °C was also included in the experiments.

In the first phase of the experiment, *G. mellonella* 7th instars of similar weight (260 ± 25 mg) placed individually into the plastic Petri dishes and exposed to variable heat shock in pre-set ovens at four different temperatures (30, 36, 38, and 40 °C) for 2 h. During this time, a pair of male and female *B. hebetor* were placed in another plastic Petri dish to mate and were fed cotton balls with honey. The sex of *B. hebetor* adults was determined based on external morphological characteristics under a stereomicroscope (Olympus SZ61). Females were identified by their larger body size and the presence of an ovipositor, whereas males were smaller and lacked an ovipositor (Alam et al., 2014). The host larvae, which were removed from the oven and placed with mated female parasitoids, were kept in the same oven for a further 2 h. Thus, in


None encapsulation;

There are no hemocytes or only a few hemocytes around the bead.

Weak encapsulation;

Hemocytes accumulated around the bead form 2 to 10 layers.

Strong encapsulation;

Hemocytes accumulated around the bead form 10 or more layers.

Fig. 1. Encapsulation stages in 7th instar larvae of *G. mellonella*.

the second phase of the experiment, the host was exposed to thermal stress for 4 h, while the parasitoid was exposed for 2 h. This experimental design aimed to assess both the direct effects of heat on the parasitoid and the indirect effects mediated through the host. To achieve this, the host larvae were first exposed to heat alone for 2 h to induce physiological stress responses and then kept for an additional 2 h together with the parasitoid under the same thermal conditions. This two-phase exposure allowed us to evaluate (1) how the parasitoid responds to a heat-stressed host, and (2) how simultaneous exposure to heat affects the host-parasitoid interaction. This two-phase exposure design, which allowed us to distinguish the direct and indirect thermal effects on the host-parasitoid relationship, was inspired by approaches used in previous studies (Ma et al., 2021; Costaz et al., 2022). At the end of the period, the *G. mellonella*-*B. hebetor* Petri dish was removed from the oven and placed in the same environment as the *B. hebetor* stock cultures. The female parasitoid was monitored daily at the same time until death to determine how thermal stress affects some biological parameters (egg fecundity and hatching time, larval spinning time, pupal time, pupal period, adult emergence time, adult sex ratio, and adult longevity) of *B. hebetor* in the host-parasitoid relationship. For unmated adult longevity, the offspring from the heat-exposed female (both males and females) were observed together, and their longevity was analyzed as combined data. All experiments were conducted in four replicates with four host larvae and four female parasitoids randomly selected from different populations at different times (16 host-parasitoid pairs for each tested group).

2.2.3.1. Egg fecundity and hatching time. The plastic Petri dishes containing *G. mellonella*-*B. hebetor* pairs exposed to thermal stress in two successive phases were checked at the same time each day. The eggs laid by the female on the host until death were counted daily under a microscope (Olympus CX21, Japan) and the total number of eggs (fecundity) was determined. To avoid recounting the eggs, the Petri dishes were marked with colored acetate pens and numbered. The hatching time (day) was recorded as the time elapsed between the day each egg was numbered and the day it was broken.

2.2.3.2. Life history traits of first-generation offspring. First-generation (F_1) *B. hebetor* individuals from the *G. mellonella*-*B. hebetor* pairs exposed to thermal stress in plastic Petri dishes were observed daily to assess changes in the larval spinning time, pupal time, pupal period, and adult emergence time. Initially, *B. hebetor* larvae hatched from the numbered eggs in the Petri dishes were allowed some time to move and attach to the surface of the dish. The duration from the day the larvae attached to the surface to the day they formed a complete cocoon was then recorded as the larval spinning time (day). The time between the day the larvae cocooned and attached to a point and the day they became pupae was recorded as the pupal time (day). The elapsed time from the pupation to the emergence of the adult was considered as the pupal period (day). Finally, the time from the day each egg was laid on the host to the day F_1 adults emerged was recorded as the adult emergence time (day).

The number of F_1 *B. hebetor* adults obtained from the eggs laid on the host was recorded to calculate the adult ratio. The sex of these adults was also identified and the percentage of females and males was calculated. The sexually identified adults were individually placed in plastic Petri dishes and were fed cotton balls with honey. The elapsed time from adult emergence until death was recorded as the unmated adult longevity.

2.3. Statistical analysis

The effects of thermal stress on the immune and the biological parameters of the insects were tested using one-way analysis of variance (ANOVA). Tukey's post hoc (HSD) test was applied for multiple comparisons based on variance homogeneity. Data for THC, DHC, and MI also were subjected to two-way ANOVA to determine the main effects of temperature, time, and their interaction on these values. SPSS software program (version 18.0 for Windows, SPSS Science, Chicago, IL) was used for data analysis. The results were considered statistically significant when $P < 0.05$.

3. Results

3.1. Cellular immune parameters of host

3.1.1. Total hemocyte counts

The impact of different temperatures and experimental time points on THC in *G. mellonella* 7th instars is shown in Fig. 2. When the changes in THC were evaluated based on the experimental observation hours, the highest hemocyte count was at the end of the second hour at 30 °C and THC decreased significantly as the temperature exposure time increased. At 38 and 40 °C, the highest THC was recorded at 24 h, with markedly lower hemocyte counts at both 2 h and 48 h. THC peaked at 42 °C after 48 h, and this increase was significant compared to 2 and 24 h. Statistical comparisons of the temperature treatments at each observation time revealed significant variations in THC at 2 h ($F = 32.418$; $df = 3, 60$; $P = 0.000$), 24 h ($F = 7.618$; $df = 3, 60$; $P = 0.000$), and 48 h ($F = 14.042$; $df = 3, 60$; $P = 0.000$). At 2 h, hemocyte counts were considerably lower at all other temperatures compared to 30 °C; moreover, the increment observed at 42 °C was also important compared to 38 and 40 °C. After 24 h of exposure, THC at 40 °C was significantly higher than at 38 °C and 42 °C, whereas at 48 h, levels at 42 °C were significantly higher than at all other temperatures (Fig. 2). The effect of thermal stress on larval hemocyte counts was temperature ($P = 0.000$) and time ($P = 0.000$) dependent, and the relationship between temperature and THC was significantly influenced by the time ($P = 0.000$) (Table 1).

3.1.2. Differential hemocyte counts

The impact of different temperatures and experimental time points on differential hemocyte type ratios in *G. mellonella* 7th instars is shown in Fig. 3. In terms of morphlogy, *G. mellonella* hemocytes were classified into granulocytes, plasmatocytes, spherulocyte, and other cell types (oenocytoids and prohemocytes) (Fig. 4). Evaluating granulocyte (GR) ratios across different observation times in all temperature applications revealed that the GR value decreased significantly at 48 h compared to 2 h for only 38 °C. Comparative assessment of GR ratios revealed insignificant differences among temperature treatments at 2 h ($F = 2.840$; $df = 3, 60$; $P = 0.045$) and 24 h ($F = 1.622$; $df = 3, 60$; $P = 0.194$) except for

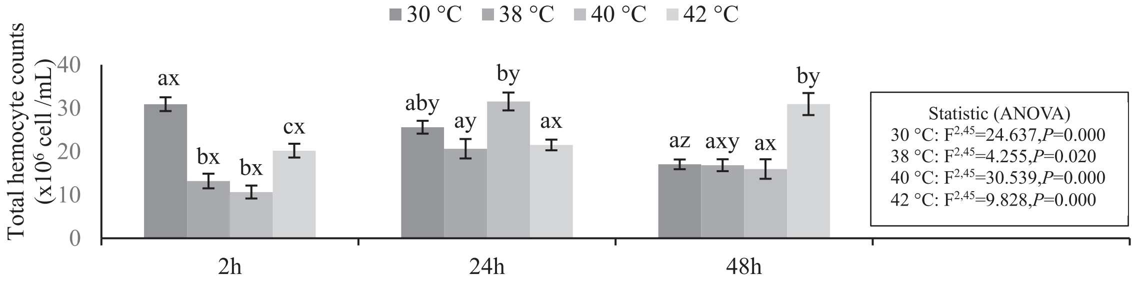


Fig. 2. Total hemocyte counts (×10^6 cells/mL) of *G. mellonella* larvae exposed to different temperature treatments. Temperatures (a–b) and exposure times (x–y) labeled with the same lowercase letter are not significantly different ($P<0.05$; Tukey's HSD test). Data are average of four replicates. Statistic (ANOVA) (F, df, and P values) refer to comparisons among exposure times within each temperature.

Table 1
ANOVAs of the effects of thermal stress, time, and their interactions on the total hemocyte counts in *G. mellonella* larvae.

Source	df	MS	F	P	r^2
Temp.	3	670.602	13.320	0.000	
Time	2	644.176	12.795	0.000	0.50
Temp.×Time	6	930.951	18.491	0.000	
Error	180	50.346			

48 h (F = 3.187; df = 3, 60; P = 0.030). The highest GR value was at 40 °C for 48 h and this increase was only significant compared to 42 °C (Fig. 3a).

Analysis of plasmatocyte (PL) ratios in 7th instars revealed that time-dependent changes were not statistically important at 30, 38, and 40 °C. At the highest experimental temperature of 42 °C, only the increment at 2 and 48 h was important compared to 24 h. Significant increases and decreases in PL ratios among temperature treatments were observed at 2 h (F = 3.406; df = 3, 60; P = 0.023) and 48 h (F = 6.037; df = 3, 60; P = 0.001) except for 24h (F = 0.472; df = 3, 60; P = 0.703). After 2 h of exposure, the highest PL value was observed at 42 °C, and this increase was statistically important only compared to 40 °C. Although PL numbers changed insignificantly with temperature at 24 h, the increase at 42 °C was significant compared to 30 and 38 °C at 48 h (Fig. 3b).

Evaluation of spherulocyte (SP) ratios over time showed an increase at 30 and 38 °C with an extended exposure duration, a time-dependent decrease at 40 °C, and fluctuating values at 42 °C. The lowest SP count (11.59 ± 2.01) was recorded at 30 °C after 2 h at all observation times, and this decrease was significant according to 24 and 48 h. At 38 and 40 °C, the changes in SP ratios were not statistically important, whereas at 42 °C, only the increase at 24 h compared to 2 h was significant (Fig. 3c). Comparative analysis of SP ratios revealed insignificant differences among temperature treatments at 24 h (F = 1.048; df = 3, 60; P = 0.378) and 48 h (F = 2.227; df = 3, 60; P = 0.094), except for 2 h (F = 4.952; df = 3, 60; P = 0.004). A significant effect on SP ratio was detected only after 2 h of exposure, with the highest value recorded at 40 °C relative to all other temperature groups (Fig. 3c). When the changes in other cell types (OCT) were evaluated according to the hours of observation, the percentages remained almost constant at all temperatures except 38 °C with only a significant increase at 48 h compared to 2 h. Although some fluctuations were observed in the ratios of OCT across temperature treatments at 2 h (F = 0.673; df = 3, 60; P = 0.572), 24 h (F = 2.014; df = 3, 60; P = 0.121), and 48 h (F = 1.886; df = 3, 60; P = 0.142), none were statistically important (Fig. 3d).

Two-way ANOVA results showed that DHC (GR, PL, SP, and OCT) in *G. mellonella* exhibited distinct responses to heat stress, depending on both cell type and experimental factor (temperature or time). GR (P = 0.021) and PL (P = 0.001) values varied with temperature but were not time-dependent (GR: P = 0.118; PL: P = 0.075). Moreover, the temperature-induced variation in PL was considerably affected by the time (P = 0.044) but not in GR (P = 0.055). The effect of heat stress on SP (P = 0.020) and OCT (P = 0.043) depended on time but not temperature (SP: P = 0.559; OCT: P = 0.214). Additionally, the relationship between temperature and SP was significantly influenced by the time (P = 0.002) but not in OCT (P = 0.096) (Table 2).

3.1.3. *Mitotic index*

Analysis of mitotic index (MI) in 7th instars across different time points revealed that 30 °C caused a significant decrease only at 48 h compared to 2 h, while at 40 and 42 °C, the decreases at 24 and 48 h compared to 2 h were also significant. Furthermore, the decline observed at 48 h compared to 2 and 24 h for 38 °C was also important (Fig. 5). Comparison of MI values across temperature treatments at each time point revealed statistically important differences at 2 h (F = 4.479; df = 3, 60; P = 0.007) and 24 h (F = 18.394; df = 3, 60; P = 0.000), but not at 48 h (F = 2.348; df = 3, 60; P = 0.082). At the end of the second hour, MI ratios were significantly lower only at 42 °C compared to 30 and 38 °C, and significantly lower at both 40 and 42 °C after 24 h (Fig. 5). The MI rates of 7th instars *G. mellonella* varied considerably depending on both temperature (P = 0.000) and time (P = 0.000), and the relationship between temperature and MI was significantly influenced by the time (P = 0.001) (Table 2).

3.1.4. *Encapsulation*

The impact of different temperatures and experimental times on the three levels of bead encapsulation in 7th instar *G. mellonella* larvae are shown in Fig. 6. Comparisons of temperature treatments following bead injection revealed insignificant variation in the number of unencapsulated beads at 24 h (F = 1.900; df = 3, 60; P = 0.139) and 48 h (F = 0.455; df = 3, 60; P = 0.715) except for 2 h (F = 4.505; df = 3, 60; P = 0.006). At 2 h, the increment in unencapsulated bead numbers was statistically important only at 40 °C compared to 30 °C. Comparative analysis of weakly encapsulated bead ratios in 7th instar *G. mellonella* larvae across different temperature treatments revealed significant fluctuations after 2 h (F = 4.742; df = 3, 60; P = 0.005), 24 h (F = 3.346; df = 3, 60; P = 0.025), and 48 h (F = 6.511; df = 3, 60; P = 0.001) (Fig. 6). The number of weakly encapsulated beads considerably reduced after 2 h only at 42 °C compared to 30 °C, and after 24 h only at 42 °C compared to 38 °C. Moreover, the increase at 38 °C compared to 30 and 40 °C after 48 h was also important. Comparisons of strongly encapsulated bead ratios among temperature treatments revealed considerable variations at 2 h (F = 5.237; df = 3, 60; P = 0.003), 24 h (F = 3.065; df = 3, 60; P = 0.035), and 48 h (F = 4.530; df = 3, 60; P = 0.006) (Fig. 6). The percentage of strongly encapsulated beads significantly increased only at 42 °C compared to 40 °C after 2 h and only at 40 °C compared to 38 °C after 24 h, whereas decreased only at 38 °C compared to 30 °C after 48 h.

3.2. *Host-parasitoid interaction*

3.2.1. *Egg fecundity and hatching time under thermal stress*

The total number of eggs laid by female *B. hebetor* exposed to thermal

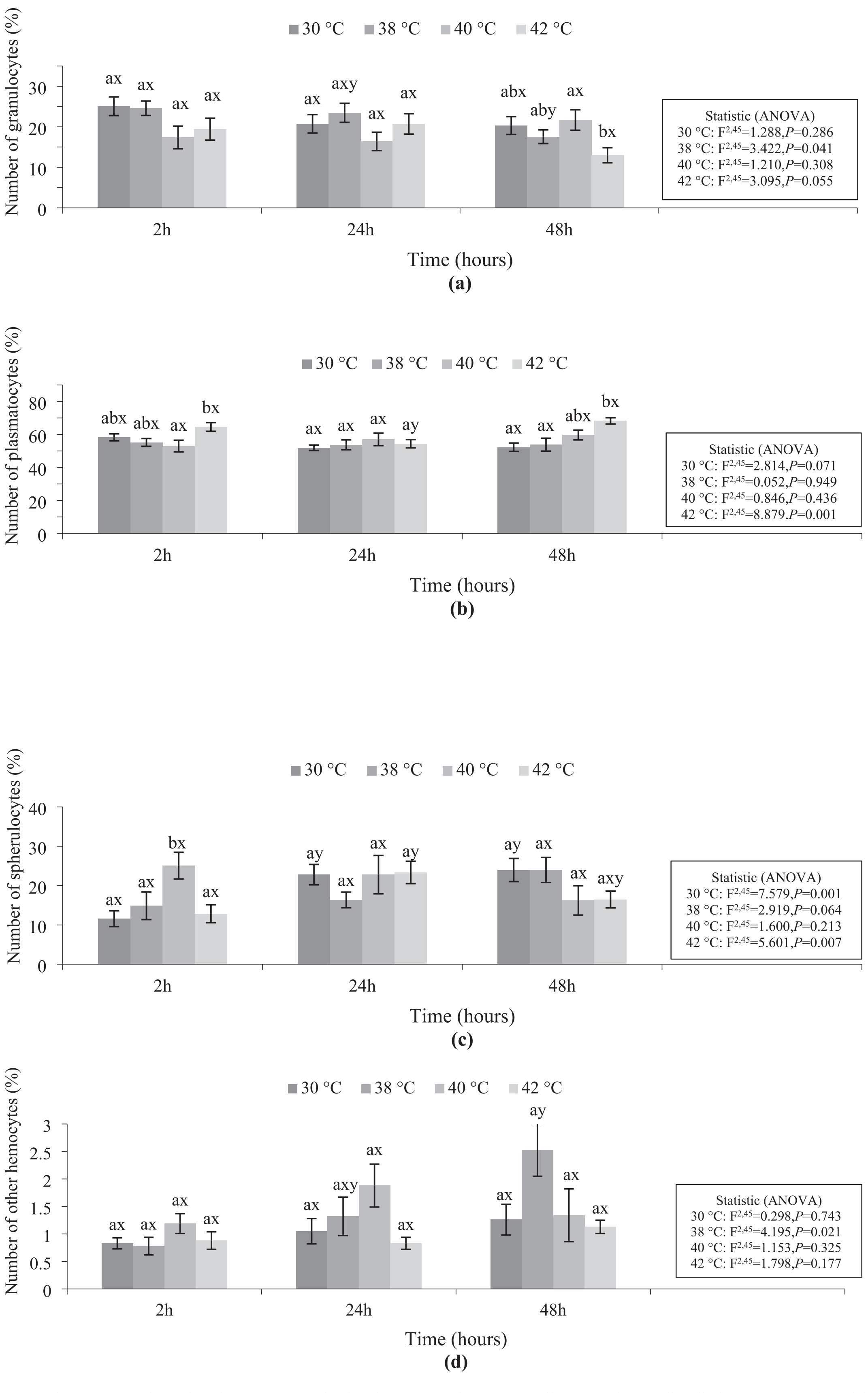


Fig. 3. Granulocyte (a), plasmatocyte (b), spherulocytes (c), and other hemocyte (d) counts (cells/100) of *G. mellonella* larvae exposed to different temperature treatments. Temperatures (a-b) and exposure times (x-y) labeled with the same lowercase letter are not significantly different ($P < 0.05$; Tukey's HSD test). Data are average of four replicates. Statistic (ANOVA) (F, df, and P values) refer to comparisons among exposure times within each temperature.

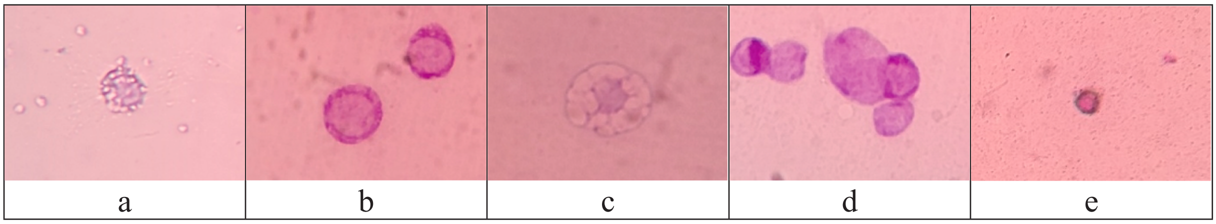


Fig. 4. Hemocyte type of *G. mellonella*; a) Granulocytes, b) Plasmatocytes, c) Spherulocytes, d) Oenocytoid, and e) Prohemocyte.

Table 2
ANOVAs of the effects of thermal stress, time, and their interactions on the differential hemocyte counts in *G. mellonella* larvae.

Hemocyte type	Source	df	MS	F	P	r^2
Granulocytes	Temp.	3	0.047	3.328	0.021	
	Time	2	0.031	2.163	0.118	0.13
	Temp.× Time	6	0.030	2.107	0.055	
	Error	180	0.014			
Plasmatocytes	Temp.	3	0.078	5.600	0.001	
	Time	2	0.037	2.628	0.075	0.16
	Temp.× Time	6	0.031	2.207	0.044	
	Error	180	0.014			
Spherulocytes	Temp.	3	0.016	0.690	0.559	
	Time	2	0.093	4.019	0.020	0.15
	Temp.× Time	6	0.083	3.596	0.002	
	Error	180	0.023			
Others	Temp.	3	0.003	1.507	0.214	
	Time	2	0.007	3.211	0.043	0.11
	Temp.× Time	6	0.004	1.827	0.096	
	Error	180	0.002			
Mitotic index	Temp.	3	0.056	15.355	0.000	
	Time	2	0.090	24.785	0.000	0.40
	Temp.× Time	6	0.015	4.219	0.001	
	Error	180	0.004			

stress within the host-parasitoid relationship revealed a significant decrease in fecundity at 38 and 40 °C compared to 30 and 36 °C (F = 8.414; df = 3, 60; P = 0.000; Fig. 7a). The hatching time of the eggs was similar at 30 and 40 °C, while the reduction at 36 and 38 °C was significant compared to these temperatures. The extension at 38 °C compared to 36 °C was also important (F = 207.054; df = 3, 1096; P = 0.000; Fig. 7b).

3.2.2. *Impact of thermal stress on F_1 offspring*

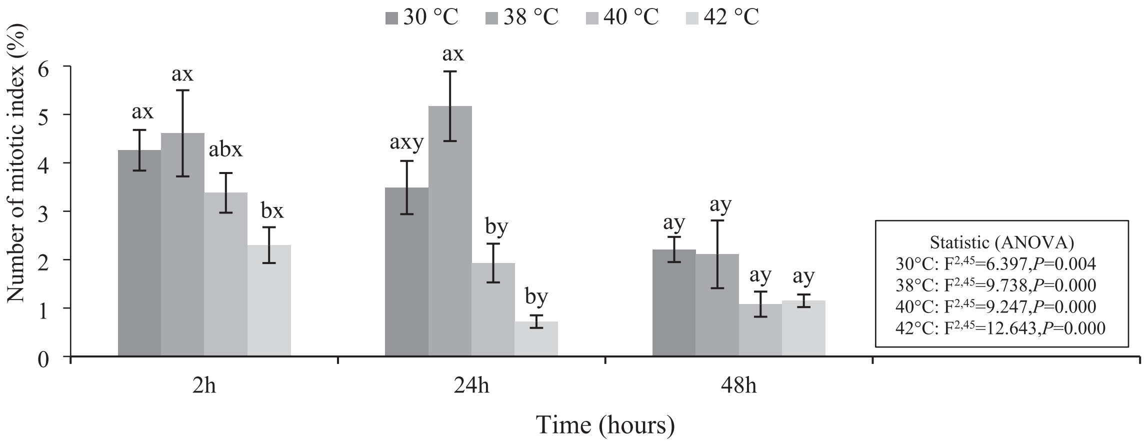
The effect of heat on the host-parasitoid relationship induced fluctuations in the larval spinning time of F_1 *B. hebetor* larvae (Fig. 8). Statistically, the larval spinning time was significantly prolonged at 38 °C compared to 30 and 36 °C. The shortening at 40 °C was also important compared to all other temperatures (F = 30.637; df = 3, 1096; P = 0.000). When the effects of thermal stress on F_1 pupal times were analysed, the decreases were significant at all other temperatures compared to 30 °C (Fig. 8). Furthermore, the elongations observed at 38 and 40 °C compared to 36 °C were also important (F = 27.784; df = 3, 1096; P = 0.000). As shown in Fig. 8, thermal stress considerably prolonged the pupal period of F_1 *B. hebetor* larvae at all temperatures compared to 30 °C. There was also a significant increment in the pupal period at 36 °C compared to 38 °C (F = 67.969; df = 3, 1096; P = 0.000). Heat exposure caused significant prolongation in the adult emergence time of F_1 progeny at all temperatures compared to 30 °C (Fig. 8). The increases at 36 and 40 °C compared to 38 °C were also important (F = 23.419; df = 3, 1096; P = 0.000).

The adult ratio of the F_1 progeny obtained from the thermally stressed parental eggs was highest at 36 °C and this increase was also significant compared to all other temperatures (F = 6.689; df = 3, 60; P = 0.001; Fig. 9). When examining the adult sex ratio, the highest percentage of females was observed at 30 °C with 80.80 ± 4.26 %, while the maximum value for males was 55.52 ± 8.35 % at 36 °C. From a statistical point of view, it was found that the female ratio was significantly lower (F = 4.409; df = 3, 60; P = 0.007) and the male ratio was significantly higher (F = 4.409; df = 3, 60; P = 0.007) at 36 and 38 °C compared to 30 °C (Fig. 9). As shown in Fig. 10, F1 adults died after an average of 24 days at 30, 38, and 40 °C, while individuals at 36 °C had a considerably longer lifespan of 36 days (F = 30.910; df = 3, 1096; P = 0.000).

4. Discussion

4.1. *Cellular immunity*

We examined the cellular immune responses of 7th instar



Statistic (ANOVA)
30°C: $F^{2,45}$=6.397,*P*=0.004
38°C: $F^{2,45}$=9.738,*P*=0.000
40°C: $F^{2,45}$=9.247,*P*=0.000
42°C: $F^{2,45}$=12.643,*P*=0.000

Fig. 5. Mitotic index (cells/100) of *G. mellonella* larvae exposed to different temperature treatments. Temperatures (a-b) and exposure times (x-y) labeled with the same lowercase letter are not significantly different (P<0.05; Tukey's HSD test). Data are average of four replicates. Statistic (ANOVA) (F, df, and P values) refer to comparisons among exposure times within each temperature.

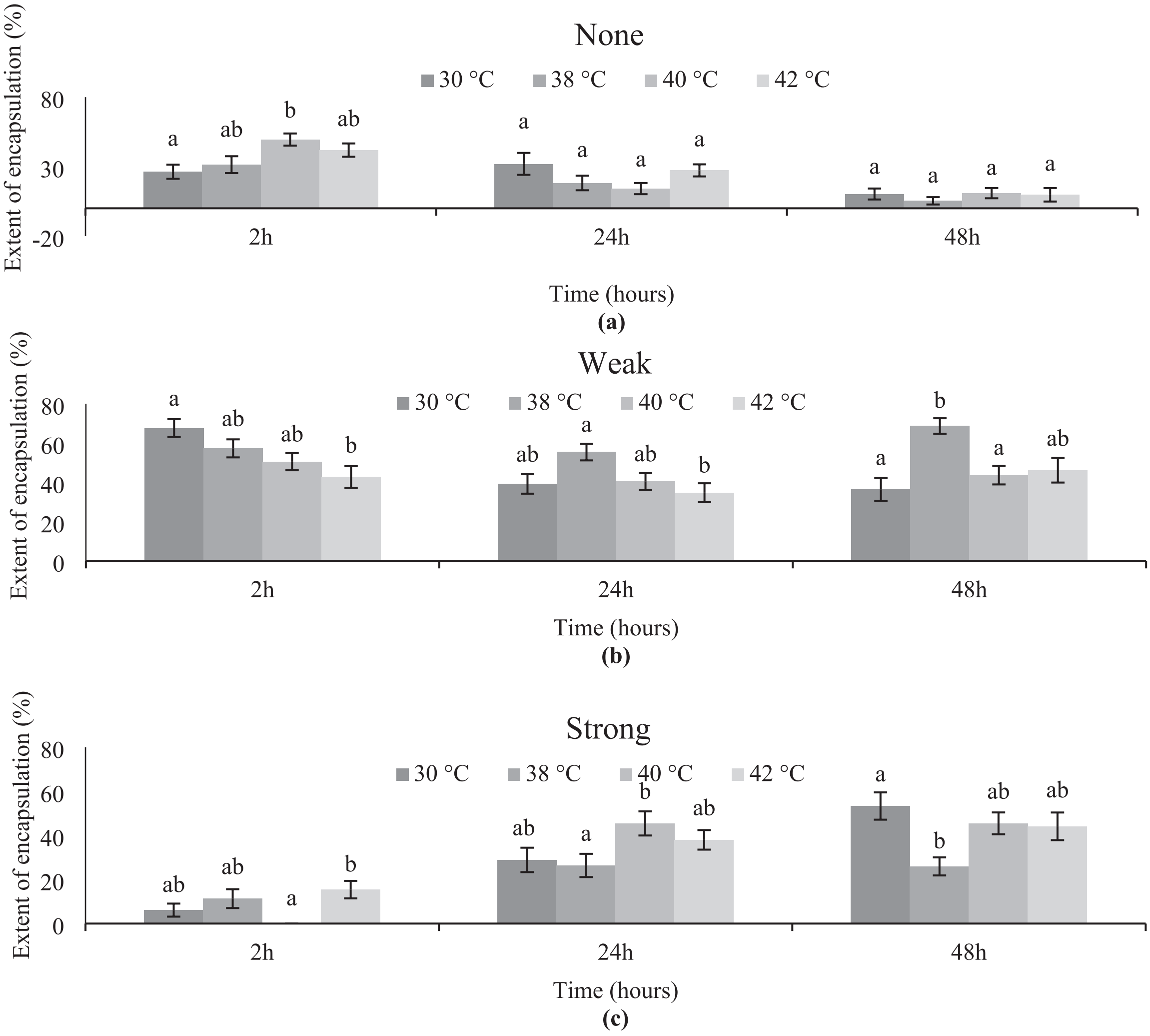


Fig. 6. Encapsulation (%) of Sephadex A-25 beads in *G. mellonella* larvae: (a) none (no or few hemocytes around the bead), (b) weak (2-10 hemocyte layers), (c) strong (>10 hemocyte layers). Temperatures (a-b) labeled with the same lowercase letter are not significantly different ($P<0.05$; Tukey's HSD test). Data are average of four replicates.

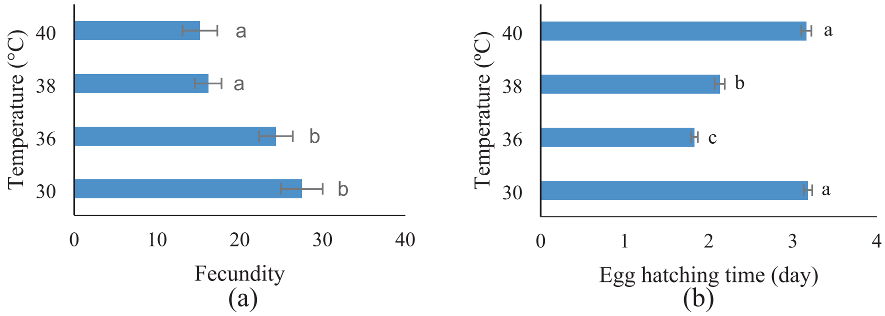


Fig. 7. Heat shock-induced variations in the fecundity (a) and the egg hatching time (b) of *B. hebetor*. The same letters are not significantly different ($P>0.05$; Tukey's HSD test). Data are average of four replicates.

G. mellonella larvae, the reactions of *B. hebetor* to thermally stressed hosts, and the changes in certain biological traits of F_1 parasitoids. This study comprised two distinct experimental setups: (1) assessing the immune responses of *G. mellonella* larvae after exposure to 30, 38, 40, or 42 °C for 2, 24, or 48 h, and (2) evaluating the biological traits of adult female *B. hebetor* when re-exposed together with their host previously subjected to 30, 36, 38, or 40 °C for 2 h, to the same thermal stress for an additional 2 h. Results from the immune assays showed that the highest total hemocyte count in *G. mellonella* was observed after 2 h of exposure at 30 °C, whereas longer or higher-temperature exposures caused a significant decline in hemocyte density. These reductions indicate that effective cellular immune responses require optimal thermal conditions (Mondal and Rai, 2001). The numbers of different hemocyte types also varied; granulocytes and spherulocytes paralleled changes in total hemocyte counts, while plasmatocytes remained the most abundant cell type across all temperatures. Similar patterns have been observed in

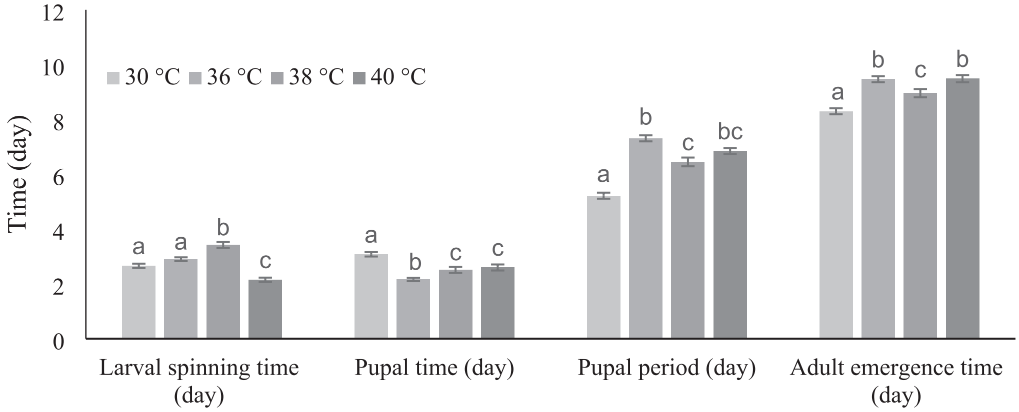


Fig. 8. Heat shock-induced variations in the larval spinning time, pupal time, pupal period and adult emergence time of *B. hebetor* (day). The same letters are not significantly different (P>0.05; Tukey's HSD test; d-day). Data are average of four replicates.

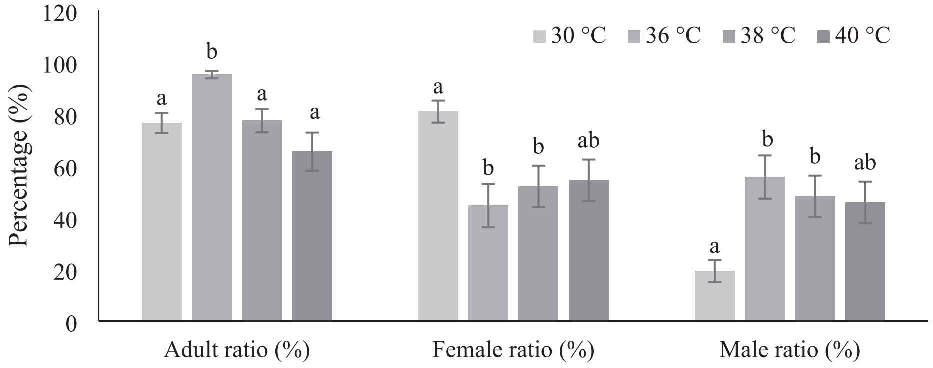


Fig. 9. Heat shock-induced variations in the adult sex ratio (%) of *B. hebetor*. The same letters are not significantly different (P>0.05; Tukey's HSD test). Data are average of four replicates.

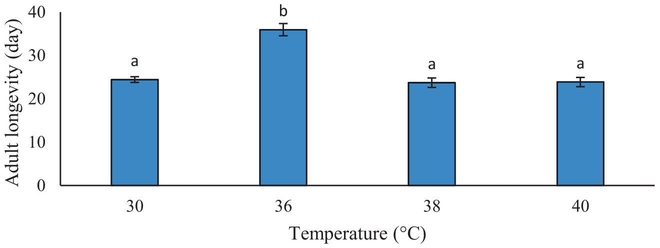


Fig. 10. Heat shock-induced variations in the adult longevity (day) of *B. hebetor*. The same letters are not significantly different (P>0.05; Tukey's HSD test; d-day). Data are average of four replicates.

other insect species, with both total and differential hemocyte counts reported to vary depending on temperature and exposure duration (Ghasemi et al., 2013; Shamakhi et al., 2019). Temperatures exceeding the thermal optimum likely impair insect development and induce physiological stress, which in turn affects hemocyte production and reduces immune competence (Karl et al., 2011; Takano et al., 2021). Moreover, hemocyte responses to thermal stress differ among insect species. Overall, our results reveal a mechanistic link between thermal tolerance and immune competence, suggesting that temperature fluctuations beyond the thermal optimum can weaken host defense and, in turn, reduce parasitoid success.

When the temperatures applied to 7th instar of *G. mellonella* larvae exceeded the thermal optimum, we observed a general increase in total hemocyte count (THC). In such cases, the numbers of plasmatocytes and spherulocytes changed more noticeably than those of granulocytes and other hemocytes (oenocytoids and prohemocytes). In particular, the increase in plasmatocyte count closely paralleled the overall rise in THC, whereas the numbers of granulocytes, spherulocytes, and other cell types were largely unaffected by temperature elevation. This increase in THC is considered an adaptive response aimed at enhancing the insect's immune defense under thermal stress (Nakahara et al., 2003; Siddiqui and Al-Khalifa, 2014; Laughton et al., 2017). Similar findings in other lepidopteran species, such as *Bombyx mori, Ephestia kuehniella* (Lepidoptera: Pyralidae), and *Phthorimaea operculella* (Lepidoptera: Pyralidae), have linked THC elevation to changes in specific hemocyte types (Nakahara et al., 2003; Ghasemi et al., 2013; Pourali and Ajam Hassani, 2018). Ghasemi et al. (2013) also suggested that the rise in plasmatocyte numbers under thermal stress may reflect an adaptive strategy to maintain immune competence. Consistent with our results, although various hemocyte combinations have been associated with THC elevation, an increase in plasmatocyte count remains the most common response. This pattern implies that *G. mellonella* may allocate physiological resources toward immune cell proliferation to counteract temperature-induced stress. Such modulation of cellular immunity could represent a key mechanism linking thermal stress to host–parasitoid interactions.

Our experiments revealed that the mitotic index of *G. mellonella* varied depending on both the intensity and duration of thermal exposure. Although previous studies have reported that the mitotic index changes with the developmental stage of the insect (Ghasemi et al., 2013), the use of larvae at the same developmental stage in our experiments allows us to attribute the observed differences solely to temperature and exposure time. In our study, prolonged exposure to 30 °C resulted in a gradual decrease in the number of mitotic cells. Moreover, at all observation time points, the mitotic index declined significantly at 40 and 42 °C compared to 30 and 38 °C. Similarly, Kiuchi et al. (2008)

observed that reduced mitotic activity in *B. mori* larvae coincided with decreased haemocyte density, suggesting that cell division may be suppressed as larvae approach molting. In line with this, the normal mitotic index in insect haemolymph is reported to be around 1 % (Jones, 1967; Jones and Liu, 1968), whereas in our study this value reached 5.17 % at 38 °C after 24 h of exposure. These findings suggest that *G. mellonella* may mount an immune response when subjected to elevated temperatures such as 38 °C.

The encapsulation response induced by thermal stress in 7th instar *G. mellonella* larvae differed from previous reports (Lynn and Vinson, 1977; Seehausen et al., 2017; 2018). Rather than a steady increase in encapsulation with rising temperatures, our study revealed a fluctuating pattern of encapsulation activity. Nevertheless, in the experimental groups where increases in both weak and strong encapsulation rates were observed with rising temperature, the number of plasmatocytes showed a tendency to increase. Specifically, weak encapsulation was higher at 48 h at 38 °C compared to 30 °C, while strong encapsulation was higher at 2 h at 42 °C compared to 40 °C, and at 24 h at 40 °C compared to 38 °C. These findings are consistent with previous studies (Seehausen et al., 2017; 2018), which suggested that elevated temperatures enhance plasmatocyte activity and peptide release, thereby promoting encapsulation. Such cellular changes may contribute to enhanced immune activity in insects under specific thermal and exposure conditions. However, in contrast to these reports, our results generally showed that increasing temperature tended to suppress overall encapsulation rates. When hemocyte composition was examined, granulocyte numbers significantly decreased at 42 °C compared to 40 °C after 48 h, whereas plasmatocyte numbers slightly increased. We propose that under high-temperature stress, encapsulation efficiency may be reduced not by cell number but by functional impairments such as disruptions in cell structure or spreading ability, rendering granulocytes and plasmatocytes less effective.

4.2. Host-parasitoid relationship

In the experiments with host-parasitoid interactions, *G. mellonella* was initially subjected to thermal stress at 30, 36, 38, and 40 °C for 2 h. Following this, both *G. mellonella* and *B. hebetor* were exposed to the same thermal stress conditions together for an additional 2 h. The results showed that female *B. hebetor* remained an active biological control agent against *G. mellonella* at all tested temperatures. Our results demonstrate that *B. hebetor* has a high thermal tolerance and can survive over a wide temperature range, which is consistent with the literature even though the temperature regimes and application methods vary (Forouzan et al., 2008; Golizadeh et al., 2017; Noor-ul-Ane et al., 2018; Wu et al., 2022). Moreover, when compared to other parasitoid species in the literature, the findings suggest that *B. hebetor* exhibits greater thermal resilience than species such as *Trichogrammatoidea bactrae* (Hymenoptera: Trichogrammatidae) (Wang et al., 2014), *Cotesia congregata* (Hymenoptera: Braconidae) (Moore et al., 2020; 2022), and *Colpoclypeus florus* (Hymenoptera: Eulophidae) (Milonas and Savopoulou-Soultani, 2000). This thermal stability suggests an ecological advantage for *B. hebetor* in variable environmental conditions, ensuring successful parasitism and potential biocontrol efficacy under future warming scenarios.

Previous studies involving parasitoids have primarily aimed at producing large numbers of healthy individuals rather than investigating the effects of high temperatures. This typically involves maximizing both egg production and the number of successfully emerging parasitoids. In our study, the highest number of eggs was recorded at 30 °C and 36 °C, whereas the lowest egg counts were observed at 38 °C and 40 °C which were significantly lower than those at 30 °C and 36 °C. Experiments at 36 °C achieved the highest adult emergence success rate (95 %), which was significantly greater than those at other tested temperatures. The observed pattern suggests that elevated temperatures may impair ovarian function or embryonic development, ultimately reducing both egg production and successful emergence. Thus, 36 °C appears to represent an optimal range for reproduction and progeny viability, confirming that even small deviations from this thermal window can strongly influence parasitoid fitness.

Another factor contributing to mass production in parasitoids is gender distribution (Jeffs and Lewis, 2013). Research has shown that sexual distribution in parasitoids varies with temperature (Colinet and Boivin, 2011; Moiroux et al., 2014). Most studies have reported that parasitoid populations tend to be male-dominated at extremely low and high temperatures, whereas at intermediate temperatures, the ratio shifts to female-dominated or a near 1:1 sex ratio (Zamani et al., 2007; Golizadeh et al., 2017). Some researchers have attributed these shifts in sex ratio primarily to temperature-induced effects on sperm quality and male mating behaviour (Nguyen et al., 2013; Moiroux et al., 2014). However, other studies have reported no significant changes in sex ratio in response to temperature (Qiu et al., 2012; Wu et al., 2022). Heat stress significantly altered the adult sex ratio of *B. hebetor* in our study. The F_1 progeny was 81 % female-dominated at 30 °C and females again outnumbered males at 38 and 40 °C, although the proportion was lower (52 and 54 %, respectively). On the other hand, at 36 °C heat stress, 56 % of the F_1 adults were male-dominated. We attribute the discrepancy between our results and the literature to three main factors. First, the temperature levels used in previous studies were considerably lower than those applied in our study. Second, we specifically examined how short-term thermal stress affected the egg-laying performance of female *B. hebetor*, and in this context, our method of temperature application differed significantly from those in the literature (Zamani et al., 2007; Qiu et al., 2012; Golizadeh et al., 2017; Wu et al., 2022). Finally, our experiments focused on the response of female parasitoids and their heat-stressed hosts, which may have uniquely influenced progeny sex allocation.

Studies in the literature indicate that temperature can influence the mechanism controlling net formation in insects. However, these studies primarily aimed to improve the cocoons of *B. mori* to enhance their productivity and quality by manipulating temperature, humidity, and light conditions. These findings revealed that larval spinning time decreases with increasing temperature, though silk quality also diminishes (Ramachandra et al., 2001; Offord et al., 2016). Other research on Lepidoptera (Çelik Biçer et al., 2025) and Hymenoptera (Radmacher and Strohm, 2011) has demonstrated that temperature application methods can affect larval spinning time. Radmacher and Strohm (2011) found that in bees, cocoon construction time decreased with increasing temperature under constant conditions. In contrast, the larval spinning time in moths fluctuated with increasing temperature under constant heat shock but remained largely unaffected under variable heat shock. Additionally, both studies (Radmacher and Strohm, 2011; Çelik Biçer et al., 2025) reported that cocoon spinning was faster under variable heat shock compared to constant heat shock. In our study, thermal stress markedly influenced the larval spinning time of *B. hebetor*. The duration initially increased with rising temperature, peaking at 38 °C, but then sharply declined at 40 °C. These findings suggest that high temperature disrupts the physiological mechanisms underlying silk secretion or spinning behavior, possibly through alterations in silk protein synthesis or enzymatic activity. The differences from previous studies likely stem from species-specific thermal tolerance and experimental conditions. Notably, the fact that variable heat shock affected larval spinning time in *B. hebetor* but not in *G. mellonella* (Çelik Biçer et al., 2025) may indicate a greater sensitivity of *B. hebetor* to thermal stress.

In nearly all studies examining the pre-adult stages of parasitoids, increasing temperature shorten both egg hatching time and other developmental stages (pupal time, pupal period, and adult emergence time) (Hailemichael and Smith, 1994; Milonas and Savopoulou- Soultani, 2000; Mohammed et al., 2006; Zamani et al., 2007; Forouzan et al., 2008; Qiu et al., 2012; Appiah et al., 2013; Wang et al., 2014; Golizadeh et al., 2017; Noor-ul-Ane et al., 2018; Moore et al., 2020; Wu et al., 2022). In our study, egg hatching time in *B. hebetor* also decreased with rising temperature, consistent with previous findings. The longest egg hatching times were observed at the lowest and highest temperatures tested (30 and 40 °C), whereas hatching was significantly faster at intermediate temperatures. However, unlike the literature, the durations of the subsequent pre-adult stages in *B. hebetor* exhibited both increases and decreases depending on the temperature. These discrepancies may result from differences in temperature application and range, as previous studies used lower temperatures (Hailemichael and Smith, 1994; Milonas and Savopoulou-Soultani, 2000; Qiu et al., 2012). In our study, the host was exposed to heat for two hours before the parasitoid was introduced. Both were then exposed to heat stress together, and the pupal time of individuals emerging from eggs laid on the host was 3.08 ± 0.08 days at 30 °C. However, as temperature increased over 30 °C, pupal time significantly decreased, reaching its shortest duration at 36 °C (2.16 ± 0.06 days). In contrast, the pupal period and adult emergence time significantly increased with rising temperatures. Additionally, the adult longevity of *B. hebetor* was approximately 24 days at 30, 38, and 40 °C, but extended to 36 days at 36 °C, which was statistically significant. Previous studies investigating the effects of thermal stress on *B. hebetor* (Forouzan et al., 2008; Golizadeh et al., 2017; Noor-ul-Ane et al., 2018; Wu et al., 2022) have reported that the species can survive and develop across a broad temperature range (16–40 °C). Our findings support these results and also demonstrate, through a different thermal stress application method, that *B. hebetor* exhibits high thermal tolerance.

Previous studies have clearly demonstrated that *B. hebetor* remains active across different temperature ranges depending on the host species: 25–30 °C on *E. kuehniella* (Golizadeh et al., 2017), 16–32 °C on *Opisina arenosella* (Lepidoptera: Xyloryctidae) (Wu et al., 2022), 15–40 °C on *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Noor-ul-Ane et al., 2018), and 18–38 °C on *G. mellonella* (Forouzan et al., 2008). In our study, *G. mellonella-B. hebetor* pairs were subjected to variable heat shock in two successive phases. *B. hebetor* remained active as a biological control agent of *G. mellonella* at all tested temperatures (30, 36, 38, and 40 °C). Furthermore, when *B. hebetor* reared on *G. mellonella* previously exposed to 36 °C for two hours, 95 % of the 24.38 ± 2.03 eggs per female successfully developed into adults, and 45 % of these were female, demonstrating that *B. hebetor* may outperform many other parasitoid species reported in the literature. Additionally, a previous study showed that exposing *G. mellonella* to variable heat shock at 36 °C resulted in significantly prolonged larval spinning time and pupal period compared to other temperatures (Çelik Biçer et al., 2025). This situation may prolong the host’s larval stage, thereby increasing the likelihood of parasitism by *B. hebetor,* which is a larval parasitoid. Another factor enhancing its parasitism success is its ability, as an ectoparasitoid, to naturally evade the host’s encapsulation response. Its broad host range may also enhance its persistence under changing climatic conditions. Overall, our results highlight that *B. hebetor* can tolerate substantial temperature variation while maintaining its parasitic performance. Moreover, our findings on hemocyte profiles and encapsulation clearly indicate that moderate thermal stress (up to 40 °C) transiently suppresses the host’s cellular immune defense. This suppression was evidenced by decreased total hemocyte counts, reduced proportions of granulocytes, and lower encapsulation efficiency at these temperatures. Such impairment of the host immune system likely facilitates parasitoid success, as reduced hemocyte activity and encapsulation limit the host’s ability to mount an effective defense against *B. hebetor*. In contrast, exposure to more extreme temperatures (≥42 °C) may impair host physiology and nutrient availability, which can negatively affect parasitoid fitness despite weakened host immunity. Understanding such host-parasitoid interactions will help clarify the temperature tolerance, physiological capacity, and adaptive potential of both species, thereby contributing to predictions of insect distribution patterns under climate change. It will also help anticipate how rising temperatures may affect pest and beneficial insect species within tritrophic interactions.

5. Author’ contributions

Aylin ER proposed the idea and they worked with Olga SAK in the design of the study. Erinç ÇELİK BİÇER performed the experiments. Olga SAK, Aylin ER, and Erinç ÇELİK BİÇER wrote the paper.

6. Consent for publication

All authors read and consent to the publication of the manuscript.

CRediT authorship contribution statement

Erinç Çelik Biçer: Writing – original draft, Validation, Investigation, Data curation. **Olga Sak:** Writing – review & editing, Supervision. **Aylin Er:** Methodology, Conceptualization.

Ethics approval

This study is a study on Arthropoda. It does not require any Ethical Committee/Institutional Review Board (IRB) approval.

Funding

This research was supported by the Balıkesir University Research Foundation [grant numbers 2022, 116].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M., Russo, A., Siscaro, G., et al., 2015. Combined non-target effects of insecticide and high temperature on the parasitoid *Bracon nigricans*. PLoS One 10 (9), e0138411. https://doi.org/10.1371/journal.pone.0138411.

Alam, M.S., Alam, M.Z., Alam, S.N., Miah, R.U., Mian, M.I.H., Hossain, M.M., 2014. Biology of *Bracon hebetor* reared on wax moth (*Galleria mellonella*) larvae. Available online: Persian Gulf Crop Protection 3 (4), 54–62 https://research.ebsco.com/c/zrhf4i/search/details/uc3dkqsi7v?db=obo#Au.

Appiah, E.F., Ekesi, S., Salifu, D., Afreh-Nuamah, K., Obeng-Ofori, D., Khamis, F., et al., 2013. Effect of temperature on immature development and longevity of two introduced opiine parasitoids on *Bactrocera invadens*. J. Appl. Entomol. 137 (8), 571–579. https://doi.org/10.1111/jen.12036.

Arifie, U., Farook, U.B., Dar, S.A., Malik, A.R., Javid, R., Khaliq, N., Wachkoo, A.A., 2024. Pests and diseases of honey bees. In: Wachkoo, A.A., Nayik, G.A., Uddin, J., Ansari, M.J. (Eds.), *Honey Bees, Beekeeping and Bee Products*. CRC Press, pp. 53–68.

Bloemkolk, J.W., Gray, M.R., Merchant, F., Mosmann, T.R., 1992. Effect of temperature on hybridoma cell cycle and MAb production. Biotechnol. Bioeng. 40 (3), 427–431. https://doi.org/10.1002/bit.260400312.

Colinet, H., Boivin, G., 2011. Insect parasitoids cold storage: a comprehensive review of factors of variability and consequences. Biol. Control 58 (2), 83–95. https://doi.org/10.1016/j.biocontrol.2011.04.014.

Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal environments. Annu. Rev. Entomol. 60 (1), 123–140. https://doi.org/10.1146/annurev-ento-010814-021017.

Collier, T.R., 1995. Host feeding, egg maturation, resorption, and longevity in the parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). Ann. Entomol. Soc. Am. 88 (2), 206–214. https://doi.org/10.1093/aesa/88.2.206.

Costaz, T.P., Gols, R., de Jong, P.W., van Loon, J.J., Dicke, M., 2022. Effects of extreme temperature events on the parasitism performance of *Diadegma semiclausum*, an endoparasitoid of *Plutella xylostella*. Entomol. Exp. Appl. 170 (8), 656–665.

Cracknell, A.P., Varotsos, C.A. (Eds.). (2021). *Understanding global climate change: modelling the climatic system and human impacts*. İngiltere: CRC Press. Available online: https://www.google.com.tr/books/edition/Understanding_Global_Climate_Change/1DkyEAAAQBAJ?hl=tr&gbpv=1.

Çelik Biçer, E., Sak, O., Er, A., 2025. Constant and variable heat shock effects on *Galleria mellonella* L.(Lepidoptera: Pyralidae) mortality and biological traits in the context of climate change. Int. J. Trop. Insect Sci. 45 (2), 759–771. https://doi.org/10.1007/s42690-025-01466-y.

Danks, H.V., 1987. Insect dormancy: an ecological perspective. In: H.V. Danks. *Biological Survey of Canada (Terrestrial Arthropods)* (pp. 439), England: National government publication. Available online: https://www.cabidigitallibrary.org/doi/full/10.5555/19870543089.

Ellis, J., Graham, J.R., Mortensen, A.N., 2013. Standard methods for wax moth research. J. Apic. Res. 52 (1), 1–17. https://doi.org/10.3896/ibra.1.52.1.10.

Forouzan, M., Amir, M.M., Sahragard, A. (2008). Temperature-dependent development of *Habrobracon hebetor* (Hym.: Braconidae) reared on larvae of *Galleria mellonella* (Lep.: Pyralidae). *Journal of Entomological Society of Iran, 28*(1), 67-78. Available online: .

Ghasemi, V., Moharramipour, S., Sendi, J.J., 2013. Circulating hemocytes of Mediterranean flour moth, *Ephestia kuehniella* Zell.(Lep: Pyralidae) and their response to thermal stress. Available online: Invertebr. Surviv. J. 10 (1), 128–140 https://www.isj.unimore.it/index.php/ISJ/article/view/295/209.

Golizadeh, A., Alikhani, M., Hassanpour, M., Enkegaard, A., Rafiee-Dastjerdi, H., Razmjou, J., 2017. Comparative biology and life table of *Habrobracon hebetor* (Hymenoptera: Braconidae) on *Anagasta kuehniella* (Lepidoptera: Pyralidae) at five constant temperatures. Int. J. Pest Manage. 63 (4), 364–370. https://doi.org/10.1080/09670874.2016.1277801.

Gupta, P., Ferkovich, S.M., 1998. Interaction of calyx fluid and venom from *Microplitis croceipes* (Braconidae) on developmental disruption of the natural host, *Heliocoverpa zea*, and two atypical hosts, *Galleria mellonella* and *Spodoptera exigua*. J. Insect Physiol. 44 (9), 713–719. https://doi.org/10.1016/S0022-1910(98)00004-3.

Hailemichael, Y., Smith Jr, J.W., 1994. Development and longevity of *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae) at constant temperatures. Ann. Entomol. Soc. Am. 87 (6), 874–878. https://doi.org/10.1093/aesa/87.6.874.

Hance, T., van Baaren, J., Vernon, P., Boivin, G., 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annu. Rev. Entomol. 52 (1), 107–126. https://doi.org/10.1146/annurev.ento.52.110405.091333.

IPCC, 2022: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., https://doi.org/10.1017/9781009325844.

Jeffs, C.T., Lewis, O.T., 2013. Effects of climate warming on host–parasitoid interactions. Ecol. Entomol. 38 (3), 209–218. https://doi.org/10.1111/een.12026.

Jones, J.C., 1967. Changes in the hemocyte picture of *Galleria mellonella* (Linnaeus). Biol. Bull. 132 (2), 211–221. https://doi.org/10.2307/1539889.

Jones, J.C., 1967. Normal differential counts of haemocytes in relation to ecdysis and feeding in *Rhodnius*. J. Insect Physiol. 13 (8), 1133–1141. https://doi.org/10.1016/0022-1910(67)90087-X.

Jones, J.C., Liu, D.P., 1968. A quantitative study of mitotic divisions of haemocytes of *Galleria mellonella* larvae. J. Insect Physiol. 14 (8), 1055–1061. https://doi.org/10.1016/0022-1910(68)90043-7.

Joodaki, R., Zandi-Sohani, N., Zarghami, S., Yarahmadi, F., 2018. Temperature-dependent functional response of *Aenasius bambawalei* (Hymenoptera: Encyrtidae) to different population densities of the cotton mealybug *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). European journal of entomology, *115*, 326-331, https://doi.org/ 10.14411/eje.2018.032.

Karl, I., Stoks, R., De Block, M., Janowitz, S.A., Fischer, K., 2011. Temperature extremes and butterfly fitness: conflicting evidence from life history and immune function. Glob. Chang. Biol. 17 (2), 676–687. https://doi.org/10.1111/j.1365-2486.2010.02277.x.

Keever, D.W., Arbogast, R.T., Mullen, M.A., 1985. Population trends and distributions of *Bracon hebetor* Say (Hymenoptera: Braconidae) and lepidopterous pests in commercially stored peanuts. Environ. Entomol. 14 (6), 722–725. https://doi.org/10.1093/ee/14.6.722.

Kiuchi, T., Aoki, F., Nagata, M., 2008. Effects of high temperature on the hemocyte cell cycle in silkworm larvae. J. Insect Physiol. 54 (2), 454–461. https://doi.org/10.1016/j.jinsphys.2007.11.004.

Klok, C.J., Chown, S.L., Gaston, K.J., 2003. The geographical range structure of the Holly Leaf-miner. III. Cold hardiness physiology. Functional Ecology 17 (6), 858–868. https://doi.org/10.1111/j.1365-2435.2003.00794.x.

Laughton, A.M., O'Connor, C.O., Knell, R.J., 2017. Responses to a warming world: Integrating life history, immune investment, and pathogen resistance in a model insect species. Ecol. Evol. 7 (22), 9699–9710. https://doi.org/10.1002/ece3.3506.

Lavine, M.D., Strand, M.R., 2002. Insect hemocytes and their role in immunity. Insect Biochem. Mol. Biol. 32 (10), 1295–1309. https://doi.org/10.1016/S0965-1748(02)00092-9.

Lynn, D.C., Vinson, S.B., 1977. Effects of temperature, host age, and hormones upon the encapsulation of *Cardiochiles nigriceps* eggs by *Heliothis* spp. J. Invertebr. Pathol. 29 (1), 50–55. https://doi.org/10.1016/0022-2011(77)90171-9.

Ma, C.S., Ma, G., Pincebourde, S., 2021. Survive a warming climate: insect responses to extreme high temperatures. Annu. Rev. Entomol. 66 (1), 163–184. https://doi.org/10.1146/annurev-ento-041520-%20074454.

Milonas, P.G., Savopoulou-Soultani, M., 2000. Temperature dependent development of the parasitoid *Colpoclypeus florus* (Hymenoptera: Eulophidae) in the laboratory. J. Econ. Entomol. 93 (6), 1627–1632. https://doi.org/10.1603/0022-0493-93.6.1627.

Mohammed, S.A., Overholt, W.A., Wharton, R.A., Lux, S.A., 2006. Effect of temperature on developmental time and longevity of *Psyttalia cosyrae* (Hymenoptera: Braconidae). Biocontrol Sci. Tech. 16 (7), 717–726. https://doi.org/10.1080/09583150600700016.

Moiroux, J., Brodeur, J., Boivin, G., 2014. Sex ratio variations with temperature in an egg parasitoid: behavioural adjustment and physiological constraint. Anim. Behav. 91, 61–66. https://doi.org/10.1016/j.anbehav.2014.02.021.

Mondal, S., Rai, U., 2001. In vitro effect of temperature on phagocytic and cytotoxic activities of splenic phagocytes of the wall lizard, Hemi-dactylus flavividis. Comparative Biochemistry and Physiology A 129, 391–398. https://doi.org/10.1016/S1095-6433(00)00356-1.

Montazeri, M.M., Goldasteh, S., Shirazi, J., Sanatgar, E., Vafaeeshushtari, R., 2019. Impact of temperature on some biological traits of *Habrobracon hebetor* (Hymenoptera: Braconidae). Journal of Entomological Society of Iran 39 (3), 271–294. https://doi.org/10.22117/jesi.2019.125695.1302.

Moore, M.E., Hill, C.A., Kingsolver, J.G., 2022. Developmental timing of extreme temperature events (heat waves) disrupts host–parasitoid interactions. Ecol. Evol. 12 (3), e8618.

Moore, M.E., Kester, K.M., Kingsolver, J.G., 2020. Rearing temperature and parasitoid load determine host and parasitoid performance in *Manduca sexta* and *Cotesia congregata*. Ecol. Entomol. 45 (1), 79–89. https://doi.org/10.1111/een.12776.

Nakahara, Y., Kanamori, Y., Kiuchi, M., Kamimura, M., 2003. In vitro studies of hematopoiesis in the silkworm: cell proliferation in and hemocyte discharge from the hematopoietic organ. J. Insect Physiol. 49 (10), 907–916. https://doi.org/10.1016/S0022-1910(03)00149-5.

Nguyen, T.M., Bressac, C., Chevrier, C., 2013. Heat stress affects male reproduction in a parasitoid wasp. J. Insect Physiol. 59 (3), 248–254. https://doi.org/10.1016/j.jinsphys.2012.12.001.

Nittono, Y., 1960. Studies on the blood cells in the silkworm, *Bombyx mori* L. Bulletin of the Sericultural Experiment Station 16, 171–266.

Noor-ul-Ane, M., Mirhosseini, M.A., Crickmore, N., Saeed, S., Noor, I., Zalucki, M.P., 2018. Temperature-dependent development of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and its larval parasitoid, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae): implications for species interactions. Bull. Entomol. Res. 108 (3), 295–304. https://doi.org/10.1017/S0007485317000724.

Offord, C., Vollrath, F., Holland, C., 2016. Environmental effects on the construction and physical properties of *Bombyx mori* cocoons. J. Mater. Sci. 51, 10863–10872. https://doi.org/10.1007/s10853-016-0298- 5.

Overgaard, J., Sørensen, J.G., 2008. Rapid thermal adaptation during field temperature variations in *Drosophila melanogaster*. Cryobiology 56 (2), 159–162. https://doi.org/10.1016/j.cryobiol.2008.01.001.

Pandey, J.P., Mishra, P.K., Kumar, D., Singh, B.M.K., Prasad, B.C., 2010. Effect of temperature on hemocytic immune response of tropical tasar silkworm, *Antheraea mylitta* D. Research Journal of Immunology 3 (2), 169–177. https://doi.org/10.3923/rji.2010.169.177.

Payne, N.M., 1934. The differential effect of environmental factors upon *Microbracon hebetor* Say (Hymenoptera: Braconidae) and its host, *Ephestia kuhniella* Zeller (Lepidoptera: Pyralidae). II. Ecological monographs 4 (1), 4–46. https://doi.org/10.2307/1943209.

Pietrykowska-Tudruj, E., Staniec, B., Wojda, I., Wagner, G.K., 2025. Insight into the larva of the greater wax moth *Galleria mellonella* as a model organism, with a pictorial key for identifying larval stages. The European Zoological Journal 92 (1), 337–360.

Pourali, Z., Ajam Hassani, M., 2018. The effect of thermal stresses on the immune system of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). J. Entomol. So. Iran 37 (4), 515–525. https://doi.org/10.22117/jesi.2018.116103.

Prozell, S., Schöller, M., 1998. Insect fauna of a bakery, processing organic grain and applying *Trichogramma evanescens* Westwood. Bulletin OILB/SROP 21 (3), 39–44.

Qiu, B.O., Zhou, Z.S., Luo, S.P., Xu, Z.F., 2012. Effect of temperature on development, survival, and fecundity of *Microplitis manilae* (Hymenoptera: Braconidae). Environ. Entomol. 41 (3), 657–664. https://doi.org/10.1603/EN11101.

Radmacher, S., Strohm, E., 2011. Effects of constant and fluctuating temperatures on the development of the solitary bee *Osmia bicornis* (Hymenoptera: Megachilidae). Apidologie 42, 711–720. https://doi.org/10.1007/s13592-011-0078-9.

Ramachandra, Y.L., Bali, G., Rai, S.P., 2001. Effect of temperature and relative humidity on spinning behaviour of silkworm (*Bombyx mori* L.). *Indian Journal of Experimental Biology*, *39*(1), 87-89. Available online: https://www.researchgate.net/profile/YLRamachandra/publication/11987162_Effect_of_temperature_and_relative_humidity_on_spinning_behaviour_of_silkwormBombyx_moriL/links/581b0e4d08aed2439387e4de/Effect-of-temperature-and-relativ-humidity-on-spinning-behaviour-of-silkworm-Bombyx-moriL.pdf.

Richards, E.H., Edwards, J.P., 2002. Parasitism of *Lacanobia oleracea* (Lepidoptera) by the ectoparasitic wasp, *Eulophus pennicornis*, disrupts the cytoskeleton of host haemocytes and suppresses encapsulation in vivo. Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America 49 (2), 108–124. https://doi.org/10.1002/arch.10013.

Schmidt, O., Theopold, U., Strand, M., 2001. Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. Bioessays 23 (4), 344–351. https://doi.org/10.1002/bies.1049.

Seehausen, M.L., Cusson, M., Régnière, J., Bory, M., Stewart, D., Djoumad, A., et al., 2017. High temperature induces downregulation of polydnavirus gene transcription in lepidopteran host and enhances accumulation of host immunity gene transcripts. J. Insect Physiol. 98, 126–133. https://doi.org/10.1016/j.jinsphys.2016.12.008.

Seehausen, M.L., Naumann, P.H., Béliveau, C., Martel, V., Cusson, M., 2018. Impact of rearing temperature on encapsulation and the accumulation of transcripts putatively involved in capsule formation in a parasitized lepidopteran host. J. Insect Physiol. 107, 244–249. https://doi.org/10.1016/j.jinsphys.2018.04.013.

Seneviratne, S.I., Zhang X., Adnan M., Badi W., Dereczynski C., Di Luca A. et al. (2021). Weather and Climate Extreme Events in a Changing Climate. In Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. In V.

Delmotte, P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger et al. (pp. 1513–1766), Cambridge: Cambridge University Press. https://doi.org/10.1017/9781009157896.

Shamakhi, L., Zibaee, A., Karimi-Malati, A., Hoda, H., 2019. Effect of thermal stress on the immune responses of *Chilo suppressalis* walker (Lepidoptera: Crambidae) to *Beauveria bassiana*. J. Therm. Biol 84, 136–145. https://doi.org/10.1016/j.jtherbio.2019.07.006.

Siddiqui, M.I., Al-Khalifa, M.S., 2014. Review of haemocyte count, response to chemicals, phagocytosis, encapsulation and metamorphosis in insects. Italian J. Zool. 81 (1), 2–15. https://doi.org/10.1080/11250003.2013.858780.

Singh, D., Singh, R.P., Tripathi, C.P.M., 2014. Effect of temperature on life table statistics of *Bracon hebetor* Say.(Hymenoptera: Braconidae). Available online: Int. J. Innov. Appl. Stud. 7 (2), 497 https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=3ebb91f627b15d25a40500a9ddc1530b982c956d.

Smith, T.L., 1965. External morphology of the larva, pupa, and adult of the wax moth, *Galleria mellonella* L. J. Kansas Entomol. Soc. 287–310.

Takano, Y., Sakamoto, T., Tabunoki, H., Yoshimura, J., Iwabuchi, K., 2021. Integrated effects of thermal acclimation and challenge temperature on cellular immunity in the plusiine moth larvae *Chrysodeixis eriosoma* (Lepidoptera: Noctuidae). Physiol. Entomol. 46 (1), 52–59. https://doi.org/10.1080/11250003.2013.858780.

Uçkan, F., Gülel, A., 2002. Age-related fecundity and sex ratio variation in *Apanteles galleriae* (Hym., Braconidae) and host effect on fecundity and sex ratio of its hyperparasitoid *Dibrachys boarmiae* (Hym., Pteromalidae). J. Appl. Entomol. 126 (10), 534–537. https://doi.org/10.1046/j.1439-0418.2002.00706.x.

Wang, D.S., He, Y.R., Zhang, W., Nian, X.G., Lin, T., Zhao, R., 2014. Effects of heat stress on the quality of *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae). Bull. Entomol. Res. 104 (5), 543–551. https://doi.org/10.1017/S0007485314000303.

Wu, Q., Yan, S., Lyu, B., Wu, X., Lu, H., Tang, J., et al., 2022. Influence of temperature on the development and reproduction of *Habrobracon hebetor* (Say), as parasitoid of *Opisina arenosella* Walker. International Journal of Pest Management 1–8. https://doi.org/10.1080/09670874.2022.2141910.

Zamani, A.A., Talebi, A., Fathipour, Y., Baniameri, V., 2007. Effect of temperature on life history of *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Braconidae), two parasitoids of *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae). Environ. Entomol. 36 (2), 263–271. https://doi.org/10.1603/0046-225X-36.2.263.

Zhang, J., Huang, J., Lu, Y., Xia, T., 2016. Effects of temperature and host stage on the parasitization rate and offspring sex ratio of *Aenasius bambawalei* Hayat in *Phenacoccus solenopsis* Tinsley. PeerJ 4. https://doi.org/10.7717/peerj.1586.