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Development of *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) from the Jazan region of Southwest Saudi Arabia under different laboratory temperatures: applications in forensic entomology

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Abstract

Background: *Chrysomya albiceps* (Wiedemann, 1819) (blowflies), family Calliphoridae, is important in forensic entomology, where the minimum and maximum postmortem intervals (PMI) are estimated on the basis of the developmental stages of Diptera larvae that consume dead tissue. The present study was designed to estimate the effects of different ambient temperatures (20, 25, and 30 °C) under controlled laboratory conditions on the developmental stages of *C. albiceps* from the Jazan region, Saudi Arabia.

Results: The present study showed that the larval body weight and length were significantly increased when larvae were reared at 30 °C compared with corresponding values at 24 h, 48 h, and 72 h at rearing temperatures of 20 °C and 25 °C; however, the weight and length were significantly decreased compared with corresponding values at 96 h at 20 °C and 25 °C. The pupation time was inversely related to the rearing temperature, occurring at 144, 124, and 120 h at rearing temperatures of 20 °C, 25 °C, and 30 °C, respectively. The pupal weight and length were significantly increased in larvae reared at 30 °C compared with those reared at 20 °C and 25 °C. At 20 °C, 25 °C, and 30 °C, larval durations of 5.00, 5.00, and 4.00 days were recorded, respectively. Pupae and adults showed gradual decreases in life stage durations, at 6.00, 5.30, and 4.80 days in pupae and 20.00, 18.70, and 16.90 days in adults, with increasing rearing temperatures. Average adult longevity at 30 °C (194.40 h) was significantly less than adult longevity at 20 °C (216.00 h) and 25 °C (204.60 h). The results showed an inverse relation between durations of developmental stages and rearing temperatures.

Conclusions: Insect laboratory colonization for the determination of biological characteristics of insects is economically viable for forensic entomology and as a technique for evaluating insect evidence.

Keywords: *Chrysomya albiceps*, Postmortem interval (PMI), Temperature, Larva, Pupa, Longevity, Development rate

Background

Forensic science is one of the most important aspects in the investigation of any crime. According to the National Institute of Justice, forensic science is the application of sciences to matters of the law (Bruinsma and Weisburd 2014). The forensic science manipulate with the usage of a broad spectrum of scientific branches like physics,

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chemistry, and biology to achieve relevant information to criminal and legal evidence. Forensic science is any scientific and specialized skills and tools that the crime scene investigators and lab technicians are able to collect and analyze as an evidence in order to solve a crime or successfully convict the offender (Morgan 2019).

Forensic entomology evaluates the succession of necrophagous insects and the larval age of relevant insects collected from decomposed remains to estimate the time elapsed since death; entomological analysis is a tool in forensic investigations and can provide evidence of the postmortem interval (PMI) in a court of law.

Forensic entomology examines insect evidence for forensic and legal purposes to estimate the minimum time since death. The collected entomological evidence can provide necessary and important information about the movement or storage of remains following death, submersion interval, time of decapitation and/or dismemberment, identification of specific trauma sites, and postmortem artifacts on the body. The use of entomotoxicology can link a suspect to the scene of a crime, determine sexual molestation, or identify a suspect by examining the insects recovered from infested wounds to determine the period of neglect of living humans and animals (Catts 1992; Campobasso and Introna 2001; Amendt et al. 2007). Determination of the time since death is temperature dependent because temperature affects insect development as well as insect access to corpses (Campobasso et al. 2001; Myskowiak and Doums 2002).

Dipterans are the most important insect order in forensic investigations. These insects utilize temporary microhabitats of the corpse to feed and lay their eggs; they are normally the first arthropods to colonize a corpse (Baia et al. 2016).

The first group of insects to arrive and lay eggs on and in dead bodies in the early stage of decomposition are usually blowflies (Diptera: Calliphoridae), which are attracted by odor (Amendt et al. 2007; Gullan and Cranston 2014). The development rates of blowflies can be used to estimate the time elapsed since death within the first few weeks after death (Anderson 2000). The stage of development of the oldest immature insects on the body and the average environmental temperatures at the crime scene while the body was in situ allow the calculation of an accurate PMI (Amendt et al. 2011).

Chrysomya albiceps (Wiedemann, 1819) (Diptera: Calliphoridae) is one of the most studied blowflies and is recognized as a pioneer species in the colonization of corpses and carcasses. Similar to other muscoids, it is attracted by the odor produced during corpse decomposition (Lane 1975; Goddard and Lago 1985; Vasconcelos et al. 2013). *C. albiceps* was the dominant species

recorded on indoor and outdoor carcasses in stages of bloating, decay, and advanced decay in a recently published study in Riyadh, Saudi Arabia (Al-Khalifa et al. 2020). Mashaly et al. (2017) found that *C. albiceps* was the most abundant species of carrion fly on sheep carcasses in seven cities.

C. albiceps (Wiedemann, 1819) is a native tropical and subtropical African species with a worldwide distribution that has expanded since the turn of the century (Laurence 1981; Hall and Wall 1995; Grassberger et al. 2003; Gomes et al. 2009; Kotrba et al. 2012; Klekovska et al. 2017; Makovetskaya and Verves 2018). Blowflies identified and represented in the Middle East (Akbarzadeh et al. 2015) have been reported, and *C. albiceps* has been previously collected and identified in the area of interest of the present study (Jazan, Saudi Arabia) (Bosly 2010; Setyaningrum and Al Dhafer 2014; Dawah et al. 2019). In addition to its forensic medical and veterinary importance, larvae can cause primary and secondary cutaneous myiasis in humans and livestock (Alahmed 2004; Alahmed et al. 2006; Stevens and Wallman 2006; Amendt et al. 2011). The worldwide distribution of *C. albiceps* has allowed its use in different important studies because of easy collection and laboratory handling (Vélez and Wolff 2008; Pujol-Luz and Barros-Cordeiro 2012). *C. albiceps* is important in forensic entomology since it can be used to determine the PMI by calculating the age of the oldest larval stage feeding on a corpse (Gomes and Von Zuben 2005; Gomes et al. 2006; Mendonça et al. 2010; Salazar-Souza et al. 2018). Hence, the developmental stage of the insect helps specialists determine the time since death, which is temperature dependent (Li et al. 2016).

To date, entomology has not been used in legal investigations in the Kingdom of Saudi Arabia and most Arab countries in the Middle East. Development data of insects associated with corpse decomposition can help estimate the PMI in forensic investigations that requires judicial requirements. Forensic entomology is recognized as an important tool for legal investigations in many countries, while in our region, it has not yet been used as legal evidence in court. The reason for the lack of application of entomological evidence may be the insect fauna, which changes from one region to another, or the lack of standardized reference protocols regarding the developmental stages of arthropods (Vasconcelos et al. 2019).

The present study aimed to explore the effect of different ambient temperatures on the developmental stages of forensically important *C. albiceps* under laboratory conditions. That is because the use of laboratory colonization technique may add for evaluating insect evidence, and the present study is the first to be done in the Jazan region, southwest Kingdom of Saudi Arabia.

Methods

Stock colony

The stock colony was established from collected flies from governmental slaughterhouses in Abu Arish (16° 58' N-42° 47' E), which is a city located in the eastern Jazan region (southwestern Saudi Arabia), with previous collection experience (Bosly 2010). *C. albiceps* flies were identified at the Biology Department, Faculty of Science, and were reared in the laboratory for ten generations. Flies were placed in polypropylene breeding cages (45 × 30 × 20 cm), and adults had access to food *ad libitum*; the diet consisted of skimmed powder milk and 10 g of sugar in 100 ml of water in Petri dishes (Al-Shareef and Al-Qurashi 2016; Salazar-Souza et al. 2019). Larvae were placed in beakers within transparent boxes containing sand and sawdust to prevent the postfeeding larvae from escaping. Larvae were provided fresh rat muscle *ad libitum* as a rearing medium/substrate in the cages. Rats were supplied by the Animal Research Center. The rearing laboratory conditions were 25 ± 1 °C, with 75% RH and a photoperiod of 12 h/12 days (light/dark).

Experimental procedures

First instars hatched from an egg mass containing approximately 50–60 eggs were transferred directly and individually with the aid of a brush (number 0) to plastic vials (15 × 12 × 11 cm³) containing 20 g of normal untreated rat muscle. The vials were covered with muslin secured with a rubber band, and the muscle tissue was replaced daily with sterilized muscle shavings. The vials were kept in climatic chambers maintained at 20, 25, and 30 °C, with the aforementioned humidity and photoperiod. The experimental procedures were performed in three replicates under the temperature conditions of interest. The replicates were observed every 24 h, and the larvae and pupal weights (mg) and lengths (mm) were calculated and recorded.

The ambient temperatures range used in the present study were chosen to investigate the most affecting temperature within this range on the insect's developmental changes because the optimal temperature range for most species of blowflies (Diptera: Calliphoridae) is between 20 and 30 °C, with development and survival compromised at temperatures outside this range (Grassberger and Reiter 2001; Richards et al. 2008a). *C. albiceps* (Wiedemann, 1819) is reported to be the most temperature-tolerant species and has been found to be capable of surviving and developing at temperatures ranging from 11 to 50 °C (Richards et al. 2008b).

Measurements of body weights and lengths were carried out regularly every 24 h. Thirty randomly selected larvae were immersed in hot water (70–80 °C) for 3–5

min to prevent shrinkage before preservation in 75% alcohol according to the method described by Adams and Hall (2003). The weight of each dry larva was recorded by using a sensitive electrical balance with a sensitivity of 0.001 g⁻¹. The larval length was measured to the nearest 0.01 mm under a stereoscopic binocular microscope. In all replicates, larval samples from each temperature group were allowed to complete their development cycle to estimate the duration of all insect stages at all temperatures under investigation. The times of pupation and adult emergence were recorded in each group, and the group mean developmental period was calculated to assess longevity.

Statistical analysis

Data analysis was performed using one-way ANOVA (with a least significant difference (LSD) test), and significant differences were defined as those with $P < 0.05$. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) for Windows software, Release 22.0 (SPSS, Chicago, IL, USA).

Results

The data in Table 1 show the means and standard errors of *C. albiceps* larval body weights every 24 h at constant temperatures. There was a significant increase in the larval body weight in the group reared at 25 °C compared with the corresponding weights in larvae reared at 20 °C at 24, 48, 72, 96, and 120 h. However, the larvae exhibited a significant decrease in body weight at 120 h compared with the recorded body weights at 96 h in larvae reared at both 20 °C and 25 °C. The larval body weights of those reared at 30 °C were significantly higher than the body

Table 1 Effect of constant temperatures on the average of *Chrysomya albiceps* larval body weight (mg) (mean ± SE) at different durations

Hours	Temperature		
	20 °C	25 °C	30 °C
24	2.30 ± 0.15	3.00 ± 0.06	5.00 ± 0.09 ^a
48	9.40 ± 0.35	12.40 ± 0.22 [*]	30.20 ± 0.37 ^{*a}
72	36.60 ± 0.52	49.00 ± 0.39 [*]	85.30 ± 0.22 ^{*a}
96	43.03 ± 0.34	79.60 ± 0.78 [*]	78.00 ± 0.36 ^{*a#}
120	39.77 ± 0.23 [#]	71.90 ± 0.84 ^{*#}	Pupation
144	Pupation	Pupation	-
Onset time of pupa (h)	144	124	120

Data represented as mean ± SE, $n = 30$. Significance at 0.5 level represented by asterisk (*) as compared with weight at 20 °C. ^aSignificance at 0.5 level as compared with weight at 25 °C within the same row. [#]Comparison between the weight at 120 h with that at 96 h (at 20 °C and 25 °C) and between the weight at 96 h with that at 72 h (at 30 °C) within the same column

weights of those reared at 20 °C and 25 °C at 24 h, 48 h, 72 h, and 96 h. There was a significant decrease in body weight at 96 h compared with the body weight at 72 h. Larvae reared at 30 °C initiated pupation at 120 h, which was earlier than the time of pupation in those reared at 20 °C (144 h) and 25 °C (124 h).

Regarding the effect of temperature on larval body length (Table 2 and Fig. 1), the results showed a significant increase in the body length of larvae reared at 25 °C compared with the body length of larvae reared at 20 °C after 48 h, 72 h, and 120 h. Moreover, the larval body length of those reared at 30 °C was significantly higher than the corresponding body lengths of larvae reared at 20 °C and 25 °C at 24 h and 48 h. There was a significant decrease in body length compared with the corresponding body lengths of larvae reared at 20 °C and 25 °C at 96 h.

The data presented in Table 3 show the means and standard errors of pupal body weights, lengths, and durations of *C. albiceps* life stages at different constant temperatures. The mean pupal weight and length in those reared at 25 °C were significantly increased compared with those in pupae reared at 20 °C. The pupal duration was significantly decreased in those reared at 25 °C compared with that in those reared at 20 °C. The mean weight and length of pupae reared at 30 °C were significantly increased compared with those in pupae reared at 20 °C and 25 °C. The pupal duration was significantly decreased in those reared at 30 °C compared with that in those reared at 20 °C and 25 °C.

The results of the effects of temperature on the mean and standard error of *C. albiceps* adult longevity are presented in Table 4. In those reared at 25 °C, the average adult longevity was significantly decreased compared with that in those reared at 20 °C. In those reared at 30 °C, the average adult longevity was decreased significantly compared with the average adult longevity in those reared at 20 °C and 25 °C.

Table 2 Effect of constant temperatures on the average of *Chrysomya albiceps* larval body length (mm) (mean \pm SE) and the onset time of pupa (h) at different durations

Hours	Temperature		
	20 °C	25 °C	30 °C
24	4.59 \pm 0.07	4.79 \pm 0.07	5.56 \pm 0.06 ^a
48	7.47 \pm 0.07	8.44 \pm 0.12 [*]	8.93 \pm 0.15 ^a
72	11.87 \pm 0.19	13.25 \pm 0.65 [*]	13.38 \pm 0.16 [*]
96	13.12 \pm 0.17	13.20 \pm 0.14	12.25 \pm 0.10 ^a
120	11.22 \pm 0.15	12.79 \pm 0.12 [*]	-

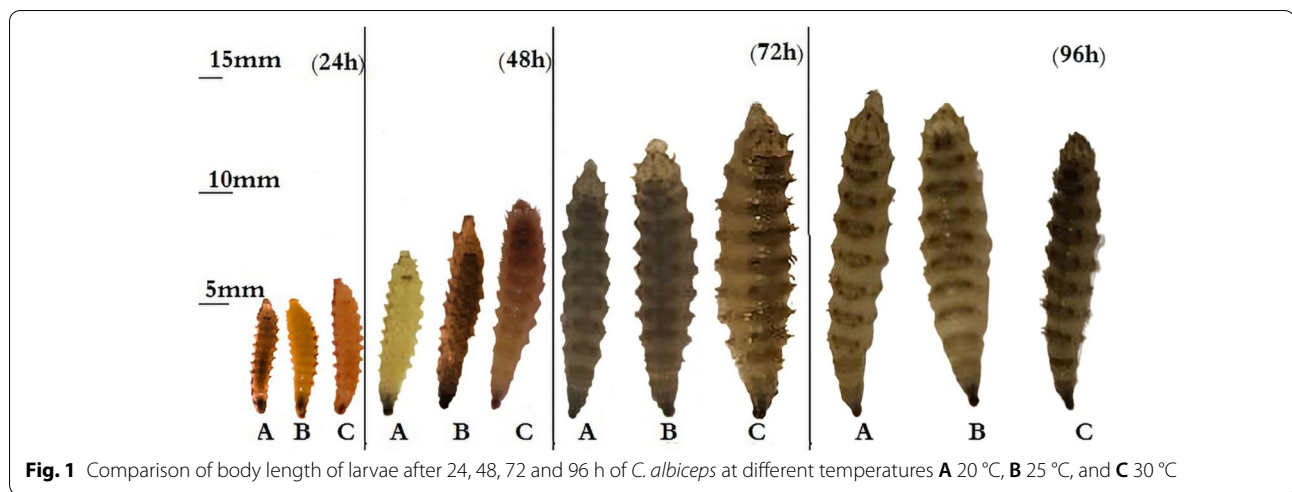
Data represented as mean \pm SE, $n = 30$. Significance at 0.5 level represented by asterisk (*) as compared with body length at 20 °C; ^a significance at 0.5 level as compared with weight at 25 °C within the same row

The results regarding the effects of temperature on the *C. albiceps* mean developmental duration in days at each stage are represented in Fig. 2. The larval period lasted 5.00, 5.00, and 4.00 days at 20 °C, 25 °C, and 30 °C, respectively. *C. albiceps* pupae and adults showed gradual decreases in developmental durations with increasing rearing temperatures. The pupal durations were 6.00, 5.30, and 4.80 days, and the adult durations were 20.00, 18.70, and 16.90 days at 20 °C, 25 °C, and 30 °C, respectively.

Discussion

The goal of the present study was to evaluate the effects of different ambient temperatures on the developmental stages of *C. albiceps* under laboratory conditions for the first time in the Jazan region, Saudi Arabia. The developmental data of forensically important *C. albiceps* under laboratory conditions should be considered reliable, as several studies demonstrated that prediction of the developmental rate at constant laboratory temperatures was quite similar to that under normal temperature conditions (Bohem 2015). Muscles were selected as the larval feeding source to provide optimal nutrition according to different studies that revealed that diet plays important roles in the development and developmental periods of forensically important *C. albiceps*. These studies also found that a diet of muscular tissue resulted in the fastest developmental rate at a constant temperature due to the availability of required nutrients (Rabêlo et al. 2011; Beuter and Mendes 2013; Thyssen et al. 2014). Regarding larval growth, the study showed that larval body weight gain and length were positively related to temperature increases from 20 to 25 °C to 30 °C. At 24, 48, 72, and 96 h, the larvae reared at 20 °C had slightly lower larval body weights, at 2.30, 9.40, 36.60, and 43.00 mg, than those reared at 25 °C at 3.00, 12.40, 49.00, and 79.60 mg, respectively. Then, the body weights decreased to 39.77 mg and 71.90 mg at 120 h in larvae reared at 20 °C and 25 °C, respectively. Larvae reared at 30 °C had significantly higher body weights than those reared at the aforementioned rearing temperatures. The larvae weighed 5.00 mg at 24 h and 30.20 mg at 48 h, with body weight peaking at 72 h (85.30 mg). The body weight was decreased at 96 h (78.60 mg), which is in line with earlier pupation in this group. Larval body length followed the same patterns as larval body weight; there was a significant increase in larval body length in larvae reared at 30 °C compared with that in those reared at 20 °C and 25 °C from 24 h until 72 h. The body length was decreased at 96 h.

These results may be explained by the nature of *C. albiceps*, which is a tropical and subtropical species that prefers high temperatures and humidity levels (Saleh et al. 2014; Greenberg and Povolny 2019); most insect species



have low activity levels at low temperatures (Scholtz and Caveney 1992). When substrate is available, a high ambient temperature may stimulate faster feeding by individual maggots, and the metabolic rate may be markedly increased, which could result in increased body weight and length. In addition, temperature has been suggested to affect the development through the larval central nervous system, promoting thermotaxis and an increased feeding rate due to the stimulation of temperature-sensitive neurons in the CNS of larvae that accelerate the larval growth (Hückerfeld et al. 2011).

The effects of temperature on larval growth in the present study are consistent with those in earlier studies on *C. albiceps* (Queiroz 1996; Vélez and Wolff 2008; Al-Shareef and Al-Qurashi 2016; Salimi et al. 2018), supporting that higher temperatures accelerate larval growth, as also observed in other forensically important flies, such as *Chrysomya megacephala* (Fabricius, 1794) (Bansode et al. 2016). The decrease in body length and weight at the last sampling period at each rearing temperature indicated the postfeeding phase, which is characteristic of the blowfly (Greenberg and Kunich 2002). The present study recorded the onset of pupation at 120 h in the larvae

reared at 30 °C, while the larval period extended to 144 h in the larvae reared at 20 °C and at 124 h in those reared at 25 °C. The larval developmental period decreased from 5 days at 20 °C and 25 °C to 4 days at 30 °C. Hence, the present results of the inverse relationship of the developmental period and temperature are in agreement with those previously reported for *C. albiceps*. Growth acceleration resulted in shortening of the developmental period due to an elevated minimum growth temperature threshold. Queiroz (1996) found that the basal temperature required for rearing *C. albiceps* under laboratory conditions at 60 + 10% RH and a 14-h photoperiod was 15.04 °C in the larval stage, 17.39 °C in the pupal stage and 15.38 °C in the larva-to-adult phase. Later studies that analyzed *C. albiceps* under four constant rearing temperatures (20, 25, 30, and 35 °C) reported total larval stage durations of 9, 6, 4.83, and 4.75 days, respectively (Al-Shareef and Al-Qurashi 2016). The larval stages of those reared at 25 °C and 30 °C were 6 and 4 days, respectively (Salimi et al. 2018); and the larval stages of those reared at 18, 22, 27, and 32 °C were 21.30, 10.61, 5.0, and 4.0 days, respectively (Queiroz 1996). These results are similar to those of studies on forensically important species of family Calliphoridae, such as *Chrysomya pinguis*

Table 3 Effect of different constant temperatures on *Chrysomya albiceps* pupal average body weight (g), length (mm), and duration (h) (mean ± SE)

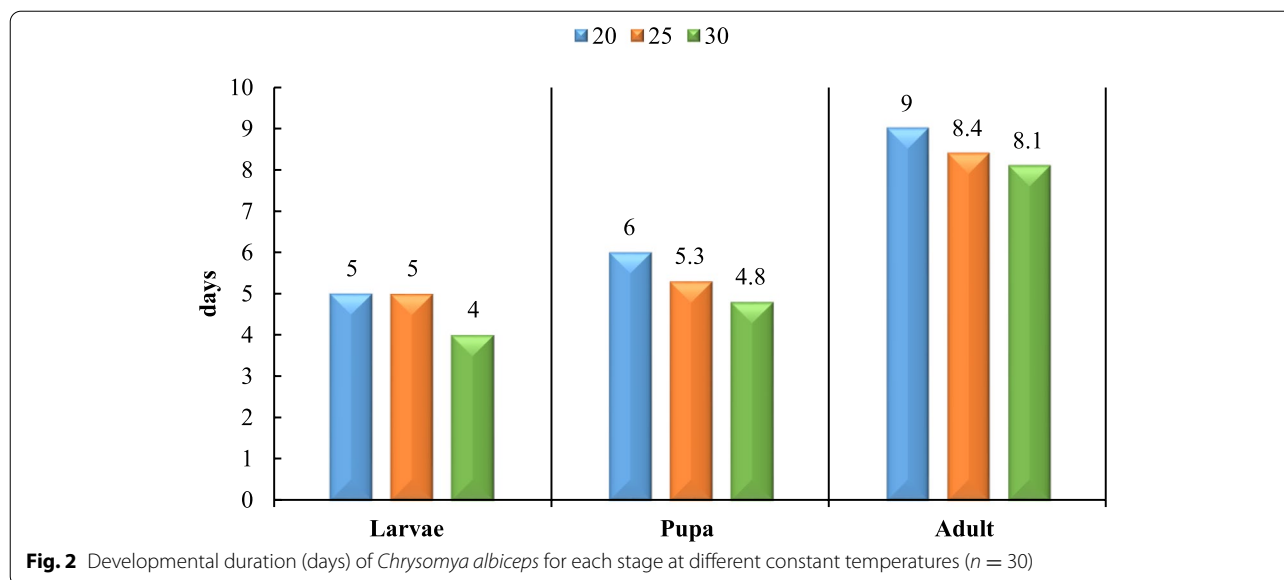
Temperature	Item		
	Weight	Length	Duration
20 °C	27.50 ± 0.52	7.92 ± 0.06	144.00 ± 1.68
25 °C	48.20 ± 0.25*	8.78 ± 0.09*	127.20 ± 1.62*
30 °C	62.03 ± 0.65 ^a	10.33 ± 0.09 ^a	115.20 ± 1.28 ^a

Data represented as mean ± SE, n = 30. Significance at 0.5 level represented by asterisk (*) as compared with the mean value at 20 °C. ^aSignificance at 0.5 level as compared with the mean value at 25 °C within the same column

Table 4 Effect of different constant temperatures on the average of *Chrysomya albiceps* adult longevity (h) at different constant temperatures

Temperature	Adult longevity (h) n = 10
20 °C	216.00 ± 0.00
25 °C	204.60 ± 4.00*
30 °C	194.40 ± 2.40 ^a

Data represented as mean ± SE. Significance at 0.5 level represented by asterisk (*) as compared with the mean value at 20 °C. ^aSignificance at 0.5 level as compared with the mean value at 25 °C



(times from egg to intrapuparial development at rearing temperatures ranging from 16 to 34 °C ranged from 388.00 to 91.20 h) (Zhang et al. 2019), *Lucilia cuprina* (time from egg to prepupal development at rearing temperatures of 20 °C, 20 °C, and 30 °C were 194.00, 128, and 83.50 h, respectively) (Bansode et al. 2016), and *Calliphora vicina* (Bharti 2009).

In the present study, increasing temperatures from 20 and 25 to 30 °C were inversely associated with the pupation period, and the lengths of the pupal period for *C. albiceps* at 20 °C, 25 °C, and 30 °C were 6.00, 5.30, and 4.80 days, respectively. The latter data are in line with those previously reported for *C. albiceps* in laboratory studies, where under four constant rearing temperatures at 20, 25, 30, and 35 °C, pupal periods of 7, 5.5, 4.00, and 1.50 days were recorded, respectively (Al-Shareef and Al-Qurashi 2016). Additionally, at 25 °C and 30 °C, the pupal periods were determined to be 5 and 3.5 days, respectively (Salimi et al. 2018). Under laboratory conditions (28 °C and 40% RH), the *C. albiceps* pupation period ranged from 4 to 6 days (Shiravi et al. 2011). Additionally, the maximum pupal periods recorded for *C. albiceps* were 5 and 4.5 days at 30 °C and 32 °C (Augul and Jassim 2009), and the developmental pupation periods recorded at rearing temperatures of 20, 25, 30, and 35 °C with 60% RH and 14:10 (light:dark) h were 12.90, 8.10, 5.9, and 4.6 days, respectively (Grassberger et al. 2003). A study on *Chrysomya megacephala* reported a reduction in the pupariation period from 168.00 h at 22 °C to 105.47 h at a rearing temperature of 31 °C (Yang et al. 2016). The present results showed that adults reared at laboratory temperatures of 20 and

25 °C had significantly increased longevity (216.00 and 204.00 h, respectively) and an increased adult duration, at 9.0 and 8.4 days, compared with those reared at 30 °C, where longevity was 194.40 h and the duration was 8.1 days, in line with previous studies (Al-Shareef and Al-Qurashi 2016; Salimi et al. 2018). Additionally, a study estimated that the biological upper limit for the complete development of *Sarcosenia chlorogaster* was approximately 31 °C (Lecheta et al. 2015). The present data from adults are in agreement with the study results of Augul and Jassim (2009), in which adult longevity at laboratory temperatures of 24, 30, and 32 °C in both sexes of *C. albiceps* decreased with increasing temperatures. Moreover, females did not deposit eggs at 24 °C, while females reared at 30 °C deposited eggs. From the recorded data of that study, the authors concluded that rearing at 30 °C was optimal for *C. albiceps*.

The results of the present study are applicable to real-life scenarios because Jazan is among the tropical cities with average temperature 30.7 ± 2.28 °C within the range of 23.0–35.1 °C (Al-Mekhlafi et al. 2021). Hence, the results of the present study confirmed the optimal rearing for *C. albiceps* at 30 °C and in line with that discussed in previous studies. In Nigeria, Ekanem and Dike (2010) identified *C. albiceps* and other flies at temperatures of 28.6 °C (unshaded area) and 26.5 °C (shaded area). Additionally, in South Africa, *C. albiceps* and others were recorded at a temperature of 30.4 °C (Richards et al. 2009). *C. albiceps* is a cosmopolitan species that is active in different regions with a similar temperature range, making it possible to compare its behavior in different locations.

Conclusions

The results showed an obvious inverse relation between developmental stage and rearing temperature, as the developmental duration in each insect stage decreased with increasing rearing temperatures. The shortest developmental durations were recorded at 30 °C, and the longest developmental durations were recorded at 20 °C in the larval, pupal, and adult stages. Hence, insect laboratory colonization can serve as a basis for different entomological studies, and determination of the biological aspects of insects is useful in forensic entomology to guide the interpretation of insect evidence. However, it is important to be cautious when applying insect data as evidence in forensic investigations.

Abbreviations

JUMRC: Medical Research Center; RH: Relative humidity (%); PMI: Postmortem interval.

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Author's contributions

The author declares that this research was designed experimentally and procedurally and written by the author. The author has read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

The Ethical approval applied and provided within submission Reference No.: REC-43/02/016.

Consent for publication

Not applicable.

Competing interests

The author declares that she has no competing interests.

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