Sains Malaysiana 53(6)(2024): 1255-1267
http://doi.org/10.17576/jsm-2024-5306-03

Post Mortem Interval Calculation of *Oryctolagus cuniculus* through Forensic Entomofauna – A Tool for Resolving Medicolegal Issues in Crime Scenes

(Pengiraan Sela Masa Pascakematian *Oryctolagus cuniculus* melalui Entomofauna Forensik – Suatu Alat untuk Menyelesaikan Isu Medikolegal di Tempat Kejadian Jenayah)

Mohammad Attaullah[1,*], Fahim Ullah[1], Imran Khan[1], Ikram IlahI[1], Muhammad Zahoor[2], Muhammad Amin[3], Riaz Ullah[4], Essam A. Ali[5], Arif Jan[6] & Sidra Bibi[1]

[1]*Department of Zoology, University of Malakand, Chakdara, Lower Dir, Pakistan*
[2]*Department of Biochemistry, University of Malakand, Chakdara, Lower Dir, Pakistan*
[3]*Department of Zoology, University of Karachi-75270, Karachi, Pakistan*
[4]*Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[5]*Department of Pharmaceutical Chemistry, College of Pharmacy King Saud University Riyadh Saudi Arabia*
[6]*Department of Fisheries, Wildlife, and Conservation Sciences, Oregon State University, Nash Hall 104, Corvallis, OR 97331, USA*

Received: 20 August 2023/Accepted: 10 May 2024

ABSTRACT

Post mortem interval (PMI) is an essential tool in crime scene investigations and is pivotal for resolving medicolegal issues. Developmental rates of insects can be utilized for the calculation of PMI of known and unknown animal and human cadavers. In the present study, PMI for rabbit, *Oryctolagus cuniculus* (Linnaeus 1758) was calculated through insect developmental rates and insect successional patterns on decomposing carcass of *O. cuniculus*. A total of 8 species of insects belonging to three orders and 8 families were identified on the *O. cuniculus* carcass. The blow fly, *Chrysomya albiceps* (Wiedemann 1819) belonging to the family Calliphoridae was the common and first visitor species to the rabbit carcass. The developmental rate of *C. albiceps* associated with the ambient climatic conditions was used as a model for the calculation of PMI. Temperature correction factor of the carcass temperature versus met station temperature was found as $y = 0.9185x + 3.3624$; $R^2 = 0.8343$. The accumulated degree days (∑ADD) were found as 535 while the accumulated degree hours (ADH) were found as 12842 with a resultant PMI of 7 days (ADD) and 167.4 h (ADH). Five decomposition stages of *O. cuniculus* were identified as fresh, bloated, active decay, advanced decay and dry remains stage. Findings of the present study can be applied for the calculation of PMI of known and unknown animal and human cadavers for resolving medicolegal issues in crime scenes.

Keywords: Calliphoridae; carcass; *Chrysomya albiceps*; degree days; forensic science

ABSTRAK

Sela masa pascakematian (PMI) sangat penting dalam penyiasatan tempat kejadian jenayah dan penting untuk menyelesaikan isu medikolegal. Kadar perkembangan serangga boleh digunakan untuk pengiraan PMI haiwan dan mayat manusia yang diketahui dan tidak diketahui. Dalam kajian ini, PMI untuk arnab, *Oryctolagus cuniculus* (Linnaeus 1758) telah dihitung melalui kadar perkembangan serangga dan corak berturutan serangga pada bangkai *O. cuniculus* yang mereput. Sebanyak 8 spesies serangga yang terdiri daripada tiga order dan 8 famili telah dikenal pasti terdapat pada bangkai *O. cuniculus*. Langau hijau, *Chrysomya albiceps* (Wiedemann 1819) daripada famili Calliphoridae adalah spesies pelawat biasa dan pertama kepada bangkai arnab. Kadar perkembangan *C. albiceps* yang dikaitkan dengan keadaan iklim ambien digunakan sebagai model untuk pengiraan PMI. Faktor pembetulan suhu karkas berbanding suhu stesen dipenuhi didapati sebagai $y = 0.9185x + 3.3624$; $R^2 = 0.8343$. Hari darjah terkumpul (∑ADD) didapati sebagai 535 manakala jam darjah terkumpul (ADH) didapati sebagai 12842 dengan PMI terhasil 7 hari (ADD) dan 167.4 jam (ADH). Lima peringkat penguraian *O. cuniculus* dikenal pasti sebagai peringkat segar, kembung, pereputan aktif, pereputan lanjutan dan peringkat sisa kering. Penemuan kajian ini boleh digunakan untuk pengiraan PMI haiwan dan mayat manusia yang diketahui dan tidak diketahui untuk menyelesaikan isu medikolegal di tempat kejadian.

Kata kunci: Bangkai; Calliphoridae; *Chrysomya albiceps*; hari darjah; sains forensik

Introduction

Calculation of PMI is an important step in resolving medicolegal issues in crime scenes. The developmental rates of forensically important insects found on dead bodies is commonly used for the calculation of PMI in the field of forensic entomology and forensic sciences (Mashaly & Ibrahim 2022). Insects are mostly used during death investigations to calculate the PMI for a variety of animals including human cadavers (Matuszewski 2021). Entomology possesses a great potential in the field of forensic investigations particularly because of its role in the estimations of minimum PMI (Bugelli et al. 2023). Pathological methods are helpful in the estimation of PMI in the initial stages of decomposition but in the later stages of decomposition, entomological indicators have been proved to be highly useful in the estimation of PMI (Bajerlein, Taberski & Matuszewski 2018). PMI can be estimated by two ways, first by using the developmental stages of flies found on corpses (Guo et al. 2023; Matuszewski & Madra-Bielewicz 2023; Zhang et al. 2023) and second through the successional patterns of carrion-arthropods, the type and composition of fauna change in predictable pattern as decomposition progresses through various stages (Akpa et al. 2021; Schoenly et al. 1996).

Ecological data of an insect that engaged in the decomposition process in a particular area must be known. Successional patterns of insect invasion may provide PMI signs over longer time intervals. For shorter periods, however, developmental rates of larvae are used (Nabity, Higley & Heng-Moss 2006). Medico-legal forensic entomology covers indication collected through arthropod studies at the scenes of killing, suicide, rape, physical misuse (Catts & Goff 1992). Due to the importance of insects in medico-legal investigations, the field of forensic entomology has grown during the past several decades (Weidner et al. 2021).

After death, the colonization of a corpse by insects persists during the evolution of decomposition from the first few minutes until the bones resemble the bleached white stage (Farag et al. 2021). There are five identifiable post mortem stages in a decomposing body as fresh, bloated, active decay, post decay and skeletonization or dry stage (Arnaldos et al. 2005).

The rate of decomposition is dependent upon the temperature. Forensic pathologists have devised a formula for estimating the time of body decomposition to a skeleton in relation to temperature (Vass et al. 1992) (Equation 1)

$$Y = \frac{1285}{X} \quad (1)$$

where Y is the number of days to skeletonization; and X is the average temperature for the days before the dead body was found (Vass et al. 1992).

For resolving medico-legal issues at the crime scenes, accurate PMI estimation is of paramount importance. Entomofaunal diversity has already been reported in Pakistan (Hussain & Umar 2021) while another study has only reported forensically important entomofauna in the study area (Ullah et al. 2019) without the application of forensically important insect fauna for the calculation of PMI. The calculation of PMI through forensically important insect fauna is a cheap, reliable, and reproducible method. To obtain a standardized estimate of the PMI, diverse studies are required using a variety of animal models in different ecosystems based on the ambient climatic conditions by using entomofauna of forensic importance. For this purpose, the present study was designed to calculate the PMI of *O. cuniculus* based on the developmental rates and successional patterns of forensically important insect fauna.

Materials and Methods

Study Area

The present study was conducted at Kakad, Wari, Dir Upper, Pakistan located between Latitude 34° 49’ 12.73” N and Longitude 72° 04’ 22.62” E with an altitude of 1000 m (3281 feet) above the sea level (Figure 1). The average annual temperature of Dir is 16.9 °C, July is the hottest month of the year with a mean temperature of 29.1 °C while the lowest mean temperature in the year is recorded in January as 4.2 °C.

Animal Model

A rabbit (*Oryctolagus cuniculus*) weighting 2 kg was sacrificed by killing through a shot gun with the help of a veterinary expert after seeking approval from the Ethical Committee of the University of Malakand vide No: E-SA-05-2019, the University of Malakand according to bye-laws 2008 Scientific Procedure Issue-I and permission from the Wildlife department, Dir Upper, Pakistan. The rabbit carcass was placed in a metal cage (0.3 m × 0.3 m × 0.6 m) and the cage was fixed to the ground using wood holders to prevent the carcass from being disturbed by other vertebrate scavengers. The study was conducted from June 24 to June 30, 2019.

INSECTS' COLLECTION AND IDENTIFICATION

Adult insects were collected using aerial and sweep nets above and around the rabbit carcass and placed in the killing jar containing chloroform. Insects were pinned using entomological pins and kept in the entomological boxes for further identification at the Entomology Laboratory, Department of Zoology, University of Malakand, Pakistan. Maggots and other immature stages were picked up by hand wearing gloves for preservation and identification. All specimens were labeled according to the date of specimen collection. The insect specimens were identified according to the previously reported identification keys (Akbarzadeh et al. 2015; Park & Moon 2020; Szpila 2012; Szpila, Richet & Pape 2015). Stages of *C. albiceps* were recorded directly on the carcass from first arrival and egg laying till emergence of the adult flies.

PMI ESTIMATION

The durations of various developmental stages of *C. albiceps* combined with the daily mean carcass temperatures were used for the calculation of ADD and subsequently the ADD data was used for the calculation of PMI of *O. cuniculus*. The same procedure can be applied for finding out PMI in unknown death scenes (Table 2). The rabbit carcass was regularly observed twice daily, once in the morning and once in the evening till complete decomposition. Five decomposition stages were recorded (fresh, bloated, active decay, advanced decay, and skeletonized stage) following (Martinez, Duque & Wolff 2007). Durations of each decomposition stage were noted and tabulated. Temperature and relative humidity of the carcass as well as the ambient environment were recorded daily using thermometer and psychrometer, respectively.

Accumulated degree days (ADD) or ⁰D were calculated by Equation (2):

$$Time_{(days)} \times (Temperature - Base\ Temperature) = ADD \quad (2)$$

Mean of the regression coefficients of the developmental rates vs. daily mean temperatures was used here instead of base temperature. This gives accurate results when applied to any sort of data in forensic entomology for the calculation of PMI.

Accumulated degree hours (ADH) or ⁰H were calculated by Equation (3):

$$Time_{(hours)} \times (Temperature - Base\ Temperature) = ADH \quad (3)$$

PMI (days or hours) was calculated by dividing the value of the sum of ADD or ADH on the mean temperature of the carcass in the study period. In case of ADD, the PMI was calculated by Equation (4):

$$PMI = \frac{\sum ADD}{Mean\ Temperature} \quad (4)$$

In case of ADH, PMI was calculated by Equation (5):

$$PMI = {}^{ADH}/_{Mean\ Temperature} \quad (5)$$

DATA ANALYSIS

The collected data was evaluated using mean, standard deviation, and regression analysis through Microsoft Excel Worksheet. Map of the study area was designed using ArcGIS (ver. 10.8).

RESULTS

Forensically important adult insects (n = 274) were collected during the decomposition of *O. cuniculus* and were then categorized into 03 different orders, 08 families and 08 genera of the class Insecta (Table 4). Five different decomposition stages of *O. cuniculus* were identified (Table 3; Figure 4). The overall daily mean values of the rabbit carcass temperature, met station temperature, wind speed and humidity were found to be 76.7±2.92 °F, 79.8±2.91 °F, 7.6±0.6 km/h and 30.8±1.5 %, respectively (Table 1).

Temperature correction factor calculated through regression of the *O. cuniculus* carcass temperature and met station temperature was found as y = 0.9185x + 3.3624; $R^2 = 0.8343$ (Figure 2). The corrected temperature values were used for the calculation of accumulated degree days (ADD) (Table 2). Biology of any insect can be used for the calculation of ADD. In this study, *C. albiceps* was first detected on the rabbit carcass on the fresh stage within 30-60 min post mortem. The daily mean corrected temperatures of *C. albiceps* from the egg laying stage on day first to the emergence of the first adult were used for the calculation of ∑ADD which was found as 535 (Table 2).

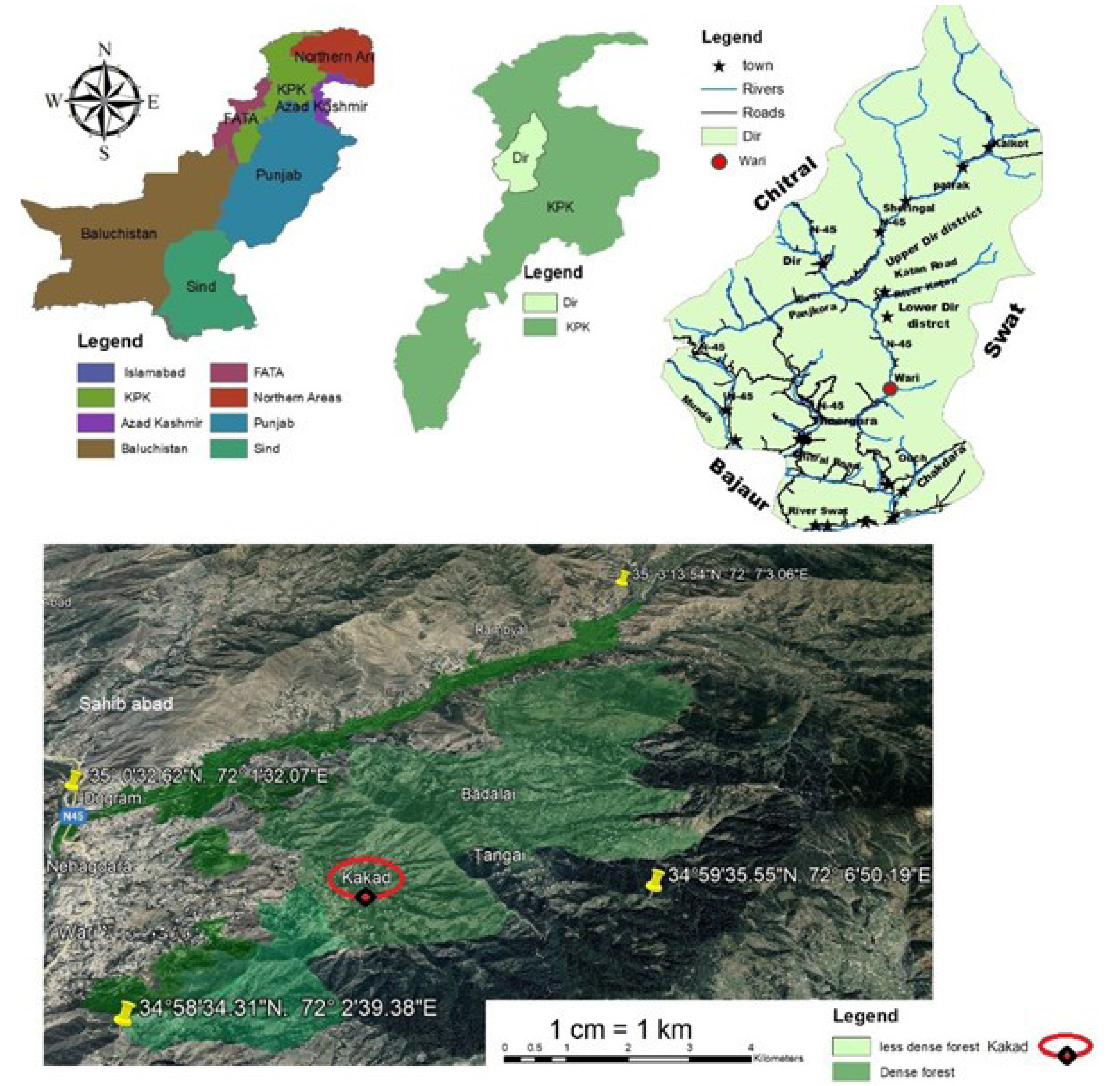

FIGURE 1. Map of the study area, Kakad, Wari, Dir Upper, Pakistan

Another important parameter for the calculation of ADD is the base temperature which can be calculated through regression of the developmental rate of any insect against the daily mean temperature of the meteorological station. In this study, we used the regression coefficient of the developmental rate of *C. albiceps* against the daily mean temperature of met station and the overall mean value calculated was 0.26 (Table 2; Figure 3).

Mean of the regression coefficients of the developmental rates vs. daily mean temperatures was taken here instead of base temperature. This gives accurate results when applied on any sort of data in forensic entomology for the calculation of PMI. The ADD in the present study was found as:

$$ADD = 7 (76.7 - 0.26) = 535$$

This value was the same as calculated through the temperature correction factor and regression coefficient of the developmental rates versus daily mean met temperatures 535.1 (Table 2).
In this case, ADH = 168 (76.7 - 0.26) = 535×24 = 12842

PMI (days) = 535/76.7 = 6.97 or 7 days.

PMI (hours) = 12842/76.7 = 167.4 h (= 7 days).

Total number of the forensically important adult insects collected from the *O. cuniculus* carcass was 274 with *Piophila casei* as the major visitor to the carcass (n = 74) followed by *C. albiceps* (n = 59), *M. domestica* (n = 42), *Hister* sp. (n = 36), *Monomorium pharaonis* (n = 29), *Wohlfahrtia magnifica* (n = 17), *Dermests maculatus* (n = 15); and *Dolichovespula* sp. (n = 02) (Tables 4 and 5). Five decomposition stages of *O. cuniculus* spanning over seven days were identified as the fresh stage, bloated stage, active decay stage, advance decay stage and the dry stage (Table 3; Figure 4).

The decomposition stage with the highest number of forensic insects was the bloated stage with 124 recorded insects followed by the active decay stage (n = 97), advance decay stage (n = 33), fresh stage (n = 17) and dry stage (n = 03) (Table 5). The collected insects belonged to 03 orders (Diptera, Coleoptera and Hymenoptera) and 08 families (Calliphoridae, Muscidae, Sarcophagidae, Piophilidae, Dermestidae, Histeridae, Vespidae and Formicidae) of the class Insecta (Table 4).

The first insect that visited the carcass was *C. albiceps* (Diptera: Calliphoridae). This can be observed within 30 to 60 min after death. The main species collected during the fresh stage (12 h period) were *Chrysomya albiceps* (n = 03), *Wohlfahrtia magnifica* (n = 11), *Piophila casei* (n = 03) (Table 5). The bloated stage lasted for two days and the collected forensic insects during this stage included *Chrysomya albiceps* (n = 27), *Wohlfahrtia magnifica* (n = 04), *Musca domestica* (n = 38) *Piophila casei* (n = 44), and *Monomorium pharaonis* (n = 11) (Table 5).

During the active decay stage (4 days old), the number of forensic insects included *C. albiceps* (n = 29), *M. domestica* (n = 04), *P. casei* (n = 27), *D. maculatus* (n = 10), *Hister* sp. (n = 25) and *Dolichovespula* sp. (n = 02) (Table 5). Advance decay stage was 6 days long and the collected adult insects included *W. magnifica* (n = 02), *D. maculatus* (n = 02), *Hister sp.* (n = 11) and *M. pharaonis* (n = 18) (Table 5). The dry stage was 7 days long and the only insect species that was collected at this stage was *D. maculatus* (n = 03) (Table 5) belonging to the order Coleoptera and family Dermistidae (Table 4). The resultant PMI calculated based on the developmental rate of *Chrysomya albiceps* and the climatic conditions during decomposition of *O. cuniculus* was 7 days (ADD) and 167.4 hours (ADH) (Figure 5).

TABLE 1. Climatic conditions (Mean±SD) during the decomposition of *O. cuniculus*

Day	Daily mean temp. (°F) of rabbit carcass	Daily mean temp. (°F) of met station, Kakad	Daily mean wind speed (km/h)	Daily mean humidity (%)
1	79	82	6.5	30
2	73	75	7.5	32
3	74	78	08	31
4	79	81	8	29
5	80	84	7.5	32
6	78	80	8.5	29
7	74	79	7.5	33
Mean±SD	76.7±2.9	79.8±2.9	7.6±0.6	30.8±1.5

TABLE 2. Calculation of ADD from the temperature data and developmental rate of *C. albiceps*

C. albiceps	Met data	Corrected Met temp.*	Regression Coefficient**	ADD***	∑ADD
	82	78.679	0.29	78.389	78.389
	75	72.2495	0.23	72.0195	150.4085
	78	75.005	0.24	74.765	225.1735
	81	77.7605	0.29	77.4705	302.644
	84	80.516	0.3	80.216	382.86
	80	76.842	0.28	76.562	459.422
	79	75.9235	0.24	75.6835	535.1055
Mean	79.8	76.7	0.26	535	535

*Corrected met data from the regression equation: $y = 0.9185x + 3.3624$; $R^2 = 0.8343$
**Regression coefficient of developmental rate of *C. albiceps* vs. daily mean temperature: $y = 0.0114x - 0.5072$; $R^2 = 0.0121$
*** ADD = Corrected met temp. minus base temperature multiplied by 1 day (ADD), 1 h (ADH)

TABLE 3. Decomposition stages with postmortem periods and climatic conditions of *O. cuniculus*

Decomposition stage	Post mortem (Days)	Temperature (^{0}F)			Relative humidity (%)
		Min	Max	Mean	
Fresh	0-0.5	70	86	78	30
Bloated	1-2	71	78	74	31
Active decay	3-4	73	75	74	30
Advance decay	4-6	72	88	80	30
Dry	6-7	69	79	74	31

TABLE 4. Abundance of the forensic insects associated with the decomposition of *O. cuniculus*

Order	Family	Genus	Species	Numbers
Diptera	Calliphoridae	*Chrysomya*	*albiceps*	59
	Muscidae	*Musca*	*domestica*	42
	Sarcophagidae	*Wohlfahrtia*	*magnifica*	17
	Piophilidae	*Piophila*	*casei*	74
Coleoptera	Dermestidae	*Dermestes*	*maculatus*	15
	Histeridae	*Hister*	sp.*	36
Hymenoptera	Vespidae	*Dolichovespula*	sp.*	2
	Formicidae	*Monomorium*	*pharaonis*	29
Total	08	08	08	274

*Identified up to the genus level

TABLE 5. Insect succession on rabbit carcass during different decomposition stages

Species	Decomposition stage					Total
	Fresh	Bloated	Active decay	Advance decay	Dry	
Chrysomya albiceps	3	27	29	0	0	59
Musca domestica	0	38	4	0	0	42
Wohlfahrtia magnifica	11	4	0	2	0	17
Piophila casei	3	44	27	0	0	74
Dermestes maculatus	0	0	10	2	3	15
Hister sp.	0	0	25	11	0	36
Dolichovespula sp.	0	0	2	0	0	2
Monomorium pharaonis	0	11	0	18	0	29
Total	17	124	97	33	3	274

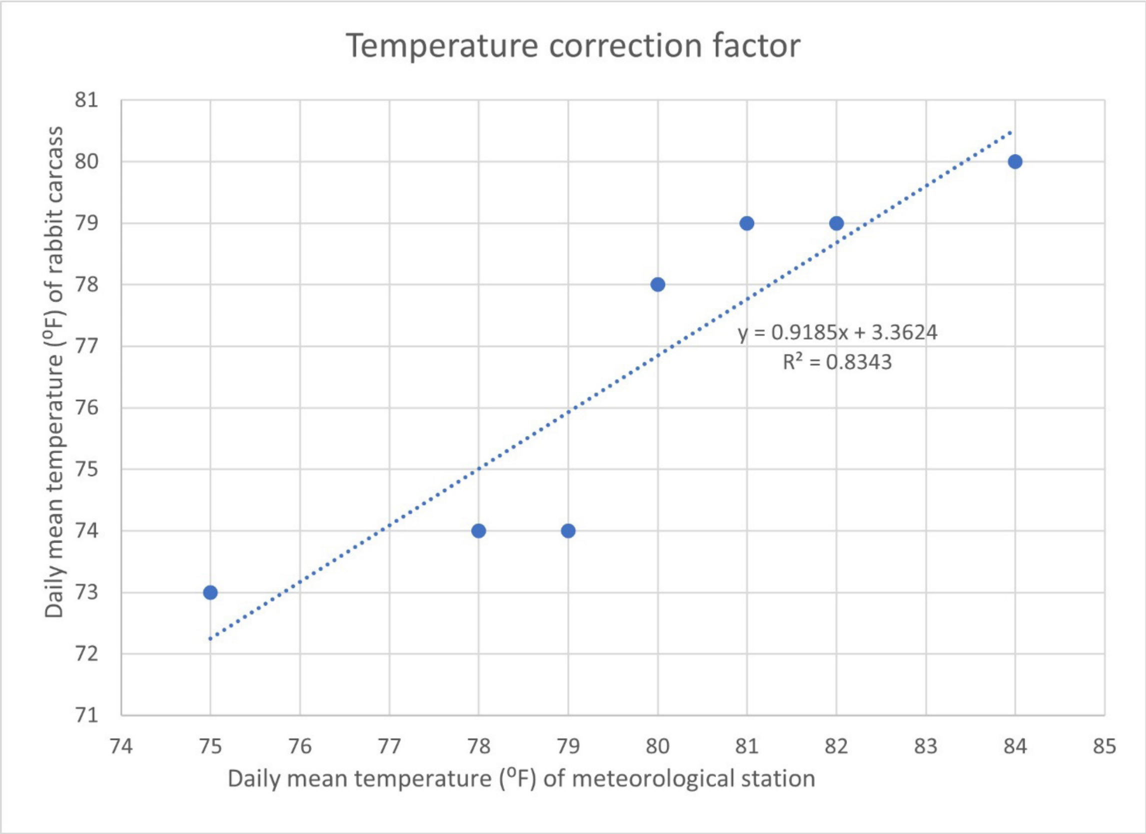

FIGURE 2. Temperature correction factor calculated through regression of the rabbit carcass temperature vs. meteorological station temperature

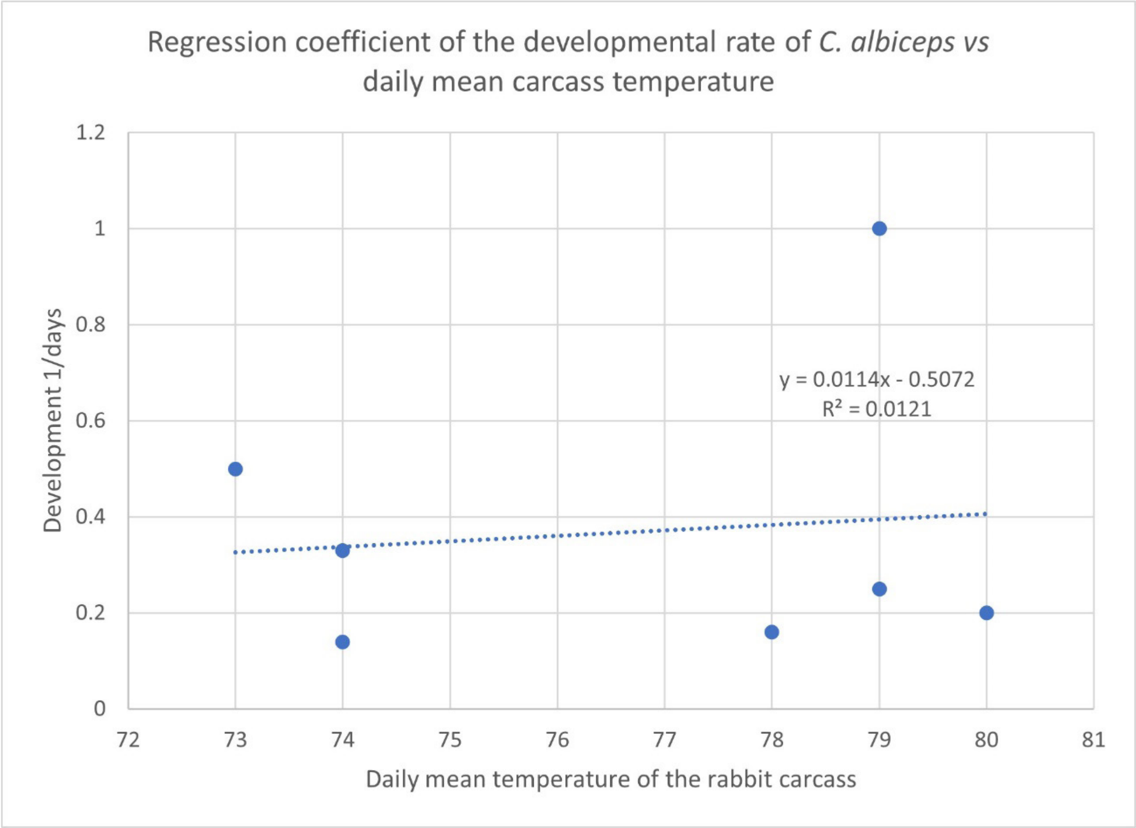

FIGURE 3. Regression coefficient of the developmental rate of *C. albiceps* vs. daily mean carcass temperature

DISCUSSION

The present study was conducted to calculate the PMI of *O. cuniculus* based on the developmental rates of forensic insects and the temperature of carcass as well as meteorological temperature data. Rabbit is a good animal model for forensic studies which is widely used in aquatic (Dalal et al. 2021) as well as terrestrial habitats (Oladejo et al. 2021). Biology, behavior, and distribution of various insect species associated with a decomposing carcass play a key role in the estimation of PMI (Goff 1993). There is a predictable and orderly pattern of insect colonization and succession of decomposing carcasses associated with the set of climatic conditions and stage of decomposition that is used as a baseline for the entomological estimation of PMI (Catts & Goff 1992).

In the present study, PMI was calculated by first finding out the temperature correction factor through regression of the *O. cuniculus* carcass temperature and met station temperature (Figure 2). The corrected temperature values were then used for the calculation of accumulated degree days (∑ADD) which were then used for the calculation of PMI (Table 2). The present study conforms with previous studies where the temperature data of the local weather stations has been corrected with the death scene data for the estimation of PMI (Hofer et al. 2020, 2017). This protocol has been supported by previous studies as one of the best tools for the estimation of PMI particularly in outdoor death scenes (Matuszewski 2021).

We used the blow fly, *C. albiceps* for the calculation of PMI on *O. cuniculus* carcass because it was first detected on the fresh stage of carcass within 30-60 min post mortem which conforms to previous studies where blow flies have been reported as the first colonizers on the carrions (Farag et al. 2021). Moreover, *C. albiceps* is a good model insect for the calculation of PMI (Bosly 2021; Gennard 2012). We used the regression coefficient of the developmental rate of *C. albiceps* against the daily mean temperature of met station and the overall mean value calculated was 0.26 (Table 2; Figure 3). This value was used as an alternative to the base temperature which gives highly accurate results for the calculation of ADD and PMI. Based on the developmental rates of *C. albiceps* and the carcass temperature along with the met station temperature, the resultant ADD was 535, ADH was 12842, and the PMI was 7 days (ADD) and 167.4 h (ADH) (Table 2; Figure 5). The value of PMI depends on the climatic conditions and varies according to the variations in

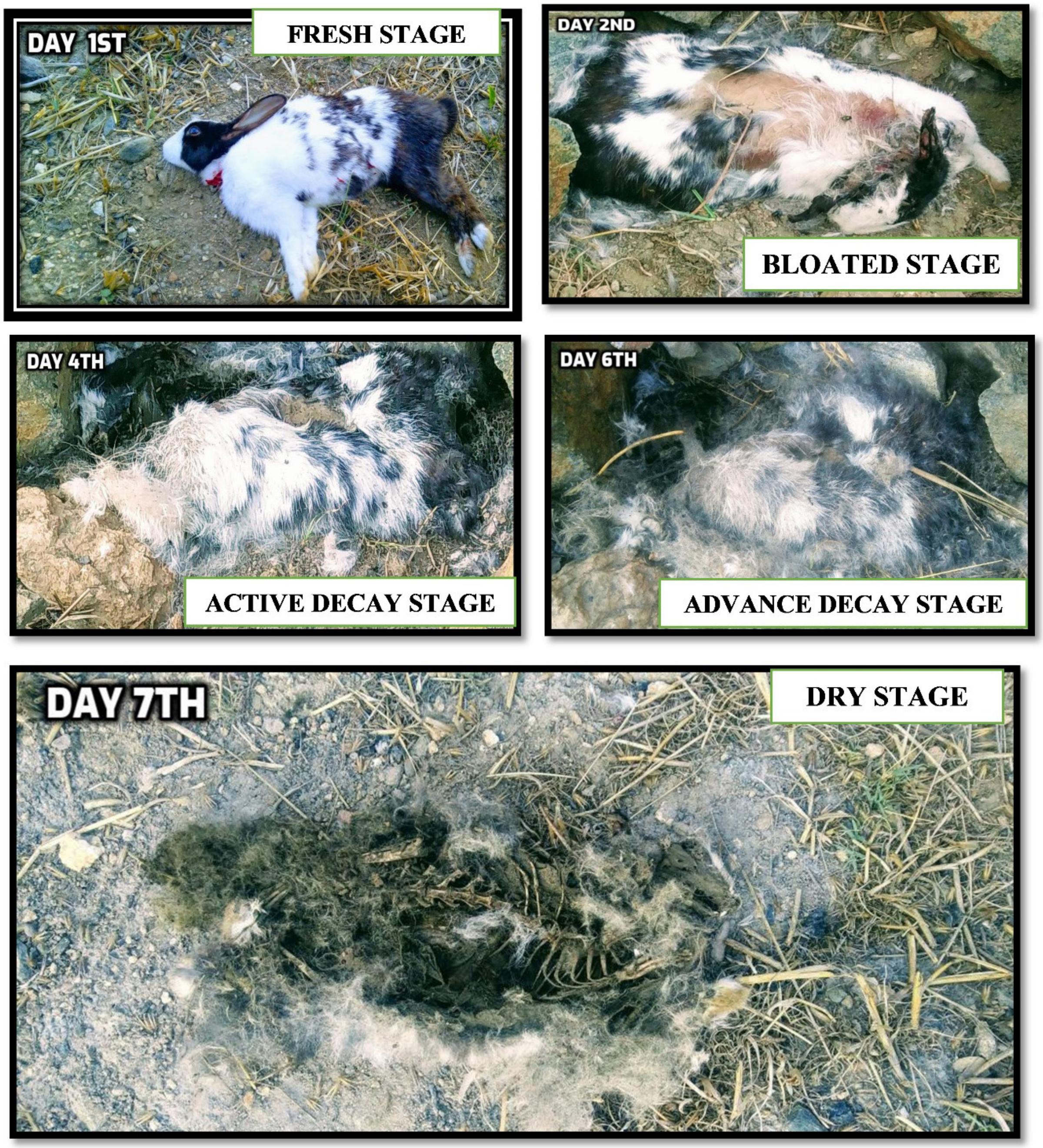

FIGURE 4. Decomposition stages of *O. cuniculus*, fresh, bloated, active decay, advance decay, and dry stage

climatic conditions (Li et al. 2016). The developmental rate of *C. albiceps* was calculated from the egg stage to the adult stage which conforms to a previous study (Marchenko 1985). The life cycle of *C. albiceps* on the decomposing carcass of *O. cuniculus* can be effectively applied in the calculation of PMI based on the ambient climatic conditions (Figure 5).

In the present study, insects belonging to 3 orders, 8 families and 8 genera were identified associated with the decomposition of the rabbit carcass. The results are different from a previous study (Maramat & Rahim 2015) where 15 species belonging to 6 families and 2 orders were identified associated with the decomposition of rabbit carcass. Another study (Farag et al. 2021) has reported forensic insects belonging to 3 orders, 9 families and 12 species. The reported insects belonged to the order Diptera, Coleoptera and Hymenoptera which conforms with the present study. Another study by Zeariya et al. (2015) has also reported variable number of forensic insects associated with two different carcasses of dog and rabbit. They categorized the collected insects into 03 orders, 05 families and 05 species during the decomposition process of the rabbit carcass. Succession of insects on carcasses is different from location to location and specific for specific environmental conditions (Payne 1965).

Calliphoridae, Muscidae, Piophilidae, and Sarcophagidae were the major families responsible for the decomposition of the rabbit carcass which supports the previous studies (De Jong & Chadwick 1999; Shi et al. 2009). Similar findings have been also reported by Abouzied (2014) with Calliphoridae, Muscidae and Sarcophagidae as the major families responsible for the decomposition but family Piophilidae was not reported. In the present study, members of the Calliphoridae were common in the initial stages of decomposition followed by members of the Sarcophagidae in the later stages of decomposition that conforms to a previous study (Carvallo & Linhares 2001).

In the present study, five decomposition stages of the *O. cuniculus* carcass were identified as the fresh, bloated, active decay, advance decay and dry remains stage which conforms to previous studies (Arnaldos et al. 2005; Martinez, Duque & Wolff 2007). Insect succession

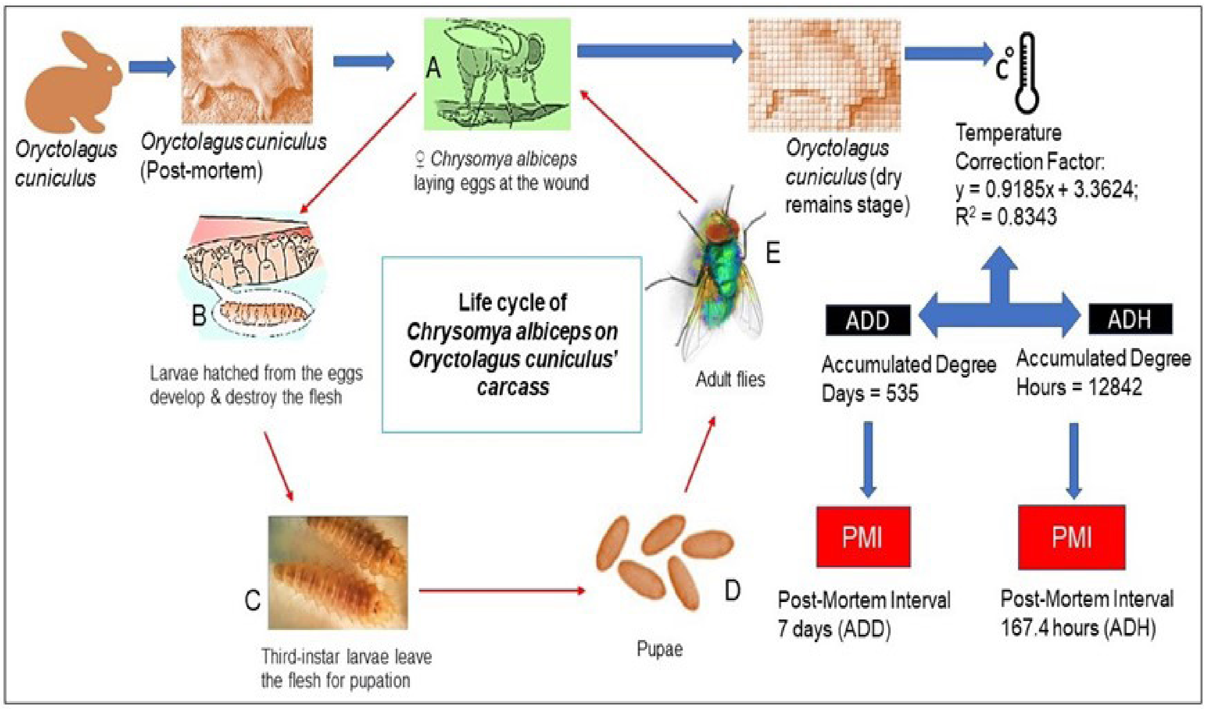

FIGURE 5. PMI calculated based on the life cycle of *C. albiceps* on *O. cuniculus* carcass

was higher in the bloated and active decay stages (Table 5) compared with other stages which supports the findings of Akpa et al. (2021) where they reported highest number of forensic insects on rabbit carcass in the active decay stage while the number of insects declined in the advanced decay stage. The fresh stage took 0.5 day during the present study which conforms to a previous study by Zeariya et al. (2015) who reported 0.5 day for the fresh stage of rabbit carcass at Egypt within a temperature range of 22 ºC to 33 ºC and a relative humidity of 56%. Another study by Al-Shareef and Al-Mazyad (2016) has reported 2 days for the fresh stage within a temperature range of 26 ºC to 32.8 ºC and 53.5% relative humidity. The differences in decomposition rates and stages are due to differences in temperature, age of corpse, structure, cause of death, ventilation, and humidity (Campobasso, Di Vella & Introna 2001).

Diptera species were the common species present in the fresh stage of rabbit decomposition which supports the previous findings by Zeariya et al. (2015). Few coleopterans were reported in the present study in the later stages of decomposition. Coleopterans arrive late in the decomposition process, so they do not lay eggs on the remains if the decomposition process is too short (e Castro et al. 2013) and the complete life cycle of coleopterans take place almost two months (Kulshrestha & Satpathy 2001) which mostly exceeds the decomposition period of the corpses.

Conclusions

Calculation of PMI through forensically important insect fauna is one of the most authentic and cheap techniques compared with other methods in use. Rabbit, *O. cuniculus* is the best animal model for the calculation of PMI through insect successional patterns and developmental rates. The blow fly, *C. albiceps* is a model insect for the calculation of PMI in the field of forensic entomology. The present findings can be used for the calculation of PMI in known and unknown death scenes. Temperature correction factors of the met station temperature and decomposing carcass temperature can be used effectively for the calculation of PMI through regression analysis. Findings of the present study can be used as a reference for calculating PMI in known and unknown animal and human models by using the developmental rates of forensically important insects with reference to the climate data of a given habitat or crime scene.

Acknowledgements

The authors extend their appreciation to the researchers supporting Project Number (RSP2024R45) King Saud University, Riyadh, Saudi Arabia, for financial support.

References

Abouzied, E.M. 2014. Insect colonization and succession on rabbit carcasses in southwestern mountains of the Kingdom of Saudi Arabia. *Journal of Medical Entomology* 51: 1168-1174. https://doi.org/10.1603/me13181

Akbarzadeh, K., Wallman, J.F., Sulakova, H. & Szpila, K. 2015. Species identification of Middle Eastern blowflies (Diptera: Calliphoridae) of forensic importance. *Parasitology Research* 114: 1463-1472. https://doi.org/10.1007/s00436-015-4329-y

Akpa, H.O., Tongjura, J.D.C., Amuga, G.A. & Ombugadu, R.J. 2021. Postmortem evaluation of rabbit carcasses using insect populations in Keffi Nasarawa State, Nigeria. *European Journal of Biology and Biotechnology* 2: 6-9. https://doi.org/10.24018/ejbio.2021.2.6.247

Al-Shareef, L.A. & Al-Mazyad, M.M. 2016. Insect faunal succession on decaying rabbit carcasses in urban area at Jeddah city, Kingdom of Saudi Arabia. *American Journal of Science* 12: 78-88. http://www.dx.doi.org/10.7537/marsjas121216.11

Arnaldos, M.I., Garcia, M.D., Romera, E., Presa, J.J. & Luna, A. 2005. Estimation of postmortem interval in real cases based on experimentally obtained entomological evidence. *Forensic Science International* 149: 57-65. https://doi.org/10.1016/j.forsciint.2004.04.087

Bajerlein, D., Taberski, D. & Matuszewski, S. 2018. Estimation of postmortem interval (PMI) based on empty puparia of *Phormia regina* (Meigen) (Diptera: Calliphoridae) and third larval stage of *Necrodes littoralis* (L.) (Coleoptera: Silphidae) - Advantages of using different PMI indicators. *Journal of Forensic and Legal Medicine* 55: 95-98. https://doi.org/10.1016/j.jflm.2018.02.008

Bosly, H.A.E.K. 2021. Development of *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) from the Jazan region of Southwest Saudi Arabia under different laboratory temperatures: applications in forensic entomology. *Egyptian Journal of Forensic Science* 11: 1-8. http://dx.doi.org/10.1186/s41935-021-00245-3

Bugelli, V., Tarozzic, I., Galante, N., Bortolini, S. & Franceschetti, L. 2023. Review on forensic importance of myiasis: Focus on medicolegal issues on post-mortem interval estimation and neglect evaluation. *Legal Medicine* 63: 102263. https://doi.org/10.1016/j.legalmed.2023.102263

Campobasso, C.P., Di Vella, G. & Introna, F. 2001. Factors affecting decomposition and Diptera colonization. *Forensic Science International* 120: 18-27. https://doi.org/10.1016/S0379-0738(01)00411-X

Carvallo, L.M.L. & Linhares, A.X. 2001. Seasonality of insect succession and pig carcass decomposition in a natural forest area in south eastern Brazil. *Journal of Forensic Sciences* 46: 604-608. https://pubmed.ncbi.nlm.nih.gov/11372997/

Catts, E.P. & Goff, M.L. 1992. Forensic entomology in criminal investigations. *Annual Reviews of Entomology* 37: 253-272. https://doi.org/10.1146/annurev.en.37.010192.001345

Dalal, J., Sharma, S., Bhardwaj, T., Dhattarwal, S.K. & Verma, K. 2021. A seasonal study of the decomposition pattern and insects on submerged rabbit carcasses. *Oriental Insects* 55: 280-292. https://doi.org/10.1016/j.jflm.2020.102023

De Jong, G.D. & Chadwick, J.W. 1999. Decomposition and arthropod succession on exposed rabbit carrion during summer at high altitudes in Colorado, USA. *Journal of Medical Entomology* 36(6): 833-845. https://doi.org/10.1093/jmedent/36.6.833

e Castro, C.P., García, M.D., da Silva, P.M., e Silva, I.F. & Serrano, A. 2013. Coleoptera of forensic interest: A study of seasonal community composition and succession in Lisbon, Portugal. *Forensic Science International* 232(1-3): 73-83. https://doi.org/10.1016/j.forsciint.2013.06.014

Farag, M.R., Anter, R.G., Elhady, W.M., Khalil, S.R., Abou-Zeid, S.M. & Hassanen, E.A. 2021. Diversity, succession pattern and colonization of forensic entomofauna on indoor rat carrions concerning the manner of death. Rendiconti Lincei. *Scienze Fisiche e Naturali.* 32: 521-538. http://dx.doi.org/10.1007/s12210-021-01009-w

Gennard, D. 2012. *Forensic Entomology: An Introduction*. John Wiley & Sons. ISBN 978-0-470-68902-8.

Goff, M.L. 1993. Estimation of postmortem interval using arthropod development and successional patterns. *Forensic Science Reviews* 5: 81-94. https://pubmed.ncbi.nlm.nih.gov/26270076/

Guo, Y., Hu, G., Li, L., Liao, M., Wang, J., Wang, Y. & Tao, L. 2023. Developmental indicators of *Chrysomya nigripes* Aubertin under different constant temperature conditions and an application case for estimating the PMImin. *Insects* 14: 729. https://doi.org/10.3390/insects14090729

Hofer, I.M., Hart, A.J., Martín-Vega, D. & Hall, M.J. 2020. Estimating crime scene temperatures from nearby meteorological station data. *Forensic Science International* 306: 110028. https://doi.org/10.1016/j.forsciint.2019.110028

Hofer, I.M., Hart, A.J., Martín-Vega, D. & Hall, M.J. 2017. Optimising crime scene temperature collection for forensic entomology casework. *Forensic Science International* 270: 129-138. https://doi.org/10.1016/j.forsciint.2016.11.019

Hussain, M. & Umar, M. 2021. Faunistic analysis of insects of Deva Vatala National Park and agroecosystem of Gujrat Pakistan. *Kuwait Journal of Science* https://doi.org/10.48129/kjs.15801

Kulshrestha, P. & Satpathy, D.K. 2001. Use of beetles in forensic entomology. *Forensic Science International* 120: 15-17. https://doi.org/10.1016/s0379-0738(01)00410-8

Li, L., Wang, Y., Wang, J., Ma, M. & Lai, Y. 2016. Temperature-dependent development and the significance for estimating postmortem interval of *Chrysomya nigripes* Aubertin, a new forensically important species in China. *International Journal of Legal Medicine* 130: 1363-1370. https://doi.org/10.1007/s00414-016-1315-6

Maramat, R. & Rahim, N.A.A. 2015. Forensically important flies associated with decomposing rabbit carcasses in mangrove forests in Kuching, Sarawak, Malaysia. *Forensic Science* 6: 79-83.

Marchenko, M.I. 1985. Characteristic of development of the fly *Chrysomya albiceps* (Wd.) (Diptera, Calliphoridae). *Entomologicheskoe Obozrenie* 64: 79-84.

Martinez, E., Duque, P. & Wolff, M. 2007. Succession pattern of carrion-feeding insects in Paramo, Colombia. *Forensic Science International* 166: 182-189. https://doi.org/10.1016/j.forsciint.2006.05.027

Mashaly, A. & Ibrahim, A. 2022. Forensic entomology research in Egypt: A review article. *Egyptian Journal of Forensic Science* 12: 11. http://dx.doi.org/10.1186/s41935-022-00272-8

Matuszewski, S. 2021. Post-mortem interval estimation based on insect evidence: Current challenges. *Insects* 12: 314. https://doi.org/10.3390/insects12040314

Matuszewski, S. & Mądra-Bielewicz, A. 2023. Field validation of post-mortem interval estimation based on insect development. Part 1: Accuracy gains from the laboratory rearing of insect evidence. *Forensic Science International* 354: 111902. https://doi.org/10.1016/j.forsciint.2023.111902

Nabity, P.D., Higley, L.G. & Heng-Moss, T.M. 2006. Effects of temperature on development of *Phormia regina* (Diptera: Calliphoridae) and use of developmental data in determining time intervals in forensic entomology. *Journal of Medical Entomology* 43(6): 1276-1286. https://doi.org/10.1093/jmedent/43.6.1276

Oladejo, A.O., Musa, I., Sikiru, G.K., John, W.C., Olori-Oke, O.O., Olorundare, O.O., Mudi, A. & Adedire, O. 2021. Succession pattern of insect of forensic importance on rabbit carrion at three locations in Federal College of Forestry of Jos, Plateau State, Nigeria. *Russian Journal of Agricultural and Socio-Economic Sciences* 9(117): 150-158.

Park, S.H. & Moon, T.Y. 2020. Carrion beetles (Coleoptera, Silphidae) of potential forensic importance and their pictorial identification key by user-friendly characters in Korea. *Korean Journal of Legal Medicine* 44: 143-149. http://dx.doi.org/10.7580/kjlm.2020.44.4.143

Payne, J.A. 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology* 46: 592-602. https://doi.org/10.2307/1934999

Schoenly, K., Goff, M.L., Wells, J.D. & Lord, W.D. 1996. Quantifying statistical uncertainty in succession-based entomological estimates of the postmortem interval in death scene investigations: A simulation study. *American Entomologist* 42: 106-112. https://doi.org/10.1093/ae/42.2.106

Shi, Y.W., Liu, X.S., Wang, H.Y. & Zhang, R.J. 2009. Seasonality of insect succession on exposed rabbit carrion in Guangzhou, China. *Insect Science* 16: 425-439. http://dx.doi.org/10.1111/j.1744-7917.2009.01277.x

Szpila, K. 2012. Key for identification of European and Mediterranean blowflies (Diptera, Calliphoridae) of medical and veterinary importance-adult flies. In *Forensic Entomology, An Introduction*, edited by Gennard, D. Chichester: Willey-Blackwell. pp. 77-81.

Szpila, K., Richet, R. & Pape, T. 2015. Third instar larvae of flesh flies (Diptera: Sarcophagidae) of forensic importance - Critical review of characters and key for European species. *Parasitology Research* 114: 2279-2289. https://doi.org/10.1007/s00436-015-4421-3

Ullah, H., Attaullah, M., Ilahi, I., Ahmad, S., Ali, H., Dad, O., Ali, L., Hussain, A., Ullah, N. & Ahmad, A. 2019. Entomofauna of forensic importance on *Canis domesticus* carcasses at Dir Lower, Pakistan. *International Journal of Biosciences* 15(2): 532-538. http://dx.doi.org/10.12692/ijb/15.2.532-538

Vass, A.A., Bass, W.M., Wolt, J.D., Foss, J.E. & Ammons, J.T. 1992. Time since death determinations of human cadavers using soil solution. *Journal of Forensic Science* 37: 1236-1253. https://pubmed.ncbi.nlm.nih.gov/1402750/

Weidner, L.M., Meeds, A.W., Noblesse, A.P. & Hans, K.R. 2021. A review of forensic entomology literature in the southwestern United States. *Wiley Interdiscip. Reviews in Forensic Science* 3: e1421. http://dx.doi.org/10.1002/wfs2.1411

Zeariya, M.G., Hammad, K.M., Fouda, M.A., Al-Dali, A.G. & Kabadaia, M.M. 2015. Forensic -insect succession and decomposition patterns of dog and rabbit carcasses in different habitats. *Journal of Entomology and Zoology Studies* 3: 473-482.

Zhang, Y., Li, L., Liao, M., Kang, C., Hu, G., Guo, Y., Wang, Y. & Wang, J. 2023. Development of *Megaselia scalaris* at constant temperatures and its significance in estimating the time of death. *International Journal of Legal Medicine* 138(1): 97-106. https://doi.org/10.1007/s00414-023-02993-4

*Corresponding author; email: m.attaullah@uom.edu.pk