Original Article

Determination of the post-mortem interval using forensically important Sarcophagidae

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ABSTRACT

Background: Estimating the age of Sarcophagidae' pupa is a challenge in forensic entomology because the postmortem anatomical and morphological changes are not easily determined. Therefore, accurate estimation of pupal age is crucial for assessing the minimum postmortem interval (minPMI).
Objective: To detect the insect concerned with decomposition of dead animal remains in the Egyptian environment, specifically focusing on the life stages at the time of collection and the time it takes for the colonizing insects to complete their development.
Material and Methods: *Sarcophaga* life cycle stages collected from distributed animal tissues and buried animal carcasses were identified. Their characteristics were recorded microscopically, and the duration needed for the initial colonizing insects to develop and reach their full size or life stage at the time of collection was determined.
Results: Collected Sarcophagidae larvae, pupae, and adults were identified. Estimation of the periods of their emergence and climate conditions showed that three larval instars appeared from day 2 till day 8, then pupae appeared on day 8 and finally adults emerged at day 16. The pupal period constituted approximately 50% of immature development.
Conclusion: Determining the pre-pupal and intra-puparial development of household Sarcophagidae enables reliable estimation of minPMI which is valuable in forensic entomology.

Keywords: flesh flies; forensic entomology; larval instars; PMI, pupa; Sarcophagidae.

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INTRODUCTION

Forensic entomology is a crucial field within forensic science that primarily focuses on estimating the time since death, *i.e.*, the post-mortem interval (PMI). This is accomplished through the identification of decomposition stages, the pattern of arthropod succession, or by estimating the age of the immature stages of the arthropods present at the crime scene[1]. Nowadays, entomological evidence has become increasingly important in death investigations. However, one of the obstacles preventing the wider use of entomological evidence is the difficulty in finding qualified forensic experts capable of identifying insect samples collected at crime scenes[2].

During the early stages of carcass decomposition, *i.e.*, typically within the first 3 to 72 h after death, the minPMI is usually estimated forensically[3]. However, as time progresses beyond this initial period, the presence and development of fly eggs, larvae, and pupae become one of the most reliable indicators for determining carcass' age[4,5]. The timing of colonization can be influenced by various factors, such as the season, and whether the body is exposed or concealed. Depending on these environmental conditions, the colonization may not occur immediately after death. The delay in colonization can affect the accuracy of the minPMI estimation based on the insect's developmental stages[6].

In fact, insects are known to colonize a body in a semi-predictable manner if sufficient access is available. The Calliphoridae (blowfly) family typically arrives first, followed by other families whose larvae feed on more decomposed tissues, such as Muscidae (house flies) or Silphidae (carrion beetles). Some families, like Phoridae (scuttle flies), may appear at multiple stages of the decomposition process. In addition, flies from the Calliphoridae, Sarcophagidae, and Muscidae families have received considerable attention in the field of forensic entomology. Since Dipteran insects are among the first to colonize dead bodies, their developmental stages are used to estimate minPMI[6]. It corresponds to the time when the body was first colonized by insects, which may not always be immediately after death. Additionally, data regarding the insects' biology, seasonal patterns,

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distribution, and habitat preferences are utilized in forensic entomology to answer investigative questions. This includes determining the season of death, especially in cold/old cases, as well as identifying if a body has been moved from its original location to a secondary site. Furthermore, global climate change and increased globalization are impacting the distribution of insect species, which can influence the interpretation of entomological evidence collected from crime scenes[7].

The pupal stage of necrophagous (carrion-feeding) flies is a crucial developmental phase that can last for up to half the total duration of the insect's immature development. This pupal period is highly significant when estimating the minPIM, as it represents a substantial portion of the insect's life cycle on the decomposing remains[8].

There are two recognized ways to estimate PMI using entomological data. One is by predicting the sequences of arrival and colonization of different species of insects on a dead body (long PMI). The second is by determining the age of immature flies (short PMI) that can be assessed by calculating the duration of larval development or body lengths or weights[9]. The guidelines for collecting insect evidence at a death scene stated that the sample should accurately reflect the insect fauna, or entomofauna, associated with the cadaver or meat pieces[10]. The targets of the present research were to detect the minPMI through exposure of dead bodies or animal tissues to environmental flies and morphological identification of their different stages through natural or laboratory rearing.

MATERIAL AND METHODS

This descriptive study was performed at Parasitology Department laboratory, Faculty of Medicine, Benha University, during the period from July 2023 till September 2023.

Study design: The study determined minPMI under extra- and intra-laboratory environmental conditions. The study utilized market-procured meat pieces that were distributed outdoors in open dishes, and inside a plastic bag with wide pored meshes to detect life cycle stages of already attracted flies. Albino rats, and meat pieces were buried under the ground, and the recorded data were analyzed with the results of meat pieces distributed outdoors. Emergence of larvae was monitored by daily examination. Emerged larvae were collected and maintained in the laboratory for 1) morphological identification of species, and 2) estimation of time required for each developmental lifestage. Albino rats, and meat pieces were buried under the ground, and the recorded data were analyzed with the results of meat pieces distributed in outdoors.

Samples collection: In the extra-laboratory experiment, meat pieces were collected in plastic bags from open air markets in Qalioubyia province, in Qalioub city as well as the livestock shopping center, and close vicinity of slaughter houses. Regarding the buried albino rats and meat pieces, larvae and pupae were collected from the surrounding shifted soil.

Rearing of emerged larvae (intra-laboratory experiment): Appearing larvae on exposed, and buried tissues were collected and transferred in specific cages made of 3 transparent plastic walls covered by a metallic net for ventilation. A thin layer of sterilized soil (2.5 cm) was placed in the cage bottom with a piece of 25 mg beef meat for larval nutrition. The cage containing 30-40 larvae was kept in an incubator (30-35°C) or at room temperature, and 75% relative humidity, as measured by digital hygrometer, and photoperiod of 12 h light, 12 h dark. About 10 newly hatched larvae were randomly collected from the larval rearing cage every 24 h. For measuring the length of the larvae, they were placed in hot water (70-80^{0}C) for 3–5 min and preserved in 75% alcohol to prevent larval shrinkage. Body length of the larvae was measured to the nearest 0. 1 mm under a binocular stereoscope. Similarly, an approximate number of larvae were transferred to minced meat and bred under controlled temperature in the laboratory until the adult stage.

To induce larviposition, pieces of fresh meat (50 gm each) were placed in a 10 cm culture dish and in rearing cages in room temperature and 75% humidity until pupation. A total of 20 pupae were sampled every 24 h until adult emergence. Adult flies were killed by freezing at –20°C and then stored in 96% ethanol. Since adults commonly gather on sweet substances for feeding, the cage was supplied with skimmed powder milk and 10 gm sugar in 100 ml water in a Petri dish.

Morphological identification: The morphological features and description are based on previous reports and keys[11-14]. About 10 larvae were randomly collected from larval rearing cage, immersed in 70-95°C hot water for 3-5 min, fixed in 70% alcohol and their body length measured under binocular stereoscope. A Nikon stereomicroscope and camera of smart phone (Galaxy S 21) were used.

Data recorded under intra- and extra-laboratory environments

- Intra-laboratory experiment included rearing emerged larvae in the laboratory up to the adult stage. Estimation of development time of each stage was assessed according to regular observations every 4 h during daytime and once at 10.00 pm then once at 6.00 am during night time[16].
- In extra-laboratory experiment, 50 meat pieces were distributed outdoors, and similar number of camel meat pieces buried in two male albino rats (>25

gm)[15] were buried under the ground in different areas and climate conditions. Protecting the buried tissues from predators was by burying them under the ground (30 cm depth), covering them by physical barriers and adding substances like wood chips, which mask the odor of buried tissues from predators. Period from burial of animal tissues till appearance of 1st larval instar as well as duration of larval instars 1st, 2nd, 3rd, and total larval duration, pupal period and the period from 1st larval instar to adult emergence were determined. In addition, larvae length and weight as well as pupa weight were measured every 24 h relative to periods of exposure.

- In both experiments, the stagnant stages (larvae and pupae) were trapped using plastic bottle fly traps or collected manually from decayed meat baits. Adults were collected by nets or adhesive tapes.

Statistical analysis: Statistical Package for the Social Sciences (SPSS) version 23.0 for windows (SPSS Inc., Chicago, IL, USA) was used. The normality of distribution for the analyzed variables were tested using Shapiro test assuming normality. The collected data were analyzed in terms of mean and standard deviation for parametric data, and median and range for nonparametric data. Student *t* and Mann Whitney tests were used for comparison between the results of larvae and pupae of distributed meat, and buried dead animals (intra- and extra-laboratory experiment, respectively). A *P* value less than 0.05 was considered significant.

Ethics approval: The animal study protocol was approved by the Research Ethics Committee (REC), Faculty of Medicine, Benha University, Egypt (Research RC 13-7-2023).

RESULTS

Meat distributed in open wide dishes showed incomplete small maggots while the plastic bags distributed outdoors showed fully developed maggots. Female flies were attracted to decomposing meat and laid their larvae on the surface.

- **On day 2:** One day after meat distribution and exposure to putrefaction, larvae appeared as pale small instars (nearly 3-4 mm) (Fig. 1). Their movement was sluggish at the beginning. Those reared on meat were scavengers and fed voraciously on the decaying flesh.

- **On day 6:** Larvae went through several instars or molting stages as they grew to their longest and last form around the meat pieces (Fig. 2a); and that was after growing through different lengths (Figs. 2 b, c). After larval transformation to pupae stages the pieces of meat appeared mummified with multipole holes (Fig. 2d). Posterior respiratory spiracles dissected from larvae and pupae had the same morphology of incomplete peritrem, peripheral button and 3 straight respiratory slits (Fig. 2e).

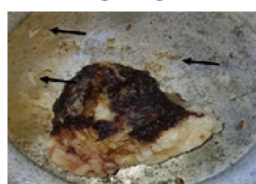

Fig. 1. Small maggots (arrows) appeared on day 2.

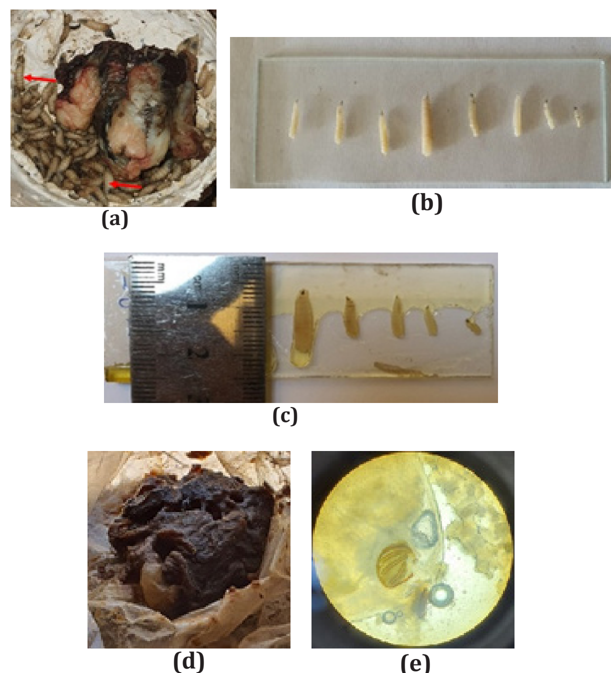

Fig. 2. On the 6th day: (a): Appearance and movement of larvae around meat pieces; **(b, and c):** Different lengths of different larval instars; **(d):** Mummified meat after larval transformation to pupae; **(e):** Similar morphology of the posterior respiratory spiracles of larvae and pupae.

- **On day 8:** With signs of advanced decay of meat, the larvae underwent metamorphosis and transformed into pupae (Fig. 3a). During this stage, the larvae encased themselves in a hardened, protective puparium shell. Inside the puparium (Fig. 3b), the larval body underwent significant changes, transforming into the adult fly. With development the pupae underwent various physiological and structural changes. The duration of the pupal stage appeared shorter (8 d) due to the hot temperature in July (35-42ºC). The mean weights of puparia ranged from 78.7 to 89.3 mg. Pupae showed similar larval posterior spiracles (Fig. 2e).

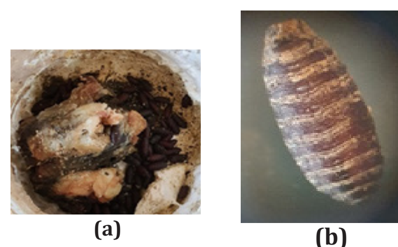

Fig. 3. (a) Transformation of larvae into pupae (pupation stage); **(b):** *Sarcophaga* brown pupa (barrel shaped) with multiple segmentations.

- **On day 16:** Adult *Sarcophaga* emergence from the puparium occurred after full pupal development, marking the completion of the life cycle. The newly emerged fly needs some time to dry and harden its exoskeleton before it becomes fully functional and capable of reproduction (Fig. 4a). Among the identifying features of the adult fly are the segmented antennae interconnected by a flexible joint allowing for movement and sensory reception. The number of segments can vary between species, but they typically consist of three main parts: the scape, pedicel, and flagellum. The scape is the basal segment of the antenna that connects to the head of the fly. It is usually the shortest segment and often appears thicker or broader than the other segments. The pedicel is the second segment of the antenna located between the scape and the flagellum. It is typically narrower and shorter than the scape. The flagellum is usually the elongated most prominent part of the antenna with an arista that is typically plumose, slender, elongated, and often tapered towards the tip, covered by hair on both sides and a bare distal third (Fig. 4b). The wings of *Sarcophaga* flies are typically transparent, relatively large compared to the body, elongated and narrow, with a slight curvature along their length. They are usually longer than the body extending beyond the abdomen when at rest and are supported and structured by a network of veins. These veins provide strength and rigidity to the wings. The venation pattern has a closed 1st posterior cell. The outer edge of the wing, known as the wing margin, is typically smooth and curved. It lacks any pronounced notches or indentations (Fig. 4c).

In our study, the same morphological shaped *Sarcophaga* larvae appeared on all buried dead animal cadavers at a depth of 30 cm, and after three days in the soil of Qalioub city, Qalioubyia Governorate, Egypt. There was no significant difference between the results obtained for larvae from distributed meat and buried dead animals regarding weight and size. In contrast, there was significant difference ($P<0.001$) regarding pupae weight (Table 1).

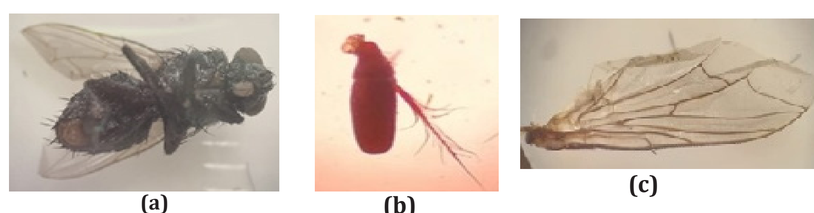

Fig. 4. *Sarcophaga* adult fly **(a);** antenna **(b)**; wing **(c)**

Table 1. Differences between the *Sarcophaga* larvae and pupa from distributed meat and buried dead animals.

Variable	Total (No.=100)	Distributed meat pieces (No.=50)	Buried dead animals (No.=50)	Statistical analysis@ (P value)
Larva				
Size (mm)	5.2 (3.0-13.5)	5.75 (3.0-13.5)	5.00 (3.0-13.5)	1.28 (0.198)
Weight (mg)	15.2 (5.1-60.9)	17.00 (5.6-60.9)	14.00 (5.1-48.6)	1.66 (0.96)
Pupa				
Weight (mg)	81.8±3.3	83.47±2.98	80.15±2.95	5.60 (<0.001)*

Data are expressed in median (range) for larvae and mean±SD for pupae. **@:** Zmann Whittney test, and Student *t* test were used in larvae, and pupae, respectively; ***:** Significant ($P<0.05$).

DISCUSSION

The collection and study of medically significant insect species, such as different life stages of *Sarcophaga* flies, can contribute to the advancement and broader utilization of entomological evidence in criminal investigations. This evidence can be particularly useful for estimating the PMI. Additionally, provision of a comprehensive image gallery with high-quality, detailed photographs can greatly facilitate the identification of fly species and enable researchers worldwide to exchange information by accessing images of Egyptian insect species. However, the entomofauna found in samples collected from death scenes may not accurately reflect the actual insect activity on the cadaver. Defining the scope of sampling errors and their potential impact on accurately estimating the PMI remains a challenging task[17].

Our study revealed that hot temperatures during July enhanced larval growth on distributed meat pieces in our Egyptian climates. This was explained by Harvey *et al.*[18] who showed that insects undergo slowdown or complete cessation of development at extreme temperatures, indicating essentiality of specific upper and lower thresholds of temperature for completion of larval development. However, as the temperature increases, the duration of development shortens[19]. As the development of immature insects depends on ambient temperatures and the heat released by maggot aggregations, PMI is normally calculated by measuring the thermal time taken to reach each development[9].

Our study revealed that after few hours the 1st visitors to meat pieces distributed in a closed room with mesh on the windows were Sarcophagidae; while Naima[20] suggested that the initial visitors to the scene after a 24-hour period belong to the family Caliphoridae, specifically two species: *Lucilia sericata* and *Chrysomya albeceps*.

Different lengths and weights were recorded in our study for larvae invading the distributed and buried meat, which agreed with the results of Tony *et al*.[21] who noted a gradual increase in the larval body length, width and weight with larval growth until the end of feeding third-stage larval phase. A key aspect in the promotion of the field of forensic entomology is the conduction of studies on the phenomenon of thermogenesis, or the generation of heat, within larval aggregations feeding on cadavers[17].

Our study recorded the pupal weights from the 8th to the 16th day at a temperature between 30ºC and 38ºC, while another study reported that the duration for *Sarcophaga crassipalpis* to complete its development from larviposition to adult emergence was at constant temperatures of 15, 20, 25, 30, 32, and 35ºC, at respective times of 1256.3±124.2, 698.6±15.1, 481.8±35.7, 366.0±13.5, and 295.8±20.5 h. However, at 35ºC, none of the pupae were able to reach adulthood. Under variable temperatures, they exhibited a duration of 485.8±5.4 h[22]. In another study at a temperature of 25°C, the pupal stage with morphological characters of *Sarcophaga peregrine*[23], persisted for a duration of 9 d.

In our study, emergence of the adult flies after the process of pupariation lasted 16 d marking the completion of the life cycle. A similar study conducted by do Couto and Queiroz[24] described the process of pupariation in dipterous flies in determining post-mortem period where the insects were differentiated based on their eye color, with variations observed as transparent eyes, pink eyes, and red eyes. The emergence of adults took place within specific time intervals, namely 162-180 h, 138-144 h, and 96-102 h, respectively. Life cycles were observed by recording notes every 8 and 24 h[25]. Despite the development of a novel and non-invasive approach for the molecular identification of adult Sarcophagidae specimens[6], the molecular methods used in a recent study focused solely on DNA extraction from the abdomens of the flies, and the morphological characteristics found on the head and thorax were not described. The intrapuparial period of *S. peregrina* was divided into distinct sub-stages based on external morphological changes like the compound eyes, antennae, thorax, legs, wings, and abdomen[26].

Our study examined sacrificed albino rats and animal tissues buried at a depth of 30 cm and collected fauna over four sampling days to identify Sarcophagidae larvae in Qalioub city, Egypt. Nearly similar results were recorded in Michigan[27] on buried pig carcasses at different depths of 30 and 60 cm, and in-between distances of 30 cm, where the process of insect succession commenced with the initial colonization by flesh and muscid flies. The researchers indicated that subsequently blow flies were the next group of insects to colonize the area, while insect succession at 60 cm did not proceed similarly.

In conclusion, this study demonstrated that Sarcophagidae flies, have a strong attraction to animal tissues and carcasses in Egypt under household conditions and temperatures ranging from 30-38°C. One of the primary contributions of forensic entomology is the estimation of the PMI, which can be determined in the early stages of death by considering the development time and rate of the initial colonizers on deceased tissues. Besides, our study indicated that PMI can be determined by finding larval stages in carcasses from 2nd day till 8th day, while pupae appear from 8th day till 16th day and adult emergence occurs on day 16.

Author contributions: All authors participated in the study design. Elawamy WE and Abdel-Basset M shared in performing all investigations, results interpretation, data analysis and writing the manuscript. Abdel-Basset M contributed to the critical revision of the final version. Elawamy WE contributed to editing of the manuscript for publication. All authors accepted the authorship and the final version of their manuscript before publication.
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REFERENCES

1. Singh R, Sharma S, Sharma A. Determination of post-burial interval using entomology: A review. J Forensic Leg Med 2016; 42:37-40.
2. Elleboudy NA, Eldin HME, Azab SM. Focus stacking technique in identification of forensically important *Chrysomya* species (Diptera: Calliphoridae). Egypt J Forensic Sci 2016; 6:235-239.
3. Bajerlein D, Taberski D, Matuszewski S. Estimation of post-mortem 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. J Forensic Leg Med 2018, 55:95–98.
4. Pittner S, Bugelli V, Weitgasser K, Zissler A, Sanit S, Lutz L, *et al*. A field study to evaluate PMI estimation methods for advanced decomposition stages. Int J Legal Med 2020; 134:1361–1373.
5. Bambaradeniya YTB, Magni PA, Dadour IR. Current status of five warm season Diptera species in estimating the post-mortem interval. Ann Entomol Soc Am 2023; 116:19–50.

6. Giordani G, Whitmore D, Vanin S. A New, Non-invasive methodology for the molecular identification of adult Sarcophagidae from collections. Insects, 2023; 14(7):365.
7. Pinto SL, Giordani G, Tuccia F, Ventura F, Vanin S. First records of *Synthesiomyia nudiseta* (Diptera: Muscidae) from forensic cases in Italy. Forensic Sci Int 2017; 276:1-7.
8. Brown K, Thorne A, Harvey M. *Calliphora vicina* (Diptera: Calliphoridae) pupae: A timeline of external morphological development and a new age and PMI estimation tool. Int J Legal Med 2015; 129:835-850.
9. Al-Shareef LA, Al-Qurashi SI. Study of some biological aspects of the blowfly *Chrysomya albiceps* (Wiedemann 1819) (Diptera: Calliphoridae) in Jeddah, Saudi Arabia. Egypt J Forensic Sci 2016;6:11-16.
10. Sanford MR, Byrd JH, Tomberlin JK, Wallace JR. Entomological evidence collection methods: American Board of Forensic Entomology approved protocols. In Forensic Entomology Utility of Arthropods in Legal Investigations; Byrd JH, Tomberlin JK, Eds.; CRC Press: Boca Raton, FL, USA, 2019; pp. 63–86.
11. Szpila K, Matuszewski S, Bajerlein D, Konwerski S. *Chrysomya albiceps* (Wiedemenn, 1819), a forensically important blowfy (Diptera: Calliphoridae) new for the Polish fauna. Pol J Entomol 2008; 4:351-355.
12. Marshall S, Whitworth T, Roscoe L. Blow flies (Diptera: Calliphoridae) of eastern Canada with a key to Calliphoridae subfamilies and genera of eastern North America, and a key to the eastern Canadian species of Calliphorinae, Luciliinae and Chrysomyiinae. Can J Arthropod Identif 2011; 11:1-93.
13. Ramaraj MP, Selvakumar C, Ganesh MA, Janarthanan S. Report on the occurrence of synanthropic derived form of *Chrysomya megacephala* (Diptera: Calliphoridae) from Royapuram fishing harbour, Chennai, Tamil Nadu, India. Biodivers Data J 2014; 2:e1111.
14. Whitworth T. Keys to the genera and species of blow flies (Diptera: Calliphoridae). America North of Mexico. 3rd ed. 2019.
15. Gunn A, Bird J. The ability of the blowflies *Calliphora vomitoria* (Linnaeus), *Calliphora vicina* (Rob-Desvoidy) and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and the muscid flies Muscina stabulans (Fallén) and *Muscina prolapsa* (Harris) (Diptera: Muscidae) to colonise buried remains. Forensic Science International 2011; 207(1):198-204.
16. Arkin H, Colton RR. Tables for statisticians (pp. 1-152). Barnes and Noble books. Inc., New York 1963.
17. Matuszewski S. Post-mortem interval estimation based on insect evidence: Current challenges. Insects 2021; 12(4):314.
18. Harvey ML, Gasz NE, Voss SC. Entomology-based methods for estimation of postmortem interval. Res Rep Forensic Med Sci 2016; 6:1-9.
19. Sert O, Örsel GM, Şabanoğlu B, Özdemir S. A study of the pupal developments of *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830). Forensic Sci Med Pathol 2020; 16:12-19.
20. Naima B. Insects involved in decomposing corpses in the Constantine region-Algeria. Open J Trauma 2020; 4(1):22-24.
21. Tony M, Zahra A, Abdellah NZ, Abdelreheem AMA, Abdel-Samad MRK. Impact of aluminum phosphide on development of the forensically important fly, *Chrysomya albiceps* (Diptera: Calliphoridae). Rev Bras DE Parasitol 2024; 33:e012323.
22. Ngando FJ, Zhang X, Qu H, Zhang C, Yang F, Feng Y, *et al*. Analysis of the influence of changing and fixed temperatures on the growth and Pteridine content in the head of adults *Sarcophaga crassipalpis* (Diptera: Sarcophagidae). Animals 2023; 13:2402.
23. Shang Y, Feng Y, Ren L, Zhang X, Yang F, Zhang C, *et al*. Genome-wide analysis of long noncoding RNAs and their association in regulating the metamorphosis of the *Sarcophaga peregrina* (Diptera: Sarcophagidae). PLoS Negl Trop Dis 2023; 17:e0011411.
24. do Couto LCM, Queiroz MMdC. Characterization of intrapuparial development of *Musca domestica* (Diptera: Muscidae), under different temperatures in laboratory conditions. J Med Entomol 2023; 61:64-73.
25. Zanetti NI, Centeno ND. Forensic significance of Roundup Full® II effect on the development of *Dermestes maculatus* (Coleoptera: Dermestidae) and *Lucilia sericata* (Diptera: Calliphoridae). J Forensic Sci 2024;69:213-221.
26. Shang Y, Amendt J, Wang Y, Ren L, Yang F, Zhang X, *et al*. Multimethod combination for age estimation of *Sarcophaga peregrina* (Diptera: Sarcophagidae) with implications for estimation of the postmortem interval. Int J Legal Med 2023; 137:329-344.
27. Pastula EC, Merritt RW. Insect arrival pattern and succession on buried carrion in Michigan. J Med Entomol 2013; 50:432-439.