

Unveiling the Impact of Entomological Evidence in Forensic Investigations: A Review

Basma H. Bedair¹, Nada H. Bedair^{2*}, Ahmed T. Enad³, Saba R. Jaafar², Ruaa H. Ali², Shahrazad H. Muhi²,
Sahar M. Ibrahim², Safa S. Mahdi⁴

¹Department of Experimental Therapy, Iraqi Center for Cancer Research and Medical Genetics, Mustansiriyah University, Baghdad, Iraq.

²Department of Forensic Biology, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq.

³Ibn Sina University for Medical and Pharmaceutical Sciences.

⁴Department of Forensic Chemistry, Higher Institute of Forensic Sciences, Al-Nahrain University, Baghdad, Iraq.

Article Info.

Keywords:

Biological evidence,
Entomology, Entomological
evidence, Forensic, Insects,
Parasites, PMI

Abstract

Background: Forensic entomology has introduced new methods that are recommended to determine the cause, manner, and time since death, particularly for deteriorated, putrid bodies discovered at unexpected places. This scientific field provides new techniques that are applicable to molecular studies, such as DNA analysis based on entomological data. Numerous techniques are used to estimate the minimum postmortem interval, depending on the location and state of the corpse as well as the circumstances surrounding its discovery.

Objectives: Since forensic pathologists always face the difficult task of excluding misleading evidence when investigating cases of unnatural deaths or when the corpse has been moved from the original crime scene, this review aimed to provide a broad overview of the potential applications and advantages of forensic entomology data in criminal investigations in courts. In addition to highlighting the factors that can negatively influence the use of these entomological evidences.

Conclusion: Various factors could affect the entomological data collected from a crime scene but careful analysis accompanied by thorough observations and evaluations could overcome such obstacles in an investigation.

Received: 12.05.2025

Accepted: 12.07.2025

Published online: 01.10.2025

Published: 01.10.2025

1. Introduction

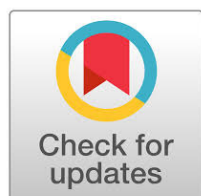
Numerous biological disciplines have contributed to forensic research by comprehending and using living things as indicators of criminal investigations, either as whole creatures or specific body parts, such as deoxyribonucleic acid (DNA), spores, feathers, etc. Since insects have been used as silent forensic markers since the 13th century, forensic entomology is the oldest of these disciplines. In addition to helping investigators look into the cases of biological attacks, gender crimes, criminal neglect, and wildlife trafficking and exploitation, insects and parasites provide information on how people and animals move across great distances [1]. Forensic pathologists have also used microbiology, another

branch of biology, to identify the cause of death in cases of pandemics or manifestations [2].

Forensic entomology is a scientific field that allows the use of evidences provided by arthropods, mainly insects collected from the decomposing remains of crime scene in order to aid legal investigations [3].

This information is used to help investigators in estimating the time since death (postmortem interval) based on the biology and ecology of these carcass-associated insects. The period between the actual death and the discovery of carcass is known as postmortem interval (PMI) [4, 5].

The origin of the word "Entomology" is from the fact that insects are characterized by having their body divided



*Corresponding author: Nada H. Bedair: nada.hassan@nahrainuniv.edu.iq

How to cite this article: Bedair, BH, et al. Unveiling the Impact of Entomological Evidence in Forensic Investigations: A Review. Baghdad Journal of Biochemistry and Applied Biological Sciences, 2025, VOL. 6, NO. 4, 183–192. <https://doi.org/10.47419/bjbabs.6i4.395>

License: Distributed under the terms of The Creative Commons Attribution 4.0 International License (CC BY 4.0), which Permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are properly cited.

Copyright: © 2025 the AuthorsCC BY license (<http://creativecommons.org/licenses/by/4.0/>).

into three segments, this gave rise to the Greek term *entomon*, which literally means “notched,” referring to the segments of their bodies. Entomology is a branch of zoology which deals with the study of behavior, ecology, genetics, morphology, taxonomy, and physiology of insects [6].

Insects play a major role in the process of natural decomposition, hence they are naturally and immediately attracted to a decomposing body [6,7]. Inside a laboratory, their growth metrics, such as the amount of time required to fully develop from one stage to the next, may be examined; the older the larva, the more time has elapsed since the time of death, which helps to determine PMI_{min} [8].

2. Aims of the current study

This review aimed to provide a broad overview of the potential applications and advantages of forensic entomology data in criminal investigations in courts. In addition, it highlights the factors that can negatively influence the use of these entomological evidences.

The study of flesh-eating insects that consume dead bodies is known as forensic entomology, and it aids law enforcement and legal agencies in the investigation of criminal cases [9]. In fact, this entomological timeline begins after 72 hours of death [9], and the typical indicators of PMI are separated, leaving the remains unidentifiable or putrefied [3].

By studying the population of insects on a body and the development stages of their larvae, forensic entomologists estimate the time between victim's actual death and discovery of the corpse or remains, known as the minimum postmortem interval (PMI_{min}) [10,11], besides the cause of death (suicide, homicide, or accidental) and whether or not the position of the corpse was changed [12,13].

This process involves the collection of adequate data about the development of forensic insects at temperatures, in addition to biotic and abiotic factors relevant to the crime scene that may have an effect on the developmental rate of different life stages [13].

The estimation of PMI was established by many studies; while Goff provided observational insights, Amendt *et al.* expanded it with statistical models, and increasing reliability [14,15]. However, environment variables, such as temperature and humidity, often overlooked in early works, were later incorporated in more advanced models by Tomberlin *et al.* [11]. These studies establish the importance of insect developmental stages, especially blowflies (Calliphoridae), in estimating time since death, but the problem was that they often used ideal laboratory conditions [16]. Real crime scenes introduce uncontrolled variables.

The first recorded case where forensic entomology was used to solve a crime was in the 13th century, in China. In this case, the body of a farmer was found in a field, the recorded cause of death was a sharp weapon, according to the investigators. For that, all the suspects were ordered to put their sickles on the ground; blowflies were attracted to one sickle only, and on it trace amount of blood was

discovered; this finding led to the confession of the murderer [17].

However, the use of forensic entomology in a courtroom was first introduced in France in the 18th century. After a child's skeletal remains were discovered in a house, entomological evidence was sufficient to clear the names of the current occupants of the house; as a result, the science of forensic entomology was established by evaluating the insect succession on corpses [14]. Greenberg and Kunich analyzed legal precedents, and noted a growing acceptance for entomology in court, but also challenges because of unfamiliarity of juror and the need for expert testimony [18]. This means that there's a gap between science and legal practice, with limited training of legal professionals on interpreting entomological data.

In the last 30 years, the application of forensic entomology in criminal investigations has been extensively recorded in America, Asia, Europe, and Australia [19]. In a reported case, a male body was found hanging in winter inside a building in agricultural area. The body showed insect activity by both adult and immature insects. Subsequent identification confirmed the presence of Coleoptera, Dermestidae, and Diptera third instar larvae, in addition to third instar mummified larvae of Calliphoridae and a puparium Calliphoridae. Dermestidae is known to be associated with stored products or cadavers in an advanced stage of decomposition, when the fats are rancid. It is found in decomposing bodies in winter, especially in late stages, when the remains are dry. The entomological evidence as a whole suggested an advanced stage of decomposition and the PMI estimation was 6½ weeks before the body was found [20].

These procedures demonstrate how well forensic entomology can be used to investigate drug consumption, cadaver transfer, victim identification, and PMI calculation [21] together with the signs of neglect in both children and elderly [22].

Insect metamorphosis comprises the following four stages: (a) first stage is the egg, (b) post-hatching, (c) larvae emergence, and (d) their feeding until fully grown. Then larvae enclose themselves into pupal shells; when ready, adult insects break these shells in a process known as eclosion, and emerge into the environment [23,24].

Insect groups such as blowflies (Diptera and Calliphoridae) use their olfactory senses to detect decomposing remains, which are considered as sources of protein as well as suitable sites for oviposition and egg development [25,26].

The age of the oldest immature insect specimen taken from the remains represents the estimated arrival time of adult females, which, in turn, indicates the minimum time that the decomposing carcass was available for insect colonization and, consequently, PMI_{min} [27]. PMI is essential in the investigation of crimes, especially untimely deaths as well as natural deaths, because such information helps to connect the missing links in relevant cases and estimate the time elapsed since death [28]. This is because the time of colonization, development, and departure of insects in the remains are linked to the progression of carcass

decomposition [27,29]. Although these methods provide a broad time window within which the death occurred, they are not as accurate as age-based estimations. Occasionally, the collected species are compared to established patterns of investigation and time frames associated with each phase and species [30]. Because laboratory techniques are advancing every day, the DNA identification of insects has become very useful in estimating the time since death (PMI) as well as providing a more accurate results of species identification than the morphological methods used previously [31].

Molecular analysis facilitated identification even through partial specimens, where several approaches are adopted for identifying wildlife species by DNA target (nuclear DNA [nDNA] or mitochondrial DNA [mtDNA]), and the techniques applied to develop genetic marker. Some techniques, such as sequencing, are applied to investigate both types of DNA (nDNA and mtDNA), while other techniques are specific to nDNA. mtDNA is often favored as a genetic marker over nDNA for species identification of wildlife because mtDNA is easier to type from highly processed and degraded tissues [32]. Other techniques involve complex procedures, such as electrophoresis, flow cytometry, immunohistochemistry, and spectroscopy [28].

Knowledge about the pattern of insect succession on a dead body provides vital information about when the person died. It is important to note that an accurate determination of PMI requires a lot of training and education, because different and numerous environmental factors—such as burial conditions, temperature, humidity, moisture, and type of clothing—must be taken into account [33].

According to the literature reviewed, a number of authors have detailed how crime scenes are altered and modified as well as how various insects and natural cadaver decomposition create postmortem damage. As shown in Table 1, these changes constitute two categories [4].

2.1. Insects as source of data

Insects are a widely distributed taxa found in both terrestrial and aquatic habitats [34]. Their mere presence provides entomological evidence at the scene of a crime, determining several forensic aspects about the nature and time of death [13]. Proper collection and handling of these specimens together with proper laboratory analysis are required to increase the accuracy of results, and helping legal authorities to solve mysteries [3].

Forensic entomology was a tedious and time-consuming field, but it helped in enigmatic criminal cases. Until the 20th century, studies and literature were

restricted to the traditional morphological identification of insects according to their class, order, and species [21]. As a result of recent scientific developments that use DNA typing in addition to barcoding to identify insects, legal investigators are now more dependent than ever on forensic entomology evidence [9].

2.2. The biological timeline of insects

A unique sequence of metamorphosis is present for each species because their succession on the carcass follows a natural timeline from the moment of death to their discovery, and these fixed timelines help entomologists to provide a rapid and accurate estimation of PMI [35].

Such timelines are utilized under controlled environment in laboratory, ensuring a careful and detailed post-colonization biological timeline [3,36].

Even though forensic insects have a set and predictable life cycles, some environmental factors can drastically shorten or lengthen the time needed for maturation [3]. The most important factors are temperature and humidity, with other minor and less important factors, such as shade, rainfall, and drug use [37]. That's why it is important that researchers must focus on understanding how weather changing patterns affect development and distribution of insects, and adapt forensic methods accordingly [38].

2.2.1. Isomegalen and Isomorphen diagrams

The entire life cycle and stages of development of insects could be studied at multiple temperature ranges. The recorded data are illustrated as an isomegalen diagram, which shows the time since egg hatching (x-axis) in relation to temperature (y-axis). In order to estimate the age of insects, graphical lines must be drawn between both axes representing the length of the feeding larvae (Figure 1). If the temperature is relatively constant, as seen in case of indoor corpses, the age of the maggot could be read instantly based on its length [3,13].

In addition, the isomorphen diagram representing all morphological stages from oviposition to eclosion should be used if maggots in the migratory phase or pupae or puparia are recovered from the scene (Figure 2) [39]. The isomegalen and isomorphen diagrams facilitate a quick and more precise estimation of PMI even for inexperienced investigators. A study done by Richards (2009) used developmental curves as a measure of age using body length for the sister species *Chrysomya chloropyga* (Wiedemann, 1818) and *Chrysomya putoria* (Wiedemann, 1830) (Calliphoridae [order: Diptera]) to estimate PMI [40]. Another study conducted on *Thanatophilus micans* larvae at 10 constant temperatures discovered that length generally increased with increased rearing temperatures but decreased at extremely high temperatures, while their development took longer at lower temperatures [41].

2.3. The faunal succession

The body begins to decompose as soon as an animal dies, its cells begin to die, and autolysis begins [42]. The

Table (1): Crime scene alterations caused by insects.

Entomological body artifacts	Crime scene entomological alterations
Skin lesions	Fly stains
Soft tissue alterations (bone and hair)	Floor and wall stripes

bacteria in the gastrointestinal tract then start to feed on the soft tissues, releasing gases and liquids, such as ammonia, carbon dioxide, hydrogen sulfide, and methane. These volatile fumes attract insects and can change their behavior [3,12,43].

Based on physical appearance, internal temperature of the carcass as well as characteristic insect populations, the decomposition process could be arranged into the following five stages [12,43]:

1. Fresh stage (days 1–2): This phase begins at the time of death and concludes when the corpse becomes swollen. Despite autolytic activity at this stage, no significant morphological alterations are observed. Since insects are drawn within the first 10 minutes of death, the PMI estimated using entomological data after 24 hours is more accurate than the medical examiners' estimation at this time. However, no eggs are laid at this time, and the scents are not readily apparent to humans.
2. Bloated stage (days 2–7): The process of putrefaction starts. The anaerobic bacteria's activity produces gases that cause the carcass's belly to expand and raise its interior temperature. Adult diptera are drawn to the scene in greater numbers at this time, and by the fourth day, first- and early second-larval stages are visible.
3. Decay stage (days 5–13): The carcass is deflated due to the penetration of abdominal walls, and the internal temperature drops significantly at the end of this stage. Decaying odors are obvious, and the weight of the carcass is decreased. At the end, the larvae migrate away to pupate.
4. Post-decay stage (days 10–23): Most of the diptera larvae have migrated by now, and only bones, cartilage, and hair are left, along with a large amount of wet and viscous matter, known as by-products of decay (BoD).

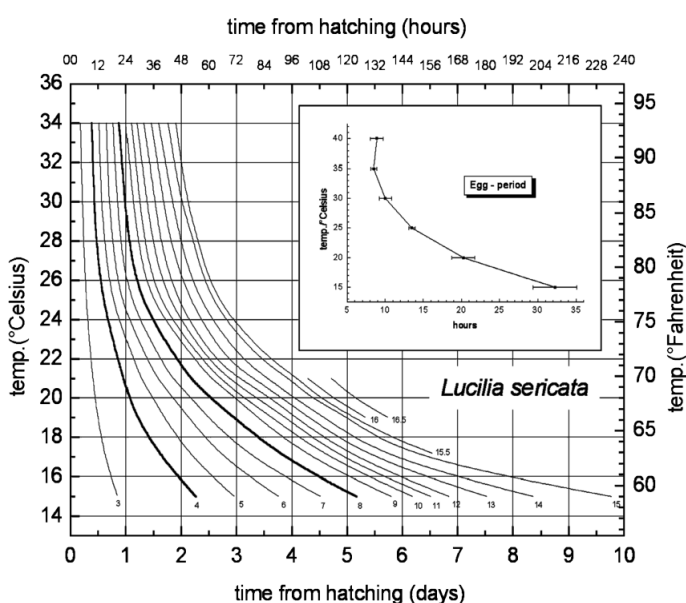


Figure (1): *L. sericata* larvae isomegalen diagram from hatching to peak feeding. Identical larval length (mm) is represented by each line. The graph displays analogous egg periods at temperatures ranging from 15°C to 40°C [39].

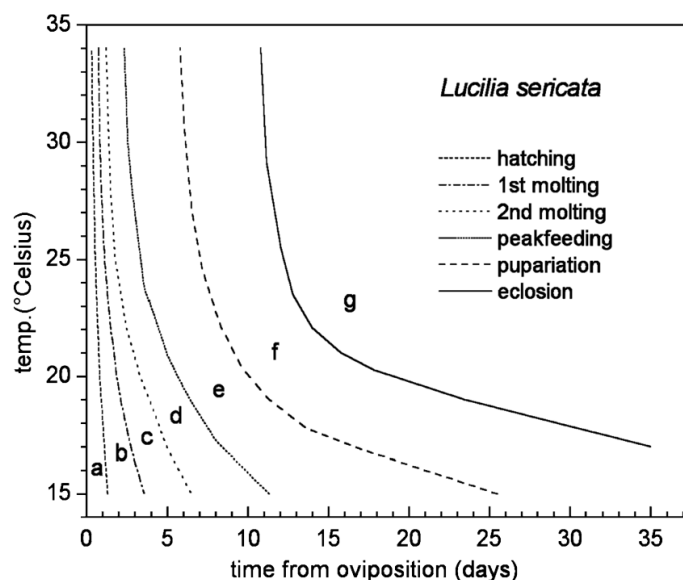


Figure (2): Isomorphen diagram for *L. sericata* showing all stages from oviposition to eclosion (15 ± 34°C). (a) egg, (b) 1st instar, (c) 2nd instar, (d) 3rd instar, (e) post-feeding larva, (f) pupa, and (g) adult. Each line represents identical morphological stage [39].

5. Remains stage (days 18–90+): This stage is characterized by dried BoD and bones with little cartilage. The adult and larval diptera populations decrease significantly.

The two most prevalent forensic species in the order are *Sacrophagidae* (flesh flies: medium-size flies with longitudinal stripes of black and gray) and *Calliphoridae* (blow-flies: usually shiny with metallic colors, usually blue or green); and *Muscidae* (house flies). The first two may arrive within minutes after death; however, the third species arrives when the body reaches the bloat stage [44].

Even after 1–3 years, when the remains have gone completely dry, there is still a chance to discover dermestid (Coleoptera) beetles [45].

2.4. Collection of entomological evidence

When a dead body or remains are found, specimens should be taken from above, below, and inside the flesh. Adult flies that are flying over the dead body should also be caught with nets. Following that, the containers are sealed and labeled with the collector's information, location, time, and the developmental stage [3,45].

While collecting insects from a child sexual assault scene, it should be noted that it is characterized by the phenomenon of myiasis within the peri-anal area in addition to the genital areas [46].

Since they do not stop insect invasion; extreme weather conditions or fires cannot destroy the entomological evidence; nevertheless, they can shorten the invasion period during the initial days [36,47].

The site of the crime scene is very important for the evaluation of the insect specimens; the climate and geography

all effect the biological timeline and hence the PMI. Careful collection and handling of such fragile data provides a better and more accurate results for solving crimes [48].

Since forensic entomology relies on the presence of insects, numerous studies report a decline in insect biodiversity and biomass on a global scale. The most important factor driving insect decline and affecting forensic entomology is probably the climate change, which has an impact on necrophagous insects, leading to reduced flight and oviposition activity, leading to modified growth rates [49].

2.5. Estimation of Postmortem Interval

2.5.1. Age-dependent variation of intestinal contents

Radiological studies on larval feeding behavior showed that maggots stop eating the instant they reach maximum length, due to which the anterior intestine becomes empty; the fullness of their intestines could help in estimating PMI_{min} [50].

2.5.2. Developmental patterns

The amount of time a certain species of insects requires to develop into a stage of growth is calculated in investigations as PMI, usually depending on a detailed timeline and a temperature range [51–53].

2.5.3. Weight of the larvae

The precise age of the larva might be determined by measuring its weight in a laboratory setting under carefully monitored conditions [3]. The time interval between egg hatching and the post-feeding stage is computed, and a statistical model is developed to determine the weight distribution by age; the population is presumed to be in the same age range and environment as the primary specimen [5].

2.5.4. Effect of body length and crawling speed

Flies arrive on corpses shortly after death, hence they provide PMI_{min}; the average size of the larvae and their developmental stage are the basic elements used to indicate their arrival time at the body [10]. After reaching their last stage of development, they migrate from the body to find a suitable site for pupation [29].

At higher temperatures, the larvae travel at a faster rate, and their speed also depend on body length; this knowledge is practical for estimating time of departure from corpse to pupation cite [54].

Sometimes larvae migrate to another nearby corpse in search of food or a more suitable environment; this data should not be underestimated when estimating PMI [55].

2.5.5. Analyses of internal morphology of pupae

Insects spend about 50% of their life cycle inside pupae; therefore, this stage is important in estimating their biological timeline [35]. During this stage, changes in the insect's gene expression are noticed as well [56].

Pupae are punctured through each of their three tagmata (head, thorax, and abdomen), because cuticle reduces solution penetration if openings are not present;

thus, piercing of the cuticle is required. After that, the pupa is immersed in hot water, and stored in 80% alcohol (Figure 3). Histological analysis of the internal changes within the puparium can yield significant and trustworthy data. Both hematoxylin and eosin (H&E) stains are applied to sections of pupal shells. This helps to determine PMI_{min} and provides information about the various stages of thoracic and brain muscle development over time, which could be used as an age indicator [57].

2.6. Factors influencing entomological evidence

The scene of the crime from which the dead remains are discovered offers a large amount of evidences about these insects. External factors, such as weather, sunlight or shade, and rainfall affect the process of identification for these forensic insects, with the most important factors being temperature and humidity [37].

A careful handling and preservation of specimens and evidence, while following correct protocols, could be very beneficial for finding out the cause, manner, and mechanism of a crime [58].

The use of insecticides, such as dimethoatecan, effect investigation of crime in a great manner, because both sarcophagidae and carrion flies (Calliphoridae flies) feed on carcasses and may ingest toxic substances found in poisoned dead body, leading to negative correlation with the concentration of the chemical and altering PMI determination [59,60].

2.7. Crime scene artifacts

In spite of being very important in collecting data about a certain crime or incident, insects can also alter and modify evidences at a crime scene together with causing changes in the dead body [4].

2.7.1. Postmortem bite wounds

When insects, such as cockroaches and ants, invade a freshly deceased person, they start biting the delicate flesh. It is possible that these bite marks are mistaken for intravenous drug use. According to Ventura *et al.*, certain larger wounds may also mimic entry or exit gunshot wounds [61].

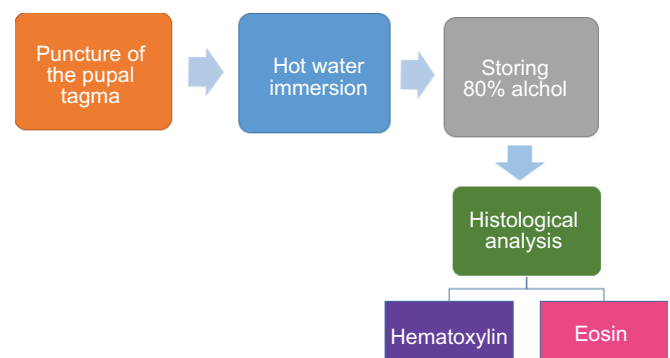


Figure (3): Procedure steps to analyze the internal morphology of pupae.

By passing through the vessel foramina opening, flesh-eating larvae can even enter the bone marrow [62]. These larvae, as well as dermestid and clown beetles, can produce larger bite wounds on the skin that resemble fire-arms wounds [63].

2.7.2. Modifying evidences

Blood marks around the crime scene could be modified due to the tread marks of adult flies feeding on the liquid blood; this leads to tadpole-like smudges that resemble blood spatter [64].

Fortunately, this problem could be solved by examining the direction, ratio, and irregular shape of these smudges [65].

Bloodstain pattern analysis (BPA) is very important in investigating a crime scene, as it provides vital information about the physical events that caused such bloodstain depositions [66]. The data obtained through BPA could be used for reevaluating the cause of death as well as other primary factors, such as the positions of both victim and killer, along with any movement before or after committing the crime [67].

1. Fly artifacts

Fly artifacts (FAs) were first described by Lassaigue [68]; they were defined as small stains transferred from the source of blood onto a blood-free surface because of the activities of blowflies [4].

When flies feed on blood, they regurgitate it as bubbles and then suck it back, causing spatter, and their appendages become blood-soaked, causing them to create new blood marks when travelling to blood-free surfaces. In addition, cockroaches can also produce blood-like droplets that are larger than those produced by flies [45,69] (see Figure 4).

2. Aquatic insects

Water-derived carcasses typically exhibit postmortem lesions from turbulence or contact with water's bottom, but aquatic insects' feeding behavior also plays a significant role in these alterations [70,71]. Aquatic insects play an important role in decomposition of water carcasses, even though their impact differs from

terrestrial decomposition. Aquatic insects primarily feed on bacteria and fungi that grow on the carcass. Water also significantly affects the rate and process of decomposition, influencing the types of insects and their activity, unlike land, where carrion insects are primary decomposers [72].

The loss of soft tissues, primarily from the facial and cervical regions, is one of these postmortem alterations that has led to theories of criminal assault [71].

3. Misleading investigations

The insects' antemortem invasion should be differentiated from the postmortem colonization while investigating a crime scene, as both can be present at the same time, especially in cases of neglect [73].

In addition, the entomological activities of feeding and moving around the crime scene, within the clothes and on the dead body, could lead to wrongful deductions [45].

Therefore, the analysis of a crime scene in order to answer questions relating to 5 Ws + 1 H (i.e., what, why, why, who, when, and how) and to understand the nature of the incident highly depends on entomological evidence and the knowledge of forensic pathologists about the damage that could be caused by arthropods [4].

2.8. Entomo-toxicological evidence

If the victim's body contained drugs or toxins, they are transferred to insects upon feeding; these drugs affect the metabolism rates of these insects, causing a change in their growth and size, plus trace amounts of these drugs could be detected in the insect flesh during laboratory analysis [74,75]. Even if actual quantity of drugs could not be determined, the mere detection of such substances should be enough to establish the diagnosis [44].

These evidences for the presence of a drug or chemical substance could be useful in cases where the dead remains are too dry or too decomposed, and a regular toxicological examination is difficult; both cocaine and heroin accelerate the development of larvae, whereas poisons, such as malathion, cause a delay in the insect colonization process [76].

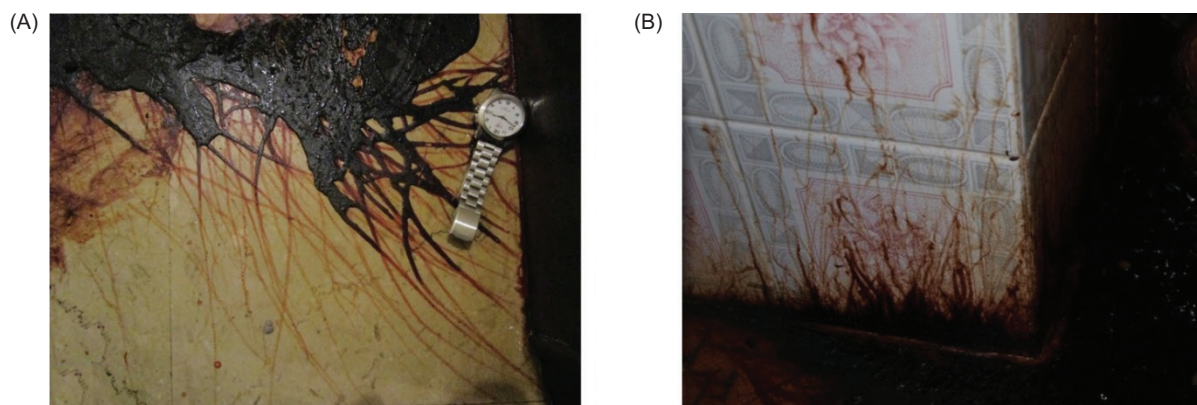


Figure (4): Fly Artifacts, (A) Floor stripes caused by wandering maggots, (B) Wall stripes caused by wandering maggots [4].

Another study tested the toxic effects of *Ecballium elaterium* fruit juice on *Lepidoptera* larvae, where molecular binding poses of Cucurbitacin-E (the most important component of *E. elaterium*) and antioxidant enzymes of *Galleria mellonella* (Lepidoptera larvae) were determined *in silico*. The results showed that it could be used as a pest control because of its toxic effect because mortality rate increased with the increasing concentration of fruit juice. Also, increasing doses of essential oil caused decrease in superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidases (GPx), glutathione reductase (GR), and acetylcholinesterase (AChE) activities [77].

2.9. Current issues

In spite of the developmental timeline used to determine the age of an insect, several obstacles could arise and hinder the analysis; for example, available references and forensic literature are usually focused on the dipteran species [30,78,79], meaning that references are neither equally nor readily available for all species in association with forensic investigations, particularly in the practices of estimating the PMI_{min}; such neglected insects include beetles and parasitic wasps [13].

3. Conclusions

Forensic entomology is an emerging field in forensic sciences, where the behaviors and biology of the insects feeding on carcasses are studied, and this has developed into an important tool in solving criminal investigations.

Comparison with similar studies provided a better understanding of the potential applications of forensic entomology and highlighted the factors that may negatively affect criminal investigations and make it more challenging.

Concurrent methods that depend on multiple factors, such as entomological timeline, could be extremely helpful in estimating PMI_{min}, location, cause, manner, and mechanism of a crime.

Entomological science has been developing over the years; it is considered essential together with other fields of forensic science, which advantageous in death investigations.

Although multiple factors could affect the growth proportions and life cycle of flesh-eating insects, the correct and careful observations could aid in solving mysterious deaths.

It is highly improbable to find a single applicable rule to solve all questions, because each case accrues different challenges and factors; however, a flexible, scientific, and well-tested solution is what forensic entomologists are hoping to achieve in order to improve the estimation of PMI_{min}.

4. Recommendations

A thorough and easy to apply technique for collecting insect from crime scenes, and rearing them in a laboratory

could be extremely useful for forensic investigators, entomologists, and forensic pathologists.

The guidelines for entomological data collection, along with their analysis, should be revised from time to time in order to insure the complying of correct protocols.

Publishing the used approaches and challenges faced when solving a difficult forensic case successfully could significantly aid the future investigators.

When entomological activity is detected at a crime scene, then cooperation between forensic pathologists and forensic entomologists is highly recommended for accurate evaluation and correct investigation.

We also recommend studying the role of other insect groups because most studies focus only on blowflies' species.

Disclosure

The authors declare no conflicts of interest or external funding.

Author Contributions

Asst. Lec. BHB: Conceptualization; Asst. Lec. RHA: Data curation; Asst. Lec. SMI: Investigation; Dr.ATE: Project administration; Asst. Lec. SHM: Resources; Dr SSM: Supervision; Asst. Lec. BHB and Asst. Lec. NHB: Writing-original draft; Asst. Lec. NHB and Asst. Lec. SRJ: Writing-review & editing.

References

- [1] Kwak, M. L., Wallman, J. F., Yeo, D., Archer, M. S., and Nakao, R., Forensic parasitology: a new frontier in criminalistics. *Forensic Sci Res*, 9(2): owae005, 2024. <https://doi.org/10.1093/fsr/owae005>
- [2] Spagnolo, E. V., Stassi, C., Mondello, C., Zerbo, S., Milone, L., and Argo, A., Forensic microbiology applications: a systematic review. *Leg Med (Tokyo)*, 36: 73–80, 2019. <https://doi.org/10.1016/j.legalmed.2018.11.002>
- [3] Mona, S., Khalid, M., Jawad, M., Noreen, S., and Rakha, A., Forensic entomology: a comprehensive review. *Advan Life Sci*, 6(2): 48–59, 2019.
- [4] Viero, A., Montisci, M., Pelletti, G., and Vanin, S., Crime scene and body alterations caused by arthropods: implications in death investigation. *Int J Leg Medicine*, 133(1): 307–316, 2019. <https://doi.org/10.1007/s00414-018-1883-8>
- [5] Wells, J.; LaMotte L., The role of a PMI-prediction model in evaluating forensic entomology experimental design, the importance of covariates, and the utility of response variables for estimating time since death. *Insects*, 8(2): 47, 2017. <https://doi.org/10.3390/insects8020047>
- [6] Benbow, M.E.; Merritt, R.W.; Pechal, J.L., Entomology. In: *Wiley Encyclopedia of Forensic Science*; Jamieson, A. and Moenssens, A., Eds. Wiley-Blackwell: New York, NY; 2013, pp. 1–14. <https://doi.org/10.1002/9780470061589.fsa072.pub2>
- [7] Shaurub, E-S.H.; Salem, A.M.; Zaher, E.E., A preliminary study on decomposition and seasonality of insect succession of decomposing rabbit carcasses at El-Sharkia Governorate,

- Egypt. Beni-Suef University J Basic Appl Sci, 13(1): 102, 2024. <https://doi.org/10.1186/s43088-024-00561-2>
- [8] Ahmad, N. W., Lim, L. H., Dhang, C. C., Chin, H. C., Abdullah, A. G., Mustaffa, W. N. W. and et al., Comparative insect fauna succession on indoor and outdoor monkey carrions in a semi-forested area in Malaysia. *Asian Pacific J Trop Biomed*, 1(2): S232–S238, 2011. [https://doi.org/10.1016/S2221-1691\(11\)60161-5](https://doi.org/10.1016/S2221-1691(11)60161-5)
- [9] Sharma, R.; Garg, R.K.; Gaur, J.R., Various methods for the estimation of the postmortem interval from Calliphoridae: a review. *Egypt J Forensic Sci*, 5(1): 1–12, 2015. <https://doi.org/10.1016/j.ejfs.2013.04.002>
- [10] Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R., and Hall, M. J., Forensic entomology: applications and limitations. *Forensic Sci Med Pathol*, 7(4): 379–392, 2011. <https://doi.org/10.1007/s12024-010-9209-2>
- [11] Tomberlin, J. K., Mohr, R., Benbow, M. E., Tarone, A. M., and Vanlaerhoven, S., A roadmap for bridging basic and applied research in forensic entomology. *Ann Rev Entomol*, 56: 401–421, 2011. <https://doi.org/10.1146/annurev-ento-051710-103143>
- [12] Joseph, I., Mathew, D. G., Sathyan, P., and Vargheese, G., The use of insects in forensic investigations: an overview on the scope of forensic entomology. *J Forensic Dental Sci*, 3(2): 89, 2011. <https://doi.org/10.4103/0975-1475.92154>
- [13] Harvey, M.L.; Gasz, N.E.; Voss, S.C., Entomology-based methods for estimation of postmortem interval. *Res Rep Forensic Med Sci*, 6: 1–9, 2016. <https://doi.org/10.2147/RRFMS.S68867>
- [14] Amendt, J.; Krettek, R.; Zehner, R., Forensic entomology. *Naturwissenschaften*, 91(2): 51–65, 2004. <https://doi.org/10.1007/s00114-003-0493-5>
- [15] Goff, M.L., A Fly for the Prosecution. Harvard University Press: Cambridge, MA, 2001. <https://doi.org/10.2307/j.ctv21hrqwk>
- [16] Amendt, J., Insects help to solve crimes. In: *Nature Helps...: How Plants and Other Organisms Contribute to Solve Health Problems*; Mehlhorn, H. Ed. Springer: Berlin; 2011; pp. 227–242. https://doi.org/10.1007/978-3-642-19382-8_10
- [17] Lei, G., Liu, F., Liu, P., Zhou, Y., Jiao, T. and Dang, Y.H., A bibliometric analysis of forensic entomology trends and perspectives worldwide over the last two decades (1998–2017). *Forensic Sci Int*, 295: 72–82, 2019. <https://doi.org/10.1016/j.forsciint.2018.12.002>
- [18] Greenberg, B.; Kunich, J.C., *Entomology and the Law: Flies as Forensic Indicators*. Cambridge University Press: Cambridge, UK, 2002.
- [19] Campobasso, C.P.; Introna, F., The forensic entomologist in the context of the forensic pathologist's role. *Forensic Sci Int*, 120(1–2): 132–139, 2001. [https://doi.org/10.1016/S0379-0738\(01\)00425-X](https://doi.org/10.1016/S0379-0738(01)00425-X)
- [20] Arnaldos, M.I., García, M.D., Romera, E., Presa, J.J. and Luna, A., Estimation of postmortem interval in real cases based on experimentally obtained entomological evidence. *Forensic Sci Int*, 149(1): 57–65, 2005. <https://doi.org/10.1016/j.forsciint.2004.04.087>
- [21] Marchetti, D., Arena, E., Boschi, I. and Vanin, S., Human DNA extraction from empty puparia. *Forensic Sci Int*, 229(1–3): e26–e29, 2013. <https://doi.org/10.1016/j.forsciint.2013.03.043>
- [22] Benecke, M.; Josephi, E.; Zweihoff, R., Neglect of the elderly: forensic entomology cases and considerations. *Forensic Sci Int*, 146: S195–S199, 2004. <https://doi.org/10.1016/j.forsciint.2004.09.061>
- [23] Tarone, A.M., Jennings, K.C., Foran, D.R., Aging blowfly eggs using gene expression: a feasibility study. *J Forensic Sci*, 52(6): 1350–1354, 2007. <https://doi.org/10.1111/j.1556-4029.2007.00587.x>
- [24] Zanetti, N.I.; Visciarelli, E.C.; Centeno, N.D., The effect of temperature and laboratory rearing conditions on the development of *dermestes maculatus* (Coleoptera: dermestidae). *J Forensic Sci*, 61(2): 375–381, 2016. <https://doi.org/10.1111/1556-4029.12965>
- [25] Voss, S.C.; Spafford, H.; Dadour, I.R., Annual and seasonal patterns of insect succession on decomposing remains at two locations in Western Australia. *Forensic Sci Int*, 193(1–3): 26–36, 2009. <https://doi.org/10.1016/j.forsciint.2009.08.014>
- [26] Matuszewski, S., Bajerlein, D., Konwerski, S. and Szpila, K., Insect succession and carrion decomposition in selected forests of Central Europe. Part 2: composition and residency patterns of carrion fauna. *Forensic Sci Int*, 195(1–3): 42–51, 2010. <https://doi.org/10.1016/j.forsciint.2009.11.007>
- [27] Matuszewski, S., Frątczak, K., Konwerski, S., Bajerlein, D., Szpila, K., Jarmusz, M. and et al., Effect of body mass and clothing on carrion entomofauna. *Int J Leg Med*, 130(1): 221–232, 2016. <https://doi.org/10.1007/s00414-015-1145-y>
- [28] Sangwan, A., Singh, S.P., Singh, P., Gupta, O.P., Manas, A. and Gupta, S., Role of molecular techniques in PMI estimation: an update. *J Forensic Leg Med*, 83: 102251, 2021. <https://doi.org/10.1016/j.jflm.2021.102251>
- [29] Nassu, M.P.; Thyssen, P.J.; Linhares, A.X., Developmental rate of immatures of two fly species of forensic importance: *Sarcophaga (Liopygia) ruficornis* and *Microcerella halli* (Diptera: Sarcophagidae). *Parasitol Res*, 113(1): 217–222, 2014. <https://doi.org/10.1007/s00436-013-3646-2>
- [30] Amendt, J., Krettek, R., Niess, C., Zehner, R. and Bratzke, H., Forensic entomology in Germany. *Forensic Sci Int*, 113(1–3): 309–314, 2000. [https://doi.org/10.1016/S0379-0738\(00\)00239-5](https://doi.org/10.1016/S0379-0738(00)00239-5)
- [31] Amendt, J., Campobasso, C.P., Gaudry, E., Reiter, C., LeBlanc, H.N. and JR Hall, M., Best practice in forensic entomology—standards and guidelines. *Int J Leg Med*, 121(2): 90–104, 2007. <https://doi.org/10.1007/s00414-006-0086-x>
- [32] Alacs, E.A., Georges, A., FitzSimmons, N.N. and Robertson, J., DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Sci Med Pathol*, 6(3): 180–194, 2010. <https://doi.org/10.1007/s12024-009-9131-7>
- [33] Jay, A.S., Forensic science. In: *Encyclopædia Britannica [Internet]*. 2025. Encyclopædia Britannica Inc. Available from: <https://britannica.com/science/forensic-science>
- [34] Chapman, A.D., *Numbers of Living Species in Australia and the World. Report for the Australian Biological Resources Study (ABRS)*. ABRS: Canberra, ACT, 2009.
- [35] Grassberger, M.; Reiter, C., Effect of temperature on development of the forensically important holarctic blowfly *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae). *Forensic Sci Int*, 128(3): 177–182, 2002. [https://doi.org/10.1016/S0379-0738\(02\)00199-8](https://doi.org/10.1016/S0379-0738(02)00199-8)
- [36] Gomes, L.; Godoy, W.A.C.; Von Zuben, C.J., A review of postfeeding larval dispersal in blowflies: implications for forensic entomology. *Naturwissenschaften*, 93(5): 207, 2006. <https://doi.org/10.1007/s00114-006-0082-5>
- [37] Adair, T., Aspects influencing the entomological postmortem interval in crime scene reconstruction. *J Assoc Crime Scene Reconstr*, 18(3): 17–19, 2012.
- [38] Thompson, T.J.U., The impact of climate change and sustainability initiatives on forensic practice. *Forensic Sci Int Synerg*, 8: 100475, 2024. <https://doi.org/10.1016/j.fsisyn.2024.100475>
- [39] Grassberger, M.; Reiter, C., Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomalen- and isomorphen-diagram. *Forensic Sci Int*, 120(1–2): 32–36, 2001. [https://doi.org/10.1016/S0379-0738\(01\)00413-3](https://doi.org/10.1016/S0379-0738(01)00413-3)

- [40] Richards, C.S.; Crous, K.L.; Villet, M.H., Models of development for blowfly sister species *Chrysomya chloropyga* and *Chrysomya putoria*. *Med Vet Entomol*, 23(1): 56–61, 2009. <https://doi.org/10.1111/j.1365-2915.2008.00767.x>
- [41] Midgley, J.M.; Villet, M.H., Development of *Thanatophilus micans* (Fabricius 1794) (Coleoptera: Silphidae) at constant temperatures. *Int J Legal Med*, 123(4): 285–292, 2009. <https://doi.org/10.1007/s00414-008-0280-0>
- [42] Sharif, S.; Qamar, A., Insect faunal succession on buried goat carcass in Aligarh region of Uttar Pradesh, India, with implications in forensic entomology. *Egypt J Forensic Sci*, 11(1): 21, 2021. <https://doi.org/10.1186/s41935-021-00235-5>
- [43] LeBlanc, H.N.; Logan, J.G., “Exploiting insect olfaction in forensic entomology”. In: *Current Concepts in Forensic Entomology*. Amendt, J., Goff, M., Campobasso, C., Grassberger, M., Eds. Springer: Dordrecht, Netherlands, 205–221, 2009. https://doi.org/10.1007/978-1-4020-9684-6_11
- [44] Verma, K.; Paul, R., Assessment of postmortem interval (PMI) from forensic entomotoxicological studies of larvae and flies. *Entomol Ornithol Herpetol*, 2(104): 2161–0983.1000104, 2013.
- [45] Villet, M.; Byrd, J.H.; Castner, J.L., Forensic entomology: the utility of arthropods in legal investigations forensic entomology: the utility of arthropods in legal investigations. *Afr Entomol.*, 18: 387–387, 2010. <https://doi.org/10.4001/003.018.0221>
- [46] Benecke, M.; Lessig, R., Child neglect and forensic entomology. *Forensic Sci Int*, 120(1–2): 155–159, 2001. [https://doi.org/10.1016/S0379-0738\(01\)00424-8](https://doi.org/10.1016/S0379-0738(01)00424-8)
- [47] Anderson, G.S., Effects of arson on forensic entomology evidence. *Can Soc Forensic Sci J*, 38(2): 49–67, 2005. <https://doi.org/10.1080/00085030.2005.10757584>
- [48] Corotti, S., Dattrino, F., Boulkenafet, F., and Lambiasi S., Altitudinal variations in forensically relevant dipterans in Trentino region Italy: implications for PMI estimation and forensic ecology. *Forensic Sci Med Pathol*, 21(6): 1–14, 2024. <https://doi.org/10.1007/s12024-024-00909-1>
- [49] Amendt, J., Insect decline—a forensic issue? *Insects*, 12(4): 324, 2021. <https://doi.org/10.3390/insects12040324>
- [50] Rivers, D.; Thompson, C.; Brogan, R., Physiological trade-offs of forming maggot masses by necrophagous flies on vertebrate carrion. *Bull Entomol Res*, 101(5): 599–611, 2011. <https://doi.org/10.1017/S0007485311000241>
- [51] Nabity, P.D.; Higley, L.G.; Heng-Moss, T.M., Effects of temperature on development of *Phormia regina* (Diptera: Calliphoridae) and use of developmental data in determining time intervals in forensic entomology. *J Med Entomol*, 43(6): 1276–1286, 2006. <https://doi.org/10.1093/jmedent/43.6.1276>[https://doi.org/10.1603/0022-2585\(2006\)43\[1276:EOTODO\]2.0.CO;2](https://doi.org/10.1603/0022-2585(2006)43[1276:EOTODO]2.0.CO;2)
- [52] Richards, C.S.; Villet, M.H., Data quality in thermal summation development models for forensically important blowflies. *Med Vet Entomol*, 23(3): 269–276, 2009. <https://doi.org/10.1111/j.1365-2915.2009.00819.x>
- [53] Baque, M., Filmann, N., Verhoff, M.A. and Amendt, J., Establishment of developmental charts for the larvae of the blowfly *Calliphora vicina* using quantile regression. *Forensic Sci Int*, 248: 1–9, 2015. <https://doi.org/10.1016/j.forsciint.2014.12.020>
- [54] Charabidze, D., Bourel, B., Leblanc, H., Hedouin, V. and Gosset, D., Effect of body length and temperature on the crawling speed of *Protophormia terraenovae* larvae (Robineau-Desvoidy) (Diptera Calliphoridae). *J Insect Physiol*, 54(3): 529–533, 2008. <https://doi.org/10.1016/j.jinsphys.2007.11.010>
- [55] Singh, D.; Bala, M., The effect of starvation on the larval behavior of two forensically important species of blowflies (Diptera: Calliphoridae). *Forensic Sci Int*, 193(1–3): 118–121, 2009. <https://doi.org/10.1016/j.forsciint.2009.09.022>
- [56] Brown, K.; Thorne, A.; Harvey, M., Preservation of *Calliphora vicina* (Diptera: Calliphoridae) pupae for use in post-mortem interval estimation. *Forensic Sci Int*, 223(1–3): 176–183, 2012. <https://doi.org/10.1016/j.forsciint.2012.08.029>
- [57] Davies, K.; Harvey, M.L., Internal morphological analysis for age estimation of blowfly pupae (Diptera: Calliphoridae) in postmortem interval estimation. *J Forensic Sci*, 58(1): 79–84, 2013. <https://doi.org/10.1111/j.1556-4029.2012.02196.x>
- [58] Kumar, A., Rajak, K. K., Yadav, A. K., Rai, V., Bhatt, M., Singh, R. P. and et al., Collection of samples, their preservation and transportation. In: *Protocols for the Diagnosis of Pig Viral Diseases*; Deb, R., Yadav, A.K., Rajkhowa, S., Malik, Y.S., Eds. Springer, Humana: New York, NY, pp. 21–30, 2022. https://doi.org/10.1007/978-1-0716-2043-4_2
- [59] Abd Al Galil, F.M., Zambare, S.P., Al-Mekhlafi, F.A. and Al-Keridis, L.A., Effect of dimethoate on the developmental rate of forensic importance Calliphoridae flies. *Saudi J Biol Sci*, 28(2): 1267–1271, 2021. <https://doi.org/10.1016/j.sjbs.2020.12.022>
- [60] Abd Al Galil, F.M., Zambare, S.P., Al-Mekhlafi, F.A., Wadaan, M.A. and Al-Khalifa, M.S., Effects of insecticide dimethoate on the developmental rate of forensic importance sarcophagid flies. *J King Saud Univ Sci*, 33(2): 101349, 2021. <https://doi.org/10.1016/j.jksus.2021.101349>
- [61] Ventura, F.; Gallo, M.; De Stefano, F., Postmortem skin damage due to ants: description of 3 cases. *Am J Forensic Med Pathol*, 31(2): 120–121, 2010. <https://doi.org/10.1097/PAF.0b013e3181d3db89>
- [62] Paczkowski, S.; Schütz, S., Post-mortem volatiles of vertebrate tissue. *App Microbiol Biotechnol*, 91(4): 917–935, 2011. <https://doi.org/10.1007/s00253-011-3417-x>
- [63] Omer, S.S.A., The Succession of Forensic Beetles on Exposed and Wrapped Carcasses during Winter and Summer in Khartoum State. Master’s thesis, Khartoum University, Sudan, 2014.
- [64] Kohlmeier, R.E., Beating the devil’s game: a history of forensic science and criminal investigation. *Am J Forensic Med Pathol*, 32(2): e12, 2011. <https://doi.org/10.1097/PAF.0b013e3181dbac81>
- [65] Benecke, M., “Arthropods and corpses”. In: *Forensic Pathology Reviews*; Tsokos, M., Eds. Springer: New York, NY, pp. 207–240, 2005. <https://doi.org/10.1385/1-59259-872-2:207>
- [66] Rivers, D.; Geiman, T., Insect artifacts are more than just altered bloodstains. *Insects*, 8(2): 37, 2017. <https://doi.org/10.3390/insects8020037>
- [67] Peschel, O., Kunz, S.N., Rothschild, M.A. and Mützel, E., Blood stain pattern analysis. *Forensic Sci Med Pathol*, 7(3): 257–270, 2011. <https://doi.org/10.1007/s12024-010-9198-1>
- [68] Lassaingne, J.B., Neue untersuchung zur erkennung von blutflecken auf eisen und stahl (New investigation into blood stains on iron and steel). *Vierteljahresschrift für Gerichtliche Oeffentliche Med*, 10: 285–289, 1856.
- [69] Gennard, D., *Forensic Entomology: An Introduction*. John Wiley: Hoboken, NJ, 2012.
- [70] Colombage, S.M.; Telisinghe, P.U., An unusual finding in a body recovered from the sea. *J Forensic Leg Med*, 17(5): 289–290, 2010. <https://doi.org/10.1016/j.jflm.2010.04.005>
- [71] Vanin, S.; Zancaner, S., Post-mortal lesions in freshwater environment. *Forensic Sci Int*, 212(1–3): e18–e20, 2011. <https://doi.org/10.1016/j.forsciint.2011.05.028>

- [72] Wang, Y., Wang, M., Xu, W., Wang, Y., Zhang, Y. and Wang, J., Estimating the postmortem interval of carcasses in the water using the carrion insect, brain tissue RNA, bacterial biofilm, and algae. *Front Microbiol*, 12: 774276, 2021. <https://doi.org/10.3389/fmicb.2021.774276>
- [73] Barbosa, R.R., Carrico, C., Souto, R.N., Andena, S.R., Ururahy-Rodrigues, A. and Queiroz, M.M., Record of post-mortem injuries caused by the neotropical social wasp *Agelaia fulvofasciata* (Degeer) (Hymenoptera, Vespidae) on pig carcasses in the Eastern Amazon region: implications in forensic taphonomy. *Revista Brasileira de Entomologia*, 59(3): 257–259, 2015. <https://doi.org/10.1016/j.rbe.2015.07.004>
- [74] Bushby, S.K., Thomas, N., Priemel, P.A., Coulter, C.V., Rades, T. and Kieser, J.A., Determination of methylphenidate in calliphorid larvae by liquid–liquid extraction and liquid chromatography mass spectrometry–forensic entomotoxicology using an in vivo rat brain model. *J Pharm Biomed Anal*, 70: 456–461, 2012. <https://doi.org/10.1016/j.jpba.2012.06.024>
- [75] Ojewumi, M.E.; Obanla, O.R.; Ataubu, D.M., A review on the efficacy of *Ocimum gratissimum*, *Mentha spicata*, and *Moringa oleifera* leaf extracts in repelling mosquito. *Beni-Suef Univ J Basic Appl Sci*, 10(1): 87, 2021. <https://doi.org/10.1186/s43088-021-00176-x>
- [76] Chen, W.Y.; Hung, T.H.; Shiao, S.F., Molecular identification of forensically important blowfly species (Diptera: Calliphoridae) in Taiwan. *J Med Entomol*, 41(1): 47–57, 2004. <https://doi.org/10.1603/0022-2585-41.1.47>
- [77] Ercan, F.S.; Baş, H.; Azarkan, S.Y., *In silico* detection of Cucurbitacin-E on antioxidant enzymes of model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae) and variation of antioxidant enzyme activities and lipid peroxidation in treated larvae. *Beni-Suef Univ J Basic App Sci*, 11(1): 130, 2022. <https://doi.org/10.1186/s43088-022-00310-3>
- [78] Richards, C.S.; Crous, K.L.; Villet, M.H. Models of development for blowfly sister species *Chrysomya chloropyga* and *Chrysomya putoria*. *Med Vet Entomol*, 23(1): 56–61, 2009. <https://doi.org/10.1111/j.1365-2915.2008.00767.x>
- [79] Tomberlin J.K.; Adler, P.H.; Myers, H.M., Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. *Environ Entomol*, 38(3): 930–934, 2009. <https://doi.org/10.1603/022.038.0347>