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Advance Techniques Used in Forensic Entomology

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Authors' contributions

This work was carried out in collaboration between both authors. Author GD conceptualized, designed, and performed the analysis and interpretation of the data. Author DP developed the initial draft of the article, revised it critically for important intellectual content, and approved the final version. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Forensic Entomology plays a very important role in forensic science. It provides valuable inputs for crime scene investigation. This involves looking into the many pest species that are commonly related to dead bodies, as well as their life cycles and biological presence in a specific environment. This review included concepts of forensic entomology, stages of decomposition and the latest techniques used by entomologists. It gives the detailed study of different techniques that are used to examine the entomological evidences, such as molecular analysis, entomotoxic analysis, Micro CT, cuticular hydrocarbon analysis, etc. by taking the references of previous researches. This review focuses light on the factors affecting PMI estimates and analyzes the uses of entomological data in PMI estimation. The main task is to determine the age of the insects. This review focuses on the advantages and disadvantages of age-determined methodologies. The aim of this study is to summarize techniques that are so far used commonly and all the latest advances made in the concerned field. All these methods have shown more reliable results, adequate accuracy and more

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effectiveness. As evidence contamination is a major setback in providing results, these techniques have less risk of contamination of evidence and have also proved to be less time consuming.

Keywords: Advance techniques; death time estimation; forensic entomology; insects; post-mortem interval.

1. INTRODUCTION

Forensic entomology is the branch of forensic science that deals with the study of insects of class arthropods in relation to criminal investigation (Sanjay Kumar Meena 2020). It focuses on the study of insects to identify the outlook of crime, like post mortem interval, position or location of body, etc. Insects are the very important evidence for the entomologist according to the time period, location, temperature, climate, etc. there are various insects that are developed on the dead body. A Forensic Entomologist examines the different stages of insects on the body as egg, larva, pupa and adults which change with respective time periods. Forensic entomologists collect all the insects from the crime scene, examine the breed of insect and analyze the stage of the insect then compare according to climate and assume the location, time since death and also provide a link of the type of crime. The entomology work on locard's principle of exchange "everything that comes in contact leaves the traces" that means the insects leaves the traces when comes in contact with crime scene, victim, etc. (Isaac Joseph 2011).

2. FORENSIC ENTOMOLOGY FIELD

Forensic Entomology is classified to three field and subfield:

- Urban entomology- It is the field of forensic entomology that included study of insects and arthropods found in urban areas, such as soil, garbage or muddy water, etc. to find out the cause and infestation in places like buildings and gardens. (Raut 2008).
- Stored products entomology- this subfield is related to investigation of cases of insects like contamination of packet food and any legal case on food quality and Safety (Ian Robert Dadour 2014).
- Medicolegal entomology- this subfield is related to study of insects and arthropods from the crime scene, to collect evidences to conclude the cause, time and location of death (Ian Robert Dadour 2014).
- These field and subfields help to get the answer of why, what and where related to any cases.

3. STAGES OF DECOMPOSITION OF CADAVERS

- Fresh stage – It is the 1st stage of decomposition that starts immediately after death and ends when the body starts to expand. It up to 1 to 2 days. This stage is called autolysis or self -digestion. That means quickly stopping the respiratory and blood circulation in the body, which increases the amount of carbon dioxide acidic in the body. Due to acidic natures the cells get damaged and released enzymes. The rigor mortis (stiffening of the body) also takes place in this stage (Raut 2008).
- Bloat stage – 2nd stage of decomposition. In these stages the body starts swelling, the enzymes released in 1st stages produce infinite gases. Due to the gases, the body becomes four times its original size. Also, the skin color is getting darker due to the sulfur compound chemical discharge by bacteria. Highly unpleasant smell is produced by enzymes called in terms of putrefaction. Smell can stay for a long time (ML 1993).
- Decay stage – In 3rd stage of decomposition the body fluids are released from the opening of the body. The organs, skin start melting and degenerating. The hard tissues of the body such as hair, cartilages, bones, etc. are left over after all soft tissue has decomposed (Raut 2008)
- Dry stage – In this stage starts when only traces of decaying tissues are left. This stage is challenging because it has many tasks and difficult to define the boundaries. Many varieties of creatures reside and some content of moisture is there due to dew, rain, etc. Very small quantities of partially decaying materials are present there (Bornemissza 1957).
- Skeletal stage – skeletonization doesn't have any exact time duration that depends

upon the decomposition of organic and inorganic materials. At this stage of decomposition, the investigators get clues from the soil samples (Vass 2022).

After the death, insects are the first present on all openings of the body. There are 4 categories of insects studied as follows:

- Necrophages – It gather the insects that comes first to feed body tissues and stay for shorter period of time like half of day or one day. They are very useful for PMI Estimation (GOFF 2000).
- Predators – When various study is done, particularly on the predators, so they are also a helpful tool for predicting the post-mortem interval according to various studies. Parasites that might consume other species that have previously occupied the body.
- Omnivorous - In this category including animals like beetles that owns the breakdown process, significantly less helpful for determining the PMI (GOFF 2000).
- Incidental or adventurous – which are only accidental visits and typically have no forensic significance. But no matters the categories all the insects need to be counted by entomologist (Mudassir Alam 2024).

4. LIFE CYCLE OF INSECTS

Most common insects found on bodies are flies and beetles; both are from the different taxa with significance in Forensic Entomology. Flies are the very previous insects to appear after the death, Around the natural orifices or injuries on body, female flies will lay eggs or live larvae. So, the larvae produce bacteria and enzymes that will aid in the soft tissue consumption of the corpse.

The life cycle of insect's results from some biological changes known as metamorphosis, which involved different stages that included the vast changes to insect body structure as result of cell growth and differentiation. The life cycle of an insect includes four stages: egg, larva, pupae, and adult. Not every bug will go through each of the four phases (Martin H Villet 2011).

Egg – In this the adult female insect that lays the eggs. While some eggs can develop through parthenogenesis without fertilization, others require coupling and fertilization in order to grow up. As the eggs hatch, larvae appear.

Larva - The insect's larval stage is when it is still juvenile and feeds. Larvae and adults have extremely distinct appearances, live in different environments, and eat different things. Maggots, grubs, and caterpillars are a few types of larvae. As they develop, larvae molt, or lose their outer shell, multiple time.

Pupa - The pupa spent much of this stage resting and not active. The pupa's adult structure is rearranged by the bursting larval tissues. The pupa molts once this procedure has concluded, appearing as an adult with two wings. During the pupa stage, the insect's wings fully mature and it becomes an adult, entering the world (Insect Life Cycle n.d.).

Adult – In this stage, insects have tactile hair which is present on the antennae, legs, and torso of adult insects, aids in the insects' ability to sense their surroundings. An insect's entire surface is covered in tactile hair. The adult insect is sexually developed at this point and mostly engages in reproduction (kualo n.d.).

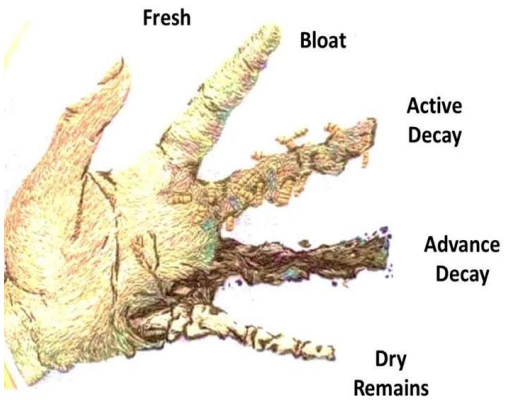

Fig. 1 Stages of Decomposition of cadavers
Reference: https://forensicfield.blog/tag/dead-body-decomposition-stages/

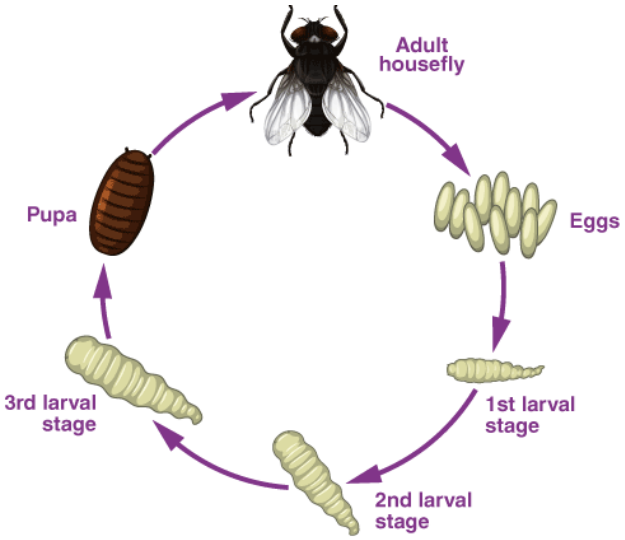

Fig. 2 life cycle of fly
Reference: https://byjus.com/biology/fly-life-cycle/

5. COLLECTION AND PRESERVATION OF ENTOMOLOGICAL SAMPLES-

Collection of entomological evidence may be in different stages of eggs, maggots, pupae, adult and different areas of cadavers and surrounding environment. Collected samples provided clues about the injuries, time since death, locations, etc. for the collection appropriate tools and techniques are used such as gold, silver, or bronze standards that depends on the experts of the crime scene investigations.

Gold Standard: In this forensic entomologist cannot attend the crime scene for collection, instead of forensic entomologist their proxy visits the crime scene they use of special tools like entomology kits, brushes, forceps, spoons, insect nets, sticky traps, vented containers and a chilled fridge for transportation. Gathers microclimatic information for ten to twelve days following discovery, including soil temperature, humidity, photoperiod, temperature of the larvae mass, and ambient temperature.

Sliver Standards: Here, involves the collection of any entomological evidence by pathologist, police officer, or medical examiner with the trained forensic examiners. They use containers, preservatives, and disposable forceps that are given by the organization in charge of the crime scene gathers information from the closest meteorological station about the surrounding temperature and rainfall (Tharindu B. Bambaradeniya 2023).

Bronze Standards: In this involves collecting eggs, larvae, and pupae with an unskilled police officer or field technician using containers, preservatives, and disposable forceps that are given by the organization in charge of the crime scene (Chada Anu Reddy 2023).

Preservation of insects or entomological evidence for analyses and to determi9ne the post mortem interval, live insects should place into ventilated containers. It can be refrigerated or frozen and for eggs should add the moist paper to avoid dehydration. Preservative used for the preservation 70-80 % ethanol, Isopropyl alcohol, formalin, methanol (Tharindu B. Bambaradeniya 2023).

Labeling inside and outside of the preservative containers should properly indicate the date, time, case reference number, and type of specimen.

The proposed downstream investigation, such as morphological identification, toxicity, or molecular approaches, will determine which preservative is best. The most used preservative for gathering general entomological data is ethanol.

6. ADVANCED TECHNIQUES USED IN FORENSIC ENTOMOLOGY

6.1 Electron Microscope (scanning electron microscope)

A wide range of morphological characteristics are available for identifying fly eggs by using the SEM. As long as there is enough time, suitable tools, and the specific fly eggs are available, the SEM procedure operates well. Considering these morphological variations, for Forensic Entomologists these is a useful tool that can assist in determining a PMI and other linked information, like whatever the body has been moved after the death. Some morphological traits that can be used to differentiate between species includes the and form of median area, the presence and lack of holes or anesthetic. One technique that can be used to identify important molecular features of eggs and maggots is scanning electron microscopy (SEM) (N. Ubero-Pascal 2010).

SEM is used to analyze fly artifacts produced by insects on rough surfaces and some fabrics also. It helps to differentiate bloodstain from the fly artifacts on the basis of amorphous crystals and absence of RBCs. It is non-destructive, allowing for analysis of evidence without damaging it. Particularly useful for biological samples like insects (Guido Pelletti 2019).

6.2 Entomotoxicology

The study of using insects as a substitute for toxicological samples is known as Entomotoxicology. The excessive use of medications, pesticides, insecticides and poison is a major cause of death globally. In this condition, the original toxicological samples like tissues, body fluids, organs are no longer available sometime, so the second reliable specimen insects are available (Rito Chophi 2019). As insects use dead bodies as food supply for their larvae, they are important to investigation. During their time of feeding on cadaveric tissues, the larvae's metabolic system absorbs all the substances from cadavers, which include medicines and other hazardous compounds like drugs, poisons found in the tissue. To detect these substances from insects Immunoassay, HPLC, LC-MS, GC-MS has been utilized commonly (Matthias Gosselin 2011). Entomotoxicology is one of the best tools to detect poisonous substances. Sometimes it is challenging to do toxicological testing on bodies that are skeletonized or in an advanced decay stage of decomposition so the larvae consume substances that can be examined using thin layer chromatography. Toxins have the ability to affect larvae’s developmental phases. The presence of heroin or cocaine in the body may speed up the colonization and poisons such as malathion postpone the colonization of insects (D. W. Sadler 1995).

6.3 Molecular Identification (DNA Analysis)

Most of the time the entomological evidence is not accurately examined for species identification. Molecular data is helpful to identify insect species, morphological identification is obtained. Here we need to extract mitochondrial DNA materials, and also need to prepare the DNA extraction samples by using various methods. By the identification of the correct species, estimate the age of larvae. In this method for determining species, we need to perform morphological comparison, which takes lots of effort and skilled people with specialized knowledge about the DNA estimation. To overcome the challenge, species identification is done by using polymerase chain reaction (PCR), random fragment length polymorphism (RFLP), randomly amplified polymorphic DNA(RAPD), Inter simple sequence repeat (ISSR). Only the suitable region of larvae’s genome is used as reference data (K Schoenly 1987).

6.4 Micro CT (Micro- computed tomography)

In Forensic entomology the microCT has become a very important tool that enhances the analysis of insect anatomy and developmental stages in post-mortem examination, it allows the visualization of insect larvae, pupae and adults inside the plant tissues to display the exact arrangement of space (Schmidt VM 2022). All this information is crucial to understand the feeding habits, growth, and ecological functions of insects. Micro CT techniques provide the high- resolution view of insect anatomy without any destruction of samples, this is essential for estimating the postmortem interval and to understand the ecological connections between insects and the crime scenes (Nur Aliah 2024). These techniques allow researchers to examine external and internal structures of insects in traditional

techniques that need to follow the dissection procedure which can destroy the valuable morphological information, but here in advance no need of dissection, it provides reliable parameters for age, time estimations. The primary advantage of the technique is its non-invasive nature, allowing detailed examination with any damage to samples (Donkó 2022).

6.5 Stable Isotope Analysis

Stable Isotope Analysis has become the most precious tool in forensic entomology, that provides a deeper view of geographic origin and life cycle of insects with respect to human remains. This technique includes the study of stable isotopic composition of elements like carbon, nitrogen, hydrogen and oxygen in insects' tissues, so the researcher gets information about diet, environment in which the insects developed. By using these techniques forensic entomologists can deduct the information about the insect diet such as whether it contained organic material or human remains. By studying isotopes, we can identify the breakdown process of insects. The implementation of this method can enhance the precision of their evaluations and provide important information about the situation surrounding a death. But the biggest obstacles to the use of isotope analyses in forensic entomology are its errors, lack of requirements and lack of reference materials (Agency 2009).

7. CONCLUSION

This review renovates more of the recent knowledge that applied in forensic entomology, or all the situations that faced by the entomologist are significantly documented in the literature. One of the key concerns of forensic entomologists is education and practical knowledge about the entomological evidence while collecting the insects and other biological fluids from the crime scene, their collection and preservation is a major part for entomologists. The review helps to make an appropriate collection and examination. Their decision making will enhance by reading this document. It cannot be overstated how important it is that these efforts be of the highest quality if the forensic entomologist and the case in general are to profit from the evidence and related data especially in the view of the court future examination of it.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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