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Forensic Entomology: A Comprehensive Review

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Abstract

Determination of post mortem interval is done with various methods depending upon the condition of the dead body and the circumstances in which it is found. Ruling out foul play in unnatural deaths has been an enigma of a sort for forensic pathologists and scientists all over the world. The methods to determine the cause and manner of death keep on emerging with ongoing researches in the fields of forensic sciences. Many methods have been devised to determine cause, manner and specifically time since death. Forensic entomology is one such field that aids in determination of time since death, especially in putrefied corpses found in different habitats in mysterious unknown circumstances. The field keeps on emerging with the advent of new techniques on molecular level, including DNA analysis and identification based on entomological data that has diversified over the years. The standards and guidelines for entomological data collection and processing needed for implication on the crime scene must be revised now and again for assistance of crime scene investigators, scientists, entomologists and pathologists. Various factors must be considered while processing entomological data that affect post mortem intervals. The keen study of life cycles, groups of similar insects, valid and standard methods of insect collection, breeding and identification can give a lead on cause, manner, time, place and circumstances of unknown or unnatural deaths.

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Introduction

Solving forensic cases complicated with identity issues of the victims and time elapsed since crime has become a lengthy task for crime scene scientists. Few circumstantial evidences can be of great value in saving time and ruling out the cause and time since death. One of the circumstantial parameters is forensic entomology. Though not a priority for crime scene investigation, it still has paramount importance in cases of unknown and unnatural deaths. Currently, mass disasters, whether natural or unnatural have been on the rise for the past decade. Forensic entomology can be positively utilized to determine post mortem intervals in a variety of cases. This article will provide an overview of basic and new techniques for insect collection, preservation and lab analysis. We will briefly describe history, old methods and new molecular advances in the study of forensically relevant insects.

Forensic entomology is the study of the insects that inhabit the flesh of dead bodies of humans and animals for purposes of law and helping legal agencies to solve criminal cases [1]. It aids in determination of time onwards since death till the sighting of the dead remains [2]. A new term arises relevant to forensic entomology study, which is an entomological post mortem interval that is defined as the time taken from the colonization of flesh-eating insects to their last developmental or adult stage [3]. This entomological timeline is utilized when the traditional markers or rate methods of post mortem intervals have weaned off and remains are putrefied or unrecognizable. Entomology provides the concurrent time line used to determine the time since death. When the time-since-death has exceeded more than 72 hours, this concurrent method of forensic entomological time line becomes an integral part of death investigations [1].

With advances in the lab techniques, DNA identification of insects is also useful in determining the time since death and species identification of the insects [4]. For species identification regarding their demographic origin and possible relevance to the site of crime, mitochondrial DNA determination utilizing the CO1 gene is also in current use. Sharma and Singh in 2017 stated that determination of Genetic Fingerprinting of flesh eating insect specimen can be of higher value if medico legal centers and labs support forensic entomologists with proper DNA settings [1,5]. Different species of insects have different DNA compositions. Insect gene expressions are unique and this fact facilitates forensic entomologists to carry out species identification and determine the postmortem interval intervals [1].

Durdle *et al.,* in 2016 stated that even the blow artifacts can be utilized to get human DNA profile. The species identification is carried out by using suitable primers of the polymerase chain reaction or locating correct sequencing sites. Most suitable targets are the STRs, RAPDs and minisatellite DNA. The non-repetitive, but unique sites on mtDNA are the most possible targets. The differential gene expression can determine the possible estimation of blow fly age [6]. Tarone *et al.*, profiled the expression of three genes, bicoid, slalom, chitin synthase (*bcd, sll, cs*) throughout the blow fly egg maturation to predict their developmental stage, detecting prominent linear trends in gene expression during insect growth [7].

Zehner *et al.*, monitored a differential gene expression in *Calliphora vicina* pupae at different ages by using a specific ddRT-PCR protocol. Insects have non-coding mtDNA region containing high numbers of adenine and thymine bases, which aids in molecular identification in forensic entomology [8]. mtDNA of flesh-eating flies provides useful molecular information that facilitates new species diverse analysis. The cytochrome oxidase subunits 1 and 2 can actively be used for determining molecular diversity in various insect species [9]. Molecular analysis allows identification through partial specimen rather than intact specimen as required in morphological identifications which are considered as lengthy and outdated methods now [10].

Methods

Literature Search Strategy and Selection Criteria

The literature was searched by using the following search terms, "Forensic Entomology", "Insects in Forensic Sciences", "Insects DNA Barcoding", "Forensic Insects Life Cycles", "Updated Methodologies of Using Insects in Forensic Sciences" and "Forensic Entomology in the History". The publications that were about insects, but with any forensic perspective were excluded while writing this review. Google Scholar services were used for searching the articles. In this study, 77 peer reviewed research articles were selected.

Discussion

Insect chronicles

Being vastly abundant taxa, insects are both land and aquatic inhabitants [11]. Not classified as animals though they are part of arthropods of the animal kingdom with as much as six to ten million extant species inhabiting vast majority of dry land on the planet [12,13]. Only a million have been identified and described. Insects have an anatomy that has been divided into segments, so is the literal meaning of the word insect whether insect or entomon [14]. From nuisance value in disseminating many animal and human diseases they have vast industrial usage and benefits ranging from food, clothes and cosmetic industries [15].

McGraw Hill in 2007 described the holometabolism of insect, ranging from egg laying to adult stage as a four-stage metamorphosis. The first stage is the egg, when hatched larvae emerge that after feeding enough enclose themselves into pupal shells that break after a certain time to emerge as adult flies or insect, a process also called eclosion [7]. The adult insects have an amazing sense of smell that makes them reach the food easily. The enclosed or embalmed bodies escape the invasion of insects to limited time and mummify but exposed are rapidly consumed by the flesh-eating scavengers including insects which are the primary and permanent residents of dead matter, owing to the smell of gases and organic fluids. The only clues they leave behind are the skeletonized remains. Though various environmental factors affect insect life cycles, but every species has a similar and unique pattern of succession [15].

Catts and Goff in 1992 explained the utilization of the foreseeable and unsurprising insect life cycles as a major timeline to determine the time elapsed since the incidents [16]. The mere physical presence of entomological evidence at the crime scene determines many other forensic aspects of the crime or unnatural occurring. Proper collection and handling of this entomological evidence and proper lab analysis is required to bring out the best in investigations at the crime scene or labs for helping legal authorities to solve the mysteries.

1981 account of Tz'u describes origins of forensic use of insects dating back as far as the 13th century. Al Mesbah in 2010 commented on the 1767 observation of insect succession by Carl Von Linne. Similar observations can be studied in the texts of 18th and 19th century [17]. Gomes and Zuben in 2006 describe first forensic case to be solved using entomological evidence by a French physician Bergeret [18]. From France the science of forensic entomology spread to Canada, United States and Europe, according to Benecke statement in 2005 [19]. This includes a famous case of Buck Ruxton murder charges in 1935 and insect life cycles association with decomposition rate of dead remains by Reed [19].

So far, the studies until the 20th century were limited to the traditional morphological identification of insect in relevance to their predictable life cycles according to their class, order and species that made entomology as a lengthy and time-wasting field in forensics but useful in mysterious and unnatural criminal cases. With further scientific advancements and utilizing DNA typing to identify insects and barcoding has encouraged legal investigators rely on forensic entomological data more than in previous times [1].

Medico legal significance of insects

The insects have constant and predetermined life cycles that have been reported extensively in the past. Each species has a unique sequence of metamorphosis. Fixed and constant entomological timeline can lead to accurate species identification, and a decent determination of time since death. The arthropods are of special forensic interest to scientists. The insect succession of the dead remains embarks a natural timeline on the crime scene until they are discovered, collected and observed till their maturation under controlled environment. This ensures an accurate post colonization biological timeline [18,20].

Though the life cycles are fixed, and predictable, certain environmental factors can shorten or prolong the time of maturation of the insects. The most significant variable is temperature and humidity. Though there are some insignificant factors like shade, rainfall and food sources and drugs [21].

The faunal succession

The assessment of putrefaction in the remains and to associate these with insects in different developmental stages needs knowledge of entomology and its training. According to Byrd the fresh dead matter attracts Blow flies (*Calliphoridae*) initially in the first 3 months. They lay eggs that resemble rice particles. The dead matter when it starts to putrefy releases a smell in the first couple of months, attracting both blow and flesh flies (*Sarcophagidae*). The flesh flies are viviparous. If the fat goes rancid in the dead organic matter in next 3-6 months, it keeps on attracting various flies and interestingly. Dermestid beetles (*Coleoptera*) also. In further 4-8 months this process of flesh eating continues, and mites can also be recovered in 1-12 months. If the dead remains go completely dry in 1-3 years there is still the possibility to discover Dermestid beetles, which are found even after 3 years of death [3].

Collection of entomological evidence

After locating the dead body or remains, evidence should be collected from above, below and inside the flesh. If there are live adult flies hovering above the dead body, they should be captured manually by using nets. Dead and alive insects are preserved separately in separate containers. The containers are sealed and labeled accordingly with details of the time of collection, location, developmental stage with names and contacts of collector scientist. The alive and immature insects are reared in the lab facility until they mature for morphological identification [3].

While collecting entomological evidence, special attention should be taken in cases of child sexual assault for the phenomenon of myasis in the peri-anal and genital areas that exist before corpse infestation, in geriatric neglect, with the history of bed sores and injuries. The insect specimen can resist many calamities like extremes of weather or fires even. The extreme fires cannot destroy the conditions for entomological evidence as they do not hinder the insect invasion or the capacity to determine the time since death; nevertheless it can shorten the time of insect invasion within the first few days [18,20].

The location of the incident or crime scene is crucial for assessment of insect specimen found there. The climate, geography and spot of discovery effectively aid the legal investigations. The complete climatic range of the terrain subtly facilitates the outcomes of probing the facts. Effective collection and handling of these delicate evidences provides better recognition of facts and discovering crimes [22].

Estimation of Post Mortem Interval

The forensic entomological clock has two observable parts.

1. Period of insect isolation
2. Time since insect colonization

The period of insect isolation is the time of invasion of flesh eating insects on the dead matter, while time since insect colonization is the developmental stage of the discovered insect from the crime scene [23].

Different insect species have a variable or unique timeline of morphological growth. The developmental stages of larvae when they increase in length, weight or shape is described as instars. The post feeding larvae isolate themselves into the soil or dark places to convert themselves into pupae. The last metamorphosed stage is the cracking of larvae, a process called eclosion when the adult insect emerges [24]. For complete developmental analysis the weather report from the nearest meteorological center should also be consulted [25].

Methods to determine PMI

Various methods are used globally to calculate post mortem interval by means of inset growth on the dead matter.

Stages of succession

Succession of insects on carrion depends upon the biogeographical region where dead matter exists. These succession patterns determine the minimum and maximum intervals of time since death. There is a variety of species with succession stages depending upon changes in decomposition stages no matter how advanced [26].

Age dependent variation of intestinal contents

Life cycle of insects can be taken as precise clocks starting even within minutes of death. The first instinct is to be attracted by the odor of a decaying corpse. The blow flies reach within a few minutes and lay eggs that hatch to form first instar larvae. After moulting they become third instars, that go in wandering stage to pupate. Radiological examination of larval feeding habits shows that maggots stop eating the moment they reach maximum length. Afterwards the anterior intestine remains empty, intestinal filling can help to estimate the postmortem interval [27].

On stage invasion

The postmortem interval can be determined from the stage of insect present on cadaver by using the following formula:

T=A+B×C

A = Stage of colonization, B = life cycle stage, C = Correction of climatic factor [28].

Developmental patterns

Developmental patterns of flies can be applied to forensic applications. The time period required for insect species to develop to the stage of growth is taken as post mortem interval. Blow flies and flesh flies provide a decent estimate, though their short life cycles are useful during first three to four weeks following the death only [29]. The larvae are reared in laboratory to adult forms and post mortem intervals are estimated. Many factors affect this growth period of insects, mainly temperature and humidity that influence certain factors like rate of oviposition and maturity [30].

Weights of larvae

Weight of larvae can be estimated and can be used to determine larval age. Under controlled condition of laboratory their weights are measured. The time interval between the hatching of eggs and post feeding stage is estimated and a statistical model is formulated that relates weight distribution to age. This formulation is termed as calibration or inverse prediction. If larval age is unknown, its weight can be compared to model by inverse prediction and a confidence interval on larval age can be made. While making confidence intervals, weight of one larva can be regarded as been sampled from a larval population at random, the population is assumed to be at the same age level and under the same conditions. Different areas have different insect species, so separate models are made for each species. Scientists have generated their own baseline data for conditions relevant to any specific location, species and environment [31].

Isomegalen/isomorphen diagrams

The whole life cycles of insects and their development can be observed at various scales of temperature. This observation can be plotted as isomegalen diagram where time since egg hatching is taken at x-axis and temperature at y-axis. Age estimation of insects can be done by graphical lines representing larval length. The isomorphen diagram represents all the structural stages from hatching of eggs to eclosion phase are taken, the area between the lines in the graph represents morphological stages of insects. It can determine the age of post feeding larvae or pupae [32].

Fly eggs

The egg laying attitude of insects can help to determine post mortem intervals. Within minutes of death, insects arrive and feed on fresh dead corpses. Observation of time of hatching of eggs in the laboratory at constant temperatures can be used to generate data to be used in actual forensic cases. This experimental and developmental data represents an egg hatching time within a period of 2 hours to determine the postmortem interval. This can infer emergence of first instar and determine a short post mortem interval [33]. These life cycle studies help in estimating their biotic potential and can be used as a basis for simulation models [34].

From insect's gut contents

Larvae after hatching, feed rapaciously on the dead carrion and rapidly increase in size. Sometimes due to inadequate food supply they wander away to other carrion nearby. In such cases, DNA typing of gut contents can determine which species fed upon which body. This is important evidence proving larval-corpse relationship and can prove a good source for determining time since death [35].

From cuticular hydrocarbons and width

The pupal shells contain hydrocarbons, their presence and wearing inside the cuticles has a direct relationship with time. These slowly changing the ratios of hydrocarbons and the biochemical changes can be evaluated by gas chromatography mass spectrometry. These changes can lead to estimations of post mortem intervals more accurately with these advanced technologies. The cuticular hydrocarbons of pupal shells have n-alkanes, methyl branched alkanes and dimethyl branched alkanes. The hydrocarbon composition and length of carbon chain from C21 to C35 represents significant uniform changes with time. The low molecular weight alkanes with even numbers such as n C22, n C24 and n C26, the abundance rises gradually and markedly with weathering time. As for n C26, for example, the abundance increases in linear pattern with weathering time. For lower molecular weight hydrocarbons like n C26 or less, the abundance decreases considerably with the passage of time [36].

From accumulated degree days/hours (ADD/ADH)
This is another method of measuring time since death. The ADH value represents a specific digit of energy hours, required for insect larval development. The degree day or hour idea describes that the rate of development is proportional to temperature within species specific temperature range. However, this relationship is typically curvilinear at high and low degrees of temperatures and is linear only in between [37].

Aging of the blow fly eggs through gene expression
The flesh-eating insects lay eggs at predictable times during decomposition of corpses. The larvae are not identifiable up to species level and must be reared up to adult stage for correct identification and for correct post mortem identification. This can cause delays in estimating time since death, even up to several weeks. As in forensic cases, quick investigations are required. One reliable method is study of expression of three genes i.e., *bcd, sll, cs*, present in blow fly eggs as a method of determining insect age. This method estimated egg age within 2hrs of actual age when all expression data is available, while presence/absence of cis-transcripts identified two age classes, predicting true blow fly age [7].

Effect of body length and crawling speed
Flies reach dead bodies very soon after death and can give minimum post mortem interval. The size of larvae and their maturity are basic elements indicating first arrival at the corpse. After completing their development, they disperse to find a proper site for pupation. If the temperature is increased, the larvae crawl at a faster speed. The speed is also a function of body length. Such findings are useful for estimating length of time since the departure of larvae from the corpse. This can help estimating time since death [38].

Ontogenetic study
Hydrocarbons are composed of carbon and hydrogen atoms contained in the insect's cuticle. These hydrocarbons have long chains with carbon atoms ranging from 21 to 35 carbons. Ontogeny of these hydrocarbons can be assessed by gas chromatography and studied in different growth stages of insects. These profiles vary in different life stages of insects as well as with the age of individuals by gas chromatography. The eggs or maggots from different species can be identified, if these profiles are different. Composition of cuticular waxes can help determine post mortem intervals [39].

New simulation model
Based on the developmental stage, measured as a function of temperature, a new simulation model has been proposed. The larval age is calculated in steps corresponding to current developmental stage [40].

Larval dispersal and length of larvae
The blow flies lay eggs in natural orifices where moisture and protection from the sun is adequate. Depending upon insect species and ambient temperatures, the blow fly eggs hatch to release first instar larvae. After moulting into second and third instar larvae, post feeding larvae go into puparial stage after moving long distances to find a suitable and dry environment. The lighter the larva, the longer the distance it covers. The way pupae disperse can help with estimation of minimum time since death. Sometimes larvae move away in search of food and may choose another nearby corpse. So, the larval dispersal should not be underestimated while estimating time since death [5]. There is a direct correlation of time since death with larval length. It can be determined using growth parameter and larval length as a biological clock. In such cases, larval specimen should be collected alive for identification and estimation of time since death. The growth parameters like egg period, instars, total larval time, pupal stage and egg to adult period can be observed inside laboratories. The older the larva, the more time has elapsed since death, and this fact helps in determining the minimum post mortem interval [41].

Pupae, internal morphological analysis of pupae
During the whole life cycle, insects spend half of their development inside the pupae. This stage is therefore valuable in estimations of entomological time lines. During the pupal stage, changes occur in gene expression too. The age-dependent differential gene expression derived from gene expression patterns of transcripts expressed differently during pupal development [42]. The color changes in pupae are not a reliable method of estimation of the postmortem interval. The internal changes in the puparium are reliable factors that can be studied using histological techniques. For studying such changes, the pupae are

submerged hot water after piercing through three tagmas and preserved in 80% alcohol. Sections of pupal shells are stained with hematoxylin and eosin stains. It shows different thoracic and brain muscle development throughout the development of pupae. This muscle developmental difference is used as an indicator of age and estimate time since death intervals [43].

Differential gene expression during metamorphosis

Evaluation of insect age is usually determined by larval lengths and weights. Such changes are not observable in cases of pupae with naked eyes, so pupae are usually ignored in these estimations. However, differential expression of genes and two other genes, i.e. actin and arylphorin receptor during the life cycle of insects is quantified by real time PCR. This is relatively new technique for estimation of pupal age. This regulation of these transcripts depends upon temperature and age [44].

Several studies have explored the mechanisms of genes that regulate larval metamorphic development. The differential expression of eight superfamily genes has been studied during the metamorphosis of *Ciona intestinalis* [45,46]. Similarly, several genes of homeobox have been found to be responsible for larval metamorphic development in *Haliotis rufescens* [47,48]. Moreover, the expression levels of dopamine and adrenaline were observed abnormal in the larval attaching stage of the *Pacific oyster*, *Crassostrea gigas* [49], while a different study observed increased expression of a molluscan growth and differentiation factor (mGDF) in the metamorphosing stage of the same organism [50]. These findings indicate the diversity of genes involved in the transitions of larval forms.

Age determination with 3D micro CT and volatile organic compounds released by larvae and pupae

Pupal stages are not observable from outside, and external as well as internal changes during metamorphosis can be assessed by a relatively recent technique called micro CT. The age of blow fly can be estimated with a higher degree of precision and accuracy with this technique. This recent technique can help in the determination of post mortem interval in cases where only pupae are found [51].

Volatile organic compounds released by larvae and pupae

Certain volatile compounds are released by larvae and pupae of insects. These volatile compounds can help in estimating age of larvae and pupae. This is done by studying headspace solid phase micro extraction. It is further processed by gas chromatography mass spectrometry. These hydrocarbons are branched and unbranched, having alcohols, esters and acids. Their profiles vary with larval and pupal age and differ in composition and quantity. This technique has enhanced accuracy of post mortem interval estimation [52].

Factors that influence entomological evidence

The crime scene from where the dead body is recovered offers maximum number of trace evidences including insects. External factors in the form of weather condition, sunlight effects, rain fall all affect the identification and determination of forensically relevant insects. The most important climatic factor is the temperature and humidity [21]. The careful preservation and handling of the entomological evidence can be fruitful in deduction of cause, manner and mechanism of crime [22].

Study of wound artifacts; some drawbacks overlooking entomological evidence

- **Post mortem insect bite wounds**

After the subtle invasion of fresh dead flesh, insects like ants and cockroaches arrive and start biting on the juicy flesh. The abraded tracks caused by the slow munching in the skin can be faultily labeled as I/V drug abusers prominent vein markings misleading the investigations or trickling of a corrosive down the skin. Some bigger punched out wounds resemble entry or exit gunshot wounds [53]. The awed presence of larvae of flesh eating insects inside the bone marrow that probably traveled through vessel foramina inside the bone to reach marrow [54]. When the larvae increase in number and dry out the flesh, Dermestid beetles arrive and feed on the corpse as well as the larvae. The larder and clown beetles cause bigger pinch out wounds that resemble firearm wounds on the skin [55].

- **Wrongful blood splash marks**

Blood drops splashed around the dead body can be faultily distorted by tread marks of adult flies trying to feed on the liquid blood leading to crisscross tadpole markings resembling blood spatter [56]. This problem can be ruled out by observing the direction of tails of spatter, ratio of the tail to the body, irregular shape of blood spots [19,57-59].

Misleading investigations

At the crime scene, the cooperation of all the forensic experts to lead the investigations to a common inference is required. The ante-mortem insect invasion must be differentiated from post mortem insect colonization at the crime scene. Both can be present at the same time on the crime scene often, specially in cases of neglected nursing care [60].

Entomotoxicological evidence

Some drugs stay in the body systems for quite some time. When arthropods feed on the soft flesh of the dead body they consume the non-metabolized drugs. These drugs alter the metabolism of the insects changing their rate of growth and size. Some of the drugs stores in insect flesh also that gives a chance of their detection in the insect flesh [61]. Even if the quantity of the drug cannot be determined, the simple detection of substance is enough to make the diagnosis [62].

DNA Typing

Morphological identification is getting outdated and rapidly replaced by molecular identification [63]. Forensic DNA typing is the most commonly performed parameter in the modern forensic science settings. The different methods and technology have improved over the time and continue to evolve as STRs, SNPs and mtDNA. These methods can be used in a wide variety of evidences and strongly rule out victims and suspects. The results are commonly presented in courts. Forensic DNA is now rendered as the "gold standard" of forensic science technologies [64]. DNA typing has following advantages over morphological entomology studies;

- Huge diagnostic information in comparison to previous outdated methods such as typing of the blood groups [65]
- Human red blood cells are devoid of nuclei so excluding them all other cells contain a nucleus and DNA [66]
- Proteins are easily degradable but DNA is sustainable [67]
- Many loci for PCR are less than 350 base pairs in length so it allows utilization of degraded DNA [68]

Forensic implications of entomological DNA Typing

- Genetic Fingerprints of insect specimen [69]
- Insect species identification [1]
- The estimation of blow flies age by differential expression of genes. Tarone *et al.*, 2007 profiled the expression of three genes bicoid, slalom, chitin synthase (*bcd, sll, cs*) to predict their age [7]
- Zehner *et al.*, monitored a differential gene expression in *Calliphora vicina* pupae at different ages by using a specific ddRT – PCR protocol [8]
- Insects have non-coding mtDNA region applied for DNA typing in forensic entomology. The cytochrome oxidase subunits 1 and 2 facilitates in species identification [9]
- Molecular analysis allows identification through partial specimen rather than intact specimen as required in morphological identifications [10]

Insects are the most abundant taxa around the globe. The fact that they scavenge most of the organic matter around us cannot be ignored. Their role in consuming unattended dead organic matter is well known. In forensics, same observation can be made in cases of ignored or dumped human dead bodies indoors or out-doors, even in farfetched locations. Usually when such bodies are found, the locations can be strange, and circumstances can be unmatched with the condition of the bodies that can be flyblown themselves. In such cases, no evidence can be ignored on the location that can lead to further investigations. Usually the bodies are so much dilapidated, that the usual rate methods to calculate the time of death are rendered difficult. So, the concurrent methods are contemplated to decipher the facts. Even using these methods, the techniques and methods of collection of data has to be suitable and proper in order to get the desired results.

The relevant facts that the life cycles of different Necrophagous species must be known or already studied must be considered before going to use such information for finding post mortem intervals. Luckily, most of these insects have been studied before and their life cycles are already known. Many studies have been done by entomologists all over the globe that can be useful for forensic entomologists and pathologists. A collection of entomological evidence is a technical issue that must be handled by experts only. Favorable evidence can prove useful in estimating the minimum post mortem interval in forensic investigations. Post mortem interval estimation can lead to identification of the deceased and help in solving a crime. The early dead body invaders like dipteran flies are used as preferred species as compared to late invaders like beetles to estimate the time elapsed since death [70].

Recent studies on the identification of the forensically relevant arthropod taxa, lengths of decomposition stage and arthropod succession sequence have shown similarities and variations in each of the biogeographical zones. The length of carrion decomposition varies markedly between different zones. The patterns of arthropod invasion of carrion are variable from one place to another [71]. Seasonal distribution of larvae on decomposed bodies has been observed along with geographical distribution [72]. The different geographical places have variable, diverse and unique fauna. Different insect species under the effect of a specific climate in a region can exhibit variations on a genetic level. Discrepancy in information of the specific geographical variation in insect species can lead to inaccurate forensic investigation [73].

The main aim for forensic entomologists is species identification at larval stages of insects because at this stage insects show many morphological similarities, especially among similar species making it difficult to differentiate and identify them. The taxonomic keys to identify these immature insect stages are not at hand yet. Rearing larvae until they reach the adult form and identifying them traditionally and morphologically can be done, but it is a time-consuming observation and it delays the results. Alternatively, live specimens may perish before they are tested in the laboratory, rendering investigations futile [74].

As compared to morphological identification, recent molecular identification is a common method, especially for evaluation of immature or badly preserved specimens [75]. The molecular tools can overcome many problems that are faced during morphological methods. More recently, mtDNA has found its place in for forensic testing because it is found in abundance in the cells as compared to nuclear DNA, making it feasible for extraction even from scanty specimen. As it is only maternally inherited and there is no genetic recombination, mtDNA haplotype is a better indicator for utilization in evolutionary and population genetics study and give diverse results. Specifically, mitochondrial cytochrome oxidase I and II (COI–COII) genes are useful as molecular indicators as they report a relatively increased degree of genetic variation in this region [76].

Forensically relevant insects can prove to be an excellent tool when probing homicide, untoward death, and other crimes of extreme nature. Forensic evaluations are of value only when desired evidence is properly obtained, preserved, and transported as soon as possible to qualified forensic entomologists for laboratory testing. The law enforcing agencies, scientists and other staff involved in solving violent crimes should be trained accordingly [22]. Traditionally, these methods are used to identify human corpses or wildlife corpses only [77].

Conclusion

Concurrent methods like entomological time line can be very helpful in determination of time since death, place of death, circumstances, causes, manner and mechanism of unnatural or neglected deaths. Various methods are available today after development of entomological science over the years that are evident from the history of its use and researches in the field to this end. Forensic entomology if regarded essential along with other fields of forensic sciences can prove to be of much help in death investigations. Though various factors affect growth and life cycle of different carrion feeding insects, still the multidisciplinary aid that it provides for solving suspicious deaths can be very valuable. A thorough and simple review of field techniques for insect collection and rearing or studying them can be useful for forensic teams, entomologists and even forensic pathologists. Compilation of results afterwards can lead to the successful evaluation of difficult forensic cases.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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