

Future Trends in Forensic Entamotoxicology

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ABSTRACT: Forensic Entomotoxicology is the branch of forensic science in the use of collecting and investigating insect and toxicology evidence. Entomotoxicology is the examination of toxins in arthropods mainly flies and beetles that feed on corpse. The examination of insect evidence for forensic and legal purposes. Insects provide several information about crimes. Insects to determine the PMI and Time since Death of a decomposing Human Body. Forensic entomotoxicology of insects as unconventional toxicological samples. Use of insects as unconventional medium for drug detection is well known and suggested when conventional mediums such as blood, urine or internal organs are no longer available. The effects of alcohol and opioids on the development of forensically important flies. Drugs within a corpse may affected the development rate of insects that feed on them. These applications are including to the determination of the place of death, cause of death.

KEYWORDS: - Entamotoxicology, Drugs, Death, Insects.

I. INTRODUCTION

There is been an increase in drug related deaths in USA and rest of the world. Many a times these deaths are discovered after a period of time and it is not unusual that the corpse could be highly de-composed or skeletonized. Under such circumstances, there will not be sufficient tissues for toxicological analysis. Even then, it is possible to detect various toxins and controlled substances by analysis of insects, larval skins or puparial skin present near the corpse [1]. From 1980 entomologists started to detect drugs in insects, hoping it would become a useful tool in forensic investigations [2, 3]. For forensic entomology to be effective in legal investigations, knowledge of local insect assemblages and their population dynamics is essential. Entomotoxicology deals with the “analysis of toxins in arthropods (mainly flies and beetles) that feed on carrion. Using arthropods in a corpse or at a crime scene, investigators can determine whether toxins were present in a body at the time of death [3]. Forensic Entomotoxicology includes the study of the effects of drugs on the development rate of carrion-feeding insects and the use of these as alternative sample in the absence of other tissues [4]. Most forensic Entomotoxicological studies have concentrated on commonly prescribed drugs. Insect specimens collected from decomposing bodies enable forensic entomologists to estimate the minimum post-mortem interval (PMI). The most common application of entomological evidence in forensic medicine [5]. Is the estimation of the time of death, i.e., of the decomposing interval [6-10]. Techniques devised recently allow experts in the field to collect robust entomological evidence that can provide vital information in a death investigation, to answer questions concerning movement or storage of the remains following death, submersion interval, time of decapitation and/or dismemberment, identification of specific sites of trauma, post-mortem artefacts on the body, use of drugs (entomotoxicology), linking a suspect to the scene of a crime, sexual molestations and the identification of suspects [11]. Forensic toxicologists qualitatively and quantitatively identify drugs and poisons which may be relevant to cause and manner of death. In most cases, toxicological specimens are collected at autopsy. Alternatively, if a body is badly decomposed, bone, hair, and insect larvae and pupae are collected and analyzed. The use of insects and insect remnants as toxicological specimens is well documented. Insect tissue or remnants (pupal cases, frass, etc.) can be used to identify drugs and toxins present in decomposing tissues. Literature to date has cited the use of arthropods as an alternative toxicological source since 1980. Beyer was one of the first to use maggots to qualitatively assess drug

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Vol. 6, Issue 4, April 2017

presence in a suspected suicide case. A body of a 22-year old female was found skeletonized except for the skin. Larvae were collected and homogenized with the proteins precipitated out of solution. Gas chromatography was used to identify a phenobarbital concentration of 100 µg/g in larval tissue. The larvae were identified as *Cochliomyia macellaria*[12]. Levine et al. (2000) described a case in which an unidentified male was found by a river and was decomposed and skeletonized. An empty bottle of secobarbital was found near the body. Calf muscle and maggots were sent for toxicological analysis. No substances or drugs were detected in the calf muscle, but secobarbital was identified in the maggots by electron ionization gas chromatography/mass spectrometry [13].

II. INSECTS: A RESERVE FOR INVESTIGATING DRUG CONSUMPTION

The accurate estimation of the postmortem interval is extremely critical to the successful completion of death investigations. At the present time, there are several methods available for estimating the postmortem interval. Forensic entomology is the well-researched method of determining the time since death in the later postmortem interval. In forensic entomology, the arthropod evidence associated with the corpse is most often used to estimate the elapsed time since death, or postmortem interval. At present, there are two approaches available for estimating the postmortem interval using insect evidence, and the application of either one largely depends on the state of decomposition of the corpse at the time of discovery by humans. The first method involves the analysis of the pattern of colonization of the carrion by successive waves of insects and other arthropods. The second method relies on the development of immature flies that are deposited on the carrion shortly after death. The use of either approach will also depend on factors such as season, climate, and location of the corpse and treatment of the corpse [14]. Furthermore, insects may serve as important alternative species for toxicological analysis in cases where human samples are not available for this purpose. Several publications have described the detection of toxic and controlled substances through analyses of arthropods [15-19]. Including different drugs. Drugs within a corpse may affect the development rate of insects [20]. that feed on them For examples drugs and poisons like Morphine [21], heroin [16], Opiates [22,23], cocaine [15], barbiturates [22], clomipramine, amitryptiline [18], nortriptyline, levomepromazine and tioridazine [23,24] Diazepam [25], hydrocortisone, Sodium methohexital [26] , Methadone [27], methamphetamine [28] , phencyclidine [29] and Malathion [30] are commonly involved in cases where forensic entomology is used.

III. INSECT SAMPLING FOR ENTAMOTOXICOLOGY

For some toxicologists, insect sampling can seem simple; just take some maggots from the corpse. However, it is a factor leading to high variability in drug detection. First of all, sampling of insects (larvae or pupa) can be carried out, around or under the body or body discovery site. When the corpse is highly decomposed, investigators must be aware that the collected insects can originate from a source other than the deceased. In addition, several authors demonstrate the importance of collecting at different bodysites, as inter-site sampling results in a high variation of drug concentrations [31-34]. This observation is logic as drugs are distributed in the body according to their physicochemical properties, leading to different drug concentrations in different organs and tissues, and thus also in insects reared on these different substrates. While most investigators sample randomly, the best sampling sites for drug detection in insects are the internal organs (e.g. liver), the head-area or muscles in cases where no internal organs are left. However, in the literature, other sampling sites such as the skin surface are observed. At the moment, standards and guidelines for insect sampling in forensic entomotoxicology are published [33, 35]. Unfortunately, the minimum number of specimens that should be sampled is not mentioned and the differentiation between insect activities and maturity is not specified [32].

IV. EQUIPMENT, TOOLS AND PRESERVATIVES

The collection of entomological evidence at the crime scene, especially from the corpse, requires the wearing of protective clothing, mainly to avoid any contamination of the scene with fibres or other material from the investigator. In particular, it is strongly recommended that FE practitioners wear overalls, gloves and shoe covers or boots. For the collection of insects and additional information at the scene, the following equipment is recommended:

- Tool box.

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Vol. 6, Issue 4, April 2017

- Protocol sheets for writing down what specimens were collected, when and where (see [Appendix](#)).
- Dark graphite pencil or pen with waterproof and alcohol-proof ink (do not use standard inks, because they will dissolve in wet surroundings or if splashed with ethanol).
- Labels.
- Fine and medium forceps (with different levels of spring tension for collecting adults and the more fragile immature insects).
- Spoons for collecting maggots.
- Fine paintbrush for collecting eggs (after moistening the brush).
- Vials and storage boxes of different sizes for preserving living and dead insects.
- Shovel or trowel for taking soil and leaf-litter samples and searching for buried larvae/puparia.
- Robust plastic or paper (double bagged) bags for soil samples and leaf litter.
- Sawdust or tissue paper for handling eggs and living larvae in vials or storage boxes.
- Thermometer for measuring the body and ambient temperatures, as well as the larval mass temperature.
- Ethanol (70–95%) for storing dead specimens.
- Camera/video for picture documentation (photographic evidence should include a measurement scale).
- Material for sealing the samples (different in each country: e.g. sticker, sealing wax).
- Cooler bag with re-usable ice packs for storing living insect samples.
- Handheld insect capture net for catching flying insects, if necessary.
- Temperature data logger for measuring the scene temperature for the 5- to 10-day period after body recovery [

V. SAMPLE PRESERVATION

Once the specimens have been removed from the body, or the crime scene, they are washed with deionizer or tap water and the specimens are then frozen for storage at a temperature ranging from -20°C to 4°C until they are needed for analyses. Specimens are prepared for analysis in a variety of ways. They differ based upon the substance that is in question. For the analysis of *inorganic substances*, the arthropods are taken out of storage, washed, and then dried to insure the removal of any foreign human fluids. They are then crushed and stored in a porcelain crucible at a constant temperature of 650°C for 24 hours. The resulting ash has a high concentration of metals, which are then analyzed by acid digestion using 70% HNO_3 (nitric acid). *Organic substances* investigation starts up with washing and drying the specimens. 1-10 grams of larvae are finely cut and an internal standard solution is added. The specimens are then homogenized, in a 0.9% saline solution, followed by centrifuged. Strong acids or bases breakdown the chitinous exoskeleton to release any toxins present and the sample is allowed to extract overnight at a temperature of 65°C . The acid solution is then removed and the organic substances are available for further analyses (8-10). Pharmacokinetics of drugs in insects depends on the species, the developmental stage as well as on their feeding activity. Apart from necrophagous species, bioaccumulations can also occur in parasitoids, predators or omnivorous species. However, this drug bioaccumulation will not be similar as these species present different feeding behaviour due to their diet or life history traits. For entomotoxicological investigations, use of necrophagous species belonging to Coleoptera and/or Diptera is recommended as they are the first to colonize the corpse. Necrophagous species are usually very common and abundantly present on the crime scene [37]. Moreover, their biology and development are well-known, as they are already used in forensic entomology to estimate Post Mortem Interval (PMI) [35]. Morphine and heroin were both believed to slow down the rate of fly development. However, closer examination of the effects of heroin on fly development has shown that it actually speeds up larval growth and then decreases the development rate of the pupal stage. This actually increases the overall timing of development from egg to adult. Cocaine and methamphetamine also accelerate the rate of fly development. Some effects of toxins on these arthropods depend on the concentration of the toxin while others simply depend on its presence. For example, cocaine (at the lethal dose) causes larvae to "develop more rapidly 36 (to 76) hours after hatching". The amount of growth depends on the concentration of cocaine in the area being fed upon. The amount of methamphetamine, on the other hand, affects the rate of pupal development [38].

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 6, Issue 4, April 2017

VI. EFFECTS OF DRUGS ON INSECTS GROWTH RATE

Flies are the most commonly used insect in Entomotoxicology. Some effects of drugs on these flies depend on the concentration of the drugs while others simply depend on its presence. Drugs can have a variety of effects on development rates of flies. Substances like Cocaine, Heroin, Morphine, methamphetamine, Methylene Dioxymethamphetamine, Triazolam, Oxazepam, Chloripriamine, Barbiturates, Malathion, Nortriptyline and Amitriptyline, and Paracetamol are commonly encountered in cases where forensic Entomotoxicology is used.

Various studies have shown that ante mortem use of various drugs and toxins affect maggot development rate, manifesting into an inaccurate PMI estimation based on insect development. Errors of up to 29 h can occur in PMI estimates with heroin containing tissues based on development of the fly *Boettcherisca peregrina*. Similar results were reported for methamphetamine and amitriptyline. Errors of up to 24 h can occur in estimates with heroin on *Lucilia Sericata*.

Cocaine and methamphetamine accelerate the rate of fly development. Cocaine causes larvae to “develop more rapidly 36 to 76 hours after hatching”. The amount of methamphetamine, on the other hand, affects the rate of pupal development. A lethal dose of methamphetamine increases larval development through approximately the first two days and afterwards the rate drops if exposure remains at the median lethal dosage. However, closer examination of the effects of heroin on fly development has shown that it actually speeds up larval growth and then decreases the development rate of the pupal stage. The differences observed in the rates of development were sufficient to alter postmortem interval estimates based on larval development by up to 29 h and estimates based on pupal development by 18 to 38 h.

Barbiturates were found to increase the length of the larval stage of the fly, which will ultimately cause an increase in the time it takes to reach the stage of pupation. Blowfly larval development is slightly impacted by paracetamol if present in the rearing foodstuff particularly during days 2-4 of development. *Chrysomya Megacephala* larvae from control group developed more rapidly than larvae feeding on tissue containing Malathion. The time required for adult emergence was significantly greater for Malathion treated colony which was 10 days compared to 7 days in control colony [14].

Life Cycle Stage	Timing	Description	Observations
Eggs	1 day	1-2 mm	Located especially around the body's natural orifices, such as the nose, eyes, ears, anus, penis, vagina, and in any wounds.
Larvae — 1st Instar	1.8 days	2-5 mm	
Larvae — 2nd Instar	2.5 days	10-11 mm	
Larvae — 3rd Instar	4-5 days	14-17 mm	
Pre pupae	8-12 days	11-12 mm	Larvae become restless and start to move away from the body, crop organ is gradually emptied of blood, and internal features are gradually obscured by the larvae's enlarged body
Pupae	14-18 days	14-18 mm Darkens with age	Presence of empty puparia an indication that the person in question has been dead approximately 20 days.
Adult flies	Emerges from pupa cases after 4-7 days	New generation	Small adult flies

Table 1: show Normal Life cycle stages of fly [14].

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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Vol. 6, Issue 4, April 2017

VII. COMPLICATING FACTORS FOR THE DETERMINATION OF PMI

One of the disadvantages of using forensic entomology is that the growth rate of the maggots can be affected by changes in the temperature, Geographic location may change developmental rates, indoor or outdoor exposure, sun or shade, time of day and season, humidity, and rain, hogan [39]. Temperature is an extremely crucial factor for the utilization of this method because flies won't be able to lay eggs below 40 degrees, nor do they seek the body after sundown. Another complicating factor is the presence of foreign substances in the decomposing body such as drugs and toxins can affect the growth rate of feeding maggots, information that can be crucial in estimating the PMI [29]. Drugs and poisons; Cocaine speeds up development, heroin down, Methamphetamine speeds up development but greatly increases maggot mortality. Bioaccumulation, Presence/absence of clothing, Changes with substrate (sand vs. soil. vs. concrete, significant errors introduced up to 29 hours. Entomologists will have to take each of these variables into consideration in order to give a more accurate estimate of PMI [14].

VIII. LIMITATIONS OF ENTAMOTOXICOLOGY

Entomological specimens make excellent qualitative toxicological specimens. Thereis, however, a lack of research in developing an assessment to quantify the concentration of a drug in tissue using entomological evidence. One reason for this is that a drug can only be detected in larvae when the rate of absorption exceeds the rate of elimination, also samples of pupae and third instars larvae does not contain concentrations of the drugs, suggesting that drugs do not bioaccumulate over the entire life-cycle of larvae. This leads entomologists to theorize that toxins are eliminated from the larvae's system over time if they are not receiving a constant supply of the toxin. This field of entomotoxicology is in its infantile stage, hence lot of active research is needed in this field [37].

IX. DISCUSSION

The status of Forensic Entomotoxicology in India is quite encouraging and it is desirable to focus on this field in future. Insects and Arthropods evidence to be valuable tools in the investigation of homicides, suicides, and other unattended human deaths. Entomotoxicology can provide alternative Samples for drug and Toxic sample detection in putrefied bodies. Entomotoxicology containing studies on drugs and toxins effects on development phase of insect growth are to be extremely occupied more frequently in the forensic examination of crime scenes in direction to get an approximation of time since death especially from the remains of putrefied bodies. PMI is well-defined as the time from death to finding of the corpse; it is the most familiar use of entomological evidence in criminal investigations. Temperature are play the most vital role in insect development, The biotic and abiotic aspects such as individual species characteristics, weather, maggot mass, food type, presence of drugs and toxins, and geographic region can affected insect development. Because, in some conditions, the flesh from the corpse can retain some kinds of drugs that had been consumed by the victim before died and which may even have been the cause of death, these drugs may be recovered by analyzing the insects.

X. CONCLUSION

Toxicological and molecular investigations of insects are found on a body it might be advantage expose the cause of death, the identity of a victim, or even relation a suspect to a crime. Insects arrive at putrefying remains of a body in probable, succeeding waves based on the stage of decomposition. It is evident from the foregoing discussion that the field of Forensic Entomotoxicology has been rapidly growing around the world for the last 10 years. At the moment, discovery of drug in insects is certainly possible. While it cannot yet be used for toxicological explanation in maximum cases, when only skeletonized remains are left, pupa can be the only hope for a toxicologist to have some information concerning drug use prior to death. A lot of work still has not to be done in India to make this field good enough to be utilized in the medico-legal investigations. So, future workers from Forensic Scientist and medico legal expert are hereby strongly encourage to select this wonderful field in their respective research and development activities.

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 6, Issue 4, April 2017

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Vol. 6, Issue 4, April 2017

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