

Utility of Entomotoxicology in Medico- Legal Investigations

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ABSTRACT: Entomotoxicology is the analysis of toxins in carrion feeding arthropods. It also examines the effects of drugs/ toxins on the insects. Diptera and other arthropods can be utilized as alternate samples for toxicological analysis where no viable specimens are available. Thorough analysis of insect community on a decomposed body along with knowledge of insect ecology, biology and distribution can provide valuable insights. It includes estimation of post mortem interval, cause of death, indication of ante mortem injuries, and presence of drugs/toxins. The identification and quantification of the present drug in the cadaver is studied through the analysis of the insects and other feeding arthropods present on the cadaver.

KEYWORDS: Entomotoxicology, Post mortem interval (PMI), Drugs/ Toxins, Carrion.

I. INTRODUCTION

Ascertaining the cause of death is one of the most important aspects in a crime investigation. Deceased in suspected drug poisoning cases are not generally recovered in appropriate time. Thus, when the bodies are recovered in an extremely decomposed or skeletonized state, the conventional samples such as biological fluids such as blood, urine, etc. or internal organs are not available for drug testing [1]. In such cases, living adult insects, insect larva and the chitinized remains of insects, such as shredded larval and puparial skins, may serve as alternative evidence and can provide a lot of vital information [2].

Forensic entomology is the application and study of arthropods that feed on cadaver in legal investigations. Entomotoxicology investigates for the presence or absence of toxic substances in various arthropods, primarily flies and beetles [3]. It also studies the impact of drugs on the development rate of carrion feeding insects [4]. Various illicit drugs (cocaine, heroin, barbiturates) can significantly alter the growth rate of insects. Insects recovered from the body can be homogenized and processed using traditional chemical extraction techniques to reveal the toxic substance they have ingested. Insect evidence enables entomologists to estimate the elapsed time since death and also determine the cause of death [5]. Post Mortem Interval (PMI) is an estimation of the time between death and corpse discovery.

II. INSECT AS EVIDENCE

The process of decomposition can be categorized in five stages i.e; fresh, bloat, active decay, advanced decay and remains. As soon as the process of decomposition sets in, a variety of insects are attracted to the dead body because of the odor released due to putrefaction. The type of flies varies from region to region. Blow flies of the Calliphoridae family are usually the first insects to colonize a cadaver within few hours after death, thus they deliver the most precise assessment of time since death. Beetles generally colonize at advanced stages of decomposition [6,7]. Temperature plays a vital role in maggot development cycle. High temperatures generally slow the growth rate of dipterans.

Insects deposit their eggs at sites from where hatched larvae can easily get access to soft tissues usually in natural orifice in the body usually around the eyes, nostrils, and mouth and later in rectal or genital area. If there are any open wounds in the body, eggs will be deposited in the exposed areas. As maggots hatch, they cluster and feed on the soft tissues, often reaching such high numbers and are referred to as "maggot masses" [8]. As the maggot emerges from eggs, they feed on the body and undergo changes from one life stage to another and this process is known as metamorphosis. At the third instar, the mature maggots migrate from the food source i.e; corpse, to a suitable pupation site usually dry habitat in order to pupate. The outer skin of maggots gets thickened form a hard protective covering during pupation. The adults emerge from the pupa and empty puparia cases are left behind which can be recovered from deceased's clothing or under the carpets of room or under the soil. The pupal case is quite resistant to any environmental degradation and thus may be found in soil for hundreds of years [9,10,11].

Larvae feeding on a tissue containing xenobiotics such as drugs and other toxic substances present in that tissue gets transferred to its metabolic system. And furthermore, these toxic substances get carried through food chain to other arthropods that predate on the larvae. This process is termed as secondary bioaccumulation.

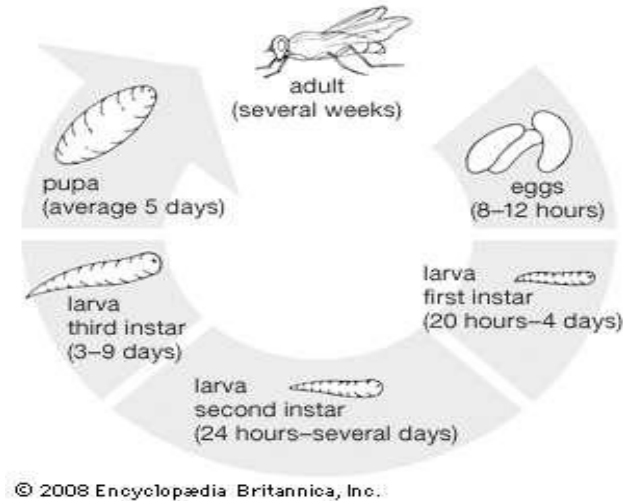


Fig 1: Life cycle of a Dipteran

III. INSECT SAMPLING AND PRESERVATION

Insect sampling and preservation is of vital importance as it determines the variability in drug detection process. The samples can be collected from the corpse and different sites of the body. The reason lies in the fact that drugs are distributed in the body according to their physiochemical properties, which leads to varying drug concentrations in different organs and tissues, thus causing variance of the same in larvae. The best sampling sites for drug detection in insects are the internal organs i.e; liver, and in cases where no core organs are present, muscles or head- area is examined [12,13]. Once the specimens have been collected from the body, they are washed with tap water and then the specimens are stored at an extremely low temperature ranging from -20°C to 4°C.

Specimens are prepared depending whether they are organic or inorganic. For organic samples, they are first washed and then dried. They are finely cut and an internal solution is added and homogenized in a saline solution. The mixture is then centrifuged and kept overnight at 65°C temperature.

For the analysis of inorganic samples, the arthropods are firstly washed thoroughly. They are then dried, crushed and stored at a constant temperature of 650°C for 24 hours [14].

IV. EXTRACTION

Traditional chemical extraction techniques such as Liquid-Liquid extraction and solid phase extraction these techniques enable extraction of different poisons and drugs to be detected. The most common analytic procedures used for processing insect samples are gas chromatography, gas chromatography-mass spectrometry (GC/MS), thin layer chromatography (TLC) and high-performance liquid chromatography-mass spectrometry (HPLC/MS) [15,16].

V. IMPACT OF DRUGS ON INSECT'S GROWTH RATE

Flies are the most commonly used insect in entomotoxicological studies as they are the first to colonize the corpse [17]. The pharmacokinetics of drugs in insects depends on a number of factors such as type of species, the developmental stage and their feeding activity. Illicit drugs such as Cocaine, Heroin, Barbiturates, Methamphetamine, Malathion, Morphine, Paracetamol etc. are of major concern to forensic entomotoxicologists because deaths due to these drug overdose is very common.

Studies show that antemortem use of various drugs and toxins can greatly affect maggot developmental rate, resulting in the erroneous estimation of postmortem interval (PMI) based on insect development. Errors of up to 29h, 48h, and 77 h were found to occur in PMI estimates with the presence of heroin, methamphetamine, and amitriptyline, respectively in decomposing tissue [18].



Investigations indicate that fly larvae exposed to cocaine start and end the pupariation process much earlier than other drug free larvae. They consume more cadaver tissue and gain body weight faster than drug free larvae. Studies conducted for heroin using *B. Peregrina* maggots concluded that there was a rapid development of maggots, the puparial period was longer than usual. It was demonstrated that an error of upto 29 h could occur in PMI determination with the presence of heroin. Similar studies on Methamphetamine showed accelerated larval development and alteration of PMI estimation by upto 48 h.

Studies of the impact of diazepam on the development of *Chrysomya Albiceps* and *Chrysomya Putoria* larvae indicated that initially, there were no changes in the rate of development of the larvae, but after several hours of the development, the larvae turned out larger than usual. It was found that diazepam can lead to an error in the estimation of the age of larvae and thus the post-mortem interval of up to 54 hours. Thus the presence of any of these drugs/ poisons may result in incorrect estimation of PMI.

Certain chemical compounds can slow down the larval development process as well. Studies show that carbon monoxide delays the colonization of corpse by binding with hemoglobin pigment in blood, thus hampering insect attack. Other chemicals that delay larval development include hydrocortisone, malathion, ethanol, etc [19].

<u>Name of chemical agent</u>	<u>Species/ stage of development</u>	<u>Effect on insect metabolism</u>
Heroin (Goff et. al., 1991)	<i>Sarcophaga peregrina</i> larvae	Accelerates development of larvae, but delays pupation
Cocaine (Goff et. al., 1989)	<i>Sarcophaga peregrina</i> larvae	Accelerates development of larvae
Diazepam (Carvalho et. al., 2001)	<i>Chrysomya albiceps</i> - larvae, pupae and adult forms	Accelerates development of larvae
Methamphetamine (Goff et. al., 1992)	<i>Sarcophaga ruficornis</i> larvae	Accelerates development of larvae, but finally the exposed larvae are smaller than control
Phencyclidine (Goff et. al., 1994)	<i>Sarcophaga ruficornis</i> larvae	No significant impact on larval growth, but pupation is slower
Malathion (Gunatilake and Goff 1989)	<i>Chrysomya megacephala</i> and <i>Chrysomya rufifacies</i> larvae	Delays laying eggs by adult forms and delays growth of larvae
Ethanol (Tabor et. al., 2005)	<i>Phormia regina</i> larvae	Delays growth of larvae
Sodium pentothal (Sadler et. al., 1997)	<i>Calliphora vicina</i> larvae	Causes death of larvae

Table 1: show effects of certain chemical substances on insect metabolism

VI. LIMITATIONS

Insects are considered as excellent samples for determining the post- mortem interval in legal investigations. However, one of the disadvantages of insect evidence is that the development rate of maggots greatly relies on various variable factors such as temperature, humidity, geography, exposure to radiation, place of death etc [20]. Another drawback is the presence of any toxic substances in the corpse as it not only affects the growth rate of maggots but also create significant difference in the evaluation of post mortem interval [21]. Certain drugs such as Cocaine and Methamphetamine speeds up their development while others such as heroin, malathion, slows down. Moreover, drug detection is only possible when the rate of absorption has exceeded the rate of elimination. Also, pupae samples and larvae (third instar) does not show drug concentration, suggesting that drugs do not bioaccumulate over the entire life-cycle of larvae.

VII. DISCUSSION AND CONCLUSION

Forensic entomology has become an inspiring field that helps in the investigation of suicide, homicide or other unnatural deaths with the help of arthropods. Entomotoxicology can provide alternative specimen for drug detection in

decomposed and skeletonized bodies. It helps to establish the link between the intoxicated and sobriety dead bodies. Forensic entomology aids in determining post mortem interval and also the cause of death. Post Mortem Interval (PMI) is an estimation of the time between death and corpse discovery. Apart from these applications, it can also be used for determination of place of death, detection of ante mortem injuries and movement of the body after death. Since it is an evolving discipline, one major lacunae of this field is that maggots offer only a qualitative role, and thorough study needs to be carried out to highlight its role in quantitative analysis in order to understand the pharmacokinetics of drugs in insects.

Using entomological evidence, there are two ways to estimate PMI. One is based on the age of oldest maggots i.e Calliphoridae feeding on human remains, which aids to determine a close approximation of the time since death. By calculating the estimated age of immature flies that have fed on remains provide a minimum PMI, however it does not provide any information about maximum PMI because the time period between death and colonization by insects is unknown. The other method is based on ecological succession of arthropods on the cadaver [22,23,24]. The succession model requires information about the time passed between death and discovery of a particular species (taxonomic group) and also stage of decomposition. The succession pattern depends on a number of factors such as climatic conditions, geographical location, exposure to sun, synanthropy, location of the body, presence of clothes, condition of body, cause of death, larval migration etc [25,26]. The presence of drugs/ poisons increase or decrease the growth rate of larvae. The larvae feeding on tissues containing toxic substances were found to be smaller than usual and it could alter PMI estimation process by significant hours.

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