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Entomotoxicology: An Alternative for Biological Specimens

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Abstract:

Forensic entomology is the utilization of insects in lawful issues and gets information about where, when, and the way crime was committed based on their distribution, ecology, biology, and conduct of insects present at the crime scene. Entomotoxicology is the assessment of poisons in arthropods that prey on the corpse. Forensic Entomotoxicology examines the assessment of insects as toxicological samples since they are being used for the poisonous content in them. The utilization of insects as an alternative matrix for drug identification is well-reported and suggested without conventional matrices for example urine, blood, or internal organs. Hence, drugs present in a decomposing body might be recognized through the investigation of maggots feeding off it. In this paper, the results for the practice of Entomotoxicology as a forensic technique are discussed in-depth and the function of insects as a toxicological sample, its collection, and preservation, examination utilizing different instrumental procedures, is being stated for the forensic considerations.

Keywords: *Entomotoxicology, Forensic Entomology, Toxins, Corpse, Insects, Investigation.*

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Introduction

Entomology springs from the Greek word Entomon (insect) and Logos (word, reason) meaning the study of insects. Forensic Entomology, is perhaps one of the oldest branches of Forensic Science, is the utilization of the insects and the relative species to arthropod that possess decomposing remains to assist legal investigations. Over the years Forensic Entomology has developed from being utilized just for discovering post-mortem interval for the season of death, the geological area of death, movement, or storage of remains after death. The involvement of Enomo-toxicology has additionally expanded and upgraded the function of entomology in forensic science. The term Entomotoxicology was first coined by Beyer and colleagues in the year 1980 and since then many entomologists have routinely started to detect drugs in insects found on the cadaver after a prolonged period **(Pounder, 1991)**.

Entomotoxicology deals with the "examination of toxins" (poisons) in arthropods (mainly beetles and flies) that prey on carrion. Utilizing arthropods in a dead body or at a scene of the crime, forensic investigators can determine that at the time of death whether the poisons were present in a body or not. Therefore, the study of the helpfulness of insects as an alternative toxicological sample is known as Entomotoxicology **(Dayananda and Kiran, 2013)**.

Entomotoxicology is a collaboration of fields of science, Zoology, and Chemistry that has been implemented as an alternative to toxicological samples. This method for drug detection in corpses is very well-reported and suggested when other biological matrices such as urine, blood, or integral organs have decomposed. One of the main applications of Forensic Science is to identify fatalities recovered after many weeks to months. Such cases arise if an individual has died in an isolated place because of substance abuse or has committed suicide. Insects prey on dead bodies that might have died because of a drug overdose and hence xenobiotics like drugs or any other toxic substances present within the tissue get introduced into the body of the larvae. The drugs then get distributed in their body and also pass onto other arthropods that predate on them **(Chophi *et al.*, 28-36)**.

The maggots may be used to determine the presence or absence of drugs when human body tissues are too badly decomposed to do toxicology on the tissue remains. Death because of abuse of drugs, insecticides, pesticides, and different poisons are normal everywhere in the world. It is reported that about 450,000 individuals died around the world because of drug abuse in 2015. About 275 million individuals aged (15–65 years) were accounted to possess abused drugs in any event once in the year 2016. It is estimated that on the brink of 800,000 individuals die per annum due to suicide, of which 20% (1,60,000) constitute death as a result of pesticide ingestion **(Bachmann, 2018; www.who.int)**. These figures and numbers indicate that massive deaths occur because of drug abuse, and in several cases, dead bodies are recovered after many weeks to months **(Carvalho *et al.*, 2001; Goff *et al.*, 1989)**.

Entomotoxicology would be an excellent tool, in solving the cases of poisoning when the dead bodies are recovered after a delayed period. The fact that the drug got introduced in the body of the insect only because of the human organism upon which the larvae was feeding is a crucial characteristic of this technique. Entomotoxicology also serves as a useful tool to identify the impacts brought about by toxic substances on arthropod development for PMI estimation. The flies lay their eggs on the corpses within a couple of days depending upon the conditions in there. The most found species and the first to show up are the *Calliphora* as wide geographic distribution is governed by them and are mostly found in urban areas. They are attracted by gases and body fluids and their developmental stages- egg, prepupal or pupal, larval stages, adulthood aid in the determination of time since death. Then as the body further decays, Sarcophagus, *Anglossa*, *Piophilacasie*, and other carrion feeders get attracted to the body. The materials of interest can be analyzed from the pupae, larvae, puparial cases, adult insects, cast beetle skins (exuviae), fly predators, beetle fecal material (frass), and scavengers. A study demonstrated that greater sensitivity is obtained by utilizing fly larvae rather than of putrefied material. Moreover, the drug concentration is more stable in the insects compared to that in tissues. Also, the main advantage is that insects are present in greater quantities and their remains are present for an extended time than the toxicological samples. Compounds such as pesticides, drugs, and metals are recognized in insects for forensic purposes. However, quantification of the drug and understanding its relationship to the cause of death observed from insects remains an issue unsolved due to a wide array of influencing factors **(Kintz *et al.*, 2001)**.

Forensic entomology is derived from an extended history of research dating back to 13th Century China. Insects are known to possess been utilized in the detection of crimes for an extended time and some other researchers have written about the history of forensic entomology **(Pounder, 1991)**.

Entomotoxicology's history is comparatively short and the field of entomotoxicology was published the primary article about the utilization of Phenobarbital drugs in1980. A female aged about 22-year-old was found in the initial skeletonized stage, fourteen-days after she had last been seen alive. No tissues or fluids were present because of advanced decomposition. For toxicological analysis as samples, the fly-larvae were utilized, and phenobarbital was present. Since then, many drugs and poisonous substances like Amitriptyline, Propoxyphene, Acetaminophen, Steroids, Trazodone, Trimipramine and Temazepam, Benzodiazepines, Barbiturates, and Meprobamate, Methylphenidate, Methamphetamine, Clomipramine, Bromazepam, Levomepromazine, Cocaine and Nortriptyline, Opiates and Opioids, Nordiazepam, Phencyclidine, Codeine, Insecticides and Pesticides, Mercury,etc. are detected in various tissues of larvae and insects **(Chophi *et al.*, 2019)**.

Sample Collection and Preservation

One of the critically important procedures in entomotoxicology is the collection and preservation of samples. This step might seem easy for most toxicologists but comes with great responsibility. The collection of all types of insects present at the crime scene is essential. Necrophagous beetles (adults/immatures) and predatory and are later arriving fly species that give extra progression information for estimation of PMI **(Gagliano-Candela and Aventaggiato, 4-5)**.

It is prescribed to collect both preserved and live samples. Preserved samples include specimens placed directly into 80% (or more prominent) Ethyl Alcohol or anesthetized utilizing an insect kill container and afterward placed into 80% Ethyl Alcohol. Live samples of fly eggs, larvae, and pupae are placed directly on a food medium (raw hamburger, beef liver) and are wrapped freely in aluminum foil. Vermiculate is added to the container of larvae and raw meat**.** These samples can be collected around or under the body or body discovery site. While most examiners randomly sample, the best sampling sites for drug identification in insects are integral organs (e.g.: liver), the head area, or muscles in cases where no internal organs are left. Even though the minimum number of specimens that ought to be examined isn't referenced and the separation between insect activities and maturity is not determined. It is described as a sampling of insignificant 30 specimens of a similar stage and activity from every area. (**Tracqui *et al.*, 2004; Bourel *et al.*, 2001)**.

Different techniques of preservation are used in different laboratories. Once specimens have been collected from the entire body of the corpse and up to 10m surrounding area, preservation of dead insects and eggs in 70-95% ethanol is done killing larvae at 80°C hot water for 30 seconds and then stored in 70-95% ethanol, pupae storing in 2°C-6°C temperature with punched-holes in the lid, killing off adult flies in vial freeze at -20°C and the dead specimen storing in 70-95% ethanol **(Campobasso *et al.*, 2004; Gojanović *et al.*, 2007)**.

For toxicological analysis, specimens are preserved at -40°C and the analysis is carried out in the same way as human fluids or tissues. Storage of specimens under the dry conditions at -20°C is done to ensure drug stability and diminish drug extraction from the matrix while storing in alcohol **(Nolte *et al.*, 1992)**.

Analysis

The preparation of the sample relies upon the nature of the insect tissue and the drugs of interest. Since the specimens of insects are solid, they will initially be macerated and pulverized or homogenized by grinding. Once the samples are pulverized, drugs are extracted from the matrix. Different extraction techniques like solid-phase extraction and liquid-liquid extraction are employed for extracting various poisons and drugs according to the chemical features of the substances to be detected. From the aqueous extracts of the entomological specimen, the solid-phase extraction accounted for to give the best organic toxicant purification. Best results are obtained depending upon the extraction technique and the adequacy of the technique employed. The technique of being employed depends upon the physicochemical properties of the drug. For accurate results, it is important to have very sensitive methods to ensure the analysis of single specimens from different locations of the body **(Karampela *et al.*, 2015; Musshoff and Madea, 2009)**.

As stated earlier, entomotoxicology is a technique used in alternative to biological specimens where insects reveal the possibility of drug exposure to the body e.g. cocaine has been detected from a

skeletonized body using skeletal muscles and larvae using GC-MS. Several research articles have proven the discovery of drugs from insects using techniques like Immunoassay, LC-MS/MS, HPLC, GC, and GC-MS **(Liu *et al.*, 2009)**. Drug concentration gets incredibly decreased in insects and larvae as compared to the substrate on which it is feeding. The feeding of larvae on a substrate on treated with a higher dose or concentration is usually found to contain a larger concentration in its tissue. However, this may not be valid for all the cases **(Parry *et al.*, 2011).**

Some few quantities of the drug are known to aggregate in the cuticle of the puparium and subsequently, drugs can be recognized from empty puparial cases. The type of technique employed must be sensitive enough to identify such low concentrations of drug in larvae, insects, and puparial case. Quantitation of low-level drugs has been proved to give the best results using LC-MS and GC-MS techniques where confirmatory identification and quantification of toxic substances can be achieved even though a profoundly degraded matrix (**Goff and Lord, 1994; Beyer *et al.*, 1980)**. A study demonstrated that recognition and quantification of the drug, Nordiazepam, and its metabolite Oxazepam could be completed from a single larva and puparium down to pg level. For techniques such as LC-MS, it is mandatory to evaluate the matrix effects as other compounds can impact the ionization of compounds of interest **(Pien *et al.*, 2004)**.

New trends of analytical techniques have been incorporated for entomotoxicological studies, where chromatographic methods coupled with mass spectrometry have been used abundantly. Recently, studies are conducted to analyze entomological samples using non-destructive techniques which are spectroscopically favored by statistical tools such as PCA (Principal Component Analysis), SPA (Successive Projection Algorithm), and GA (Genetic Algorithm). Spectroscopic Techniques are preferred as they have a huge amount of advantages than compared to other instruments such as GC-MS. A study conducted utilized UV-Spectrophotometry for the successful detection of Fluoxetine, a type of antidepressant from the insect specimens from all developmental stages of Exuviae and *D. maculatus* **(Zanetti *et al.*, 2016)**. Another study used Near-Infrared Spectroscopy in combination with statistical tools for analysis of Flunitrazepam in entomological specimens. The tool provided useful potential differences between the drug and biochemical alterations of the insects. Thus, this technique proves to yield exact outcomes that are reliable and reproducible. Although a wider range of studies should be established in this context **(Oliveira *et al.*, 2014; De Lima *et al.*, 2018).**

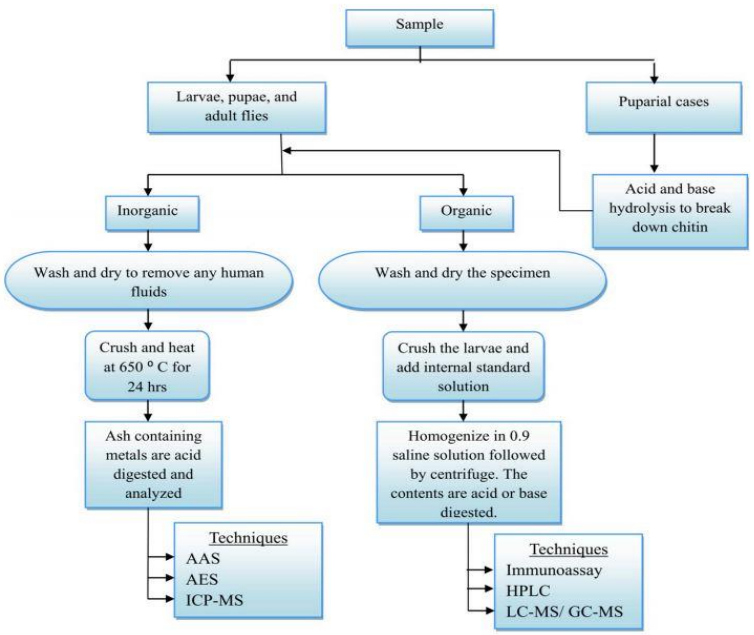

Figure No. 1 – Analysis of Insect Specimen for Toxicological Analysis
Source: (Chophi *et al.*, 2019)

Limitations

At present, it is accepted as a fact that entomotoxicology cannot be interpreted as conclusive evidence as the pharmacokinetics of the drug in insects is not fully understood. The absorption, elimination, and accumulation of the drug in insects depend upon the type of species, their developmental stage, and their feeding activity. Other factors that play an important role are stability, temperature, and humidity. The major limitation of this field is the detection of drug concentration from insect specimens **(Chophi *et al.*, 2019)**.

Discussion

All these studies above have illustrated the possibility of qualitative and quantitative analysis between drug and the insects. Although the use of such insects for the study of drug intake in a decomposed body is well documented, it still has certain limitations. A study conducted demonstrated no relationship between the drug concentration in the insect and in the tissues on which the insects were feeding. He also stated that the current research study does that simplify exact quantitative interpretations. Wilson *et al.* conducted a study using HPLC and found out that the drugs do not bioaccumulate throughout the larval cycle and get eliminated through malpighian tubules. Hence, the rate of absorption should exceed the rate of elimination. In this respect, Sadler *et al.* concentrated on drug accumulation and elimination and observed that the larvae metabolize and eliminate drugs with varying levels depending upon their developmental stages that ultimately lead to a decrease in drug concentration. This suggested that fully developed larvae should be used as a toxicological sample as they yield the best source of drug residues **(Introna *et al.*, 2001)**.

Conclusion

From the elucidated uses of insects in forensic entomology above, it is obvious that forensic entomology is an interesting aspect with useful application to investigate and in aiding justice. This field has always proven to be beneficial in estimating the time since the death of a corpse. Although this area requires a little lighter as before one could conclude that insects offer only qualitative analysis and no quantitative results. Drug overdose, insecticides, and pesticide poisoning cases are very prevalent in our society and therefore entomotoxicology is a great field to look into. Further entomotoxicological research should be carried out focusing on physiological processes during the feeding stage, metabolism of the drug, redistribution of the drug, post-mortem stability, and other mechanisms.

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