Indian Journal of Forensic Medicine and Pathology
Volume 14 Number 3, July - September 2021 | Special Issue
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.14321.44

REVIEW ARTICLE

Entomotoxicological Studies: A Review Report

[1]Usha Sisodia, [2]Sally Lukose

ABSTRACT

Toxicology and forensic entomology are mixed to determine the minimum postmortem time (PMI-min) and circumstances of death in cases where toxins and hazardous substances are suspected causes of death. Forensic entomotoxicology proves the existence of toxicants in insects feeding on corpses, but it also investigates their impact on insects' bio-morphometry and growth rate. Isolation of larvae and pupae of real flies (Dipteran) and/or adult forms of, for example, beetles (Coleopteran) located on or around the corpse can provide information about harmful compounds potentially present in the body. This review aims to examine current knowledge in the subject of entomotoxicology, including cases from the research, and to demonstrate the effects of various toxic compounds and medications on the growth of insect larvae.

KEY MESSAGES: Insects are significant in forensics because their larva feeds on dead bodies. Insects (larvae) are also a suitable toxicological sample because they are present in high concentrations on the cadaver and the puparia case remains unchanged and unaffected for an extended period.

KEYWORDS | X-ray entomotoxicology, post-mortem time, insects

Author's Credentials:
[1]Research Scholar, [2]Professor, School of Allied Health Sciences, Sharda University, Greater noida, Uttar Pradesh 244001, India.

Corresponding Author:
Sally Lukose, Professor, School of Allied Health Sciences, Sharda University, Greater noida, Uttar Pradesh 244001, India.

Email:
sally.lukose@sharda.ac.in

How to cite this article
Sisodia Usha. Entomotoxicological Studies: A Review Report. Indian J Forensic Med Pathol. 2021;14(3 Special):723-729.

INTRODUCTION

Forensic entemology is a discipline under forensic science concerned with the investigation of numerous insects and arthropods found on human and animal cadavers. It is the application of entomology in the case of legal investigations, to solve a crime.[1] Insects are involved in the decomposition process of a cadaver and can be an important aid in evaluating the various phases of decomposition. Because of the known history of insects, their developmental stages, and the habitats in which species live, the investigation of insects to determine criminality is helpful. These factors aid in the identification of the scene of the crime, as well as for determining the possible cause of the crime and identifying postmortem and antemortem injuries.[1] The most typical kind of civil lawsuit using forensic entomology is those involving bug infestations in metropolitan areas or pests of stored commodities. The majority of these insects are located in man-made constructions. In medico-legal instances, insect inspections are conducted to determine the period since the death. The medicolegal investigation includes determining if the crime was homicide, suicide, or an accident (Merritt and Higgins, 2000). Since the 1990s, forensic entomology has been employed to solve crimes (Jamieson *et al.,* 2009). A forensic entomologist aids in the assessment of the postmortem interval, which is defined as the time between an individual's death and the body's recovery.[6] The post mortem interval can be calculated by estimating the pattern of insect succession on corpses and calculating the developmental period for each stage of an insect's life cycle. Fresh, bloated, decay, advanced decay, and dry stages of decomposition are observed as per insect activity and are useful in predicting the post mortem interval, based on the pattern of insect succession.[6] The fresh stage ends with the bloated stage, which involves the degradation and metabolism of proteins, fats, and carbs. This stage is expected to last between 0 and 3 days. Adult blowflies, flesh flies, muscid flies, and yellow jackets were the most frequent insects found in this stage. Putrefaction happens in the bloated stage which leads to the evolution of gases and the swelling of the corpses. This time lasts between 4 and 7 days. Adult and larval blowflies, flesh flies, muscid flies, rove beetles, hister beetles, and yellow jackets are the most frequent insects found. The body deflates and ruptures during the decomposition stage, releasing unpleasant fumes. This step is expected to take 8-18 days.[6] Adult and larval blowflies, rove beetles, carrion beetles, and cockroaches are among the insects discovered. The dominant group of insect families present during different days of decomposition was as follows: early succession by insects of dipterans family Calliphoridae, sacrophagidae and Muscidae families, and late succession is done by insects of histeridae, Cleridae, Dermestidae, Scarabaeidae, and Staphylinidae families. The succession patterns and the presence of the insect families were vary in different regions. The skin becomes dry and just cartilage and bones remain in the advanced deterioration stage.[7] This stage lasts between 19 and 30 days. Dermestid, hister beetles, mites, and other insects are commonly found at this stage. Only the bones, cartilage, and hair remain in the dry stage. This stage lasts for more than 30 days. Dermestid beetles, soldier bugs, and ground beetles are some of the insects discovered at this stage.[7] The blowflies are the first to colonize the corpses, laying thousands of eggs in the natural orifices and around the cadaver's wounds. Flesh flies leave tiny larvae in the same area. The flies' larvae go through three stages of development. The size of a larva upswings after each instar due to feeding on the cadaver mass, and by the third and final instar, the larva assumes ten times larger size than the first, indicating the start of the dry stage of decomposition.[10] After the third instar, the larvae metamorphose away from the decomposing site and enter the pre-pupal stage. During the prepupal stage, the larva immobilizes and darkens, and the pupa begins to develop within the hard exoskeleton covering. The pupa goes through metamorphosis and becomes an adult fly before emerging like a flying adult beginning its new cycle (Fig. 1). Many factors influence insect growth and colonization, including temperature, weather conditions, food exposure, burial, photo duration, and so on.[10] Insects are cold-blooded animals, which means their temperature is affected by the temperature of their surroundings. The temperature of the environment should be ideal for both the insects' growth and their development. The optimal temperature is closely related to insect development. Maximum insect development can be observed at optimum temperature, because if the temperature is either too hot or too cold, the rate of development of the insects becomes slow, and the rate of development cannot be observed below the optimum temperature range. Insect developmental rates differ from species to species and geographical location to location. The rate of metabolism of insects increases as the temperature rises, and thus the rate of decomposition rises with each successive stage of insect development.[11] For a more accurate estimation of the post mortem interval, forensic entomologists focus on determining the accumulated degree hours of different species, which was unique to each species.[8] The accumulated degree hour (ADH) is defined as the amount of thermal energy required by the insect to progress from one stage of its life cycle to the next. It can be calculated as the product of time and the difference between the average and threshold temperatures. A hot summer day will have more ADH than a cool autumn day. The post mortem interval index can be calculated in two ways: the first is to determine the age of the fly by calculating the growth stage of each stage of the insect's life cycle.[8] The ADH can be calculated and compared using the same method under temperature-controlled laboratory conditions. The second method is to identify the pattern of insect succession at each stage of decomposition. The arrival of various insect groups can be determined by the body's natural decomposition process. During the first stage of decomposition, flies arrive, lay eggs, and the larvae develop. After that, many types of beetles arrive, feeding on eggs, maggots, and soft tissues of the carcasses. After that, omnivore insects such as ants and wasps arrive on the body to feed on other insects or vegetation, and lastly, indigenous insects such as spiders come. Rain, darkness, varying temperatures, and delayed oviposition are among the elements that influence post mortem interval estimation.[9] Many circumstances, including burial below, submersion underwater, wrapping or enclosure in a freezer, building, or closed vehicle, have been blamed for the delayed oviposition and colonization of insects. Seasonal and habitat interest were used to estimate the season of death and a second location of the remains (Dekeirsschieter et.al, 2011). While Forensic Entomology is concerned with the investigation of numerous insects and arthropods found on human and animal cadavers, Entomotoxicology refers to the application of entomology to the detection and estimation of drugs or toxins. The field of entomotoxicology is separated into two subfields: entomological analysis of compounds that alter insect developmental rates and the post mortem interval index. The second is a toxicological examination of medicines, toxins, or drugs in the body of insects3. Because of the bioaccumulation of medications inside the insects, the concentration of drugs will be higher inside the insect's body than in the surrounding areas. Benzodiazepines, antidepressants, amphetamines, cocaine, organophosphate, barbiturates, heavy metals, and other drugs or toxins are some of the commonly detected toxins in entomotoxicological cases. Insect samples are usually gathered from natural orifices, wounds, under the body, and wrapping materials, among other places. The toxins are examined from the body of the insects from the adult, larvae, pupa, and skin of the insects discovered on or around the cadavers[3] Several studies have been done in forensic entomotoxicology such as quantification of Phenobarbital in blowfly larvae and its detection by gas chromatography (Beyer.et.al, 1980), quantification of mercury in larvae of various blow flies' species (Nuorteva and Nuorteva, 1982), detection of the arsenic in the insect's species Muscidae, Piophilidae (Brahy et.al). Goff and Gunatilake (1989) in their study showed the detection and quantification of Malathion in the Calliphoridae species using gas chromatography. Studies have shown that the rate of development of the insects and their survival were affected by the presence of drugs (Magni et.al 2014, 2016b). The rate of development and survival of the insects was also found to be dependent upon the varieties of tissues or diet on which the rearing of the insects was done.

One of the studies which were done by Clark et.al (2006) compared the development rate of Lucilia sericata on different tissues such as the lung, liver, and heart of animals like cows and swine. Their study showed variations of larval growth rate between lung, liver, and heart. The entomological evidence determines the cause of death, manner of death and medico-legal aspects of not only humans but also in wildlife investigations. In wildlife investigations, the death of the animal is caused due to illegal cruelty, trade, possession, and poaching. The accuracy of the post mortem interval was more at the advanced decay stage of decomposition because the changes in the insect succession were slow and most of the body mass was lost. The sites of trauma on the body were estimated by measuring the larva activity on areas of the body except for eyes, nose, ear, and mouth before the decay stage of decomposition. Few of the fly species lay eggs on the living tissue and their presence help in determining the duration of being alive before death.

Entomotoxicological Researches

Gunn *et al.,* (2006) determines morphine in

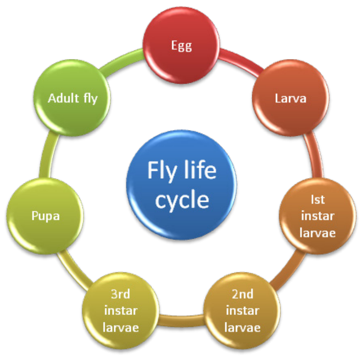

Figure 1: Life cycle of Blowfly

the larvae of Calliphora Stygia using HPLC chemiluminescence detection. Potassium permanganate was used to detect the morphine in larvae in low concentrations and the chemiluminescence detection was used for the effectiveness and robustness.[2] Strong chemiluminescence was detected when acidic potassium permanganate reacted with morphine. Results of the study showed that the minimum limit of detection for morphine was 2500ng/g. The control samples tested negative for morphine. The presence of morphine was reported at high concentrations. The detection of morphine was negative or absent at low concentrations. No chemiluminescence was detected when the substrate was reared with the potassium permanganate. The concentrations of morphine increased with the increased concentration of morphine in the larvae.[2] The concentrations of drugs in the insects were stable and several kinds of research were also done for detecting the drugs in the larvae, pupa, and puparia of insects. The quantification of drugs in the larvae helps in the estimation of drug effects on insect development. The most investigated insect sample for the quantification of the drug was the third instar larva. Various instrumental techniques were used for the quantification but because of the heterogeneity of insect tissues, the G/LC coupled with MS was used for their sensitivity and selectivity.

Matthias Gosselin *et al.,* (2010) in their research determine the concentrations of methadone and its metabolites in third instar larvae using LC-MS. The quantification of the methadone and its metabolites were done in the feeding and post-feeding stages of Lucilia sericata insects.[3] The findings of their study showed that the feeding stage marked the higher presence of methadone's metabolites than the post-feeding stage because of the different chemical structures and physiological properties. The more excretion of metabolites was because of higher polarity and more solubility resulting in less concentration in a post-feeding stage. In both the feeding and post-feedingstages, the concentration of methadone was similar because of the bioaccumulation in adipose tissues. The pupal stage marked the low concentration of methadone and its metabolites.[3]

Magni, Paola *et al.,* (2019) describes the method for the quantification of the Endosulfan and its isomers in the Calliphora vomitoria using the HPLC/MS technique. The results showed that the quantification of the Endosulfan was different in the test samples when compared with the control samples.[4] When the Endosulfan concentration was less than 10ng/mg and 25ng/mg in the food substrate, no effect was noticed in the development time and survival of insects but when the concentration was 50ng/mg, the developmental stage of the larva was observed.[4] The larvae and pupae length of the insects in the test sample has no differences when compared with the control samples. When the concentration of Endosulfan was 50ng/mg, the larvae length was smaller and they don't undergo pupation.

Paola et al. (2019) describes a method

for the quantification of the ketamine in Calliphora vomitoria using HPLC/MS and its validation based on the various parameters. The findings of their study showed that the concentrations of ketamine were absent in all control samples, L2 and adult samples.[5] In terms of developmental time especially from oviposition to eclosion, the time was different between control and larvae. The ketamine also affects the survival of the instar's development but during the metamorphosis this effect was significant. During the instar development, the survival was 15% and in metamorphosis, this survival was 85%.

Thomas *et al.,* (2016) describe a study held in Texas, the USA to determine and estimate the temperature and tissue types on the development of various stages of the life cycle of the insects. The larval length and growth rate of insects were examined on the different temperature ranges and tissues types. The method was validated to determine the accuracy by estimating the post mortem interval index. The findings of their study showed that the development rate of the insect was affected by temperature range. An increase in temperature promotes faster development.[7]

The study was done by Harnden and Tomberlin (2016) for studying the effect of temperature and tissues types on the developmental stages of H. illucens with a comparison between laboratory and field reared larvae. The results showed that the development rate of the concerned insect was directly affected by the temperature and the tissue diet. The pork and beef-based diet estimated the larval age in most of the sampling units but it was impossible by grain-based diet.[8] When compared the larval length of laboratory-reared larvae, it was less than the larval length of the field-reared larvae in most of the samples. In the case of pupal development, the temperature and tissue diet have no effects on accumulated degree hours.

The present study done by Wang et.al (2020) mainly focused on determining the developmental stages of H. spinigera at seven different temperatures ranging from 16, 19, 22, 25, 28, 31 to 34°C, and developed the various models that help in determining the post mortem interval index based on the developmental data of the insect H. spinigera. Their study showed that with the help of the Isomorphen diagram it was estimated that the rate of development of insects from egg-laying to adult emergence decrease with an increase in the temperature range. With the help of the Isomegalen diagram, it was determined that with an increase in the temperature range, the larval development rate also increased.[9]

The development of insects was depending mainly on the temperature ranges. Research has shown the effect of temperature on the developmental stages of flies. Previous many types of research also showed that the time of colonization of different flies was also affected by the temperature ranges. The geographical ranges and the densities of blowflies were also affected by the temperature. There is less information about the threshold temperature required for oviposition for an individual blowfly species. The temperature variations on the probability of oviposition behavior of individual blowfly species remain undetermined and also to determine how the temperature of the remains affects the oviposition behavior and the survival of the eggs lying.

Considering all these points, Ody, *et al.,* (2017) describe a study for the determination of various temperature ranges of oviposition in individual blowflies and also the probability of oviposition behavior and survival of eggs in these temperature ranges.The colonization of the species in the cadavers occurs predictably. The necrophagous dipterans are the primary colonizers of the remains colonized in the early stages of decomposition while the coleopteran was the late colonizers of the remains colonized in the late stages of decomposition. The insects were found in every habitat and at all times on cadavers, whether it may be outdoor/indoor, open/forest habitat, high/low elevation, cold/warm, and small or large cadavers.[10] The changes in the composition of the insects and their succession patterns were associated with the changes in the geo-climatic conditions and ecological features of the crime scene. The patterns of a succession of necrophagous insects help in the estimation of post mortem interval index. The succession patterns of the insects and their developmental stage were found to be affected by the local geographical and environmental conditions, so their estimation was an important part of investigations.

The studies done by Abd El-bar and Sawaby (2011) to determine the stages of decomposition and insect succession pattern of cadavers in Egypt and compared these data with the data of stages of decomposition and insect succession patterns of cadavers killed with organophosphate poisoning.[11] The results of their study showed that the rate of decomposition was rapid in the control carcass and takes around 19 days to reach the skeletal or dry stage. The time taken by the test carcass to reach the decay stage was 40 days but only the lower part gets decayed and the upper part remains as it is because of the slow rate of decomposition. The presence of toxins in the decomposing body changes the succession patterns of insects and their developmental stages but it does not affect the arrival of the insects.

CONCLUSION

From the past, many years of research have been done on entomotoxicology to establish a relation between the concentration of drugs and toxins in the substrate to their concentrations in the insects reared on that substrate. The detection of drugs and toxins in the human remains by using various analytical techniques helps estimate the post mortem interval of cadavers.[1] Various methods and procedures have been systematically developed and validated for entomotoxicological analysis. The effectiveness of methods and procedures depends on the ability to detect and identify the drugs present in the insects. The most accepted technique used for detection was chromatography coupled with mass spectrometry. The fate of drugs or toxins in insects depends on their feeding activities and developmental stages.[6] Every insect has different feeding habits because of its diet and life histories that affect the accumulation of the drug in different species of insects. With the help of entomological evidence, the post mortem interval index can be estimated in cadavers by using two primary ways – one by determining the insect succession pattern since the patterns of their succession depict the various stages of decomposition. The other one is depending on the rate of development of immature insects on the corpses. The rate of development of immature insects depends on various natural and anthropogenic factors like climatic conditions, the temperature of the cadavers, humidity, nature of burial, clothing, presence of drugs or toxins, and many more.[8] The thermal growth of flies is a useful technique in forensic entomology, and more research will improve its utility. Because of the intricacy of insect growth and the numerous factors that affect it, determining insect development is inevitably an estimating process. With more research, we can expect our estimates to become more consistent. However, until we have highly exact temperature measurements, thermal development predictions are unlikely to ever yield correct post-mortem interval estimations.[7] When combined with other data, such as successional patterns or direct age marking of insects, temperature growth estimations are a powerful line of independent evidence. The predictable pattern of insect succession on a body has long been regarded as a reliable way to calculate the time after death. However, a variety of factors may affect the frequency and species richness of the carrion fauna. It's critical to be aware of all the circumstances that can affect insect colonization of remains and to account for them while examining a death.[11] Studies need to be done, in particular, to establish more spatial datasets of insect succession on carrion in a wide range of habitats and circumstances across all countries where forensic entomology is applied. IJFMP

Acknowledgment:
The author would like to thank all his colleagues and family who encouraged him to do this review.
Conflict of Interest:
The authors declare that there is no commercial or financial links that could be construed as conflict of interests.
Source of Funding: *None*

REFERENCES

1. ***Choph Rito, Sharma Spriha, Sharma Sahil, Singh Rajinder.*** *Forensic entomotoxicology: Current concepts, trends, and challenges. Journal of forensic and legal medicine 2019;67:28-36.*
2. ***Gunn Joshua A, Shelley Cara, Lewis Simon W, Toop Tes, Archer Melanie.*** *Quantification of the morphine using HPLC in the immature stage (larvae) of the Calliphora Stygia insect. Journal of analytical toxicology 2006;30:519-523.*
3. ***Gosselin Matthias, Fernandez Maria del Mar Ramirez, Wille Sarah M.R, Samyn Nele, Boeck Gert De, Bourel Benoit.*** *Determination of the parent toxin and its metabolites of methadone in the instar larva of insect L.Sericata and its quantification using LC-MS.Journal of analytical toxicology 2010;34:374-380.*
4. ***Magni, Paola, Pazzi, Marco, Vincenti, Marco, Converso, Valerio, Dadour, Ian.*** *Analytical method validation of Endosulfan metabolites in the Calliphora vomitoria insect (Diptera: Calliphoridae). Journal of medical entomology 2019;55:51-58.*
5. ***Magni, Paola, Pazzi, Marco, Vincenti, Marco, Dadour, Ian.*** *Analytical method validation of ketamine drug in Calliphora vomitoria insect using HPLC-MS/MS. Journal of forensic and legal medicine 2018;58:64-71.*
6. ***R Dayananda, J Kiran.*** *Entomotoxicology. International journal of medical toxicology and forensic medicine 2013;3(2):71-74.*
7. ***Joshua K. Thomas.*** *Effects of Temperature and Tissue Type on the Development of Megaselia scalaris (Diptera: Phoridae).Journal of medical entomology 2016;0:1-7.*
8. ***Harnden.M. Laura, Tomberlin K Jeffery.*** *Effects of temperature and diet on black soldier fly Hermetia illucens (L.) (Diptera: Stratiomyidae) development. Forensic science international 2016;266:109-117.*
9. ***Wang, Yu, Zhang Yingna, Wang Man, Hu Guoliang , Fu Yangfan , Zhi Rong, Wang Jiangfeng.*** *Development of Hydrotaea spinigera (Diptera: Muscidae) at Constant Temperatures and Its Significance for Estimating Postmortem Interval.Journal of medical entomology 2020;20(20):1-8.*
10. ***Ody, Helen, Bulling Mark T, Barnes Kate M.*** *Effects of environmental temperature on oviposition behavior in three blowflies species of forensic importance. Forensic science international 2017;275-138.*
11. ***Abd El-bar M.M.*** *A preliminary investigation of insect colonization and successionon remains of rabbits treated with an organophosphate insecticide in El-QalyubiyaGovernorate of Egypt. Forensic Science International 2011;208:26–30.*