MEDICOLEGAL PERTINENCE OF CADAVER ENTOMOFAUNA FOR DETERMINATION OF TIME SINCE DEATH AND CAUSE OF DEATH

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By

MUSKAN

Registration Number: 11916715

Supervised By

Dr. Saurabh Shukla (UID-26174)

Department of Forensic Science

(Assistant Professor)

PhD Forensic Science

Co-Supervised by

Dr. Jaskaran Singh

Forensic Science & Sciences

Geeta University

(Associate Professor & Head)

PhD Forensic Science

&

Dr. Dasari Harish

Department of Forensic Medicine &

Toxicology, GMCH-32, Chandigarh

(Professor & Head)

MD Forensic Medicine

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2024

DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Medicolegal Pertinence of Cadaver Entomofauna for Determination of Time Since Death and Cause of Death**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision **Dr. Saurabh Shukla**, working as **Assistant Professor**, in the **Department of Forensic Science, School of Bio-engineering and Bio- Sciences** of **Lovely Professional University, Punjab**, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

Name of the scholar: Muskan

Registration No.: 11916715

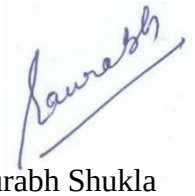
Department/school: Department of Forensic Science, School of Bio- Engineering and Bio- Sciences

Lovely Professional University,

Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Medicolegal Pertinence of Cadaver Entomofauna for Determination of Time Since Death and Cause of Death**" submitted in fulfilment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the **Department of Forensic Science, School of Bio-engineering and Bio- Sciences**, is a research work carried out by **Muskan**, (Registration No.) **11916715**, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



(Signature of Supervisor)

Name of supervisor: Dr. Saurabh Shukla

Designation: Assistant Professor

Department/school: Department of Forensic Science, School of Bio-engineering and Bio- Sciences

University: Lovely Professional University, Phagwara, Punjab, India.

(Signature of Co-Supervisor)

Name of Co-Supervisor: Dr. Jaskaran Singh

Designation: Associate Professor & Head

Department/school: Department of Forensic Science

University: Geeta University, Naultha, Panipat, Haryana, India.

(Signature of Co-Supervisor)

Name of Co-Supervisor: Dr. Dasari Harish

Designation: Professor & Head

Department/school: Department of Forensic Medicine & Toxicology

University: Govt. Medical College & Hospital, Chandigarh, India.

Dedicated to all the victims killed due to toxic substances.

ABSTRACT

Forensic entomology and entomotoxicology are two charming and specialized fields that have recently emerged as important tools in the investigation of criminal cases. These fields provide invaluable assistance to forensic investigators in determining the time of death, as well as the presence and type of toxic substances in a victim's body.

Forensic entomology involves the study of insects and their behaviour in the context of a criminal investigation. This field is particularly useful in cases where the time of death is unknown, and no physical markers are available to estimate it. In such cases, forensic entomologists can observe the growth of insect on the corpse to estimate the time of death with a high degree of accuracy. By studying the types of insects present on the body and their development stages, entomologists can determine how long the body has been in the location where it was found. Entomotoxicology, on the other hand, involves the analysis of toxins present in insects that have fed on a corpse.

This field is useful in cases where a victim has been poisoned, and the type of toxin is unknown. By analysing the insects found on the corpse, entomotoxicologists can determine the presence of toxic substances and their quantities. This information can then be used to determine the cause of death and assist investigators in identifying potential suspects. Both forensic entomology and entomotoxicology are highly specialized fields that require extensive training and expertise. However, the information that they provide can be invaluable in solving criminal cases and bringing perpetrators to justice. As these fields continue to develop and evolve, they will undoubtedly become even more important in the field of forensic science.

There are always three major questions in front of investigator to solve the mystery of crime which are when, where and how. Forensic entomology and entomo-toxicology can answer all three of them in any kind of homicide, suicide, or accidental deaths. And Forensic Entomology and Entomotoxicology can answer these queries by estimating PMI (Post mortem Interval), insects identification helps to find the place of crime and drug identification helps in finding the manner of crime. The toxic substance available

in tissues can alter the estimation of mPMI (minimum Post mortem Interval) as different drugs or toxic substance can affect the growth rate of insects in different manner. Efficient detection of drugs and understanding their effects on an insect's life cycle are the subjects of numerous global research projects. Unfortunately, there is a scarcity of data available on entomo-toxicology studies in India. While previous research has examined the effects of drugs such as heroin, morphine, and dolpezam, yet many drugs remain unexplored in terms of their entomo-toxicological effects.

Insects feeding on a cadaver can act as valuable indicators of the presence of drugs in the body. Through entomo-toxicology studies, researchers can determine which drugs are present in the insect's system and analyze the potential effects on its life cycle. While various global research projects have explored the entomo-toxicological effects of a range of drugs, there has been limited research conducted in India. The limited availability of entomo-toxicological data in India represents a significant challenge in terms of determining the presence of drugs in criminal investigations. Although previous studies have examined the effects of drugs such as heroin, morphine, and dolpezam on insect populations, there remains a vast pool of drugs yet to be studied.

In-depth entomo-toxicology research on these drugs is essential to improve our understanding of their effects on insects and to aid forensic investigators in identifying the presence of drugs in the body. In conclusion, entomo-toxicology research is a crucial aspect of forensic science, particularly in the investigation of criminal cases. While there are numerous global research projects exploring the effects of drugs on insects, there is a pressing need for additional entomo-toxicology research in India. A more extensive body of research will enable investigators to detect unidentified drugs which are not yet identified via entomotoxicological samples and to better understand their effects on insects' life cycles.

Likewise, the drug Asenapine maleate has been linked to numerous cases of suicide and accidental deaths. The drug is commonly prescribed for the treatment of bipolar disorder and schizophrenia. Surprisingly, there is scanty literature available on the use of this drug in entomo-toxicological studies. This study is a blessing as it focusing on investigation of the entomo-toxicological effects of Asenapine maleate, and no specific

insect species were targeted for the experiment. The objective was to cover a wide range of insect species and study the effects of the drug on their growth patterns.

To achieve this objective, experiments were conducted at five different locations during four different seasons. The study aimed to obtain a comprehensive understanding of the impact of the drug on various species of insects. The significance of the study lies in the fact that Asenapine maleate has been identified as a major cause of deaths and this research can help identify the potential risks associated with its usage in the environment.

In conclusion, the entomo-toxicological effects of Asenapine maleate have not been studied before, despite its significant use in psychiatric treatment and association with numerous deaths such as drug abuse/ drug overdosage. This study aims to fill this gap by investigating the effects of the drug on a wide range of insect species in different locations and seasons.

There were four different species identified from Diptera order which is common in Northern India. Those four species were *Chrysomya megacephala* (Fabricius, 1794), *Calliphora vicina* (Robineau-Desvoidy, 1830), *sacrophaga albiceps* (Meigen, 1826), and *Musca sorbens* (Wiedemann, 1830). The experiments were conducted on substrate such as goat meat, after spiking drug Asenapine maleate with different concentrations. The same was applied on real forensic matrix such as viscera- human post mortem liver.

The findings establish the fact that drug Asenapine maleate effects the growth cycle of insects but not the body dimensions at any stage of life. From this study, it is observed that the presence of Asenapine maleate in the corpse colonized by dipteran insects can lead to variations in estimating the minimum PMI. If the PMI estimation will be based on duration of larval and pupal stage, the variables of the PMI would be longer than the actual interval, as due to asenapine maleate; the rate of development of insects increases.

Furthermore, the mortality at the larval stage can be higher during the summer season in northern India. Also, the concentrations spiked and extracted from the larvae of insects are highly co-related and proves that the determination of dose of Asenapine

maleate taken by a person prior to death is possible from the larvae feeding on cadaver. Thus, the larvae of *Chrysomya megacephala, Calliphora vicina, Sacrophaga albiceps* and *Musca sorbens* are proven to be reliable alternative specimes for toxicological investigation involving the drug Asenapine maleate.

Keywords: Forensic entomology, entomotoxicology, Postmortem Interval, Asenapine maleate, *Chrysomya megacephala, Calliphora vicina, Sacrophaga albiceps, Musca sorbens*

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Table of Contents

Sr. No.	Title	Page No.
	Abstract	IV
	Table of Contents	XI
	List of Tables	XV
	List of Figures	XVII
	List of Abbreviations	XIX
	List of Appendices	XX
Chapter 1	Introduction	1
1.1	History	1
1.2	Biology of Insects and their significant	4
1.3	Use of Insects in Medico-Criminal Investigation	6
1.4	The Faunal Succession	7
1.5	Stages of Decomposition of cadaver	7
1.6	Calculation of Time Since Death	10
1.7	Entomotoxicology	10
1.8	Role of Insects in body decomposition and PMI estimation	13
1.9	The common flies which are generally found in India	14
1.10	Insect succession on Cadavers	18
1.10.1	Life Cycles of Insects Associated with Cadavers	19
1.11	Detection of drugs and toxins	20

1.12	Effects of drugs in tissues on development of insects	21
1.13	Determination of PMI in Entomotoxicology	22
1.14	Forensic entomology in Court of Law	24
1.15	Introduction to the drug used for study: Asenapine maleate	24
Chapter 2	Literature Review	28
Chapter 3	Material and Method	43
3.1	Study area and Period of study	43
3.1.1	Site Selection	44
3.2	Preparation of working and stock solution	45
3.3	Experimental food substrate	45
3.4	Drug Used	46
3.5	Environmental Conditions	46
3.6	Experimental Set up	46
3.6.1	Collection of insects	47
3.7	Chemicals and Reagents	48
3.8	Tools & Techniques used for toxicological analysis	48
3.9	Research Gap	49
3.10	Aim & Objectives	50
Chapter 4	Results and Discussion	51
4.1	Objective 1	51
4.1.1	Introduction	51

4.1.2	Methodology	51
4.1.3	Outcomes	51
4.1.3.1	*Chrysomya megacephala* (Fabricius, 1794)	54
4.1.3.2	*Calliphora vicina* (Robineau-Desvoidy, 1830)	56
4.1.3.3	*Sarcophaga* (parasarcophaga albiceps, Meigen 1826)	58
4.1.3.4	*Musca Sorbens*, Wiedemann, 1830)	61
4.2	Objective 2	65
4.2.1	Introduction	65
4.2.2	Methodology	65
4.2.3	Outcomes	67
4.3	Objective 3	84
4.3.1	Introduction	84
4.3.2	Methodology	84
4.3.2.1	Preparation of specimen for toxicological examination	84
4.3.2.2	Clean-up, Extraction and Purification process	85
4.3.2.3	Extraction by Solid Phase Extraction	85
4.3.2.4	Gas chromatography and Mass spectrometry	85
4.3.3	Outcomes	86
4.4	Objective 4	97
4.4.1	Introduction	97
4.4.2	Methodology	97

4.4.3	Outcomes	97
4.5	Application of developed study on Human Post-mortem Liver sample	109
4.5.1	Introduction	109
4.5.2	Methodology	110
4.5.2.1	Preparation of working and stock solution	110
4.5.2.2	Experimental food substrate	110
4.5.2.3	Collection of insects	111
4.5.2.4	Chemical and Reagents	111
4.5.2.5	Tools & Techniques used for toxicological analysis	111
4.5.3	Outcomes	112
4.5.3.1	Duration of experiment	112
4.5.3.2	Flies collected and identified	112
4.5.3.3	Drug Analysis	113
4.5.3.4	Clean-up and Purification procedure	114
4.5.3.5	Extraction by Solid Phase Extraction	114
4.5.3.6	Gas chromatography and Mass spectrometry	115
4.6	Discussion	121
Chapter 5	Conclusion	125
	References	129

List of Tables

Table No.	Title	Page No.
1.1	Relation of insects with different decomposition stages	7
3.1	The duration of experiments conducted in Mohali and Jalandhar	47
4.1	Temperature and ADD (Accumulate degree days calculated) of Mohali, Punjab during	68
4.2	Time taken by insects to grow in to successive stage during 1st Experiment	69
4.3	Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 2nd experiment	71
4.4	Time taken by insects to grow in to successive stage during 2nd Experiment	72
4.5	Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 3rd experiment	73
4.6	Time taken by insects to grow in to successive stage during 3rd Experiment	73
4.7	Temperature and ADD (Accumulate degree days calculated) of Mohali, Punjab during 4th experiment	75
4.8	Time taken by insects to grow in to successive stage during 4th Experiment	75
4.9	Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 5th experiment	77
4.10	Time taken by insects to grow in to successive stage during 5th Experiment	77
4.11	Temperature and ADD (Accumulate degree days calculated) of Mohali, Punjab during 6th experiment	78
4.12	Time taken by insects to grow in to successive stage during 6th Experiment	78
4.13	Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 7th experiment	79
4.14	Time taken by insects to grow in to successive stage during 7th Experiment	79
4.15	Temperature and ADD (Accumulate degree days calculated) of Mohali, Punjab during 8th experiment	81
4.16	Time taken by insects to grow in to successive stage during 8th Experiment	81
4.17	Validation of Asenapine maleate analysis using Gas Chromatography- Mass spectrometry	95

4.18	Time taken by insects to grow in to successive stage during 1st Experiment	98
4.19	Analysis by Single factor ANOVA on data of 1st experiment	98
4.20	Time taken by insects to grow in to successive stage during 2nd Experiment	99
4.21	Analysis by Single factor ANOVA on data of 2nd experiment	99
4.22	Time taken by insects to grow in to successive stage during 3rd Experiment	100
4.23	Analysis by Single factor ANOVA on data of 3rd experiment	100
4.24	Time taken by insects to grow in to successive stage during 4th Experiment	102
4.25	Analysis by Single factor ANOVA on data of 4th experiment	102
4.26	Time taken by insects to grow in to successive stage during 5th Experiment	103
4.27	Analysis by Single factor ANOVA on data of 5th experiment	103
4.28	Time taken by insects to grow in to successive stage during 6th Experiment	104
4.29	Analysis by Single factor ANOVA on data of 6th experiment	104
4.30	Time taken by insects to grow in to successive stage during 7th Experiment	105
4.31	Analysis by Single factor ANOVA on data of 7th experiment	105
4.32	Time taken by insects to grow in to successive stage during 8th Experiment	106
4.33	Analysis by Single factor ANOVA on data of 8th experiment	106
4.34	Temperature data and ADD (Accumulate degree days calculated) during the experiment	112
4.35	Time taken by insects to grow in to successive stage during Experiment	118
4.36	Analysis by Single factor ANOVA on data of experiment	118
4.37	Comparative data of Mutton substrate and Post-mortem Liver Substrate studies	119

List of Figures

Figure No.	Title	Page No.
1.1	Categories of applications of Forensic Entomology	2
1.2	Image of insect on flower taken during experimentation	5
1.3	Calliphora vicina	14
1.4	Chrysomya megacephala	15
1.5	Chrysomya rufifacies	15
1.6	Calliphora vomitoria	16
1.7	Lucilia sericata	17
1.8	Life cycle of Blow fly	19
1.9	Chemical structure of Asenapine maleate	25
3.1	Study area map of entomological sample collection regions	43
3.2	Spiking drug	45
3.3	Spiking drug	45
3.4	Samples kept in cage	45
4.1	Pinned insects	52
4.2	Microscopic image of dorsal view of *Chrysomya megacephala*	54
4.3	Microscopic image of front view of *Chrysomya megacephala*	54
4.4	Microscopic image of wing of *Chrysomya megacephala*	55
4.5	Microscopic image of dorsal view of *Calliphora vicina*	57
4.6	Microscopic image of front view of *Calliphora vicina*	57
4.7	Microscopic image of wing of *Calliphora vicina*	57
4.8	Microscopic image of dorsal view of *Parasarcophaga albiceps*	59
4.9	Microscopic image of front view of *Parasarcophaga albiceps*	60

4.10	Microscopic image of wing of *Parasarcophaga albiceps*	60
4.11	Microscopic image of dorsal view of *Musca sorbens*	61
4.12	Microscopic image of front view of *Musca sorbens*	61
4.13	Microscopic image of wing of *Musca sorbens*	62
4.14	Spiked and control meat	66
4.15	Jars in cage	66
4.16	Attack of fly on fresh meat	66
4.17	Fly laying eggs on substrate	66
4.18	Eggs on substrate	66
4.19	1st instar larvae	66
4.20	2nd instar larvae	66
4.21	3rd instar larvae	67
4.22	Pupa	67
4.23	Adult fly	67
4.24	Thermometer	67
4.25	Glass jars covered with muslin cloth	67
4.26	Clean up and purification procedure	85
4.27	Calibration curve of Asenapine maleate standard	95
4.28	Post-mortem investigation of deceased	109
4.29	Spiking drug in post-mortem liver	110
4.30	Attack of flies on liver	110
4.31	Clean-up and purification procedure	114

List of Abbreviations

PMI	Post mortem Interval
ASPM	Asenapine Maleate.
M	Molar
UV	Ultraviolet
GC-MS	Gas Chromatography/Mass Spectrometry
LC MS	Liquid Chromatography/Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
HPTLC	High-Performance Thin Layer Chromatography
UPLC	Ultra Performance Liquid Chromatography
UFLC	Ultra-Fast Liquid Chromatography
NA	Not Available
ACN	Acetonitrile
LOD	Limit of Detection
LOQ	Limit of Quantification
mg	Microgram
mL	Microliter
C	Degree Celsius
v/v	Volume by Volume
CE	Capillary Electrophoresis
Rf	Retention factor
MeOH	Methanol
ng	Nanogram
nm	Nanometre
LTZ	Letrozole
SWGTOX	Scientific Working Group for Forensic Toxicology
SPE	Solid Phase Extraction
FDA	Food and Drug Association

List of Appendices

Appendix A Ethical permission letter

Appendix B Permission letter from ZSI, Kolkata

Appendix C Insects' Identification Report

Chapter 1

Introduction

The scientific study of insects is known as entomology and it is focused on all kinds of insects including domestic and parasites. Insect identification, categorization, behaviour, ecology, physiology, and evolution are the main topics of this subfield of Zoology. With over a million species yet known, insects are the most diverse group of creatures on earth and are essential components of many ecosystem. Pest control, crop protection, disease vector control, and forensic science (forensic entomology, which utilizes insects to predict the time of death in criminal investigations) are all areas where the study of insects has practical applications. Additionally, insect are essential part of food web and pollinators for many plant species, Therefore, entomology is an important for conservation (Catts and Goff, 1992).

Forensic entomology is a field within forensic science that focuses on the utilization of flies and invertebrates that feed on leftovers as a means of evidence in criminal investigations. It plays a significant role in human and wildlife cases. The purpose of forensic entomology is to employ forensic information gathered from the morphology, growth patterns, species distribution, and toxicological content of insects and their larvae in human or animal tissues as admissible evidence in civil and criminal investigations (Amendt *et al.,* 2004). It aims to determine the circumstances surrounding a crime scene area, including the time, location, and manner. The primary application of forensic investigation is to determine the minimum post mortem interval in suspicious death cases. The field of forensic entomology is divided into three branches: urban, stored products, and medicolegal, thus, each branch focus on a specific area of insect involvement in criminal investigations and for legal purposes (Farhan *et al.*, 2018).

1.1. History

Insects played a vital part in facilitating the decay of organic matter and natural processes. Their importance in detecting and solving crimes has been recognized for a

significant period, with numerous researchers contributing to the field of forensic entomology (Mark, 2005). The earliest known practical application of this field is documented in the Chinese book "The Washing Away of Wrongs," which provides insights about a criminal investigation in which the number of blow flies found on a sickle led to the culprit's confession to killing a fellow farm worker (Sung *et al.*, 1981). Over time, various terminologies have been used to describe the presence of maggots on corpses, including "dances of the death," and ivory carvings, such as the "skeleton in the Tumba" (Mark, 2001). Two significant studies in Italy are particularly noteworthy. In 1668, Redi and colleagues utilized the meat of numerous animal species to show that flies' eggs evolved into larvae. A categorization system for insects was created by Linnaeus in 1775, offering a way of identification, including flies of forensic significance. The duration of the development of the insect life cycle and indications of the time since death may be calculated (Gill, 2005; Sharma, 2016).

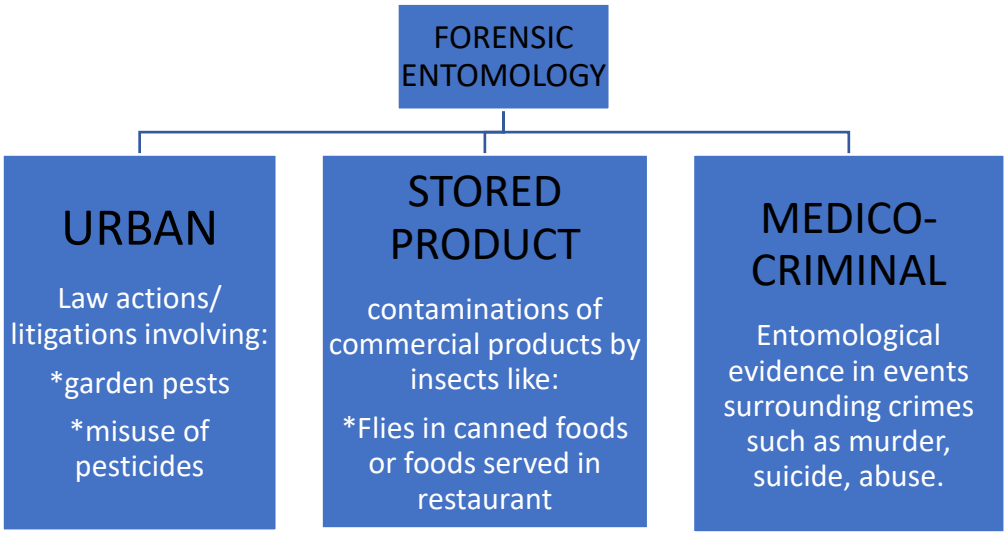


Figure 1.1: Categories of applications of Forensic Entomology (Farhan *et al.*, 2018)

In the first half of the 19th century, necrophagous insects, including beetles, flies, and other taxa, were identified for their potential use in forensic investigations. In 1855, French doctor Bergeret used blowfly pupae and larval moths to check the time since death and ascertain age of a child's skeletonized corpse. With the taxonomic identification of Phorid flies and beetles from unearthed remains in Saxonia, the first systematic study in forensic entomology was published in 1881. This was followed by a similar report from Hofmann in 1886 (Amendt *et al.*, 2004). These studies indicated

the possibility for employing entomological evidence to establish PMI in criminal investigations and emphasize the significance of necrophagous insects in forensic investigations.

Firstly, Forensic entomology was founded by Yovanovich and Megnin, who were the first forensic investigators to analyse insect succession on dead bodies. The important phase in the history of forensic entomology that followed was brought about by Megnin's discoveries and conclusions in 1894. In 'La Faune des Cadavres: Application de l'Entomologie a la Médicine Légale,' he divided the eight stages of decomposition that followed the path of insects that attracted the body after death. The surrounding environment, which includes the clothing worn and the temperature, affects these stages of decomposition, which advance at varying speeds. The information on the fly succession on a cadaver served as the foundation for forensic entomologists' assessment of the corpse's minimal PMI (Gupta and Setia, 2004; Abajue and Sylvanus, 2016). Mégnin's work was crucial to the development of forensic entomology, providing a framework for determining the PMI based on the presence and activity of insects on a corpse.

The comprehensive knowledge published about the creatures present on decomposed cadavers sparked the interest of scientists in the subject and as a tribute to Mégnin, the mold Endoconidium megnini was named after him (Triplehorn *et al.*, 2005). New insect species were found in the 20th century, and their life cycles and taxonomy were thoroughly investigated. Several conventional and innovative approaches have been documented in literature and journals with regard to the medicolegal importance of insects recovered from crime scenes, the duration of colonization, and ultimately determining the time elapsed since death. The endorsement of forensic entomology as a respectable scientific field has been contingent upon the backing of scholars, professionals, law enforcement, and legal authorities. This field's advancement and growth in the late 20th and early 21st centuries have contributed to its reputation as a trustworthy and practical instrument for criminal investigations (Abajue and Sylvanus, 2016).

1.2. Biology of Insects and their significance

The word insect is derived from a Latin word "insectum," which means "split into segments". Entomology, which means "split into two or sliced in segments," is the name given to the study of insects. In the phylum Arthropoda, class Insecta, Animalia kingdom, and Eukaryota domain, insects are members of the phylum Insecta. Insects, which aren't considered animals yet account for more than half of known living things. Thus, there are between 6 and 10 million species in existence even if only one million have been discovered. Insects are characterized by their chitin exoskeleton, three pairs of legs, a compound eye, a pair of antennae, and a body composed of the head, thorax, and abdomen (Illingworth, 1923).

Insects are highly adaptable and diverse creatures that can thrive in any environment and habitat. They undergo three distinct developmental stages, namely, egg, larva, and pupa. They are present in diverse species at various developmental stages and are essential for the decay of corpses. Among the arthropods connected to the decomposition of dead bodies are harvestmen (Opiliones), mites (Arachnida), flies (Diptera), beetles (Coleoptera), and lice (Isopoda). Biological preferences, the degree of decomposition, and geographic location affect their prevalence and abundance. The study of insects is essential in forensic science, they provide vital information about the time since death and help in criminal investigation. In the late 20th and early 21st century, several new species have been found, and their life cycles and taxonomy were thoroughly examined to expand forensic entomology as a scientific discipline (Lord and Rodrigues, 1989; Tuzun *et al.,* 2009). Many insects are useful in forensic entomological investigations, including cheese skippers, blowflies, flesh flies, rove beetles, hide and skin beetles, and clown beetles.

While some of these insects are necrophages both as juveniles and adults, others are necrophages while they are young. Insects are the first to attack from outside on a decomposing body because they are drawn to dead animals and human bodies. By analyzing the sequential colonization of a corpse and the development rates of the offspring, forensic entomologists can estimate the time of death, also known as the

minimum postmortem interval (mPMI). This information may be used to estimate the period between the death and the finding of the corpse, in addition to the method and mode of death and the relationship of any suspects from the crime scene.

For example, blowflies are often the first insects to appear at a corpse and lays eggs, which eventually hatches into maggots. As maggots develop, their size, stage of growth, and number can indicate the PMI. A further indicator of the time of death is the emergence of specific bug species at various stages of decomposition. For example, certain species of flies are drawn to corpses in the diptera stage of decomposition, whereas other species are drawn to the coleoptera stage.

Figure 1.2 Insect on flower found during experimentation

Forensic entomology is a well-known and important field, mainly in the investigation of criminal matters, due to the accuracy and reliability of insect evidence in determining the PMI. By understanding the lifecycle and habits of different insects, forensic entomologists can provide valuable information to law enforcement and legal authorities in solving crimes (Sukontason *et al.,* 2007; Lord and Rodrigues, 1989). Forensic entomology provides valuable information that can be used as circumstantial evidence at a crime scene, whether the victim is dead or alive. This information also enables forensic scientists to exclude various aspects of crime, such as suspicious activity, addiction, neglect, infestation, suicides, and murders (Smith, 1986).

1.3. Use of Insects in Medico-Legal Investigation

Entomologists often rely on insects and other arthropods in solving criminal cases where the time of death or cause of death are unknown, because insects are the first organisms to attack and colonize a corpse after death (Sukontason *et al.,* 2007). Within a short time after death, insects lay eggs on the cadaver, and the process of egg-laying by fly species found on or near the corpse can reveal changes in putrefaction development. The biological roles of these insects are classified into four categories based on the ecology of decomposition (Byrd and Castner, 2009).

a. **Necrophages** These species grow by eating the corpse and mostly belong to order Diptera (Coleoptera (silphids and dermestids) and calliphorids and sarcophagids) included in this and PMI is estimated by determining the insect's age.

b. **Omnivores -** These include wasps, beetles, and ants that feed on the cadaver as well as the surrounding fauna. The availability of these omnivorous insects may decelerate the decomposition rate of corpse.

c. **Parasites and Predators** - In the early stages of the maggot development, fewer species are necrophagous; instead, they become predaceous. Mites are also the members of this category as they are recognized as hunters of other developing insects such as mites, nematodes andinsects probably also belong in this category as hunters of other mites, insects, and nematodes (e.g. Hymenoptera, Uropodidae, Macrochelidae, Parasitidae, Parholaspidae)

d. **Incidentals** - arthropods use carcass as an intense resource and also they are specific to the habitat. For examples - Springtails, pillbugs, spiders, centipedes

1.4. The Faunal Succession

To accurately identify the developmental stages of insects, present in decomposing remains and their relation to the process of putrefaction, expertise in entomology is necessary and requires appropriate training (Henssge and Madea, 2004).

Table 1.1. Relation of insects with different decomposition stages

Succession	Decomposition stage	Time of decomposition	Insects
1	Fresh	First 3Months	Flies: Blowfiles
2	Odour	-	Blowfiles & Flesh Flies
3	Rancid Fats	3-6 months	Dermestid beetles (Coleoptera)
4	-	-	Various flies
5	Ammonia/fermentation	4-8 months	Various flies and beetles
6	-	1-12 months	Mites
7	Completely dry	1-3 years	Dermestid beetles
8	-	3= years	Beetles

1.5. Stages of Decomposition of cadaver

When a freshly deceased human or animal body experiences post-mortem changes, such as rigor mortis (body rigidity), livor mortis (blood pooling), and algor mortis (body temperature drop), forensic pathologists can use these changes to reasonably estimate the time since death within the first one to three day post-mortem period. Forensic pathologists need to have a thorough grasp of these post-mortem effects and the order in which they occur in order to properly identify the time of death. (Vass *et al.,* 1992).

Three distinct processes are identified in the decomposition of a body during the late postmortem period: skeletal bone degradation, putrefaction, and autolysis. Enzymes like lipases, proteases, and sugars break down bodily cells naturally during a process called autolysis. This process occurs most rapidly in organs like the brain and liver (Tullis and Goff, 1987). Putrefaction is a process which is done mainly by microorganism and alongside source of protein broke into fatty acids by bacteria. After the superficial skin or tissue is decomposed, the remaining skeletal components undergo further breakdown under environmental conditions and eventually become

part of the soil. The decomposition rate is affected by the environmental heat and humidity.

a. On land

The categorization of a corpse can be identified within five distinct stages of decomposition, each of that can be associated with the 8 different phases of arthropod colonization first exclusively presented by Mégnin in 1894. The physical characteristics of the body, the internal temperature, and the typical insect populations are used to define these phases. Previous research has shown that the decomposition process can be separated into these five stages, which are as follows (LeBlanc and Logan, 2009; Haefner *et al.* 2004):

Stage 1- Fresh stage: The process begins shortly after ones to be and continues until the cadaver begins to bloat. During this time, blowflies are the first attracted to the body and begin to lay eggs in specific areas, including around the eyes, nostrils, mouth, and eventually the anus or genital opening.

Stage 2- Bloated stage (Days 2-7): At this stage, breakdown of the body occurs as a result of bacterial activity, or putrefaction, which is easily identifiable. The gases are produced by the anaerobic bacteria (mainly metabolic activities) that produce gases that cause the abdomen to inflate, giving the entire body a stretched and balloon-like appearance later on. As this process continues, the smell of the breakdown gases attracts more blowflies to the body. Additionally, the activities of arthropods, combined with the putrefaction process, can raise the internal temperature of the carcass. Rove beetles, specifically those from the Staphylinidae family, may be drawn to the body at this point due to the presence of eggs and maggots. However, it is important to note that other predators may also affect the counts and life stages when feeding on removed pupa and larvae and difficult to interpret it.

Stage 3: Active decay stage (5-13 days): During this stage, there is breakage in the skin of the corpse that causes increase in the weight of the corpse due to gas production. The sign of putrefaction shows when temperature reaches 14 ◦C or above and decay stages ended. The next phase is characterized by advanced putrefaction, during which

a different set of decomposers are attracted to the decomposed dead-body. By 10^{th} day, the carcass's weight gradually drops.

Stage 4: Post-decay stage (10-23 days): During the later stages of decay, all that's left are the bones, cartilage, and skin, along with some leftover meat. In this stage, the most prominent signs that there is increment of beetles presence and a shows lesser number of flies (Diptera) on the dead corpse.

Stage 5: Skeletonization (20-90 days): The body is limited to hard tissues at this point, such as teeth, hair, and bones; no other noticeable insect families are present, with the exception of the Nitidulidae beetle family. The last stage of decomposition is indicated by the disintegration of the body's toughest parts, such as the ribs, limbs, and skull bones.

b. On water

When a dead body is found in water bodies such as rivers, lakes, or wells, the five stages of decomposition still occur but with some additional changes. One major difference is that the body may be moved by the flow of the river or floated to the water surface after the decay stage and in such situations, aquatic insects may also colonize or feed on the body. The length of the PMI becomes very relevant, after the body is discovered and recovered by police and will rely on the heat present on the water. As per Giertsen (1977), the rate at which the body loses heat in water is twice as fast as the rate at which it loses heat in air, which accounts for the difference in decomposition between the two environments (Haskell *et al.,* 1989; Villet and Amendt, 2011). Further work is required to determine the decomposition of corpses in various types of water bodies and its geographical information, so that a deep understanding of PMI can be established.

1.6. Calculation of Time Since Death

The estimation of the PMI can be significantly aided by the presence of carrion insects in human cadavers. Forensic entomologists have developed, validated, improved, and

applied various methods based on insect evidence for PMI estimation. This topic is growing as a result of the consistent rise in scholarly publications and the extensive application of entomology-based PMI estimate in international death investigations. (Samnol *et al.,* 2020). There are mainly two ways to determine time since death-

Period of insect isolation- The time during which the dead matter is invaded by flesh-eating insects is known as the period of insect isolation, while the developmental stage of the insects found at the crime scene represents the time since insect colonization. This technique is utilized to determine the season of death and to gain knowledge about insect succession and habitat. The determination can be made within a few weeks after death (Zanetti *et al.,* 2016).

Time since insect colonization the initial stage of insect colonization on a corpse involves the arrival of maggots, which are immature flies or larvae that deposit their eggs on wounds, natural orifices or openings in the body. The growth stages of maggots, which are characterized by changes in weight, length or shape, are referred to as instars. Since insects are ectothermic, their metabolism accelerates with rising temperatures. The final stage of metamorphosis is the emergence of the adult insect, which is known as eclosion. A comprehensive developmental analysis of the maggots requires access to weather data from the nearest meteorological center (Disney *et al.,* 2014; Pounder, 1991).

1.7. Entomotoxicology

Entomotoxicology is a discipline derived from Greek words, with "entomon" meaning insect, "toxikos" meaning poisonous and "logos" meaning subject matter. The field primarily deals with the study of xenobiotics that impact insects. The term "entomotoxicology" was coined by Pounder in 1991. As the name implies, entomotoxicology mainly concerns the accumulation of xenobiotics in insect bodies and their physiological effects. According to Pounder's definition, forensic entomotoxicology refers to the identification of drugs present in a decomposed body, as well as the examination of the maggots feeding on it. Nowadays, this field of forensic science is becoming increasingly popular due to its focus on the investigation of toxins

present in insects for use in medico-legal contexts. Goff and Lord provided an additional definition of entomotoxicology as the utilization of insect samples to perform toxicological assessments when the typical tissues and fluids necessary for death investigations are unavailable (Goff and Lord, 1994). Detecting the existence of specific xenobiotics like insecticides, heavy metals, drugs, or repellents in various surroundings such as a landscape, carrion, or river can be vital for analyzing.

a. Forensic Entomotoxicology

Forensic entomotoxicology is an area of forensic science that involves comprehending the impact of various foreign materials (living or non-living) on different insect species of medico-legal significance and its potential applications. Several authors have provided different definitions based on their approaches. Silva *et al.,* (2017) have provided a suitable and straightforward explanation of Forensic entomotoxicology, is the examination of insect specimens as secondary evidence if no primary evidence, such as blood, urine, soil or water, is unavailable, in order to analyse xenobiotic presence in river, or even land or in corpse.

b. Insects as toxicological indicators

In 1980, an article discussing phenobarbital drug in the area of entomotoxicology was published. Briefly, dead body of a young female was found in initial skeletonization phase, two weeks after she was last seen alive (Beyer *et al.,* 1980). The majority of insects that are significant in forensic investigations undergo three developmental stages (egg, larva, and pupa) prior to becoming adults. Flies that are valuable for estimating the PMI can consume the decaying body within four weeks. The species found include the Calliphoridae and the Sarcophagidae (the first insects to colonize a corpse after death) (Byrd and Castner, 2009).

Blowflies have the ability to lay eggs on the corpse within a few hours of death. The eggs are deposited directly onto the corpse and subsequently hatch into maggots that feed on the decaying tissue. The maggots pass through three larval instars and then migrate to a suitable site for pupation, which is usually in the soil. After pupation, adult blowflies emerge and leave behind empty pupal cases, which can sometimes be found

on the clothing of the deceased or hidden in the soil or under carpets (Gagliano and Aventaggiato, 2001). During the phase of growth and development of cuticle of larvae, drugs and toxics can accumulate as they are transformed into sclerotized puparium during pupariation. When the remains are retrieved at the skeletonized stage, the empty puparium case may be the only viable alternative for toxicological tests. Various organic or inorganic constituent can be analysed, which is directly or indirectly related to insects found (Gosselin *et al.,* 2011).

c. Sample collection, preservation, and extraction

Preserving the samples accurately and reliably is a crucial step in estimating the PMI. To achieve this, collection of samples from the crime scene and the surrounding area within 10 meters is to be done. Drug concentration in larvae may vary due to drug redistribution; therefore, samples should be collected from different areas of the body. According to studies by Midgley and Millet, among various preservation methods like -(10% formalin, 80% ethanol, and 95% ethanol), are the most effective. Larvae can be preserved by directly putting them into the preservative, or sacrificing them in hot water at 80°C for 30s and then storing them in at least 70% ethanol. Pupae can be stored in a temperature range of 2°C–6°C with punched holes in the lid, and adult flies can be killed in a vial and stored in denatured ethanol (Midgley and Villet, 2009; Nolte *et al.,* 1992). While performing analysis, specimens should be refrigerated at −4°C and analyzed. According to available studies, samples are stored in dry conditions to speed-up the process and reduce extraction (Pounder, 1991).

Once collected and preserved, the subsequent step is to extract and prepare the larvae samples, which is carried out using similar techniques as for human tissue samples. Various extraction methods, including liquid-liquid extraction and solid-phase extraction, are utilized to extract different poisons and drugs based on the chemical characteristics of the substances being detected. Solid-phase extraction is considered the most effective method for purifying organic toxicants from aqueous extracts of entomological specimens, as reported by several studies (Gosselin *et al.,* 2011; Karampela *et al.,* 2015; Adair, 2012).

d. Factors that influence entomological evidence

There are many different kinds of evidence at the crime scene, including insects, when a dead corpse has been discovered. The identification and analysis of insects that are significant to forensics can be impacted by outside variables including the temperature, amount of sunshine, and rainfall. The two most important meteorological variables that affect the analysis are temperature and humidity. As a result, processing and preserving entomological evidence is essential to figuring out the crime's motive, mode, and method. However, some limitations of entomological evidence, such as postmortem insect bites and mistaken blood splash marks, are often overlooked (Lord and Rodrigues, 1989; Erzinclioglu, 1987).

1.8. Role of Insects in body decomposition and PMI estimation

Flies and insects are the first ones that arrive on both an exposed body and buried remains, and colonize them and play a vital role in decomposition. All flies like blow flies, release specific kinds of odours in every decomposition stage will further attract a large number of flies and variety of species. Identification of the insects present and their taxonomy, the data generated will surely provide an extra edge in calculating PMI (Byrd and Castner, 2009; Rivers and Dahlem, 2013). After death, female insects will usually lay eggs within the first few hours of the decomposition process. However, the location, environment, climate, and temperature can all affect the type of insects found on an exposed body. For instance, a body found in water, a vehicle, buried, indoors or outdoors, exposed to the elements, or wrapped in blankets or carpets will have different faunal colonization. In forensic cases, the estimation of the PMI can be calculated by analyzing the stage of insect development on the body and correlating it with the temperature. Insect identification through their morphology, appearances, and microscopic details on the cadaver can provide information about the species and their habitat (Perez *et al.,*2016; Badenhorst and Villet, 2018).

1.9. Common flies which are generally found in India

a. Calliphora vicina

This species is one of the most important blowflies from forensic point of view. It is a large, slow flying, loud buzzing blue bottle fly found in urban/suburban areas and may enter our house. It is typically a winter fly 'available in Punjab during December to March and this could provide an important clue if a body is found infested with maggots of this fly in mid-summer in a place where it is unlikely to be present during that season. This fly prefers shady and cold habitat, and this species is sexually dimorphic and larvae of this fly develops in carrion. Internal fertilization is the method of sexual reproduction used by blue blowflies. They breed seasonally and lay eggs inside of carrion. Female fly tends to live longer than the male fly. It mainly consumes carrion, detritus, and garbage. They prefer to consume carrion and plants in cold and shaded locations. If this fly is found at the scene of crime, the forensic scientists can use the growth of larvae and the known temperature to determine the time of death (Castner *et al.,* 2001).

Figure 1.3 *Calliphora vicina*

b. Chrysomya megacephala

This fly is active throughout the year in the state of Punjab. With the help of this fly, physical factors such as temperature, wind, rainfall, or moisture played their part and greatly altered the gross appearance of these corpses. However, in every case when the calculated PMIs based on entomological data matched the circumstantial evidence, they dropped between 4 and 5.5 days. There was a progressive rise in the amount of time needed for the life cycle to be finished when the average daily temperature dropped from summer to winter (Linnaeus, 1758).

Figure 1.4 *Chrysomya megacephala*

c. Chrysomya rufifacies

Blow flies or bottle flies are the common names used for insects belonging to the Calliphoridae family. The species known as *Chrysomya rufifacies* is the first of its kind to be in the United States. These flies have a robust build and exhibit a greenish-blue hue as adults. They are among the earliest species to arrive at decomposing remains, and continue to lay eggs for approximately 4 to 5 days during the decomposition process. *Chrysomya rufifacies* can be a valuable forensic indicator due to the shorter development time of its maggot and larvae compared to other species, and their predatory nature can alter the estimated PMI based on the prey species (Anderson and Kaufman, 2011).

Figure 1.5 *Chrysomya rufifacies*
https://tse2.mm.bing.net/th?id=OIP.iF9lzPKSmgZRgnGI0u7TwgHaFSandpid=ApiandP=0

d. Calliphora Vomitoria

The blue bottle fly, which is significantly larger than the housefly, can be easily distinguished by its shiny blue body. This type of fly undergoes complete metamorphosis, including egg, larva, pupa, and adult stages, with a development period of approximately 2 weeks, the longest stage being the pupal stage. The same area where they feed—decomposed meat, trash, or excrement—is also where the female blue bottle fly lays their eggs. Since their development rate rises with temperature, temperate zones are home to the majority of these flies. They are also the first species to deposit eggs on dead bodies. There are two methods for determining the PMI: killing the insects or calculating the accumulated degree hours/days (Smith, 1986).

Figure 1.6 *Calliphora vomitoria*

https://www.naturamediterraneo.com/Public/data7/ff57/6539.JPG_201018151353_6539.JPG

e. Lucilia sericata

Lucilia sericata, also known as sheep blowflies or green bottle flies, can be found all over the northern hemisphere and are commonly found in areas such as carcasses, feces, and garbage in both temperate and tropical biomes. The female fly typically lays 200 eggs in a cluster on a host, which hatch 18-21 hours later, and the larvae then mature over a period of 3-4 days, undergoing three instars. The amount of time spent in the larval stage can be impacted by factors such as food source and environmental humidity. These flies play an important role in the ecosystem as they consume carrion and contribute to biodegradation. They also have significant applications in various fields of science such as forensic, medical, and veterinary science. For instance, in

medical science, green bottle fly larvae are used to treat infections that are resistant to other forms of treatment. In forensic science, analyzing the development of green bottle fly larvae can be useful in determining the time gap of death of discovered bodies (Varatharajan and Sen, 2000).

Figure 1.7 *Lucilia sericata*

https://www.diptera.info/forum/attachments/210509alf537luciliaw.jpg

Other forensic important flies: (Hinton, 1981; Byrd and Castner, 2009)

f. Lucilia illustris

This is a common Sarco saprophagous fly with a metallic green color that is usually found in urban or suburban areas and is of medium size. Lord *et al.,* (1986) utilized the maggots, which were collected from the body and then raised to adulthood. The developmental time of the species was calculated based on the temperature in the area, and this data was used to accurately determine the PMI.

g. Blowflies (Family Calliphoridae)

The insect family known for their metallic blue and green appearance are the first to interact with a corpse, often within minutes due to their ability to detect the odour of a dead body. They will quickly search for a suitable location to lay their eggs, often choosing exposed body openings. This makes them useful in estimating the PMI.

h. Flesh Flies (Family Sarcophagidae)

The Sarcophagidae family, also known as flesh flies, consists of at least 2,000 species ranging from 2 to 14mm in length. They are typically found on decomposing carrion, but will not lay eggs on corpses. When estimating the PMI, the time it takes for their eggs to develop is subtracted. Examples of flesh flies include Sarcophaga bullata and Sarcophaga haemorrhoidalis, also known as the red-tailed flesh fly. During the summer, female Sarcophaga haemorrhoidalis will deposit larvae on human corpses.

i. Carrion Beetles (Family Siliphidae)

With a flat body and antennae, carrion beetles' range in size from 10 to 35mm. Their larvae also come in various sizes and shapes. These beetles are drawn to decomposing matter and adult siliphids have been observed consuming maggots.

1.10. Insect succession on Cadavers

Various stages of decomposition on cadavers or corpse that have been observed and also noted as per their succession pattern in estimating PMI. But the most important factors that influence the succession pattern are seasons, geographical location, body size and shape. The other factors are habitat, vegetation, soil type and climatological conditions of an area or region. These above factors have major influence on the composition of insect species and their seasonal availability as well as their diversity (Villet, 2011). Some measures can be taken while data collection for a specific region with care and when evaluating the time since death in alternative region which further helps in establishing better interpretation. observed in experimental investigations that the primary factor for the change from the fresh stage to the bloated stage is microbial activity, which produces gasses by eating into the tissues and, ultimately, the interior tissue colonization by maggots. The amount of species present affects the relative measures of the swollen and decay phases (Greenberg, 1991; Mabika, 2014).

1.10.1. Life Cycles of Insects Associated with Cadavers

Whether an organism is oviparous, viviparous, or develops in another way, the life cycle illustrates how an organism develops and offers a comprehensive picture of an individual's existence. Similarly, when we discuss insects in entomology, we are talking about their four stages of metamorphosis in life cycles, each of which is distinct from the others. These insects have life cycles in which the adult females lay eggs, which then grow into the larval stage, which is followed by the pupae stage and, finally, the adult stage (Gennard, 2007).

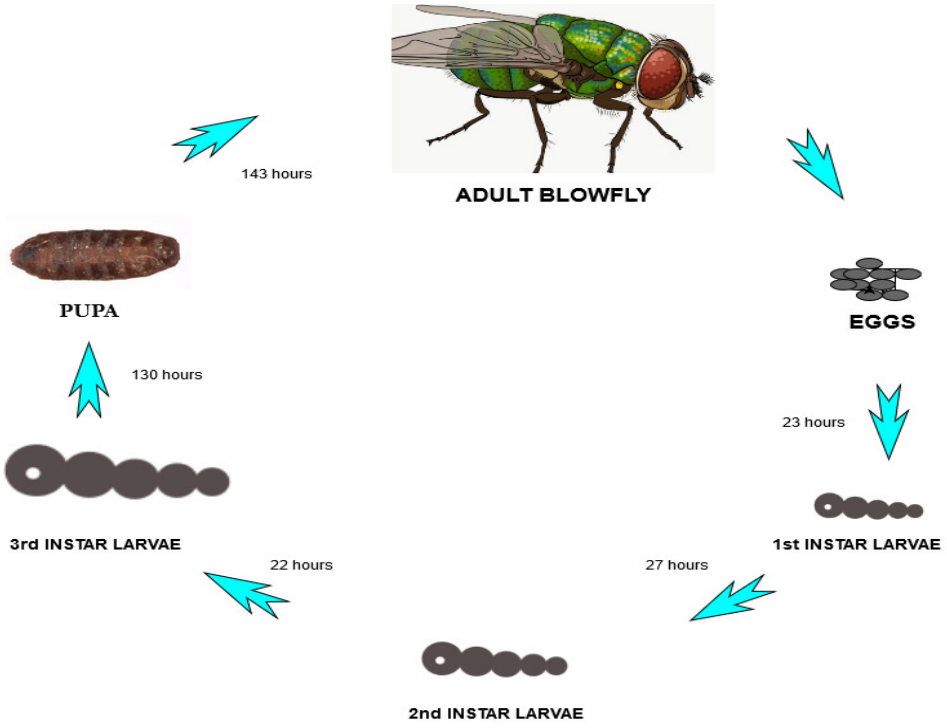


Figure 1.8 Life cycle of Blow fly

One term for this sort of hatching is eclosion, which is the process by which first instar larvae emerge from their eggs. These larvae travel through three different phases, or instars, and each one is identified by the number of slits it has. The first instar has one slit, the second has two, and the third has three. Insects and flies usually have erroneous measures and sizes since their numbers and kinds rely on the amount, sort, and quality of food that is accessible to them. In the latter phase of development, late third instar larvae usually cease eating and migrate as flies, looking for a dry place to pupate and become the adult stage. The "larval post-feeding stage" is the term for this period.

Beetles experience complete metamorphosis in their development, passing through from 3rd to 5th larval stages which varies from species to species, and a pupal stage before reaching adulthood from the egg stage. The shape of beetle eggs during the pupation stage varies from oval to round (Mabika, 2014; Beyer *et al.,* 1980).

1.11. Detection of drugs and toxins

Numerous publications have attempted to explore the ability to identify, quantify, and correlate drugs and toxic substances across flies and insects. These publications have used selection of mammals -rabbits, pigs, chicken, goat and rats and spiked meat substrates with drugs and other toxic substances to alleviate the actual development of decomposition and taken out the samples of toxicants from larvae that feed on dead carcasses. The effectiveness of the extraction technique used is crucial to successfully detect and recover drugs from insect specimens (Goff *et al.,* 1989; Kintz *et al.,* 1990; Moody, 2006).

Qualitative analysis and Quantitative analysis

Various techniques, including Immunoassay, High Performance Liquid Chromatography, Liquid Chromatography-Mass Spectroscopy, Gas Chromatography, Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy, and Gas Chromatography-Mass Spectroscopy were used to conduct the analyses. Although results obtained from the immunoassay technique are not confirmatory, it is a valuable tool for screening a larger number of drug classes (Hedouin *et al.,* 1999). This technique offers a prompt analysis of samples and determines whether further confirmatory techniques should be used. The immunoassay technique has primarily been utilized in forensic entomotoxicology to identify morphine drugs from different substrates and specimens (Liu *et al.,* 2009). According to conventional wisdom, the concentration of a substance tends to decrease compared to its original source. When larvae and insects feed on a substrate containing drugs, the concentration of the drug in their bodies decreases. However, if the larvae feed on a substrate treated with a higher concentration of the drug, they will likely have a higher concentration of the drug in their tissues.

As an illustration, when larvae were studied on two food substrates containing morphine at concentrations of 17.5 nmolg−1 and 7 nmolg−1, larvae that consumed the former diet had a higher concentration of morphine. Furthermore, as the larva matures into an adult, it effectively eliminates drugs and other toxic substances from its body, resulting in lower concentrations (Parry *et al.,* 2011; Pien *et al.,* 2004). To quantify low-level drugs, LC-MS and GC-MS techniques are recommended. Pien *et al.,* comprehend that Nordiazepam and its metabolite Oxazepam could be detected and measured from a single larvae and pupa at levels as low as pg. In general, drug concentration is higher in the liver than in other organs such as the skin, blood, urine, and brain, as xenobiotic metabolism primarily takes place in the liver. Therefore, the results should be interpreted with caution (Charabidze *et al.,* 2017).

1.12. Effects of drugs in tissue on development of insects

Forensic entomology can be used to determine various factors related to a carcass, such as the time since death, climatic conditions, the surroundings, and the any finding of toxicants in the body (Bourel *et al.,* 2001). However, the presence of drugs in the carcass can affect the growth and morphology of insects on the body, potentially leading to errors in the estimation of the PMI. The development of reared insects on the substrate is correlated with the concentration of drugs present. However, the pharmacokinetics of drugs in insects are influenced by factors such as species, developmental stage, the mode of drug taken and its consequence on body after intake (Carvalho *et al.,* 2001; Goff *et al.,* 1991).

Several studies have demonstrated that the use of drugs and toxins before death can affect the rate of maggot development, leading to inaccurate estimates of the PMI based on insect development. For instance, in the case of heroin-containing tissues, errors of up to 29 hours can occur in PMI estimations based on the development of Boettcherisca peregrina flies, and similar results have been reported for methamphetamine and amitriptyline (Goff *et al.,* 1989). In contrast, cocaine and methamphetamine can accelerate the rate of fly development, and a lethal dose of methamphetamine can increase larval development for the first two days, followed by a drop in the rate if exposure remains at the median lethal dosage. The presence of barbiturates can prolong

the larval stage, resulting in a longer time to reach the pupation stage. The presence of paracetamol in the rearing foodstuff can slightly impact blowfly larval development during days 2-4 of development. Furthermore, the first oviposition might be delayed by 01 to 03 days and the pupation time can be extended by 2 to 3 days if malathion is present in the corpses. Toxins in the body at the moment of death can therefore make estimating the PMI more difficult. In one particular instance, a man's systemic concentration of organophosphate malathion caused a few-day delay in oviposition (Sadler *et al.,* 1997; Obrien and Turner, 2004; Gunatilake and Goff, 1989; Rivers and Dahlem., 2014).

1.13. Determination of PMI in Entomotoxicology

In legal investigations, insects are utilized to determine time elapsed since death. This is accomplished after collection or taken sample of immature larvae, pupae, and insects from the corpse, which can be identified based on their size and stage of development. Insect succession on the cadaver also plays a role in determining PMI and is influenced by several factors, such as climatic conditions, season, geographical area, sun radiation, synanthropic, type of sample (Oliveira *et al.,* 2014; Amendt *et al.,* 2011).

a. Forensic Implications

It relates mainly to arrive at an opinion regarding the cause and time since death, which includes insects and other arthropods, and its involvements helps in

a) Homicide, suicide, and assault investigation
b) Destructions such as bodily abuse and contraband trading
c) Find out how long it has been since the death or how long humanoid remnants have gone undiscovered
d) Whether the body was disturbed at any point (either by animals or by the murderer going back to the scene of the misbehavior) or if the cadaver had been moved after death
e) Analyze the manner and cause of death (the circumstances around a crime)
f) Examining the connection of suspects with the scene of death

g) Detection of poisons or drugs through examining the insect larvae

h) Species determination in terms of molecular analysis

i) Identification and characterization of human DNA in fly larvae

j) Gene Expression studies

k) If supported by rigorous statistical analysis, investigations on the growth of insects and their succession can produce a reliable estimate of the least amount of time from death in court (Gosselin *et al.,* 2011)

b. Limitation and Inconsistency

a) Our understanding of the pharmacodynamics and pharmacokinetic of drugs and toxicants is still incomplete

b) Various factors, including drug stability, temperature, and humidity, can influence the results of the analysis

c) The presence of drugs within a corpse can affect the use of insects as evidence, as chemicals can slow down or accelerate insect growth

d) The main concern is that interpretation of analyzed drug concentration

e) The developmental time of necrophagous insects can be altered by xenobiotics

f) The reaction time of insects, whether of the same or different types, may vary

g) The absence of methods, standards, and validation is an issue

h) There is no correlation between the toxic substance levels in larvae and the amount detected in the cadaver

i) Our knowledge of the metabolism of toxic substance in insect bodies is inadequate

j) Using research lab animals such as rabbits and rats may lead to incorrect assumptions due to the production of different metabolites in humans

k) Forensic entomology cannot provide immediate results since rearing insects takes time (Joseph *et al.,* 2011; Subedi, 2016; Campobasso and Introna, 2001; VanLaerhoven, 2010)

1.14. Forensic entomology and admissibility in Court

The process of forensic investigation requires the expertise of many individual fields-like fundamental science to medical personnel. They are all aware of and cognizant of the evidence gathered at the postmortem and at the crime scene. The integration of forensic evidence in a medico-criminal inquiry necessitates the presentation of the physical characteristics, distinctive morphology, and composition of arthropod species discovered on a deceased person. Standard operating procedures and standards have been proposed to offer quality assurance, accreditation, and quality testing of forensic entomology in court in order to guarantee the validity, repeatability, and reliability of entomological evidence from a crime scene (Lord and Burger, 1983).

Several researchers have recommended rules for collecting, preserving, and presenting entomotoxicological evidence in court. Lord and Burger provided a guide for collecting entomological evidence from corpses in 1983 (Lord and Burger, 1983), which was later elaborated by Smith who formulated additional guidelines for sample handling (Smith, 1986). Catts and Haskell developed an inclusive specification for crime-scene entomotoxicologists and post-mortem pathologists in 1991 titled "Entomology and Death: a procedural guide." However, because to varying environmental conditions, the criteria for jurisdiction systems may alter across various geographic locations.

Introduction to the Drug used for Study: Asenapine Maleate

Drug Name: Asenapine Maleate

IUPAC name : (3aR,12bR)-rel-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenz (2,3:6,7) oxepino[4,5-c] pyrrole (2Z)-2-butenedioate (1:1)

Molecular Formula: C17H16ClNO·C4H4O4 (and enantiomer)

Molecular weight: 401.84(285.8 as free base.)

Category: Antipsychotic Agents

1.15. Chemical structure of drug

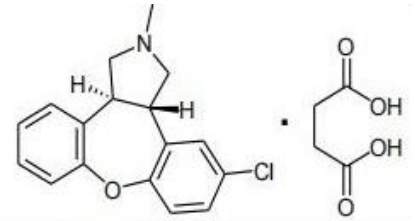


Fig 1.9. Chemical structure of Asenapine maleate

Description: SAPHRIS is a novel antipsychotic, belonging to the dibenzo-oxepino pyrroles. It has antagonist activity on the dopamine 2 (D2) and serotonin (5-HT)-2A receptors.

Solubility: The solubility of asenapine (active entity) in water is 3.7 mg/mL, in0.1M HCl is 13 mg/mL and in aqueous buffers of pH 4.0 and 7.0 the solubilityis 3.8 mg/mL and 3.0 mg/mL, respectively.

pKa: is 8.6 (determined in water/methanol). Asenapine has a log P (noctanol/water) of 4.9 for the neutral species and 1.4 for the cationic species.

Melting point: The melting point of asenapine is 139.9 ºC, with a melting range of 141-145 ºC.

Indication: Asenapine is indicated for the treatment of psychosis, schizophrenia, and schizoaffective disorders, manic disorders, and bipolar disorders, either as a monotherapy or in combination with other medications.

Pharmacodynamics: Asenapine is an antagonist for serotonin, dopamine, noradrenaline, and histamine receptors, with stronger activity on serotonin receptors than on dopamine receptors. Its antagonistic activity on histamine receptors is associated with sedation in patients. Asenapine has a lower incidence of extrapyramidal effects, which can be attributed to its ability to upregulate D1 receptors in a dose-dependent manner. Asenapine achieves this upregulation by influencing glutamate transmission in the brain. Asenapine does not have any significant activity on muscarinic or cholinergic receptors, so it is unlikely that patients will experience dry mouth or constipation as symptoms of anticholinergic drug activity. Compared to first-

generation and second-generation antipsychotics, Asenapine has a higher affinity for all of the aforementioned receptors, except for 5-HT1A and 5-HT1B receptors.

Mechanism of action: It has been shown that the atypical antipsychotic drug asenapine increases dopamine (DA) and acetylcholine (Ach) efflux in rat brains. Asenapine is a powerful 5HT2A (serotonin) and D2 (dopamine) receptor antagonist. Asenapine may improve cognitive function and adverse symptoms in schizophrenia patients.

Absorption: one dosage of 5 mg = 4 ng/ml (within an hour); Bioavailability when administered sublingually is 35%; when administered orally (swallowed), it is 2%; 5 mg over 3 days to reach steady state; Within 0.5 to 1.5 hours, the plasma concentration reaches its maximum. Asenapine's maximum concentration and exposure rise 1.7-fold with a doubled dosage. Water consumption between 2 to 5 minutes after asenapine injection reduces exposure.

Volume of distribution: 20-25 L/kg.

Protein binding: 95% protein bound.

Metabolism: Asenapine is oxidized via CYP1A2 and undergoes directglucuronidation via UGT1A4. Oxidation via CYP1A2 is asenapine's primary mode of metabolism.

Route of elimination: Urine (50%) and feces (50%).

Half-life: 24 hours (range of 13.4 - 39.2 hours).

Uses: Certain mental or mood problems (such as schizophrenia and bipolar disorder) are treated with this medicine. We can think more clearly and feel less anxious thanks to this medication. Additionally, it could aid in reducing hallucinations and averting extreme mood changes. Atypical antipsychotics are a class of medications that includes asenapine as a member. It functions by assisting in the reestablishment of the proper ratio of certain natural brain chemicals, or neurotransmitters.

Side effects: Possible side effects include sleepiness, lightheadedness, and weight gain. Mouth tingling or numbness is possible but normally goes away after an hour. Rarely, there may be sores, blisters, or soreness under the tongue. The usage of this medication

may result in uncontrolled movements, twitching of the face or muscles, and spike in blood sugar.

Overview of Development

A second-generation (atypical) antipsychotic drug called asenapine is used for individuals with bipolar disorder and schizophrenia for both acute and maintenance therapy. Sublingual administration is used to administer it. Organon International conducted phase 3 studies to produce asenapine. The US Food and Medicine Administration (FDA) approved Schering-Plough'snewdrug application for commercialization in August 2009, following the company's merger with Organon in November 2007. Merck and Co, Inc. markets asenapine under the trade name Saphris in the US (Tarazi and Shahid, 2009).

Chapter 2

Literature Review

Kaneshrajah *et al.*, (2004) investigated and reported on Calliphora vicina larvae (laboratory growth model), which develop on different pig body tissues at different speeds. The Calliphora vicina larvae were reared at 20 °C on either pig liver, brain, heart muscle, lung, or kidney tissue (approximately 30 g pieces, on which larvae were kept in groups of ten). After the sample larvae were collected, cleaned, and prepared, the statistical analysis was carried out using Stat View v5 for SAS. According to this study, the pace at which larvae grow on lung, kidney, heart, or brain tissue is up to two days faster than it does on pig liver.

Kimberly *et al.*, (2005) through an experimental setting, detected the alcohol content of the maggots. In this study, 4 pigs were used as samples, and 2 of the pigs received alcohol intravenously and orally while the other two were controls. Blood was drawn from both the pre- and post-mortem periods, and 1 kg of meat was taken from both the alcoholic and non-alcoholic samples. Meat was given the best circumstances for breakdown, and analysis was carried out at various time intervals. Alcoholic samples contained a concentration of alcohol in both the antemortem and postmortem blood as well as in the maggots, but non-alcoholic samples only included a concentration of alcohol in the postmortem blood.

Joshua *et al.*, (2006) determined the morphine content in flies. In this study, blow flies were raised. After some time, the blow flies lay eggs, which are then placed to several substrates that have variable levels of morphine intoxication. The substrates were 100g of beef mince. Six concentrations were available as 0, 500, 1000, 2500, 5000, and 10000ng/g. After collecting (around 50), the larvae were then cleaned with distilled water. The same procedure was repeated three times after drying with a paper towel. 0.5g of homogenate was added to 5ml of 1% acetic acid, and the mixture was centrifuged for 15 minutes at 3500 rpm. Analysis of flow injection was then performed. In the presence of acidic potassium permanganate, chemiluminescence was conducted. HPLC proved successful in detecting morphine. For morphine, all

substrates proved positive.

Gojanovic *et al.*, (2007) detected different drugs using GC-MS. In this study, he used decomposed bodies as his study model. Maggots were collected from the body's interior and skin. Additionally, blood, liver, and kidney were taken and kept at 4°C. Solid phase extraction was used to remove the materials from the maggots. Using a flame ionization detector and head space, GC-MS was used for the analysis. A test for amphetamine yielded positive results in the form of maggots, but liver, kidney, and blood quantification were also carried out.

Rashid *et al.*, (2008) observed the amounts of malathion and its impact on the growth of Chrysomya megacephala. They utilized three rabbits for their experiment, injecting varying concentrations of the drug, exposing the rabbits' livers for fly rearing, and observing the fly larvae's development over time along with the presence of the drug in various concentrations. The entire length of the larvae was measured in order to assess the development of the exposed larvae. The larvae's growth rate was reduced when malathion was used, and the rate at which the larvae developed varied markedly depending on the drug's dosage. The control sample larvae's growth rate did not differ significantly.

Kelly *et al.*, (2009) noted the impact of induced morphine drug Calliphora stygia growth rate. The sample utilized in this study was pet mince, which includes lamb fry, kangaroo mince, and heart. The larvae in the four treatment groups were fed different amounts of morphine. After morphine was initially examined using high performance liquid chromatography on four different samples, it was found that the substance was present in the two chosen samples but not from the control samples. Furthermore, no statistically significant variations were seen in the length or width of pupae among treatments. Additionally, there were no appreciable variations in the larvae measurements among the four distinct groups.

Shi *et al.*, (2009) From 2006 to 2008, researchers in Guangzhou, China, examined the faunal succession of insect on rabbit corpses. During these four seasons, 49 species derived from 15 families were gathered on four phases of decomposition. In these

stages, the insects were most abundant during the bloated and subsequently the decay stages. Among other significant species, Chrysomya megacephala was the most common and abundant in all four seasons. Its various phases of growth on carrion served as the most significant markers for PMI assessment in Guangzhou. Differences in insect activity throughout the year and by season were compared. This kind of research also reveals that arthropod succession and insect growth rates are influenced by the climate.

Saymsa *et al.*, (2010) The Department of Parasitology and Medical Entomology, Universiti Kebangsaan Malaysia, performed research in which a total sample size of 10 bodies were examined. These instances were all tracked down using forensic entomological samples. Numerous death scenarios were acquired, including both interior and outdoor settings including shrubs, fields, landfills, and water bodies. Identified fly species gathered from the death sites were blow flies and unknown larvae, however in Malaysia, *Chrysomya megacephala* and *Chrysomya rufifacies* of family Calliphoridae have been the predominant species of fly identified on corpses, while the places found were fairly varied, including rural, residential, and aquatic settings. The assessment of the PMI, based on the entomological samples obtained, varied from one to five days. According to the author's analysis, more than half of the specimens collected, came from indoor corpses. Even though, insects may need several hours to find and reach a body if it is in a confined room. The assessment of the PMI, based on the entomological samples obtained, varied from one to five days.

Tüzün *et al.*, (2010) conducted a preliminary investigation and identification of insect species of forensic value in Urmia, Iran. In this study, various bodily tissues and viscera from certain vertebrate animals, including sheep, cows, fish, and hens, were sampled. Then, for 53 days, their organs—including the liver, intestines, skin, spleen, and head were left out in the open. Throughout the research study period, the lowest and highest temperatures, humidity, and rainfall were recorded. After 3,197 specimens were collected for examination, it was discovered that there were 11 families, 16 genera, 18 species, and five insect orders (Diptera, Coleoptera, Hymenoptera, Dermaptera, and Blattaria). The author of this study concentrated on two topics: insect species and animal tissues.

Thevan *et al.*, (2010) reported that the flies named- Phoridae were identified in this case studies from June (2007-2009) in Penang, Malaysia. All samples were taken during the autopsy at the Penang Hospital's Forensic Medicine Division. The first example was a 78-year-old corpse that was discovered in the mummified stage of decay while still wearing all of his clothes. The insects that were there were gathered and tested. Four puparia and two mature female *Megaselia scalaris* were identified. In the second example, a completely dressed corpse that was experiencing active decomposition was found to have pupae of the Phoridae family below the garment, on the surface of the abdomen, and on the right foot. It is clear from the aforementioned case studies that more Megaselia species than only *Megaselia scalaris* are found in warm areas than has been previously documented. The time frame that includes PMI is defined by the victim's last known location and the victim's earliest oviposition dates (EODs).

Almeida *et al.*, (2011) mentioned how understanding an insect's anatomical structure is essential for understanding its growth and function. Traditionally, methods like dissection or obtaining histological slices have been used to understand anatomy and morphology. The device that is frequently used to visualize 2D specimens includes a stereo or compound microscope as well as an SEM (Scanning Electron Microscope). De Ameida and associates studied the microanatomy of Rhodhinus prolixus (Hemiptera, Reduviidae) using x-ray computed tomography. The discussion leads to the conclusion that the useful energy ranges are 8 to 35 keV.

Taylor and Chandrasena, (2013) reported a case of an intended asenapine overdose involving a 49-year-old male who had arrived to the emergency room after consuming 74 asenapine pills outside. He just had electroconvulsive treatment, during which a borderline personality disorder and bipolar disorder were identified. Examination results for the heart, lungs, and abdomen were unremarkable. Amphetamines, marijuana, opiates, and benzodiazepines were detected in a urine drug test as being present. No consequences were seen and no first aid was given. All of the test readings for electrolytes and troponin I were recorded within the range values. Asenapine overdoses have been reported in six prior occurrences at dosages up to 400 mg, although the precise dosage and mode of administration were unknown.

Miller *et al.*, (2013) presented four case studies, where they created a method for validation study of GC-MS, and distributed postmortem samples of Asenapine (Saphris), a medicine that was authorized for use in the USA in 2009 to treat schizophrenia and bipolar disorder. The toxicological department of Los Angeles Laboratory devised and organized this study and created an practical and chemical technique for the detection and quantification of asenapine by GC-MS in several postmortem specimens. By using a straightforward liquid-liquid extraction technique using an internal standard- of D5-fentanyl and asenapine was extracted from specimens and quantitated. SWGTOX, developed a practical method for drug asenapine and determined the four case studies' tissue distribution. The authors believe that by sharing their experiences, they will assist other forensic toxicologists in determining the cause and manner of death in their cases. They believe that these are the first occurrences that have been reported in the literature.

Zou *et al.*, (2013) examined how the unusual medication ketamine affected *Lucilia sericata*'s development phase. For the aim of the investigation, ketamine hydrochloride injections at varying doses were administered to ten New Zealand rabbits. Tissues were left out in the open to continue decomposing after they had died. The bug Lucilia sericata arrived to feed on the dead tissue, and several stages of larval development were watched. Ketamine was found to decrease larval development in its early stages. While the surviving larvae developed into the second instar stage, the larvae that were taken out of the tissue and administered a fatal dose of ketamine after 12 hours remained in the first instar stage. Chi-square tests between various samples of specimens at different concentration and controls samples revealed significant differences. The respective measurements of larvae body were also compared between standard and analyzed samples, and significant differences were discovered between them.

Oliveira *et al.*, (2014) NIRS, a cutting-edge non-destructive technique, was used to determine its effectiveness in detecting flunitrazepam in Chrysomya megacephala (Fabricius) (Diptera: Calliphoridae) larvae, puparia, and adults. As an alternative toxicological sample, necrophagous insects in the larvae were utilized as they can also aid in the qualitative identification of misused medications found in the body. The

author then coupled a portable NIR spectrometer with variable selection approaches like successive projection algorithm (SPA-LDA) and genetic algorithm-linear discriminant analysis (GA-LDA) to identify this medicine in the insects based on the distinctive spectral fingerprints of their biochemical makeup. Flunitrazepam was produced and tested in larval, puparium, and adult calibrators at doses ranging from 0, 4, 8, 16, and 32 pg g-1. With just 9 wave numbers, the resultant GA-LDA model correctly identified adult females according to their concentration. This alternate method for studying entomotoxicology produced findings that point to the potential utility of NIR spectra in identifying illicit drugs in insects.

Bugelli *et al.*, (2014) revealed eight cases in central Italy between June and November where remains separated from people were recorded. Insects were collected during the autopsy and body recovery. The potential of this species to colonize indoors was confirmed by the presence of Sarcophagidae and Calliphoridae species in 75% of the cases, Lucilia sericata and Chrysomya albiceps in 50% of the cases, and scuttle flies (Phoridae) in 37.5% of the cases.

Patel *et al.*, (2015) reported solubility test, external diffusion experiments, stability-indicating HPTLC, a quick and sensitive technique for quantitative assessment of asenapine maleate, a typical antipsychotic medication (ASPM) in pharmaceutical tablets. Silica Gel plates were used as the stationary phase in the development of this HPTLC technique, while ethyl acetate and methanol were used as the mobile phase in a 1:1 (v/v) ratio. At 235 nm, the ASPM Rf value was 0.43 0.02. The linearity range was 300–1800 ng/band, and new HPTLC technique was verified, and statistical analysis of the data showed that it was linear, accurate, exact, repeatable, and selective for the study of Asenapine maleate.

Bala *et al.*, (2016) submitted a case report in which the Accumulate Degree Hours (ADH) approach was used to determine the PMI. In 2014, a mummified female body was found in a rice field in the Punjabi hamlet of Kakrala, Police Station Ghagga, District Patiala. From the body's clothing and skull, blow flies’s eggs and pupa, were recovered. In a glass container, the pupae, adult flies, and beetles were put before being preserved in 80% ethanol. Taxonomic keys were used in the Department of Zoology

and Environmental Sciences at Punjabi University, Patiala, to identify insects. The results of the current investigation revealed that the PMI of the female corpse was judged to be 9.6 days, but the autopsy surgeon expected a PMI of 10 to 12 days. The results of the ADH calculation were more accurate than the forensic examiner's statement and agreed with the outcome of the police inquiry.

Zanetti *et al.*, (2016) conducted an experimental investigation using entomological samples of Dermestes fed on pig muscle with the primary goal of detecting and quantifying fluoxetine, an antidepressant medicine. The dosage chosen, 2000mg/kg, simulates a fatal fluoxetine overdose in both people and lab animals. Then, 50 adults, 30 larvae in their fourth and fifth stages, and a number of exuviae were collected and their fluoxetine content was examined. By using UV spectrophotometry at wavelengths of 270 and 277nm, fluoxetine was detected. Fluoxetine was detected in D. maculatus and exuviae at all developmental stages. They measured the drug as well. In the general model, there were no appreciable variations between the days or the stages, but it was noted that the concentration changed over time at a wavelength of 277 nm. Fluoxetine concentrations at 277 nm were nearly equivalent to or higher than those at 270 nm.

Gião *et al.*, (2017) conducted a study evaluating the effects of amphetamine and phenobarbital on the population dynamics of Chrysomya albiceps (Wiedemann) using a combination of laboratory tests and mathematical modeling. They have studied the factors that influence the ecology of blow flies in this, especially the dynamic population equilibrium, which is important for forensic entomology. Occasionally, experts' opinions in medico-legal reports may be influenced by their lack of knowledge of how drugs affect these flies' population dynamics.

Ramadan *et al.*, (2017) proposed the TLC approach for ASPM measurement, employing a produced silica gel plate as the stationary phase and a combination of toluene, methanol, chloroform, and 33% ammonia as the mobile phase. The plates underwent development up to 180 mm in length and were scanned at a wavelength of 220 nm. For ASPM, the TLC method's limits of detection were 1.284, and its limits of quantification were 3.891. This technique, combined with densitometric analysis,

provides the advantages of rapid processing, huge sample throughput, and low solvent use. This technique may be used to analyze ASPM on a regular basis and for quality control without being hampered by additives or degradation products that are frequently seen in dosage forms.

Another similar HPTLC method was reported by **Avasarala *et al.*, (2018)** for estimating ASPM and compounds that are connected to it. Asenapine maleate, a common antipsychotic medication, was quantified using a quick and accurate high performance thin layer chromatography (HPTLC) approach. The procedure calls for dilution of the medication with methanol and immediate HPTLC analysis. Ethyl acetate and methanol were used in a 1:1 (v/v) ratio as the mobile phase for the separations on a Silica gel 60F254 TLC plate. After derivatization under UV light with a 254 nm wavelength, the formed plate was scanned using the CAMAG-TLC SCANNER-3 apparatus. Over the concentration range of 3 to 7 g/5 L, the standard curve was linear (r = 0.999). 1.85% was the intra-assay precision percentage relative standard deviation. 96.7 to 99.31% accuracy was the range. The upper and lower bounds of quantification and detection were 0.052 and 0.15, respectively. The estimation of asenapine maleate and its associated components may be successfully accomplished using the suggested analytical approach.

Lima *et al.*, (2018) developed two approaches based on fluorescent excitation-emission matrix (EEM) spectroscopy and differential pulse voltammetry (DPV) to distinguish dead colarvae. The findings of their study proved that the methods DPV and EEM in combination with chemometrics may be used to classify flunitrazepam in fly larvae and provide novel applications. This provided a quick, cheap, and accurate approach for classifying specimens where the resultant GA-QDA model effectively recognized flunitrazepam with 100% accuracy and specificity. This method requires minimal sample preparation. It was fully validated and demonstrated that it could be used as an approved technique for entomological procedures.

Açıkgöz *et al.*, (2018) showed spiking research that was created with the goal of analyzing and determining the effects of over-the-counter drugs on the growth of *Chrysomya albiceps* larvae. To do this, a farmed pig was gavaged with amitriptyline

(5100 mg), citalopram (476 mg), and diazepam (510) before receiving a toxic dose of morphine (54.4 mg). Using gas chromatography/mass spectrometry (GC/MS), it was feasible to determine any residual drug content in the 500 maggots that were collected. The early investigation revealed that the dominant species discovered is Chrysomya albiceps. Amitriptyline, citalopram, and morphine were all measured in developing larvae at concentrations of 91.45 mu g/kg, 52.03 mu g/kg, and 5.37 mu g/kg, respectively. Furthermore, our findings demonstrated that Chrysomya albiceps achieved the third larval stage in just 72 hours at an average temperature of 35 °C. The discovery also suggested that poisonous chemical had an impact on the biological cycles of insects, a crucial for specific quantitative investigations and possible post-mortem impacts.

Magni *et al.*, (2018) published a study that detailed for the first time the creation and approval of an analytical technique suitable for finding ketamine in Calliphora vomitoria L. and adults. Ketamine has been linked in recent years to unexplained animal deaths as well as to recreational drug usage, sedative use in sexual assault, and infrequent use as a sedative. The larvae were raised on liver substrates that had been laced with 300 ng/mg of ketamine at concentrations similar to those discovered in recreationally using individuals. HPLC-MS/MS was used to complete the study. published a study that detailed for the first time the creation and approval of an analytical technique suitable for finding ketamine in the selected insects, and adults. Ketamine has been linked in recent years to unexplained animal deaths as well as to recreational drug usage, sedative use in sexual assault, and infrequent use as a sedative. The larvae were raised on liver substrates that had been laced with 300 ng/mg of ketamine at concentrations similar to those discovered in recreationally using individuals.

Patel *et al.*, (2018) developed the LC-MS/MS technique in order to estimate asenapine maleate in human plasma. In the estimate of ASPM, it was discovered that this approach was very sensitive and selective. The technique was effectively applied for the first time to a bioequivalence study of asenapine in healthy Indian participants.

The same method was used by **Boer *et al.*, (2012)** to measure ASPM and three of its

metabolites in human plasma, including N-desmethylasenapine (DMA), asenapine-N-glucuronide (ASG), and 11-osulfate-asenapine (OSA), researchers used LC-MS/MS with automated solid-phase extraction.

Protti *et al.*, (2018) published a study that examined four different micro-matrixes for enantio-separation and ASPM measurement using HPLC and DAD diode array detection in hematic samples. They examined four micro-matrices, two of which were made from dried blood and plasma spots and the other two from volumetric absorptive micro sampling of blood and plasma.

Cruise *et al.*, (2018) used four species of scarab beetles, six species of blow flies, flesh flies, house flies, and ten taxa of beetles were included in this study. In contrast to beetle populations that remained low until day 4, fly activity predominated vigorously throughout the early phases of decomposition, and within a week, half of the pig carcasses were skeletonized. This study also recorded the taxa and their ecological differences, which vary and are mostly influenced by seasonal and regional factors.

The study by **Singh *et al.*, (2018)** highlights the unique characteristics of the regional insect fauna found on human remains in Punjab, Northern India, and how it interacts with human remains. The insects were gathered during the autopsy of five corpses. The insects were taken during the autopsy process from human bodies. The five human case studies consisted of four male and one female subjects, with ages ranging from 26 to 52. Common insects were seen or noted in all of the corpses, including Chrysomya megacephala eggs, adults, and maggots, and Chrysomya rufifacies larvae. Necrobia rufipes, Dermestes maculatus, Chrysomya rufifacies, Phormia regina, and Chrysomya albiceps maggots. Last but not least, the main goals of this study were to collect necrophagous insects inhabiting human bodies and to learn more about their possible contribution to criminal investigation.

Al-Qahtni *et al.*, (2019) presented two unique case studies of natural deaths in Riyadh, Saudi Arabia. The two corpses displayed several stages of colonization, one of which being the colonization and disintegration of insects. The body of a 65-year-

old man who had been dressed and mummified was discovered in an outdoor habitat in the first occurrence. The carcass contained several life stages of Dermestes maculatus and because of the advanced stage, the PMI was determined to be three months. The second corpse, that of a 40-year-old man, was discovered in an indoor environment and it had reached the end stage. Dermestes maculatus and Musca domestica, the only two kinds of insects found from the cadavers in both experiments. In the first scenario, the advanced decomposition stage and the interior atmosphere both have an impact on insect activity. Average temperatures for the first body were 23.3 ± 1.6 °C, while for the second body they were 27.5 ± 1.7 °C. These two investigations allow us to draw the conclusion that environment was the main element in luring insects to the two cadavers. This study focuses on the idea that understanding the effects of various factors on insects located over and around the carrion is crucial for providing accurate estimations of the time since death.

Wang *et al.*, (2019) Presented an original case study of four cadavers whose entomological data was used to estimate PMI. **In the first case** The body was discovered outside at 26°C and was still in the fresh stage. The PMI was calculated using maggots removed from the corpse, and was found to be more than 68 hr. Due to the body's mild degree of decomposition and the fact that flies may have invaded the corpse before death, this example provided an erroneous assessment. **In the second case**, A dead adult guy with severe decomposition and mummification was discovered in a semi-enclosed chamber. Dermestes maculatus DeGeer was utilized in this instance to successfully estimate the PMI, which was around 29 days.

The third case portrayed a woman whose body was discovered in her room after using clozapine and too much alcohol to commit suicide. Fly larvae were detected mostly on the head and face, following the species key. Third-instar maggots with a maximum body length of 12 mm were found. The PMI determined from entomological data did not match the time of death suggested by the other evidence. Our findings suggest that clozapine and alcohol may inhibit the growth and development of *C. megacephala* larvae. As a result, this instance implies that we should take into account how chemical substances affect insect development in future work to more accurately estimate PMI in the presence of chemicals. **The fourth case**

occurred in the winter, where charred remain of female were located in which killer killed the victim and burnt and discarded the corpse. Samples were therefore collected, and the PMI, which was about 26 days, was determined using this data.

Sampson *et al.*, (2020) released the first ever research piece on forensic entomology study of beetles sin Alaska in 2019. One piglet corpse was cut in half to form two carcass plots. Each corpse was surrounded by four pitfall traps, while four control pitfall traps were set 40 meters apart. The adult fly off was the subject of this instance. They concentrated subfamilies of the Coleoptera. Researchers collected and processed 621 specimens in all. A one-way analysis of variance (ANOVA) showed no statistically significant difference in the mean numbers of staphylinines or carabids collected in corpse traps. In corpse traps, the average number of beetles taken was much greater than in silphid traps as compared to control traps. Two species of Nicrophorus made up the majority of the specimens in the Silphidae family, which has four recognized species. Among the four species, three of the target taxa showed an increase in the number of specimens collected in the swollen phase.

Griffithsa *et al.*, (2020) acquired preliminary data of decomposition and faunal succession on pig corpses in Australia and internationally. Throughout the summer, adult insects were sampled every day for 30 days. If we compare with other places, it was the quickest to decompose; by Day 5, all carcasses had reached the dry stage. They saw and noted the discrepancy between the arrival and departure times. In contrast to temperate locations where Diptera seem to be most effective, tropical Australia benefits more from the later introduction of coleopteran taxa. Overall, this study reveals a thorough grasp of how insect succession pattern affects PMI estimation. Even a tiny bit of disinterest can have a big impact on this research.

Palavesam *et al.*, (2020) conducted a study using necrophagous flies that were gathered from 24 medical-legal cases in Tamil Nadu, India, between 2011 and 2018. Based on morphological characteristics, the fly stages were recognized and presented in tabular form. Pre-autopsy and indoor/outdoor fly incidence were also noted. The human corpses are where Chrysomya megacephala, Chrysomya ruffacies, Sarcophaga sp., and Musca domestica were found and collected.

Chrysomya megacephala was the most common (70.8%) fly species among all those seen, and it is a forensically significant bug that may be discovered in both indoor and outdoor settings, as well as on drowned and burned human remains. Only outdoor and interior habitats were home to Chrysomya ruffacies and Sarcophaga, respectively. This kind of research will contribute to our understanding of the variety and eco-geographical distribution of insects, as well as the forensic significance of insect identification and its use as proof of the movement of corpses and cadavers.

Cox *et al.*, (2021) showed the ability to comprehend and evaluate the medication in question, as well as the components that are present at every stage of the fly's life cycle, and the effects that synthetic opioids have on the development of blow fly (*Lucilia sericata*) larvae. For this reason, the researchers allowed blow fly larvae to feed on human tissue by injecting it with fentanyl at four different treatment levels. To extract fentanyl and its metabolites, Lucilia sericata larvae, pupae, pupa casings, and adults were considered. A rapid, simple, inexpensive, secure, and effective extraction technique was created, examined, and implemented. Following this extraction procedure, LC-MS/MS was used to quantitatively assess the target medicines and their metabolites. The results of this investigation indicate that the growth of flies influences the development of insects, the assessment of PMI, and the degree to which the concentration of fentanyl and its metabolites in insect tissue may be connected to the starting concentration of the feeding medium.

Istiqomah *et al.*, (2021) published a review paper on the use of insects to detect toxins and ascertain the cause of death in human fatalities. The authors provided details on the crime's scene and victims' conditions. Insects that consume corpse tissue will transfer xenobiotics, such as pharmaceuticals and other harmful compounds, to the larval body's metabolic system where they may be easily found using high-tech equipment. Xenobiotics from the body, larvae, and insects have been frequently detected using methods such immunoassays, HPLC, LC-MS, and GC-MS. If the drug's pharmacokinetics in insects and the identification of insects and larvae are each known, the quantitative and qualitative roles may be determined. Finally, they noted that the correlation of observed drug concentration is an area of weakness for entomotoxicology and later recommended the creation of standard procedures that

must contain models of organisms (for example, accessible insect species), standard matrices for feeding substrates, determining sample sizes, and applying advanced analytical technologies.

Hameed *et al.*, (2021) used the four antipsychotics in parallel, in pure and tablet form: aripiprazole, asenapine maleate, quetiapine fumarate, and chlorpromazine HCl. This was done using the HPTLC method, a green analytical technique. The separation process in this novel method used silica gel 60 F254 HPTLC plates, and the mobile phase consisted of a binary mixture of green solvents (ethanol: water, 9: 1 v/v). This resulted in a compact band with Rf values for the medications ranging from 0.14 to 0.70. According to ICH criteria, the method's linearity, accuracy, precision, selectivity, and robustness were all confirmed. The suggested HPTLC method might be effectively used in laboratories to test these antipsychotics on a regular basis.

Arnaldos and Garcia, (2021) highlighted many forensic incidents that took place in the Murcia area of Southeast Spain, where the use of entomological evidence contributed to the complete resolution of the challenges encountered both in the course of the legal inquiry and in the actual forensic cases. Insects discovered at a crime scene that provide entomological evidence of the victim's time and place of death, probable pre- or post-mortem care, and the location of the origin of drugs and narcotics. According to this study, many forensic investigators lack specialized training in fundamental entomology techniques, which might provide challenges when attempting to compile an accurate expert report.

Pawar, (2021) obtained Lucilia cuprina (Diptera: Calliphoridae), including eggs, three instar stages, pupa, and adult stages, from the meat sample of the Aurangabad region for an experimental investigation on the impact of benzodiazepines on their development. In this study, Lucilia cuprina alterations and the impact of the medication on the length of life cycles were examined. It was discovered that greater concentrations of diazepam slowed the process of decomposition and that adult flies emerged based on concentration.

Hamdy *et al.*, (2022) has investigated the identification of faunal swarms that inhabit the decomposing carrion of rabbits and guinea pigs treated with the medication

tramadol in Cairo, Egypt. Six animals were used for the sample, both pigs and rabbits were split into two groups, one group was given the tramadol medicine to kill them, while the other group was given hypoxia to kill them as a control. In comparison to summer, the pace of disintegration was slower in winter. In this research investigation, a total of 12,966 individuals (immature and adults) were gathered and documented for the class Insecta. It was discovered that throughout the winter and summer seasons. For both rabbits and guinea pigs, the quantities of adult insects varied in the control sample and when compared to samples that had taken tramadol while drunk. Finally, we may draw the conclusion that medicines and poisons present in a decaying body may affect the tramadol drug's impact and the rate of insect growth in the body, both of which are relevant to forensic research.

Pawar and Deshmukh, (2022) examined how the most often prescribed sedative, alprazolam, affected Chrysomya megacephala, a blow fly with forensic significance. When food is laced with the chosen medicine, the larvae's growth rate is slowed down. Larval and pupal development is slowed down when alprazolam content in the diet rises. The several phases of the blow fly life cycle and the morphometric characteristics of these stages aid in criminal investigations.

Chapter 3

Materials and Methodology

3.1. Study area and Period of study

This study was conducted in five cities - Mohali, Fazilka, Anandpur Sahib, Amritsar and Jalandhar of Punjab state of India.

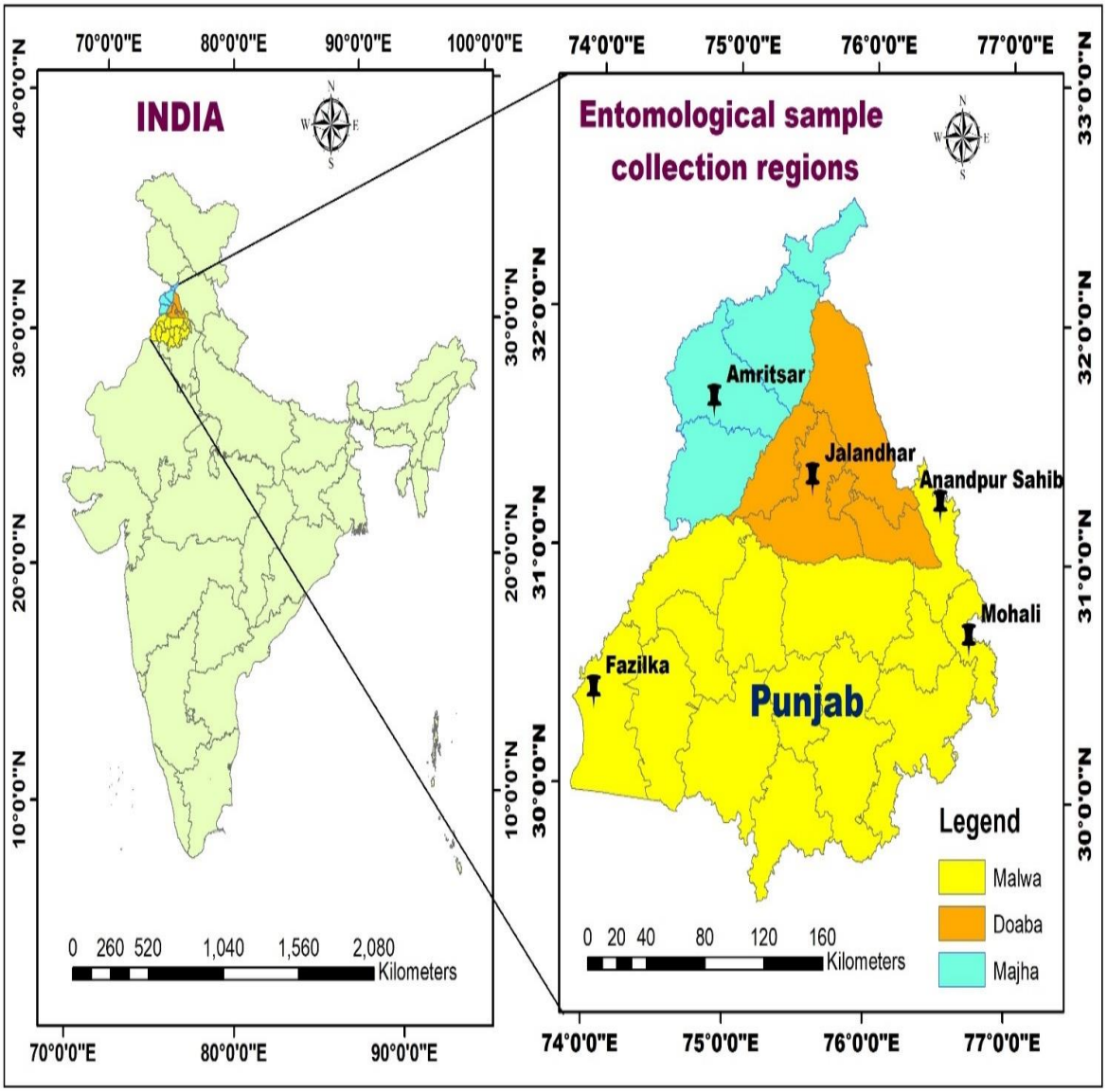


Fig 3.1. Study area map of entomological sample collection regions

The coordinates of locations-

1. Mohali - Northern Latitude 30.4551°N; 763740 Eastern Longitude
2. Jalandhar - Northern Latitude 31.31022°N; 75.63939 Eastern Longitude
3. Fazilka- Northern Latitude 30.2312°N; 74.211 Eastern Longitude
4. Anandpur Sahib- Northern Latitude 31.234961°N; 76.498808 Eastern Longitude
5. Amritsar- Northern Latitude 31.382°N; 74.515 Eastern Longitude

The complete studies were done only in Mohali and Jalandhar in all four seasons i.e., Winter, Spring, Summer, and Autumn with all 5 different concentrations of drug and control sample. Other three cities i.e., Fazilka, Amritsar, and Anandpur Sahib were captured to look after the maximum number of flies identification and to capture the maximum area of Punjab.

3.1.1. Site Selection

It was based on the following criteria like,

- Sites were selected to cover the major regions of Punjab i.e., Amritsar from **Majha**, Jalandhar from **Doaba**, Mohali, Anandpur Sahib, Fazilka from **Malwa.**
- It needs to guarantee the carrion's unrestricted accessibility to various insects.
- At least twice or three times a day, observations should be possible to take.
- There shouldn't be a chance that people or other animals, especially carnivores or scavengers, would disrupt the peace.
- Each was examined during the day so that, at each visit, the sample's physical state and level of decay could be seen.

3.2. Preparation of working and stock solution

The drug Asenapine Maleate was procured from Indian Pharmacopoeia, Uttar Pradesh, India in quantity of 200mg with purity of 99.99%. Using asenapine that was acquired from the Indian Pharmacopeia, asenapine maleate was created as a 1.0 mg/ml stock solution in methanol. This was utilized to create functioning solutions with 10µg, 20µg, 30µg, 40µg, and 50µg, in that order. The above aliquots were subjected for spiking in blank matrices for estimating quantitative and qualitative assessments.

3.3. Experimental food substrate

The food substrate was goat mutton (1.5Kgs.) were taken from shop and used in this study. Then, the mutton sample were divided into 6 parts, each of 250 grams (named- A, B, C, D, E and F), and transferred into medium-sized glass jar with a mouth that is 7.5 centimetres (3 inches) diameter and 15 centimetres (6 inches) tall., in which five of them were spiked with 5 different Concentration of drug i.e., 10µg, 20µg, 30µg, 40µg and 50µg referred as A, B, C, D, E and one Jar (named F) was used as control or blank.

A- 10µg ASPM
B- 20µg ASPM
C- 30µg ASPM
D- 40µg ASPM
E- 50µg ASPM
F- No ASPM (As a control)

Fig 3.2. Spiking drug

Fig 3.3. Spiking drug

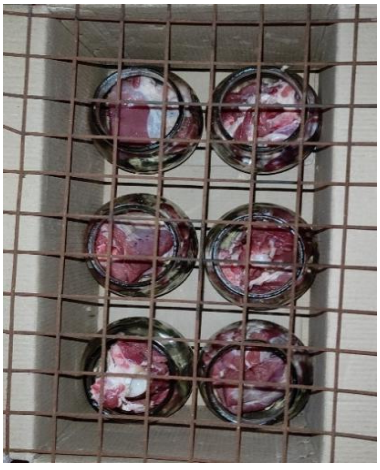


Fig 3.4. samples kept in cage

3.4. Drug Used

The drug Asenapine Maleate was spiked in mutton tissue sample and post-mortem liver sample. It was brought from Indian Pharmacopoeia with purity of 99.99%. To make the stock solution, 10 mg of the medication were dissolved in 10 ml of methanol. Thereafter, five different concentrations of drug were prepared by diluting with methanol. Then, this solution was preserved at 4℃ till experiment.

3.5. Environmental Conditions

The experimental part began on 15 January 2021. Temperature was noted by hand-held thermometer at the selected location (Mohali and Jalandhar) and the internal and external temperature was measured. The temperature was recorded on a regular basis during all experimental studies. The summary of weather information as follows-

This research study was divided into four major seasons i.e.- winter, spring, summer, and autumn season. Using a hygro-thermometer, ambient site temperatures and humidity were continually monitored. A typical rain gauge was used to measure the amount of rain, and it was also monitored. Conditions of the local cloud cover were observed visually. All observations of the ambient temperature and rainfall were compared to data from the Jalandhar Cantt. Observatory and the Indian Meteorological Department.

3.6. Experimental Set up

For experimentations, two different cities of Punjab i.e., Mohali and Jalandhar were used. The experimental study inculcates the analysis and comparison of the entomological data of insects, insets succession, detection of drug, effect of drug, temperature and environment and eventually, its usage in PMI estimation. Eight experiments were conducted to cover all four seasons- winter, spring, summer and autumn seasons of Mohali and Jalandhar. One experiment was done on human post-mortem liver for the validation of process and another experiment was done for the quantitative analysis.

The arrival time of flies in summer season is about 3-4 hours and in winter season is about 24-30 hr. The main point of observation was noted in this study that the insects

were died in the 3rd instar stage (mainly in summer season) at the concentration of 40µg and 50µg Here, the observation was presented in tabular format (chronologically) of both locations, and the results were derived as per experimental setup.

Table 3.1. The duration of experiments conducted in Mohali and Jalandhar

Mohali	**Jalandhar**
15th January 2021 - 17th February 2021	18th February 2021- 15th March 2021
10th April 2021-22nd April 2021	20th March 2021-5th April 2021
5th July 2021- 11th July 2021	25th June 2021- 2nd July 2021
25th September 2021- 9Th October 2021	7th September 2021- 20th September 2021

The above table shows the period of experiment study according to the location (Mohali and Jalandhar) and these eight-experiment study is divided into two groups (each four experiment for both locations). This table will assist the research in order to observe at what time the eggs, larvae, pupae growth will appear and help in estimate the PMI. All these eight-experiment study was observed regularly, recorded the time, and documented all these observation in tabular formats.

3.6.1. Collection of insects

A) **Adults Insects**- Insect collection net was used to collect adult flies and they were further preserved in 70and ethanol solution, so they won't shrink till the time of identification. Labels for vials included the site name, city location, date, time, sample number of insect.

B) **Larvae** - The larvae were collected with the use of forceps. From each experimental setup, around 50 maggots from the feeding and post-feeding stages were collected in vials and kept at -10°C in a deep refrigerator until toxicological examination. Additionally, the collected maggots were cleaned using boiling water to prevent surface contamination before being kept in 70% ethanol for microscopy purposes.

The control jar/sample consisted of insects of the same type and age but fed with fodder not treated with drug.

3.7. Chemicals and Reagents

All solvents and reagents were of analytical grade and were of high-quality standards.

Reagent	Procured from
Asenapine Maleate (for standard/spiked)	Indian Pharmacopoeia
Potassium Di hydrogen ortho phosphate (Phosphate buffer)	Sigma-Aldrich
Methanol	Thermofisher Scientific
Ammonia solution	Sigma-Aldrich

3.8. Tools and Techniques used for toxicological analysis.

- Solid phase extraction Superclean Ultra 2400 model with C8 Column
- GC–MS Shimadzu QP-2020 NX (Kyoto, Japan) coupled with auto-sampler Shimadzu AOC-20N Plus.

Injector- Splitless mode

Column- SH- RXi-5Sil-MS fused silica capillary column

Column dimensions- 30 m×0.25 mm ID×0.25µm

Laboratory tools- Injector vial, RIA vial, micro pipette, electronic balance, vortex, centrifuge, beaker, flask, evaporator, spatula, refrigerator, viscera cutter etc.

3.9. Research Gap

After conducting an extensive review of existing literature in the field, it was established that a significant number of research has already been conducted using various drugs and species. However, the current study aims to investigate the effects of the drug asenapine maleate, which has not been previously explored in literature. Additionally, the present study seeks to investigate the seasonal variations of all four seasons, which has also not been examined before.

The primary objective of this study was to determine the effects of the drug on different species of entomological samples. This will provide significant insights into estimating the time since death with a higher level of accuracy than previously possible. One of the unique aspects of this study was the use of asenapine maleate, a drug that has not been investigated in previous studies. The use of this drug will provide valuable insights into its potential applications in forensic science. Furthermore, the study of the drug's effects on different entomological species samples will help to improve our understanding of the mechanisms involved in estimating PMI.

In addition to the novel use of asenapine maleate, the study will also investigate seasonal variations. This is an important consideration as environmental factors, such as temperature and humidity, can significantly impact the rate of decomposition. By considering the effects of the drug under different seasonal conditions, the study will provide a more comprehensive understanding of its potential applications in forensic science.

The study's focus on entomological samples is also noteworthy as insects are often used to estimate the PMI in forensic investigations. Insects colonize a body soon after death, and the development of their larvae can be used to estimate the time of colonization, which can, in turn, provide an estimate of the time of death. By investigating the effects of asenapine maleate on various species of insects, the study will contribute to our understanding of the developments involved in estimating the PMI using entomological evidence.

3.10. Aim and Objectives

The primary objective of this research is to pioneer a method that not only possesses an exceptional degree of sensitivity but also offers a precision and accuracy that can significantly contribute to the estimation of the minimum PMI and the identification of Asenapine Maleate (ASPM) within entomological samples. These samples originate from instances where insects have predated upon animal meat or human viscera intentionally spiked with the aforementioned compound.

The objectives of research are as follows-

- **Identification of species of predators in different geographical locations of Punjab**
- **Time since death assessment from predators on cadaver**
- **Identification and estimation of drug from different forensic entomo-toxicological Samples**
- **Impact of drug on forensic entomo-toxicological samples**

Chapter 4

Results and Discussion

4.1. OBJECTIVE-1- Identification of species of predators in different geographical locations of Punjab

4.1.1. Introduction

The need of identification of insects in forensic entomology is for the better understanding of their life cycle. Whenever a dead body is found without the known time of death and physical markers of estimating time since death are also not available, then forensic entomology is the only helpful field. To estimate the time since death with forensic entomology, the investigator needs to observe the life stage of insect. The insect identification is done only by expert taxonomist of that particular order. In India, there is only one organisation which certifies the insect identification i.e., Zoological Survey of India, Kolkata. In present study, the preliminary identification was done using taxonomic keys which was further re-checked and certified by Zoological Survey of India.

4.1.2. Methodology

The specimens were collected from animal tissue (Goat meat) samples and human post-mortem liver samples. The flies were collected using insects' collection net and preserved in 70% ethanol. Larvae were collected with the help of forceps which were then transferred to diced animal tissue sample (50gms) for the purpose of identification. The identification was done priorly by using entomological identification keys and confirmed with the help of Zoological Survey of India, Kolkata.

4.1.3. Outcomes

The identified species are as follows:

a. Jalandhar- **Chrysomya megacephala, Calliphora vicina, Musca Sorbens**
b. Mohali- **Calliphora vicina, Sarcophaga parasarcophaga albiceps, Musca Sorbens**
c. Amritsar- **Calliphora vicina, Sarcophaga parasarcophaga albiceps**

d. Fazilka- **Chrysomya megacephala, Calliphora vicina**

e. Anandpur- **Calliphora vicina, Sarcophaga parasarcophaga albiceps**

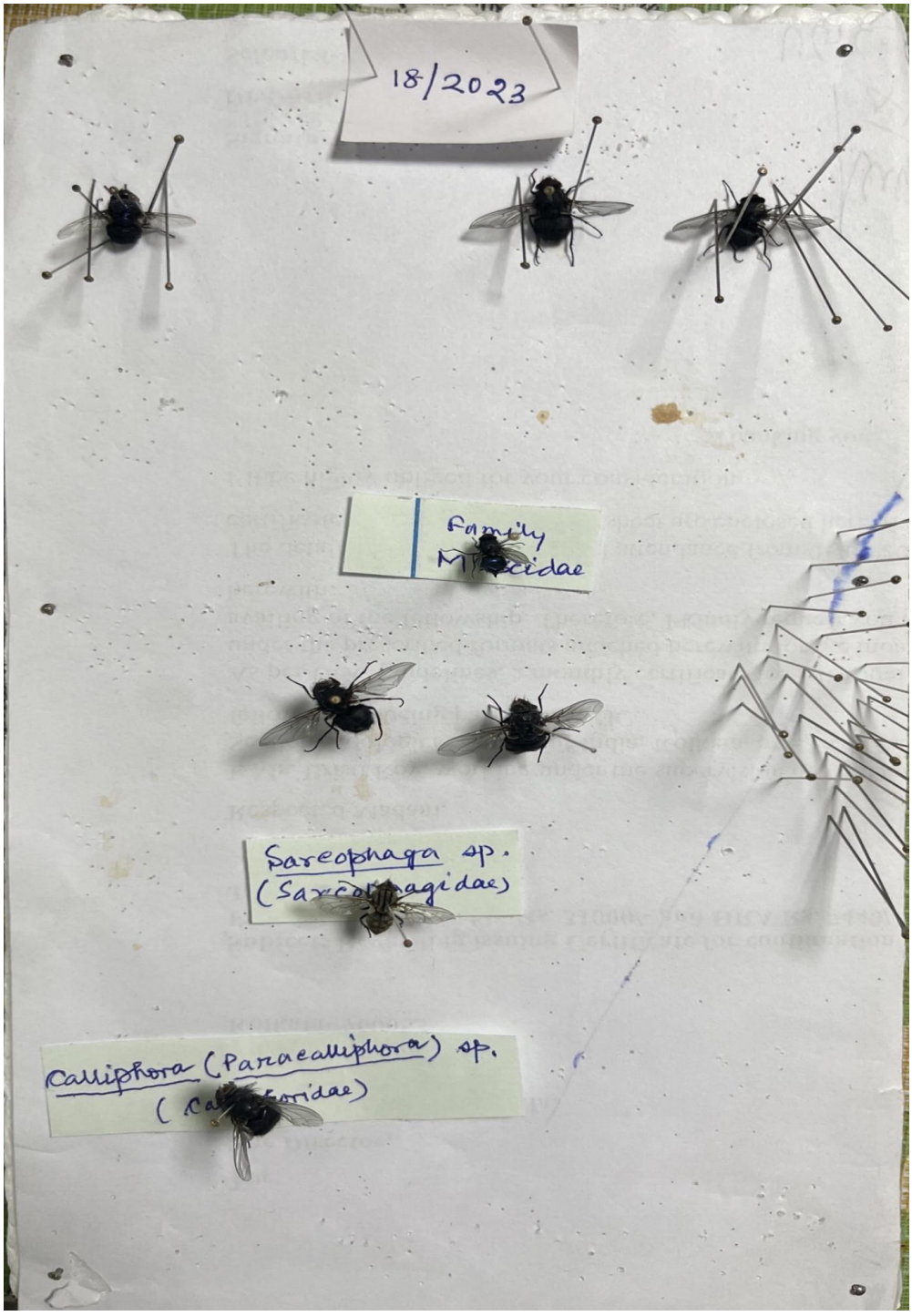


Figure 4.1. Pinned insects.

Mohali	Calliphora vicina 	Parasarcophaga albiceps 	Musca sorbens 
Jalandhar	Chrysomya megacephala 	Calliphora vicina 	Musca sorbens 
Anandpur Sahib	Calliphora vicina 	Parasarcophaga albiceps 	
Fazilka	Chrysomya megacephala 	Calliphora vicina 	

Amritsar	Calliphora vicina 	Parasarcophaga albiceps 	

4.1.3.1.*Chrysomya megacephala* (Fabricius, 1794)

Chrysomya megacephala (Fabricius, 1794), due to its propensity to lay its eggs in latrines, is also referred to as the Oriental Latrine Fly and is valuable in both medicine and forensics. It typically feeds on organic waste, including feces, corpses, decaying meat, and fish. As a result, it is regarded as a pest that infests fish products and a human disease vector (de Oliveira *et al.,* 2008).

Figure 4.2. Microscopic image of Dorsal view of *Chrysomya megacephala*

Figure 4.3. Microscopic image of Front view of *Chrysomya megacephala*

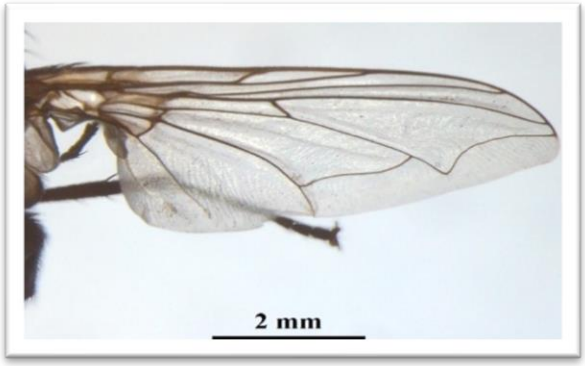


Figure 4.4. Microscopic image of wing of *Chrysomya megacephala*

A) Morphological identification

I. Egg

There is a shortage of data on *Chrysomya megacephala* eggs. The eggs are sausage-shaped, whitish, and 1.5–1.6 mm long before turning cream in colour. Although their eggs resemble those of other blow flies, especially the closely related Chrysomya pacifica, they are said to have a broad, flattened strip along the length of one side with projections that resemble leaves (Sukontason, 2003).

II. Larva

Studies of the structure of the larval skeleton and photographs of the larvae are available. The anterior spiracle, the bands of spines on each segment, and the mouth parts are typically used for identification. The size of larvae in the first instar stage ranges in size from 1.7 to 3.5 mm, and one slit is seen in the posterior spiracles. The posterior spiracles of second-instar larvae have two slits and are 6–8 mm long. This instar's morphology (specifically its mouth parts) differs slightly from that of the first and third instars, but there is no clear identification key. Depending on the temperatures they experience, third-instar larvae can reach a length of about 16 mm, but they quickly contract just before pupariation, and the posterior spiracles have three slits. The last segment has a dorsally incomplete band of spinules, and there are 11–13 branches on

the anterior spiracle (Mendonça *et al.*, 2012; Szpila and Villet, 2011; Amorim and Ribeiro, 2001).

III. **Puparium**

The third instar larva gives rise to the puparium, which resembles the adult larva in appearance. Its anterior spiracles are yellow and brown in colour. The third-instar larva's mouth hooks, which help identify pupae, are typically found adhering to the interior of the enclosed puparium (Yang and Shiao, 2012).

B) Life cycle

Chrysomya megacephala follows a blow fly's normal life cycle. An adult female may deposit a mass of 220–325 (mean ~254) eggs on substrates in a single day under ambient circumstances. Although they are mostly active during the day, females have been seen to deposit eggs at night in warm environments. It appears that *Chrysomya megacephala* females prefer to reach sites of nocturnal oviposition. It is unknown whether precocious eggs—eggs that have been fertilized before being laid—occur in *Chrysomya megacephala*. These considerations are crucial for forensic entomology when it comes to qualifying estimates of minimum PMIs (Esser, 1990; Smith *et al.*, 2016; Reiter, 1984).

4.1.3.2.*Calliphora vicina* (Robineau-Desvoidy, 1830)

Calliphora vicina (Diptera: Calliphoridae), commonly referred to as "meat flies," has a restricted range of distribution to regions where summertime temperatures do not consistently exceed 30 °C. In the subtropical region, this insect species typically only becomes active in the winter. A prepupa, a pupa, an adult stage, three larval stages, and an egg stage are all parts of the calliphoridae life cycle. Only the three juvenile stages of the larva are when it feeds (Gennard, 2007).

Figure 4.5. Microscopic image of dorsal view of *Calliphora vicina*

Figure 4.6. Microscopic image of front view of *Calliphora vicina*

Figure 4.7. Microscopic image of wing of *Calliphora vicina*

A) Morphological identification

Calliphora vicina (blowfly) measures 9 to 11 mm in length, orange colour characterizes the front thoracic spiracle. The front half of the cheek (bucca) is reddish orange and the top of the head is black. Black dominates the lower third of the face. Regardless of the jowl's colour, there are black hairs there. The thorax is black, and its top, or dorsum, is

covered in a thick layer of pubescent (a greyish shine). The centre of the thorax has two thick bristles arranged in a row. The acrostically bristles are what they are known as. The hypo pleural bristles are a fan-shaped group of bristles that are found on a plate located above the coxa of each hind (third) leg, close to the posterior spiracle, just like other blowfly species. You'll find them if you keep an eye out for this spiral. The abdomen is blue with a tessellation pattern that looks like a silvery checkerboard. The basicosta on the wing is initially yellowish, but it can eventually turn yellowish-brown (Krikken and Huijbregts, 2001).

The most well-known species are undoubtedly those of the genus *Calliphora* and *Lucilia*. They undergo a complete metamorphosis, beginning as an egg and progressing through three larval stages, post-feeding and prepupa stages, and a pupal stage at the end. Although the speed of development is greatly influenced by temperature, new generation can be produced in three weeks at summertime temperatures. When eggs and tiny first instar larvae are seen, PMI estimation may occasionally be made in hours rather than days as this indicates that between 12 and 18 hours have passed since oviposition. Blowing insects only lay their eggs during the day and some, like Lucilla, only do so at high body surface temperatures (above 30°C), so the oviposition period and, consequently, the PMI, may actually be fairly well limited to hours rather than days (Greenberg and Tantawi, 1993; El-Moaty and Kheirallah, 2013).

4.1.3.3.*Sarcophaga* (*parasarcophaga albiceps*, Meigen 1826)

A) Morphological identification

Males can range in size from being somewhat big to being huge (11-17 mm). The frons are roughly 3/5 the breadth of an eye. Para-frontal, para-facial, and frontal vittae are all black and covered with silvery pollen. The adult flies have dark brownish antennas and the first antennal segment seems to be blackish brown, followed by the second and third segments, which are somewhat brown with whitish pollen.

Thorax has three longitudinal black lines and is greyish in colour with well-developed pro stigmatic and pro pleural bristles are present, together with short hairs. Brown

latero-scutella, apico-scutellar, and disco-scutellar bristles were seen in the pro and mesothoracic spiracles, respectively (Chakraborty *et al.,* 2015; Levot *et al.,*1979).

Generally, females are 7 to 12 mm shorter than males. The men' biometric differences are not that different. In females, apico-scutellar bristles were missing and the leg chaetotaxy is identical to that in males, whereas the second and third sternites have four bristles each, the fourth and fifth have two long bristles, the sixth is wider than the others, the seventh has no hairs but a row of robust peripheral bristles, and the eighth is naked, membranous, and somewhat concave. The anal sternite has small hairs, and the seventh tergite has long bristles (Chakraborty *et al.,* 2015; Levot *et al.,*1979).

Special focus on 3rd instar larvae and pupa of Sarcophaga (P) albiceps due to their specific physical characteristics as juvenile stages. The light microscopy of the anterior and posterior spiracles as well as the second and third integuments of the pupa were focused on in order to distinguish the larval stages. The presence of long, pointed spines on the interband area of the thoracic and abdominal segments served as the diagnostic feature for the third instar larvae and pupae of Sarcophaga (P) albiceps (Tayyib *et al.,* 2006).

Figure 4.8. Microscopic image of dorsal view of *Parasarcophaga albiceps*

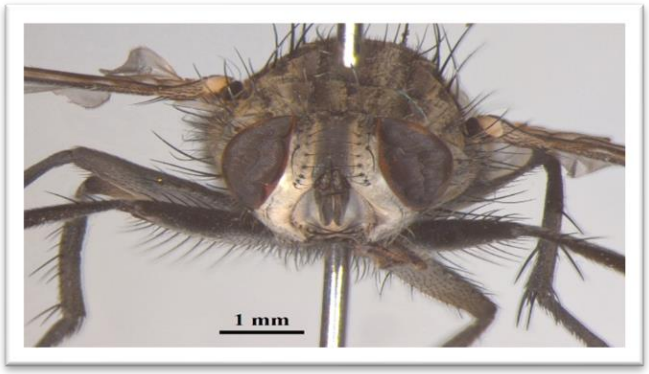


Figure 4.9. Microscopic image of front view of *Parasarcophaga albiceps*

Figure 4.10. Microscopic image of wing of *Parasarcophaga albiceps*

B) Ecology and Life cycle

Since no eggs were observed from the cadaver in any season, it appears that the Sarcophaga (P) albiceps is larviparous. In the pre-monsoon, monsoon, and post-monsoon season, *Sarcophaga* (P) *albiceps* completes its life cycle in 326.11 hr, 336.38 hr, and 343.09 hr, respectively and the weight of the three corpses is the same across the seasons. As a result, *Sarcophaga* (P) *albiceps* typically takes 335.43 hours to complete its life cycle in a Gallus corpse. It was noticed that how the various stages of *Sarcophaga* (P) *albiceps*' life cycle varied according to the season and according to literature and other sources of collected data, a distribution map of *Sarcophaga* (P) *albiceps* can be produced to determine how this data may be used in the future (Singh and Bharti, 2008; Ma and Huang, 2018).

4.1.3.4.*Musca Sorbens*, Wiedemann, 1830

The bazaar fly, or *Musca Sorbens* Wiedemann, 1830, is a member of the Dipteran family Muscidae. It is a pest of medical and veterinary significance that spreads all over the world. This vast genus now contains 561 species, including Musca domestica Linnaeus, 1758, one of the most crucial species for medicine (Thompson, 1993). The family of flies that humans most commonly encounter is the Muscidae, or housefly family. Schiner provided the first definition of the Muscinae, a subfamily of the Muscidae, and one of the most primitive groups in this subfamily is thought to be the tribe Muscini. There are 351 species in 18 genera that make up the entire range of the Muscini tribe (Schiner, 1862; Ronald, 1978). When compared to bovine dung, dog and cat excrement were shown to be superior medium for larval growth, earning *Musca sorbens* its common name of dog dung fly. The *Musca sorbens* fly is now recognized as a mechanical vector of the Chlamydia trachomatis bacterium, the major agent cause of trachoma and a contributing factor in the spread of this blinding human illness (Hafez and Attia, 1958; Sareef, 2016).

Figure 4.11. Microscopic image of dorsal view of *Musca sorbens*

Figure 4.12. Microscopic image of front view of *Musca sorbens*

Figure 4.13. Microscopic image of wing of *Musca sorbens*

A.) Morphology

The size of *Musca sorbens* flies ranges from tiny to medium (7 to 11 mm). The mesonotum had a pale grey to gold hue with two dark grey vittae that, in females, were divided in the shape of a Y in front of the suture. light-colored or white anterior spiracle The eyes of the male flagellomere are consistently sized and relatively close together, with the male front being smaller than the first flagellomere. In females, the space between compound eyes is more than one-third the diameter of the head. In males, the fourth segment's edge and the majority of the fifth segment are darker. With the exception of the medial abdominal strip, most female abdomen segments were darkened by dusty setae and brown transverse lines, creating the appearance of grey and gold checks (Mau *et al.,* 1981).

B.) Morphological identification

I. **Eggs**

As they complete three ovarian cycles in a two-week period, females lay their eggs in clusters of few to over one hundred in the dung's cracks and crevices. Each cycle saw an average of 42 eggs laid by each female. Size, lifespan, and food quality of a female can all affect how many eggs she produces. Larger clusters were caused by many female adults depositing eggs. Within ten days, these eggs began to hatch (Ronald, 1978; Miller *et al.,* 2013).

II. **Larvae**

Larvae in their first instar (growth stage) hid in the excrement until they reached adulthood. There are three larval instars, and each takes 4-5 days to mature fully. Within a day, mature larvae emerged from the dung (typically at night) and pupated (into an immobile development stage). The adults appeared in the late afternoon or early evening for approximately five days, peaked in the morning, and disappeared by midday (Ronald, 1978; Miller *et al.,* 2013).

III. **Pupae**

Often at night, mature larvae emerged from the lab's excrement and pupated the next day. In around five days, adults began to emerge in the late afternoon or early evening, peaked in the morning, and ended by lunchtime (Ronald, 1978; Miller *et al.,* 2013).

IV. **Adults**

Male and female adults mate between 4–7 days, and after fertilization, the females oviposit (laid eggs) within 1–2 days. With enough food and moisture, adults of this pest may survive for about two months; without food and water, adults do not last more than 45 hours (Ronald, 1978; Miller *et al.,* 2013).

4.1.3.5.Microscopy

Larvae and pupae were collected, inspected under stereomicroscopes, and samples were illuminated with transmitted light to aid with sampling thereafter. Digital cameras were used to take pictures of each species. Adult insects were taken out of the ethanol and left to dry for a little while.

Conclusion

Based on the data provided, it can be inferred that the various species found in different locations share similarities, with only one or two species being unique to particular areas. This information is crucial in identifying the location of a crime, as the presence or absence of certain species can provide valuable clues for forensic investigations. The identification of these species is a critical component of this study, as it can provide insight into their normal life cycles and aid in distinguishing one insect from another. A total of 185 insects were collected and classified into four different species, with three flies initially suspected to belong to a different species. However, it was later

determined by the Zoological Survey of India in Kolkata that these flies were in fact part of the *Calliphora vicina* species.

Understanding the life cycles and behaviours of these insects is crucial in forensic entomology, as it can provide data and facts regarding the time of death and other important details of a crime. Since insects are among the first to inhabit a decomposing body, their growth and presence can be utilized to calculate the PMI, or the time elapsed since death. The identification of these insects is often done through morphological analysis, where various characteristics of the insects are examined to determine their species. This can include their body shape, coloration, and the presence of certain appendages or features. In addition to their importance in forensic investigations, insects play a vital role in ecological processes. They are important pollinators and decomposers, helping to break down organic matter and recycle nutrients in ecosystems. Many insects are also important food sources for other animals, including birds, reptiles, and mammals. However, insect populations around the world are facing significant threats from habitat loss, climate change, and pesticide use. This has led to declines in many insect species, with potential impacts on ecosystem function and food webs.

In conclusion, the data presented in this study highlights the importance of insect species identification in forensic investigations. By understanding the life cycles and behaviours of these insects, investigators can gain valuable insights into the timing and circumstances of a crime. However, it is also important to consider the broader ecological context in which these insects exist.

4.2. OBJECTIVE-2- Time since death assessment from predators on cadaver

4.2.1. Introduction

There are numerous incidents happen around the world where the dead body of a person generally found in vulnerable conditions and the time of death is unknown. To estimate time since death, forensic pathologists use physical markers such as algor mortis, rigor mortis and others, but in cases where body is partially or completely skeletonized, then it becomes difficult to estimate. In such scenario, forensic entomology plays an important role as the time since death or PMI can be estimated by observing the life stage of insects found on body or ground. Still there are many factors which effects the life cycle of insects like temperature, rain, humidity, clothing, drugs etc. Forensic entomology can give an estimate only but there are chances of error. This particular study is done to provide better understanding of insect succession and life cycle.

4.2.2. Methodology

In this study, goat meat was used as substrate and total 8 experiments were conducted to get better accuracy, moreover, Sandu *et al.;* Sharif *et al.*and Jeffrey *et al.* have also used goat meat as a substrate [206-208]. Different species of flies were collected by using meat sample placed in open environment and specie of choice which was *Calliphora vicina* was used during the study as *Calliphora vicina* was the first to feed during all the experiments and was larger in number than any other specie came to feed. A total of 1.5 kg meat was used during each experiment which was further divided in to 6 equal parts of 250 gms. Five of them were spiked with different concentrations of drug and one was used as control or blank. This objective was accomplished with the control or blank meat sample only. The meat was kept in jar in the balcony of rented flats in Mohali and Jalandhar and flies of specie *Calliphora vicina* were introduced to all the different jars. The complete life cycle was observed during all four seasons and recorded through photographs. All the climatic factors were also observed with the help of hygro-thermometer and direct observation which was further validated with the data taken from Meteorological Department of India, Chandigarh, and Jalandhar Cantt. Observatory.

Figure 4.14. spiked and control meat

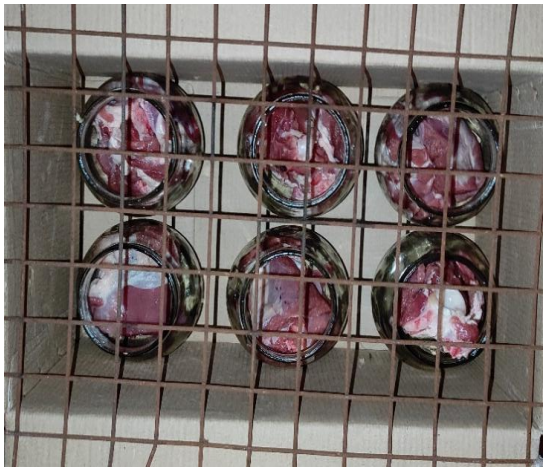


Figure 4.15. Jars in cage

Figure 4.16. Attack of fly on fresh meat

Figure 4.17. fly laying eggs on substrate

Figure 4.18. Eggs on substrate

Figure 4.19. 1st instar larvae

Figure 4.20. 2nd instar larvae

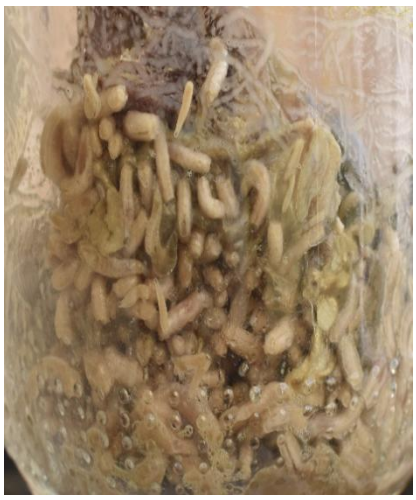


Figure 4.21. 3rd instar larvae

Figure 4.22. Pupa

Figure 4.23. Adult fly

Figure 4.24. Thermometer

Figure 4.25. Glass jars covered with muslin cloth.

4.2.3. Outcomes

In tables 4.1- 4.16, all the eight experimental studies as well as its date and location and after that, recorded the time taken by insects to grow into successive stage of their life in hours after the oviposition of flies have been mentioned. Among all the experimental studies, the earliest time recorded for the arrival of insects and flies in the month of June and July and the time delayed in the month of January and February.

At last, the arrival time of flies in winter is delayed as comparison to the summer season of experimental studies.

Calculation of ADD (Accumulate Degree Days)

To calculate the accumulated degree days, the minimum and highest temperatures were added, and the total was divided by two, to determine the daily average temperature. If the resulting average temperature surpasses the specified threshold temperature, which serves as the base temperature for development, the accumulated degree days for that 24-hour period were determined by subtracting the threshold temperature from the calculated average. For the next day, Calculated the average temperature of that day and added it to ADD of previous day. The ADD of subsequent days was then calculated with the same formula and the appearance of subsequent stages of insects was validated by dividing the ADD of that particular day with the mean of average temperature of complete duration.

Table 4.1. Temperature and ADD (Accumulate degree days) of Mohali, Punjab during 1st experiment

1. Experimental setup at Mohali (Winter)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	17.7	7.2	12.45	12.45
2	20.8	5.8	13.3	25.75
3	11.3	8.3	9.8	35.55[1]
4	19.2	7.6	13.4	48.95
5	22.6	7.1	14.85	63.8
6	23.0	9.7	16.35	80.15[2]
7	18.4	7.2	12.8	92.95
8	18.9	7.8	13.35	106.3
9	18.2	12.6	15.4	121.7[3]
10	20.8	11.2	16	137.7
11	14.5	11.0	12.75	150.45
12	17.7	5.7	11.7	162.15
13	20.0	6.2	13.1	175.25

14	21.0	5.2	13.1	188.35
15	17.4	4.2	10.8	199.15[P]
16	19.5	7.0	13.25	212.4
17	20.6	4.0	12.3	224.7
18	21.8	6.8	14.3	239
19	21.9	6.2	14.05	253.05
20	24.2	12.6	18.4	271.45
21	23.8	12.6	18.2	289.65
22	16.5	6.1	11.3	300.95
23	21.2	6.6	13.9	314.85
24	21.9	7.6	14.75	329.6
25	22.8	7.6	15.2	344.8
26	23.4	9.6	16.5	361.3
27	24.1	10.2	17.15	278.45
28	24.9	10.1	17	395.45
29	25.0	11.6	18.3	413.75
30	24.5	11.1	17.8	431.55
31	23.5	9.8	16.65	448.2
32	25.0	9.8	17.4	465.6
33	26.0	10.3	18.15	483.75
34	27.0	9.2	18.1	501.85[F]

Table 4.2. Time taken by insects to grow in to successive stage during 1st Experiment

Life Stage	Control
1st Instar	60 hr
2nd Instar	70 hr

3rd Instar	72 hr
Pupa	170 hr
Fly	445 hr
Total Time	817 hr

Table 4.3. Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 2nd experiment

2. Experimental setup at Jalandhar (Winter)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	24	8	16	16
2	22	5	13.5	29.5
3	23	8	15.5	45[1]
4	24	8	16	61
5	24	8	16	77[2]
6	21	12	16.5	93.5
7	22	12	17	110.5[3]
8	24	12	18	128.5
9	16	11	18.5	147
10	23	10	16.5	163.5
11	23	9	16	179.5
12	23	12	17.5	197
13	24	10	17	214
14	20	13	16.5	230.5[P]
15	24	10	17	247.5
16	24	11	17.5	265
17	23	12	17.5	282.5
18	26	14	20	302.5
19	28	14	21	323.5
20	29	12	20.5	344
21	27	14	20.5	364.5
22	27	14	20.5	385
23	28	16	22	407
24	30	14	22	429

25	32	17	24.5	453.5
26	32	17	24.5	478[F]

Table 4.4. Time taken by insects to grow in to successive stage during 2nd Experiment

Life Stage	Control
1st Instar	52 hr
2nd Instar	48 hr
3rd Instar	52 hr
Pupa	143 hr
Fly	330 hr
Total Time	625 hr

Table 4.5. Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 3rd experiment

3. Experimental setup at Jalandhar (Spring)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	32	15	28.5	28.5
2	33	19	26	54.5[1]
3	34	17	25.5	80[2]
4	35	18	26.5	106.5
5	34	20	27	133.5[3]
6	35	20	27.5	161
7	34	18	26	187
8	33	17	25	212
9	35	20	27.5	239.5[P]
10	33	19	26	265.5
11	35	21	28	293.5
12	37	21	29	322.5
13	37.5	21.5	29.5	352
14	35.5	22	28.75	380.75
15	38	22	30	410.75
16	38	22	30	440.75
17	37	22	29.5	470.25[F]

Table 4.6. Time taken by insects to grow in to successive stage during 3rd Experiment

Life Stage	Control
1st Instar	37 hr

2nd Instar	33 hr
3rd Instar	30 hr
Pupa	137 hr
Fly	173 hr
Total Time	410 hr

Table 4.7. Temperature and ADD (Accumulate degree days calculated) of Mohali, Punjab during 4th experiment

4. Experimental setup at Mohali (Spring)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	35.1	15.4	25.25	25.25[1]
2	36.6	17.2	26.9	52.15[2]
3	37.5	21.9	29.7	81.85[3]
4	36.8	19.6	28.2	110.05
5	37	20.8	56.9	166.95
6	39.2	22.9	31.05	198
7	37.8	20.5	29.15	227.15
8	39	20.8	29.9	257.05[P]
9	32.2	19.6	25.9	282.95
10	34.6	19.5	27.05	310
11	36.5	18.6	27.55	337.55
12	28.2	17.4	22.8	360.35
13	30.7	17.2	24	384.35[F]

Table 4.8. Time taken by insects to grow in to successive stage during 4th Experiment

Life Stage	Control
1st Instar	20 hr
2nd Instar	22 hr
3rd Instar	20 hr

Pupa	128 hr
Fly	131 hr
Total Time	321 hr

Table 4.9. Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 5th experiment

5. Experimental setup at Jalandhar (Summer)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	36	28	32	$32^{1/2}$
2	42	32	37	69^{3}
3	37.6	31	34.3	103.3
4	38	28	33	136.3
5	38	28	33	169.3^{P}
6	39	25	32	201.3
7	30	26	28	229.3
8	33	25	29	258.3^{F}

Table 4.10. Time taken by insects to grow in to successive stage during 5th Experiment

Life Stage	Control
1st Instar	12 hr
2nd Instar	10 hr
3rd Instar	11 hr
Pupa	84 hr
Fly	80 hr
Total Time	197 hr

Table 4.11. Temperature and ADD (Accumulate degree days calculated) of Mohali, Punjab during 6th experiment

6. Experimental setup at Mohali (Summer)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	37.5	29.4	33.45	$33.45^{1/2}$
2	38.2	29	33.6	67.05^{3}
3	39	29.5	34.25	101.3
4	40.2	30.6	35.4	136.7^{P}
5	40.5	28.6	34.55	171.25
6	36.8	30.3	33.55	204.8
7	37.5	26.7	32.1	236.9^{F}

Table 4.12. Time taken by insects to grow in to successive stage during 6th Experiment

Life Stage	Control
1st Instar	9 hr
2nd Instar	8 hr
3rd Instar	8 hr
Pupa	70 hr
Fly	77 hr
Total Time	172 hr

Table 4.13. Temperature and ADD (Accumulate degree days calculated) of Jalandhar, Punjab during 7th experiment

7. Experimental setup in Jalandhar (Autumn)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	34	27	30.5	30.5[1]
2	35	28	31.5	62[2]
3	34	27	30.5	92.5[3]
4	35	26	30.5	123
5	33	27	30	153
6	34	26	30	183
7	33	26	29.5	212.5
8	32	24	28	240.5
9	33	26	29.5	270[P]
10	32	26	29	299
11	34	26	30	329
12	33	25	29	358
13	34	25	29.5	387.5
14	33	26	29.5	417[F]

Table 4.14. Time taken by insects to grow in to successive stage during 7th Experiment

Life Stage	Control
1st Instar	22 hr
2nd Instar	23 hr
3rd Instar	22 hr

Pupa	133 hr
Fly	136 hr
Total Time	336 hr

Table 4.15. Temperature and ADD (Accumulate degree days calculated) of Mohali, Punjab during 8th experiment

8. Experimental setup in Mohali (Autumn)

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	34	24	29	29[1]
2	33.8	25.6	29.7	58.7[2]
3	33.4	24.6	29	87.7[3]
4	35	25.1	30.05	117.75
5	35.8	25.5	30.65	148.4
6	35	25.4	30.2	178.6
7	33.1	24.6	28.85	207.45
8	33.9	24.7	29.3	236.75
9	33.2	23.7	28.45	265.2[P]
10	33.8	24.6	29.2	294.4
11	34.1	24.7	29.4	323.8
12	34.4	23.1	28.75	352.55
13	33.9	22.3	28.1	380.65
14	33.7	22.1	27.9	408.55
15	33.8	22.4	28.1	436.65[F]

Table 4.16. Time taken by insects to grow in to successive stage during 8th Experiment

Life Stage	Control
1st Instar	24 hr
2nd Instar	26 hr
3rd Instar	25 hr

Pupa	137 hr
Fly	143 hr
Total Time	355 hr

Where,

[1] means appearance of 1st Instar Stage

[2] means appearance of 2nd instar stage

[3] means appearance of 3rd instar stage

[P] means appearance of Pupa stage

[F] means development of Pupa as fly

Conclusion

Based on the information presented, it can be inferred that the life cycle of insects is greatly influenced by temperature, humidity, and precipitation, particularly, during summer season when both the temperature and humidity were high, entire life cycle of insects was completed in only seven days in contrast to winter season, when the life cycle took approximately 32 days, which is four times longer than the summer season. This significant difference can be attributed to the colder temperatures and lower humidity levels during the winter months.

The appearance of species on meat or corpse is directly linked with the stage of decomposition and during present study, *Calliphora vicina* was the first to attack on the substrate within 1-2 hours during summer and 5-6 hours in winters which was followed by *Chrysomya megacephala* by attacking in 3-4 hours in summer and 10-12 hours in winter season. *Parasarcophaga albiceps* came after 48 hours during the summer season and after that *Musca sorbens* took nearly 72 hours to attack the substrate. It is important to note that these findings have significant implications for the field of entomology and forensic science. Insect colonization on a dead body is a common method used to estimate the time since death in forensic investigations. Interestingly, during the autumn

and spring, the life cycle of insects was found to be similar and this can be attributed to the fact that the weather condition during these seasons are generally similar, with moderate temperatures and levels of precipitation and humidity. The ADD shows the validation of study model as the time of appearance of different stages of insects are in accordance with the ADD calculation. Furthermore, these findings may have broader implications for our understanding of insect ecology and their role in natural ecosystems. Insects play important roles in pollination, decomposition, and nutrient cycling, and their life cycles are closely linked to environmental conditions.

In conclusion, this study highlights the impact of temperature, humidity, and precipitation on the life cycle of insects. The findings from this research indicate that the period for insect colonization can exhibit notable variation based on prevailing weather conditions. This variability is a crucial factor to consider when determining the time of death. The margin of error in estimating the time since death in this study was ±10 minutes for the 1st and 2nd instar stages, ±20 minutes for the 3rd instar stage, ±50 minutes for the pupa stage, and ±65 minutes for the fly stage.

4.3. OBJECTIVE-3- Identification and Estimation of Drug from Different Forensic Entomotoxicological Samples

4.3.1. Introduction

In most of the investigations, the cause of crime remains mystery and is the most important question to answer. In cases where the body of deceased is found partially or completely skeletonized, the toxicological analysis becomes difficult as there is a need of visceral organs to perform the same. But Entomo-toxicology can be of great help and mostly the only option available. Insects feeding on a cadaver can act as valuable indicators of the presence of drugs in the body. In this present work, solid phase extraction and Gas Chromatography- Mass spectrometry for the extraction and analysis of drug have been used. While various global research projects have explored the entomo-toxicological effects of a range of drugs, there has been limited work conducted in India. The limited availability of entomo-toxicological data in India represents a significant challenge in terms of determining the presence of drugs in criminal investigations.

4.3.2. Methodology

4.3.2.1. Preparation of specimen for toxicological examination-

Forceps were used to collect the larval insects from the source of the feed, and then they were rinsed with distilled water to prevent surface contamination and dried with paper towels. In order to prevent changes in Asenapine maleate content due to drug metabolism in larval bodies, insects were then stored at -10°C and kept in deep refrigeration at that temperature until analysis. From each substrate— 10µg, 20µg, 30µg, 40µg and 50µg, referred to as A, B, C, D, and E—as well as from substrate F, which was free of drugs, about 50 larvae of the feeding and post-feeding stages were collected.

4.3.2.2. Clean-up, Extraction and Purification procedure

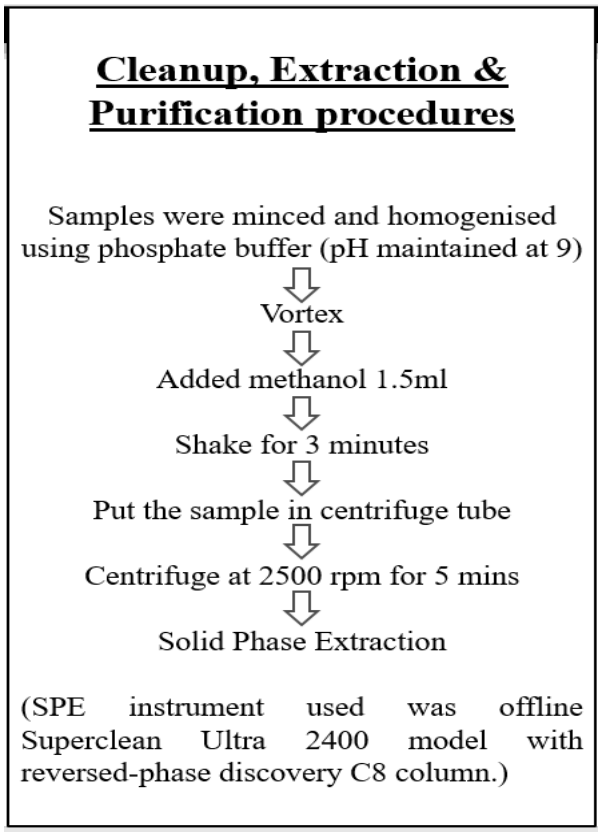


Figure 4.26. Clean up and purification procedure.

4.3.2.3. Extraction by Solid Phase Extraction

For the preparation of spiked samples, cartridges underwent conditioning with the addition of 2 ml of methanol and 2 ml of water. The sample was loaded onto the cartridge and passed through it. Subsequently, the cartridge underwent washing with 2 ml of water and 2 ml of 10% methanol. The elution process was conducted based on the pKa value of asenapine, utilizing acid and base. Given the pKa value of asenapine as 8.6, elution was performed with acetic acid into new vials. The eluted sample was then injected into the GC-MS for the generation of chromatograms.

4.3.2.4. Gas chromatography and Mass spectrometry

A) GC-MS Conditions

The drug analysis was carried out in a Shimadzu AOC-20N Plus auto-sampler connected to a GC-MS Shimadzu QP-2020 NX with a split/splitless injector in the splitless mode. The analysis was performed using a Shimadzu SH-RXi-5Sil-MS fused

silica capillary column, which is 30 meters long, has an inner diameter of 0.25 mm, and a stationary film thickness of 0.25 µm. The column was manufactured by Shimadzu in the USA. During the analysis, Helium (He) gas was used as the carrier gas, with a constant flow rate of 1.2 ml/minute. The injection volume was 5 µL (LVI), which was injected onto a Carbofrit plug in the liner with an open purge valve (30:1 split ratio) for 10 seconds. The inlet temperature was initially set at 90°C, which was ramped up to 290°C at a rate of 200°C/minute after a 30-second delay. The oven temperature program was set to 85°C for 3 minutes, followed by a 25°C/minute ramp to 180°C, then a 10°C/minute ramp to 300°C and held for 4 minutes. The total time for one GC-MS run was 23 minutes. The MS instrument transfer line temperature was set to 240°C, while the ion source temperature was set to 230°C. The ionization mode used was electron impact at 70eV full-scans (30-550 m/z). The optimization of the retention times and chromatographic resolution were conducted in the scan mode using all prepared standard concentrations.

4.3.3. Outcomes

I. QUALITATIVE ANALYIS

GC-MS #1 - 5 Sample (A, B, C, D, E) with different spiked concentration (10µg, 20µg, 30µg, 40µg, 50µg) of Asenapine maleate

Study # 1 was created to examine the degree of drug compound detection and capability in meat samples. To optimize sensitivity, an injection quantity of 2µL was used. GC-MS analyses confirmed that the asenapine maleate which were spiked to the fodder sample was present in the insects' larvae with the concentration of 20µg, 30µg, 40µg, 50µg.

As it can be said that asenapine maleate could not be detected in homogenised sample of 10µg ASPM (jar A sample). However, GC-MS chromatograms qualitatively and quantitatively confirmed its presence rest of the samples (B, C, D, and E).

Among all the six chromatograms (of both the location- Mohali and Jalandhar) shown here, it is inferred that the asenapine maleate drug, the peak of the drug was detected and highlighted that peak in the respective chromatogram.

The Retention time (R_t) of all the sample jars (A, B, C, D, and E) as follow- (the data of retention time is of the winter season, mentioned as figure 1 to figure 5)

- R_t of sample A - no detection
- R_t of sample B- 14.886
- R_t of sample C- 14.87
- R_t of sample D- 14.883
- R_t of sample E- 14.855

The Retention time (R_t) of all the sample jars (A, B, C, D, and E) as follow- (the data of retention time is of the summer season, mentioned as graph 7 to graph 11)

- R_t of sample A – no detection
- R_t of sample B - 14.870
- R_t of sample C - 14.857
- R_t of sample D- 14.857
- R_t of sample E- 14.859

GC-MS study #2- blank sample (named F jar)

The term "blank" denotes a sample devoid of the targeted drug, which can either consist of the solvent or mobile phase alone (referred to as an aqueous blank) or an extracted blank without any added analyte. Conducting a blank sample is essential to validate baseline stability and ascertain the absence of contamination in the sample or solvent. Injection of the blank samples produced no peak, which indicates that there is no contamination present.

The conclusion of this study #2 is that the asenapine maleate concentration was absent (lower than the LOD) in the blank samples and in the jar F analysed by GC-MS. GC-MS chromatogram qualitative determined the absence of ASPM in control meat sample. The chromatogram of control sample having no ASPM of winter season is shown in graph 6 and in graph 12 of summer season.

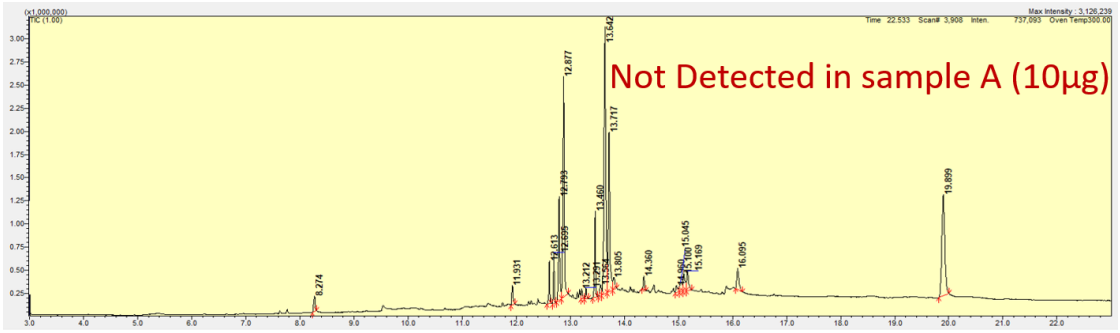
GC-MS study #3- standard sample of Asenapine maleate (ASPM)

Working standard sample of ASPM was prepared by diluting with methanol at 1mg/ml and were stored in the absence of light at 4 °C. The GC-MS of standard sample of ASPM is shown here, and peak of ASPM is highlighted.

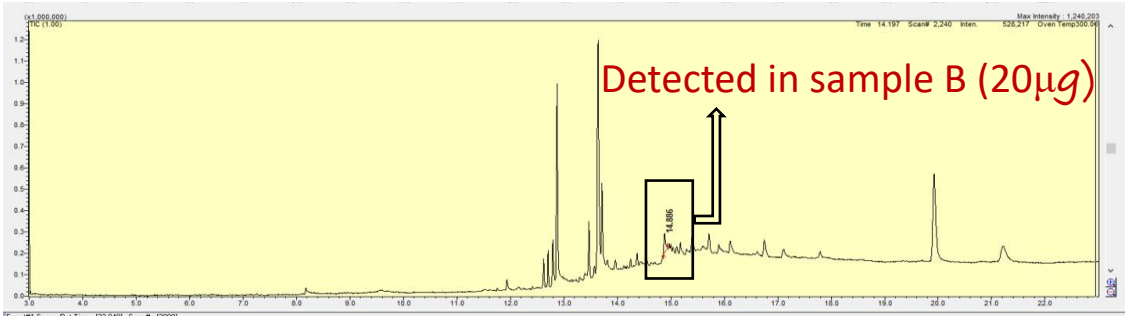
The retention time (R_t) of ASPM in standard solution is - 14.9

The GC-MS chromatogram of all six sample of winter season is as follow accordingly the concentration of ASPM from lower to higher. From this objective, it can be said that the drug can be found in insects' sample or evidence by GC-MS if the drug concentration is about 20µg.

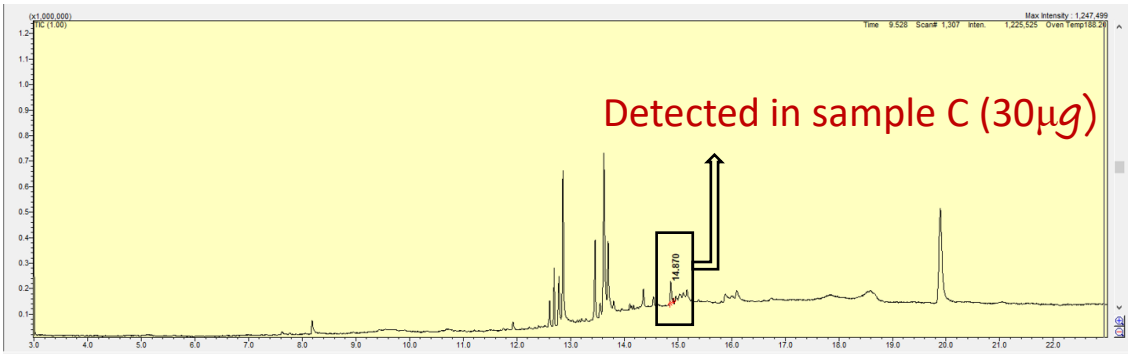
The graph from 4.1 to 4.6 showing below were represented the samples taken in winter season.



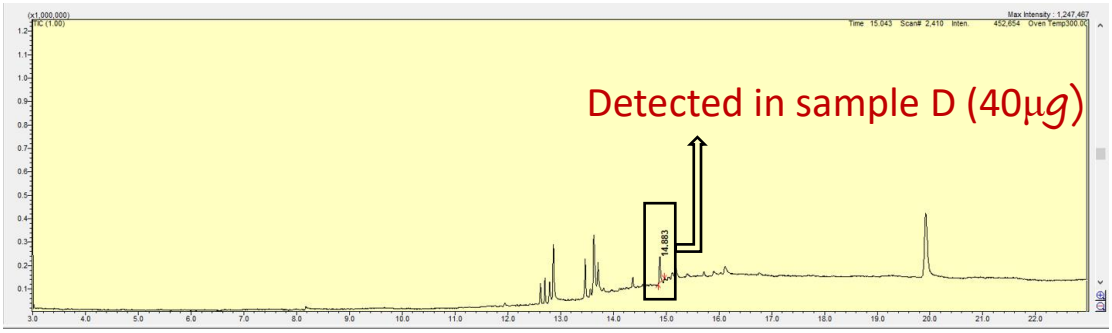
Graph 4.1. showing GCMS graph of sample A (10µg)



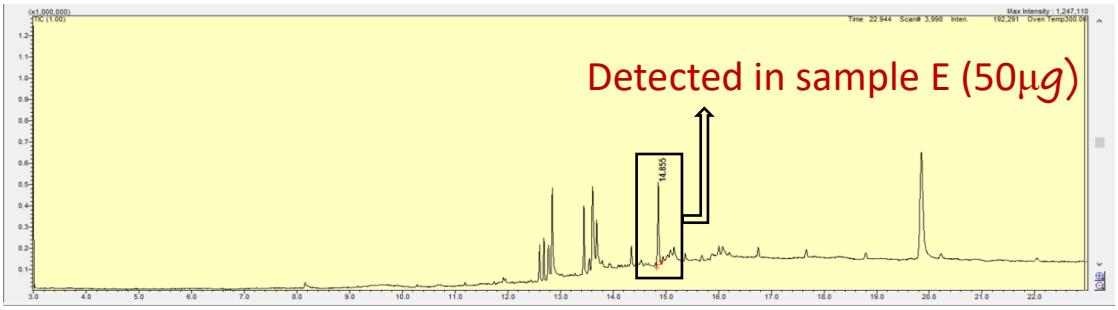
Graph 4.2. showing GCMS graph of sample B (20µg)



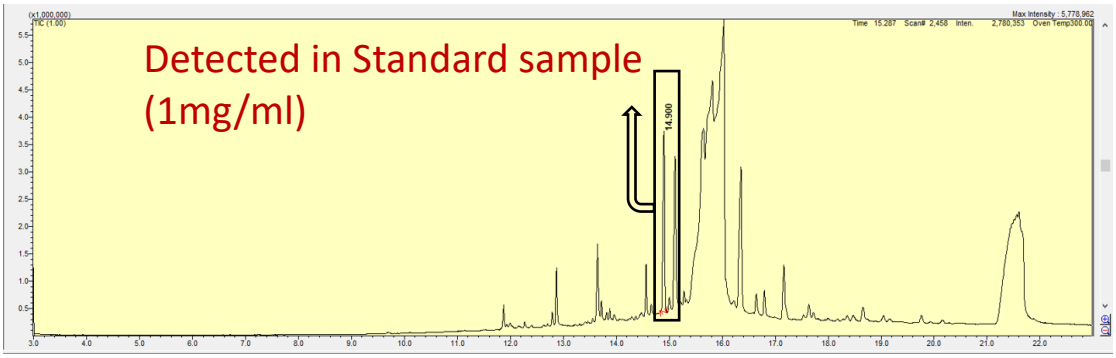
Graph 4.3. showing GCMS graph of sample C (30µg)



Graph 4.4. showing GCMS graph of sample D (40µg)

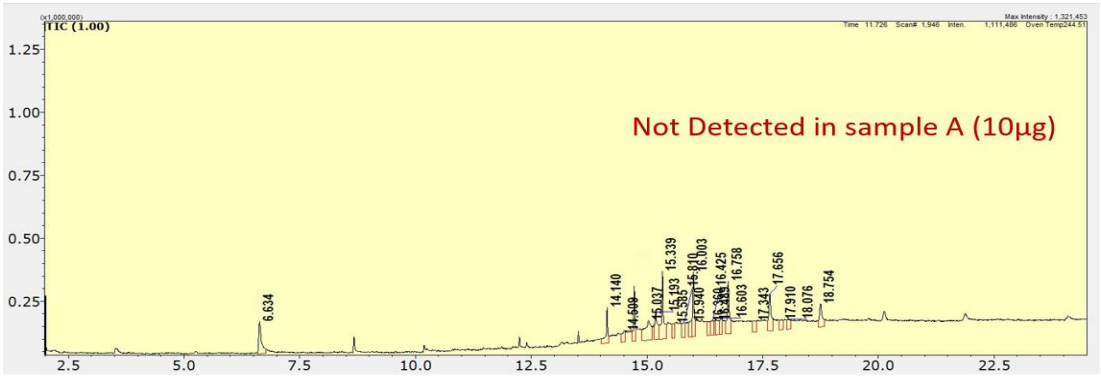


Graph 4.5. showing GCMS graph of sample E (50µg)

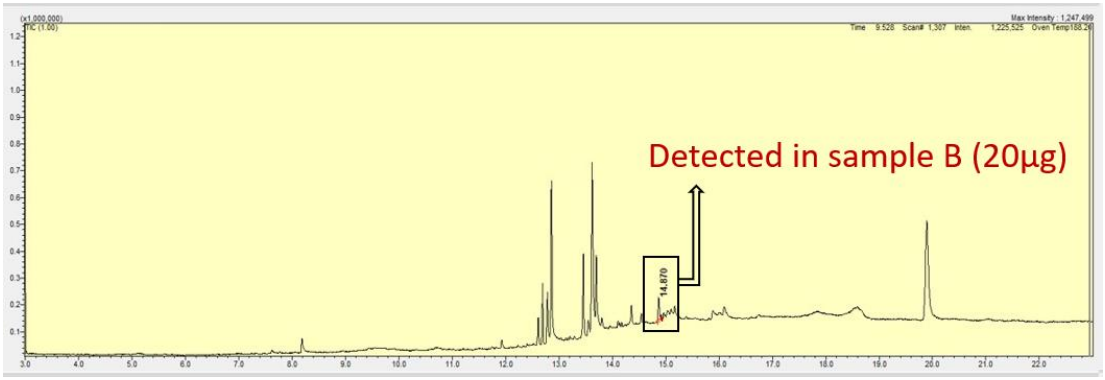


Graph 4.6. showing GCMS graph of Standard (1mg/ml)

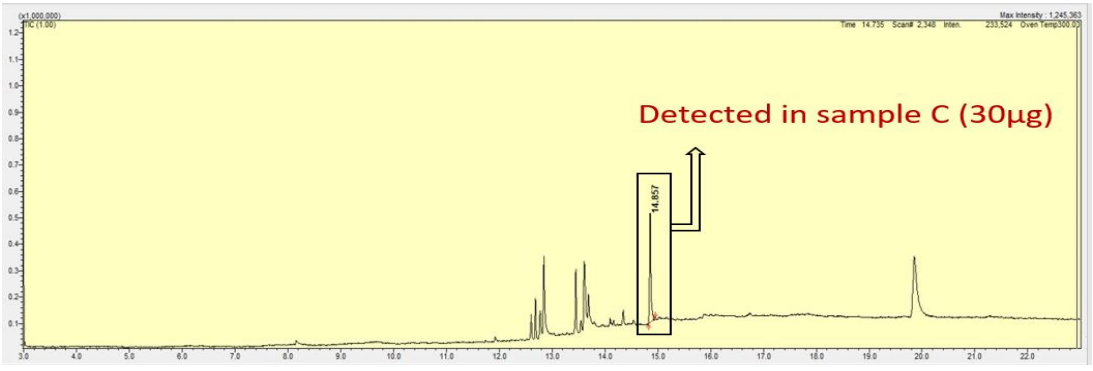
The graph from 4.7to 4.11 showing below were represented the samples taken in summer season.



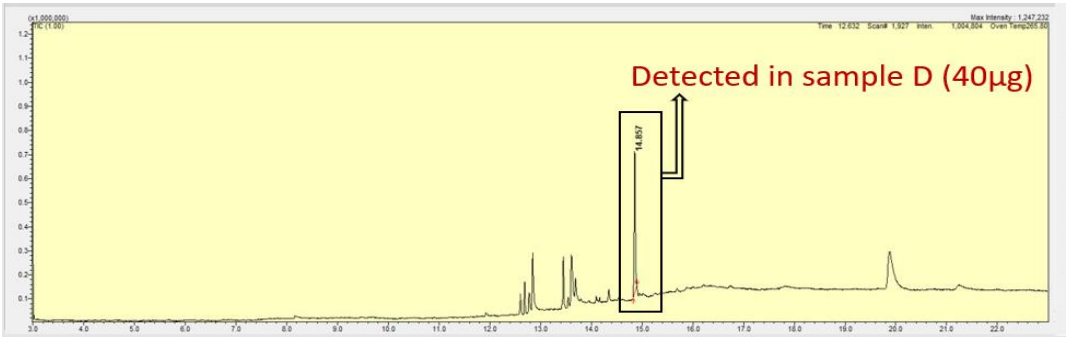
Graph 4.7. showing GC-MS graph of sample A (10µg)



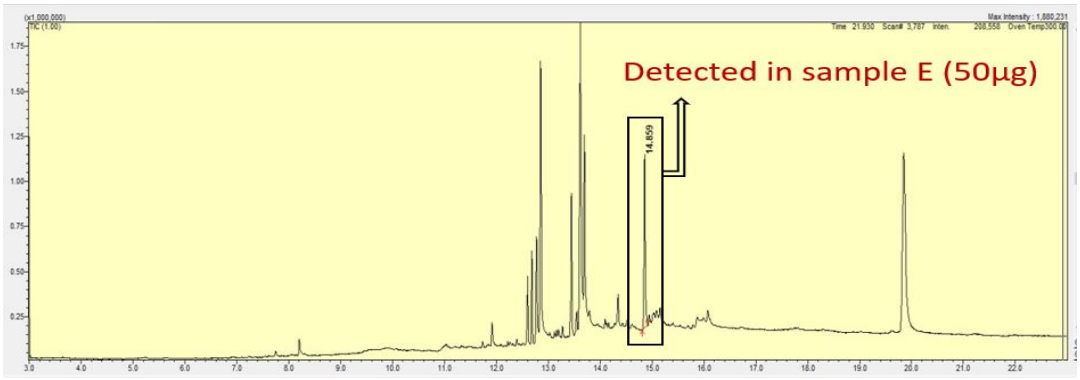
Graph 4.8. showing GC-MS graph of sample B (20µg)



Graph 4.9. Showing GC-MS graph of sample C (30µg)



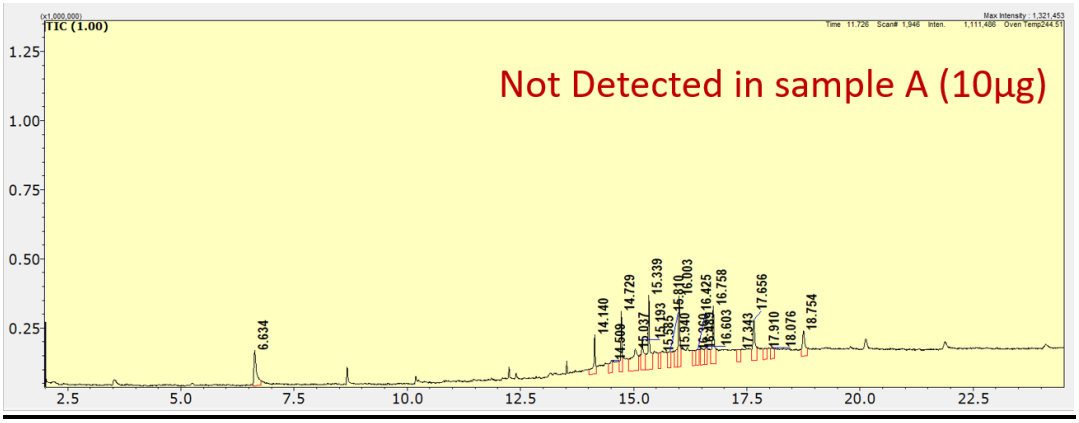
Graph 4.10. showing GC-MS graph of sample D (40µg)



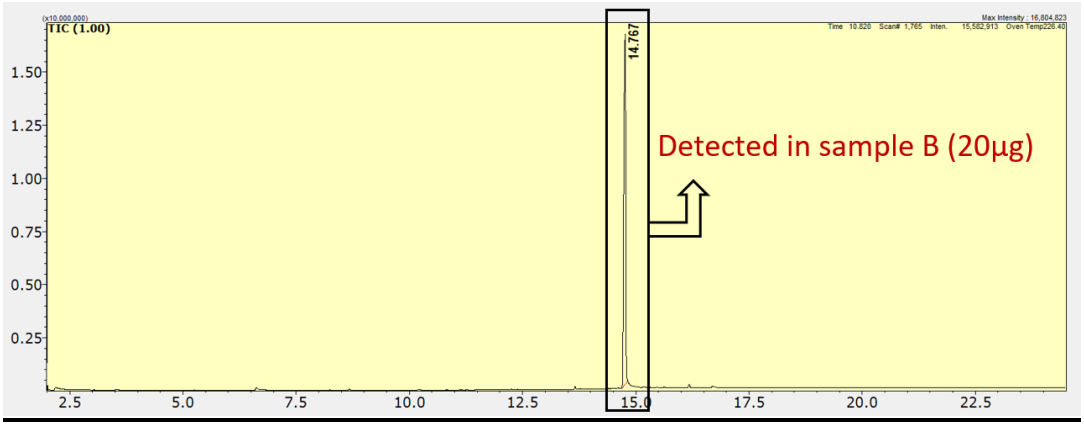
Graph 4.11. showing GC-MS graph of sample E(50µg)

II. QUANTITATIVE ANALYSIS

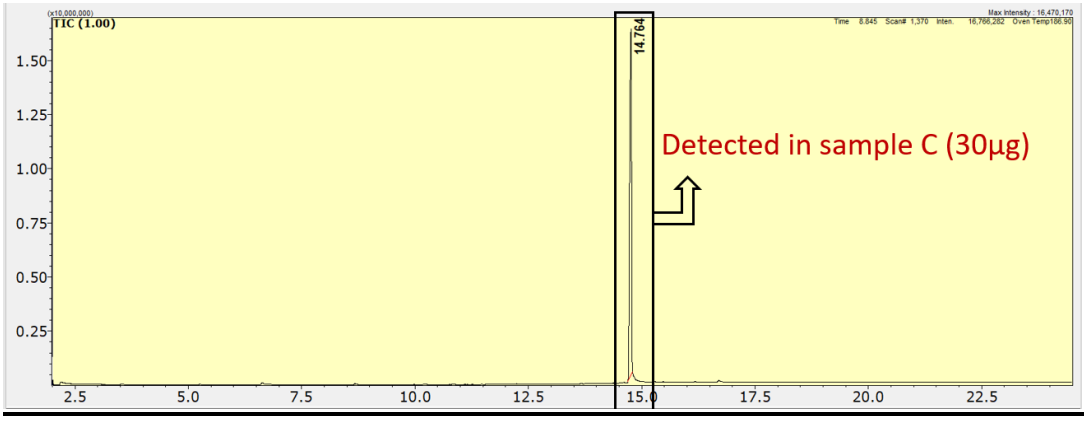
The quantitative analysis was done to gain the more knowledge about the analysis. The same process was followed as before but only 50 eggs were transferred to each substrate this time so that the total number of feeding maggots to be known. Priorly a meat was kept and *Calliphora vicina* flies were introduced to lay their eggs, then a total of 250 eggs were collected with the help of a paint brush and 50 eggs were then transferred to each substrate named A, B, C, D, and E with concentrations of drug 10µg, 20µg, 30µg, 40µg, 50µg respectively. After their growth to 3rd instar larvae stage, both feeding and post feeding larvae were collected by counting 50 each. The same procedure of extraction and analysis was followed, and it was found that the concentration of drug extracted was more this time.



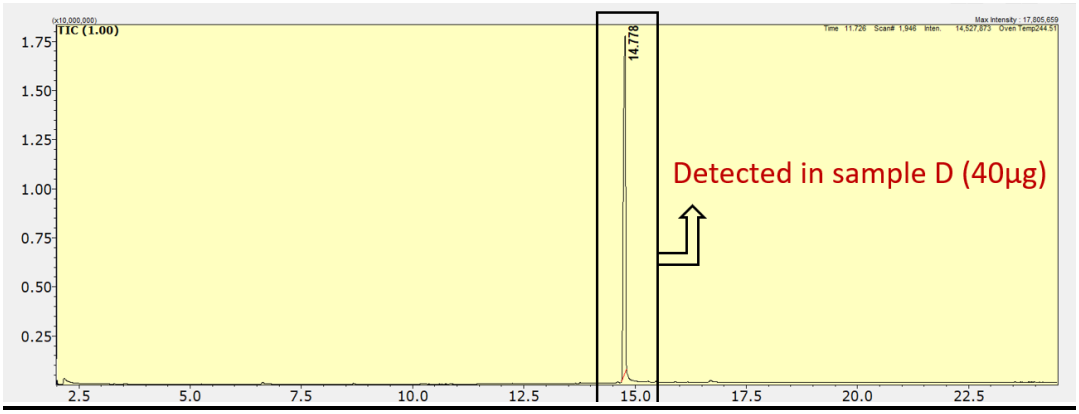
Graph 4.12. showing GC-MS graph of sample A (10µg)



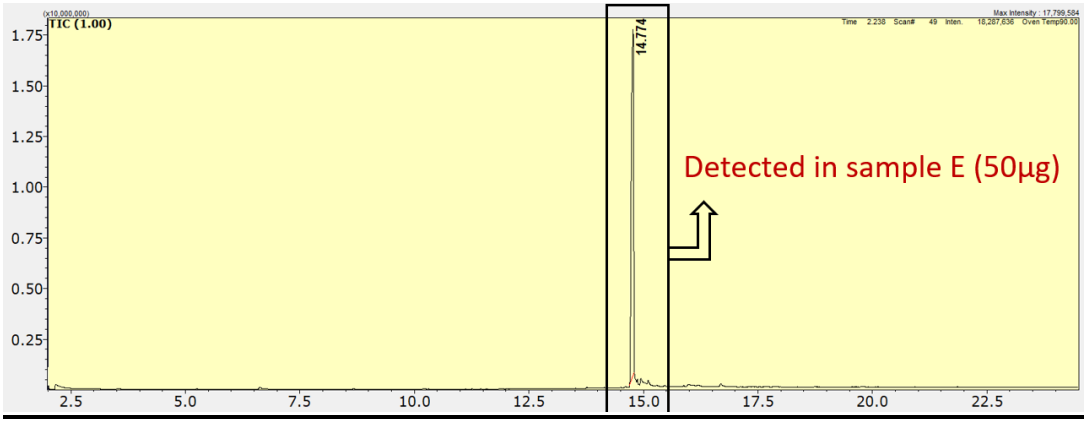
Graph 4.13. showing GC-MS graph of sample B (20µg)



Graph 4.14. showing GC-MS graph of sample C (30µg)



Graph 4.15. showing GC-MS graph of sample D (40µg)



Graph 4.16. showing GC-MS graph of sample E(50µg)

The method of analysis was validated by using entomo-toxicological and biological samples. The method selection was done by analysing the control samples in identifying the interference of asenapine maleate as well as absence of asenapine maleate. The calibration curve of asenapine maleate was constructed using 5 points ranging from 10µg/ml to 50µg/ml. The area response was plotted against the concentration which generated the regression analysis of y= 924499x + 1E+07 with R^2= 0.9957. Table 4.17. and Figure 4.18. shows the validation method and standard calibration curve respectively. In analysis through GCMS, the drug was detected successfully from the four higher concentration samples i.e., 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml only, but not in 10µg/ml concentration sample due to lower than the limit of detection. This

particular drug was previously reported in many deaths (Boer *etal.*2012). The developed protocol of analysis will be helpful in the field of entomo-toxicology.

Asenapine maleate has been the subject of spiking research in human plasma and urine. HPLC-MS/MS and LC-TMS were used in the analysis, even though the pattern of analysis was different from the current study (Boer *et al.,* 2012; Prasad and Aneesh, 2022). However, asenapine was injected into post-mortem human liver in the current study, and Solid Phase Extraction and Gas Chromatography-Mass Spectrometry were used for analysis. There are different cases reported regarding entomo-toxicology where deceased were found skeletonised, both PMI estimation and drug and drug analysis by done with the help of insects (Groth *et al.,* 2022). In a recent case study, two deceased were found in their respective apartments and their body was completely skeletonized. It was suspected that cause of death can be drug overdose and the analysis was done taking maggots as sample using LC-TMS and Liquid Chromatography-quadruple time of-flight mass spectrometry (El-Ashram *et al.,* 2022).

In another study, aluminium phosphide was used to check the forensic entomo-toxicological significance and larvae of known insect species *Chrysomya albiceps* were allowed to rear on spiked rat substrate. The researchers had noticed the significant reduce in body length of larvae due to aluminium phosphide and it was concluded that higher dose of aluminium phosphide can cause length reduction in larvae whereas in present study, the drug analysis was done using GCMS and there was no difference found in length of larvae from control samples and spiked samples (Miller *et al.,* 2013). There are numerous cases where Asenapine maleate have been used for drug abuse and 4 such cases are reported in a study where the analysis was done using GCMS same as present study but the drug was extracted from biological matrices like liver, heart, jugular vein etc (Boer *et al.,* 2012). There are always three major questions in front of investigator to solve the mystery of crime which are When, Where and How? Forensic entomology and entomo-toxicology can answer all three of them in any kind of homicide, suicide, or accidental deaths. And this present study is solving answering these questions perfectly by estimating PMI, insects identification helps to find the place of crime and drug identification helps in finding the manner of crime.

Table 4.17. Validation of Asenapine maleate analysis using Gas Chromatography-Mass spectrometry

Parameter	Value
Linearity	10 - 50µg/ml
R^2	0.9957
LOD	9.26469µg/ml
LOQ	28.0748µg/ml
Precision (%RSD)	4.25%
Recovery	67%

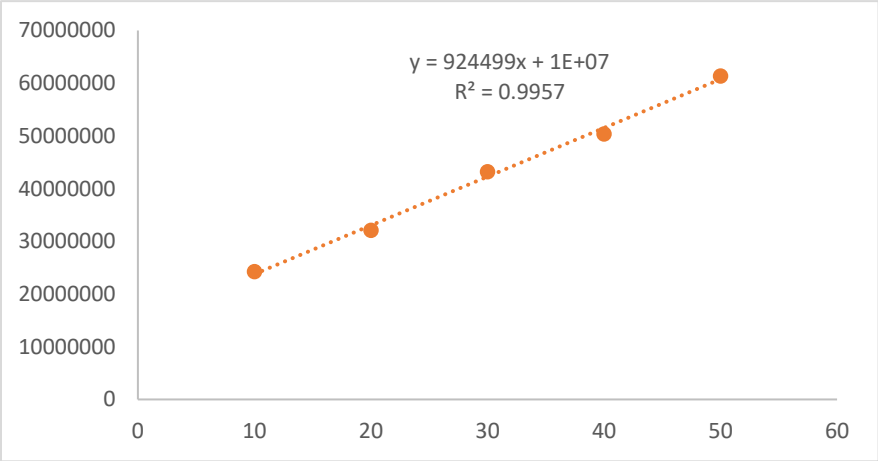


Figure 4.27. Calibration curve of Asenapine maleate standard

Conclusion

The presented data illustrates the outcomes of a study focused on detecting and quantifying Asenapine maleate in different samples with varying concentrations. Samples A, B, C, D, and E underwent analysis with drug concentrations of 10µg, 20µg, 30µg, 40µg, and 50µg, respectively, utilizing a GC-MS instrument. The study aimed to identify and measure the drug in each sample. The results revealed that out of the five samples examined, only samples B, C, D, and E exhibited drug detection. Sample A,

with a concentration of 10µg, did not register any drug detection due to its concentration falling below the instrument's limit of detection.

Additionally, the study sought to quantify the drug in each sample using the same GC-MS instrument. The quantitative analysis demonstrated higher drug recovery compared to the qualitative study, indicating that drug quantification was more accurate and reliable than detection. Drug recovery, representing the percentage of the drug recovered from the sample during analysis, correlated with more accurate quantification. These findings have significant implications for the detection and quantification of Asenapine maleate in diverse samples.

The study underscored that drug detection is constrained by instrument sensitivity and the drug concentration in the sample. Therefore, maintaining the drug concentration above the instrument's limit of detection is crucial for accurate detection. Moreover, the research highlighted the superior accuracy and reliability of drug quantification compared to detection. Consequently, for researchers and scientists studying the drug's effects and requiring precise concentration measurements in various samples, quantitative analysis is recommended over qualitative analysis.

In conclusion, the data presented emphasizes the dependence of detecting and quantifying Asenapine maleate in various samples on instrument sensitivity and drug concentration. The study revealed limitations in drug detection linked to instrument sensitivity, with quantification proving to be a more accurate and reliable approach. These findings hold significance for researchers studying the drug's effects and aiming for precise concentration measurements in diverse samples.

4.4. OBJECTIVE-4-Impact of drug on forensic entomotoxicological samples

4.4.1. Introduction

Although previous studies have examined the effects of drugs such as heroin, morphine, and dolpezam on insect populations, there remains a vast pool of drugs yet to be studied. In-depth entomo-toxicology research on these drugs is essential to improve our understanding of their effects on insects and to aid forensic investigators in identifying the presence of drugs in the body.

4.4.2. Methodology

As already mentioned, all Jars (A, B, C, D, E) were spiked with Asenapine maleate (ASPM) with different concentration like 10µg, 20µg, 30µg, 40µg, 50µg and Jar F kept as a blank sample without spiked with ASPM.

The development of 1^{st} Instar, 2^{nd} Instar, 3^{rd} Instar, Pupa and fly in various concentration of ASPM and its effects in life cycle of insects (*Calliphora vicina)* shown in tables and also mentioned the time (in hr).

The below tables show about the Estimation of PMI and effect of ASPM drug on insects' life cycle is presented below in accordance with experiment duration and location-

4.4.3. Outcomes

Table 4.18. Time taken by insects to grow in to successive stage during 1st Experiment

1. Winter Season (Mohali)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	60 hr	30 hr	27 hr	27 hr	26 hr	25 hr
2ND INSTAR	70 hr	47 hr	46 hr	46 hr	45 hr	45 hr
3RD INSTAR	72 hr	60 hr	55 hr	55 hr	48 hr	48 hr
PUPA	170 hr	165 hr	161 hr	161 hr	161 hr	147 hr
FLY	445 hr	440 hr	437 hr	435 hr	426 hr	426 hr
Total Time	817 hr	742 hr	726 hr	724 hr	692 hr	691 hr

Table 4.19. Analysis by Single factor ANOVA on data of 1st experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	1634	272.3333	92621.07
10µg	6	1484	247.3333	82211.07
20µg	6	1452	242	79690.4
30µg	6	1448	241.3333	79148.27
40µg	6	1398	233	73106.4
50µg	6	1382	230.3333	73439.87

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6791.222	5	1358.244	0.01697	0.999879	2.533555
Within Groups	2401085	30	80036.18			
Total	2407877	35				

Table 4.20. Time taken by insects to grow in to successive stage during 2nd Experiment

2. Winter Season (Jalandhar)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	52 hr	27 hr	24 hr	24 hr	21 hr	20 hr
2ND INSTAR	48 hr	36 hr	33 hr	32 hr	30 hr	28 hr
3RD INSTAR	52 hr	45 hr	40 hr	39 hr	34 hr	31 hr
PUPA	143 hr	135 hr	133 hr	129 hr	126 hr	122 hr
FLY	330 hr	313 hr	310 hr	305 hr	303 hr	299 hr
Total Time	625 hr	556 hr	540 hr	529 hr	514 hr	500 hr

Table 4.21. Analysis by Single factor ANOVA on data of 2nd experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	1250	208.3333	53453.87
10µg	6	1112	185.3333	44657.87
20µg	6	1080	180	42850.8
30µg	6	1058	176.3333	41213.47
40µg	6	1028	171.3333	39649.47
50µg	6	1000	166.6667	37952.67

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6521.333	5	1304.267	0.030124	0.999504	2.533555
Within Groups	1298891	30	43296.36			
Total	1305412	35				

Table 4.22. Time taken by insects to grow in to successive stage during 3rd Experiment

3. Spring Season (Jalandhar)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	37 hr	23 hr	21 hr	21 hr	20 hr	18 hr
2ND INSTAR	33 hr	29 hr	26 hr	25 hr	23 hr	22 hr
3RD INSTAR	30 hr	28 hr	27 hr	27 hr	25 hr	24 hr
PUPA	137 hr	127 hr	125 hr	124 hr	121 hr	118 hr
FLY	173 hr	163 hr	162 hr	160 hr	156 hr	156 hr
Total Time	410 hr	370 hr	361 hr	357 hr	345 hr	338 hr

Table 4.23. Analysis by Single factor ANOVA on data of 3rd experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	820	136.6667	21617.87
10µg	6	740	123.3333	18097.07
20µg	6	722	120.3333	17431.07
30µg	6	714	119	17050.8

40µg	6	690	115	16041.2
50µg	6	676	112.6667	15545.07

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2163.667	5	432.7333	0.024545	0.999699	2.533555
Within Groups	528915.3	30	17630.51			
Total	531079	35				

Table 4.24. Time taken by insects to grow in to successive stage during 4th Experiment

4. Spring Season (Mohali)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	20 hr	20 hr	19 hr	18 hr	17 hr	16 hr
2ND INSTAR	22 hr	20 hr	20 hr	19 hr	18 hr	18 hr
3RD INSTAR	20 hr	21 hr	19 hr	18 hr	18 hr (70% maggots died)	16 hr (70% maggots died)
PUPA	128 hr	128 hr	127 hr	127 hr	128 hr	127 hr
FLY	131 hr	128 hr	125 hr	124 hr	124 hr	125 hr
Total Time	321 hr	317 hr	310 hr	306 hr	305 hr	302 hr

Table 4.25. Analysis by Single factor ANOVA on data of 4th experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	642	107	13835.2
10µg	6	634	105.6667	13501.07
20µg	6	620	103.3333	12981.87
30µg	6	612	102	12745.2
40µg	6	610	101.6667	12741.07
50µg	6	604	100.6667	12598.27

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	183.2222	5	36.64444	0.002804	0.999999	2.533555
Within Groups	392013.3	30	13067.11			
Total	392196.6	35				

Table 4.26. Time taken by insects to grow in to successive stage during 5th Experiment

5. Summer Season (Jalandhar)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	12 hr	12 hr	11 hr	11 hr	11 hr	10 hr
2ND INSTAR	10 hr	12 hr	11 hr	11 hr	10 hr	11 hr
3RD INSTAR	11 hr	11 hr	11 hr	10 hr	9 hr (70% maggots died)	10 hr (70% maggots died)
PUPA	84 hr	82 hr	81 hr	83 hr	82 hr	82 hr
FLY	80 hr	80 hr	80 hr	81 hr	82 hr	83 hr
Total Time	197 hr	197 hr	194 hr	196 hr	194 hr	196 hr

Table 4.27. Analysis by Single factor ANOVA on data of 5th experiment

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	394	65.66667	5351.467
10µg	6	394	65.66667	5293.867
20µg	6	388	64.66667	5173.867
30µg	6	392	65.33333	5319.467
40µg	6	388	64.66667	5259.067
50µg	6	392	65.33333	5347.867

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.222222	5	1.244444	0.000235	1	2.533555
Within Groups	158728	30	5290.933			
Total	158734.2	35				

Table 4.28. Time taken by insects to grow in to successive stage during 6th Experiment

6. Summer Season (Mohali)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	9 hr	9 hr	9 hr	9 hr	8 hr	9 hr
2ND INSTAR	8 hr	7 hr	6 hr	8 hr	7 hr	7 hr
3RD INSTAR	8 hr	8 hr	9 hr	8 hr	7 hr (70% maggots died)	8 hr (70% maggots died)
PUPA	70 hr	66 hr	65 hr	68 hr	68 hr	69 hr
FLY	77 hr	76 hr	77 hr	76 hr	75 hr	75 hr
Total Time	172 hr	166 hr	166 hr	169 hr	165 hr	168 hr

Table 4.29. Analysis by Single factor ANOVA on data of 6th experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	344	57.33333	4179.867
10µg	6	332	55.33333	3902.267
20µg	6	332	55.33333	3907.467
30µg	6	338	56.33333	4025.867
40µg	6	330	55	3897.2
50µg	6	336	56	3997.6

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	22.22222	5	4.444444	0.001115	1	2.533555
Within Groups	119551.3	30	3985.044			
Total	119573.6	35				

Table 4.30. Time taken by insects to grow in to successive stage during 7th Experiment

7. Autumn Season (Jalandhar)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	22 hr	20 hr	19 hr	18 hr	17 hr	17 hr
2ND INSTAR	23 hr	20 hr	20 hr	19 hr	18 hr	18 hr
3RD INSTAR	22 hr	20 hr	19 hr	18 hr	18 hr	17 hr
PUPA	133 hr	128 hr	127 hr	127 hr	128 hr	127 hr
FLY	136 hr	128 hr	125 hr	124 hr	124 hr	123 hr
Total Time	336 hr	316 hr	310 hr	306 hr	305 hr	302 hr

Table 4.31. Analysis by Single factor ANOVA on data of 7th experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	672	112	15062.8
10µg	6	632	105.3333	13450.67
20µg	6	620	103.3333	12981.87
30µg	6	612	102	12745.2
40µg	6	610	101.6667	12741.07
50µg	6	604	100.6667	12512.27

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	519.6667	5	103.9333	0.007845	0.999982	2.533555
Within Groups	397469.3	30	13248.98			
Total	397989	35				

Table 4.32. Time taken by insects to grow in to successive stage during 8th Experiment

8. Autumn Season (Mohali)

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	**24 hr**	**22 hr**	**22 hr**	**21 hr**	**19 hr**	**19 hr**
2ND INSTAR	**26 hr**	**21 hr**	**20 hr**	**18 hr**	**18 hr**	**17 hr**
3RD INSTAR	**25 hr**	**21 hr**	**19 hr**	**18 hr**	**15 hr**	**15 hr**
PUPA	**137 hr**	**128 hr**	**127 hr**	**127 hr**	**126 hr**	**125 hr**
FLY	**143 hr**	**132 hr**	**129 hr**	**128 hr**	**126 hr**	**126 hr**
Total Time	**355 hr**	**324 hr**	**317 hr**	**312 hr**	**304 hr**	**302 hr**

Table 4.33. Analysis by Single factor ANOVA on data of 8th experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	710	118.3333	16620.67
10µg	6	648	108	14033.2
20µg	6	634	105.6667	13502.27
30µg	6	624	104	13210
40µg	6	608	101.3333	12693.47

50µg	6	604	100.6667	12555.47

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1258.667	5	251.7333	0.018282	0.999854	2.533555
Within Groups	413075.3	30	13769.18			
Total	414334	35				

Conclusion

According to the data presented in the tables, following observations can be made:

During the summer months of June and July, both in Jalandhar and Mohali locations, at least 70 percent of maggots died when exposed to concentrations of 40µg and 50ug. In April, the life cycle of insects was completed in 18 days and 16 days when exposed to concentrations of 40µg and 50µg, respectively. Similarly, in June, the life cycle of insects was completed in 9 days and 10 days when exposed to concentrations of 40µg and 50µg, respectively. In the following month of July, the life cycle of insects was completed in 7 days and 8 days when exposed to concentrations of 40µg and 50µg, respectively.

The Single factor Analysis of Variance (ANOVA) test results indicate that there is a gradual increase in the growth rate of decomposers with an increase in the concentration of the drug. This trend is consistent across all the seasons.

To summarize, the data indicates that the drug concentration has a significant effect on the growth rate of decomposers, particularly maggots, during the summer months in both Jalandhar and Mohali locations. The higher the drug concentration, the shorter the life cycle of insects, and the higher the mortality rate. This finding has implications for the management of waste and the control of insect populations, particularly during the summer months. Future research could investigate the impact of other environmental

factors, such as temperature and humidity, on the efficacy of the drug in controlling insect populations.

4.5. Application of developed study on Human Post-mortem Liver sample

4.5.1. Introduction

The present research work was done on goat meat samples which makes it necessary to replicate the same process on human post-mortem sample for the validation of process. Complete process was applied on human post-mortem liver which was collected from mortuary of Govt. Medical College and Hospital, Chandigarh. The liver was of an unknown dead body died due to accident. The ethical permission was priorly taken from Institutional Ethical committee of GMCH, Chandigarh. A total of 600 gms liver was collected which was further divided in to 6 pieces and complete experiment was done in old mortuary of GCMH, Chandigarh

Location- Old Mortuary, Govt Medical College and Hospital, Sector 32, Chandigarh, India

Location co-ordinates- Northern Latitude 30.4230°N; 76.4652°E Eastern Longitude

Figure 4.28. Post-mortem investigation of deceased

4.5.2. Methodology

4.5.2.1. Preparation of working and stock solution

Asenapine maleate, sourced from the Indian Pharmacopeia, was acquired and subsequently formulated into a stock solution with a concentration of 1.0 mg/ml in methanol. Utilizing this stock solution, working solutions of 10µg, 20µg, 30µg, 40µg, and 50µg were prepared in methanol. These working solutions were then employed in quantitative analyses to fortify blank matrices and establish calibration levels.

4.5.2.2. Experimental food substrate.

The food substrate was human post-mortem liver (750gms.) taken from mortuary of GMCH Chandigarh and used in this study. Then, the liver sample was divided into 6 parts, each of 125 grams (named-A, B, C, D, E and F), and transferred into medium sized jars approximately 15 centimetres (6 inches) tall and 7.5 centimetres (3 inches) wide at the mouth, in which five of them were spiked with 5 different Concentration of drug i.e., 10µg, 20µg, 30µg, 40µg and 50µg referred as A, B, C, D, E and one Jar (named F) was used as control or blank.

A - 10µg ASPM
B – 20µg ASPM
C – 30µg ASPM
D – 40µg ASPM
E – 50µg ASPM
F – No ASPM (As a control)

Figure 4.29. Spiking drug in post-mortem liver

Figure 4.30. attack of flies on liver

4.5.2.3. Collection of insects

I. **Adults Insects**- Insect collection net was used to collect adult flies and they were further preserved in 70% and ethanol solution so they won't shrink till the time of identification.

   Labels for vials included the site name, city location, date, time, sample number of insect.

II. **Larvae** - Collection of larvae was accomplished using forceps. Approximately 50 maggots were of feeding and post feeding stage were collected in vials from each experimental set up and preserve in deep refrigerator at -10°C till toxicological analysis. And for the purpose of microscopy, the collected maggots were preserved in 70% ethanol after washing with boiling water to avoid the surface contamination.

The control jar/sample consisted of insects of the same type and age but fed with fodder not treated with drug.

4.5.2.4. Chemical and Reagents-

All solvents and reagents were of analytical grade and were of high-quality standards.

Reagent	Procured from
Asenapine Maleate (for standard/spiked)	Indian Pharmacopoeia
Potassium Di hydrogen ortho phosphate (Phosphate buffer)	Sigma-Aldrich
Methanol	Thermofisher Scientific
Ammonia solution	Sigma-Aldrich

4.5.2.5. Tools and Techniques used for toxicological analysis:

Solid phase extraction Superclean Ultra 2400 model with C8 Column

GC–MS Shimadzu QP-2020 NX (Kyoto, Japan) coupled with auto-sampler Shimadzu AOC-20N Plus

Injector- Splitless mode

Column- SH- RXi-5Sil-MS fused silica capillary column

Column dimensions- 30 m×0.25 mm ID×0.25µm

Laboratory tools- Injector vial, RIA vial, micro pipette, electronic balance, vortex, centrifuge, beaker, flask, evaporator, spatula, refrigerator, viscera cutter etc.

4.5.3. Outcomes

4.5.3.1. Duration of experiment

The total time taken during this study was 318 hours approx. 14 days. It began on 1st September 2022 and completed on 13th September 2022. The liver was collected in the morning and was divided and spiked with drug immediately.

4.5.3.2. Flies collected and identified-

Calliphora vicina, Chrysomya megacephala and *Sarcophaga parasarcophaga* albiceps were collected during the study, which was priorly identified using taxonomic keys and confirmed by Zoological Survey of India. These species are similar to those found in Mohali. The flies attacked this time only in 3-4 hours which is similar to other experiments with same weather condition.

Table 4.34. Temperature data and ADD (Accumulate degree days calculated) during the experiment

Day(s)	Min. Temperature (in °C)	Max. Temperature (in °C)	Average Temperature (in °C)	ADD
1	35.5	27.1	31.3	31.3[1]
2	35.4	26.9	31.15	62.45[2]
3	34.5	26.2	30.35	92.8[3]
4	33	25.7	29.35	122.15
5	36	26.6	31.3	153.45
6	35.6	24.8	30.2	183.65
7	35	25.6	30.3	213.95

8	35.1	25.5	30.3	244.25[P]
9	36.2	26.6	31.4	275.65
10	35.6	27.4	31.5	307.15
11	35.1	27	31.05	338.2
12	35	26.3	30.65	368.85
13	34.3	25.5	29.9	398.75[F]

Where,

[1] means appearance of 1st Instar Stage

[2] means appearance of 2nd Instar stage

[3] means appearance of 3rd Instar stage

[P] means appearance of Pupa stage

[F] means development of Pupa as fly

Calculation of ADD (Accumulate Degree Days)

To calculate the accumulated degree days, the minimum and highest temperatures were added, and the total was divided by two, to determine the daily average temperature. If the resulting average temperature surpasses the specified threshold temperature, which serves as the base temperature for development, the accumulated degree days for that 24-hour period were determined by subtracting the threshold temperature from the calculated average. For the next day, Calculated the average temperature of that day and added it to ADD of previous day. The ADD of subsequent days was then calculated with the same formula and the appearance of subsequent stages of insects was validated by dividing the ADD of that particular day with the mean of average temperature of complete duration.

4.5.3.3. Drug Analysis

Preparation of specimen for toxicological examination-

Larval insects were detached from the fodder source using forceps, washed with distilled water to avoid the surface contamination, and dried using paper towel. Insects were then sacrificed by freezing at -10°C and preserved in deep refrigerator at same temperature till analysis in order to avoid the modification of Asenapine maleate

concentration by metabolism of drug in larval body. Approximately 50 larvae were collected from each substrate i.e., 10µg, 20µg, 30µg, 40µg and 50µg referred as A, B, C, D, E and also from substrate F which was drug free.

4.5.3.4. Clean-up and Purification procedure

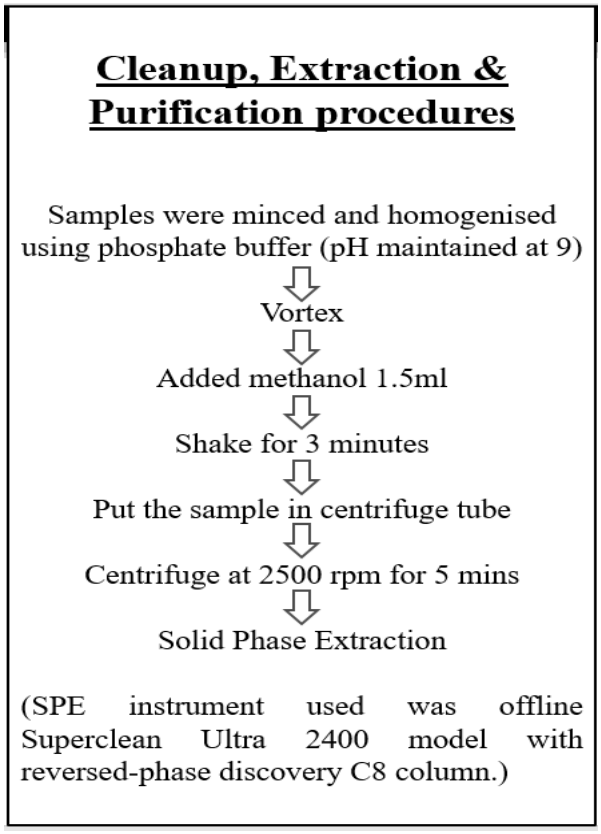


Figure 4.31. Clean-up and purification procedure

4.3.5.5. Extraction by Solid Phase Extraction

To prepare the spiked samples, 500 µL of plasma was vortexed, followed by the addition of 50 µL of internal standard (IS) into RIA vials, which were then vortexed. Cartridges were conditioned by adding 2 ml of methanol and 2 ml of water. The sample was loaded onto the cartridge and passed through it. The cartridge was then washed with 2 ml of water and 2 ml of 10% methanol.

Based on the pKa value of asenapine, the elution process was carried out using acid and base. Since the pKa value of asenapine is 8.6, it was eluted with acetic acid into new vials. The eluted sample was then loaded into injector vials and injected into the GC-MS to obtain the chromatogram.

4.3.5.6. Gas chromatography and Mass spectrometry

a) GC-MS Cconditions

The drug analysis was conducted using a Shimadzu AOC-20N Plus auto-sampler connected to a GC-MS Shimadzu QP-2020 NX instrument, which is equipped with a split/splitless injector in the splitless mode. The analysis was performed using a Shimadzu SH-RXi-5Sil-MS fused silica capillary column, which is 30 meters long, has an inner diameter of 0.25 mm, and a stationary film thickness of 0.25 μm. The column was manufactured by Shimadzu in the USA.

During the analysis, Helium (He) gas was used as the carrier gas, with a constant flow rate of 1.2 ml/minute. The injection volume was 5 μL (LVI), which was injected onto a Carbofrit plug in the liner with an open purge valve (30:1 split ratio) for 10 seconds. The inlet temperature was initially set at 90°C, which was ramped up to 290°C at a rate of 200°C/minute after a 30-second delay. The oven temperature program was set to 85°C for 3 minutes, followed by a 25°C/minute ramp to 180°C, then a 10°C/minute ramp to 300°C and held for 4 minutes. The total time for one GC-MS run was 23 minutes.

The MS instrument transfer line temperature was set to 240°C, while the ion source temperature was set to 230°C. The ionization mode used was electron impact at 70eV full-scans (30-550 m/z). The optimization of the retention times and chromatographic resolution were conducted in the scan mode using all prepared standard concentrations.

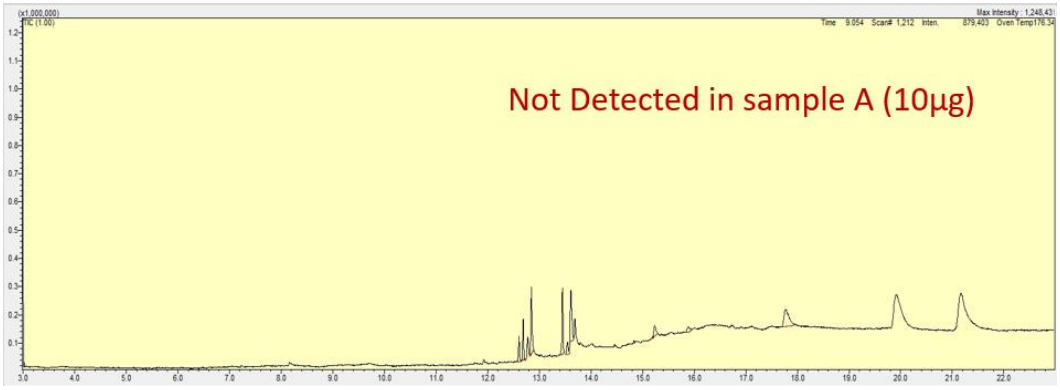
Overall, this study used precise and standardized techniques for the analysis of drugs, ensuring the accuracy and reproducibility of the results.

b) Chromatograms

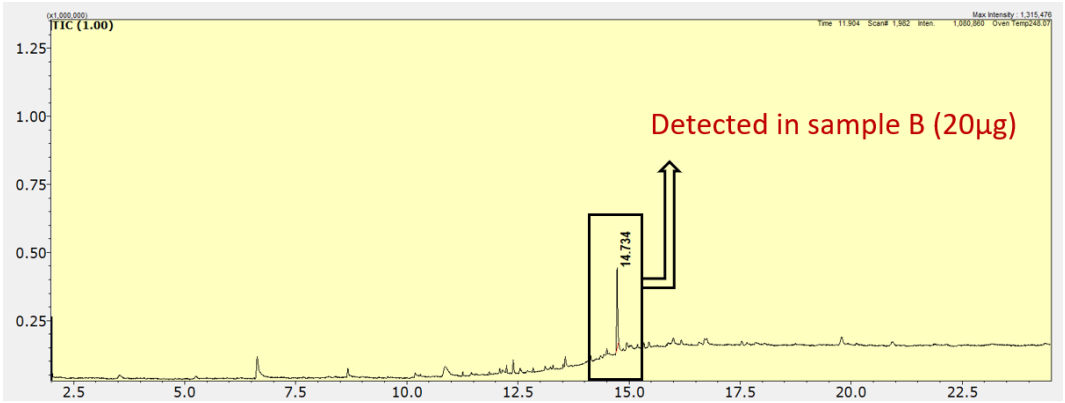
Chromatogram are obtained as a by-product of the GC-MS instrument. The graphical representation is directly related to the concentration of the sample used and the peak forms at the retention time which is characteristic of an analyzed molecule and peak area that represented the concentration of the respective components of sample analyzed.

The Retention time (R_t) of all the sample jars (A, B, C, D, and E) as follow-

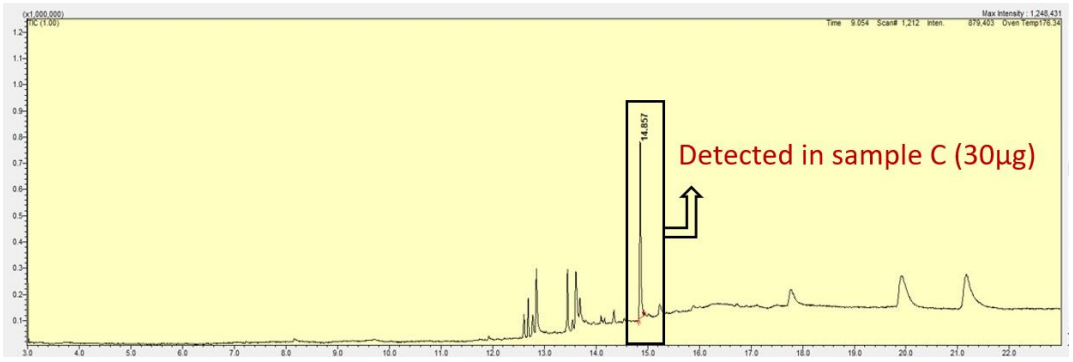
- R_t of sample A – no detection
- R_t of sample B - 14.734
- R_t of sample C - 14.857
- R_t of sample D- 14.749
- R_t of sample E- 14.753
- R_t of sample F – no detection



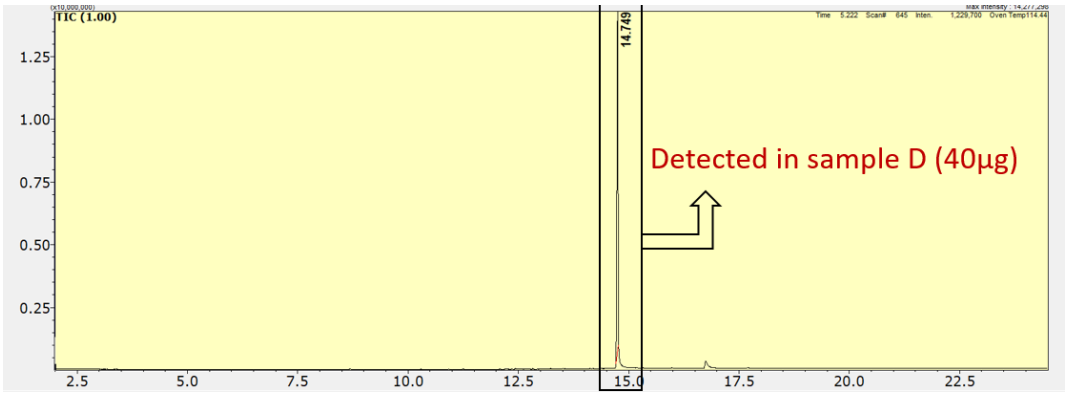
Graph 4.17. showing GC-MS graph of sample A(10µg)



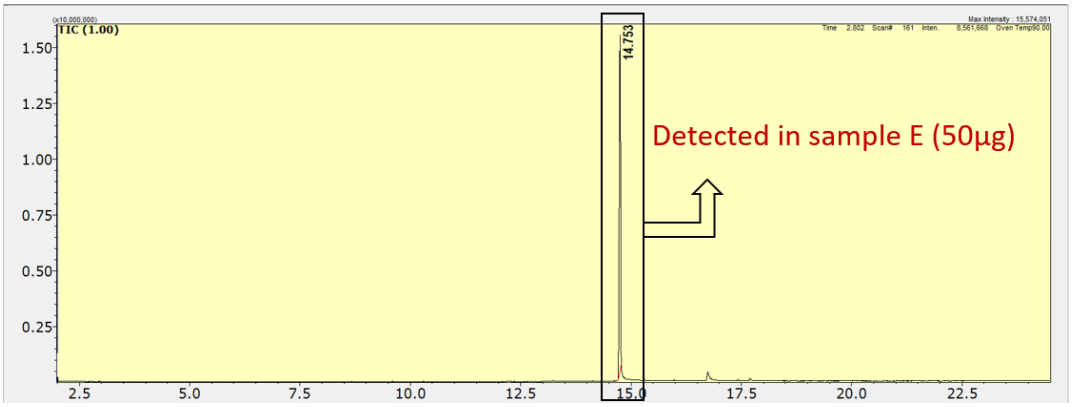
Graph 4.18. showing GC-MS graph of sample B(20µg)



Graph 4.19. showing GC-MS graph of sample C(30µg)



Graph 4.20. showing GC-MS graph of sample D(40µg)



Graph 4.21. showing GC-MS graph of sample E(50µg)

Table 4.35. Time taken by insects to grow in to successive stage during Experiment

	CONTROL	10µg	20µg	30µg	40µg	50µg
1ST INSTAR	18 hr	18 hr	18 hr	16 hr	16 hr	15 hr
2ND INSTAR	23 hr	20 hr	18 hr	17 hr	17 hr	16 hr
3RD INSTAR	19 hr	19 hr	19 hr	18 hr	17 hr	16 hr
PUPA	126 hr	122 hr	120 hr	119 hr	118 hr	115 hr
FLY	132 hr	130 hr	128 hr	128 hr	127 hr	125 hr
Total Time	318 hr	309 hr	303 hr	298 hr	295 hr	287 hr

Table 4.36. Analysis by Single factor ANOVA on data of experiment

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
CONTROL	6	636	106	13644.4
10µg	6	618	103	12939.2
20µg	6	606	101	12479.2
30µg	6	596	99.33333	12203.07
40µg	6	590	98.33333	11979.07
50µg	6	574	95.66667	11408.67

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	396.8889	5	79.37778	0.00638	0.999989	2.533555
Within Groups	373268	30	12442.27			
Total	373664.9	35				

Table 4.37 Comparative data of Mutton (M) substrate and Post-mortem Liver (PML) Substrate studies

	CONTROL (in hrs)		10µg (in hrs)		20µg (in hrs)		30µg (in hrs)		40µg (in hrs)		50µg (in hrs)	
	M	PML	M	PML	M	PML	M	PML	M	PML	M	PML
1ST Instar	22	18	20	18	19	18	18	16	17	16	17	15
2ND Instar	23	23	20	20	20	18	19	17	18	17	18	16
3RD Instar	22	19	20	19	19	19	18	18	18	17	17	16
PUPA	133	126	128	122	127	120	127	119	128	118	127	115
FLY	136	132	128	130	125	128	124	128	124	127	123	125
Total Time	336	318	316	309	310	303	306	298	305	295	302	287

Conclusion

The findings of this study indicate that the analysis of post-mortem liver samples provide similar results to those obtained from goat meat samples. There were also similarities observed in the life cycle of insects, GC-MS analysis, and the effects of the drug on the life cycle of insects. These results demonstrate the effectiveness of our study model for the determination of Asenapine maleate in insects that have fed on a cadaver contaminated with the same drug. Furthermore, the results of the single factor ANOVA analysis on the post-mortem liver sample were comparable to those obtained from the goat meat sample.

In essence, the study has shown that it is possible to use insects' sample as an alternative to post-mortem liver samples for forensic entomotoxicology investigations. The results obtained from the liver samples were found to be consistent with those obtained from the goat meat samples, indicating that the use of liver samples can be a valid and reliable approach for determining the presence of drugs in insects that have nourished on a contaminated corpse.

Moreover, the study highlights the importance of understanding the life cycle of insects, as well as the effects of toxic substances on their growth and progress. This knowledge can aid in the determination of the PMI, which is a critical factor in forensic investigations.

All things considered, the study highlights the need for more research in this subject and offers insightful information on forensic entomotoxicology. Forensic investigators might improve their capacity to solve crimes and provide victims and their families with justice by persistently investigating novel and inventive methods of examining insects and their relationship with drugs.

4.6 Discussion

The Food and Drug Administration (FDA) approved asenapine, a second-generation atypical antipsychotic—in 2009. Its long-term efficacy in controlling acute manic/mixed episodes associated with bipolar I illness, lowering the risk of relapse, and treating schizophrenia has been demonstrated. The main mechanism of action of the medication is the inhibition of certain dopamine and serotonin receptors in the central nervous system, which reduces psychotic symptoms. More than 10 years ago, the USFDA authorized asenapine as a second-class atypical antipsychotic medication with the potential to prevent relapses in schizophrenia and bipolar disorder.

Although asenapine has been relatively accepted in the USA as a typical antipsychotic therapy for bipolar disorder and schizophrenia, there is little to no literature accessible for researchers or specialists who have their own interpretation of the drug, including post-mortem data or case studies. The current approaches use various instruments, hyphenated methodologies, and extraction procedures that were first designed for therapeutic applications (www.swgtox.org).

At present, there is enough literature available, or methodology has been developed for clinical purposes, but no work has been done on post-mortem samples or medicolegal purposes or criminal cases yet. Many methods has been there for its extraction process as well as for qualitative and quantitative analysis in pharmaceutical formulations, human plasma and urine through various chromatographic and spectroscopic techniques.

The finding of species as certified by Zoological Survey of India, Kolkata, there is a variation and similarity of species in different location of Punjab, India

Overall, in this study 4 species commonly found in all selected location. The name of species identification along with their locations-

Jalandhar- **Chrysomya megacephala, Calliphora vicina, Musca Sorbens**
Mohali- **Calliphora vicina, Sarcophaga parasarcophaga albiceps, Musca Sorbens**

Amritsar- **Calliphora vicina, Sarcophaga parasarcophaga albiceps**
Fazilka- **Chrysomya megacephala, Calliphora vicina**
Anandpur- **Calliphora vicina, Sarcophaga parasarcophaga albiceps**

However, as presented in tables - the one life cycle of each species was completed within 7 days in summer, but in winter, the life cycle of each species was completed in at least 30 days. The results were seen better, when pH = 9 was maintained, as in lower or higher than the above-mentioned pH, the results were not shown clearly. Next the effect of drug where morbidity was shown mainly in summer, and it would somehow rely on concentration of ASPM. As per data mentioned in all the table, significant differences were observed among development times in larvae/insects reared on different drug concentrations.

The presence of ASPM in the food substrate had significant effects on fly development time and The ASPM drug spiked in the food substrate/sample significantly affected the early instar of development while it has delayed affect in the control sample (Jar F). The time of development of fly (1^{st}, 2^{nd}, and 3^{rd} instar) was mostly similar in Jar B, C, D and E as compared to Jar A, but its almost very delay in Jar F (control sample) As per the data mentioned in table, the immature insects first seen in Jar E (spiked with 50µg concentration) almost after one day on the food substrate, then in Jar D and so on as concentration decreases, the time of rearing of insects increases.

Prominent variation was shown in the mean width and average length of larvae and pupae between standard and spiked jars. Mainly, the differences showed only in the length of Jar E with respect to the Jar F (control). the early development of larvae, the more the length and the width were. (More in Jar E samples, and less in Jar F) The effect of temperature is clearly visible in both the season, i.e., winter and summer. As in summer season where temperature is high, the onset of flies in this condition is earlier while in winter season where temperature is low, the onset of flies was delay (in every experimental setup).

In conclusion, it was also observed that the drug concentration impacts the growth rate of insects directly in both the winter and summer season and higher concentration of

drug increase growth rate of insects while the control sample showed slower rate of growth. In 2012, SWGTOX developed a validation method for the detection of ASPM in postmortem samples and applied in the regular casework in which D5-fentanyl taken as the internal standard and further, detected by a GC–MS. This process proved to be accurate, sensitive and robust for toxicology analysis and also adopted by laboratory.

Several experiments have been conducted by researchers to detect ASPM using spectrophotometry and determine its limit of quantification (LOQ). The primary quantification of ASPM was carried out by Halima *et al.,* in 2012, who used a UV spectrophotometer to measure the UV absorbance of ASPM at 270 nm in both bulk samples and pharmaceutical formulations. They established method linearity in the concentration range of 10-60 mg/ml of ASPM, and the method was found to be sensitive with LOD and LOQ values of 1.40 mg/ml and 4.26 mg/ml, respectively. Furthermore, the method was deemed accurate, precise, and specific, with no interference from excipients.

Subsequently, Mrudulesh *et al.,* (2013) published a research article on the detection of ASPM in bulk samples. Their method showed linearity within the range of 2-10 mg/ml at 220 nm and demonstrated better sensitivity compared to previous methods with LOD and LOQ values of 0.20 mg/ml and 0.61 mg/ml, respectively. The UV spectrophotometry of ASPM has the potential to be useful in the analysis of small amounts, particularly in dissolution profile studies. While UV analysis provides information about drug and metabolite absorption, it is not considered to be highly advanced.

The analysis of ASPM has advanced over time, and one of the earliest HPLC methods for its characterization was reported by Chhalotiya *et al.,* (2012). The study employed a mobile phase comprising of a blend phosphate buffer and acetonitrile (95:5 v/v) with phosphoric acid used to adjust pH to 3.5. The stationary phase used was Sunfire C18 (250 x 4.6 mm) 5 mm, and ASPM was estimated in tablet formulation. ASPM quantification was carried out at a wavelength of 232 nm using a photodiode array (PDA) detector, and ASPM was eluted at 5.51 minutes at room temperature.

LC-MS/MS methods have been published for the detection of ASPM in biological matrices and human urine using a SPME was developed by Boer *et al.* (2012). This method successfully quantified ASPM and its two main metabolites, N-desmethyl asenapine and Asenapine N-glucuronide in human urine obtained from a clinical trial. Reddy *et al.,* (2013) reported another LC-MS/MS method for ASPM in human plasma. In this study, Phenomenex C18 column was utilized as a stationary phase and the mobile phase consisted of ammonium format: acetonitrile (5:95 v/v) in the isocratic mode. This approach has the potential to be utilized for clinical drug surveillance owing to the relatively short chromatographic run time (2.5 minutes) and simple sample pre-treatment protocol.

The LC-MS/MS method developed by Patel and colleagues allowed for the estimation of ASPM in human plasma in the presence of its inactive metabolites, N-desmethyl Asenapine and Asenapine N-glucuronide, and demonstrated high selectivity and sensitivity. De Boer and colleagues utilized automated LC-MS/MS to quantify ASPM and three of its metabolites in human plasma. Various studies have used GC/MS, LC/MS, or LC/MS/MS methods to determine the plasma concentration of Asenapine and its metabolites, with a clinical therapeutic blood concentration of 4 ng/ml. However, concentrations up to 62 ng/ml represented a value for blood, as reported in the literature.

The ingestion of drugs or other chemicals by insects can lead to disruptions in their physiology, growth rate, and development. These alterations can either accelerate or prolong various stages of the insects' life cycle, with the duration and development of flies being particularly affected by the concentration of the ingested substance. In the current study, the life of maggots was found to be impacted mainly in the summer season. The ingestion of such chemicals can also affect carrion colonization and oviposition in a measurable way, following a distinct pattern of succession. Toxicological analysis of insects that have fed on carrion can be used to identify and quantify these toxins or drugs.

Chapter 5

Conclusion

This study aimed to demonstrate the usefulness of investigating the entomological fauna of forensic interest using a psychotropic drug called Asenapine maleate (ASPM). The main objective was to identify the insects/fly species in Jalandhar and Mohali and estimate the PMI, analyze the effects of ASPM drug and perform taxonomic and morphological identification. However, it is generally believed that the results of such researchs are variable and indirectly applicable to forensic science since they depend on various factors such as the condition of the corpse, its size and shape, any injuries, understanding the type of insects and their habitat, ecological requirements, seasons, and climatic conditions. These factors are critical in crime scene investigation and for deriving accurate conclusions, expert testimony or reports when needed.

The study involved collecting various insect species from the forensic entomological fauna, including blow flies and flesh flies, and feeding them with ASPM drug. The impact of environmental elements on insect larval growth, such as temperature and humidity, is also taken into account in this study. The season and the drug's concentration both affected how ASPM affected the insects, the researchers discovered. The effects of summertime on the life cycle of maggots led to modifications in their length and growth.

The principal investigator also conducted toxicological analysis on insects that had fed on carrion to determine the presence of ASPM and its metabolites. The analysis was performed using GC-MS, a widely used analytical method that provides accurate and reliable results. The results showed that the presence of ASPM in the insects indicated that the drug had a measurable effect on carrion colonization and oviposition and followed a distinctive pattern of succession.

The findings of this study suggest that entomological studies using ASPM as a psychotropic drug can be useful in determining the PMI and in toxicological analysis. The research also emphasizes how crucial it is for forensic investigations to take into

account a variety of elements that affect the growth and behavior of insects. The results of such studies can provide valuable information for crime scene investigators, forensic experts, and law enforcement agencies.

In conclusion, the study presented here demonstrates the usefulness of investigating the entomological fauna of forensic interest using ASPM as a psychotropic drug. The study emphasizes how crucial it is for forensic investigations to take into account a variety of elements that affect the growth and behavior of insects. The results of such studies can provide valuable information for crime scene investigators, forensic experts, and law enforcement agencies. Further research in the field of forensic entomotoxicology is attracted more interest and developing as broader field with easily acceptance as evidence in court of law globally.

From Objective 1, it can be concluded that the various species found in different locations share similarities, with only one or two species being unique to particular areas. The other species found in different studies at different locations are *Chrysomya megacephala, Chrysomya rufifacies* from Punjab during the spring season with temperature ranging between 15-33◦C (Bala and Sharma, 2016), *Chrysomya megacephala* and *Calliphora vicina* were found during all the seasons i.e., summer, winter, spring and autumn from Punjab and in this study ethanol and cannabis were used (Verma, 2013; Singh and Bharti, 2000) whereas, in present study the flies found were *Chrysomya megacephala, Calliphora vicina, parasarcophaga albiceps and Musca Sorbens* with the drug Asenapine maleate used for study. *Calliphoridae and Sarcophagidae* were found from Rohtak, Haryana and the temperature during study was ranging from 14.1-34◦C, and the decomposition was studied using pig model (Dalal *et al.,* 2020) and both Punjab and Haryana experience hot and dry summers, with temperatures often soaring above 40°C (104°F) during the peak months of May and June.

Both states have cold winters, with temperatures dropping significantly between December and February. During this time, temperatures can range from 5°C (41°F) to 20°C (68°F), and foggy conditions are common, particularly during the early morning hours. The tropical climates of Thailand and India produce hot, muggy weather all year

round for *Chrysomya megacephala, Chrysomya rufifacies, Chrysomya villeneuvi, Chrysomya nigripes, Chrysomya bezziana, Chrysomya chani, Lucilia cuprina,* and *Hemipyrellia ligurriens.* Average temperatures in both countries tend to range between 25°C (77°F) and 35°C (95°F), with coastal areas experiencing higher humidity levels. Whereas, the transition between seasons tends to be more pronounced in India compared to Thailand. India experiences distinct spring and autumn seasons, which are characterized by moderate temperatures and changes in vegetation. Thailand's seasonal transitions are less distinct, with a more gradual shift between the wet and dry seasons (Sukontason *et al.,* 2007).

In another studies, *Lucilia sericata, Musca stabulans* were found during summers and *Necrobia rufipes* were found during winters from 2 case studies from Russia (Benecke and Biol, 1998). Russia generally experiences more extreme temperature variations compared to India. Russia may have severely frigid winters with temperatures falling below -40°C (-40°F) in locations like Siberia, whereas India can have scorching summers with temperatures reaching above 40°C (104°F) in some parts. In an unrelated investigation, summertime finds of *Chrysomya megacephala* and *Phormia regina* were made in the eastern parts of the Kingdom of Saudi Arabia (Shaalan *et al.,* 2017). India and Saudi Arabia have some weather similarities, including hot summers and dry climates. However, there are significant differences between the two countries. India experiences a distinct monsoon season with heavy rainfall, while Saudi Arabia has low rainfall and a predominantly arid climate. India has more pronounced seasonal variations and higher humidity levels compared to Saudi Arabia. India also exhibits more varied temperature extremes, including cold winters in some regions, while Saudi Arabia has milder winters. This information is essential for pinpointing the scene of a crime since the existence or absence of a certain species might offer important hints for forensic examinations.

From Objective 2, we may thus infer that temperature, humidity, and precipitation all have a significant impact on the life cycle of insects. In particular, during the summer season when both the temperature and humidity were high, the entire life cycle of insects was completed in only seven days. In contrast, during the winter season, the life

cycle took approximately 32 days, which is four times longer than the summer season. This significant difference can be attributed to the colder temperatures and lower humidity levels during the winter months. The ADD (accumulate degree days) based data shows the validation of study model as the time of appearance of different stages of insects are in accordance with the ADD calculation.

From Objective 3, it can be concluded that out of the six samples analyzed, only four samples (B, C, D, and E) showed detection of the drug. Sample A, with a concentration of 10µg, did not show any detection of the drug. This is because the concentration was below the limit of. The limit of detection is the lowest concentration of the drug that can be detected by the instrument.

From Objective 4, it can be concluded that there is a gradual increase in the growth rate of decomposers with an increase in the concentration of the drug. This trend is consistent across all the seasons.

However, there are some limitations in Quantitative study of drug in Forensic Entomo-toxicology as there are N number of maggots which feed on deceased and it becomes difficult to estimate the quantity of drug present in body of deceased. In future, more focus should be on quantitative analysis as it will help in determining the cause of death. Also, the effect of other drugs should be considered as it will make the estimation of minimum PMI more reliable.

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Appendix A

INSTITUTIONAL ETHICS COMMITTEE GMCH-32, Chandigarh.
Sector 32-B, Chandigarh-160030, Ph. 0172-2665253-57, Fax No. 0172-2609360
IEC Regd. No.ECR/658/Inst/PB/RR-20

No. GMCH/IEC/2021/314. Dated: -19.08.2021

To

Dr. Dasari Harish,
Prof. & Head,
Department of Forensic Medicine,
GMCH-32, Chandigarh.

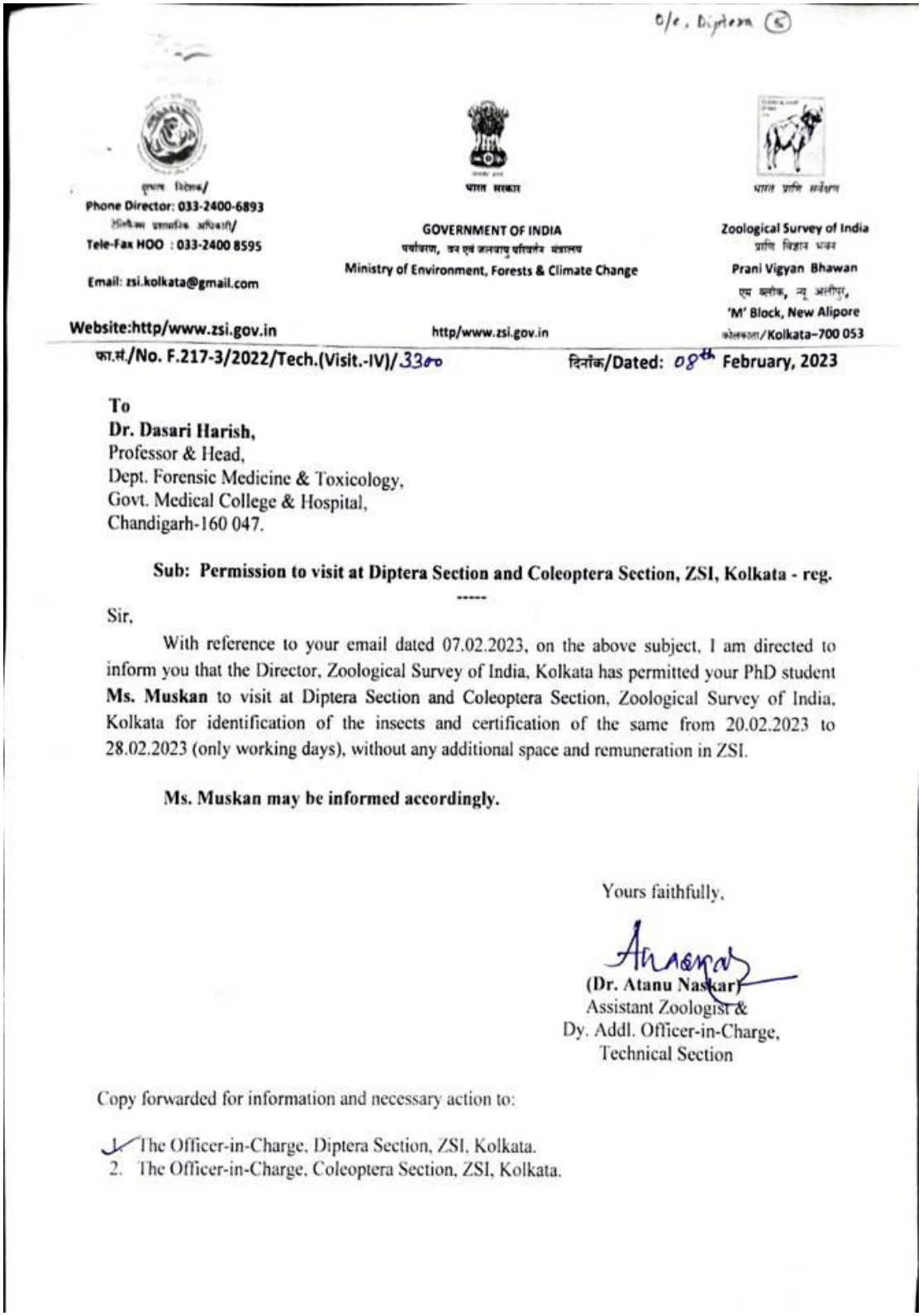
Subject: Approval of thesis.

Sir,

With reference to your letter No:GMCH-FM-2021/1852, dated19.08.2021 requesting approval letter titled **"Medico-legal Pertinence of Cadaver Entomofauna for determination of time since death and cause of death"** By Ms. Muskan, Registration no. 11916715, Lovely Professional University, Jalandhar, under supervision of Dr. Saurabh Shukla and Dr. Dasari Harish has been approved by the Institutional Ethics Committee vide its communication no. GMCH/IEC/2021/63 dated 02.03.2021 by the Chairman.

Dr. Jagjit Singh
Member Secretary, IEC

Appendix B



O/o. Diptera (5)

भारत सरकार

GOVERNMENT OF INDIA
पर्यावरण, वन एवं जलवायु परिवर्तन मंत्रालय
Ministry of Environment, Forests & Climate Change

http/www.zsi.gov.in

भारत प्राणि सर्वेक्षण

Zoolological Survey of India
प्राणि विज्ञान भवन
Prani Vigyan Bhawan
एम ब्लॉक, न्यू अलीपुर,
'M' Block, New Alipore
कोलकाता/Kolkata-700 053

निदेशक/
Phone Director: 033-2400-6893
टेलिफैक्स प्रशासनिक अधिकारी/
Tele-Fax HOO : 033-2400 8595

Email: zsi.kolkata@gmail.com

Website:http/www.zsi.gov.in

फा.सं./No. F.217-3/2022/Tech.(Visit.-IV)/3300 दिनांक/Dated: 08th February, 2023

To
Dr. Dasari Harish,
Professor & Head,
Dept. Forensic Medicine & Toxicology,
Govt. Medical College & Hospital,
Chandigarh-160 047.

Sub: Permission to visit at Diptera Section and Coleoptera Section, ZSI, Kolkata - reg.

Sir,

With reference to your email dated 07.02.2023, on the above subject, I am directed to inform you that the Director, Zoological Survey of India, Kolkata has permitted your PhD student **Ms. Muskan** to visit at Diptera Section and Coleoptera Section, Zoological Survey of India, Kolkata for identification of the insects and certification of the same from 20.02.2023 to 28.02.2023 (only working days), without any additional space and remuneration in ZSI.

Ms. Muskan may be informed accordingly.

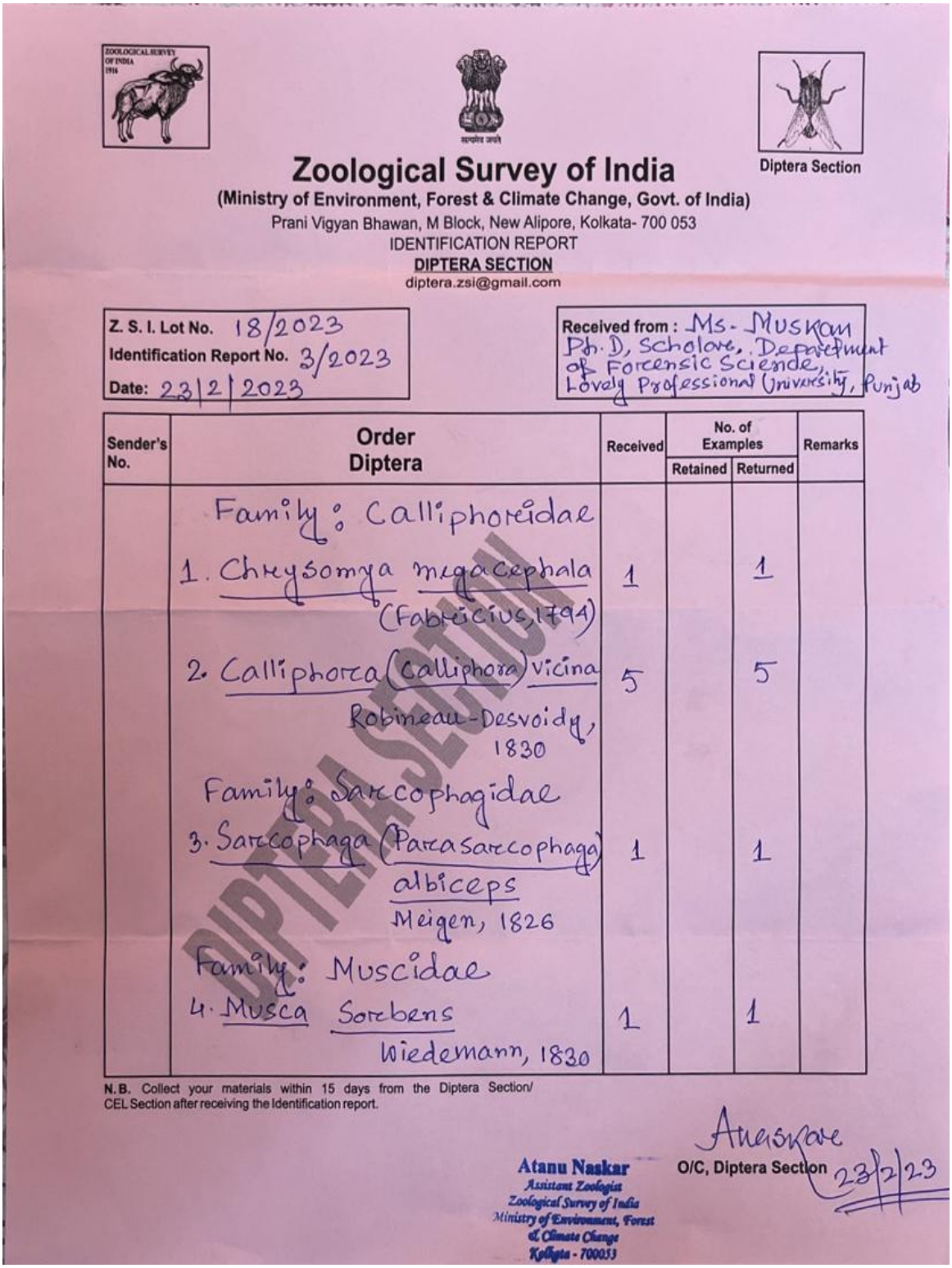
Yours faithfully,

(Dr. Atanu Naskar)
Assistant Zoologist &
Dy. Addl. Officer-in-Charge,
Technical Section

Copy forwarded for information and necessary action to:

1. The Officer-in-Charge, Diptera Section, ZSI, Kolkata.
2. The Officer-in-Charge, Coleoptera Section, ZSI, Kolkata.

Appendix C



Zoological Survey of India

(Ministry of Environment, Forest & Climate Change, Govt. of India)

Prani Vigyan Bhawan, M Block, New Alipore, Kolkata- 700 053

IDENTIFICATION REPORT

DIPTERA SECTION

diptera.zsi@gmail.com

Diptera Section

Z. S. I. Lot No. 18/2023

Identification Report No. 3/2023

Date: 23/2/2023

Received from : Ms- Muskan Ph. D, Scholar, Department of Forensic Science, Lovely Professional University, Punjab

Sender's No.	Order Diptera	Received	No. of Examples		Remarks
			Retained	Returned	
	Family: Calliphoridae				
	1. Chrysomya megacephala (Fabricius, 1794)	1		1	
	2. Calliphora (Calliphora) vicina Robineau-Desvoidy, 1830	5		5	
	Family: Sarcophagidae				
	3. Sarcophaga (Parasarcophaga) albiceps Meigen, 1826	1		1	
	Family: Muscidae				
	4. Musca sorbens Wiedemann, 1830	1		1	

DIPTERA SECTION

N.B. Collect your materials within 15 days from the Diptera Section/ CEL Section after receiving the Identification report.

Atanu Naskar
Assistant Zoologist
Zoological Survey of India
Ministry of Environment, Forest & Climate Change
Kolkata - 700053

O/C, Diptera Section 23/2/23

List of Publications

- Muskan, Vimukti Chauhan, Jaskaran Singh, Saurabh Shukla, Harish Dasari. Determination of Asenapine Maleate from Maggots by Solid-Phase Extraction and Gas Chromatography-Mass Spectroscopy: A Forensic Entomo-toxicological approach. Journal of Forensic Medicine Science and Law 2023;32(1):48-53. (Published) https://www.mlam.in/pdf/2023_V32_I1/JFMSL20233201104853.pdf
- Muskan, Saini Shubham, Singh Jaskaran, Dasari Harish, Sharma Neeta Raj. Drugs and their Effects on Development Rate of Decomposers: An Entomotoxicological Approach. Journal of Punjab Academy of Forensic Medicine and Toxicology 2020;20(2):180-183. DOI:10.5958/0974-083X.2020.00126.0 (Published)
- Saini Shubham, Muskan, Shukla Saurabh, Singh Jaskaran, Rana Prashant Singh. Identification of Wild Animals from Pugmarks- A Review. Journal of Punjab Academy of Forensic Medicine and Toxicology http://dx.doi.org/10.5958/0974-083X.2021.00080.7 (Published)
- Muskan, Harish Dasari, Gaurav Kumar Singh, Vimukti Chauhan, Shweta, Jaskaran Singh, Saurabh Shukla. Small Size, Big Impact: Insects for Cadaver Examination. In: Singh J., Sharma N.R. (eds) Crime Scene Management within Forensic Science. Springer, Singapore 2022:75-92. https://doi.org/10.1007/978-981-16-6683-4_3 (Published)
- Gaurav Kumar Singh, Ankita, Muskan, Shubham Saini, Dr. Ridamjeet Kaur. Review on Clinical Forensic Medicine. In: Singh J., Sharma N.R. (eds) Crime Scene Management within Forensic Science. Springer, Singapore 2022:211-239. https://doi.org/10.1007/978-981-16-6683-4_9 (Published)

List of Conferences

- Participated in Oral Presentation on topic "Medico-Legal Importance of Insects in Estimation of PMI & Detection of Drug" in "Annual International Conference on Forensic Sciences (AICFS-2023)" at Geeta University, Haryana and won Second Prize for same. (https://drive.google.com/file/d/1J6HeJ3qgsYjyK8YZvaUMOAHhZsbuAaOK/view?usp=drivesdk)
- Participated in Oral Presentation on topic "Forensic Entomology- The Study of Effect and Detection of Drug" in "National Conference on Forensic Advancements & Technological Explorations (FATE-2022)" at Lovely Professional University, Punjab and won First Prize for same. (https://drive.google.com/file/d/121TKEs_8YpxflFsxuak52skmjw-8pORr/view?usp=drivesdk)
- Participate in Oral Presentation on topic "Biography of Dead through Insects: A prospective study in Punjab, India" in "Mid year CME-cum-Conference 2022" of Punjab Academy of Forensic Medicine & Toxicology at Kalpana Chawla Govt. Medical College, Karnal, Haryana. (https://drive.google.com/file/d/1djnrG9Sy84M2t55hrp8k6WMhSGKBjvd1/view?usp=drivesdk)
- Participated in Oral presentation on topic "Cadaver Biography through Insects: A Prospective Study in Northern Region of India" in "International Forensic Forum 2021: Traversing through the Crime, Criminal Mind to the Courtroom" at Sharda University, Greater Noida. (https://drive.google.com/file/d/12pS0HPaxdNR8zyi-JtW68IegL21Lco03/view?usp=drivesdk)
- Presented poster in "National conference on Forensic Science" on topic "Entomotoxicology as a tool of drug detection" at Amity University, Gurugram. (https://drive.google.com/file/d/12WJ2hD7bsDMNUrJu_zkInXG_3Elq-sqp/view?usp=drivesdk)