Ref: **Aggarwal AD. Estimating the postmortem interval with the help of entomological evidence (thesis submitted to the Baba Farid University of Health Sciences, Faridkot, 2005 for MD in Forensic Medicine).** ***Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology*****, 2005; Volume 6, Number 2, (July - December 2005) : http://www.geradts.com/anil/ij/vol_006_no_002/others/thesis/akash_thesis.pdf; Published: December 31, 2005**

Akash Deep Aggarwal

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Contact Dr. Akash Deep Aggarwal at "**toakashdeep@yahoo.com**"

Contact the Journal office at "**dr_anil@hotmail.com**" or "**anil.aggrawal@gmail.com**"

ESTIMATING THE POST-MORTEM INTERVAL WITH THE HELP OF ENTOMOLOGICAL EVIDENCE

A

THESIS FOR

M.D. (FORENSIC MEDICINE)

GOVT. MEDICAL COLLEGE, PATIALA

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

2005

AKASH DEEP AGGARWAL

CERTIFICATE

This is to certify that this thesis titled "**Estimating the postmortem interval with the help of entomological evidence**" embodies the work carried out by **Dr. Akash Deep Aggarwal** himself under our direct supervision and guidance, in the Department of Forensic Medicine, Government Medical College and Rajindra Hospital, Patiala and that it is worthy for the award of the M.D. (Forensic Medicine) degree.

Dr. O.P.Aggarwal, MD
Additional Professor,
Deptt. Of Forensic Medicine,
Govt. Medical College, Patiala.

Dr. R.K.Gorea, MD
Professor & Head,
Deptt. Of Forensic Medicine,
Govt. Medical College, Patiala.

Dr. Devinder Singh, PhD
Reader,
Deptt of Zoology,
Punjabi University, Patiala.

ACKNOWLEDGEMENTS

It is my great honour and proud privilege to acknowledge with gratitude, the constant supervision, keen interest and an affectionate guidance rendered to me at every step by my revered teachers Dr.O.P.Aggarwal, MD, Additional Professor, and Dr.R.K.Gorea, MD, Professor and Head, Department of Forensic Medicine, Government Medical College, Patiala; which made it possible for me to pursue this study and complete this thesis. I am extremely indebted to them for their devotion, valuable time, timely advice and suggestions.

It is with great pleasure that I take this opportunity to express my gratitude to the entomologist Dr.Devinder Singh, PhD, Reader in Department of Zoology, Punjabi University, Patiala for his guidance, suggestions, the unfailing interest and enthusiasm, for this research.

I feel obliged to Dr.S.S.Oberoi, MD, Associate Professor for his encouragement and generous help during this study. I also extend my sincere thanks to Dr.D.S.Bhullar, MD, known forensic expert.

Thanks are also due to Sh. M.S.Tomar, In-charge, Govt. Meteorological Station, Patiala for supplying the weather data for the area.

I acknowledge whole heartedly the cooperation of my dear colleagues and other staff members in the department during this work.

I am deeply indebted to my respected parents for their constant enthusiasm, support and encouragement in various ways, without which it could not have been possible on my part to complete this study.

Last but not the least, I pay my regards to those honorable deceased without whom I could not have completed my study.

Akash Deep Aggarwal

CONTENTS

ABBREVIATIONS

°C	degree Celsius
%	percent
AD	of the Christian era [*Anno Domini*]
ADD	accumulated degree days
ADH	accumulated degree hours
a.m.	Before noon [*ante meridiem*]
Avg.	Average
BASIC	Beginner's All-purpose Symbolic Instruction Code
DNA	Deoxyribo Nucleic Acid
Dr	doctor
et al	and others; et cetera [*et allii*]
Govt.	Government
i.e.	that is
MD	Doctor of Medicine [*Medicinae Doctor*]
p., pp.	page, pages
PhD	Doctor of Philosophy [*philosophiae doctor*]
p.m.	After noon [*post meridiem*]
PMI	post mortem interval
PCR	polymerase chain reaction
RAPD	rapid analysis of polymorphic DNA.
RCMP	Royal Canadian Mounted Police
RFLP	restriction fragment length polymorphism
Temp.	Temperature
US	United States

INTRODUCTION

INTRODUCTION

The earth is a predominantly arthropod world, and thus it is not uncommon that we mere humans come in contact with these creatures. They make the world go round, as they pollinate, eat other arthropods, eat living plants and trees, dead plants and trees, living vertebrates, dead vertebrates and vertebrate dung and urine and a lot of other things. One of the most important feature of arthropods is that they are an important carrion feeder i.e. they eat dead vertebrate bodies, including that of man. Thus they perform a valuable recycling of organic matter in our ecosystem.

Since time immemorial man has tried to dominate this planet by sheer intelligence. He tries to dominate the ecosystem and interferes with the natural processes. But nature has her own ways and one of its finest intricacies is the food web, that is, the process of eating and being eaten. This cycle is so immaculate that even dead bodies of animals, including that of humans, are decomposed by other creatures, with insects playing a predominant role**.** The maggots and corpses go together. For many years, the "worms" crawling in the eyes, nose, and other orifices and wounds on dead bodies were considered just another disgusting element of decay - something to be rinsed away as soon as the corpse was placed on the table for autopsy. While ballistics, firearm examination, bitemarks, gunpowder residue chemistry, blood spatter analysis, and other elements of scientific criminology were studied and refined, the insects associated with death scenes were largely ignored (Catts and Haskell 1990). Entomology is derived from the Greek word *entomon* (insect) and *logos* (word, reason) meaning the study of insects (Gupta and Setia 2004).

Through the years, however, a few scientists have researched forensic entomology, which has become a fascinating, and at the same time a more arcane, field of biological study. However, the scope of the field is broad. The potential for contributions of entomology to legal investigations has been known for at least 700 years, but only within the last two decades or so has entomology been defined as a discrete field of forensic science.

The forensic application of entomology has a long, although sporadic, written history reaching back to 13^{th} century China (McKnight 1981), then more recently, more than a century ago, associated with medical professionals and pathologists interested in entomology in France (Bergeret 1855, Megnin 1894) and Canada (Johnston and Villeneuve 1897). The first entomological society in North America was the Entomological Society of Ontario, many charter members of which were medical practitioners where entomology was their hobby. The field of entomology became professionally developed. The most recent 30 to 40 years have seen a revival of the science in the criminal area, using various techniques of data analyses founded through agriculture and forestry. Currently, there are few forensic entomologists that handle criminal cases, but this is expected to change as litigators continue to challenge forensic identification methodology.

In India the earliest study on this subject is believed to be by Mackenzie in Calcutta (Indian Medical Gazette, 1889; 167) where he made observations on dead bodies about the times of appearance of eggs and maggots (Modi 1999) Though not much record is available about this study in the contemporary literature.

Forensic entomology is a growing field of study, incorporating an entomologist's expertise in insects including their identification, life cycles and habitats, with an arm of law enforcement. One of the earliest documented cases was in 1235 AD in China, where the knowledge of a fly's attraction to blood was used in the aid of solving a murder investigation (Goff 2000, Benecke 2001). Today, forensic entomology can be defined as the use of insects, and their arthropod relatives, that inhabit decomposing remains to aid legal investigations (Byrd 1997).

Practical use of insects or arthropods in solving crime led to the development of a separate branch of science, now-a-days known as Forensic Entomology. Forensic is the use of scientific techniques to solve crimes, and is used to describe the work of scientists who examine evidence in order to help the police solve crimes. Entomology is the study of insects. (Collins 2001) Forensic Entomology uses insects to help law enforcement determine the cause, location and time of death of a human being. Insect life cycles act as precise clocks, which begin within minutes of death. They can be used to closely determine the time of death when other methods are useless. They can also show if a body has been moved after death (Catts and Haskell 1990).

Forensic entomology is the name given to any aspect of the study of insects and their arthropod counterparts that interacts with legal matters (Hall and Doisy 1993). Lord and Stevenson (1986) divided it into three components:

- Urban entomology (legal proceedings involving insects and related animals that affect manmade structures and other aspects of the human environment),
- Stored products entomology (proceedings involving insects infesting stored commodities such as cereals and other kitchen products), and

- Medicolegal entomology, sometimes termed “forensic medical entomology,” and in reality “medicocriminal entomology” (because of its focus on violent crime), relates primarily to
    1. Determination of the time of death (postmortem interval)
    2. Site or place of death
    3. Mode of death (Anderson 1997)
    4. Cases involving sudden death
    5. Traffic accidents with no immediately obvious cause
    6. Possible criminal misuse of insects (Leclercq 1969)

Forensic entomology is inextricably linked with the broader scientific fields of medical entomology, taxonomy, and forensic pathology.

Forensic (or medico-legal) entomology is the study of the insects associated with a human corpse in an effort to determine elapsed time since death. Insect evidence may also show that the body has been moved to a second site after death, or that the body has been disturbed at some time, either by animals, or by the killer returning to the scene of the crime. (Anderson et al 1996)

Forensic entomology can be said to be the application of the study of insects and other arthropods to legal issues. It can be divided in three subfields: urban, stored-product and medicolegal. Medicolegal forensic entomology includes arthropod involvement in events such as murder, suicide and rape, but also includes physical abuse and contraband trafficking. (Staerkeby 1997)

Forensic Entomology, as defined by University of Florida entomologist Jason Byrd (1997), is the use of the insects, and their arthropod relatives that inhabit decomposing remains to aid legal investigations. As per the American Board of Forensic Entomology

(1997), Forensic Entomology, or Medicocriminal Entomology, is the science of using insect evidence to uncover circumstances of interest to the law, often related to a crime.

Forensic entomology proceeds on the common observation that exposed remains present a temporary and progressively changing habitat and food source for a variety of organisms ranging from microbes like bacteria and fungi, to vertebrate scavengers. Out of these, arthropod fauna comprises the major element and insects form the most constant, diverse and conspicuous group. These six legged creatures dominate the terrestrial and fresh water carrion fauna (Payne 1965). He reported 522 species on decomposing pig carcasses out of which 84% were insects. Goff et al (1986) while studying carcass decomposition reported 140 arthropod taxa out of which 83% were insects. These insects were attracted in the first instance to the body fluids oozing from natural opening and to blood or serum escaping from the wounds. The overwhelming majority of the insects are the flies and beetles. Dipteran families namely Calliphoridae, Sarcophagidae, Muscidae, Sepsidae, Sphaeroceridae, Piophilidae, Phoridae and Coleopteran families namely Histeridae, Staphylinidae, Silphidae, Cleridae, Dermestidae, Tenebrionidae predominate the scene. (Reed 1958, Payne 1965, Braack 1986, Goff et al 1986, Goff 1993, Tantawi et al 1996) These insects form a complex food web within the carrion.

Carrion is the decaying flesh of dead animals. It supports a characteristic assemblage of insects which are attracted to it due to their well developed sense organs. Calliphorids can arrive within few minutes (Payne 1965, Tullis and Goff 1987) or even a few seconds (DeJong 1995) following corpse exposure. If given access the females will oviposit on carrion within first few hours (Hall 1948, Catts 1992). These flies have a strong physiological drive to go to their food resource or oviposition site.

Out of all the insects visiting a dead body, the maggots of blowflies (Calliphoridae) and fleshflies (Sarcophagidae) are responsible for the maximum consumption of terrestrial carrion (Fuller 1934, Payne 1965, Putman 1977, Putman 1978, Braack 1981, Early and Goff 1986). Adults of these flies use carrion for feeding, mating and breeding. Blowflies lay eggs while fleshflies deposit larvae in natural body orifices. These larvae quickly invade most of the regions of the dead body (Putman 1978). According to Smith (1986) the carrion insects are grouped into following categories on the basis of their food preferences which reflects their ecological role.

1. Necrophagous species: feed on the corpse tissue.
    a) Sarco-saprophages: feed on decomposing flesh and imbibe in the blood and body fluids, e.g. Calliphoridae, Sarcophagidae, Muscidae and Dermestidae.
    b) Coprophages: are attracted to the rumen contents of herbivores, e.g. Scarabaeidae, Muscidae.
    c) Dermatophages: feed on dried skin, hair, ligament and bones, e.g. Dermestidae, Tineidae.
2. Predaceous species: feed only on carrion entomofauna, especially on Dipteran larvae, e.g. Histeridae and Staphylinidae.
3. Parasitic species: parasitize fly larvae and pupae, e.g. wasps.
4. Adventives or Incidental species: use carrion as a concentrated resource extension of their normal habitat, e.g. spiders, millipedes, centipedes, mites, etc.

Since many insects are associated with the human body after death, they are always a potential source of evidence in cases of murder or suspicious death. They are also potentially useful as evidence in situations other than murder cases. Despite this great potential, however, the field of forensic entomology remains obscure in our country, largely because of lack of awareness of the benefits that may accrue from its application. Some questions that may be answered using forensic entomological techniques include time, place, and cause of death; when burial occurred; whether the body was mutilated after death; how long a body remained submerged under water; and when the body was placed in a certain given spot. This involves the application of forensic entomology for crime scene investigations based on the use of insect evidence in estimating post-mortem interval. It involves examination of the insects involved in the decay of corpses, the collection of insect evidence and its use in predicting the post-mortem interval.

Investigators are slowly becoming more and more aware of the invaluable contributions made by the silent testimony of insects. Insects found at the crime scene, traditionally ignored and considered a nuisance to investigators, are now being studied using new technologies to provide valuable clues to legal investigations of death. This previously overlooked field of science is reaching new heights.

Because of advancements in technology and increasing knowledge of science, investigators are applying new techniques to find previously unseen clues. Although a grim subject matter, the study of insect infestation and behavior has recently come into prominence as valid scientific data. Forensic Entomology isn't just limited to the study of insects interacting with a corpse; it also delves into other diverse applications of the science. Criminal investigators must be professionally-trained and follow strict guidelines in their investigations.

Stages of Decomposition.

The process of decomposition has been defined into four or five fairly distinct stages, encompassing all the changes that take place after death until only the bones, hair and dried skin remain. Research has enabled approximate time intervals for each stage to be determined; it is these values that are used in PMI calculations.

Post-Mortem Interval Calculations.

The predominant use of insects in forensic entomology is in the aid of determining the time of death, also known as the post-mortem interval (PMI). It is important to know how long it takes for the insect to arrive on a corpse, the stage of decay to which it is attracted, its life cycle and its rate of development (Turner and Wiltshire 1999, Dadour et al 2001). Insect development is dependent on environmental temperature, where the higher the temperature, the faster the rate of development (Anderson 2000, Brewer 2001).

It is this in depth knowledge of the developmental pattern, as well as of the succession of insects, during the various stages of a decomposition that is used in calculation of the interval between death and the discovery of the body, the postmortem index. This is the most common task of the forensic entomologist. To obtain an accurate figure, the environmental conditions of the area prior to discovery must be taken into consideration. Also two major principles must be obeyed: -

1. “That the colonization of a corpse by insects follows a reasonably predictable succession of species.”
2. “That developmental time for each stage of each species of insect is predictable.”

The determined PMI is, generally, less than the actual time elapsed, and is therefore not a definitive figure on which to base the time of death. However, it can be vital in providing the police with a possible time so that they can concentrate on finding the victim's whereabouts and companions prior to this time. This can lead to the pinpointing of possible suspects as in the Ruxton case. Even though this case occurred over sixty years ago it clearly displays the use of forensic entomology to obtain a PMI in a criminal case.

When a body is found or a crime scene is investigated, the presence of insects, or lack thereof, can provide many clues as to what actually happened. Time of death can be ascertained, many clues about the cause of death or events just prior to death can be determined based on insect behavior and interaction with a corpse. However, after three days, insect evidence is often the most accurate and sometimes the only method of determining elapsed time since death. (Anderson 1996)

Entomological knowledge can reveal the manner or location of death, but is most often used to estimate the time of death, or postmortem interval. Two time-dependent processes may be involved here. The first is the growth of insect larvae that feed upon the victim. Most of the carrion insects rarely deposit offspring on a live person, therefore the age of a larva provides a minimum time since death. The second is the succession of carrion-arthropod species found in the body, which has the potential of providing both a minimum and maximum estimated post-mortem interval. In general, determining the stage of immature stages of insects found on a dead body is helpful if the latter is up to one month old. On the other hand, the succession pattern is important when death occurred several months before the discovery of the dead body. (Greenberg and Kunich 2002)

One of the first groups of insects that arrive on a dead body is the blowflies (Diptera: Calliphoridae). Usually the females oviposit within hours after death of the vertebrate. The blowfly goes through the following stages during its life history: egg, 3 instar larvae, pre-pupae, pupae within puparium, imago. If we know how long it takes to reach the different stages in an insect’s life, we can calculate the time since the egg was laid. This calculation of the age of the insects can be considered as an estimate of the minimum time of death. But even if the estimate of the insect age is correct, the death of the victim (usually) occurred before the eggs were laid. This period is quite variable and depends on temperature, time of day the death occurred, time in year the death occurred, whether the corpse is exposed or buried in soil or immersed in water. As a general rule insects will lay eggs on a corpse within few hours after the corpse is available for insects. Insects can also be of help in establishing whether the corpse has been moved after death, by comparing local fauna around the body, and the fauna on the body. (Lord 1990)

Stages of Insect Development.

The developmental stages of fly larvae are well documented and research has shown the effect of various environmental conditions on the rate of maggot development. Eggs laid by an adult fly quickly hatch into miniscule first instar larvae, which feed rapidly and shed their skins to become second instar larvae. Again these feed, grow and moult to become third instar maggots. These feed voraciously and increase in size approximately five fold before migrating from the food source to pupate. After roughly two weeks an immature adult fly emerges from the rigid pupal case. (Erzinclioglu 2000)

Knowledge of the succession of insects, from the initial colonization of a corpse until complete skeletonization, is vital for a forensic entomologist. Necrophagous insects, which feed directly from the corpse, are known to arrive within minutes of death and hence provide the most accurate post-mortem data within the first two weeks of decomposition. These are followed by species that are both necrophagous and predatory on the necrophagous organisms, as well as organisms which have no relation to the body such as those feed on leaking bodily fluids, use the body as part of their environment or may have just fallen onto the body. (Goff 2000)

Factors influencing PMI calculations.

It has been documented that the insect invasion of a corpse is influenced by a variety of environmental factors such as air temperature and sheltering. Research has been undertaken within these areas to increase the accuracy of PMI calculations by comparison with data from laboratory-based experiments.

Air Temperature.

Recent scientific literature has reported that the oviposition of species within the insect order Diptera (flies) is negligible at temperatures below 10^{o}C. This is due to the poikilothermic (cold-blooded) nature of insects. Therefore, it can be inferred that the decomposition process will also be considerably slower in the winter months due to the lower average temperatures. Also the developmental speeds of the resulting larvae will be also decreased due to the low temperatures again lengthening the decomposition time. Laboratory experiments involving a range of constant temperatures have shown that the time from oviposition to adult emergence is inversely proportional to temperature. One such experiment demonstrated that the development time of organisms of the blow fly species *Protophormia terranovae* varied from 9.19 ±0.3 days at 35^{o}C to 37.78 ±2.96 days at 15^{o}C (Grassberger and Reiter 2002). This species is abundant in Arctic regions and is thought to be the most cold tolerant of the calliphorid species. Even so, there is a considerable variation between the development times at the different temperatures. (Byrd and Castner 2001)

The effects of temperature are a vital consideration in the calculation of the post mortem interval. If it is ignored, a wildly inaccurate value will result, an example of which is shown in a case from Hungary. Testimony by the expert witness, who examined the larvae and eggs associated with the body, clearly demonstrated that, due to the low temperatures after 6pm in September, no flies would have been active. This, along with other evidence, led to the accused release from jail and highlighted the need for temperature considerations in PMI calculations. (Erzinclioglu 2000)

Quantity of larvae.

Another factor, which must be considered alongside air temperature, is that of the number of maggots feeding together in one place. A “maggot mass” can cause an increase in temperature, due to the feeding activities of the maggots. Controlled laboratory experiments have indicated that within an actively feeding mass of second and third instar maggots an increase of 1 – 1.3^{o}C from the surrounding air temperature can occur (Grassberger and Reiter 2002). This increase in temperature may lead to an increase in the speed of development and ameliorates the effect of cold climatic conditions and therefore may have a detrimental effect on the accuracy of PMI calculations if not taken into consideration (Evans 1936, Deonier 1940, Digby 1955, Kamal 1958, Payne 1965, Davison 1971, Ash and Greenberg 1975, Vinogradova and Marchenko 1984, Williams and Richardson 1984, Early and Goff 1986, Nishida et al 1986, Tullis and Goff 1987, Greenberg 1991, Catts 1992, Catts and Goff 1992, Turner and Howard 1992, Tantawi et al 1996).

Refrigeration.

The effects of refrigeration must also be considered in this manner. The insects feeding on a corpse are usually sampled in situ at the site of discovery and again during the post mortem. These samples are then refrigerated and transferred to a laboratory for analysis. Investigations have shown that maggots of the species *Protophormia terranovae* show significant changes in developmental duration following refrigeration (Myskowiak and Doums 2002). This suggests that even though low temperatures have been reported to stop insect development they in fact just disrupt the developmental pattern.

Extreme temperatures can also be indicated by the presence of dead larvae on a corpse. These larvae can be useful in the determination of the time of death of the victim provided that species identification is possible along with the time and cause of death of the larvae. (Staerkeby 2001)

Nocturnal behaviour.

The possibility that death occurred at night must be considered due to the significant effect it has on the behaviour of insects. Conventionally blowflies are believed to be inactive at night and so oviposition would not be expected to occur during this period. However, experiments by Greenberg involving the placement of bait on the ground, under bushes, showed that nocturnal oviposition does occur, but at a substantially reduced rate. Greenberg’s choice of environment for the placement of bait material presented a problem as the ovipositing insects may have crawled onto the bait media rather than having flown in after detecting the food source from some distance away. (Greenberg 1990)

Further experiments were necessary to investigate whether species of the genus Calliphoridae actively fly during the interval between sunrise and sunset. This in turn shows that the attraction of a new food source is enough to make these insects fly during the night. Recent literature has reported that studies undertaken in March and September have shown that flies will fly to a fresh food source at night, but at a rate vastly reduced from the attraction measured during daylight hours. Oviposition was also shown to be significantly reduced (~33% success rate), with fewer eggs being laid (Singh and Bharti 2001) Hence it has been shown that the possibility of nocturnal oviposition must be considered in PMI calculations.

Sheltering.

It has recently been suggested that the sheltering of a corpse can affect the rate of decomposition over the different seasons due to changes in insect succession. (Bornemissza 1957).

Studies undertaken in the humid warm-temperate climate of Argentina on both sheltered and unsheltered pig carcasses have produced quantitative results showing the differences in decomposition rates and insect oviposition between the two environments.

In all seasons, for both sheltered and unsheltered environments, blowflies were the first species to colonize the corpse. In autumn, spring and summer, oviposition occurred on the first day after death whereas, in winter, eggs were not laid until day two. Further oviposition by blowflies occurred for varying amounts of time depending on the season. In the autumn fresh egg-laying was noted on the sheltered corpse until forty-one days after death and up to day forty-eight on the unsheltered corpse. In winter the effect of sheltering was even more pronounced with oviposition ending on day fifteen on the sheltered corpse but continuing until day forty-five when the corpse was unsheltered. In spring and summer no difference was observed between the two different environments with oviposition occurring until day five.

Each stage of decomposition was characterized by the developmental stages of the insects present on the corpse. Blowfly eggs and first instar larvae were present during the fresh stage, leading to the presence of the second and third instar blowfly larvae in the bloated stage, along with adult carrion beetles. Third instar blowfly larvae were also observed in the active decay stage, with adult carrion beetles and their larvae. In the advanced decay stage mainly post-feeding blowfly larvae and carrion beetle larvae were present. The fifth and final remains stage showed the presence of an abundance of beetles with very few blowfly larvae remaining due to a lack of food.

The different seasons and environments also affect each stage of decomposition. Data obtained in autumn revealed a marked decrease in the overall decomposition time of the sheltered corpses compared to those in an unsheltered situation, due to the shorter bloated and advanced decay stages experienced by the sheltered corpse. During the winter months there was no difference in the total decomposition time between the two environments. However, the unsheltered corpse displayed a much longer bloated stage along with shorter active and advanced decay stages. Spring results also showed no difference between the decomposition times for both situations. Even so there was some difference between the stages in that the unsheltered corpse reached the remains stage much quicker than the sheltered one. The data collected in the summer months again displayed an equal decomposition time for the sheltered and unsheltered corpses. Also in this season there was little variation in the length of the individual decomposition stages. These results show that sheltering is another vital factor that must be considered in PMI calculations due to the variations in decomposition shown over the various seasons (Centano et al 2002).

Entomotoxicology.

Bodies discovered in an advanced state of decomposition present a different problem, as the specimens usually taken for toxicological analysis, such as blood, urine and internal organs, are not present (Goff 2000). However, the insects related to the corpse can be sampled as reliable alternate materials for analysis for toxins and drug substances (a process known as entomotoxicology.)

When Diptera larvae feed on intoxicated tissue, they in turn metabolize the substance and incorporate it into their own tissue. Predatory beetles such as Coleoptera can also be useful in toxicological analysis as a secondary bioaccumulation occurs. Toxins are transferred to the beetles via their consumption of larvae that have previously metabolized the drug substance from human tissue. During experimental studies a large number of poisonous chemicals have been recovered from maggots that feed upon animals which died due to intake of such chemicals. To name a few, cocaine (Goff et al 1989), triazolam, oxazepam, alimemazine, chlorimipramine, and phenobarbital (Kintz et al 1990), methamphetamine (Goff et al 1992), lead arsenate (Leclercq and

Valliant 1992), coproxamol and amitriptyline (Wilson et al 1993) have been found to be present in maggot tissues.

It is often the case that only insect remains are left on a skeletonised corpse. In particular puparial cases are usually abundant. These can withstand changes in climatic conditions and are therefore unchanged a long time after the adult insect has emerged. Techniques developed from hair analyses can be used to examine the insect remains for possible toxicological evidence. Insects of the order Diptera are of particular use. Even though post-feeding larvae eliminate the majority of the metabolized drug substance or toxin prior to pupation, some is incorporated in to the puparial case. Controlled experiments carried out by Bourel *et al* (2001) indicated that the puparial cases of larvae fed on food sources containing known concentrations of morphine contained higher levels of morphine than the desiccated remains of the adult flies. Also, insects reared on the muscle tissue from the cadavers of heroin-abusers again contained a higher concentration of morphine in their puparial cases than in the adult flies, but still in very low concentrations.

In addition to providing useful toxicological analysis, insects can be used to indicate the presence of drug abuse.

All these factors demonstrate that insects and insect remains can be vital in gaining toxicological data about a highly decomposed corpse when useful tissue samples have decayed.

Insects feeding on the corpse could be used in determining any drugs or toxins that were present in human tissue before death (Insect in Legal Investigations 2001). Drugs and toxins accumulate in body tissue that becomes assimilated in insect tissue. Similarly, insects also feed on plant material. The country or origin of this material could also be determined using toxicological analysis.

Myiasis.

So far only the uses of necrophagous insects in forensic entomology have been discussed. However, some insects also feed on living tissue; an activity called Myiasis, and has been previously used to identify cases of neglect in children and elderly people. The discovery of such larvae on a body can suggest that infestation occurred prior to death and can therefore be used as evidence to prove neglect (Benecke and Lessig 2001). This different branch of forensic entomology portrays how vital forensic entomological evidence can be in identifying cases of neglect and in determining how long it has occurred.

Using the knowledge that certain insects (such as *Muscina stabulans* and *Fannia canicularis*) are attracted to bodily waste (faeces and urine), the insect fauna found on a body can be used to indicate cases of abuse (children, rape, neglect) (Benecke 2001, Insects in Legal Investigations 2001). Some of these insects are known to be associated primarily with living bodies and others to carrion. Thus, if persons are not properly cared for and cleaned, some insects are attracted to their bodily wastes, where they deposit their eggs.

Blood spatter artifacts.

Forensic entomologists must consider the blood spatter artifacts that are caused by insects moving around a crime scene. In a double homicide investigation in Nebraska in 1997 complex blood stain patterns were discovered and characterized as fly droppings. On initial examination it was thought that the various stains were due to low, medium and high velocity blood spatter, caused by gunshot wounds and considerable movement by the victims. However, laboratory experiments and field observations suggested they may have been caused by flies feeding on pools of blood and then transferring the blood to other areas of the room. (Benecke et al 2000).

If this possibility had not been identified the investigation into the homicide would have been misled by the extensive blood spattering, again indicating the vital part forensic entomologists play in criminal investigations.

Flies can also digest blood and faecal matter contaminated with blood. Any regurgitated matter, or flyspecks, could interfere with bloodstain analysis (Insect in Legal Investigations 2001). Flies and other crawling insects can also confuse bloodstain analysis by leaving "footprints" and spatter on the floor, wall and ceiling of a room.

Around the world, insect scientists (entomologists) are being called upon with increasing frequency to apply their knowledge and expertise to criminal and civil proceedings and to become recognized members of forensic laboratories and medical / legal investigation teams. Insect evidence gathered from and around the corpse, when properly collected, preserved and analyzed by an experienced and appropriately trained forensic entomologist, can provide an objective estimate of the time of death as well as other valuable information concerning the circumstances surrounding the victim's demise. (Lord, 1990)

This science has emerged as a major discipline with the passage of time in the developed countries and its role in criminal investigations has become more and more relevant. Nowadays forensic entomologists are hired by prosecutors and defenders like lawyers and expert witnesses. Even some of the well known agencies like FBI of USA have employed entomologists as special agents. Hundreds of research papers dealing directly or indirectly with forensic entomology have been published so far and this number is increasing day by day. Five books dealing solely with forensic entomology are now available.

The above presented text gives a fair idea about the potential importance of this branch of science. However a detailed working knowledge is a must for the use of insects in solving crimes.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Application of entomological knowledge during criminal investigations requires an in depth information about the life history, habits, immature stages, geographical distribution, taxonomy etc. regarding the insects which can be potentially important as forensic indicators. Large numbers of workers have contributed in this direction.

Workers around the globe have laid stress on the importance of entomological evidence and its implications in criminal cases (Nuorteva et al. 1974; Keh 1985; Erzinclioglu 1986; Turner 1987; Greenberg 1991; Turner 1991; Singh et al 1999). Moreover, five books dealing exclusively with forensic entomology have been published; “A Manual Of Forensic Entomology” by Smith (1986), “Entomology and Death : A Procedural Guide” by Catts and Haskell (1990), “Forensic Entomology- The Utility Of Arthropods In Legal Investigations” by Byrd and Castner (2000), “A Fly For the Prosecution : How Insect Evidence Helps Solve Crimes” by Goff (2000) and “Entomology and the Law: Flies as Forensic Indicators” by Greenberg and Kunich (2002). Overall, there is a sizable representation of about 500 papers dealing with various aspects of forensic entomology. The prestigious journal Forensic Science International has also published a special issue on forensic entomology (2001) including research papers of many renowned forensic entomologists of the world.

Due to non-availability of human bodies for decomposition studies, a wide array of different animal carcasses has been used to simulate humans by various workers from time to time. In most of these studies, insect fauna mainly comprises of order Diptera (blow flies, and flesh flies), Coleoptera (Silphidae, Staphylinidae, and Dermestidae) and Hymenoptera.

The first published account of the application of entomology to legal medicine is of Bergeret (1855) where the occupiers of a house were exonerated of murder charges on the basis of insect evidence.

European pioneers, Bergeret (1855) and Yovanovitch (1888) emphasized the importance of faunal studies from dead bodies during investigations. It was expanded later on by Megnin (1894) in his monumental book entitled "*La Fauna des Cadavers: Application entomologie a la Medicine Legale*." While making observations on exposed human corpses, Megnin concluded that the corpse undergoes a series of changes that can be grouped under different stages and arthropods characteristic of each stage appear in a regular succession. He put forth that each of these stages during decay of a dead body is expected to last a certain specific period of time, until the last stage when only debris remains. This revelation came at a time when scientific methods of crime investigation were gaining ground.

Hough's (1897) work also confirms and extends observations of the earlier workers. He studied the fauna of dead bodies with special reference to Diptera and also compared the insect fauna of America with that of Europe.

Johnston and Villeneuve (1897) also worked on exposed human corpses. They agreed with Megnin, except regarding the durations of various stages which according to them vary from place to place. So they emphasized that before putting Megnin's method into universal practice, experiments should be performed at particular localities.

Prompted by these works Motter (1898) started studies on the fauna associated with buried corpses. He observed 150 disinterments where dates of original interments were known to him and provided a detailed list of the fauna found on the buried bodies.

Glaister and Brash (1937), solved a classical case, famously known as Ruxton case. Entomological evidence on the basis of immature stages of *Calliphora vicina* led to the conviction of Dr. Ruxton.

Mearns (1939) studied larval infestation and putrefaction of the human body in order to check the accuracy of the estimate of the time interval between death and the examination of the human remains. He identified the maggots that may be present upon the body, since having identified them, it becomes possible to determine the age reached in their cycle of development, and thus determine the post-mortem interval.

Deonier (1940) studied the relationship of carcass temperature to winter blow fly population and activity. He pointed out that after establishment of larvae on carcass, its temperature rises than that of the ambience.

As Chapman and Sankey (1954) put "the dipterous larvae were the only scavengers of any consequence in the decomposition of the carcasses. These provide the basis on which the rest of the fauna, mainly predaceous beetles and parasitic Hymenoptera depend."

Bornemisssza (1957) studied the effect of carrion decomposition on the soil fauna. This study showed that soil fauna plays only a minor role in decomposition of the carcasses. Ants and earwigs were the only members of the soil fauna which fed on carrion.

Kamal (1958) investigated effect of controlled temperature and humidity on the life history, rate of development and other biological activity of both adults and immature stages of thirteen species of flies, representing nine genera within the families Sarcophagidae and Calliphoridae. He also studied the nutritional aspects of larval forms with special emphasis on how the quantity and quality of food affect life span, rate of growth and size of both individuals and populations.

One of the best studies on insects and their relationship to decay rates was undertaken by Reed (1958). His study of 45 dog carcasses placed at intervals of about two weeks in hot weather and less frequently in cooler weather, revealed much information on the ecological process and stages of decomposition. He found that the total arthropod populations were greater in summer; however, certain species reached their maximum population during the cooler periods of the year. Reed also discovered that insect populations in general were larger in wooded areas than in the non-wooded areas.

Burger (1965) studied the succession of saprophagous Diptera in different seasons of the year on mammalian carcasses.

With regard to maggot development rate Payne (1965) found that carcass temperatures often are elevated due to heat generated by maggot masses in corpses and ambient temperatures often do not reflect those to which maggots are exposed. According to him a definite ecological succession occurred among the fauna of carrion. Each stage of decay was characterized by a particular group of arthropods, each of which occupied a particular niche. Their activities were influenced by physical properties of carrion, rapidity of putrefaction, time of day and weather.

According to Gilbert and Bass (1967) the presence of fly pupae or empty pupal shells on the buried bodies can tell us something about the season of burial, if not the exact age of the body.

Easton and Smith (1970) pointed out that the appearance and abundance of fauna are related more to the season than to the stage of decay.

Nuorteva et al. (1967) solved three cases with the help of entomological evidence. In one case he calculated the PMI of a body lying indoors. In another case the body was badly burnt. He also solved the case of a suicide with sedatives of a woman found dead in her bed. In all the three cases the ‘witness’ was *Calliphora vicina*.

Nuorteva (1970) has reported that histerid beetles and other predators of blow fly larvae cause remarkable decrease in numbers of developing blow flies. According to him, these beetles can be important in the biological control of Calliphorids. Similarly, Payne and Mason (1971) reported 82 species of Hymenoptera associated with pig carrion. Payne and King (1972), while studying the decomposition of pig carcasses in water, observed that water limited the number and kind of arthropod scavengers reaching the animal carcasses. So the insects disposed off the carrion at a rate approximately one third of that for carcasses on soil surface. A definite succession occurred in about 102 insect species inhabiting water carrion and each stage of decay has its particular group of scavengers.

Wasti (1972) carried out carrion study of common fowl in relation to the incidence and possible succession of arthropods, fauna. He also observed that in carrion free from arthropods, the rate of decay was very slow and no distinct decompositional stages were recognizable.

Japanese Association of Criminology (1973) described insect encroachment traces for purposes of distinguishing them from other artificial antemortem and postmortem injuries, by use of two corpses left for 10 days under conditions of continuous ventilation and low humidity, with photographs.

Cornaby (1974) observed that adults and larvae of Calliphoridae and Sarcophagidae and adult Formicidae and Scarabaeidae are most important in reducing (Lizard/Toad) carcasses to dry skin stage. He identified 172 species which are important reducers of carrion.

Nuorteva (1974) estimated the age of the blood stain on the shirt of the suspect found in a dustbin near the dead body with the help of fly larvae and puparia of *Muscina stabulans.*

Johnson (1975) examined microseral and seasonal variations in insect population on 39 small mammal carcasses. He observed that Coleoptera. Diptera, Hymenoptera and Acarina constituted 90% of the arthropod fauna that visit carcasses.

Lane (1975) mainly concentrated his studies on blow fly succession on white mice, so as to calculate precisely and accurately time of death within the period of 4 to 5 days after death during which blow flies are the most significant insects present.

Denno and Cothran (1976) studied the competitive interactions and ecological strategies of Sarcophagid and Calliphorid flies inhabiting rabbit carrion. They artificially reduced the calliphorid population density by selectively interfering with oviposition and noticed that Sarcophagid population density increased six fold. So, they could conclude that the competition from Calliphorids was the principal factor limiting the population size of Sarcophagids.

Kitching (1976) described and illustrated the eggs, three larval instars and puparia of *Chrysomya bezziana*. He also compared them with 5 other species of *Chrysomya* found in Africa and Oriental region. The most common stage found on the corpse is the larva and so, its identification is a must from forensic point of view.

Nuorteva (1977) has described the use of sarco-saprophagous insects as forensic indicators with particular importance to the determination of the post-mortem interval. He has included descriptive case studies. He concludes that forensically important conclusions can be drawn from the identification of the successional stages of the arthropod fauna and of the stage of development of the sarco-saprophagous insects on human corpses. Nuorteva calculated the PMI as well as place of death of a partly covered decayed corpse of a 17 year old girl.

Coe (1978) observed that in the absence of vertebrate scavengers dipterous larvae consume up to 5% of the soft tissue of elephant carcasses, the remaining being utilized by microorganisms.

McKinnerney (1978) studied carrion community composition in 40 rabbit carcasses. He described four decompositional stages and collected 80 arthropod species, out of which 63 were identified as participants in the carrion community.

Putman (1978) studied the role of carrion frequenting arthropods in decay process in temperate woodland and grassland habitats in 4 different seasons. A distinction is drawn between those occurring by chance and those positively associated with carrion. He also discussed the role of the latter group in actually bringing out decay of the carcasses.

Flynn and Moorhouse (1979) studied blow fly succession on carrion in order to observe the primary flies of summer and winter seasons and their importance in forensic entomology.

Levot et al (1979) studied the larval growth of some calliphorid and Sarcophagid flies. According to them the relative success of each species depended upon the ability of the larvae to attain quickly the minimum weight for viable pupation. *Calliphora nociva, Calliphora augur, Chrysomya megacephala* and *Chrysomya rufifacies* were the species best adapted to pupation at low larval weight i.e., their food requirement for successful pupation were less than those of other species.

Broadhead (1980) and Erzinclioglu (1980) independently studied the role of Trichocerid larvae in decomposition of carrion in winter. They found that these larvae can withstand low temperature and continue to breed in carrion.

Jiron and Cartin (1981) studied decomposition of dog carcasses in Costa Rica and divided it into four stages of decay. However, they did not take into account the different species of insects visiting the carrion.

Teskey (1981) described in detail the morphology and terminology of Dipteran larvae including those of sarcophagous families.

The work by Abell et al (1982) is one of the very few studies made on reptilian carrion. According to their studies, blow flies and Dermestid beetles play a major role by acting as primary consumers, and Staphylinid and Histerid beetles are the major predators found on the carcasses.

The opportunities to study human decomposition and insect fauna associated with it are rare because of ethical, religious, cultural, social and legal reasons. One such study is of Rodriguez and Bass (1983) in Tennessee, U.S.A. They concluded that decaying human cadavers undergo four basic decomposition stages; fresh, bloated, decay and dry. The succession of each decompositional stage was found to be well correlated with the succession pattern of various carrion frequenting insect groups.

Beaver (1984) compared the insect community that inhabits carrion with other type of ephemeral habitats like dung and dead wood in tropical and temperate regions.

Greenberg and Szyska (1984) reared 15 species of Peruvian blow flies to study the structure of their egg plastrons, larval instars and puparia. They also gave a key to the larvae of known species of Peru apart from studying their developmental rates and diet activity.

Lord and Burger (1984) also studied the arthropods associated with Herring Gull and Great black backed Gull. According to them, carcasses located in vegetated habitats; having less extreme environmental conditions, support significant number of predatory insects, particularly ants, and fewer carrion consumers, thereby slowing carrion consumption and decomposition rate. On the other hand, carcasses located on beaches lack protective vegetation resulting in their rapid dehydration. These drier carcasses were found to support greater populations of dried tissue consumers, especially Dermestid beetles and fewer predators, leading to rapid rate of carrion consumption.

Nishida (1984) developed exact growth tables and growth curves for seven species of blow flies and fleshflies at 15°C, 20°C, 25°C, 30°C and 35°C. On the basis of this study date of molting, pupation or emergence of each species at different temperatures could be calculated.

Smeeton et al (1984) also worked on exposed human corpses. They tried to determine whether certain species were consistently associated with specific time elapsed after death, and if so, to determine their uses and limitations as forensic indicators.

The stabilizing effect of maggot mass generated heat was studied in human remains by Vinogradova and Marchenko (1984). They presented threshold developmental temperatures for nine species of blow flies.

Williams (1984) developed a computer model for determining the time of blow fly egg hatching based on maggot weight and temperature record.

Williams and Richardson (1984) observed that higher temperature of mixed species maggot mass was tolerated by *Chrysomya rufifacies* but the population of *Lucilia cuprina* was burned out.

Erzinclioglu (1985) gave descriptions based on a detailed comparative study of the structural features of all three larval instars and puparia of the six British *Calliphora* and single *Cynomya* species. Results demonstrated that certain characters, singly or in combination, enable reliable species separation. He has also provided keys to third instar larvae and puparia of all the above species.

Goddard and Lago (1985) studied blow fly succession on carrion in order to observe the primary flies of summer and winter seasons and their importance in forensic entomology.

Greenberg (1985) solved three cases on the basis of immature stages and adults, present on the dead bodies and was able to determine the time since death and the place of death.

Rodriguez and Bass (1985) further expanded their research to include the decomposition of buried corpses under conditions that simulated burial death scene.

While working on the insect succession on Impala Ram, Braack (1986) concluded that blow flies are the important determinants of community structure owing to the dominance of their larvae in utilizing carcass soft tissues. The larvae also serve as a link in the food chain as they are an important food item for the predators. Arthropods were capable of reducing a medium sized carcass to keratinous remains and bone within 5 days in summer and within 14 days in winter.

Early and Goff (1986) recorded differences of up to 22^0C between carcass temperature and ambient air temperature in Hawaiian Islands. They studied arthropod succession patterns in two different habitats i.e. one in xerophytic habitat away from human dwelling and other in wetter habitat (mesophytic) near human dwelling. They concluded that the early stages of decay were prolonged in case of cat carrion present in xerophytic habitat due to the presence of predatory ant *Solenopsis geminata*, and latter stages of decay were lengthened due to rainfall in case of wetter habitats. Moreover, there are differences in species composition of the two habitats. Similar types of observations have also been made by Erzinclioglu (1986) with regard to carrion lying in forest and the other in an open area.

Goff et al (1986) estimated a post mortem interval of 19-20 days for remains of a female recovered from a ditch on the Islands of Oahu, Hawaiian Islands. They also based their analysis on the insects present on the remains and the surrounding area. They provided a checklist of arthropods associated with exposed carrion in Hawaiian Islands though they did not concentrate on the succession patterns.

Another case of homicide from Fort Lewis, Washington was solved by Lord et al (1986) on the basis of 3^{rd} instar larvae of green blow fly, *Lucilia illustris* found on the decomposing body, emphasizing that immature stages of this blow fly can be used to determine the accurate time of death.

Lord et al (1986) also demonstrated the importance of ADH in situations with small faunal populations, variable weather and presence of multiple species. According to these authors it is applicable to indoor situations only.

Smith (1986) described the faunal succession on dead bodies that include exposed, buried, mummified, and burnt bodies. He also discussed how the environmental conditions as temperature and humidity, light and shade influence the fauna on the body. Eighteen case histories were cited, and a large part of the work dealt with methods to study the different taxa occurring on dead bodies. Blow flies are among the earliest arrivals at a corpse, often ovipositing on it within hours of death. As a result, eggs of these flies are commonly found on the bodies of murder victims. The correct identification of species involved in such cases will often give valuable insights into the circumstances of murder and thus may be of great forensic value.

Erzinclioglu (1987) described diagnostic features of 3^{rd} instar larvae of 10 species of blow flies of medical and veterinary importance. He also described characters for recognition of the early instar larvae of genera *Calliphora* and *Lucilia*. Continuing with his studies on blow fly larvae he worked on the larval instars of *Calliphora croceipalpus* and provided keys for separation of this species from other African congeners. In the same paper a key to the 3^{rd} instars of all genera of Calliphoridae known to breed in carrion in Africa is also provided.

Goff and Odum (1987) used entomological techniques for estimating PMI in 3 homicide cases on the Hawaiian Islands. These cases represent different stages of decomposition and range from 8 to 53 days postmortem interval.

Houston (1987) studied the effect of ant predation on carrion insect communities in a Brazilian forest. According to him this predation substantially reduced the number of dipteran larvae in carrion and prevented the baits to be totally consumed by maggots.

Sifting of literature reveals that very little work dealing with forensic entomology has been done in India and only few research papers have been published till date. Kulshreshtha and Chandra (1987) while dealing with 25 death cases collected different immature stages of insects found on the dead bodies. They reared them in the laboratory at constant temperatures of 30^0C and 40^0C. From the results obtained they tried to calculate the time since death of infested bodies. One of the major draw back of this work is that they didn't even know the identity of species reared by them which is a must in such studies.

Erzinclioglu (1988) described three larval stages of the blow flies, *Phormia terranovae, Phormia regina* and *Borellus atriceps* and discussed biology of these flies which according to him are adapted to cold climate.

Goff et al (1988) have reported that almost identical PMI was estimated in three cases of homicide which differed in habitat (Xerophytic, Mesophytic and Swamp) as well as in gross appearance. Hence they have emphasized that in the absence of entomological technique wrong PMI's would have been calculated.

Greenberg (1988) points out that as much as 40% of the blow fly life span is spent as a puparium and valuable time line evidence can be gained by aging this stage in detail. He has also studied the chronological changes occurring on the surface of the pupa within the puparium and the dispersal distances traveled by post feeding maggots prior to pupariation. Greenberg also discussed the importance of ADH (accumulated degree hours, which is the product of temperature in °C with time taken by fly egg to reach adult stage at that constant temperature) for making time-line estimates.

Skinner et al (1988) studied the partial skeleton of a young person recovered below a mountain road in southeastern British Columbia in the late spring of 1985. Postcranial bone fragments were widely scattered and severely chewed by scavenging animals. By contrast, the skull was found by the roadside in many pieces, which were free of chew marks. This damage was interpreted as due to firearms trauma. Insect larvae on the decomposing remains were reared at Simon Fraser University until taxonomically identifiable adults were obtained. Analysis of insect life cycle information, combined with meteorological data, indicated that the victim's body had been dumped between October, 1983 and June 1984. Subsequent analysis showed that the victim was a sub-adult male who had been shot in the head on March 17, 1984. It is concluded that careful field collection and analysis of animal and insect activity by a coordinated team involving police and university professionals can provide sophisticated forensic evidence in homicide investigations.

Erzinclioglu (1989) did not delve so deeply into the life cycles and ecological successions. His study has a far more wide ranging scope and unleashes far more interesting aspects of forensic science than just flies, though obviously, as part of the central theme of the study they are present throughout. He studied in depth the human fly sphere of interaction throughout history. He charts the history of maggots and medicine, an interesting look at the way flies have affected human history. He described chorionic structure and size of eggs of 10 species of blow flies found in Britain. He also emphasized that most useful features are the shape and structure of platonic area.

Haskell et al (1989) felt that although its potential was great, the use of aquatic insects in determining submersion intervals at death-scene investigations had not been exploited in the past. Aquatic environments have no known true specific indicator species, as do terrestrial habitats. However, aquatic environmental studies showed that organisms may colonize a substrate dependent on factors such as size, position, exposure to current, water temperature, current speed, water depth, the presence of algal communities, or detritus. Certain aquatic insects such as the Chironomid midges (Diptera, Chironomidae) and caddis flies (Trichoptera) were capable of colonizing immersed bodies; and with the known biology of a specific species of insect for a certain geographic area, time intervals of submersion could be established.

Hawley et al (1989) noticed and recovered a peculiar red 'fiber' during preliminary examination of the body of a homicide victim. Initially believing this to be a carpet fiber, the item was subjected to fiber analysis. It was found a short coiled particle not like any known natural or synthetic fabric fiber. Subsequent examinations determined this 'fiber' to be larva of a common freshwater midge (Diptera; Chironomidae). Chironomid larvae have been observed on other bodies recovered from freshwater environments. Entomological studies of this organism led to the conclusion that the presence of Chironomid larvae indicates submersion of the body.

Introna et al (1989) studied life cycles of *Lucilia sericata* reared in the field with continuous registration of temperature, humidity and luminosity. When compared to parallel life cycles reared in a growth cabinet, the results showed no statistical difference between the field and laboratory conditions. They reared blow flies in a programmable growth chamber and were able to recreate closely the changing heat and light cycles of a field situation.

Kashyap and Pillay (1989) discussed the role of insects in solving crime. They examined and analysed sixteen insect infested cadavers to evaluate the reliability of entomological method in estimation of time elapsed since death, in relation to other medico legal approaches. They found the entomological method to be statistically more reliable and superior when compared to other prevalent methods. They recorded eight successive waves of invasion from the beginning of putrefaction right up to skeletonization. Initially two winged flies such as blue bottles, fleshflies, house flies, etc. predominate but later there was involvement of other insects such as beetles. They emphasized that the role of the doctor while doing autopsy confined to collection, preservation and sending these to a qualified forensic entomologist. Kashyap and Pillay (1988) calculated the time since death by using the formula: T = A + B x C where ‘A’ was stage of invasion, ‘B’ was stage of life cycle and ‘C’ represented climatic factor correction.

Liu and Greenberg (1989) provided identification keys and diagnostic descriptions for eggs, three larval stages and puparia, of seventeen species of forensically important flies.

Lord and Rodriquez (1989) found that insect scientists are being increasingly called upon to apply their knowledge and expertise in criminal and civil proceedings and to become part of forensic laboratories and medicolegal investigation teams. Based on the analysis of insects and other invertebrates that sequentially colonize a corpse as decomposition progresses and on the rates at which various stages of their offspring develop, entomological information also can provide information useful in determining the manner of death, length of the postmortem interval, and the movement of a cadaver from one site to another. They gave instances when, blowflies frequently arrive within a few minutes to a few hours after death, while other types of insects such as carrion and scarab beetles colonize drier remaining tissues. They emphasized that representative samples of all adult and immature insects should be collected from, on, in, and beneath the corpse. Two samples should be collected - one for rearing to adult stages and one for preservation. Their case histories illustrate how forensic entomology aided investigators in determining the time of death and more narrowly focusing efforts to identify the victim and bring the case to a reasonable conclusion.

Apart from the studies on succession, insect fauna visiting carcasses, their biology etc. workers have also focused on the detection or drugs, toxins, poisons and other chemicals through analysis of maggots that had fed on intoxicated tissues. Gunatilake and Goff (1989) detected organophosphate Malathion poisoning in a putrefying body by analyzing arthropod larvae present on it. Goff et al (1989) investigated the effect of cocaine and its metabolite benzoyl-cocaine on the rate of development of Sarcophagid *Boettohersca peregrine* and found out that cocaine accelerated the growth rate of maggots feeding on the tissue.

Willey and Snyder (1989) found that time-since-death estimations are usually based on physical decomposition of the corpse, insect succession, and contextual associations. The rates of change and succession were based on decomposition studies, most of which control access of scavengers to the corpse; however, many naturally exposed corpses are subject to scavenger modification. These modifications changed the rate of decomposition, the patterns of insect succession, and the context of associations, thus altering estimations of time-since-death.

Blackith and Blackith (1990) observed that there are about 29 species of insects (excluding Coleoptera) which infest small corpses.

Catts and Haskell (1990) have discussed various techniques employed in forensic entomology and they have quoted several cases to substantiate their observations.

Erzinclioglu (1990) studied the larvae of two closely related blow fly species of the genus *Chrysomya*.

Kintz et al (1990) performed toxicological analysis of fly larvae found on putrefied body to calculate its PMI. They identified five drugs i.e. triazolam, oxazepam, Phenobarbital, alimemazine and clomipramine in the fly larvae. They solved another case of death due to poisoning by morphine and Phenobarbital by doing the toxicological analysis of fly larvae present on the putrefied body. In another case they detected Bromazepam and Levomepromazine in remains of cerebral tissue and clavicle of putrefied cadaver and in the fly larvae found on the corpse. They demonstrated that triazolam, oxazepam, alimemazine, chloripiamine and Phenobarbital could be detected from the maggots.

Lord (1990) studied cases of forensic entomology and found that insect evidence gathered from and around the corpse, when properly collected, preserved and analyzed by an experienced and appropriately trained forensic entomologist, can provide an objective estimate of the time of death as well as other valuable information concerning the circumstances surrounding the victim’s demise.

Mann et al (1990) observed the decay of about 150 corpses and pointed out the three most important environmental factors in corpse decay i.e., temperature, access by insects and depth of burial.

Erzinclioglu (1991) felt that since many insects are associated with the human body after death, they are always a potential source of evidence in cases of murder or suspicious death. Also, the fact that many species are associated with various objects of interest to man, such as drugs and other products, means that they are potentially useful as evidence in criminal situations other than murder cases.

Goff (1991) examined different insect populations associated with decomposing remains in indoor and outdoor environments in the Hawaiian Islands through analyses of cases from the Forensic Entomology Laboratory, University of Hawaii at Manoa, Honolulu from 1983 through May 1990. Over 60 cases were referred to the laboratory by the Department of the Medical Examiner for determination of the postmortem interval. Of these, 14 were indoor situations and presented sufficient data to allow comparison. The confirmed postmortem interval ranged from 2 to 21 days in these cases. Twenty-one cases were selected from the outdoor situations in which the postmortem intervals corresponded to those of the indoor ones for comparison of insect species present and the numbers of taxa present. A total of 22 insects, representing 3 orders and 12 families, were recovered from the decomposing remains of the 35 cases considered. Of these, five were recovered in both indoor and outdoor situations. A variety of Diptera species was associated with indoor colonization during the initial stages of decomposition, whereas other Diptera appeared late in the process. In contrast, outdoor situations had fewer Coleoptera species in the beginning of the process, 10 species in 6 families were recovered outdoors. Significant differences in the insect colonization of remains in indoor and outdoor situations can provide useful information about the history of the remains.

Goff and Flynn (1991) determined the PMI of the remains which were in an advanced stage of decomposition process. The estimate in this case was based primarily on an interpretation of succession pattern of insects.

Studies on the effect of heroin on maggot metabolism were carried out by Goff et al (1991) which showed accelerated growth. Pounder (1991) laid stress on importance of entomotoxicology and its possibility in solving drug related cases where no body tissue is left for analysis.

Haskell and Williams (1991) emphasized that record of ambient air conditions of at least 3-5 days preceding the collection is important in determining age of maggots.

Hegazi et al (1991) studied carrion insects of Egyptian Western Desert. He took snail's flesh, and fish as baits and found that these attacked insects belonging mainly to the orders Coleoptera, Diptera and Hymenoptera.

Holloway (1991) provided a key for identification of 3rd instar larvae of 9 species of Calliphorids causing fly strike and also associated with carrion in New Zealand.

While studying carrion fly activity, Introna et al (1991) observed that different species were active during spring and summer seasons. *Calliphora livida*, *Calliphora vicina* and *Lucilia illustris* were observed only during spring time while *Phaenicia sericata* and *Sarcophaga sarraceniodes* were observed only during summer seasons. They also calculated the developmental time for the species encountered during spring and summer season. They reared flies in screen topped glass jars in the field during spring and summer to develop a local baseline developmental table.

Meskin (1991) provided a key to eggs of five calliphorid species present in Afro-tropical region with detailed description of the egg of *Calliphora croceipalpus*.

Peterson and Newman (1991) studied in detail the chorionic structure of egg of screwworm fly *Cochliomyia hominivorax*.

Schoenly et al (1991) proposed an experimental field protocol for determining postmortem interval using an immigration emergence trap for insects and using human and pig cadavers. This protocol can provide useful baseline data regarding medicolegal considerations of causes, manner, and time of death. This protocol was achieved through a multidisciplinary approach encompassing forensic entomology, pathology and anthropology with the apparatus and collecting methods described. Simultaneous information was obtained regarding the sequential arrival and variety of insects in the decay process, the character and manner of soft tissue decomposition, the sequence and nature of bone exposure and order of disarticulation of skeletal remains, and the influence of climate and season on decay rates and arthropod successional trends. The central feature of this protocol was the construction and uses of this dual function trap that captured both arriving and emerging insects while the process of decomposition proceeds uninterrupted on the cadaver. Field procedures and results of trap microclimate tests in an arid climate in 1990 were presented together with preliminary arthropod data collected from field-exposed pig carcasses.

Catts (1992) discussed the influence of maggot generated heat on corpse community development and on subsequent analysis of entomological data.

Goff (1992) demonstrated experimentally that the wrapping of the remains in blankets delayed invasion for oviposition by flies by 2.5 days.

Goff et al (1992) studied the effect of methamphetamine available in decomposing tissues on development rate of *Parasarcophaga ruficornis*. For this, rabbits were given 37.5, 71.4 and 142.9 mg of methamphetamine via ear vein infusion and it was found that larvae feeding on tissues from rabbits receiving 71.4 and 142.9 mg of methamphetamine developed more rapidly than larvae from the control colony and from those feeing on tissues from the rabbit receiving 37.5mg of methamphetamine.

Isiche et al (1992) compared the decomposition of mouse placed directly in sun and other under shade and observed that the insect fauna was quite different in these situations.

Leclercq and Valliant (1992) observed that lead arsenate is repulsive to certain insects and their absence from a dead body led them to conclude that the deceased had been poisoned.

Lord et al (1992) used *Synthesiomyia nudesta* (Muscidae) and *Calliphora vicina* (Calliphoridae) to estimate the time of death of a body buried under a house for 14 days.

Nolte et al (1992) solved a case of death due to cocaine poisoning by toxicological analysis of fly larvae found in the decomposed body.

Schoenly (1992) proposed a set of statistical protocols, for the analysis of carrion-arthropod succession, in forensic entomology investigations. A total of 23 carrion-arthropod data sets from temperate, tropical, desert, and coastal environments were assembled in a standard format and analyzed by the use of randomization tests and methods derived from quantitative community ecology. The data were analyzed in three ways. First, patterns of arthropod visitation on non-human carcasses were analyzed in each of the 23 cases. Second, temporal changes in the taxonomic composition of the carrion-arthropod community were studied by quantifying the degree of taxonomic similarity between pair-wise combinations of time-specific samples of the succession. Third, Monte Carlo simulation was applied to each of the 23 assemblages to test specific hypotheses about community-wide patterns of arthropod visitation times on non-human carcasses. Findings from each of these methods are reported. Possible uses of these methods to detect or validate claims of between-site, between-treatment, or between-taxon differences in carcass decay rates are also discussed. The proposed methods and their findings may prove useful to forensic entomologists in hypothesis testing, field studies of carrion-arthropod succession, and time-of-death estimates for human remains.

Schoenly et al (1992) presented a computer algorithm, implemented in the BASIC language, for calculating the postmortem interval (PMI) from arthropod successional data. They felt that entomology-assisted determination of the PMI promises to be a reliable technique in cases of homicide, suicide, accidental death, and unattended death due to natural causes. The program required, as input, the identity of arthropod taxa recovered from human remains in a death scene investigation and machine-readable data on carrion-associated arthropod taxa and their known successional patterns of activity for the same geographical area. The program performed rapid comparisons of these lists and, on output, calculated an upper and lower estimate of the PMI, identified the definitive taxa for these limits, and determined if the remaining corpse taxa have known successional patterns that were consistent for this estimate. An alternate output was provided if one or more corpse taxa did not overlap all the others at any single time in the succession. In that event, the user was prompted to recheck the identity of the non-overlapping taxon or taxa or reevaluate the environmental circumstances surrounding the case in question. Results of the analysis were saved to a file for output to a printer for making paper copies useful for the entomologist's Case Study Final Report. This program may make possible wider use of this technique in law enforcement and medical investigator offices that utilize both forensic entomologist expertise and compatible computers.

The level of heat generated in a maggot mass can also selectively affect maggots of two different species as recorded by Turner and Howard (1992).

Gaur (1993) solved a case with the help of blow fly larvae (species unidentified) found on the dead body.

While working on another case Lord et al (1993) reported that black solider fly, *Hermetia illucens*, initiates oviposition 20-30 days postmortem, and its life cycle would require at least 55 days (27-28^{0}C) for completion. So it can provide medicolegal investigators with valuable parameters for estimating PMI for badly decomposed remains.

Loew and VanderLinde (1993) studied insects frequenting decomposing corpses in South Africa. Flies related to 17 different cases and their role in solving PMI is discussed.

Shean et al (1993) studied difference in decomposition rates of two pig carcasses, kept in close proximity to each other, one exposed to sun and other under shade. They concluded that ambient air temperatures are extremely important in influencing carrion decomposition primarily through the activities of calliphorid larvae. Air temperatures can vary significantly between areas as little as 300 m apart and tend to show more extreme maximum and minimum temperatures at exposed locations than do areas that are more shaded. These differences in temperature patterns can have profound effect upon the decomposition rate of a corpse.

Tantawi and Greenberg (1993) checked the effect of killing and preservative solution and estimates of maggot age in forensic cases. They experimented on the maggots of *Protophormia terranovae* and *Calliphora vicina* and found that there was shrinkage in all the 15 solutions used as preservatives. They concluded that maggots killed in boiling water and then placed in preservative solution did not shrink and recommended such treatment of maggots collected at crime scene and at autopsy if their length is to be interpreted in a valid and consistent way. They measured the minimum developmental rates of *Protophormia terranovae* and *Calliphora vomitoria* at four different temperatures i.e. 12.5°C, 23°C, 29°C and 35°C. They also suggested that these species prefer breeding in larger carcasses as survival tactic.

Wilson et al (1993) solved a case of death due to co-proxamol and Amitriptyline poisoning by analyzing the maggots present on a putrefied body.

Goff and Lord (1994) again laid stress on the importance of toxicological analysis of fly larvae or other insects in solving drug related deaths.

Miller et al (1994) gave first report on the detection of drugs like Amitriptyline and nortriptyline from chitinized insect tissues (Fly puparia and Beetle).

Singh and Greenberg (1994) studied survival after submergence in the pupae of five species of blow flies and found that 25% of the pupae of three species produced normal adults after four days of submergence but none after five days. These data are potentially useful in estimating duration of submergence of a corpse in forensic investigations where the corpse gets submerged after pupariation.

Sperling et al (1994) described a molecular method of DNA analysis for the rapid identification of insect larvae found on a corpse so as to determine the time of death. Specific insect DNA fragments were amplified using the polymerase chain reaction (PCR), followed by direct DNA sequencing of the amplification products. A total of 2,300 base pairs of mitochondrial DNA were sequenced from each of three blowfly species; all three species are important in forensic entomology, because they lay their eggs at predictable times in the decay cycle of a corpse. Results revealed 118, 186, and 196 differences between the sequences of different pairs of the three species. These abundant DNA sequence differences made it possible to clearly identify the immature larval stages of these insects. These DNA sequence differences were also used to predict species specific, diagnostic restriction sites in the amplified DNA; these predictions were verified by digestion with nine restriction enzymes.

Wells and Greenberg (1994) used goat, rabbit and rat carrion as resource to identify conditions under which native taxa might avoid interaction with the invaders. For this, they captured post-feeding larvae as they left a carcass and found that native carrion flies belonging to Calliphoridae are regularly the first and *Chrysomya rufifacies*, the invader fly, the last to appear as post feeding larvae. Sarcophagidae was also found to precede *Chrysomya rufifacies* in succession.

Wells and Kurahashi (1994) studied the development of *Chrysomya megacephala* at 27^0C. They observed that there were differences in the length of post feeding larvae and attributed these differences to the sensitiveness of post-feeding larvae to environmental conditions.

Anderson (1995) solved 42 cases from British Columbia (Canada) on the basis of available entomological data. She highlighted the need for further work to develop complete database in various habitats, geographical regions and seasons. The objectives of this study were to begin a database of insect succession on carrion for British Columbia; to indicate the type and range of cases seen by a forensic entomologist in British Columbia; to determine the situational, regional, and seasonal distribution of the insects found; and to indicate the presence or absence of drugs or alcohol in the bodies. In each investigation, trained death investigators at the scene, autopsy, or both collected insect evidence. Samples of all species of insects present were collected directly from the remains, the clothing, if present, and the surroundings where the body was found. Samples were collected from all regions of the body. Photographs of the death scene, together with precise details of weather conditions at the time the insects were collected. Results of toxicological examination and determination of manner of death were obtained from investigators. Meteorological data were obtained for the weather station nearest to the death scene. At the end of each investigation, a report of the insects collected from each set of remains and an estimation of the time of death were submitted to the British Columbia Coroners Service. The insects collected from the remains were identified based on species, developmental stage, and age of remains to begin a database for British Columbia. Habitat and geographical region, toxicology, manner of death, and season of discovery also summarized cases. Most of the cases were from the Vancouver region and were found in primarily rural areas, although many were found inside dwellings or enclosures. Drugs and/or alcohol were detected in 80 percent of the cases tested. Most of the 42 cases were the result of homicide, and although detected year-round, occurred primarily between June and October.

Greenberg and Singh (1995) studied eggs of eleven forensically important calliphorid species belonging to six genera with the help of scanning electron microscope for useful diagnostic characters.

Patrician and Vaidyanathan (1995) studied arthropod succession in rats which were euthanized in different ways. They noticed that arthropod succession is influenced by the manner of death. Chemicals like sodium pentobarbital delayed the oviposition of Calliphorids by seven days and rat carrion took twice as long to decompose.

VanWyk et al (1995) studied the effect of ethanol in dipterous maggots on decomposing rabbit carcasses.

Vance et al (1995) worked on the decomposition of carrion under water and described a device for sampling aquatic insects associated with carrion.

Anderson (1996) conducted a practical exercise in forensic entomology as how a study of insect activity on corpses and other crime-scene materials can yield valuable evidence. She found that date of death estimation is probably the most common and best-known use of forensic entomology in criminal investigations. If the death has occurred within a few weeks of the body being found, the entomologist will be able to estimate a date of death by using data on the life cycle of the species of fly larvae found on the body.

Johl and Anderson (1996) studied the effect of refrigeration of immature stages on the development of the blow fly, *Calliphora vicina* at a temperature of 3^0C for 24 hours and concluded that such a treatment of any stage (egg, larva, pupa) induced a 24 hour delay in adult emergence because the insects did not appear to develop while chilled.

Staerkeby (1996) conducted study on determination of the time of death with the means of forensic entomology. He studied various methods that can be used to determine the post-mortem interval. He studied the succession patterns on the dead body, various insects on the body, various stages of the insects and how to determine the age of the different stages of insects so as to determine the post-mortem interval from them.

Tantawi et al (1996), while studying arthropod succession on exposed rabbit carcasses, recognized four decompositional stages namely fresh, bloated, decay and dry. They found that dipterous larvae of family Calliphoridae, and to lesser extent of families Sarcophagidae and Muscidae, were responsible for the process of carrion degradation. Similar study on insect succession on pig carrion was carried out in British Columbia (Canada) by Anderson and Vanlaerhoven (1996) to generate a data base for that province.

American Board of Forensic Entomology (1997) gives most of the information required about the field. It discusses the history, the case studies, the professional status and the science of forensic entomology.

Anderson (1997) worked on a strange case in which insects were used to determine time of decapitation which was done after death.

Goff et al (1997) studied the effect of 3, 4 methylene dioxy-methamphetamine on the development of larvae and pupae of *Parasarcophaga ruficornis*.

Goff and Win (1997) solved a very interesting case in which human remains were found inside a metal tool box. The PMI of 14+ months was calculated by determining development time required for a Stratiomyid fly (Diptera: Stratiomyidae) *Hermetia illucens* in combination with time required to establish a colony of ant *Anoplolepsis longipes* (Hymenoptera: Formicidae).

Benecke (1998) did DNA fingerprinting of larvae found outside the body bag in an effort to relate them to the batch of larvae present on the body. He calculated the PMI in six different cases. The parameters used for this purpose were ranging from larvae found on the body, to drug (heroin) detection and to bacteria found in red *Musca stabulan*s pupae etc. He studied and found insects to be useful in estimating the postmortem interval and several cases are reported that show medicolegal and hygiene questions can be answered using forensic entomology techniques, including close observation of larval development.

Greenberg (1998) checked sex ratios and reproductive status of over wintering domestic species of Muscidae and Calliphoridae and found that all the three species under observation normally over winter in the nulliparous condition. This and the inability of *Pollenia* species to breed in carrion, minimize the forensic use of these flies when found wintering indoors at a murder scene.

Greenberg and Wells (1998) reported *Megaselia Sp* as forensic indicators from human corpses in two forensic cases in Chicago. It is an important fly from forensic point of view at it could be the only evidence available especially in sealed apartments and concealed corpses inaccessible to larger insects.

Introna et al (1998) presented three cases of forensic interest regarding the estimation of postmortem interval (PMI) by entomological data. The Entomological Laboratory of the Institute of Forensic Medicine performed the three cases concerning criminal investigations in Southern Italy at the University of Bari. For each case, the authors present a detailed description of the remains as observed at the crime scene and a description of the arthropods collected from the remains. The PMI estimation was based on comparison of data from autopsy reports (rate of decay), local environmental conditions (temperature, humidity, and rainfall) and development times for the immature stages of each species of local arthropod and succession patterns. The collection of insects was performed at the discovery site and during autopsy procedures. In the first case a PMI of 5 to 8 days was established based on the presence of adult specimens of *Saprinus aeneus* (family Histeridae), and mature larvae of *Chrysomya albiceps* and *Sarcophaga carnaria* (3rd instar). In the second case, on the charred remains of a corpse, larvae of *Sarcophaga haemorrhoidalis* (3rd instar) and *Protophormia terranovae* (2nd instar) were observed in different developmental stages, as indicated, giving a PMI of 3 to 4 days based on entomological data. In the third case, a PMI of 36 to 48 hours was defined from the evidence of *Calliphora vicina* 2nd instar on the two burnt bodies. In all cases the entomological evidence alone led to conclusions on PMI.

Komar and Beattie (1998) studied clothed pig carcasses of approximate human size showed clothing disturbance patterns produced by postmortem insect activity mirrored those associated with a perimortem sexual assault. When the pigs' decomposed, insect activity generated substantial clothing changes, primarily during bloated and active decay stages of decomposition. Maggot masses were observed shifting the position of clothes several centimeters over a period of minutes. The arrangement of clothing on pig carcasses changed daily from the onset of the first maggot masses through to the advanced decay stage, at which time maggots were no longer observed on pig carcasses. An important observation was the ability of maggot masses to move clothing located on the underside of the body, despite the weight of the body overlying it and direct contact with the ground. Maggots in mass also completely removed articles of clothing, particularly snug- fitting items such as socks, hose, shoes, and underwear. Pig carcass size was an important variable in the frequency and type of clothing changes observed. No significant differences in the degree of clothing displacement were observed between and shade exposed pig carcasses, although variations in the onset and timing of changes were noted, and natural decay processes produced additional clothing changes.

Allen and Byrd (1999) developed computer modeling of insect growth for the purpose of improving accuracy in estimating time of death in the field of forensic entomology. They felt that the use and application of computer modeling in forensic entomology is largely unexplored. This is because many widely used entomological computer models do not provide acceptable results for predicting development times of insect field populations under variable temperature. The purpose of their study was to determine the mean and standard deviation of the development of immature life stages, compare the development of immature insects under constant and cyclic temperature regimes, and compare the various development model theories with the baseline data to determine the optimum model format. They advocated the development of advanced computer models that compensate for adult activity periods, ambient temperature fluctuations, interspecies competition, and the excess metabolic heat generated by actively feeding second and third instar larvae. In discussing computer modeling theory, they profile linear development rate models and nonlinear development rate models. Also described are the creation of a computer model, followed by discussions of the importance of computer modeling, the practical applications of a computer model, and further studies in computer modeling.

According to Parikh (1999) flies are attracted to the putrefying body or even a debilitated live body and lay their eggs especially in open wounds and in exposed moist and sheltered natural orifices such as the nose, mouth, vagina, anus, by about 18 – 36 hours. Further these crawl into the interior of the body and help to destroy the soft tissues. In the course of about 4 – 5 days, maggots develop into pupae, and in another 4 – 5 days, pupae into adult flies. This is of some importance in estimating the time since death. Maggots may also reveal the presence of drugs. He mentions that maggots are preserved by dropping into boiling absolute alcohol or hot 10% formalin which kills them in extended condition, while some live larvae are kept for rearing. Subrahmanyam (1999) has mentioned similar findings.

Singh et al (1999) have emphasized the need to generate the much needed basic data so that this science can be put to proper use in India as well. This whole scenario prompted the author to undertake the present proposal.

After studying insect succession on exposed pig carcasses Vanlaerhoven and Anderson (1999) studied succession on buried carrion in two biogeoclimatic zones which were chosen on the basis of different soil type and vegetation. They concluded that succession of insect species on carrion varies according to temperature, habitat and geographic location and emphasized on the need to generate a database on insect succession for exposed and buried carrion for each major biogeoclimatic zone.

To assist forensic entomologists, ecologists and public health workers, Wells et al (1999) provided a key to 3rd instars of 8 Chrysomyinae species reported from or likely to occur in carrion within the continental United States.

Anderson (2000) has studied the newer concept of accumulated degree days (ADD) and accumulated degree hours (ADH). For a more precise PMI, insect development rates at a specific temperature were converted to accumulated degree days or accumulated degree hours, which are thermal units. To calculate the ADD or ADH, the length of time taken for each developmental stage was to be multiplied by the temperature at which the insect was reared. The ADD and the ADH were valid only at the rearing temperature. He also indicated that the further away the temperature gets from this rearing temperature, the less accurate the ADD and ADH.

Byrd and Castner (2000) studied insects and other arthropods found at a death scene can provide corroborating evidence regarding time and place of death, as well as possible antemortem and postmortem treatment of the victim. They instruct even individuals without a background in entomology on what to search for when recovering entomological evidence at a crime scene.

Erzinclioglu (2000) shows his fascination with blowflies and their kin through his excellent volume for the Naturalist's Handbook Series on Blowflies. It does not delve so deeply into the life cycles and ecological successions. His book has far more wide ranging scope and unleashes far more interesting aspects of forensic science than just flies, though obviously, as part of the central theme of the book they are present throughout. Erzinclioglu displays not only his fascination with the lowly flies that are attracted to human corpses, but also an in depth knowledge of the human fly sphere of interaction throughout history. There is a chapter charting the history maggots and medicine, an interesting look at the way flies have affected human history. He gives the gruesome details of rape, murder and pedophilia.

Goff (2000) using actual cases, on which he worked, showed how knowledge of these insects and their habits allows forensic entomologists to furnish investigators with crucial evidence about crimes. Even when a body has been reduced to a skeleton, insect evidence can often provide the only available estimate of the postmortem interval, or time elapsed since death, as well as clues to whether the body has been moved from the original crime scene, and whether drugs have contributed to the death. He shows considerable expertise in both the practical and research sides of this little known science. He details the introduction to the science and the crime scene. He has been practicing 'Forensic Entomology' for at least 15 years before writing this book and therefore brings considerable expertise in both the practical and research sides of this little known science. The book also brings into the light some of the more disturbing aspects of humanity in the very nature of the crimes involved. The author's success in determining the time of death from the insect evidence is phenomenal, awe inspiring even and it was intriguing to see just how formalized this science has become. The first part of the book contains details of the author's introduction to the science and the crime scene. From there in he gradually shows us how it is done as he relates the story of his own growing expertise and acceptance by the police. The number of cases the author has been involved in is impressive. The author lays out his evidence well and the reader is left in no doubt that forensic entomology is a reliable and useful science, albeit a young one with much to learn. The forensic entomologist turns a dispassionate, analytic eye on scenes from which most people would recoil—human corpses in various stages of decay, usually the remains of people who have met a premature end through accident or mayhem. As per him each body recovered at a crime scene is an ecosystem, a unique microenvironment colonized in succession by a diverse array of flies, beetles, mites, spiders, and other arthropods: some using the body to provision their young, some feeding directly on the tissues and by-products of decay, and still others preying on the scavengers.

LaMotte and Wells (2000) using statistical methods worked out the probability of the calculation of post-mortem interval on the basis of arthropod succession data.

Singh and Bharti (2000) enlisted the species of blowflies available in the state of Punjab, which can be important from the forensic point of view.

Mulzac (2001) reviewed the increased use of forensic entomology for crime scene investigations based on the ability of insect evidence in determining postmortem interval estimation. He felt that the processing of a crime scene may typically include the recovery of fingerprints, weapons, and trace evidence, and it might also include, on occasion, the examination of an entomological factor. He examined the stages involved in the decay of a corpse, how insects aid in predicting the PMI, and the collection of insect evidence. Issues for the future of forensic entomology, such as automation were presented and discussed.

Schoenly et al (2001) confirmed the conclusion that carrion-arthropods formed indistinct communities on human and pig subjects on select sets of days in the succession. Study objectives were to test a size range of pigs against replicate human bodies for differences in arthropod succession and decomposition; determine what collection techniques recovered the largest majority of forensically important insects; monitor the insect fauna present in eastern Tennessee during the summer for their potential use in future casework; and determine whether carrion-arthropod populations are indeed "saturated" at the Anthropological Research Facility in Tennessee, by virtue of its repeated and historic use as an outdoor forensic sciences laboratory. Replicate human (two) and pig subjects (six) were placed side-by-side on the same day and subjected to identical collection methods and exposure conditions over a 32-day period during summer. Several sampling methods for obtaining arthropod specimens were used; however, the results of only two methods (aerial net sweeps and pitfall traps) are reported, since they constituted the only "quantitative" samples deemed by the researchers to be amenable for statistical analysis. The tests showed little or no preferences by the carrion-arthropod community as a whole for pig or human tissues during most days of the succession. This result held even when comparisons involved pigs of different weight classes combined with human subjects. Rarefaction results confirmed the field intuitions of forensic entomologists that 50-lb pigs are reliable models of human corpses, insofar as having comparable densities of arthropod species is concerned. Consequently, the use of 50-lb pigs over the past 15 years by different research groups working in different habitats and latitudes apparently have provided opportunities for reliable cross-site comparisons as well as pig-human comparisons at least in those habitats where pig carcasses have been used.

Singh and Bharti (2001) studied the nocturnal oviposition behaviour of blowflies and confirmed the findings of Greenberg (1990) that blowflies do lay eggs at night also and this factor should be taken into consideration while drawing conclusions on the basis of entomological evidence.

Vij (2001) has admitted that it is the field of a specialist when the issue of time since death is involved in the criminal investigations and it is essential, wherever practicable, the forensic entomologist should attend the scene and collect the material. It also enables him to study the environment. He emphasizes on the need of sensitizing the doctors to the significance and technique of collection and dispatch of samples. He also stresses on the fact that, in Indian setup especially, practicality demands the knowledge of collection, preservation and dispatch of specimens rather than the study of life cycles of the various species.

Bharti and Singh (2002) reported the occurrence of larval stages of blowflies during different stages of decay in rabbit carcasses.

Greenberg and Kunich (2002) have detailed all aspects of use of flies as forensic indicators. They have described the history, forensic biology, courtroom procedures, and insect as evidence, the problems and various other aspects relating to the subject. Of particular interest and practical use are the keys and tables of the important flies.

Bharti and Singh (2003) studied the succession of insect communities in rabbit carrion in the state of Punjab. They reported 38 species of insects belonging to 13 families from different stages of carrion decay during various seasons of the year.

Arnaldos et al (2004) investigated a case in which the time of year when death occurred was established from entomological evidence after medical examination had been unable to offer any precise information regarding the same, proffering instead a time that ran from three months to one year or more.

Campobasso et al (2004) studied the first Italian case of *Megaselia scalaris* breeding in a human corpse exhumed from southern Italy. They found that based on the predilection of some Phoridae for older carrion and their delayed arrival at a corpse, the scuttle flies are relegated to a secondary forensic role. However they may occur even in the early stages of decay as the only insect evidence especially in bodies that have somehow been at least partially sheltered from colonization by larger flies through burial.

Carvalho et al (2004) exposed carcasses of domestic pig in an urban area to determine stages of decomposition and insects of forensic significance exploiting the carcasses. Four species of Calliphoridae were collected. Unlike many other studies they found Sarcophagidae to be relatively late arrivals, while Muscidae species arrived early. Ant activity retarded the rate of biomass removal.

Klotzbach et al (2004) studied a dead body with larvae, but the lack of information produced difficulties in entomological PMI estimation. The determination of age of organisms was made by PCR-RFLP identification. They emphasized the necessity of comprehensive investigation of the environmental condition influencing the growing rate of blowfly larvae and a more widespread education and training of persons involved in crime scene work as a possible task for forensic entomologists.

Leccese (2004) sampled numerous insects of species *Calliphora vicina*, *Sarcophaga africans*, *Megaselia scalaris* and Coleopteran Dermestidae and Histeridae using pig meat as bait. The first two species were attracted to fresh meat and the third one was attracted to rotting meat and had a complete development cycle indoors. Environmental parameters such as temperature and relative humidity variation affected the presence and abundance of Dipteran specimens and their life cycle as well as other arthropods.

Oliva and Ravioli (2004) unraveled an attempt to cover up the death of a young convict by presenting medical evidence of brutal battering and by entomological evidence of a 20 -30 day post mortem interval which implied concealment of the body.

OliveiraCosta and MelloPatiu (2004) conducted three case studies in which estimates of PMI were based on the concept of accumulated degree days. In two of the cases estimates were close to that obtained by other methods.

Turchetto and Vanin (2004) carried out entomoforensic investigation on a decaying corpse found indoors and it showed an unusual insect activity that created some problems in estimating time since death. The possible implications of the parasitoids on the fly population, growth and density, the life cycle length, the maggots' and pupae survival and their hindering the PMI evaluation and other entomologic-forensic inferences were studied.

Wolff et al (2004) used high performance liquid chromatography to determine and quantify parathion in insects collected from decomposing rabbits previously injected with commercial methyl parathion. Its effect on succession of medically and legally important insects was studied.

Keeping in view the importance of this work, it is lamentable that in a developing country like India this research is in pathetic condition where as in other nations it has reached such a level that forensic entomologists have become an integral part of investigation agencies.

AIMS & OBJECTIVES

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The study of forensic entomology has not received the required systematic attention in our country by the medical professionals. The present study was done to inculcate the awareness regarding the field of medical forensic entomology for the appreciation of medical profession, forensic experts, police and legal personnel. Collection and evaluation of data regarding the cases having insect evidence has served to form proper guidelines to police and medical profession to aid reaching at the correct conclusion. Moreover the study has been of paramount importance in assisting the medical profession towards interpretation of certain cases of death. All the cases were studied having the following aims and objectives:-

1. To find the post-mortem interval by a scientific method of forensic entomology.
2. To check the accuracy of the post-mortem interval calculated by forensic entomology by comparing it with that determined by other methods like various changes of decomposition and eye-witness accounts.
3. To test the data available with entomologists by applying it practically on human dead bodies.
4. Toxicological studies on maggots in suspected poisoning cases, if any.
5. To determine the post-mortem interval, where it cannot be determined by other methods, thereby helping in crime investigations.
6. To determine the usefulness and applicability of forensic entomology in the Indian perspective.

MATERIAL & METHODS

MATERIAL & METHODS

The material for the present study comprised 54 cases of human dead, where the dead bodies had evidence of presence of the insects or their immature stages on them. The study included the dead bodies brought to the mortuary of the Deptt. Of Forensic Medicine & Toxicology, Govt. Medical College and Rajindra Hospital, Patiala, Punjab for post mortem examination during years 2003 and 2004. The department covers not only the district of Patiala but the whole of the Malwa region including the districts of Ludhiana, Fatehgarh Sahib, Sangrur and Ropar, in such cases of decomposed dead bodies.

Study and collection of entomological evidence was done under the guidance of an experienced forensic entomologist. The exact procedure varies with the type of habitat, but in general, all the bodies were received in the mortuary and then examined as under.

1. Observation and notations of the scene.
2. Climatological data collection.
3. Collection of specimens from the body before post mortem examination.
4. Post-mortem examination was done to know the cause, manner and mode of death, and time since death as determined from other findings.
5. The insect evidence collected was studied to determine the time since death.
6. All the data and findings were recorded in the proforma and the data so collected was analysed to achieve the aims and objectives of the present study.

About the scene, the data were recorded in the proforma which was the modified Forensic Entomology Data Form (Byrd 1997). Method of the study comprised detailed particulars and circumstantial evidence of the deceased from the police inquest papers and from the relatives and other persons accompanying the dead body. The type of habitat, rural/urban/suburban, aquatic, forest, roadside, closed/open building, pond/lake/river was recorded. The locations of major infestations associated with the body were noted and also whether these were eggs, larval, pupal or adult stages, or empty pupal cases. Insect predation such as beetles, ants and wasps was also noted. The exact position of the body was noted. Also observed flying, resting or crawling insect adults, larvae, or pupae within this proximity to the body. Noted any unusual naturally occurring, man-made, or scavenger-caused phenomenon, which could alter the environmental effects on the body (trauma or mutilation of the body, burning, covering, burial, movement, or dismemberment). Photographs of the different stages of insect found were taken, before collecting. Other important data related to the climate, weather and geography of the site was collected.

Collection of climatological data is critical as the length of the insect life cycle is determined mostly by temperature and relative humidity in the environment development takes place. Maggot mass temperatures were obtained by inserting the thermometer into the center of the maggot mass. Weather data were collected and recorded in the mortuary using a dial type gauge and these were correlated with the meteorological station of the Meteorological Department of Govt. of India located at the Punjabi University, Patiala. The maximum and minimum temperature and relative humidity were recorded.

Collection and processing of specific material evidence in the form of the insects, maggots, pupae and related material was done from various parts of the body. Eggs, larvae, pupae and adults of insects on the surface of the human remains were collected and preserved to show the state of the entomological data at the time of discovery. Light tension rubber tipped forceps were used to collect the delicate and soft fly larvae without damage.

Both dead and live insects at all stages of development were collected for study. In collecting larvae we followed the common practice established by Fisher (1980) and Mearns (1939), that the minimum post mortem interval should be assessed in terms of the age of the largest (oldest) larva. Precise methods were used to collect and present the evidence correctly; a vital precursor to accurate interpretation. After determining the facts about the location of the body and its accessibility to flies at the scene of the crime, the largest and therefore oldest maggots which are the most important specimens and the complete range of maggots present were sampled because they shed light on different aspects of the investigation.

The maggots were plunged into water heated to just below boiling point for 10-15 seconds. Once dead, they were rapidly transferred to a solution of 70-80% ethanol. This prevented the discoloration and shrinkage that would occur if they were placed directly into common preservative solutions such as ethanol and formaldehyde whilst still alive. Shrinkage would make the maggots appear younger than they actually were.

For determining the age of the larvae one of the methods used is the measurement of larvae (Nuorteva 1970) which provides very useful findings for the forensic estimations. The increase in body length of fly larvae over corresponding days has been recorded. On the basis of variation in the length of larvae, a scale relating to maturation of larvae can be drawn. Fisher (1980) observed that the scale enables the examiner to estimate the age of the larva by studying the specimens.

The second method adopted in the study was the rearing of fly larvae into adults to calculate time since death of the infested body on the basis of total developmental time. Nuorteva (1970) and Easton and Smith (1970) successfully used this method inside the laboratory. Subsequently Fisher (1980) also found it easier to identify the species by this method.

A few samples were collected alive and placed on a rearing medium, which in most cases was a piece of liver/tissue of the same deceased. Rearing to the adult stage made identification easier and gave vital clues to the post mortem estimation. The plastic rearing jar/container were kept covered with cloth on lid and left undisturbed in the open in the mortuary itself. The containers were labeled and observed several times to note changes such as hatching of eggs or larvae, pupariation of adult insect.

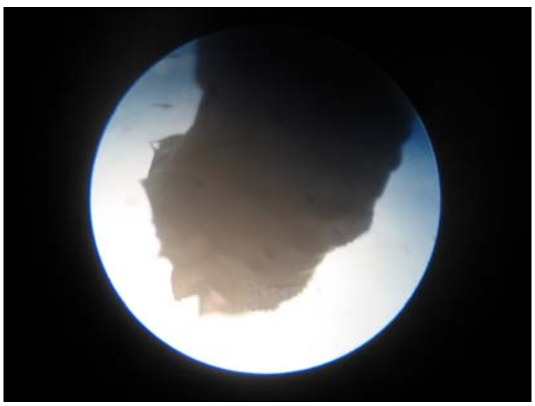
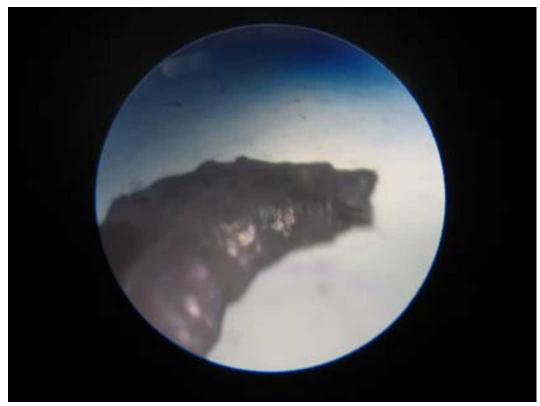
Each kind of larvae and adult were determined to genus and species where possible. This required the assistance of an expert. Taxonomic identification of the insects found on corpses was essential to the reconstruction of events surrounding criminal cases involving death. Systems of classification of biological organisms were used to facilitate their identification.

The age of the specimens was then determined to provide evidence as to when the flies first found the dead body and laid their eggs - the minimum estimate of the post-mortem interval. This was taken as the latest time by which death must have occurred. The estimation of maggot age relied on detailed knowledge of the fly lifecycle and the factors that influence it. Blowflies have four life stages—egg, larva (maggot), pupa and adult. The larval stage is divided into three instars. Between each instar, the larva sheds its cuticle (skin) to allow for growth in the next instar. The pupa is a transition stage between larva and adult. It is found inside a barrel-shaped puparium, which is actually the hardened and darkened skin of the final instar larva.

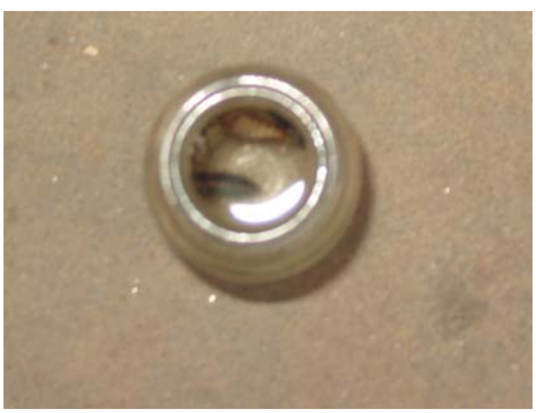
When all the data was processed conclusions were made and determined whether the remains have been disturbed or disarticulated during the post mortem interval. If presence of any antemortem administered drugs or poisons were suspected viscera of the case were sent to Chemical Examiner to the Govt. of Punjab. Estimation of the age of as many specimens as possible, based on presence of drugs, temperature and humidity conditions was done. The data so collected was analysed to achieve the aims and objectives of the present study.

PHOTOGRAPHS


PHOTOGRAPHS SHOWING THE FLY EGGS UNDER LIGHT MICROSCOPE

PHOTOGRAPHS SHOWING ANTERIOR AND POSTERIOR PARTS OF LARAVE UNDER DISSECTION MICROSCOPE



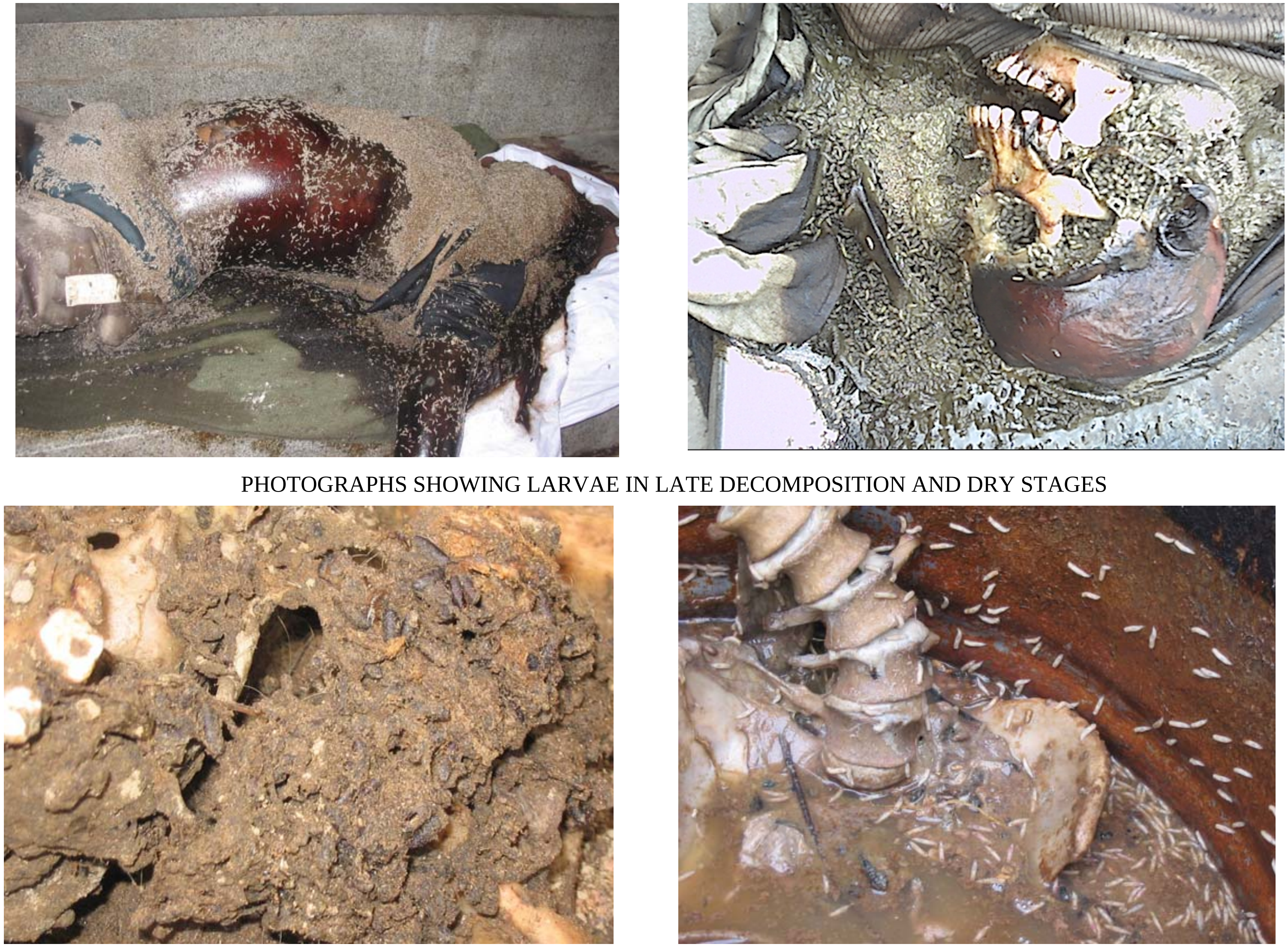

PHOTOGRAPHS SHOWING LARVAE COLLECTED IN A VIAL AND OBSERVATION UNDER A DISSECTING MICROSCOPE

PHOTOGRAPHS SHOWING REARING OF LARVAE IN JARS

PHOTOGRAPH SHOWING PUPAE IN A REARING JAR

PHOTOGRAPHS SHOWING EGG LAYING BY FLIES IN THE FRESH STAGES OF DECOMPOSITION

PHOTOGRAPHS SHOWING LARVAE IN EARLY DECOMPOSITION / BLOATING STAGES



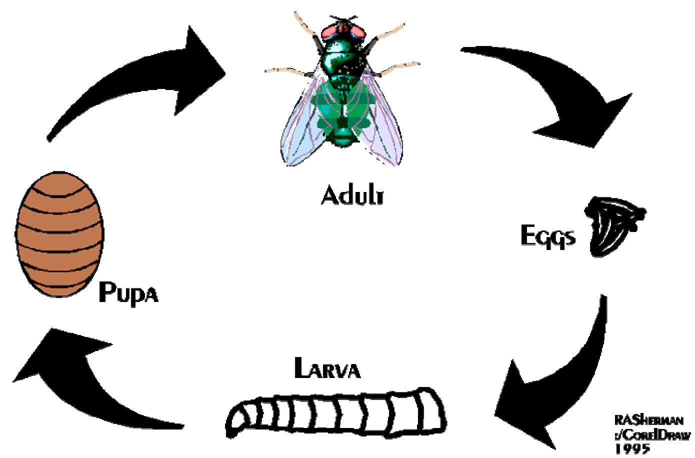
PHOTOGRAPHS SHOWING LARVAE IN LATE DECOMPOSITION AND DRY STAGES


PHOTOGRAPH SHOWING A BEETLE AMONG MAGGOTS


PHOTOGRAPH SHOWING MAGGOTS MORE OVER FACE

PHOTOGRAPH SHOWING PINNING OF THE ADULTS

DIAGRAMATIC REPRESENTATION OF BLOWFLY LIFE CYCLE



OBSERVATIONS & DISCUSSION

OBSERVATIONS & DISCUSSION

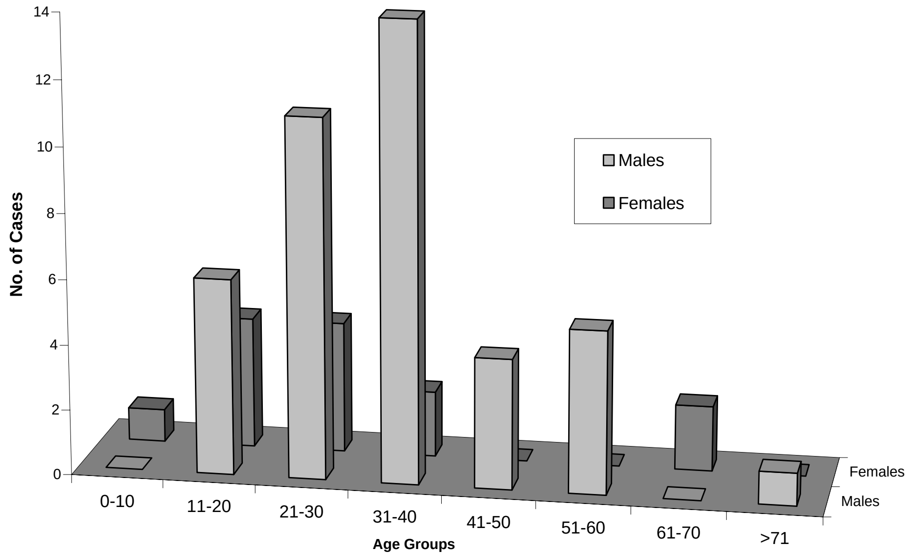
The present study was conducted to find out the relevance and use of entomological evidence found on the dead bodies and to compare the findings with the various other studies. Detailed observations were made from 54 dead bodies brought for autopsies performed in the mortuary of Department of Forensic Medicine, Government Medical College and Rajindra Hospital, Patiala during the period from December 2002 to November 2004 and various results were drawn.

The observations and the findings wherefrom have been discussed under the following relevant headings:

1. Stages of decomposition
2. Insect invasion
3. Evidence collection and processing
4. Stages of insect development
5. Species identification and Succession patterns
6. Season / temperature variations
7. Post mortem interval calculations
8. Maggot mass effects
9. Nocturnal activity
10. Entomotoxicology
11. Cause of death and Injuries
12. Indian perspective

The data collected in respect of the 54 cases showed that 18 cases were of unknown persons and in the rest 36 cases identity was known.

Of these, 13 were females and 41 were males. The ages were 10-20 years in 10 cases, 20-30 years in 15 cases, 30-40 years in 16 cases, 40-50 years in 4 cases, 50-60 years in 5 cases and 3 cases were more than 60 years of age. The age and sex distribution of cases is represented below.



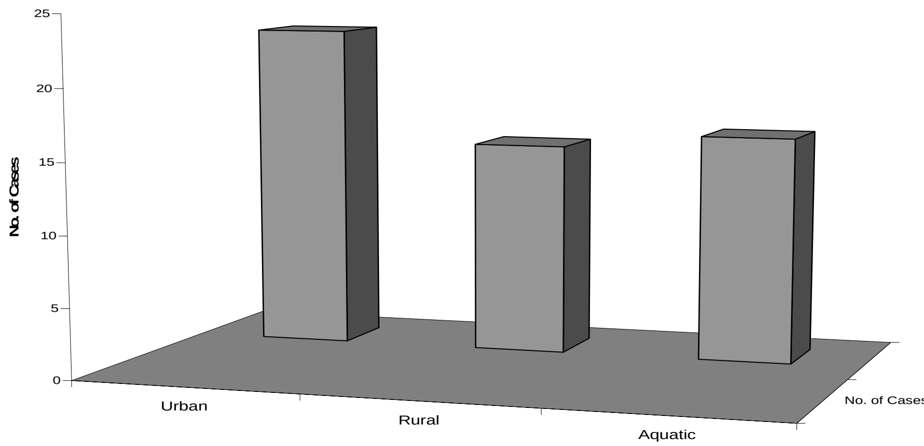
BAR DIAGRAM SHOWING NUMBER, AGE GROUP & SEX OF DIFFERENT CASES

Of the 54 cases, clothing was partial in 24 cases, complete in 21 cases and no clothes were present on 9 bodies.

According to stage of decomposition 9 cases showed dry stage (skeletonization), 12 were actively decaying, 24 cases showed bloating and 9 cases were in fresh stage.

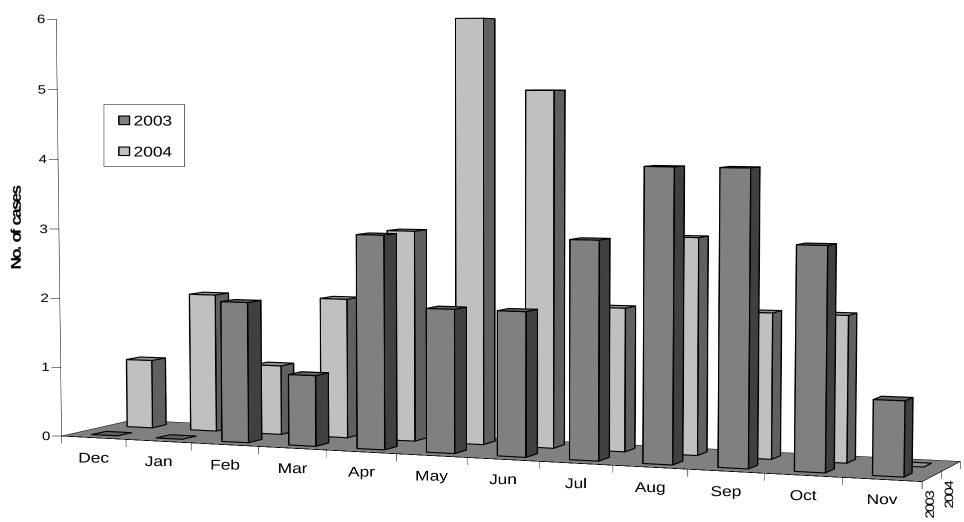
Injuries were present in 22 cases and more infestation of maggots was found in these areas of the body and also the dark moist areas and orifices of the body like nostrils, eyes, genitals and hairy areas.

The areas where the bodies were found were urban open areas in 15 cases, urban closed buildings in 8 cases, flowing water or irrigation canals in 11 cases, stagnant water in 5 cases and rural open areas in 15 cases. This is represented in the bar diagram below.



BAR DIAGRAM SHOWING SCENE AREA OF DIFFERENT CASES

Two cases were reported in January, 3 cases were reported in February, 3 in March, 6 in April, 8 in May, 7 in June, 5 in July, 7 in August, 6 in September, 5 in October and one case each were reported in November and December. This is represented below, which shows a clear biphasic trend according to seasonal variations and extremes of temperature show lesser cases.

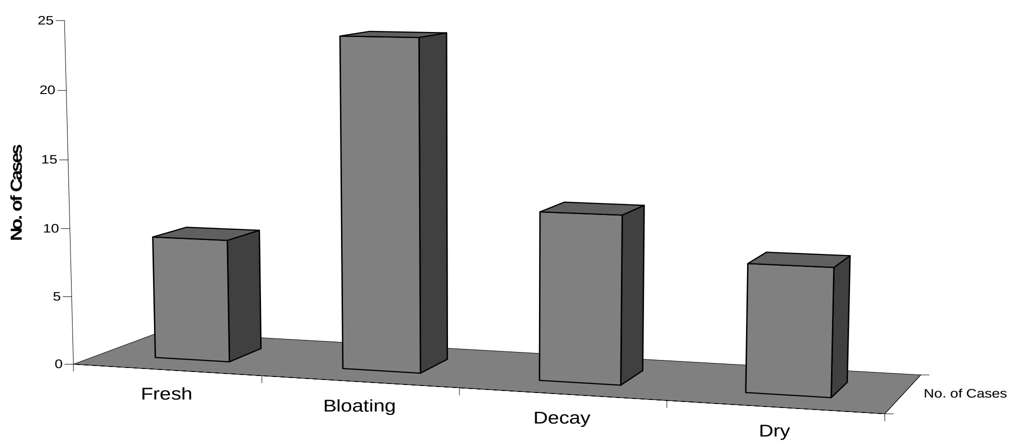


BAR DIAGRAM SHOWING MONTHLY DISTRIBUTION OF CASES

STAGES OF DECOMPOSITION

During the period of December 2002 to November 2004 studies on insect fauna on human dead bodies at Patiala yielded significant information concerning the decomposition process. Though a continuous process, it is divided into various stages by different workers for better understanding.

In the present study the decomposition process was evident in the form of four successive stages. These were fresh, bloated, decay and dry stages; and are discussed as follows. In the present study it was easy to mark out the various decompositional stages by the presence, absence or activity of the insects and their immature stages.



BAR DIAGRAM SHOWING DECOMPOSITION STAGE OF DIFFERENT CASES

The fresh stage begins at the moment of death and continues until bloating is first evident. This stage includes the immediate changes and early changes following death. The immediate changes include the complete and irreversible cessation of functions of the brain, heart and the lungs. Then the early changes are evident which comprise changes in the skin and eyes, algor mortis, livor mortis and rigor mortis. This stage lasts for one to three days and varies depending upon the season and exposure of the body. Neither gross morphological changes nor odour of decay is detectable at this stage. This stage presents the least opportunity to the various fauna to invade the body. During fresh stage only adult flies were present and that too at the later stages. Most of the flies were seen feeding and ovipositing in natural body openings or injured areas of the body. The eggs were usually found laid in the natural body openings, folds of the body, hairy regions and especially in the injuries.

The bloated stage commences with the onset of corpse swelling and ends when corpse deflates and usually lasts up to a week after death. Discoloration and odour of decay becomes noticeable as fluids begin seeping from the natural body openings. Internal temperature of the corpse begins to rise during this stage as a result of putrefaction process combined with the onset of insect activity. In the bloated stage early larval instars were observed especially in the injuries and the natural openings like mouth, eyes, ears, nostrils, perineal region, etc.

The beginning of decay stage is marked by release of gases and corpse deflates. The skin is eaten away at places by the feeding larvae. This hastens decomposition. In the later part of this stage the colliquative putrefaction of organs begins and the odour of decay becomes strong. This stage usually indicates one to three week post mortem interval. The third instar larvae were swarming over and occupying the corpse in this stage. The maggots were observed in teeming masses with their pointed anterior ends immersed in the semi-liquid flesh of decaying corpses and the posterior ends were projected above the surface. In the present study the end of decay stage and the onset of the dry stage were marked by the cessation of feeding activity of the last of the maggots and their dispersal from the carrion. Feeding insects pierced the skin and flesh had been removed from the points of initial attack, such as the eyes.

The dry stage is the final stage of decomposition at which most flesh has disappeared with some soft tissue remaining in the abdomen and the odour starts to fade. The corpse consists of only dry skin, cartilage and bones. After the decaying away of liquefied mass from its bony attachments the skeletonization is completed in one to three months. The early dry stages were marked by the presence of numerous unenclosed pupae and on later stages empty fly puparia were found scattered under and around the corpses.

TABLE SHOWING DECOMPOSITION STAGES OF A CORPSE

Stage	Features	PMI	Insect stages
Fresh	Immediate & early signs, no odour	1-3 days	Eggs
Bloating	Swelling, bloating, leaking fluids, odour	2-7 days	Larval stages
Decaying	Strong odour, colliquative putrefaction	1-3 weeks	All stages
Dry	Dry skin, cartilage, bones	>1-3 months	Empty Puparia and Beetles

Thus the rate and stage of decomposition was found to be in direct relation to the insect activity. These findings are consistent with the conclusions of Schoenly and Reid (1987) who analysed statistically the data of eleven decomposition studies performed in different parts of the world. Similar types of observations have been made by Tantawi et al (1996) and Bharti and Singh (2003) who worked on rabbits.

The decomposition pattern discussed concurs with that described in most of the previous studies like Reed (1958) and Tantawi et al (1996). Though many standard texts like Pillay (2004), Parikh (1999) and Vij (2001) have described the changes after death under three headings of immediate, early and late changes. Fuller (1934) described three stages where first stage combined fresh and bloating stages. Bornemissza (1957) and Payne (1965) described five stages dividing the decay stage into active and advanced decay. Early and Goff (1986) added another stage which they termed remains stage after the dry stage. Benecke (2001) also described a fifth and final “remains” stage when all odours have gone and just the bones, hair and dried skin remnants are left. Morris (1988) found the four stage classification most satisfactory and recommended its adoption by all researchers concerned in the medicolegal application of entomology. This description and terminology is also identical to that of Rodriguez and Bass (1983) who also worked on human cadavers.

INSECT INVASION

The fresh cases where exact time of death is known help in knowing the earliest time of invasion of the body by the insects in the form of either deposition of eggs or the larvae. The many cases of hospital deaths received in the mortuary helped in this regard. In cases of fresh dead bodies where exact time of death was known, clusters of creamy white eggs were observed on most corpses. The most common sites where eggs were deposited were among hairs of scalp and beard, followed by the natural orifices like eyes and nostrils. Similar findings have been reported by Kulshreshtha and Chandra (1987). However, Greenberg and Kunich (2002) have summed this up with an acronym EENT – eyes, ears, nose and throat – the usual portals of entry; along with other sites including anus, wounds, breaches in the body wall and underbody.

TABLE SHOWING OVIPOSITION TIME IN RELATION TO WEATHER

Case	Date	Clothes	Scene	Season	Temp °C	RH %	Oviposition
KS/48	11/5/03	Entire	Urban	Summer	39.4–22.5	26.6	12 hrs
RL/67	13/11/03	Entire	Urban	Autumn	25.6–9.0	64.2	18hrs15min
KS/54	24/6/04	None	Urban	Summer	35.6–26.5	59.8	21 hrs
KS/122	10/9/03	Partial	Urban	Rainy	32.7-24.2	76.6	36 hrs
SSO/16	18/2/04	Partial	Urban	Winter	26.8-11.2	64.4	4days13hrs
HKS/14	30/1/04	Entire	Urban	Winter	20.6-7.5	74.1	44 hrs
SSO/HS	12/2/03	Partial	Urban	Winter	22.9-10.2	69.6	47 hrs

The earliest deposition of eggs by the flies was found to be at 12 hours after death in a case (KS/48) of hospital death shifted to the mortuary in the summer season. The eggs were found to be deposited earliest in the eyes and nostrils in this case. The clothing was complete in this case. While in another case (KS/54) in summer oviposition was recorded at 21 hours after death in the hair, eyes and nostrils, though this dead body was nude. In another case (RL/67) with complete clothing in the autumn season oviposition was seen as the fly was laying the eggs in the eyes and nostrils at 18 hours and 15 minutes after death.

All these cases were shifted to mortuary after death where they were lying completely undisturbed but with a moderate human movement around. In all these cases the exact time of death was recorded from the medical and police files. In 6 other cases, in spring, summer, rainy and autumn season, oviposition was found to have occurred within 18 to 36 hours after death. This is also in agreement with the observation of Parikh (1999) and Pillay (2004).

Mearns (1939) assumed that the eggs of the insects concerned are laid soon after death. Eggs on pig carcasses were found laid within an hour (Anderson and Vanlaerhoven 1996) and within two to three hours (Shean et al 1993). On nude bodies placed in an open wooded area oviposition occurred within two to three hours (Rodriguez and Bass 1983). Greenberg and Kunich (2002) have reported that in an urban surrounding with near actual settings Lothe (1964) observed oviposition as early as 12 hours after death. The findings of the current study are in concurrence with this study.

NO FLY CASES

These cases labeled so by Greenberg and Kunich (2002) as in these cases complete absence of flies and their immature stages became an issue.

TABLE SHOWING THE CASES WHERE NO FLY STAGES WERE FOUND

Case	**Date**	**Cause of death**	**Season**	**Temp °C**	**RH %**	**PMI**
KS/63	9/7/2004	Injury	Rainy	35.9-27.1	63.4	36-48 hrs
VG/20	26/7/2004	Nonviable	Rainy	35.0-26.6	67.9	2-3 days
KS/06	8/1/2004	Cold	Winter	18.9-5.8	80.5	4-5 days
KS/01	6/1/2004	Injury	Winter	16.8-5.7	83.9	2-5 days
KS/12	19/1/2004	TB	Winter	18.0-9.9	84.5	3-4 days

The above mentioned cases were found to be without any entomological evidence even after sufficient time had elapsed since death, though all these cases had provided ample opportunities to the flies for oviposition. Not much is said in various studies about such cases but it is believed to be consequence of various factors such as extreme weather, scene, adverse cause of death, etc. Further research needs to be conducted in this field and thus correlate the findings.

EVIDENCE COLLECTION AND PROCESSING

This study concentrated more on finding larvae and pupae, that is, the oldest stages available. Larvae are usually white and look more like worms than insects. Pupae look like seeds or tiny mummies, perhaps even pills, and do not move. Also a few representative adults from the corpse were collected.

Larvae, pupae, and adults were easily killed and preserved with 70-80% ethyl alcohol. The use of higher concentrations made the insects brittle, fragile and they disintegrated easily. Attempts were made to kill the insects by putting in boiling water before putting into alcohol. Cheap ethanol e.g. methylated spirit that is easily available was used without any problems, though due to its color it caused staining of the larvae. Easily available 10% formalin and isopropyl alcohol (hand cleaning alcohol) were also found suitable for use, after the insects had been killed in boiling water. Also direct boiled alcohol or formalin was found useful for killing and preservation.

Each vial / container held only the insects collected on a particular part of the body, and was labeled accordingly.

Some larvae and pupae were kept alive to be reared to adults. It was easier to identify species using the adults. Living larvae and pupae were kept in plastic ‘Pearl-pet’ type jars in which the lid has been replaced with an easily available face mask whose strings were used to tightly fix it into place of lid. The purpose of this was to allow the insects to breathe. Without it, they died from lack of oxygen in a very short time. A piece of organ or tissue from the deceased body was also kept in the jar on a base of leaves and mud so as to provide food to the insects. The jars were labeled on the outside.

Similar collection techniques have been advocated by most of the researchers like Fisher (1980), Kulshreshtha and Chandra (1987), Morris (1988), Parikh (1999) and Vij (2001). Catts and Haskell (1990) have tried various combinations of glycerin (Hood’s solution), formaldehyde (Kahle’s solution), acetic acid and kerosene (KAAD solution), xylene (XAAD solution), etc. with ethanol; however 70-80% ethanol was suitable for most insects in most cases.

STAGES OF INSECT DEVELOPMENT

By far the most important insects found on corpses are the metallic green or blue flies of the family Calliphoridae. An observation of their life cycle, habits and habitat is as below.

Egg: The eggs were white and sausage-shaped and were usually laid in clumps of hundreds to thousands. On fresh corpses, these clumps were usually present in the hairs, mouth, nasal openings, ears, and where mucus membranes are exposed, and also on wounds and bruises. Eggs can be identified and aged by entomologists. The eggs were found to be about 0.1-0.2 cm in size.

The hatching period of eggs was observed to be between 20-22 hours in five cases. The egg hatching periods of the cases along with the weather data are give in the table below. Not many studies have been undertaken to determine the age of this stage in the life cycle of a fly. Adelson (1972), Fisher (1980) considered the egg hatching period to be 24 hours. Kulshreshtha and Chandra (1987) found the egg hatching period to be between 20-24 hours. They observed that hatching will not take place or will be delayed by one or two days if weather is cold; and the warm weather, on the other hand, may advance the process. The findings of this study bring forth similar observations regarding the hatching periods of the eggs of the flies.

TABLE SHOWING THE EGG HATCHING PERIOD IN DIFFERENT CASES

Case	**Date**	**Season**	**Temp °C**	**RH %**	**Egg hatching period (hours)**
SSO/HS	1/6/2003	Summer	40.9-27.3	35.0	18 hrs
AS/6	21/5/2004	Summer	38.1-25.3	37.0	20 hrs
KS/54	24/6/2004	Summer	35.6-26.5	59.8	20 hrs
SSO/103	8/8/2003	Rainy	34.1-26.5	77.0	21 hrs
AD/5	29/8/2003	Rainy	32.9-25.4	81.7	22 hrs
KS/122	10/9/2003	Rainy	32.7-24.2	76.6	22 hrs
RL/67	13/11/2003	Autumn	25.6-9.0	64.2	35 hrs
HKS/14	30/1/2004	Winter	20.6-7.5	74.1	40 hrs
SSO/16	18/2/2004	Winter	26.8-11.2	64.4	36 hrs

Larva: Larvae or maggots hatched from the eggs. They were white and shaped more like a cone. The mouth is at the pointed end of the cone, and the maggots were found to use a pair of “hooks” there to attach itself to the corpse while it feeds. Maggots also used the hooks to help themselves move. Normally they move by extensions and contractions of their segmented, legless body. They are the most obvious life stage on corpses that have been lying out for several days to a week or so.

Differences in the fine structure of the mouth hooks and spiracles can help tell a forensic entomologist both the species the maggot belongs to and how many times it has shed its skin. Maggots shed their skin three times. Grossly, the three stages of the larvae were found to be more easily identifiable and differentiable based on their length measurements.

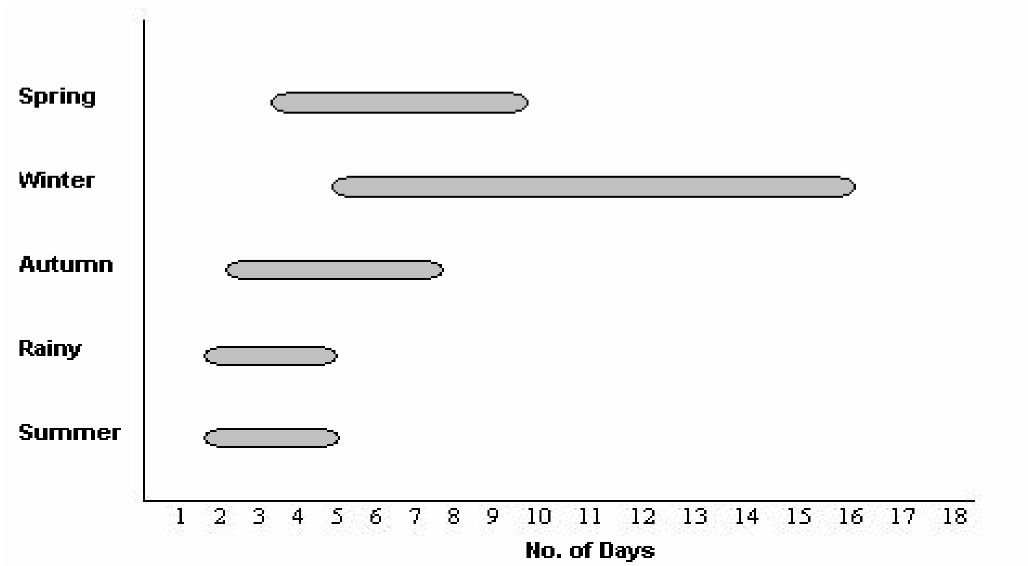
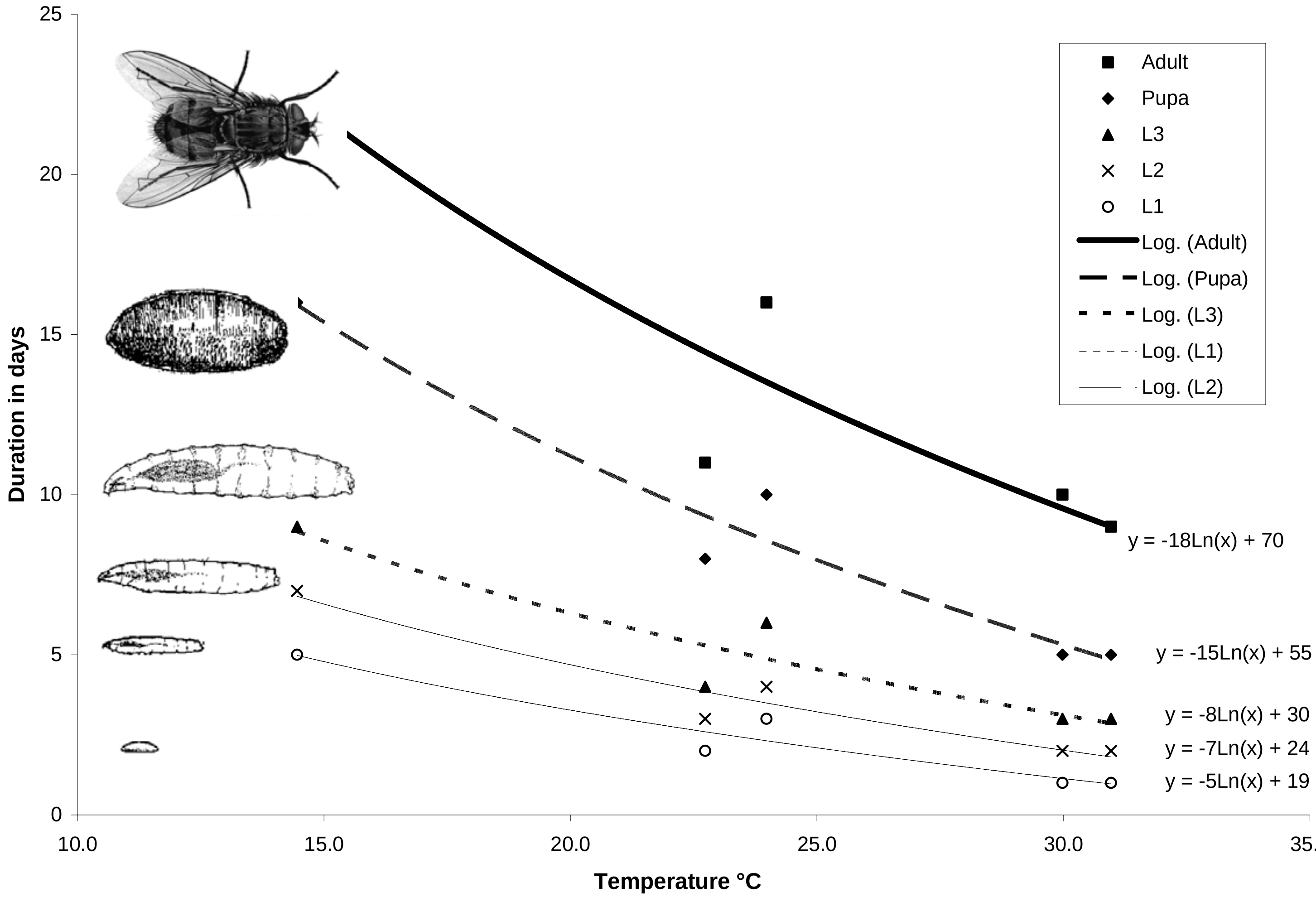


CHART SHOWING DURATION OF LARVAL ACTIVITY IN VARIOUS SEASONS

Puparium: The third time a maggot "sheds its skin" the skin contracts to a capsule-like form and becomes rigid and hardened. It is not actually shed, but remains covering the newly molted insect inside. This mummy-like form is a pupa and the hardened skin surrounding it is puparium (plural *puparia*).

Adult: The puparium has a cap-like lid that can be popped off by the emerging fly. The newly emerged flies are at first pale, soft, and with crumpled wings. They later expand their wings and turn green or blue.



GRAPH SHOWING LOGARITHMIC RELATIONSHIP BETWEEN THE TEMPERATURE AND DIFFERENT STAGES IN THE LIFE CYCLE OF A BLOWFLY (*Chrysomya megacephala*)

Blowflies do not fly much for a day or two while their body is hardening. Adult flies are obvious on most corpses.

The duration (in days) of each of the stages after oviposition varied in different seasons and the table above lists the findings which can be safely concluded to be applicable to this geographical area.

TABLE SHOWING APPROX. DURATION OF ALL STAGES OF FLY (IN DAYS)

Season	**Duration**	**Temp range °C**	**RH %**	**First instar larva ~0.5cm**	**Second instar larva ~1.0cm**	**Third instar larva ~1.5cm**	**Pupae**	**Adult**
Summer	Mid-April to June	38.0-23.9	38.3	1	2	3	5	9
Rainy	July to September	33.7-26.3	74.1	1	2	3	5	10
Autumn	September to November	30.3-15.2	63.7	2	3	4	8	11
Winter	December to February	20.1-8.8	78.6	5	6	9	16	23
Spring	March to mid-April	32.1-15.8	47.3	3	4	6	10	16

SPECIES IDENTIFICATION AND SUCCESSION PATTERNS

During the present study chronological insect succession was observed on the human dead bodies. Corpses undergoing decomposition showed a characteristic insect succession pattern according to the time of the year. The sequence of insect succession observed in this study follows the same general pattern found in both tropical and temperate areas, which is in agreement with the views of Schoenly and Reid (1987).

Three major sequential events were involved among the seasonal insect succession pattern. The first event included the rapid invasion of corpse by adult Diptera (Calliphoridae) and ants. The second event was characterized by the presence of dipterous larvae and adult Coleopterans during which insect diversity reached its peak. A distinct decline in insect richness marked the third event, when most species, especially Dipterans leave the corpse. As in most studies the order Diptera was the predominant group.

In the present study, warmer temperatures during summer and rainy seasons speeded up the succession by accelerating the development and activity of dipterous larvae which in turn produced faster degradation of corpse. Whereas the cooler temperatures of winters retarded the development and activity of dipterous larvae, hence speed of succession was slowed, as was degradation of corpse.

The various arthropods collected and identified from the dead bodies were the flies (Diptera), beetles (Coleoptera) and ants (Hymenoptera). The species of each are discussed below. Their seasonal availability is tabulated on next page.

DIPTERA / TRUE FLIES

The order Diptera contains the true flies. Four species belonging to two families were recorded frequenting the corpses during the present study. Calliphoridae were found to be responsible for maximum corpse consumption. These were predominant flies in all the seasons and were found to be the first to reach the corpse. Similar observations were made by Reed (1958), Johnson (1975), Tantawi et al (1996) and Bharti and Singh (2003). The most significant of these are the Calliphoridae (blowflies) and Muscidae (house flies).

TABLE SHOWING INSECT FAUNA IN DIFFERENT SEASONS

Insect Fauna			Seasons				
Order	**Family**	**Species**	**Summer**	**Rainy**	**Autumn**	**Winter**	**Spring**
Diptera	Calliphoridae	*Chrysomya megacephala*	+	+	+	+	+
		Chrysomya rufifacies	+	+	+	+	+
		Calliphora vicina	-	-	-	+	+
	Muscidae	*Musca domestica*	+	+	+	+	+
Coleoptera	Histeridae	*Saprinus sp.*	+	+	+	+	+
	Cleridae	*Necrobia rufipes*	+	+	-	-	+
	Dermestidae	*Dermestes maculatus*	+	-	+	-	+
	Scarabaeidae	+	-	+	+	-	-
Hymenoptera	Formicidae	+	+	+	+	+	+

In the present study Calliphoridae family was represented by *Chrysomya megacephala, Chrysomya rufifacies* and *Calliphora vicina*. It had generally been believed that Chrysomyinae flies can act as primary species in the absence of Calliphorinae only (Coe 1978, Braack 1981). However Flynn and Moorhouse (1979) and Bharti and Singh (2003) reported that members of subfamily Chrysomyinae can act as primary flies even when Calliphorinae is also available. During the present study too, *Chrysomya megacephala* and *Chrysomya rufifacies* were present in all the seasons and both Calliphorine and Chrysomyinae were found to be the primary species whenever they breed on corpse.

Chrysomya megacephala, the oriental latrine fly is widely distributed in the Oriental and Australian regions. Adults are common around human habitations (Zumpt 1965) and prefer warmer conditions (Das et al 1978). They are primary invaders of human corpses in Malaysia (Cheong et al 1973). During the present study the species was present the year round indicating that they can withstand extreme temperature fluctuations. The adults are large, thick flies of dark green or blue coloration. Goff (1988, 1992) used these species as forensic indicators in four cases.

Chrysomya rufifacies is also widely distributed throughout the Australian and Oriental regions. It is more adapted to tropical conditions and is found throughout the year (Greenberg and Povolny 1971). Similar observations were made in this study. This species has been used as a forensic indicator by many scientists (Goff et al 1986, Goff and Odum 1987, Singh and Bharti 2000).

Calliphora vicina, the blue bottles and are large, slow flying, loud buzzing, and bristly flies. They are found in urban and suburban areas and enter houses in cooler seasons (Anderson 1995). During the present study this fly has been recorded only in winter and spring seasons. This fly is a good forensic indicator and has been put to this use in several cases (Greenberg 1985, Smith 1986, Catts and Haskell 1990, Lord et al 1992, Nolte et al 1992). Since it is present only in the cooler months of the year, it could provide an important clue during investigations.

Muscidae family has a worldwide distribution and several species are of medical importance because of their relationship with man and his dwellings. During the present study *Musca domestica*, the common house fly was collected from the corpses though it was not found to breed on them. It is found all over Asia and is especially common in India and Sri Lanka.

COLEOPTERA / BEETLES

The order Coleoptera is the largest order of the insects. The beetle fauna of corpse has received adequate attention during studies though its true assessment as an indicator of forensic value has often been overlooked. Due to different roles played by different species of beetles, there is no characteristic time of appearance for this order in the decomposition process (Goff and Catts 1990). During the present study, beetles belonging to four families were collected, namely, Histeridae, Cleridae, Dermestidae and Scarabaeidae. Most of these beetles have been observed from the late bloating and early decay stages till two to three days in the dry stages.

Dermestidae (*Dermestes maculatus*) are the skin beetles which are well known to feed on dried skin and bones (Payne and King 1970). They are considered true carrion feeders, playing an important role in carcass degradation (Putman 1983). They have been reported in most of the studies and have been used as forensic indicators (Goff et al 1986, Goff and Odum 1987, Benecke 1998, Greenberg and Wells 1998, Bharti and Singh 2003).

Histeridae (*Saprinus sp.*) are the hister beetles which occur wherever there is decay and putrefaction. They arrive early in the decomposition process feeding primarily on the maggots (Payne and Crossley 1966). Fuller (1934) also recorded histerid beetles as predators on blowfly maggots and pupae. These were not found to breed on the corpses. Nuorteva (1977) used these along with other insects to solve a case.

Cleridae (*Necrobia rufipes*) are the checkered beetles which feed on maggots and corpse. This is the genus reported by majority of the earlier workers from carrion. This species is available from spring till rainy season. They have been used as forensic indicators (Goff et al 1986, Goff and Odum 1987, Lord 1990, Benecke 1998, Bharti and Singh 2003).

Scarabaeidae are the dung beetles and are primary scavengers. They arrive early on the dead body and have been noted as early as second day after death. Similar findings have been noted by Reed (1958), Payne (1965) and Catts and Haskell (1990).

HYMENOPTERA / ANTS

Ants (Formicidae) can be present at all the stages of corpse decomposition. They are opportunistic feeders on whatever is most readily available. Fuller (1934) felt that they are unimportant element in the carrion fauna as they were present all the time. Ants as predators were found to be significant in the decomposition process and retarded decomposition by decreasing the maggot population. Similar opinion was also voiced by Early and Goff (1986) and Singh and Bharti (2001).

SEASON / TEMPERATURE VARIATIONS

Duration of each decomposition stage varies during different seasons. In general the duration of the stages depends on the climatic conditions and reflects primarily the seasonal temperature changes. Five seasons were recognized in the state of Punjab (Mavi and Tiwana 1993). Average minimum and maximum temperatures and relative humidity are shown for each season, in the table below.

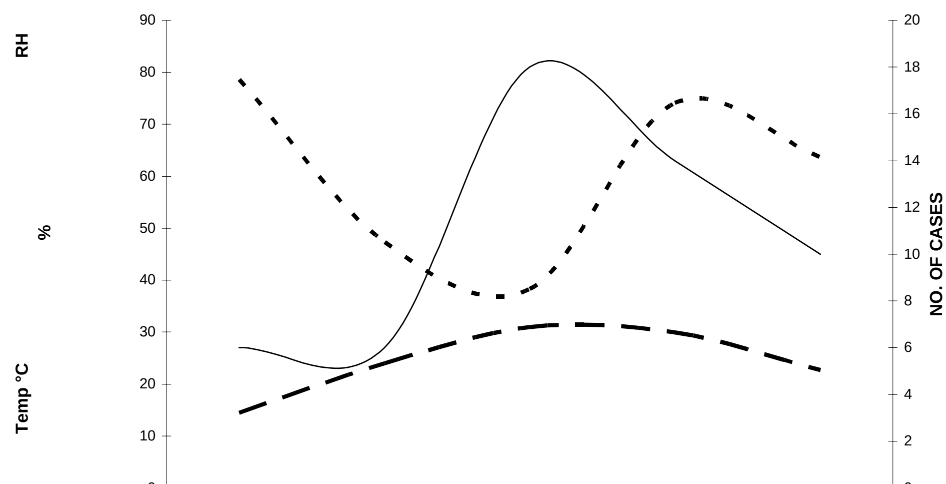
. TABLE SHOWING NO. OF CASES IN DIFFERENT SEASONS

Year	**Season**	**Months**	**Max. Temp °C**	**Min. Temp °C**	**Avg. Temp. °C**	**Relative Humidity %**	**No. of cases**
2003	**Winter**	Dec to Feb	19.8	8.8	14.3	78.7	2
	Spring	Mar to midApr	30.2	15.0	22.6	52.1	1
	Summer	mid-Apr to Jun	38.9	24.0	31.4	34.2	7
	Rainy	July to Sept	33.3	26.3	29.8	78.9	9
	Autumn	Sept to Nov	30.3	15.2	22.7	63.7	6
2004	**Winter**	Dec to Feb	20.5	8.7	14.6	78.5	4
	Spring	Mar to midApr	34.1	16.7	25.4	42.5	5
	Summer	mid-Apr to Jun	37.1	23.9	30.5	42.3	11
	Rainy	July to Sept	34.1	26.2	30.2	69.2	5
	Autumn	Sept to Nov	30.3	15.2	22.7	63.7	4

Decay was found to be faster in summer and rainy season than in winter and spring seasons. This is in agreement with the previous seasonal decomposition studies on dogs (Reed 1958), squirrels and rabbits (Johnson 1975), humans (Rodriguez and Bass 1983) and rabbits (Tantawi et al 1996).

Thus there was an increase in the number of cases showing the entomological evidence in the summer and rainy seasons. Also there was paucity of such cases in extreme weather conditions such as in the months of December and July.

The following graph shows the yearly seasonal variation in the average daily temperature and relative humidity in relation to the number of cases received having entomological evidence.

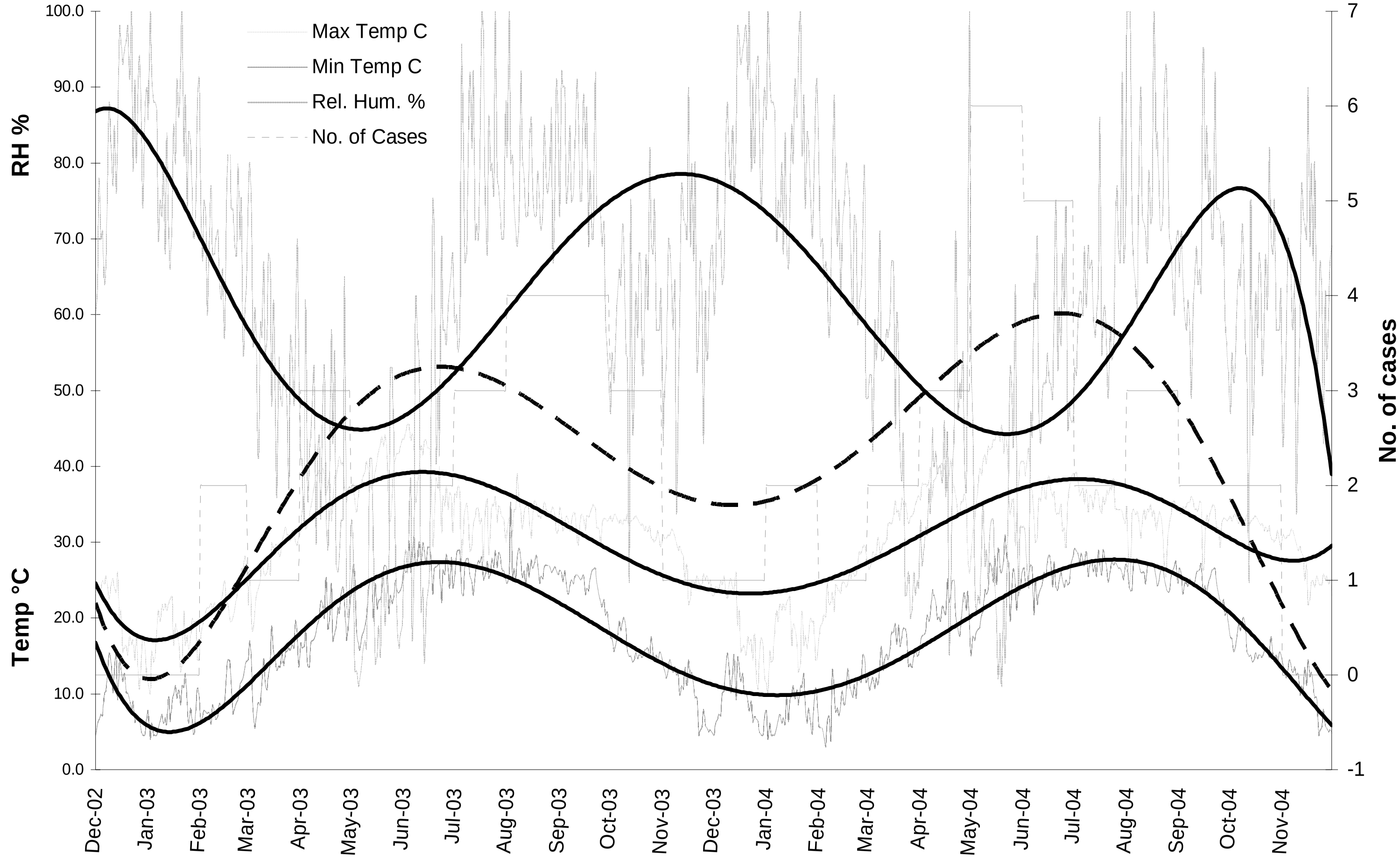


	Winter	Spring	Summer	Rainy	Autumn
Relative Humidity %	79	47	38	74	64
Mean Temperature	14.5	24.0	31.0	30.0	22.7
No. of cases	6	6	18	14	10

SEASONS

This graph clearly brings forth the relationship of the weather conditions with the number of cases showing insect evidence and thus increased decomposition. It is clear that relative humidity has an inverse relationship, whereas temperature has a direct exponential relationship.

During the present study, fly adults were found the year round indicating that they can withstand extreme temperature fluctuations. During summers there was a faster rate of development due to higher temperatures and thus ideal conditions. During winters there was a restricted fly activity for first few days due to low temperatures.



GRAPH CORRELATING THE SEASONAL WEATHER CONDITIONS
AND THE NO. OF CASES WITH ENTOMOLOGICAL EVIDENCE

It was evident from the study that the fly life cycle is temperature dependent and is prolonged with a decrease in temperature. As the average temperature decreases from summer to winter there is a gradual increase in the time required for the completion of the life history of the flies.

POST MORTEM INTERVAL CALCULATIONS

Post mortem interval is the primary aim of any investigation involving insects. This is obtained by combination of two major methods, firstly maggot age and development, and secondly succession based. The first case is used in cases where a full fly life cycle has not been completed. As soon as the pupae have hatched and the adult flies have emerged, the second succession based approach is employed. The time of oviposition until the stage of larvae recovered from the body can be determined and the post mortem interval estimate made. Succession based approach uses the assemblage of insects found on the body at the time of discovery. Different fly and beetle species prefer different stages of decomposition, from fresh to skeletonization. As the body progresses through the various stages, different species find the body attractive and lay eggs or larvae. This assemblage is specific for the particular duration of decomposition, season, habitat and geographic region.

From the graph on page 96 it is clear that the temperature has a logarithmic relationship with the stage of the fly (in days). There is determined a specific formula for each of the stages whereby for a specific average ambient temperature the age of the specific stage can be determined in days thus giving an estimate of the post mortem interval. This method is better utilized when the stages have been correctly identified by an entomologist.

TABLE SHOWING THE FORMULAE FOR CALCULATION OF AGES OF INSECTS

Stage of life cycle	**Formula to determine age (D) of the stage (in days) where T is temperature in °C and ln is inverse log**
Young Fly (Teneral)	D = 70 – 18 lnT
Pupa	D = 55 – 15 lnT
Larva 3rd	D = 30 – 8 lnT
Larva 2nd	D = 24 – 7 lnT
Larva 1st	D = 19 – 5 lnT

Many researchers have advocated the use of software, program tools and mathematical formulae to calculate the post mortem interval using Microsoft® Excel, but most have developed the formulae on the basis of ADD and ADH concepts for which data are not available worldwide for many species. Thus this is an easier method of determining the age of the maggot as a variable of temperature when the maggot has been identified by entomologist.

TABLE SHOWING THE COMPARISON OF PMI CALCULATIONS

Post Mortem Interval	Number	Percentage
Matched or smaller range	40	74.1%
Not matched (Drowning cases)	6	11.1%
Not matched (Further investigation needed)	8	14.8%
Total	54	100%

This table summarizes the main aim of this study, whereby in 74.1% of the cases the post mortem interval determined by using entomological evidence was matched or was of smaller range as compared to that determined by other methods as given in post mortem reports. In such cases it would have been better to give the post mortem interval as the duration of overlap between the two. In 11.1% cases the calculated PMI did not match as these cases were of drowning where infestation occurred after the body was removed from water and in these cases the PMI matched with the duration of removal of dead body from water. While in 14.8% cases there was a gross mismatch of the PMI and most of these cases were of dry stage and larger PMI duration. Thus it is evident that in conjunction with other methods of estimation of post mortem interval the entomological evidence does give a better and smaller ranging post mortem interval. Hence it is essential not to miss such an important evidence.

MAGGOT MASS EFFECTS

The presence of elevated temperatures in the actively feeding third instar larval aggregations on decomposing dead bodies is well documented. Temperature data from the maggot masses were recorded ranging from 1°C to 3°C above the ambient temperature. These high temperatures were found to persist from late 2nd / early 3rd instar stages through to the mass moving off the remains. The difference were found to be lesser when ambient temperatures were high, but when temperatures were low the maggot mass temperatures were impacted greatly. This has important implications when the calculation of post mortem interval is based on the development at ambient temperatures. It was observed that there was occurrence of maggot masses, the tendency of maggots to congregate together and this maggot mass resulted in an increase in temperature over the ambient temperature, thereby affecting the developmental rate of the larvae.

NOCTURNAL ACTIVITY

Post-mortem interval of a body was used to be calculated by forensic entomologists in the light of a conventional belief that blowflies are neither active nor do they lay eggs at night (Nuorteva 1977; Greenberg 1985; Smith 1986). Cessation of oviposition at night is of forensic importance because it could change an estimate of the PMI by as much as 12 hours.

This method of estimating the time of oviposition got modified when Greenberg (1990) and Singh and Bharti (2001) reported nocturnal oviposition by the calliphorid species which are used as forensic indicators. Keeping this point in view special lookout was kept for such cases. Out of the total of 18 cases in which oviposition was observed at the mortuary, nocturnal (7pm to 7am) oviposition was observed in only 2 cases which are described in the table below. This comes out to be 11.1% of the cases.

TABLE SHOWING CASES WITH NOCTURNAL OVIPOSITION

Case	**Date**	**Clothes**	**Season**	**Temp °C**	**RH%**	**Species**
RL/18	16/4/03	Entire	Summer	38.1-20.7	37.7	*Chrysomya megacephala*
SSO/25	23/3/04	Entire	Spring	36.1-18.0	32.7	*Chrysomya rufifacies*

Both these cases were in mortuary (urban scene) with closed rooms. Though the success percentage of cases showing nocturnal oviposition is less than the 33% success rate of Greenberg (1990) and Singh and Bharti (2001) but they had observed the cases kept in the open, while the present cases were indoors. The species observed are similar to the ones observed by Singh and Bharti (2001) in their study. Thus this study substantiates the report of above mentioned both the studies, that the calliphorid flies can lay eggs during night time as well. It can thus be safely concluded that, while determining the age of maggots, the possibility of nocturnal oviposition by blowflies should always be taken into consideration.

ENTOMOTOXICOLOGY

Typically this type of toxicological analysis is conducted in the event that the body is skeletonised over a short period of time. In this study however all the cases of poisoning did not have such an advanced stage of decay. Also most of the cases being of aluminium phosphide poisoning, which does not accumulate in skin, it was not useful exercise in such cases. The toxicological study from insects requires very high sensitivity instruments, equipment and methods such as gas chromatography and high performance liquid chromatography. Such facilities were not available with the chemical examiner to the Govt. of Punjab, which further restricted this part of the study. Various studies have confirmed the reliability of entomological specimens for the qualitative analysis only.

CAUSE OF DEATH AND INJURIES

The cause of death invariably affects the post mortem interval as is determined from various decompositional changes. This is a well documented fact and has been a subject of various studies. Thus cause of death also influences the development of the maggots on the dead bodies.

It has been observed that the flies are attracted earlier to the dead bodies with open wounds and injuries. The eggs are laid earlier in the wounds and thus earlier invasion shortens the total time of a maggot on the corpse. It was also observed in a case that the antemortem wounds had the maggots in them while the post mortem wounds either did not have maggots or had very few maggots. Thus this is an important finding which can have important forensic implications and needs to be further explored.

In cases of drowning in this region it was observed that when drowning was in the Bhakhra irrigation canal the insect invasion invariably occurred immediately after the body had been fished out from the canal and thereafter the decomposition proceeded at a faster rate than normal. Whereas in cases of drowning in seasonal rivulets or ponds the insect invasion took place as per normal pattern.

In cases of hanging or strangulation more maggot infestation was seen around the ligature marks on the neck and also on the face which was highly suffused and congested.

In cases of poisoning, most of them being due to aluminium phosphide, no significant difference in the insect invasion or development was observed as it is not accumulated in skin, and also as the time between poisoning and death is very less in such cases.

INDIAN PERSPECTIVE

Forensic entomology is a little used tool and a largely ignored field in India. This is perhaps because this being densely populated area putrefaction is detected at early stages. Long term, concealed bodies are uncommon and are usually the result of domestic violence or spur of the moment action.

The investigating officers and the doctors alike are not interested in recognition of the entomological specimens as evidence. In both cases either the entomological evidence is not collected or is intentionally removed so as to clean and sanitize the dead body and make it look presentable. In many cases it was observed that either the entomological evidence is dusted away or is washed away. In a few cases it was found that insecticide sprays like 'Hit' and 'Baygon' had been used to clear away and kill the maggots. In one case it was the worst as the body had been washed in 'Phenyl'.

Such is the level of ignorance, which prompted this study. Forensic scientists need to sit up and take interest in this field. There is a dire necessity of imparting proper training to the crime scene investigators and the doctors doing post mortem examinations. Also more and more entomologists should undertake research in this field in collaboration with the forensic medicine specialists.

There is a dearth of forensic medicine experts in the country, but the number of forensic entomologists in India can be counted on fingers itself. It is only with the team work of entomologists and forensic medicine specialists that this field will start getting its due. Until then it can only be hoped that forensic entomology will some day rise to the status of a specialized field in India, as in other developed countries.

SUMMARY & CONCLUSIONS

SUMMARY & CONCLUSIONS

Studies on the entomological evidence available on the dead bodies were carried out at the mortuary of Govt. Medical College and Rajindra Hospital, Patiala (Punjab) during 2003-2004. The aim of these studies was to test the data available with forensic entomologists by applying it practically on human dead bodies. The important results of these investigations are summarized as follows:

1. 54 cases of human dead bodies were studied for entomological evidence covering all the 5 seasons of the year recognized in the state of Punjab i.e. summer, rainy, autumn, winter and spring. Cases that were brought to the mortuary were taken onto account.
2. The most important aspect of this study was the comparison of the post mortem intervals calculated by using entomological versus other evidence. This revealed that in 74.1% cases the post mortem interval estimates were better and smaller ranging when used in conjunction with other findings.
3. The decomposition process described in the present work concurred with that occurring in both tropical and temperate areas. Four decomposition stages were recognized; fresh, bloated, decay and dry. The duration of the decay process depended on climatic conditions and reflected yearly temperature changes. Corpses in summer and rainy season decayed at much faster rate than those in winter and spring.
4. Three major chronological events were noticed among the seasonal insect succession patterns in this study. The first event included rapid invasion of Calliphorids and ants. The second event was characterized by the presence of dipterous larvae and adult Coleoptera, during which insect diversity reached its maximum. A distinct decline in arthropod richness marked the third event, when most species migrated away from the corpses.

5. Dipterous larvae of the family Calliphoridae, plus beetles belonging to families Cleridae and Dermestidae were mainly responsible for the process of degradation.
6. Warmer temperature in summer speeded up succession while low temperatures in winter retarded succession by slowing down the development of dipterous larvae. Higher temperature of the corpse due to maggot activity also leads to quick decay.
7. The presence of *Calliphora vicina* was unique to the winter and spring seasons. *Chrysomya megacephala* and *Chrysomya rufifacies* were the two Calliphorids, which were found in all the seasons of the year.
8. Histerids were the coleopteran predators of dipterous larvae during the present studies. Whereas Dermestids fed on carrion only. Clerids were observed feeding on maggots as well as dipterous larvae.
9. Ants (Formicidae) were observed on most bodies during the present studies. All of them were opportunistic visitors.
10. Nine insect species have been recovered from dead bodies during the present study. Life histories of the blow flies, which bred on dead bodies, were studied. Observations for nocturnal behaviour of blow flies revealed oviposition occurred in 2 cases.
11. Various problems were experienced that affected the estimate of post mortem interval from entomological evidence. Long time exposure of developing insects beyond their thresholds caused significant prolongation of the development and the calculated post mortem intervals were shorter in those cases. A visit to the crime scene or at least photographs of the scene was found to be essential to know the exact scene. It was observed that time of death may not be equal to the time of first oviposition. Insect activity during rainfall and at night was found to be highly variable.

12. The development peculiarities of forensically important insects might differ in various regions of the world, so that the seasonal variations and the thresholds of different species may not be the same everywhere.
13. Finally it was an important finding that as the post mortem interval increases, the less accurate or wider ranging the calculated post mortem interval becomes.

This work is the one of the few attempts of this kind in India and the results will have direct application while using insects as evidence in forensic investigations. It will also form a model to undertake similar studies in different parts of the country besides increasing general awareness about forensic entomology, a field yet to gain popularity in India.

The determination of post mortem interval from entomological evidence though very useful, does not meet the expectations it aroused a decade ago, unless enormous advances occur in the knowledge of factors influencing, and have remained at best a lab curiosity in the absence of interest in practical case work by the forensic medicine experts in collaboration with the entomologists.

It is true that as a scientist everything is falsifiable and facts are fleeting moments on a sliding scale. To the investigators and the lawyers, the forensic entomologists like many experts present an interpretation of an event based on an existing knowledge base. Unfortunately laymen do not understand that scientists interpret facts as possibilities and not certainties.

Admittedly, in the words of Dr. Vij (2001), it is the field of a specialist when the issue of time since death is involved in criminal investigations and it is essential that, wherever practicable, the forensic entomologist should attend the scene and collect the material. It also enables him to study the environment. Practicality here, demands the knowledge of collection, preservation and dispatch of the specimens by the doctors to an entomologist, rather than the study of life cycles of the various species.

Thus the use of entomological evidence in medicolegal death investigations promises to be a reliable technique for estimating the post mortem interval in both the early and advanced periods of corpse decomposition. It is a powerful tool in the hands of an experienced and well trained forensic entomologist.

RECOMMENDATIONS

RECOMMENDATIONS

The application of entomology to forensic science, in spite of its great potential, continues to be only an occasional exercise in India. There are several reasons for this lapse. Data about the carrion fauna in different climatic and geographical regions of this vast country is virtually non-existent. Because of bankruptcy of knowledge and lack of awareness, entomological investigations are not carried out as a matter of routine in our country, and therefore, a body of knowledge is not being built up based on accumulated experience from actual cases. There is absolute dearth of works dealing with various kinds of biological information about the fauna of decay, which is prerequisite before any conclusions can be drawn from the available entomological data (Singh et al 1999). As a consequence, the potential value of entomology to forensic science has not been fully realized and little active research work is being undertaken in India.

Workers like Smith (1973), Nuorteva (1977), Erzinclioglu (1983, 1985, 1986), Lord and Burger (1984), Meek et al (1983), Keh (1985), Smith (1986), Catts and Haskell (1990), Goff (1993), Byrd and Castner (2000), Goff (2000) etc. have repeatedly laid stress on routine entomological investigations in criminal cases and for separate entomological sections in government forensic laboratories. It cannot be overemphasized that only by the adoption of these measures, will this subject be able to develop satisfactorily. In the mean time, however, there is much that can be done by doctors in mortuaries and entomologists in universities and other research organizations. The purpose of this chapter is to define the areas that need scientific and systematic investigations. It is hoped that other workers will follow up some of the ideas discussed.

1. The main problem in forensic entomological research is the choice of a research substitute for human cadavers which cannot, of course, be used experimentally. Animals carcasses must be used instead, but the choice of carcass will depend upon the exact purpose of the research.
2. The pattern of decay and consequently of insect succession should be studied thoroughly in different climatic zones of the country. In such experiments it is essential to keep a careful record of the prevailing environmental conditions i.e. vegetation, locality (altitude and latitude), climate conditions (temperature, rainfall), season etc. As in any experiment, it will be useful to study insect succession under varied situations. Comparisons of this sort can contribute greatly to the understanding of how the habitat can affect the succession.
3. The entomological literature is replete with references to the ecology, behaviour and biology of insects associated with corpses and such works are of great value to the forensic entomologists. This type of information must be made available for the Indian species as well.
4. In forensic investigations the scene of death is, of course, very commonly a house or some other indoor situation. Such locations would, therefore, be of particular interest as a subject for study.
5. A potentially very fruitful field is the study of changes that occur in the composition of the insects in the soil beneath a corpse. The changes are in need of investigations in a precise manner, since

they can be potentially useful in determining how long a body had been lying in the place where it was discovered.

6. Investigations on the fauna of buried corpses are one more important are of research in forensic entomology. Various workers like Motter (1898), Gilbert and Bass (1967), Payne et al (1968) etc. have contributed valuable observations on the subject. Nevertheless, there is still a need for detailed, quantitative experimental work in this field particularly in the Indian scenario.
7. Adult insects have been studied taxonomically, but their larvae are poorly known. The larvae and puparia of the species belonging to families Calliphoridae, Sarcophagidae, Muscidae and others are in particular need of taxonomic study. Such studies are needed because very often in forensic cases identification has to be done on the basis of larvae alone; in such cases, valuable information remains locked away because of lack of knowledge.
8. Precise experimental studies on development rates of blow fly maggots under different environmental conditions are badly needed. Such studies have been carried out in different parts of the world but this field is absolutely unexplored in India.
9. As we already know, maggot activity will raise the temperature of the corpse, but little is known of the relationship between this temperature and the ambient temperature. This requires investigation particularly.
10. Sometimes, due to competition or scarcity of food, larvae may leave the carcass. In some cases they may shift on to another carcass lying in the vicinity and this may lead to wrong estimation of time since death. Therefore, the migratory distance of post

feeding larvae of different species should be investigated to nullify this possibility.

11. In certain indoor cases, the corpse may be discovered only after the maggots have finished feeding and left it to pupate. In these cases it is not possible to estimate time of death by examination of larvae, but structural and morphological changes in pupae or pupal cases should, in principle, enable a useful estimate to be reached. This field has hardly been explored.
12. It often happens that a maggot infested corpse is discovered at a time when the ambient temperature is too low for fly activity. In such cases, oviposition must have occurred at a time when the minimum temperature was higher. Knowledge of the minimum temperature at which oviposition and hatching will occur would, therefore, assist in minimum time of death estimation. It will also be a useful attempt to identify the eggs of various species, particularly of blow flies. Greenberg and Singh (1995) studied the eggs of North-American species with the help of electron microscope and such studies can be undertaken for the Indian species as well.
13. Corpse-associated insects may yield information on chemical contaminants present in the corpse before death. This is because maggots feeding on the tissue can accumulate certain substances and thus are more sensitive to tests. Experimental work needs to be undertaken for studying the transfer of various kinds of poisons from a dead body into the maggots that have been feeding upon it.
14. No correlation has been studied between the concentrations of chemicals in the larvae versus human samples. Such studies will be essential to estimate the cause and circumstances of death.

15. Many kinds of clues can be obtained during actual case investigations. This type of knowledge cannot be gained from research under experimental conditions.

A thorough understanding of insects and their habits should enable entomologists to contribute in great measure to the reconstruction of events of a death scene. It cannot be overemphasized that a visit by the entomologist to the scene of crime would yield much more information than it is possible to glean from samples collected and submitted to him by a non-entomologist. It is hoped that, before too long, entomology will become a fully incorporated branch of forensic science in India also.

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PROFORMA

PROFORMA

FORENSIC ENTOMOLOGY DATA FORM

DATE: __________CASE NUMBER: __________POLICESTATION:______
DECEASED: ___________________________ AGE: ________ SEX: ______
ADDRESS: ____
Last Seen Alive: _________________Date and Time found: _______________
Date Reported Missing: __________Time Removed from Scene: __________
Site Description: ____
____

Death Scene Area:

Rural:	forest	field	pasture
	Barren area	closed building	open building
	Brush	roadside	other
Urban:	closed building	open building	trash container
	Vacant lot	pavement	other
Aquatic:	pond	lake	swampy area
	Small river	large river	canal
	Salt water	fresh water	brackish water
	Ditch drainage	ditch	other
Exposure:	Open air	burial/depth	
	Clothing	entire partial	nude

Portion of body clothed______________________________
Description of clothing______________________________
Type of debris on body______________________________

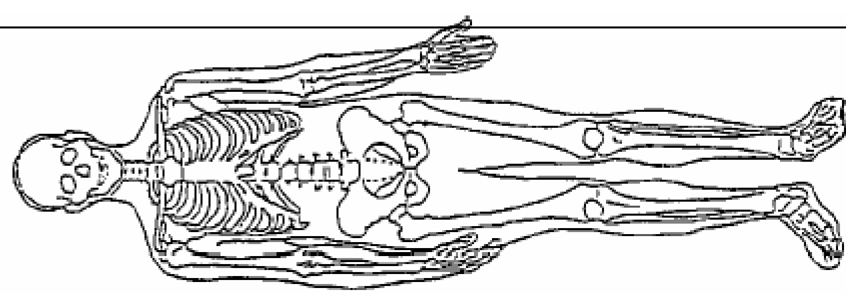
Scene temperatures:

Ambient	body surface	ground surface
Enclosed structure	ceiling fan-on/off	AC/Heat-on/of

Stage of decomposition:

Fresh	bloat	active decay
Advanced decay	skeletonization	saponification
Mummification	dismemberment	other

Evidence of scavengers: ______________________________________
Number of preserved samples ___________Number of live samples________
Possible traumatic injury sites: (Comment or draw below)
____
____



MASTER CHART

ABBREVIATIONS USED IN MASTER CHART

Insect Species	Oldest Stages	Others
M *Chrysomya megacephala*	E Eggs	PMR Post Mortem Report
R *Chrysomya rufifacies*	1 First Instar larvae	PMI Post Mortem Interval
V *Calliphora vicina*	2 Second Instar Larvae	Yrs Years
F *Musca domestica*	3 Third Instar Larvae	Wks Weeks
D *Dermestidae*	P Pupae	Hrs Hours
H *Histeridae*	S Pupal Shells (empty)	Avg Average
C *Cleridae*	A Adults / Tenerals	Temp Temperature °C
S *Scarabaeidae*		RH Relative Humidity %

MASTER CHART

No.	PMR No.	Date	Age (yrs)	Sex	Scene	Decomposition stage	Clothing	Cause of death	Season	Avg. Max. Temp.	Avg. Min. Temp.	Avg. RH %	Insect species	Oldest stage	Estimated PMI	PMI as in PMR
1	SSO/HS	12/02/2003	18	Male	Urban	Decaying	Partial	Septicemia	Winter	22.9	10.2	69.6	HVM	B2	7-9 days	1 wk
2	DSB/23	21/02/2003	35	Male	Urban	Dry stage	Partial	Injuries	Winter	22.8	11.5	71.1	MV	P	16-23days	2-4 wks
3	KS/23	17/03/2003	25	Male	Aquatic	Bloating	None	Drowning	Spring	29.3	14.9	52.4	RVD	B3	6-9days	1 wk
4	RL/18	16/04/2003	25	Male	Rural	Fresh	Entire	Injuries	Summer	38.1	20.7	37.7	R	E	18-36hrs	1-2 days
5	RL/20	18/04/2003	70	Female	Aquatic	Bloating	Entire	Drowning	Summer	37.7	21.5	35.6	HR	B1	1-2days	1-2 days
6	KS/41	29/04/2003	19	Male	Aquatic	Bloating	Partial	Drowning	Summer	37.7	21.1	24.5	PM	2	2-3days	2-3 days
7	KS/48	11/05/2003	35	Male	Urban	Fresh	Entire	Injuries	Summer	39.4	22.5	26.6	R	E	18-36hrs	12-24 hrs
8	SSO/70	27/05/2003	27	Male	Rural	Decaying	Partial	Strangulation	Summer	41.3	26.2	33.3	DM	BS	2-4wks	1-3 months
9	SSO/HS	01/06/2003	19	Female	Urban	Fresh	Partial	Poisoning	Summer	40.9	27.3	35.0	M	E	18-36hrs	1-2 days
10	HS/KS	06/06/2003	25	Male	Rural	Dry stage	Partial	Injuries	Summer	39.9	27.3	40.4	MHC	BS	2-4wks	1-3 months
11	DSB/80	13/07/2003	40	Male	Rural	Dry stage	None	Poisoning	Rainy	32.9	26.6	79.7	MRH	BS	5-10days	1 month
12	KS/94	18/07/2003	35	Male	Rural	Decaying	Entire	Injuries	Rainy	33.2	26.9	79.9	MC	BS	5-10days	1-2 wks
13	KS/103	21/07/2003	55	Male	Urban	Dry stage	Partial	Injuries	Rainy	33.2	27.0	80.8	MH	BS	2-4wks	1 month
14	RL/38	08/08/2003	32	Male	Aquatic	Decaying	Partial	Drowning	Rainy	34.1	26.5	77.0	R	3	4-5days	1 wk
15	SSO/103	08/08/2003	60	Male	Urban	Bloating	Partial	Septicemia	Rainy	34.1	26.5	77.0	RS	BP	4-5days	5-6 days
16	KS/106	11/08/2003	52	Male	Urban	Bloating	Partial	Strangulation	Rainy	33.9	26.5	78.0	R	3	3-4days	3-5 days
17	AD/5	29/08/2003	25	Male	Urban	Bloating	Partial	Septicemia	Rainy	32.9	25.4	81.7	MC	BP	5-10days	1 wk
18	KS/122	10/09/2003	30	Male	Urban	Fresh	Partial	Injuries	Rainy	32.7	24.2	76.6	MS	BE	18-36hrs	1-2 days
19	KS/128	13/09/2003	40	Male	Aquatic	Bloating	None	Drowning	Rainy	32.8	23.0	72.2	M	1	1-2days	1 wk
20	AD/6	21/09/2003	26	Male	Urban	Bloating	Entire	Drowning	Autumn	33.0	20.9	65.8	M	1	2-3days	2-4 days
21	SSO/125	29/09/2003	55	Male	Urban	Bloating	Partial	Injuries	Autumn	32.7	18.3	59.8	M	2	3-4days	4-5 days
22	KS/141	05/10/2003	30	Female	Rural	Decaying	Partial	Injuries	Autumn	32.7	16.4	58.9	RS	B3	4-5days	3-5 days
23	SSO/136	20/10/2003	13	Male	Rural	Bloating	Partial	Strangulation	Autumn	31.5	14.7	61.9	R	3	4-8days	4-5 days
24	RL/59	27/10/2003	32	Female	Rural	Decaying	Entire	Injuries	Autumn	30.6	13.9	61.5	MH	B3	4-8days	2 wks
25	RL/67	13/11/2003	16	Female	Urban	Fresh	Entire	Hanging	Autumn	25.6	9.0	64.2	M	E	18-36hrs	12-24 hrs
26	KS/155	01/12/2003	35	Male	Rural	Decaying	Partial	Injuries	Winter	22.6	9.6	77.4	MRV	2	7-9days	7-10 days
27	SSO/2	11/01/2004	13	Male	Rural	Dry stage	Partial	Strangulation	Winter	18.8	8.2	80.1	HRV	BP	16-23days	3 wks

MASTER CHART (continued)

No.	PMR No.	Date	Age (yrs)	Sex	Scene	Decomposition stage	Clothing	Cause of death	Season	Avg. Max. Temp.	Avg. Min. Temp.	Avg. RH %	Oldest stage	Insect species	Estimated PMI	PMI as in PMR
28	HKS/14	30/01/2004	28	Male	Urban	Decaying	Entire	Injuries	Winter	17.4	7.1	82.8	MVH	BP	15-16days	2 wks
29	SSO/16	18/02/2004	17	Female	Urban	Decaying	Partial	Burns	Winter	18.6	8.1	78.6	MVH	BA	23-28days	1 month
30	HKS/35	14/03/2004	40	Male	Aquatic	Bloating	Entire	Drowning	Spring	26.4	10.8	64.9	V	1	3-4days	3-5 days
31	SSO/25	23/03/2004	35	Male	Urban	Bloating	Entire	Aspiration	Spring	29.0	13.1	59.9	V	1	3-4days	2-3 days
32	HKS/38	07/04/2004	45	Male	Aquatic	Bloating	Entire	Drowning	Spring	32.7	15.8	45.0	RC	B3	6-9days	5-10 days
33	SSO/34	15/04/2004	65	Female	Urban	Bloating	Partial	Poisoning	Spring	35.3	17.5	35.8	MH	B3	6-9days	5-7 days
34	SSO/35	15/04/2004	75	Male	Urban	Bloating	Partial	Poisoning	Spring	35.5	17.7	34.8	MH	B3	6-9days	5-7 days
35	KS/42	04/05/2004	30	Male	Rural	Decaying	Entire	Poisoning	Summer	37.8	21.2	40.5	M	P	5-8days	3-7 days
36	KS/43	04/05/2004	30	Male	Aquatic	Dry stage	None	Poisoning	Summer	37.8	21.2	40.5	DM	BS	2-4wks	1 month
37	SSO/42	09/05/2004	26	Female	Aquatic	Bloating	Entire	Drowning	Summer	36.5	21.3	45.4	R	1	1-2days	4-5 days
38	KS/46	12/05/2004	0	Female	Rural	Decaying	None	Still born	Summer	35.9	20.7	44.3	MDC	BP	5-8days	1 wk
39	AS/6	21/05/2004	32	Male	Urban	Bloating	Entire	Injuries	Summer	36.5	21.2	41.2	R	2	2-3days	2-4 days
40	HKS/54	27/05/2004	55	Male	Rural	Dry stage	Partial	Injuries	Summer	38.2	22.3	34.4	HR	BS	2-3wks	1-3 months
41	SSO/54	11/06/2004	44	Male	Aquatic	Bloating	Partial	Drowning	Summer	40.2	25.6	30.5	R	1	1-2days	1 wk
42	PAR/7	19/06/2004	35	Male	Rural	Bloating	Entire	Injuries	Summer	37.5	25.0	39.6	MR	1	1-2days	2-3 days
43	PAR/8	19/06/2004	40	Male	Aquatic	Dry stage	None	Injuries	Summer	37.5	25.0	39.6	MD	BS	2-3wks	1-3 months
44	KS/54	24/06/2004	12	Male	Urban	Fresh	None	Injuries	Summer	36.5	24.8	45.0	M	E	18-36hrs	12-24 hrs
45	KS/57	25/06/2004	22	Male	Urban	Bloating	Entire	Injuries	Summer	36.6	25.0	45.2	M	P	5-8days	5-10 days
46	SSO/62	28/07/2004	28	Female	Aquatic	Dry stage	None	Injuries	Rainy	36.1	27.1	59.9	HR	BS	2-4wks	1-3 months
47	KS/74	31/07/2004	40	Female	Rural	Decaying	Entire	Injuries	Rainy	36.5	27.3	59.7	CR	B3	3-5days	5-10 days
48	HKS/102	18/08/2004	30	Female	Aquatic	Bloating	Entire	Drowning	Rainy	33.7	26.4	70.6	MR	1	1-2days	3-5 days
49	SSO/71	28/08/2004	19	Male	Aquatic	Bloating	Partial	Drowning	Rainy	32.8	26.1	78.0	MS	B1	1-2days	3 days
50	SSO/76	31/08/2004	40	Male	Urban	Fresh	Entire	Poisoning	Rainy	32.4	26.1	78.1	M	1	1-2days	3-4 days
51	KS/87	16/09/2004	17	Female	Aquatic	Bloating	Partial	Drowning	Autumn	33.3	25.4	71.5	DM	B1	2-3days	1 wk
52	HKS/114	17/09/2004	50	Male	Aquatic	Bloating	None	Drowning	Autumn	33.3	25.2	70.7	MR	3	4-7days	4-5 days
53	KS/89	01/10/2004	40	Male	Urban	Fresh	Entire	Injuries	Autumn	34.0	25.2	69.3	R	E	1-2days	1-2 days
54	SSO/85	15/10/2004	50	Male	Urban	Fresh	Entire	Injuries	Autumn	32.7	21.7	68.3	MR	E	1-2days	1-2 days

PLAN OF THESIS

PLAN OF THESIS

ESTIMATING THE
POST-MORTEM INTERVAL
WITH THE HELP OF
ENTOMOLOGICAL
EVIDENCE

DR. AKASH DEEP AGGARWAL
J.R., FORENSIC MEDICINE,
GOVT. MED. COLLEGE,
PATIALA.

ABSTRACT

Title of thesis:	Estimating the post-mortem interval with the help of entomological evidence.
Name of the student:	Dr. Akash Deep Aggarwal (Batch 2002-2005)
Name of the supervisor:	Dr. O.P.Aggarwal
Name of the co-supervisors:	Dr. R.K.Gorea Dr. Devinder Singh

Since many insects are associated with the human body after death, they are always a potential source of evidence in cases of murder or suspicious death. They are also potentially useful as evidence in situations other than murder cases. Despite this great potential, however, the field of forensic entomology remains obscure in our country, largely because of lack of awareness of the benefits that may accrue from its application. Some questions that may be answered using forensic entomological techniques include time, place, and cause of death; when burial occurred; whether the body was mutilated after death; how long a body remained submerged under water; and when the body was placed in a certain given spot. This study will involve the application of forensic entomology for crime scene investigations based on the use of insect evidence in determining post-mortem interval estimation. This study will involve examination of the insects involved in the decay of corpses, the collection of insect evidence and its use in predicting the post-mortem interval.

INTRODUCTION

Forensics involves the determination of the cause, location and time of death. Entomology is the study of insects. Forensic Entomology, or Medico-criminal Entomology, is the science of using insect evidence to uncover death of a human being.

The examination of entomological evidence or insect infestation on human remains can provide estimates of the time of death or postmortem interval. Forensic entomology has received increased interest by investigators, coroners, medical examiners, and pathologists, focusing on the criminal component of the legal system and dealing with the necrophagous, feeding insects. Insects can be of significant importance in cases of badly decomposed and unidentified remains and with an undetermined time of death. (Greenberg and Kunich)

Forensic entomology is the name given to any aspect of the study of insects and their arthropod counterparts that interacts with legal matters. This branch of science is also called as medicolegal entomology, sometimes termed “forensic medical entomology,” and in reality “medico-criminal entomology” (because of its focus on violent crime), relates primarily to

1. determination of time (post-mortem interval) or site of human death and
2. Possible criminal misuse of insects.

Forensic entomology is inextricably linked with the broader scientific fields of medical entomology, taxonomy, and forensic pathology. (Catts and Haskell)

The typical death scene investigator learns quickly that maggots and corpses go together. For many years, the “worms” crawling in the eyes, nose, and other orifices and wounds on dead bodies were considered just another disgusting element of decay, something to be rinsed away as soon as the corpse was placed on the table for autopsy. While ballistics, firearm examination, bitemarks, gunpowder residue chemistry, blood spatter analysis, and other elements of scientific criminology were studied and refined, the insects associated with death scenes were largely ignored. (Catts and Haskell)

Through the years, however, scientists have researched forensic entomology, which has become a fascinating, and at the same time a more arcane, field of biological study. However, the scope of the field is broad. The potential for contributions of entomology to legal investigations has been known for at least 700 years, but only within the last decade or so has entomology been defined as a discrete field of forensic science. (Goff)

Forensic Entomology, as defined by University of Florida entomologist Jason Byrd, is “the use of the insects, and their arthropod relatives that inhabit decomposing remains to aid legal investigations.”(Byrd and Castner) Said in other words by the American Board of Forensic Entomology, “it’s using insect evidence to uncover circumstances of interest to the law.” (American Board of Forensic Entomology)

Insect life cycles act as precise clocks which begin within minutes of death. They can be used to closely determine the time of death, especially useful when other methods are useless. They can also show if a body has been moved after death. The time of death, can usually be determined using insect evidence gathered from and around a corpse, if the evidence is properly collected, preserved and analyzed by an appropriately experienced forensic entomologist. It includes arthropod involvement in events such as murder or suicide, physical abuse and contraband trafficking. (Nuorteva)

Entomological knowledge can reveal the manner or location of death, but is most often used to estimate the time of death, or postmortem interval. Two time-dependent processes may be involved here. The first is the growth of insect larvae that feed upon the victim. Most of the carrion insects rarely deposit offspring on a live person, therefore the age of a larva provides a minimum time since death. The second is the succession of carrion-arthropod species found in the body, which has the potential of providing both a minimum and maximum estimated post-mortem interval. In general, determining the stage of immature stages of insects found on a dead body is helpful if the latter is up to one month old. On the other hand, the succession pattern is important when death occurred several months before the discovery of the dead body. (Greenberg and Kunich)

One of the first groups of insects that arrive on a dead body is the blowflies (Diptera: Calliphoridae). Usually the females oviposit within hours after death of the vertebrate. The blowfly goes through the following stages during its life history: egg, 3 instar larvae, pre-pupae, pupae within puparium, imago. If we know how long it takes to reach the different stages in an insect's life, we can calculate the time since the egg was laid. This calculation of the age of the insects can be considered as an estimate of the minimum time of death. But even if the estimate of the insect age is correct, the death of the victim (usually) occurred before the eggs were laid. This period is quite variable and depends on temperature, time of day the death occurred, time in year the death occurred, whether the corpse is exposed or buried in soil or immersed in water. As a general rule insects will lay eggs on a corpse within few hours after the corpse is available for insects. Insects can also be of help in establishing whether the corpse has been moved after death, by comparing local fauna around the body, and the fauna on the body. (Lord)

When a body is found or a crime scene is investigated, the presence of insects (or lack thereof) can provide many clues as to what actually happened. Time of death can be ascertained, many clues about the cause of death or events just prior to death can be determined based on insect behavior and interaction with a corpse. However, after three days, insect evidence is often the most accurate and sometimes the only method of determining elapsed time since death. (Anderson)

Forensic entomologists are being increasingly called upon to apply their knowledge and expertise in criminal and civil proceedings and to become part of forensic laboratories and medicolegal investigation teams. Insect evidence collected from, in, on, and around the body of a victim of untimely death or homicide, when properly collected, preserved, and analyzed by an experienced and appropriately trained forensic entomologist, can provide an accurate estimate of the victim's time of death and other valuable information.

REVIEW OF LITERATURE

A large number of studies have been done on determination of post-mortem interval from forensic entomology. A few of them have been reviewed as follows:-

Mearns (1939) studied larval infestation and putrefaction of the human body in order to check the accuracy of the estimate of the time interval between death and the examination of the human remains. He identified the maggots that may be present upon the body, since having identified them, it becomes possible to determine the age reached in their cycle of development, and thus determine the post-mortem interval.

Nuorteva (1977) has described the use of sarco-saprophagous insects as forensic indicators with particular importance to the determination of the post-mortem interval. He has included descriptive case studies. He concludes that forensically important conclusions can be drawn from the identification of the successional stages of the arthropod fauna and of the stage of development of the sarco-saprophagous insects on human corpses.

Smith (1986) described the faunal succession on dead bodies that include exposed, buried, mummified, and burnt bodies. He also discussed how the environmental conditions as temperature and humidity, light and shade influence the fauna on the body. Eighteen case histories were cited, and a large part of the work dealt with methods to study the different taxa occurring on dead bodies.

Lord and Rodriquez (1989) found that insect scientists are being increasingly called upon to apply their knowledge and expertise in criminal and civil proceedings and to become part of forensic laboratories and medicolegal investigation teams.

Erzinclioglu (1989) did not delve so deeply into the life cycles and ecological successions. His study has a far more wide ranging scope and unleashes far more interesting aspects of forensic science than just flies, though obviously, as part of the central theme of the study they are present throughout. He studied in depth the human fly sphere of interaction throughout history. He charts the history of maggots and medicine, an interesting look at the way flies have affected human history.

Catts and Haskell (1990) have discussed various techniques employed in forensic entomology and they have quoted several cases to substantiate their observations.

Lord (1990) studied cases of forensic entomology and found that insect evidence gathered from and around the corpse, when properly collected, preserved and analyzed by an experienced and appropriately trained forensic entomologist, can provide an objective estimate of the time of death as well as other valuable information concerning the circumstances surrounding the victim's demise.

Anderson (1996) conducted a practical exercise in forensic entomology as how a study of insect activity on corpses and other crime-scene materials can yield valuable evidence. She found that date of death estimation is probably the most common and best-known use of forensic entomology in criminal investigations. If the death has occurred within a few weeks of the body being found, the entomologist will be able to estimate a date of death by using data on the life cycle of the species of fly larvae found on the body.

Staerkeby (1996) conducted study on determination of the time of death with the means of forensic entomology. He studied various methods that can be used to determine the post-mortem interval. He studied the succession patterns on the dead body, various insects on the body, various stages of the insects and how to determine the age of the different stages of insects so as to determine the post-mortem interval from them.

American Board of Forensic Entomology (1997) gives most of the information required about the field. It discusses the history, the case studies, the professional status and the science of forensic entomology.

Benecke (1998) studied and found insects to be useful in estimating the postmortem interval and several cases are reported that show medicolegal and hygiene questions can be answered using forensic entomology techniques, including close observation of larval development.

Singh et al (1999) have studied the forensic entomology in the Indian perspective, finding insects to be important forensic indicators. They studied the relationship between insects and corpse decomposition.

Byrd and Castner (2000) studied insects and other arthropods found at a death scene can provide corroborating evidence regarding time and place of death, as well as possible antemortem and postmortem treatment of the victim. They instruct even individuals without a background in entomology on what to search for when recovering entomological evidence at a crime scene.

Goff (2000) using actual cases, on which he worked, showed how knowledge of these insects and their habits allows forensic entomologists to furnish investigators with crucial evidence about crimes. Even when a body has been reduced to a skeleton, insect evidence can often provide the only available estimate of the postmortem interval, or time elapsed since death, as well as clues to whether the body has been moved from the original crime scene, and whether drugs have contributed to the death. He shows considerable expertise in both the practical and research sides of this little known science. His success in determining the time of death from the insect evidence is phenomenal. He details the introduction to the science and the crime scene. He lays out his evidence well and is left in no doubt that forensic entomology is a reliable and useful science, albeit a young one.

Greenberg and Kunich (2002) have detailed all aspects of use of flies as forensic indicators. They have described the history, forensic biology, courtroom procedures, and insect as evidence, the problems and various other aspects relating to the subject. Of particular interest and practical use are the keys and tables of the important flies.

AIMS & OBJECTIVES

This study aims at increasing the awareness regarding the field of medical forensic entomology for the appreciation of medical profession, forensic experts, police and legal personnel. All the cases will be studied having the following aims and objectives:-

1. To find the post-mortem interval by a scientific method of forensic entomology.
2. To check the accuracy of the post-mortem interval calculated by forensic entomology by comparing it with that determined by other methods like various changes of decomposition and eye-witness accounts.
3. To test the data available with entomologists by applying it practically on human dead bodies.
4. Toxicological studies on maggots in suspected poisoning cases, if any.
5. To determine the post-mortem interval, where it cannot be determined by other methods, thereby helping in crime investigations.
6. To determine the usefulness and applicability of forensic entomology in the Indian perspective.

MATERIAL & METHODS

The material for the present study will comprise a minimum of 30 cases of human dead, where the dead bodies have evidence of presence of the insects or their immature stages on them. The study would include the dead bodies brought to the mortuary of the Deptt of Forensic Medicine & Toxicology, Govt. Medical College, Patiala. To widen the scope of the study, cases at other places may be studied for collecting the evidence and the related data.

Collection of specific material evidence in the form of the insects, maggots, pupae and related material will be done from various parts of the body. Both dead and live insects at all stages of development will be collected for study. The samples will be preserved in 70% alcohol. The larvae will be killed by putting them in boiling water before preservation. The live samples will be kept with food and air in separate vials.

Method of the study will comprise detailed particulars and circumstantial evidence of the deceased from the police inquest papers and from the relatives and other persons accompanying the dead body. Other important data related to the climate, weather, temperature and geography of the site will be collected. Post-mortem examination will be done to know the cause, manner and mode of death, and time since death as determined from other findings. The insect evidence collected will be studied to calculate and determine the time since death.

All the data and findings will be recorded in the proforma and the data so collected will be analysed to achieve the aims and objectives of the present study.

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ANNEXURE-1

PROFORMA

FORENSIC ENTOMOLOGY DATA FORM

DATE: __________CASE NUMBER: __________POLICESTATION:______
DECEASED: ____________________________ AGE: ________ SEX: ______
ADDRESS: ____
Last Seen Alive: _________________Date and Time found: _______________
Date Reported Missing: __________Time Removed from Scene: __________
Site Description: ____

____

Death Scene Area:

Rural:	forest	field	pasture
	Barren area	closed building	open building
	Brush	roadside	other
Urban:	closed building	open building	trash container
	Vacant lot	pavement	other
Aquatic:	pond	lake	swampy area
	Small river	large river	canal
	Salt water	fresh water	brackish water
	Ditch drainage	ditch	other
Exposure:	Open air	burial/depth	
	Clothing	entire partial	nude

Portion of body clothed_______________________________
Description of clothing_______________________________
Type of debris on body_______________________________

Scene temperatures:

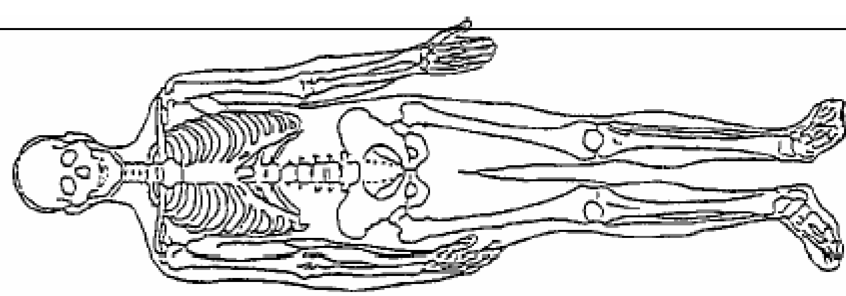
Ambient	body surface	ground surface
Enclosed structure	ceiling fan-on/off	AC/Heat-on/of

Stage of decomposition:

Fresh	bloat	active decay
Advanced decay	skeletonization	saponification
Mummification	dismemberment	other

Evidence of scavengers: ____
Number of preserved samples ___________Number of live samples________
Possible traumatic injury sites: (Comment or draw below)

____
____



ANNEXURE-2

MASTER CHART

S. No.	Case No.	Death Scene Area	Temperature	Stage of Decomposition	Stage of Insect	Insect Species	Age of Insect Stage	Estimated Post-Mortem Interval	Post-Mortem Interval from other methods