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Integrating Blowfly Data for Accurate PMI Estimation

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Abstract: Blow fly play imperative role for estimating the PMI in forensic entomology, these vary their response with variation in geographical area because of species specific and had a great impact of environmental factors (temperature, pH, humidity, food availability, decomposition) on its developmental stages. Research work was conducted in Mullanpur, Punjab and Chrysomium megacephalum was the key component of our research work. Thermometer, pH meter, hygrometer and digital thermometer was used for measuring the environmental temperature, soil pH, humidity, carcass body temperature, ambient temperature, larval development and decomposition rate of the carcass. Succession based and developmental based model was used for PMI estimation. Life cycle of blow fly and decompositions stages are affected by the temperature, moisture and pH, if one of them also not measured and calculated properly then there will misconception in the PMI appraise. The rise in the temperature results in the increase in growth rate and decomposition rate of the corpse, by calculating these factors we can calculate the life span of the blow fly, through which we can estimate the PMI. Therefore, our research concludes that all the three factors known as temperature, growth rate and decomposition rate are interdependent on each other. All these factors are influenced by the environmental factors such as environmental temperature, humidity, pH and food availability.

Keywords: Blow fly, Chrysomium megacephalum, environmental factor, forensic entomology, decomposition, PMI estimation.

Introduction

Blow fly is the first to colonise on the carcass, there are the various species of the blow flies which lay eggs on the dead body and play substantial role in the forensics to estimate the PMI but there are some exceptional species also who lays eggs on the living organism such as Calicerinae, Toxotarsinae, Cylindromyia, due to which the organism suffers from flydtrike. Blow flies are species specific and their development is affected by the environmental elements known as the geographical temperature temperature, moisture, soil pH, moisture, carcass (temperature, size, colour, odour), sunlight, food availability and decomposition rate (Slone et al.2004). As the blow fly are species specific, therefore their response will be different for different geographical area. Forensic entomology is the study of science on dead body with the help of the specific insects to appraisal the insensitive time called (PMI) post-mortem interval. This branch of

biology is the key component in investigating the criminal cases and in legal matters, it is essential to examine all the necessary conditions and factors because any kind of delusion is underused in litigation, sometimes which can also even lead to the retribution for the vitrous and culpable will be at liberty and results in destructive society. Two groups of insects were found on cadavers and provide the most reliable information in forensic science i.e., blow fly and beetles, among which blow flies were the first to found on the dead body. (Castner et al.2001).Blow fly life cycle undergoes the different larval stages such as: egg, larva, pupa and imago stage, although they are more chances for qualitative measurement which can be responsible for error in age appraisal of minimum PMI. To calculate the non-linear developmental larval age of Lucilia sericata different methods have been used such as isomegalen diagrams and ADH. (Reibe et al.2010). As time since death (TSD) plays an indispensable role in criminal cases and forensic science because of the dead body undergoes number of chemical and physical changes known as decomposition, which can be measured with different parameter such as total body score (TBS), accumulated degree-days (ADD).Decomposition plays silent role in estimating the PMI as it involves various processes such as autolysis, putrefaction, decaying etc. Estimating PMI is the challenging task and its accuracy depend on the technique and evidences used for the investigation. The study of swine is included in knowing the decomposition rate for the investigation through which we can collect large no. of sample data (Pittner et al.2020).

Importance of estimating Post-Mortem Interval (PMI):

The post-mortem interval (PMI) is the unerringly time since last living activity of the person before death. To appraisal the time of death is foremost obstacle and holds the significant momentousness in forensic pathology. Forensic entomology is the branch of science which has the capability of providing the direction to estimate the PMI, and the most reliable method by using the insect blow flies (Diptera: Calliphoridae) because they have the capability of colonizing on the death body within short period of time, for more precise and accuracy gene expression information was used to decisive the PMI, various techniques known as PCR and (GAMs) generalised additive models are to estimate PMI (Foran et al.2007). Many studies have been done in order to appraisal the PMI of the carcass with the help of blow fly life cycle. Insects found on the human remains are the indication of succession and the stages of the decomposition, and by identifying the immature stages or age of the insects to estimate post-mortem interval. (Smith et al.1986), evidences received from the crime scene gives the information not only about the time and place but also the reason of the death (Haskell et al.1997), (Slone et al.2004). For longer period of time successional patterns of insect invasion and for shorter period of time developmental rates of larvae are used for estimating the PMI. One drawback in estimating the PMI comes is that the assumption of the oviposition which occurs within a short period of time just after

the death (Nabity et al.2006), this has been found that the larval development is delayed because of the fluctuating temperature as compared with the constant temperature (Greenberg et al.,1991, Byrd and Allen et al.,2001, Clarkson et al.,2004).Two models are used for estimating the PMI: succession based and developmental based model, genetic and environmental factors affect the growth of the instar stages of the blow fly (Picard et al.2013). Blow fly is known as the decomposer, and larval length are used as phenotype for the appraisal of minimum PMI (Tomberlin et al.2011), because it helps in determination of the age of the larval length (Greenberg et al.,1991, Byrd and Castner et al.,2001, Greenberg and Kunich et al.,2002). The genomic size of male and female is different due to which there will be variation in their development (Gregory and Johnston et al.2008). A case study was done by Villet and Spafford where he studied that the variation in the result or PMI for the corpse with blow fly feeding and the corpse with blow fly, these variability in the result creates the error in estimating the PMI. Microbes play an important role in forensic science, also when they are used as evidences during investigation, another way to find the evidences to appraisal the PMI is that microbial succession during the process of decomposition. PMI estimation is helpful in many cases such as in accident, natural death, suicide and unnatural death. Megyesi et al studies that the person died at the before the time thought by the people. PMI also helps us to recreate the crime scene with the help of evidences collected from the crime spot. PMI estimation is substantial because it helps in receiving the death certificate can also be estimated with the help of different methods such as call history, geographical location, climatic conditions and human body. Each method can be considered according to its accuracy in result (Metcalf, J 2019).

Role of blowfly data in PMI estimation:

Forensic entomologist indicates the momentousness of using blow fly in estimating the PMI in future because it is easy to culture at laboratory conditions and more in number. Blow fly has the capacity to facilitate the good computing of (ADH) accumulated degree hours. Blow fly data play vital role in estimating the accurate PMI at both indoor and outdoor carcass. Blow fly is used on the large scale in estimating the PMI because of its life cycle and its decomposition factor. The antennae on the calliphorid flies have very sensitive sense organs which are prove to be very helpful in post-mortem decomposition (Slone et al.2004). Blow flies search the place on the dead organism where they can find the moisture as well as safety for laying eggs in the natural opening of carcass such as ear, anal region, mouth within a day (Smith and Wall et al.1997). The minimum average temperature required for the development of the Diptera: Calliphoridae on carcass is 12- 13°C. the accuracy in the result for the indoor blow fly data is less as compared with the outdoor blow fly data.

Blow fly life cycle:

Blow flies are having more than1000 species, some of them consists of Calicerinae, Toxotarsinae, and some Cylindromyia, Calliphoridae, Lucilia sericata and Calliphora vicina, Chrysomium megacephalum, Calliphoridae erythrocephala among these species Calliphoridae was the first to colonies on the corpse within few hours after the death (Castner et al.2001) and lays about 300 to 600 eggs, by knowing the duration of the blow fly life cycle and decomposition rate of the carcass we can find the minimum PMI. The life cycle and colonization of the blow fly depends on the environmental factors such as temperature, relative humidity, pH, food availabilityand have the intricate life cycle. Blow flies are heliotropic and active only during the day time (Slone et al.,2004, Wall et al.,1997, Hall et al.,1995). Blow flies search the place on the corpse where they can find the moisture as well as safety for their eggs and then lay their eggs in natural opening of the corpse (Smith and Wall et al.1997). Blow fly colonization starts after they lay eggs on the dead organism then they go through the embryonic development stages (Martín-Vega et al.2016), then the larva is hatched and starts feeding on the soft tissue of the dead body and the development takes place into three larval stages, (Donovan et al.2006), this methods of estimating the PMI with the help of larval stageis known as the larvae comparison.Four stages of blow fly life cycle are as follows: egg, larva, pupa and imago stage (Tao S et al.1927). Among them larval stage is divided into three instars, which are further divided on the bases of their behaviour in feeding and non-feeding larva. 1st three instar goes under a number of changes to reach to next developmental stage which is further divided on the bases of respiratory slit where the third instar live longer than first and second (Reibe et al.2010). After the growth of third instar larva, stops feeding and leave the dead organism body for the pupariation, then it contracts irreversibly and its outer covering changes in colour as well as in thickening which ultimately becomes harden and darker, known as puparium (Ždárek et al.1972). In mammals we can measure the growth, age of the corpse by its bone length, size of unmerged teeth, and ossification of bones, there is no direct method to determine the PMI. Therefore, we can measure the PMI with the help of insect such as blow fly and this estimation varies across the continents.

Egg stage:

Blow fly lays eggs in the opening of the corpse (ears, mouth and anal regions), where they can find the availability of the food source, (Smith and Wall et al.1997) which is usually of two types such as; decomposing carcass and open wounds. The larval development starts inside the egg and its developments from egg to adult depends upon the specific species and geographical conditions. Determining the age of the larva is difficult inside the egg or when it is hatched. The softness or harness of the egg is species dependent with white to yellow colour and 1mm of its length, it takes about 8-24 hours for hatching into 1st instar larval stage. The

number of the eggs depends on the size of the carcass. Usually, it takes three to four days to reach into 2nd instar larva from 1st instar larva depends upon the environmental temperature, soil pH, and humidity if all these factors are high in measurement, then it will turn into next stage earlier and if they are low then it will take longer time, these factors also vary across the county as they increase near the equator region and decreases as we move towards the polar region because of the accessibility of more sunlight at equator.

Larval stage:

The development of the larval stage includes the 2nd and 3rd instar larva, they require high temperature for its growth and for decomposition of the carcass, during the same time fluctuation in the temperature more than 28°C decreases the growth of the larval stages. 2^{nd} instar larva will take about 22 hours for its development from 1^{st} instar larva and 3^{rd} instar larva will take about 27 hours for its development from 2^{nd} instar larva, which varies with variation in geographical locations because of its species dependent. The duration of third instar larva is more than that of first and second instar larva. For indoors blow fly can arrive even at the temperature of 20-25°C, with the larval development of the 5mm in length. When they are completely matured in the 3rd instar larva, search the dry and moist place in soil where they can pupate. Chrysomya rufifaces larva needs 13 days for next stage development. Distance variation will affect the larval density and migration development of Lucilla sericata. It was observed that at low density the migration will be appreciable from the food source to pupate or the pupation will occur at the longer distance from the carcass, while at high density the migration will occur close to the carcass for the pupation, which was considered as puparial development was density dependent.

Pupal stage:

Pupa required less oxygen as compared with the 3rd instar larva. They are more resistant to decay and buffering external conditions. Pupa stage development is species dependent but influenced by the ambient temperature of the carcass. Growth of the larva depend on the temperature of the particular dead body and affected by the climatic conditions of that geographical area, after turning into the pupal stage, stops feeding on the carcass and migrate takes place according to the larval density and founds inside the soft layers of the soil near the carcass, which changes its colour and become harder, thicker and darker (usually black in colour or colour of pupation is species dependent), this process is called puparium (Ždárek et al.1972). In puparium the insect will undergo metamorphosis followed by the pupal stage and then pharate adult (Fraenkel et al.,1973, Martín-Vega et al.,2017). Also be based on the age, colouration, degree of melanisationa should be measured accurately, although they are more chances qualitative measurement which chances for error in age estimation in minimum post-mortem interval (minPMI) (Brown et al.2015). Larva empty their gut to find

the proper place for the pupation (Arnott et al.2008). One-third time is taken by the post-feeding phase for its development. Total half time of the life cycle was taken by the last stage. (Greenberg et al.1991). Lucilia sericata, pupa stage development in the laboratory by providing the environmental conditions, takes lasts 143.3 ± 2.5 h temperature as explained by Keck in1999. Blow fly life cycle data is very convenient for estimating the PMI, which is known as larval developmental timing. This is substantial to understand the integrating blow fly data for fidelity PMI estimation.

Adult stage:

Once the blow fly emerges from the pupa, the empty shell of the pupa is left behind, these empty shells can be found on the depth of 1-4inches, near the carcass which is species dependent. As in Chrysomium megacephalum, during our research study we found the empty pupa shell in the depth of 1 to 4 inches and in vertically direction because of specific species. The two most active species of the blow fly during the summer season are: Cochliomyia macellaria (F.) (Diptera: Calliphoridae), Chrysomya rufifacies, Macquart (Diptera: Calliphoridae) (Tenorio FM et al.,2003, Deonier CC et al.,1940), and during the winter season the most active species of the blow fly are Calliphora vicina Robineau-Desvoidy (Diptera: Calliphoridae) and Phormia regina Meigen (Diptera: Calliphoridae), this species can even colonize on the oldest to oldest carcass (Deonier CC et al.,1940, Gruner SV et al., 2007, Hall RD et al., 1993). Blow fly morphological characters include: metallic blue or green colour, with bristly hairs and about 8-12mm in length. Having the wings with distinct veins and yellow or reddish hue near the base, all these characters of the blow fly are species dependent, they can vary with variation in species. Once the blow fly emerges from the pupa and search another partner for mating, after that the blow will find the place or carcass with the help of smell to lay the eggs. adult stage of blow fly represents the most mobile phase among the blow fly development stages, which operates the overall geographical factors distribution, these activities influence the speed of the blow fly to approach the carcass and colonize it (Mohr, R.M et al.,2015). Adult blow fly is attracted towards the organic decomposition matter, which includes: feces, carrion, decaying vegetable, dead humans and animals. The time duration of emerging the flow fly and laying her eggs depend on the particular species of the blow fly, which can vary for the ranges. By knowing the age of the blow fly we can predict the first colonization of the blow fly on the carcass. With the help of the information gained from the age calculation of the blow fly and the decomposition stags of the carcass we can estimate the PMI. The relationship between the decomposition rate of the corpse and the temperature of the blow fly plays significant role in appraisal the PMI.

Decomposition of the carcass:

Decomposition plays important role in estimating the PMI as it involves various processes such as autolysis, putrefaction, decaying etc, decomposition rate is influenced by the abiotic and biotic factors, endo and exogenous microbes which reflect the relationship between weather and climatic conditions. The study of swine is included in knowing the decomposition rate for the investigation through which we can collect large no. of sample data (Pittner et al.2020). Insects found on the human remains are the indication of succession and the stages of the decomposition, and by identifying the immature stages or age of the insects to estimate post-mortem interval (Smith et al.1986), evidences received from the crime scene gives the information not only about the time, place, reason of death but also the sexual harassment, drug intake and many more (Haskell et al.1997), (Slone et al.2004). Decomposition stages are described in different numbers by the different scientist such as: Payne describe decomposition in six stages as follows: flesh, bloated, active, advanced, dry, and remains. Megyesi describes decomposition in four stages as follow: fresh, early, advanced and skeletonization (Metcalf, J 2019). Fresh stage of decomposition include rigor, livor, aglor mortis and eggs of blow fly (Deel et al.,2020). In bloated, early, active decay, fermentation and proteolysis a lot of changes takes places such as: microbial decomposition, larva hatch from egg and forms maggot masses (Gill-King et al.,1997, Galloway et al.,1997, J. Pinheiro et al.,2006, Deel et al., 2020). In advanced stage decomposition is sunken flesh, dry and remains and skeletonization decomposition stages contains no soft tissue and there will be no activity of insects (M.S. Megyesi et al.,2019, Payne et al.,1965). During the initial weeks of the decomposition, we can draw the temperature curve which will be used to estimate the PMI (J. Amendt et al., 2011, L.M. Weidner et al.,2014). The rate of microbial decomposition is affected by the particular geographical area because of mammalian decomposition, collection of microbiome data also helps in estimating the PMI (Metcalf, J 2019).

Factors Affecting Blowfly Development:

The various factors that affect the lifecycle or development of the blow fly includes: temperature, manner of death, carcase (size, colour, odour), sunlight, geographical area, food availability, moisture, relative humidity, pH (Slone et al.2004). Temperature plays incredible role in the development of blow fly on corpse, they behave such as poikilothermic whose body temperature can vary, as they can rase their body temperature as the same temperature of the environment. This conclude that the temperature requirement for the larval developmental of the blow fly should be above 10°C, even they cannot survive for more than few hours on 0°C, but blow fly pupal stage can survive at the temperature above freezing point. Blow fly will not survive at the temperature more than 40°C or can survive hardly for an hour above then this extreme temperature. Rearing of the culture within chambers by providing the same environmental temperature (Byrd

and Butler et al.1996, 1997). Moisture has enormous effect on the development of the blow fly. Blow fly is strongly disinclining from oviposition (which occurs within a short period of time just after the death) (Nabity et al.2006), on carcass that are exposed to the environmental conditions such as: dry or wet.

Temperature:

Temperature is one of the crucial factors that influence the life cycle and the development of the blow fly (Slone et al.2004). Growth of the larva depend on the temperature of the particular dead body and affected by the climatic conditions of that geographical area (Reibe et al.2010). Blow fly is the important factor in the decomposition of the carcass, which ultimately helps in the increasingly credible of the PMI. The two main species present in Victoria are Calliphora stygia and Chrysomya rufifacies both have same development pattern but there is the difference in their behaviour towards variation in temperature. The information gained from this is used for the estimation of death time, place, cause, especially in legal cases, decomposition rate also depends on the number of factors along with the temperature (Franceschetti et al.2021). A century ago (Perez et al.1910) noticed that there is the constant corelation between the morphological changes in the body and the active influence of the temperature on pupation process. (Lowe et al.2013) was the first who describe the quantitively study of the insect development with the help of micro-CT technique. There should be rough estimate of the temperature as it is difficult to find accurate data for insect development in different micro-environments. The various stages of the blow fly life cycle i.e. egg, larval instars, pupa, and adult, each stage development time duration largely depends upon the temperature. Larval development can also have negative impact because of the fluctuating temperature (Greenberg et al..1991, Byrd and Allen et al.,2001, Clarkson et al.,2004). Although we can also culture the same in the chambers by providing all the necessary environmental conditions (Byrd and Butler et al.1996, 1997). Also to calculate the accumulated degree hours, the temperature reading of the death geographical area along with the dead body must be known.

Moisture:

Moisture is the silent environmental factors that affects the life cycle of the blow fly(Slone etal.2004), the amount of moisture requirement varies within different species. The moisture content varies in the various stages of the blow fly which is dependent on the environmental condition in which the carcass is available, moisture from the carcass evaporate, to reach to the equilibrium state in the environment. The evaporation rate depends upon the water ratio availability in the carcass and its surroundings, weather the carcass is placed in the wet ground or in the dry ground because wet soil losses the water faster as compared to the dry soil. When the carcass is in the rainwater, then it will reach to the state of hydration dur to immersion in water and when the carcass is in the hot dry conditions then it

becomes dehydrated because of the water loss. Water is essential for the metabolic process in the blow fly life cycle which ultimately affects its stages of the development. Blow flies search the place on the dead organism where they can find the moisture as well as safety for their eggs and then lay their eggs on that particular place (Smith and Wall et al.1997).

Availability of food:

The density of the maggots will depend on the availability of food source, which ultimately affects the developmental rate of the blow fly larval stages (Slone et al.2004). If the food source availability such as organic decomposer such as carrion, decaying, dead humans and animals is abundant then the maggots will develop with the faster rate (Ash and Green) and even we can find the different larval stage on the same tissue of the carcass or when there is insufficient food it results into the death of the maggots and the developmental rate of the blowfly will disturb which ultimately gives error in the PMI estimation. The consumption rate of the carcass varies in variation of the difference in species which intricate the fidelity in appraise the PMI with the help of the blow fly data. For example, a body with a large wound site that has been colonized by blowflies, and subsequently removed from the environment after only a few hours' exposure, will still contain viable food in the form of the maggot masses and some remaining tissue. By estimating the veracity of the age determination of maggots or the tissue remaining tissue of the carcass can be used for appraise the PMI (Smith et al.1986), and the PMI must be most accurate and reliable. (Pittner et al.2020).

Collection Methods:

We conducted our research work at Mullanpur, Garibdass,Punjab, India. We kept the carcass with the complete setup at this site and visited daily for the measurements of the environmental temperature, carcass body temperature, ambient temperature, pH of the soil and the carcass, humidity measurement and decomposition rate of the carcass. With the help of parameters, we had collected the primary data for one month i.e. 17 March to 17 April because there will not be much variability in the geographical factors.This site selection plays the key role in this research work because of the species of blow fly Chrysomium megacephalum is temperature dependentand the difference between climatic conditions as we move across the geographical factors. Representative ecosystems were another key consideration in site selection to reflect the variety of habitats where blow flies are found. This includes the urban, suburban, rural, and natural landscapes, each with the unique characteristics influencing blow fly distribution and their behaviour. Urban areas provided insight into the adaptability of blow flies to anthropogenic environments, while natural habitats such as forests and wetlands offered opportunities to study undisturbed ecosystems. For our experiment we have taken thecarcass of the calf, having the age of about 2.5 to 3 years and we visit the site daily to collected the all required

data by recording the climatic factors measurements and take the records of each and every day of environment temperature, carcass body temperature, ambient temperature of the body, different body parts temperature, like eye anal region and mouth. Relative humidity, pH of the soil and carcass. Measuring its decomposition rate and the larval, pupa activities in response to all the above factors, then we can estimate the age of the blow fly which ultimately help us to know the PMI.

Integration with Blow Fly Data:

When the blow fly lay eggs on the dead body, they turn into the maggots but maggots don't stay maggots forever once they get the proper nutrients, they migrate away from the body then the larva change into pupae after finding a protective shelter with the favourable environmental factors finally the blow fly emerges from the pupae, and continues its life cycle by laying the eggs on another dead body. Many studies have been done to identify the importance of Chrysomium megacephalum in forensics. Our study highlights the contribution of Chrysomium megacephalum in appraise the PMI.The essential step in estimating the age of the larva is to identify the species of the blow fly because the growth rate of the larva is different in different species and also varies in relation with the temperature of that particular area. In our study we also identified that the larva found feeding on the carcass is of various instar stages, when the temperature is high, we can even find the larva of 1st, 2nd, 3rd, 4th instar stages all together which ultimately indicates that the growth of the carcass is dependent on the temperature. From 17 March to 17 April the maximum temperature rise during our study was 38℃ and the minimum temperature recorded was 27℃. The temperature range between 17 march to 23 march was recorded as maximum was 32℃ and minimum was recorded as 27℃, between 24 march to 30 march the maximum temperature range was recorded as the 35℃ and minimum temperature range was recorded as 29℃, between 31 march to 6 April the maximum temperature range was recorded as the 36℃ and the minimum temperature range was recorded as the 31℃, between 7 April to 13 April the maximum temperature range was recorded as the 38℃ and the minimum temperature range was recorded as the 34℃ and between 14 April to 17 April the maximum temperature range was recorded as the 35℃ and minimum temperature range was recorded as the 30℃. As the temperature is high due to less rainfall because of which there will be rapid growth in blow fly stages and therefore, blow fly will complete its life cycle early.

Estimating the PMI:

Chrysomium megacephalum (Blow fly) was the key component of our study, which undergoes through different life stages, during our study wecalculated that depending upon the time availability we can estimate the PMI by identifying the age of the larva and on the bases of the succession rate or decomposition rate.

Life stage of Chrysomium megacephalum include the egg, larva, pupa and adult stage. Larval stages i.e. 1st instar, 2nd instar, 3rd instar, 4th instar larva and pupae. 1st instar larva grow within 23 hours after the laying of the egg, 2nd instar larva grows within 27 hours after 1st instar, 3rd instar larva grows within 22 hours after 2nd instar, 4th instar larva grows within 130 hours after 3rd instar and finally 4th instar stops feeding on the dead body and turns into pupae stage which is dark black in colour with cylindrical shape found near the dead body under the depth of 1 to 4 inch and in vertical position only due to species specific Chrysomium megacephalum, it will take about 143 to 145 hours for the blow fly to originate from the pupa and the hollow cylindrical shape pupa will remains behind in the soil.

Decomposition rate for appraise the PMI with decomposition stages i.e. fresh stage, bloated stage, active decay stage, post decay stage and skeletonization stage.

Fresh stage: in this stage blow flies were the first to arrive on the dead body, this stage lasts for three days, blow fly lay their eggs in the open cavity of the dead body such as mouth, anal region, nose, ear and the pH of soil was 8 and the presence of 1st instar larva was noticed. The ambient temperature of the body was 32℃.

Bloated stage: this is the easiest stage to distinguish, several bacterial activities are there which is known as the putrefaction in which the dead body swell like a balloon because of the certain gas formation and the presence of 2nd instar larva was noticed and the smell released from the dead body attracts more and more blow flies. Body temperature of different body parts such as: anal-25℃, mouth-32℃, stomach-26℃.

Active decay stage: in this stage the gases were released with a foul smell around the dead body and the skin breakdown starts, due to the later stages of the putrefaction fermentation occurs and the two acids are generated known as caseic and butyric acid. Followed by the advanced putrefaction which include the ammoniacal fermentation of the body to which the distinct carrions of insect were fascinate such as sulphide, Bettles. Here the presence of 3rd instar larva was noticed.

Post decay stage: all the remains of the body are decomposed and all the remaining body tissue are dried. The main indicator of this stage is the presence of the increased in the number of the Bettles and decreased in the number of flies. Here the presence of 4th instar larva was noticed.

Skeletonization stage: this is known as the final decay stage in which only the hairs and the bones are left, no group of insects were noticed, only the presence of some Bettles is there. Soil pH was recorded as 7, the body temperature was recorded as 31.8℃, the ambient temperature was recorded as 34℃.

Result:

(Table-1.1)

DATE	TEMPERATURE	HUMIDITY	pH
17-03-2024	30°C	36%	7
18-03-2024	31°C	37%	8
19-03-2024	31°C	39%	6.6
20-03-2024	32°C	36%	7
21-03-2024	27°C	40%	7
22-03-2024	31°C	47%	6
23-03-2-24	32°C	38%	7
24-03-2024	29°C	34%	6
25-03-2024	31°C	39%	7
26-03-2024	32°C	35%	7.5
27-03-2024	34°C	36%	7
28-03-2024	35°C	40%	6.5
29-03-2024	35°C	50%	6
30-03-2024	34°C	42%	7
31-03-2024	34°C	44%	6
01-04-2024	31°C	38%	7
02-04-2024	31°C	38%	6.5
03-04-2024	35°C	32%	7
04-04-2024	36°C	40.5%	6
05-04-2024	36°C	33.2%	6.5
06-04-2024	35°C	30%	7
07-04-2024	35°C	29%	8
08-04-2024	37°C	28%	6
09-042024	37°C	26%	7
10-04-2024	38°C	27%	6.5
11-04-2024	37°C	29%	7
12-04-2024	38°C	27%	7
13-04-2024	34°C	34%	6.5
14-04-2024	30°C	50%	8
15-04-2024	34°C	52%	6
16-04-2024	35°C	44%	7
17-04-2024	32°C	29%	7

(Table-1.2):

Environmental factor	Maximum	Minimum
Temperature	38℃	27℃
Relative humidity	52%	26%
Soil pH	8	6
Dead body pH	8	6

(Table-1.3):

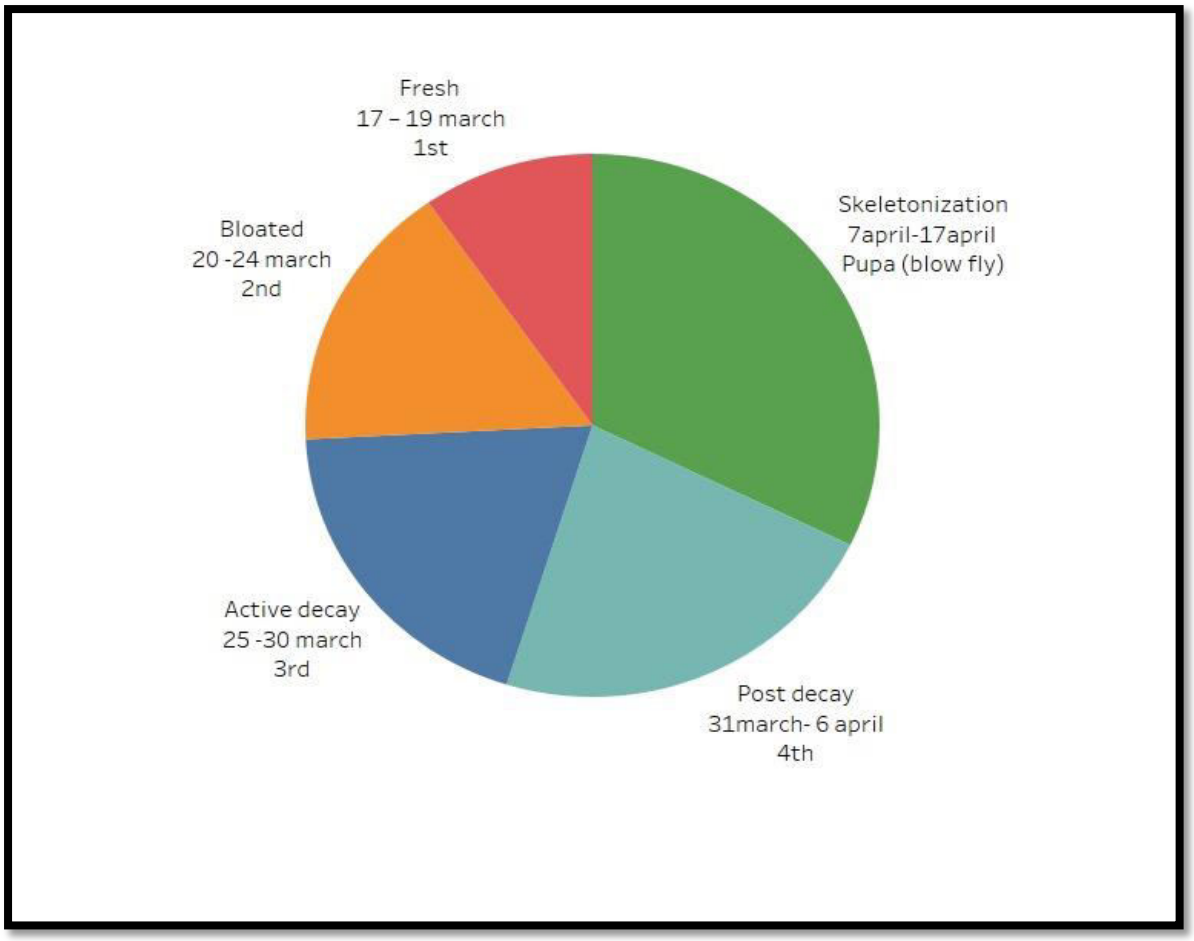
Blow fly was the key component of our study, which undergoes through different life stages:

Stages of blow fly	Time taken (hour)
1st instar larva	23
2nd instar larva	27
3rd instar larva	22
4th instar larva	130
Blow fly	143

(Table-1.4):

Here the decomposition undergoes the 5 different stages:

S.NO	Stages	Time interval	Instar larva
1	Fresh	17 – 19 March	1st
2	Bloated	20 -24 March	2nd
3	Active decay	25 -30 March	3rd
4	Post decay	31March- 6 April	4th
5	Skeletonization	7April-17April	Pupa (blow fly)



(Figure-2): pie chart representation of the decomposition stages with time interval

In our study we concluded that the growth factor if highly affected by the temperature, as the temperature increases the growth also increases with the same ratio and it was proved that growth is directly proportional to the temperature.

$G \propto T$ where, G=growth rate and T= temperature …. (1)

As the temperature increases the growth rate of the larval stages also increases rapidly and the decomposition rate of the carcass also increases with increase in the temperature, which states that the temperature is directly proportional the decomposition:

$T \propto D$ where T= temperature and D= decomposition …. (2)

The increase in the number of the larva on dead body increases the inside temperature of the body which is higher than the ambient temperature. We found that ambient temperature of the carcass lies between 32 to 35℃

Therefore,

$G \propto T$ where, G=growth rate and T= temperature …. (1)

$T \propto D$ where T= temperature and D= decomposition …. (2)

From the above two equation we concluded that:

$G \propto T \propto D$ …… (3)

Growth, temperature, and Decomposition are interdependent with each other. If temperature increases then growth of the blow fly also increases, with the increase in the temperature the decomposition rate also increases respectively.
During our study in the contribution of Chrysomium megacephalum for appraise the PMI in forensic entomology we conclude that the Growth, Temperature, Decomposition all are the key factors for the blow fly life cycle, if one of the factors also don't work properly or cannot get favourable environmental conditions then there will be error in appraise the PMI, to avoid the error we should collect the data meticulously for appraise the unerring PMI.

Discussion:

Many studies have been done to identify the importance of Chrysomium megacephalum in forensics. Our study highlights the contribution of Chrysomium megacephalum in estimating the post-mortem interval (PMI). The essential step in estimating the age of the larva is to identify the species of the blow fly because the growth rate of the larva is different in different species and also varies in relation with the temperature of that particular area. In our study we also identified that the larva found feeding on the carcass is of various instar stages, when the temperature is high, we can even find the larva of 1st, 2nd, 3rd, 4th instar stages all together which ultimately indicates that the growth is dependent on the temperature. From 17 March to 17 April the maximum temperature rise during our study was 38℃ and the minimum temperature recorded was 27℃. The temperature range between 17 march to 23 march was recorded as maximum was 32℃ and minimum was recorded as 27℃, between 24 march to 30 march the maximum temperature range was recorded as the 35℃ and minimum temperature range was recorded as 29℃, between 31 march to 6 April the maximum temperature range was recorded as the 36℃ and the minimum temperature range was recorded as the 31℃, between 7 April to 13 April the maximum temperature range was recorded as the 38℃ and the minimum temperature range was recorded as the 34℃ and between 14 April to 17 April the maximum temperature range was recorded as the 35℃ and minimum temperature range was recorded as the 30℃. As the temperature is high due to less rainfall due to which there will be rapid growth in blow fly stages therefore blow fly will complete its life cycle early, in our study we concluded that the growth factor if highly affected by the temperature. Here it was proved that growth is directly proportional to the temperature. $G \propto T$ where, G=growth rate and T= temperature …. (1)
Chrysomium megacephalum (Blow fly) was the key component of our study, which undergoes through different life stages, during our study we calculated that depending upon the time availability we can estimate the PMI by identifying the age of the larva and on the bases of the succession rate or decomposition rate. Life stage of Chrysomium megacephalum include the Larval stages i.e. 1^{st} instar larva, 2^{nd} instar larva, 3^{rd} instar larva, 4^{th} instar larva and pupae. 1^{st} instar larva

grow within 23 hours after the laying of the egg, 2nd instar larva grows within 27 hours after 1st instar, 3rd instar larva grows within 22 hours after 2nd instar, 4th instar larva grows within 130 hours after 3rd instar and finally 4th instar stops feeding on the dead body and turns into pupae stage which is dark black in colour with cylindrical shape found near the dead body under the depth of 1 to 4 inch and in vertical position only due to species specific Chrysomium megacephalum, it will take about 143 to 145 hours for the blow fly to originate from the pupa and the hollow cylindrical shape pupa will remains behind in the soil. Decomposition rate for estimating the PMI with all the 5 stages of decomposition i.e. fresh stage, bloated stage, active decay stage, post decay stage and skeletonization stage. Fresh stage stays for 3 days blow flies arrive first on the dead body and lay their eggs by finding the safe place such as mouth, anal cavity. Bloated stage stay for 5 days, body undergoes putrefaction process with foul smell and gases with swollen body, during our study we noticed that the body temperature of different regions such as: anal-25℃, mouth-32℃, stomach-26℃. Active decay stage stays for 6 days, here body starts generating the acids known as caseic and butyric acid. Post decay stage stay for 7 days, complete decomposition takes place and the increased number of Bettles and decreased in the number of flies. Skeletonization stage stay for 11 days and can also stay for months. Only the presence of hairs and bone are there, no tissue are left. Soil pH was recorded as 7, the body temperature was recorded as 31.8℃, the ambient temperature was recorded as 34℃.During our research work we noticed that as the temperature increases the growth rate of the larval stages also increases rapidly and the decomposition rate also increases with increase in the temperature, which states that the temperature is directly proportional the decomposition:

$T \propto D$ where T= temperature and D= decomposition …. (2)

The increase in the number of the larva on dead body increases the inside temperature of the body which is higher than the ambient temperature. We found that ambient temperature lies between 32 to 35℃. The maximum pH during our study was 8 and the minimum pH was 6. The maximum relative humidity was recorded as 63.1% and the minimum relative humidity was recorded as 33.2%. The maximum pH of the dead body was 8 and the minimum pH of the dead body was 6, the soil moisture was recorded as 70%.

From equation 1 and 2 we concluded that:

$G \propto T$ where, G=growth rate and T= temperature …. (1)

$T \propto D$ where T= temperature and D= decomposition …. (2)

$G \propto T \propto D$ …… (3)

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