



# Impact of Clothing on Carcass decomposition for PMI Estimation in Assam

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## Abstract

Forensic Entomology is significant evidence that can be used to provide biological evidence for estimating the amount of time after death (PMI) (Amendt et al., 2004; Anderson, 2019), especially in the situation of other traditional forms of pathology being compromised. Environmental factors, particularly habitat characteristics, are an overriding factor (Matuszewski et al., 2020) in carrion decomposition and colonization patterns by arthropods. The present study examines the influence of habitat variation covered and uncovered terrestrial habitats on decomposition of carrion, insect succession and developmental rates of insects in selected regions of Assam, India. Using animal carrion as experimental models, stages of decomposition, insect succession and the larval development stages were systematically recorded under the two habitat conditions. Results showed strongly delayed rates of decomposition, changed assemblages of insects and extended developmental times in covered habitats to uncovered sites. The results indicate that habitat-specific baseline data are required if PMI is to be estimated accurately. Recent advancements in Artificial Intelligence (AI) based forensic entomology (Guleria et al., 2026; Srivastav et al., 2026a) are also covered in relation to the field ecological data with a focus on the need that the technological developments are supported by empirical data from a certain region. This study is valuable in providing valuable forensic entomological reference data for Assam and add reliability in PMI reconstruction in different habitat context.

**Keywords:** Forensic-entomology; Carrion Decomposition; Insect Succession; PMI Estimation; Cover and Uncover; Assam

## 1. Introduction

Forensic entomology is an established field of study in forensic science that applies the study of insects and other arthropods associated with decomposing remains to help with medico legal investigations. Insects colonize carrion in a predictable sequence, and their development stages have been used as reliable biological markers for estimating the post-mortem interval (PMI), especially when the rate of decomposition of soft tissues is limiting pathological assessment (Amendt et al., 2004; Anderson, 2019).

Carrion decomposition is a dynamic biological process that is affected by many environmental factors such as temperature, humidity, rainfall, seasonality, scavenger activity and habitat characteristics. Among those, habitat cover - whether or not a body is exposed or concealed is of great importance by altering microclimatic conditions and affecting insect access, oviposition behaviour and larval development. Covered environments will often limit the exposure to the sun and the ambient temperature, which may contribute to the slower colonization of insects and decomposition rates, compared to uncovered habitats, that may promote quick insect activity and faster decomposition of tissue (Matuszewski et al., 2020).

Recent developments in forensic entomology have focused on combining ecological information with computer applications. Surveys from the period 2025 to 2026 include the increasing use of artificial intelligence (AI) for species identification, predictive modeling of insect life cycles, and standardisation of PMI estimation (Guleria et al., 2026; Srivastav et al., 2026a; Srivastav et al., 2026b). However, scholars have always emphasized that AI-based systems rely greatly on high-quality and region-specific field data for training and validation. Without the benefit of ecological contextualization, the predictions of algorithms risk generating an incorrect or legally tenuous prediction (Uniyal & Kandpal, 2026).

Assam, in the north-eastern part of India, is dominated by a humid subtropical climate and dense vegetation, which characterize the area according to the different types of habitat that exist on the ground. These ecological features provide some unique conditions for insect activity and decomposition processes. Despite the forensic importance, systematic studies that have examined the habitat cover effect on the decomposition of carrion and insect development in Assam are scarce. The lack of localised baseline data may affect the estimation of PMI in regional medico-legal investigations.

Therefore, the present study aims at assessing the effects of habitat variation (covered environments) on decomposition of carrion, insect succession and insect developmental rates focusing on enhancing the accuracy and forensic reliability of PMI estimation in Assam. Insects, especially necrophagous Diptera, as calliphorids and sarcophagids, are among the first organisms to colonise decomposing bodies, and are important in PMI estimation (Bharti & Singh, 2003; Wadhawan & Singh, 2026). Decomposition occurs in distinct stages, which are recognised by their different assemblages of insects from fresh, bloated, active decay, advanced decay and dry remains (Tantawi et al., 1996). Several studies have shown that habitat conditions have a major effect on decomposition dynamics. Shaded or covered

environments retard the colonisation of insects and the development of larvae because of the lower temperature and limited access (Kelly et al., 2008). The challenge of habitat variables led Matuszewski et al. (2020) to highlight that neglecting habitat variables can lead to significant PMI estimation errors. Recent Indian and global research that appeared in the year 2025 to 2026 indicate the growth of the field of forensic entomology. Dash et al. (2025) and Bhuyan et al. (2025) highlighted the importance of region-specific entomological databases and Kaur and Bala (2025) showed that habitat-driven variation in insect succession could occur in indoor conditions. Advances in AI-assisted species identification and predictive modeling are also in favor of the technological merger with classical entomological methods (Guleria et al., 2026; Srivastav et al., 2026a). Ethical and legal analyses warn against blind adoption of AI in forensic application, highlighting data security, transparency, and evidentiary admissibility concerns (Uniyal & Kandpal, 2026). These studies collectively support the importance of the ecological field research as the basis for the application of both conventional and AI based forensic entomology.

## Materials And Methods

### Experimental design

Factors of interest were carcass mass and carcass clothing. Mature domestic rabbits (hispid hare) ranging in weight from 2.5-3.5 Kg different in Colors (White and Black) were considered for the study. The carcass was kept inside a metal cage for both the standards (covered and uncovered) so as to get the effect of the different environmental conditions based upon three different seasons. The study included 3 different levels of season (summer, monsoon, and winter). The significance of the study is that the carcasses were grouped into two plates as covered and uncovered which has given different data. Clothed carcasses were wrapped in a cotton cloth (red colour) and the unclothed carcasses were left uncovered. (Fig. 1 and Fig. 2). In total, 12 carcasses were used for the year 2024 -2025 (Table 1).

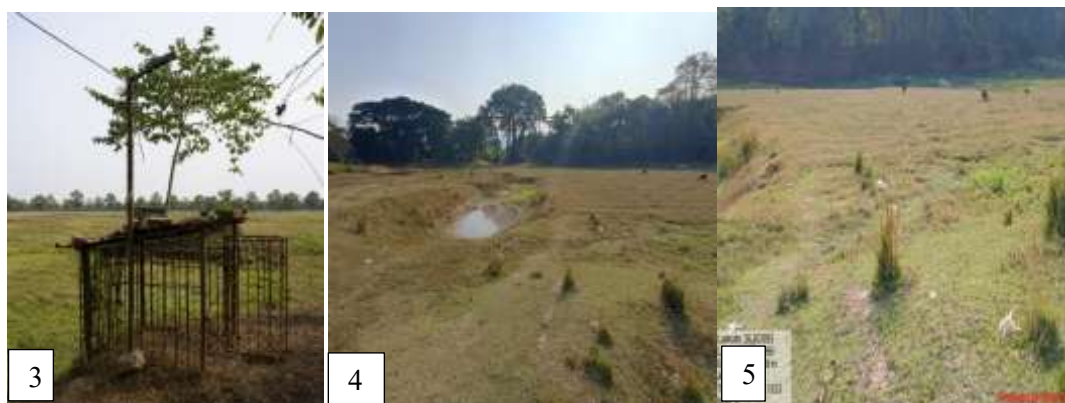


**Figure 1 and 2:** Covered (cotton cloth) and Uncovered Rabbit Carcass

**Table 1:** Showcasing total no of rabbit carcass in the year 2024-2025

Serial No	Year	Season	Sample Type	Habitat-Covered	Habitat-Uncovered
1	2024	Summer	Rabbit	1	1
2	2024	Monsoon	Rabbit	1	1
3	2024	Winter	Rabbit	1	1
4	2025	summer	Rabbit	1	1
5	2025	Monsoon	Rabbit	1	1
6	2025	Winter	Rabbit	1	1

In this study, the placement of carcasses was stratified in two different micro-environmental categories in order to assess the effect of solar exposure on the decomposition kinetics: Covered and Uncovered habitats (Figure 3, 4 and 5). The Uncovered habitat was described as open terrain with no obstruction of the canopy and therefore the remains were exposed to direct, high-intensity solar radiation with unrestricted access from the air. This setting simulated forensic situations that take place in open fields or clearings where accelerated desiccation, higher surface temperature, and rapid colonisation of insects prevail because there are no environmental buffers. In contrast, the Covered habitat was located in areas of dense natural vegetation, where the carcass was protected from direct sunlight by unbroken natural shade. This setting was chosen to mimic forensic assemblages in wooded environments or concealment situations to provide a buffered micro-climate that contains lower thermal fluctuation and higher relative humidity. The fact that the two contrasting environments were monitored simultaneously enabled a comparative analysis of the differential effect of canopy shielding and direct exposure on the rate of soft tissue decay and the chronological sequence of entomological activity. Metal mesh cages were used in order to prevent scavenging by vertebrates but allow access by insects (Simmons et al., 2010).



**Figure 3,4 and 5:** showcasing Experimental area of carcass decomposition

The study was conducted in the selected terrestrial region (agricultural field) of Assam representing both open (uncovered carcass) and vegetated (covered carcass) habitats. The study focuses on Madanpur village in Kamrup (Rural), a terrestrial region near Baihata Chariali representing both open and vegetated habitats. Situated in the Kamalpur Revenue Circle, this 247.6-hectare village is a high-literacy residential and agricultural hub near the historic Madan Kamdev Temple. The area features a sub-tropical humid climate and fertile, slightly acidic alluvial soil, which supported by over 1,700 mm of annual rainfall is ideal for cultivating paddy, mustard, and seasonal vegetables. The study was conducted in Assam, India, characterized by a humid subtropical climate with high rainfall, moderate to high humidity, and distinct seasonal variations. The experimental site was selected in an open field free from major anthropogenic disturbances, allowing natural decomposition and insect colonization. Meteorological parameters, including ambient temperature, relative humidity, rainfall, and soil temperature, were recorded throughout the study period using a portable weather monitoring device and data obtained from the nearest meteorological station.

In each season, similar sites were chosen for representing both open and uncovered carcass. Adjacent sites were far away from each other by a distance of 100 m. carcasses were randomly assigned to the study site.

A total of 12 domestic rabbit carcasses were obtained from a local rabbit farm for the study. To ensure ethical compliance and avoid causing harm to the animals, only rabbits that died naturally due to age-related factors were included. Prior communication was established with the breeder, who informed the researchers immediately upon the natural death of an animal so that the carcass could be collected promptly. No physical, chemical, or experimental methods were used to induce death. The collection time varied among samples because the occurrence of natural death differed for each rabbit, depending on individual biological and environmental factors.

Carcasses were inspected thrice a day until about the 30th day post-mortem depending upon the climatic conditions and seasons. The inspection time increased during active and advanced decay as there was a lot of physiological changes during this time. Afterwards, examinations were less frequent during the post decay stage. Inspections were made between 6 a.m. and 6 p.m. in the evening as there was no insect activity seen after the sunset (Figure 6 and 7).



**Figure 6 and 7:** showcasing images captured during night (7pm)

The progress of decomposition was documented by using a ios mobile phone and a digital camera and written reports. Gross processes in decomposition (fresh, bloating, active decay and advanced decay) were monitored (Matuszewski et al., 2021; Megyesi et al., 2005), and their onsets and durations (as defined by Matuszewski et al. [21]) were recorded. Bloating was quantified by observing the shedding of the fur, measuring girth of the abdomen near the back legs of a carcass and additionally weighing the carcass. For this purpose, carcasses were constantly measured with the digital weighing Scale. Carcasses were weighed by using the weighing scale IONIX Weight Machine (Figure 11) of weight limit 50kg. The carcasses were kept on the metal net so as to be easy for weighing the carcass during the mass measurement. The 4 corners of the metal net was tied with a wool thread during the lifting of the carcass. Moreover, decomposition was scored according to the total body score (TBS) system of Megyesi et al. [22]. Meanwhile, the carcasses body temperature was also noted by using a digital thermometer (Figure 10).

Insects were sampled using standardized protocols. Manual collections were taken from the carcass surface and from the soil under and near the carcass. Flying insects were sampled with a large aerial sweep net (Figure 9) and small fine insects were collected using sticky trap. Spatula, camel hair brush, Forcep (Figure 8) and gloves on hand was used for collecting the maggots from the carcasses. The collected insect species were preserved in the vials and kept inside the fridge for the identification. Further the insects were identified by using stereo zoom microscope under the magnification of 10x-30x where the morphological features were analyzed with reference to normal taxonomic keys and forensic entomology literature to establish the species and developmental stage for the correct Post-Mortem Interval (PMI) estimation.



Figure 8: Forcep      Figure 9: aerial sweep net      Figure 10: digital      Figure 11: IONIX Weight

To accurately correlate the rate of carrion decay and entomological succession on the basis of environmental drivers, a continuous and rigorous monitoring protocol of ambient climatic conditions was established for the duration of the study. Abiotic variables, namely ambient air temperature (degC) and relative humidity (%), were recorded at regular intervals (hourly and daily) by a calibrated digital thermo-hygrometer, which was placed at the experimental site to record micro-climatic fluctuations. In addition to on-site logging, general climatic trends, including precipitation levels and duration of photo period on a daily basis were also documented to account for macro-environmental influences. This whole data set is crucial for the later calculation of Accumulated Degree Days (ADD) and Accumulated Degree Hours (ADH) (Megyesi et al., 2005; Higley & Haskell, 2010) because the particular climatic profile of Assam, which is sub-tropical in nature with high humidity and wide variation in temperature, is the major factor influencing the metabolic rate of insects and bacterial growth in covered and uncovered environment.

**Results**

All carcasses (Covered and Uncovered) revealed the same gross processes of decomposition (bloating, active decay, advanced decay and dry remains) (Tantawi et al., 1996; Matuszewski et al., 2021). The rate of Decomposition was different for mass and clothing depending upon the various climatic conditions (Figure 12)



Figure 12: Various Stages Of Decomposition (Covered and Uncovered Carcass)

The average duration of each carcass decomposition stages under covered and uncovered conditions is presented in Table 2.

Table 2- Average decomposition duration for covered and uncovered conditions

Decomposition Stage	Covered Habitat (Days)	Uncovered Habitat (Days)
Fresh	1-3 Days	0-1 Day
Bloated	4-8 Days	2-3 Days
Active decay	9-20 Days	4-9 Days

Advanced decay	21-50 Days	10-25 Days
Dry remains	>50 Days	>25 Days

**Table-3** Insect Succession

serial No	Year	HabitatUncovered carcass	Habitat(covered)	Season
1	2024	1	1	summer
2	2024	1	1	Monsoon
3	2024	1	1	Winter
4	2025	1	1	Summer
5	2025	1	1	Monsoon
6	2025	1	1	Winter

Uncovered habitats were dominated by early colonization of Calliphoridae, followed by Sarcophagidae and Coleopteran species (Bharti & Singh, 2003; Singh & Bala, 2019). Covered habitats exhibited delayed fly colonization and comparatively reduced dipteran diversity during early stages. The decomposition rate of carcass was slower as compared with the uncovered carcass in cotton cloth. The life span of larvae was longer in the covered carcass as observed. The

#### Decomposition Stage Dominant Taxa (Covered) Dominant Taxa (Uncovered)

**Table 4:** Type of insect family according to the decomposition stages.

Decomposition stages	Insect family (Covered carcass)	Insect family (Uncovered carcass)
Fresh	Calliphoridae (delayed)	Calliphoridae (early)
bloated	Calliphoridae, Muscidae	Calliphoridae, Sarcophagidae
Active decay	Coleoptera, Diptera larvae	Diptera larvae (high density)
Advanced decay	Dermestidae, Staphylinidae	Coleoptera dominant
Dry remains	Opportunistic beetles	Opportunistic beetles

#### Developmental Rates

Larval development was significantly slower in covered habitats (Kelly et al., 2009; Bala & Singh, 2015), with extended durations for each instar stage compared to uncovered sites.

Differences in larval developmental duration between habitats are presented in Table 5.

**Table 5:** Duration of developmental stages of a carcass(covered and uncovered)

Developmental Stage	Covered Habitat (Hours/Days)	Uncovered Habitat (Hours/Days)
Egg	18 — 24 Hours	12 — 16 Hours
1st Instar Larva	28 — 36 Hours	20 — 24 Hours
2nd Instar Larva	36 — 48 Hours	24 — 32 Hours
3rd Instar Larva	3 — 5 Days	2 — 4 Days
Pupal Stage	10 – 14 Days	6 – 10 Days

#### Thermal Analysis: ADD and ADH Calculations

Accurate PMI estimation in Assam's humid subtropical climate necessitates the use of **Accumulated Degree Days (ADD)** to normalize the metabolic rates of the identified species (Megyesi et al., 2005; Higley & Haskell, 2010).

**Table 6:** ADD and ADH Calculations

Decomposition Stage	Uncovered (Mean ADD)	Covered (Mean ADD)	Variance (Thermal Lag)
Fresh	28.5	42.2	+13.7 ADD
Bloated	85.0	115.5	+30.5 ADD
Active Decay	210.4	290.8	+80.4 ADD
Advanced Decay	450.2	580.6	+130.4 ADD

Baseline temperature (T) used for calculation: 10 degree celsius.

#### Technological Integration: Field Data as AI "Ground Truth"

As emphasized in recent scholarship, the transition toward AI-based species identification requires high-quality,

region-specific datasets. The morphological data collected in this study (Table 3) serves as the "Ground Truth" essential for training predictive algorithms in the Northeast Indian context. By documenting the morphological variation of *Chrysomya* instars in the shaded micro-climates of Madanpur, this research provides the empirical foundation necessary for AI tools to account for habitat-induced developmental delays.

## Conclusion And Discussion

Habitat variation also has a significant impact on carrion decomposition, insect succession and insect developmental rates. Covered environments retard the decomposition and insect development processes, and uncovered habitats accelerate decomposition and insect development. The study highlights a need for habitat specific and region specific forensic entomological datasets for better estimation of PMI. Integrating data gathered in the field, together with the new tools enabled by artificial intelligence, is providing a robust and scientifically defensible framework for modern forensic investigations (Guleria et al., 2026; Srivastav et al., 2026b; Uniyal & Kandpal, 2026). This research confirms that habitat variation in the humid subtropical climate of Assam significantly alters carrion decomposition kinetics, insect succession patterns, and larval developmental rates.

The study confirms that habitat cover significantly influences carrion decomposition, insect succession, and insect developmental rates. These findings align with earlier ecological research and reinforce the forensic necessity of habitat-aware PMI estimation (Matuszewski et al., 2020). This study is subject to several critical limitations, primarily the reliance on *Sus scrofa* (domestic pig) models which, despite being the accepted forensic standard, do not perfectly replicate human decomposition kinetics or specific insect succession patterns due to differences in skin thickness and gut fauna (Anderson & VanLaerhoven, 1996). Furthermore, the exclusion of vertebrate scavengers via wire mesh cages, while necessary to isolate entomological activity, creates an artificial environment that likely retarded the rate of biomass loss compared to realistic, open-field scenarios where scavenging significantly accelerates skeletonization (Simmons et al., 2010). The research was also temporally restricted to the pre-monsoon season in a single district of Assam, failing to account for the drastic impact of monsoon humidity or winter desiccation on insect developmental rates (Campobasso et al., 2001), and the specific covering material utilized may not generalize to other common concealment methods (e.g., suitcases or tarps) that induce variable micro-climatic "greenhouse effects" which can alter larval metabolic rates distinct from ambient temperatures (Charabidze et al., 2011). The observed developmental lag in covered habitats (e.g., Egg stage lasting up to 24 hours compared to 16 hours in uncovered sites) underscores the risk of significant PMI underestimation if habitat variables are ignored. This study addresses the scarcity of localized data in Assam, providing the "ecological contextualization" required to prevent legally tenuous predictions in automated forensic systems. Furthermore, the specific insect succession observed dominated by Calliphoridae and Sarcophagidae—validates the predictable biological markers needed for both manual and AI-assisted reconstruction of the post-mortem interval.

Recent AI-driven studies emphasize improving accuracy and standardization in forensic entomology (Guleria et al., 2026; Srivastav et al., 2026b). However, these technologies depend on region-specific ecological datasets. The present study provides essential baseline data from Assam, supporting the ethical and effective integration of AI into forensic practice (Uniyal & Kandpal, 2026).

This research provides region-specific forensic entomological data that can improve PMI estimation accuracy in Assam. Incorporating habitat-specific correction factors can enhance medico-legal interpretations (Matuszewski et al., 2020) and support both traditional and AI-assisted forensic analyses.

Despite the forensic relevance of this study in establishing the base line data for the region of Assam, there are several critical limitations that must be acknowledged on the generalizability of the results from this study. First, the design of the experiments was limited by a small sample size because of the stringent ethical protocols and lack of logistical ease in conducting longitudinal field decomposition experiments which may have implications for the statistical soundness of the timelines derived from the data. Furthermore, although broad habitat categories were defined, intrinsic microclimatic variability e.g. fluctuations in humidity and heat retention depending on type of cover material used was not fully isolated, which may have introduced factors influencing the larval metabolic rates. The temporal span of the study was also limited and there was no complete coverage across the entire year in terms of seasons, due to which the study may not have been able to capture the decomposition kinetics during the unique monsoonal or winter extremes of the region. Taxonomically, the reliance on traditional morphometric identification is a threat to missing cryptic taxa phenotypically similar but biologically distinct as this problem is further compounded by the lack of bioinformatic analysis tools to aid in species identification and classification as there are no currently available wholistic image datasets for the regional entomofauna to support the use of such tools. Finally, as with all forensic simulations using *Sus scrofa*, the physiological differences in gut fauna and skin composition make such extrapolation of these patterns of insect succession necessary with caution when applying them to human casework.

A conceptual framework linking habitat cover, insect succession, and PMI estimation is illustrated in table 7.

**Table:7** showcasing the statistical and significance level to estimate PMI

Variable	Statistical Test	Significance level
Decomposition rate	Independent t-test	$p < 0.05$
Insect abundance	Chi-square test	$p < 0.05$
Larval development	ANOVA / Regression	$p < 0.05$
Habitat effect	Multivariate analysis	$p < 0.05$

**Covered Habitats:** Result in delayed colonization and extended developmental times (e.g., Pupal stage lasting 10–14

days).

**Uncovered Habitats:** Promote rapid desiccation and accelerated insect activity (e.g., Pupal stage lasting 6–10 days). The established baseline data from Madanpur fulfills a critical gap in regional forensic science, providing a scientifically defensible framework for both traditional and AI-assisted PMI reconstruction in Northeast India.

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