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Impact of Burn Intensity on Decomposition Timeline and Entomofaunal Assemblage in Spring Season

تأثير شدة الحرق على الجدول الزمني للتحلل وتجمع الحشرات في فصل الربيع

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Abstract

Forensic entomologists face several challenges in determining the post-mortem interval (PMI) while dealing with burnt remains. It is important to consider the succession and colonization patterns of insects on such remains while arriving at minimum post-mortem interval (PMImin). The purpose of the present study was to investigate the decomposition rate and insect succession pattern on burnt and unburnt pig carcasses during the spring season (March-May 2022) in a semi-arid region of Haryana with replication in the subsequent year. A total of eight pig carcasses were used during the study, out of which six pig carcasses were burnt at the Crow-Glassman Scale (CGS) level 1, 2, and 3, while two were left unburnt (Control) during both the years. The average accumulated degree days (ADD) for control carcass was estimated 1232.1, while for burnt level 1, 2, and 3 carcasses it was 1392.5, 1524, and 1943.9 respectively. *Chrysomya megacephala* was the earliest visitor on the control carcasses, whereas Vespa orientalis was the first one to visit on all the burnt carcasses. As the only Indian study focused on burnt carcasses, this study reveals that more severe the burns, the longest is the dry stage and slowest is the decomposition rate. The abundance of coleopterans on burnt carcasses may hold potential value in exploring their effectiveness for estimating an approximate post-mortem interval (PMI).

المستخلص

يواجه علماء الحشرات الجنائية العديد من التحديات في تحديد الفترة الزمنية بعد الوفاة (PMI) عند التعامل مع الرفات المحروقة. من المهم مراعاة أنماط تعاقب الحشرات واستيطانها على مثل هذه الرفات عند الوصول إلى الحد الأدنى للفترة الزمنية بعد الوفاة (PMImin). كان الغرض من الدراسة الحالية هو التحقق من معدل التحلل ونمط تعاقب الحشرات على جثث الخنازير المحروقة وغير المحروقة خلال موسم الربيع (مارس - مايو 2022) في منطقة شبه قاحلة في هاريانا مع تكرار التجربة في العام التالي.

تم استخدام ما مجموعه ثماني جثث خنازير خلال الدراسة، حُرق منها ست جثث وفقًا لمقياس كرو-غلاسمان (CGS) للمستويات 1 و 2 و 3، بينما تُركت اثنتان دون حرق (الضابط) خلال كلا العامين. قُدِّر متوسط الدرجات الحرارية المتراكمة (ADD) للجثث الضابطة بـ 1232.1، بينما كان 1392.5 و 1524 و 1943.9 للجثث المحروقة من المستويات 1 و 2 و 3 على التوالي. كانت ذبابة Chrysomya megacephala هي الزائر الأبكر على الجثث الضابطة، في حين كانت Vespa orientalis هي أول من زار جميع الجثث المحروقة.

باعتبارها الدراسة الهندية الوحيدة التي تركز على الجثث المحروقة، تكشف هذه الدراسة أنه كلما كانت الحروق أشد، طالت مرحلة الجفاف وتباطأ معدل التحلل. قد تحمل وفرة الخنافس (Coleopterans) على الجثث المحروقة قيمة محتملة في استكشاف فاعليتها لتقدير فترة زمنية تقريبية بعد الوفاة (PMI).

Keywords: forensic sciences, forensic entomology, decomposition, burnt carcasses, succession pattern, spring season

الكلمات المفتاحية: علوم الأدلة الجنائية، الحشرات الجنائية، التحلل، الجثث المحروقة، نمط التعاقب، موسم الربيع

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1. Introduction

Forensic entomology is the scientific study of the insects and other arthropods used in legal investigations [1]. In criminal cases, this field can provide evidence that aids in determining the time elapsed since death. After death, a body goes through various decomposition changes and the odours released during the decomposition process attract different type of insects. The decomposing body is a good food source for the insects, and they use carcasses to complete their life cycle. Dipterans are often the first one to arrive and lay eggs on bodies after death [2, 3, 4]. Their development rate, age, and stages provide crucial timeline estimation in criminal investigations [5]. Flies are typically succeeded by the beetles and then followed by various other insect species. Their succession and colonization patterns on the decomposing body offer valuable insights in the determination of time elapsed since death [6]. Thus, forensic entomology can play a significant role in the estimation of minimum post-mortem interval (PMImin) by studying the insect succession and colonization patterns at a particular geographical location and under certain environmental conditions [7, 8, 9].

The estimation of PMI is one of the most challenging tasks during the investigation of homicides and other unnatural deaths. It is the interval between death and the retrieval of a deceased body [5, 10]. A forensic pathologist plays a major role in estimating post-mortem interval in the medico-legal cases [11, 12]. Usually, the PMI can be calculated by using post-mortem changes such as immediate, early, and late decomposition in the corpse [13]. Furthermore, early post-mortem changes persist only for the first 72 hours, but when it comes to the late decomposition changes, it is challenging to arrive at a PMI. However, entomofauna feeding on a corpse may play a crucial role in such cases [14, 15]. There are two methods which have been used in the estimation of time since death by using the insects and their development stage. The first one is the employment of succession pattern and colonization by insects on dead bodies, while the second one uses insect development which is affected by accumulated degree hours (ADH) or accumulated degree days (ADD) [16, 17, 18]. Forensic entomologists can utilize these methods to estimate the PMImin in deaths that occur under different conditions and environments, including drowning, burning, or burial. The determination of time since death is a crucial part of any death investigation. Although, it becomes exceptionally challenging in the case of burnt corpses due to the severe destruction of tissues by fire. When a body is subjected to extreme heat from flames, the usual methods of evaluating the body through the study of livor and rigor mortis to estimate time since death are rendered ineffective [10, 19]. However additional forensic analysis is required to narrow down the PMI.

Burnt corpses, particularly those found in India, are linked to a wide range of social, religious, and environmental factors. The tradition of burning bodies is profoundly ingrained in India, particularly in Hinduism, where cremation is considered as a sacred ritual. Furthermore, the burning of corpses has been linked to tragic circumstances in recent years, such as mass cremations throughout the COVID-19 outbreak [20]. Additionally, situations of communal violence, mob lynchings have sometimes resulted in the burning of bodies, either to cover up crimes or as an act of retaliation [21, 22].

The act of burning human remains after death to eliminate physical evidence has been recorded at several places [15, 23]. The identification of human remains and the determination of the cause and manner of death of such burnt remains is an arduous task for forensic examiners [24]. A burnt corpse differs from normal one because burning affects the physical appearance of the dead body. A burnt corpse undergoes several post-mortem changes such as leathery or cracked skin, which can be used as pathological parameters in burn deaths [9]. A forensic examination of burnt corpses can also reveal whether the victim was exposed to the fire before or after death based on the presence of soot particles in the trachea [25]. Thus, estimating the PMI in burnt remains can be significant because burning can alter or obscure traditional indicators of time since death [26, 27]. Moreover, in some instances, the dead body remains partially burnt, and no pathological changes can be observed, in such cases, the presence of entomofauna feeding on the corpse becomes crucial for the investigators to ascertain the PMImin. The physical state of the burnt remains and insect accessibility can significantly impact both the decomposition and insect succession pattern [9]. Thus, to establish an accurate PMI, a comprehensive understanding of how burning influences insect succession patterns is essential. The research on the insect arrival, colonization and succession pattern on the burnt remains is scanty. As far as India is concerned, the insect succession pattern on the burnt remains is yet to be explored. So, the purpose of the present study was to evaluate the impact of varying levels of burns on decomposition and insect succession patterns of pig carcasses during the spring season of a semi-arid region of India.

2. Material & Methods

2.1. Study site

The study was conducted in an open area adjacent to the forest of Maharshi Dayanand University, Rohtak (28°52'14.4' N and 76°37'04.6' E). The city of Rohtak is located in the northern Indian state of Haryana. The present study was carried out in the spring season (March-May 2022) and to ensure the scientific validity, a replication study was carried out in the same season of the following year (March-May 2023). Rohtak has a semi-arid climate with moderately hot temperature during the spring season. The study area was surrounded by large Eucalyptus trees with bushy shrubs.

2.2. Experimental set up

A total of eight pig carcasses (Sus scrofa Linnaeus), weighing approximately 40 kg each were used in the present study. Four pig carcasses were used in each year. Pig carcasses were purchased from a nearby slaughterhouse at Rohtak. They were wrapped in a tarpaulin to avoid the invasion of the insects prior to the experiment setup and transported within 45 minutes to the study site. The three carcasses were burnt at different levels, and one was kept unburnt (control). To classify the levels of burn, they were categorized as level 1, 2, and 3 according to the Crow-Glassman scale (CGS) [28]. Three pig carcasses were burnt on a hard cemented surface at CGS level 1, 2, and 3 with a specific quantity of gasoline. Level 1, 2 and 3 burns were achieved with a gasoline volume of 46.875 ml/kg, 156.25 ml/kg, and 312.5 ml/kg respectively [29]. An appropriate amount of gasoline was sprayed intermittently over the carcass to control the fire and to achieve that specific level of burn. In order to prevent scavenging by vertebrates, each carcass was enclosed in an iron cage with dimensions of 91× 60× 45 cm (l×b×h). The carcasses were placed on the terrestrial surface in an open area on silt soil, approximately 50 meters away from each other to avoid the cross-contamination of insects. The ambient temperatures and relative humidity were recorded daily at logging intervals of 6 hours using a digital data logger (Elitech RC-4HC).

2.3. Insect sampling

During the experiment, carcasses were observed daily from the placement of the carcasses to the end of the experiment. Insect sampling was conducted twice a day randomly (morning and evening). The mature and immature stages of insects were collected from different decomposition stages using an insect sweep net, forceps and brush. Each insect sample collected from different carcasses was stored separately for morphological examination. Unidentified insect larvae were collected for rearing. The insect rearing of immature insects was conducted at room temperature to identify the unknown species. After rearing, newly emerged adults were collected for the morphological analysis. The morphological identification of adults was conducted in the laboratory using the standard morphological keys [30, 31, 32]. The morphological analysis of the unidentified insects was also confirmed with the help of the Zoological Survey of India (ZSI), Kolkata.

2.4. ADD calculation

The ADD was calculated by using the temperature-based method [33]. To calculate the accumulated degree days, minimum and maximum temperatures of the day were added and then divided by two to get the average or mean temperature. The threshold temperature was then subtracted from the average temperature to find the accumulated degree days. 0°C was taken as the threshold temperature since it is the temperature at which a biological process ceases, and it has been utilised in forensic entomological research in ADD calculation [33]. The actual PMI represents the duration of decomposition observed for the present experiment for all the burnt and unburnt carcasses, whereas the estimated PMI is derived from the calculated accumulated degree days (ADD) based on temperature data.

2.5. Statistical analysis

A statistical analysis was conducted to examine the substantial differences of burns on decomposition rate of burnt and unburnt pig carcasses. The Excel and SPSS (Version 30.0 (172), IBM, 2024) software were used to perform the statistical tests. A two-way Analysis of Variance (ANOVA) was used to evaluate the impact of different levels of burns on the carcass decomposition. This statistical approach was employed as it allows for the evaluation of two independent factors simultaneously as well as their interaction effect on the dependent variable. In the present study, levels of burn and decomposition stages were the independent variables, while the decomposition duration was the dependent variable. The statistical test was performed to find the statistical difference (p-value was set to 0.05) in the decomposition durations of burnt and unburnt pig carcasses. Additionally, Standard error (SE) was also calculated for the temperature and humidity data recorded during the study.

3. Results

3.1. Temperature and Humidity

During the spring season in India the temperature is moderate, the sky is clear, and the breeze is gentle. The climate is pleasant with warm and sunny days. The average temperature recorded throughout the season during the year 2022 was 32.1°C ±2.7 (S.E), with an average humidity of 40.5% ±1.5 (Figure- 1). During 2023, the recorded average temperature and humidity were 31.5°C ± 2.9 and 40.8 % ±1.2 respectively (Figure- 2). The average temperature and humidity fluctuated within the months as well. The average temperature recorded in March, April, and May of

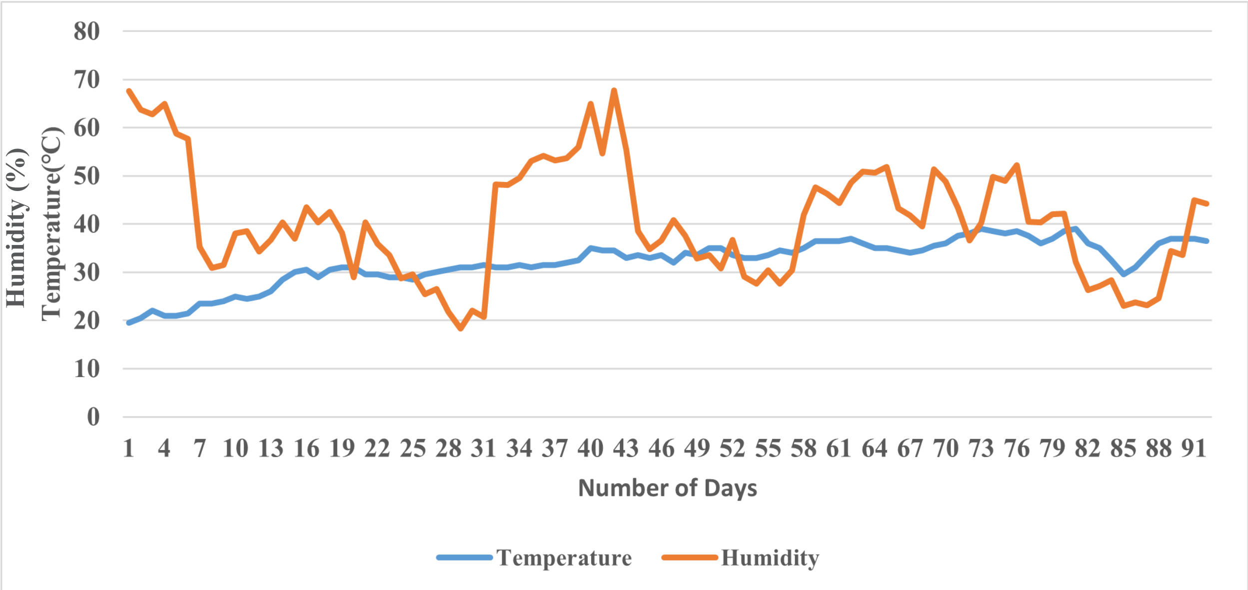

Figure 1- *A comparison of average daily temperature and humidity during the spring season of 2022*

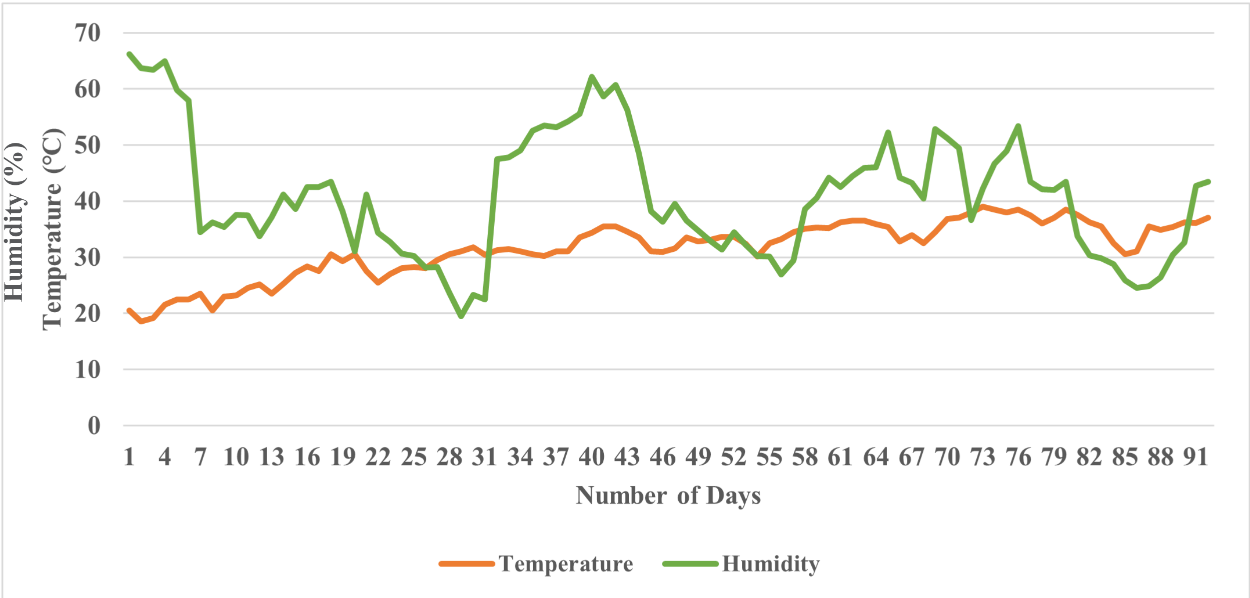

Figure 2- *A comparison of average daily temperature and humidity during the spring season of 2023*

2022 was 26.9°C, 33.4°C, and 36°C respectively with a relative humidity of 38.5%, 43.5%, and 39.6% respectively. Similarly, the average temperature during 2023 was 25.9, 32.9, and 35.5°C for March, April, and May with a relative humidity of 39.3, 43.2, and 40% respectively. The total rainfall recorded during this season of year 2022 and 2023 was 41.6 and 190.8 mm respectively.

3.2. Decomposition and insect succession pattern on the burnt and unburnt pig carcasses

The decomposition of carcasses was characterized by physical changes, odors, and the presence of insects during each stage. The burnt and unburnt carcases showed significant changes at the onset of each decomposition stage. The decomposition durations substantially varied from

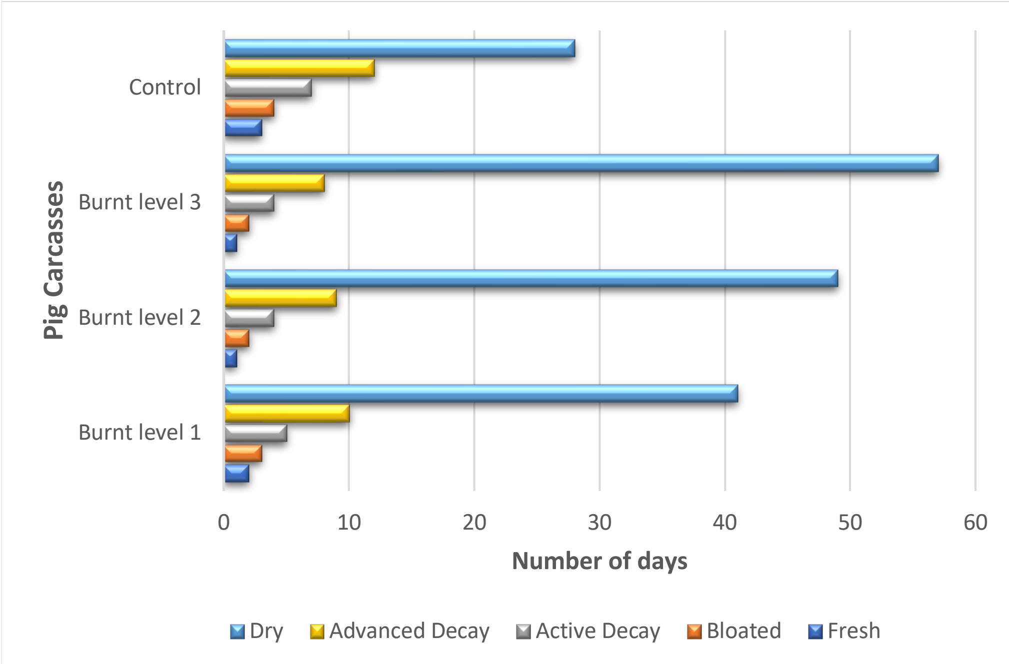

Figure 3- *The duration of decomposition stages (in days) of all the burnt pig and control carcasses*

the fresh to dry stage in all the burnt and unburnt pig carcasses as shown in Figure- 3. Average ADD and PMI (in days) based on the temperature for the burnt level 1, 2, and 3 was calculated for all the decomposition stages. The decomposition stages and associated insects can be described as follows:

Fresh Stage: During this stage, physical changes, such as blackened and dry skin, and epidermal or dermal level damage were observed in burnt level 1, 2, and 3 carcasses. Whereas no such changes were observed in the control carcass. This stage lasted for 1 day (ADD 19.5) in burnt level 2 and 3 carcasses, whereas it lasted for 2 (ADD 40) days in burnt level 1, and for 3 days (ADD 61.3) in the control carcasses in both year studies at an average temperature 20°C.

Vespa orientalis Linnaeus (Hymenoptera: Vespidae) visited all the burnt carcasses on the first day. However, dipteran flies *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae), Chrysomya rufifacies Macquart (Diptera: Calliphoridae), Sarcophaga haemorrhoidalis Fallen (Diptera: Sarcophagidae), and Musca domestica Linnaeus (Diptera: Muscidae) were recorded on the burnt level 1 and control carcasses on 2nd day. On the same day, C. megacephala, C. rufifacies oviposited, and S. haemorrhoidalis larviposited on the control carcass. On the 3rd day, 1st and 2nd instar larvae of these species were seen feeding on the control carcass. A similar observation was observed during the replicated study.

Bloated stage: The abdominal area of all the carcasses inflated at the onset of this stage. The bloated stage appeared earlier on all the three burnt carcasses as compared to the control carcass. The first bloating sign was observed on the 2nd day on burnt level 3, and after an hour it was observed on burnt level 2 carcass. This stage appeared 3rd day on burnt level 1 whereas 4th day on the control carcass at an average temperature 21.3°C. All the burnt carcasses bloated with more prominent changes compared to the control carcass. An apparent seepage of fluid was observed from the orifices of the control carcass, while fluids were

Table 1- *The entomofaunal succession pattern of all the burnt and unburnt pig carcasses during different stages of decomposition*

Order	Family	Species	Pigs	Decomposition stages				
				Fresh	Bloated	Active Decay	Advanced decay	Dry
Diptera	Calliphoridae	Chrysomya megacephala	1	A	A, L	A, L, P, E	-	-
			2	-	A, L	A, L, P, E	-	-
			3	-	A, L	A, L, P, E	-	-
			C	A, L	A, L, P	A, E	-	-
	Sarcophagidae	Sarcophaga haemorrhoidalis	1	A	A, L	L, P, E	-	A
			2	-	A, L	L, P, E	-	A
			3	-	A, L	L, P, E	-	A
			C	A, L	L, P	E, A	-	A
	Calliphoridae	Chrysomya rufifacies	1	A	A, L	A, L, P, E	-	-
			2	-	A, L	A, L, P, E	-	-
			3	-	A, L	A, L, P, E	-	-
			C	A, L	A, L, P	E, A	-	-
	Muscidae	Musca domestica	1	A	A, L	L	P, E	-
			2	A	A, L	L	P, E	-
			3	A	A, L	L	P, E	-
			C	A	A, L	L	P, E	-
	Phoridae	Megaselia scalaris	1	-	A	-	-	-
			2	-	A	-	-	-
			3	-	A	-	-	-
			C	-	-	-	-	-
	Piophilidae	Piophila casei	1	-	-	A, L	P, E	-
			2	-	-	A, L	P, E	-
			3	-	-	A, L	P, E	-
			C	-	-	A	-	-

Order	Family	Species	Pigs	Decomposition stages				
				Fresh	Bloated	Active Decay	Advanced decay	Dry
Coleoptera	Dermestidae	Dermestes maculatus	1	-	-	-	A, L, M	L, E, A
			2	-	-	-	A, L, M	L, E, A
			3	-	-	-	A, L, M	L, E, A
			C	-	-	-	A, L, M	L, E, A
	Cleridae	Necrobia rufipes	1	-	-	-	A, L	L, E, A
			2	-	-	-	A, L	L, E, A
			3	-	-	-	A, L	L, E, A
			C	-	-	-	A, L	L, E, A
	Histeridae	.Saprinus sp	1	-	-	-	A	A
			2	-	-	-	A	A
			3	-	-	-	A	A
			C	-	-	-	A	A
Hymenoptera	Formicidae	Camponotus .sp	1	-	-	A	A	-
			2	-	-	A	A	-
			3	-	-	A	A	-
			C	-	-	A	A	-
	Vespidae	Vespa orientalis	1	A	-	-	-	-
			2	A	-	-	-	-
			3	A	-	-	-	-
			C	-	-	-	-	-

1-Burnt level 1, 2- Burnt level 2, 3- Burnt level 3, C- Control, A-adult, L-larva, P-Pupa, E-Emergence, M-Molting, - species absent

purging from both skin fissures and orifices of the burnt level 2 and 3 carcasses. Similar changes were observed in the replicated study. The average ADD for the bloated stage was found to be 40, 40, 62 and 85 for the burnt level 3, 2, 1 and control carcasses respectively.

Dipteran flies *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae), Sarcophaga haemorrhoidalis Fallen (Diptera: Sarcophagidae), Chrysomya rufifacies Macquart (Diptera: Calliphoridae), and Musca domestica Linnaeus (Diptera: Muscidae) were recorded on the burnt level 2 and 3 carcasses on 2nd day. During this stage, all the burnt carcasses were colonized by flies i.e., *Chrysomya megacephala*, Chrysomya rufifacies, and Sarcophaga haemorrhoidalis. After 24 hours, the 1st and 2nd instar larvae of these species start feeding on the burnt carcasses. On control carcass, 2nd and 3rd instar larvae of *Chrysomya megacephala*, Chrysomya rufifacies, and Sarcophaga haemorrhoidalis were seen feeding at this stage on 5th day of decomposition. By the end of this stage, these larvae were found to be on pupation stage for the control carcass. Megaselia scalaris Loew (Diptera: Phoridae) also visited all the burnt carcasses during this stage.

Active decay stage: This stage started with the commencement of skin peeling off in each carcass. Owing to the severe burning effect, all the burnt carcasses displayed active decay signs more conspicuously. It was observed that all the burnt carcasses purged more fluid than the control carcass. This stage arrived earlier on all the burnt carcasses as compared to the control carcass. This stage arrived on the 4th day in burnt level 2 and 3, on the 6th day in burnt level 1, and on the 8th day in the control carcass at average temperature of 23.5°C. The average ADD for the burnt level 3, 2, 1 and control carcasses during this stage was 85, 85, 107.5 and 150.5 respectively

During this stage, Calliphoridae and Sarcophagidae's 3rd instar larvae started feeding abundantly on all the burnt carcasses. After 48 hours, their post-feeding larvae were seen on all the burnt carcasses. On the control carcass, emergence of Calliphoridae and Sarcophagidae was recorded. Piophila casei Linnaeus (Diptera: Piophilidae) visited all the carcasses but oviposited only on the burnt carcasses. Larvae of Piophila casei were seen feeding below the carcasses. In middle of this stage, Calliphoridae and Sarcophagidae larvae of all the burnt carcasses were in pupation. Camponotus sp. (Hymenoptera: Formicidae) was also observed on 7th day, and found predating on the fly larvae on all the carcasses. P. casei 2nd and 3rd instar larvae were actively feeding on all the burnt carcasses on 7th and 8th day. After three days since pupation, most of the Dipteran emergence was recorded on all the burnt carcasses. Sarcophaga haemorrhoidalis fly emerged first followed by *Chrysomya megacephala*, and Chrysomya rufifacies. At the end of this period, P. casei larvae were in post feeding stage.

Advanced decay stage: During this stage, most of the tissue was decomposed, bones and cartilage became visible. All the burnt carcasses had undergone extensive breakdown and purging ceased. This stage appeared on the 8th day for burnt level 3 and level 2, on the 11th day for burnt level 1, and on the 15th day for the control carcass at average temperature 27.2°C. The ADD was found to be 179.9, 208.2, 201.5, and 235 for the burnt level 3, 2, 1 and control carcasses respectively.

Piophila casei larvae were in pupation, on day 11th the emergence of new adults was observed. On day 15th, beetles Dermestes maculatus De Geer (Coleoptera: Dermestidae) started visiting all the carcasses followed by Necrobia rufipes Fabricius (Coleoptera: Cleridae), and Saprinus sp. (Coleoptera: Histeridae). During the replication experiment, these Coleopterans visited at this stage with the same succession pattern. On 20th day, Dermestes maculatus and Necrobia rufipes larvae were seen feeding below the carcasses. Their abundance was highest on all the burnt carcasses as compared to the control carcass which was also observed during the replicated study.

Dry remains: In this stage, all tissue had been eaten up, leaving only skin, hair, and bones. This stage appeared earlier in all the burnt carcasses followed by the control carcass. This stage appeared on the 16th, 17th, 21st, and 27th days respectively on the burnt level 3, 2, 1, and Control carcasses at an average temperature of 32.5°C. The average ADD for the burnt level 3, 2, 1 and control carcasses during this stage was 1619.5, 1171.3, 982 and 701 respectively. This stage was found to be prolonged for all the three burnt carcasses as compared to the control carcasses in both year studies. Therefore, the overall duration of decomposition was shorter for the control carcass as compared to the burnt pig carcasses. Beetles dominated this stage. Sarcophagidae were again seen visiting the carcasses at the dry stage after the rain.

The control carcass decomposed in 54 days with no insect activity thereafter, while the burnt level 1, 2, and 3 carcasses decomposed in 61, 65, and 72 days respectively. There were substantial variations between the burnt and unburnt carcasses in terms of insects' arrival, as Megaselia scalaris and Vespa orientalis were only seen visiting on the burnt carcasses. Dermestes maculatus was the earliest colonizer on all the burnt carcasses in later stages of decomposition during consecutive years. Table-1 indicates the succession pattern of insects on all the burnt and unburnt pig carcasses during both the years.

All the Dipterans preferred the unburnt areas for oviposition and only fed on the unburnt tissues. The orifices of all burnt and unburnt carcasses were the main sites of oviposition during both years of study. Sarcophaga haemorrhoidalis, *Chrysomya megacephala*, and Chrysomya rufifacies were the Dipteran species that colonized all the carcasses from the fresh to active decay stage of decomposition. S. haemorrhoidalis, C. megacephala, C. rufifacies, D. maculatus, and N. rufipes were the common species that colonized all the carcasses during both years. Coleopteran species Necrobia rufipes and Dermestes maculatus dominated throughout the advanced decay to the dry stage on all the carcasses of both years. Insect richness associated with burnt and unburnt carcasses was inconsistent, as D. maculatus and N. rufipes arrived on burnt carcasses multiple times.

3.3 Data Analysis

The calculated average ADD was found to be 1392.5 (58 days), 1524 (63.5 days), and 1943.9 (81 days) for the burnt level 1, 2, and 3 respectively, while for the control carcass it was 1232.1 (51.3 days). The two-factor analysis of variance (ANOVA) showed significant impact of levels of burning on the duration of the decomposition stages of all the burnt (level 1, 2, and 3) pig carcasses. The independent variables (levels of burn and decomposition stages) significantly affected the dependent variable (decomposition duration). The independent variable demonstrated a statistically significant influence, as indicated by its higher sum of squares (SS = 524.9), degrees of freedom (df = 3), F-value (37.7), and corresponding p-value (0.0003). The obtained p-value, which is substantially lower than the conventional significance threshold of 0.05, demonstrated that the impact of burn level 1, 2, and 3 on the carcass decomposition is statistically significant. The levels of burn on the carcasses had an impact on the overall decomposition duration as well as stage wise decomposition duration of the carcasses. When comparing the different burn levels, both burnt level 1 and burnt level 2 (p value-0.0004) exhibited statistically significant effects on the decomposition duration. Similarly, comparison among burnt level 2 and burnt level 3 (0.0001) as well as burnt level 1 and burnt level 3 (0.003) also revealed significant differences, indicating that each burn intensity uniquely influenced the rate of decomposition. Therefore, it can be stated that the differences in the dataset are statistically significant, demonstrating that the examined parameters significantly affect the decomposition durations and are crucial for forensic interpretation, such as in PMI estimation of burnt remains.

3.4 Insects abundance

During the present study, it was observed that 10 families of three orders Diptera, Coleoptera, and Hymenoptera were found associated with all the burnt carcasses. In contrast, only 8 families of the same three orders were found associated with the

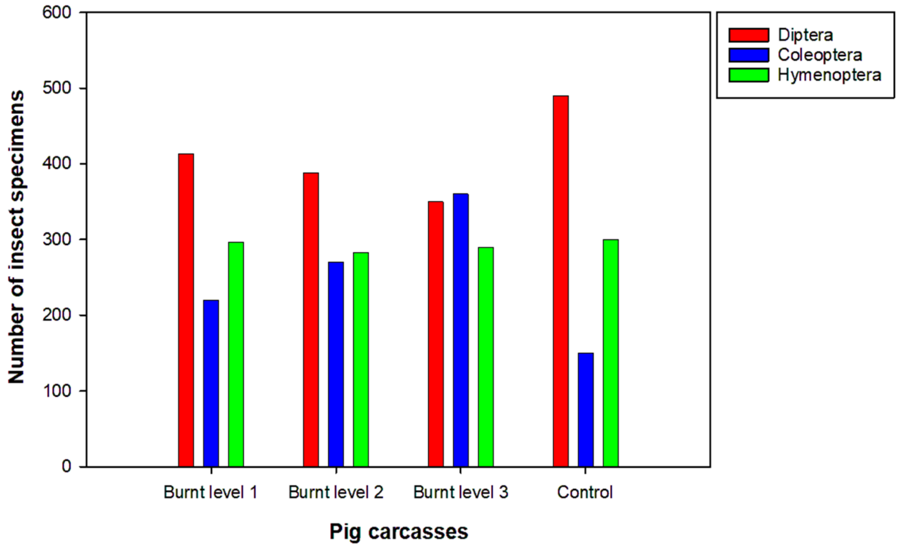

Figure 4- *Entomofaunal specimens collected from all the burnt and unburnt pig carcasses*

control carcass. A total of 3811 insect specimens were collected from burnt and unburnt carcasses throughout the study (Figure- 4). The insect specimens collected from the burnt level 1 carcass included 413 Dipteran, 220 Coleopteran, and 297 Hymenopteran, while from level 2, 388 Dipteran, 270 Coleopteran, and 283 Hymenopteran were collected. A total of 350 Dipteran, 360 Coleopteran, and 290 Hymenopteran were collected from the burnt level 3 carcass, whereas from the control carcass 490 Dipteran, 150 Coleopteran, and 300 Hymenopterans were collected. Richer abundance of Coleopteran species was seen on the burnt carcasses as compared to the unburnt carcasses.

4. Discussion

The abiotic factors such as temperature and humidity significantly affect the carcass decomposition rate [11, 34, 35]. According to Grimida et al. [36], the temperature speeds up the decomposition rate, especially in the warm season which was quite noticeable in the present study too. Increase in ambient temperature alters carcass decomposition rate as well as insect activity because the metabolic activity of bacteria increases as temperature rises [37]. These abiotic effects are significant for understanding the rate of decomposition in different climates on burnt remains. Therefore, a careful on-site examinations and consideration of all environmental factors are crucial for precise forensic analysis.

The present study found differences in the decomposition duration of burnt and unburnt pig carcasses. Conversely, a study conducted by Mashaly [15] did not find any differences in the rate of decomposition between burnt and unburnt rabbit carcasses in three different habitats (agricultural, desert, urban) of Riyadh at mean temperatures of 23.6 °C, 36.4°C, 34.3°C respectively. These differences may be attributed to use of rabbit as an animal model to simulate the human decomposition as size and hair on the body may have significant effects especially with relevance to burn deaths [38]. Barnes [39] found the decomposition stage duration of the burnt (Level 2) and unburnt pig carcasses is nearly similar, and they observed that the onset of each decay stage varied by only one day during the summers in Montana. While during the present study the onset of initial stages of decomposition varied in the range of 1-2 days, whereas the variation became more pronounced during the later stages (Figure- 3) among the burnt carcasses due the burns. The variations in these findings may have resulted due to the study being conducted in a different season and the overall temperature ranges in that season.

During the current study, noteworthy differences were observed in the fresh and bloated stages of all the burnt carcasses as compared to control carcass. In the fresh stage, the skin of burnt carcasses became black and cracked because of burns, which has also been reported by the Caserio et al. [40], Whitaker [41] and Pacheco [42]. Ellingham et al. [43] reported that exposure to heat caused the skin to tighten, split, and develop a hard, leathery appearance. Burnt experimental pigs revealed decomposition pattern variations. The prevalent variances included severe cracks in the flesh, abnormal bloating and intestinal herniation [44]. All the burnt carcasses bloated earlier than the control as burning produced more gases inside the carcasses [24]. It has also been suggested by Costa et al. [45] that the combustion may also play a role in the early onset of the bloating. Tissue damage produced by fire has quantifiable impacts on decomposition rates [46]. However, dispute exists on the extent of fire exposure, and the severity of fire can speed up decomposition for forensic situations [47].

The duration of the bloated stage was the shortest in burnt level 2, and 3 carcasses. Similar findings were reported by Avila and Goff [48], who attributed the shortened duration of this stage to the leakage of gases through cracks present in the carcass. Thus, a burn induces not only an accelerated onset of bloating but also a quick wear off.

During the active and advanced decay stages, the burnt carcasses were observed to exhibit more pronounced bursting-open signs as compared to the control carcass. Because in burnt carcasses cracks lead to the breakdown of tissues primarily due to thermal stress and dehydration, which has also been observed by McIntosh et al. [9] during the autumn of Australia. Ellingham [43] also mentioned that due to continuous high temperatures, body fluids are vaporized, and pressure is built up in closed cavities, causing the body's walls to rupture and breakdown of tissue. Therefore, observing extensive tissue breakdown signs on corpses can provide an important insight into the decomposition process and its interactions with fire.

Previous studies conducted by Avila and Goff [48] reported a consistent decomposition rate between burnt and unburnt carcasses on Hawaiian Islands, but the present study found that the decomposition rate was faster in the burnt carcasses until the arrival of dry stage. They have reported the total duration of the decomposition of burnt and unburnt carcasses, while in the present study the decomposition duration was observed during each stage along with the total duration from fresh stage to dry stage. The dry stage arrived earlier in the burnt carcasses, but the duration of this stage got prolonged for the burnt carcasses. Similar results have also been reported by Costa et al. [45], who found that the dry stage of decomposition had the slowest rate in burnt carcasses as compared to unburnt carcass during the summers of Brazil. Likewise, during the present study of both years, the dry stage was found to be the slowest, and the most prolonged in all the burnt carcasses, which can be attributed to the burns that cause rapid dehydration of tissues, leading to desiccation. These observations have important implications in forensic science in cases whereby corpse tissue doesn't decompose completely. Such corpses, recovered in the dry stage can be investigated for fire encounters before or after death.

During the present study all the burnt and unburnt carcasses were not skeletonized completely due to dryness of tissue in the semi-arid region of study site. Conversely Rigsby [47] found that charred and uncharred carcasses were entirely skeletonized by beetles. This skeletonization could be owing to the advanced decomposition, making it simpler for beetles to consume the flesh off the bone. This study revealed that in a semi-arid climate, tissue dryness stops the breakdown process and inhibit the later-stage insect activity necessary for complete skeletonization. Thus, climate of a region is a key factor during examining decomposing remains.

Chin et al. [49] suggested burning doesn't affect the insect succession pattern at a mean temperature of 29.72°C in Malaysia. Whereas there are various studies on the different insect species associated with the burnt and unburnt carcasses and which have reported that the burning of remains can affect the insect succession on the carrion [27, 39, 45, 49]. Vela et al. [50] also reported that the burn affected the arrival pattern of necrophagous insects on decomposing burnt pig carcasses in winters of Peru. The burning impacts on succession and colonization pattern can vary across different regions due to various environmental and physical factors such as temperature, humidity, and season along with the other available sources of food for the forensically relevant insects. In general burning can alter normal decomposition process by causing charring, dehydration, and destruction of soft tissues, which can obscure trauma and delay insect colonization [9]. Avila and Goff [48] found differences in the succeeded species of entomofauna in burnt and unburnt carcasses as Atherogonia orientalis (Muscidae) only visited on burnt carcass. The difference in the succession pattern of the insects on the burnt and unburnt carcasses has also been observed in the present study, where species such as Vespa orientalis and Megaselia scalaris visited only the burnt carcasses during the fresh and bloated stage respectively in both the years. While on the unburnt carcasses, Calliphoridae (C. megacephala) species were the primary visitors during the fresh stage followed by the Sarcophagidae and Muscidae species. Pai et al. [51] suggested that the *Chrysomya megacephala* and Chrysomya rufifacies were the earliest species on burnt level 2 carcasses during the summer season of Taiwan city. Observations of the present study suggest that Vespa orientalis, *Chrysomya megacephala*, Sarcophaga haemorrhoidalis, Chrysomya rufifacies, Musca domestica, Megaselia scalaris, Piophila casei, Camponotus sp., Dermestes maculatus, Necrobia rufipes, and Saprinus sp. were found to be visiting in successional waves on all the burnt carcasses. Whereas on the control carcass, the succession pattern involved subsequent arrival of C. megacephala, S. haemorrhoidalis, C. rufifacies, M. domestica, and P. casei. The rest of the species were the same as on the burnt carcasses except V. orientalis. The primary visiting of V. orientalis on burnt carcasses can be credited to its behaviour of feeding on the decomposition fluids oozing from the carcass's fissures [52]. According to Bharti and Singh [53], the insect succession observed on a rabbit carcass during the spring season included *Chrysomya megacephala*, Chrysomya rufifacies, Sarcophaga albiceps, Sarcophaga misera, Sarcophaga princeps, Saprinus species, Dermestes maculatus, and finally Necrobia species. The succession is nearly the same on control carcasses of the present study with a little variance owing to the varying species richness of that particular geographical area. This underlines the value of local data on insect succession for utilization of entomological approaches in death investigations [42, 54]. Therefore, an understanding of the succession pattern of burnt and unburnt pig carcasses in a semi-arid region during the spring season can be significant for estimating the PMI for similar areas.

Camponotus sp. was observed predating on fly larvae feeding on all the carcasses. Mashaly et al [55] had similar observations during the study of three different habitats of Riyadh. Furthermore, they also suggested that ant species vary based on the state of the cadaver. There was a greater diversity of ant species visiting unburnt rabbit carcasses than those that had been burnt in Malaysia [56]. This indicates that the presence and behaviour of ants might largely alter the species richness. Ants are omnivorous in nature; they feed upon the decomposing tissue as well as on the larvae of the dipteran flies [57]. In forensic entomology these interactions point to the importance of considering predatory species as complicating factors in the analysis of insect data from a corpse.

In the present study there was a greater abundance of dipterans on unburnt carcass and coleopterans on burnt carcasses. This may be due to the fact that flies prefer fresh and unburnt remains for feeding, while beetles prefer burnt areas [58]. A study conducted by Kruger et al. [27] also found that the unburnt carcass attracts more dipterans as compared to burnt ones during summers in Brazil. Rigsby et al. [47] reported that the burnt rat carcasses attracted more beetles compared to the flies in the Oklahoma. Burnt carcasses attracted lesser number of dipterans, which was consistent with the study conducted by Chin et al. [49], who suggested that this could be credited to the dryness of the skin or the persistent smell of fuel on the burnt carcass, which made these carcasses unfavourable habitat for dipterans. Thus, the greater abundance of Coleopterans as compared to Dipterans on a corpse may sometimes be indicative of burning.

Oviposition behaviour of dipterans is one of the most crucial factors for estimation of PMI. Pacheco [42] reported that the dipterans oviposited on the cracks present on the burnt carcasses. Caserio [40] also reported that cracks on burnt carcasses are a suitable site for oviposition during the springs season of Texas, but Avila and Goff [48] reported that cracks on carcasses were the alternative site for oviposition in addition to the natural orifices. Catts and Goff [17] also reported that the oviposition by dipterans in the cracks could be unfavoured due to the incineration of tissue. The present study is in congruence with Catts and Goff [17] as the oviposition was only found on the natural orifices of burnt and unburnt carcasses. Oviposition was observed earlier on the unburnt carcasses as compared to the burnt carcasses. Similar observations have been made by Grimida et al. [36] and Chin et al. [49]. This results due to the drying of skin during burning as dipteran have been known to prefer food sources that are moist with a protein content that is not coagulated [59]. A study by Charabidze et al. [60] have also pointed to the fact that the female blowflies have an ability to evaluate the humidity of the surface, and they have a preference to wetter locations. Similarly, a study by Archer and Elgar [61] also supports our observations that these flies not only prefer moist but also hidden areas. The cracks on the skin of carcasses are unable to provide such advantages and hence the preference for orifices is favourable for them. Thus, the oviposition behavior of dipteran on burnt and unburnt carcasses can also provide initial valuable information for forensic investigations.

The preceding heavy rainfall may have contributed to the rapid decomposition [29]. Lensing and Wise [62] reported that the decomposition can also be indirectly affected by rainfall by direct impacts on entomofauna. Griffiths et al. [35] also

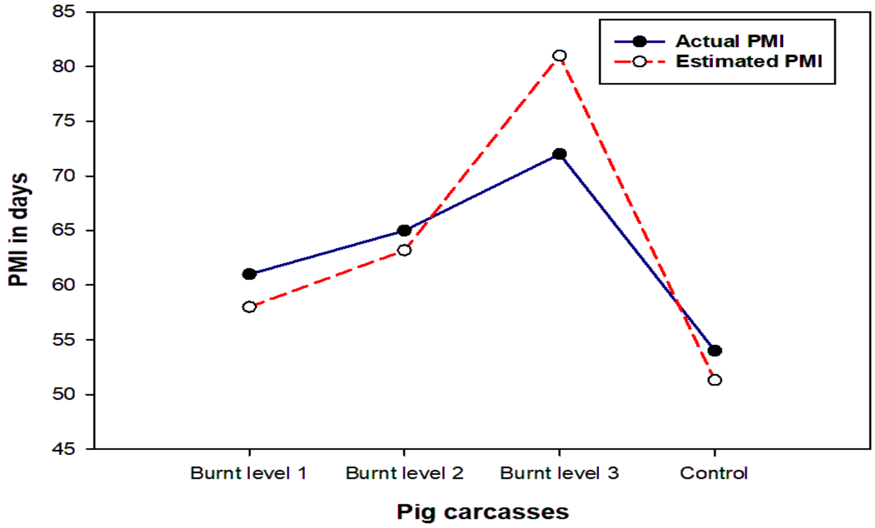

Figure 5- *A comparison of actual and estimated PMI recorded on all burnt and unburnt pig carcasses*

suggested that rainfall impacts decomposition rate and insect succession patterns, which was also observed in the present study of both years as after rain the Sarcophagidae were again seen visiting the carcasses at the dry stage. During rainfall, dry tissue becomes wet again, which is ideal for Dipterans to consume. Thus, the presence of dipteran larvae at the dry stage should also be taken into consideration during examination because it suggests that rain may also alter the succession patterns.

The actual and estimated PMI for the control and burnt level 2 carcasses showed less differences in terms of days but burnt level 1 and 3 carcasses showed greater differences. Therefore, PMI for the burnt level 1 and 3 pig carcasses based on ADD calculation alone can be misleading. But it gave better estimate for burnt level 2 remains, thus all levels of burns cannot be treated as the same. Since PMI is often estimated using ADD, a caution must always be exercised, and all levels of burns should not be considered under the same umbrella. In the present study, the estimated PMI was found to be moderately underestimated for the control and burnt level 1, slightly underestimated for burnt level 2, while overestimated for the burnt level 3 carcass as shown in Figure- 5. While a study by Cockle and Bell [63] reported that the PMI was overestimated for the carcass placed on the terrestrial surface. They also suggested that in addition to temperature the other conditions may also impact the decomposition and must be addressed for PMI estimation. Research on burnt pig carcasses must be enhanced for reconciling the disparity between estimated and actual PMI, which makes forensic work more reliable [64]. The two-factor analysis of variance revealed that different levels of burn employed statistically significant effects on the decomposition process. The individual levels of burn showed a significant impact on the overall duration of carcass decomposition. Furthermore, decomposition stage-wise comparisons revealed statistically significant differences in decomposition duration across different burn levels and control carcass.

Abundance of insect species was also found variable among the burnt and unburnt carcasses in the present study. Whereas study conducted by Medeiros et al. [65] reported that the abundance of fly species remains similar across burnt, unburnt and gasoline treated liver samples. According to this study, there was a difference in insect abundance among burnt and unburnt carcasses. The abundance of coleopterans was greater on burnt carcasses whereas dipteran abundance was greater on unburnt carcasses, which clearly indicates that burning also impacts the attraction of the types of insects to the carcasses. The results are not in agreement throughout the world; thus, the microclimatic conditions of a particular area should be taken into account to reach empirical conclusions.

The findings of this study demonstrate that the rate of decomposition on all the burnt carcasses was faster in the early stages, still the overall decomposition duration of the burnt carcasses was slower as compared to the unburnt carcasses, because of the longer dry stage. This study reveals that more severe the burns, the longest is the dry stage and slowest is the decomposition rate. Additionally, the study revealed that Dipteran insects exhibit a preference for feeding on unburnt carcasses over those that had undergone burning. It is crucial to acknowledge that burnt regions of the body have an impact on the decomposition, abundance, species composition, arrival and colonization pattern of insects. It is essential for forensic entomologists to accurately interpret insect evidence from burnt remains and provide reliable information for criminal investigations.

These findings emphasize the need to consider the influence of fire on decay duration, entomofauna composition and succession when estimating the PMI of burnt corpses. Although this study identified several factors that can alter the PMI estimation of burnt carcasses such as temperature, rain, and insects' behaviour still more in-depth research is needed to study the effects of these attributes in different climatic zones on increased number of replicates and human carcasses wherever possible.

5. Conclusion

Burn death is a unique death scenario that is often overlooked in medico-legal cases due to the paucity of evidence accessible for forensic examinations. The forensic entomological analysis is crucial in cases involving burnt corpses because it can yield significant details about the time elapsed since the death. Forensic entomology in the context of burnt remains contributes to the broader understanding of how decomposition progresses under the influence of fire and insects. This knowledge is essential for developing more accurate forensic methods for dealing with burnt remains.

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Conflicts of interest

The authors have no conflicts of interest

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