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Original article

Impact of Cause of Death on Insect Succession and Forensic Decomposition Dynamics: Insights from Poisoned, Slaughtered, and Burned Carcasses in Tripoli.

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Abstract
This study aimed to compare the dynamics of carcass decomposition and insect succession on the remains of domestic chickens, *Gallus gallus domesticus,* subjected to different modes of death: pre-mortem exposure to an insecticide, slaughter, and slaughter followed by burning. The study was conducted in the Souq Al-Juma'a and Al-Hadba Al-Khadra districts of Tripoli, Libya, between August 16 and September 5, 2024. Field observations revealed variations in the duration of decomposition stages across the different modes of death; specifically, the "fresh stage" lasted longer in carcasses treated with an insecticide compared to those that were slaughtered or slaughtered and subsequently burned. Several insect families were recorded in association with the various stages of decomposition, including dipterans (Calliphoridae, Sarcophagidae, and Muscidae) and coleopterans (Dermestidae, Histeridae, and Cleridae), with notable differences in the timing of their appearance and distribution throughout the decomposition process. Furthermore, variations in temperature ranges and relative humidity were observed between the two study sites, which coincided with differences in field observations regarding decomposition rates and insect activity. These findings provide preliminary field data on decomposition patterns and insect succession associated with various postmortem conditions within the local environment. Furthermore, they highlight the importance of conducting broader studies encompassing a larger number of cadavers and diverse geographical locations to help develop a regional database. Such a database could be utilised in forensic entomology applications and assist in estimating the postmortem interval (PMI) in Libya.
Keywords. Forensic Entomology; Insect Succession; Cadaver Decomposition; Postmortem Conditions.

Introduction

Forensic entomology has witnessed significant development over the past few decades, emerging as a vital scientific field in criminal investigations. It involves the study of insects and arthropods associated with decomposing animal or human remains, the analysis of their colonisation patterns and succession, and the use of the resulting evidence to estimate the postmortem interval (PMI). Foundational studies have established a scientific basis for understanding the succession patterns of arthropods on cadavers and the various stages of decomposition, demonstrating that the composition of insect communities and the timing of their arrival are linked to the decomposition stage and the environmental conditions surrounding the remains [1]. Furthermore, research has contributed to advancing knowledge regarding the forensic applications of insects, including their utility in estimating the postmortem interval [2, 3]. The potential role of the black soldier fly *Hermetia illucens* in this field has also been explored through various observations and case studies [4]. As the discipline has evolved, subsequent research has examined its potential and applications, while also addressing its methodological limitations and the challenges associated with utilising entomological evidence in criminal investigations [5 - 7]. The process of decomposition in human or animal remains unfolds through a series of successive biological stages: the fresh stage, the bloat stage, the active decay stage, the advanced decay stage, and finally, the dry remains stage. Each stage is characterised by specific changes in the physical, chemical, and biological properties of the remains and is often associated with a distinct assemblage of insects and arthropods that colonise or feed upon the body [8 -10].

Flies from the families Calliphoridae and Sarcophagidae are among the most significant insect groups associated with the early stages of decomposition. Meanwhile, the insect communities found on remains also include various beetles in the order Coleoptera, which may appear at different stages of decomposition depending on their biological and dietary traits, as well as the surrounding environmental conditions. The variation in the composition of these communities and the timing of their arrival are critical aspects in the study of insect succession, providing valuable evidence for use in forensic investigations [2, 11].

Environmental factors, most notably temperature and relative humidity, play a significant role in influencing the decomposition rates of cadavers and the activity of associated insects. Temperature directly affects the growth and development rates of insects, which in turn impacts the accuracy of estimating the postmortem interval (PMI) based on entomological evidence. [12,13] Models based on cumulative thermal units expressed as Accumulated Degree Hours (ADH) or Accumulated Degree Days (ADD) are used to estimate the age and developmental stage of insects, relying on the established relationship between temperature and growth rates. While these models are essential tools for estimating the time elapsed since a cadaver was colonised by insects, the accuracy of such estimates depends on the availability of temperature data and the suitability of the growth models for the specific environmental conditions under study [14].

Beyond thermal factors, the conditions surrounding a cadaver, such as its location, the nature of the environment, the degree of enclosure, and the accessibility of the body to insects also influence decomposition rates, the timing of colonisation, and patterns of insect succession. Studies have demonstrated differences in both decomposition rates and succession patterns between cadavers located in open-air environments and those found inside vehicles. [15]

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Furthermore, patterns of insect succession can vary according to the season and local environmental conditions; for instance, one study examined the succession patterns of insects associated with pig carcasses during different months in the Yangtze River Delta region of China [16]. A recent study conducted in southern Nigeria examined patterns of insect succession on cadavers, highlighting the importance of studying these patterns in light of environmental and seasonal variations across different regions [17].

Consequently, when interpreting findings to estimate the post-mortem interval (PMI), it is essential to consider environmental conditions and thermal data alongside entomological evidence [18, 19]. Applied studies underscore the significance of accounting for thermal conditions when utilising entomological evidence to estimate the post-mortem interval, given the influence of temperature on the growth and development rates of larvae. In this context, one study investigated the impact of varying temperatures on the development of *Lucilia sericata* larvae, while also exploring the potential application of the Isomegalen method for estimating the post-mortem interval [20]. Another critical aspect involves reconstructing the thermal conditions to which a cadaver was exposed at the location where it was discovered. Climatic data derived from regional or global databases such as NASA POWER may not accurately reflect the actual thermal conditions at the precise site of the body, due to localised variations in sun exposure, shade, ventilation, and the nature of the surrounding environment [21]. Furthermore, the interpretation of entomological evidence and the estimation of the post-mortem interval require the consideration of a range of environmental and biological factors; one should not rely on a single factor when evaluating the findings [22].

Practical applications of forensic entomology have demonstrated the importance of analysing entomological evidence in cases involving decomposed remains, as well as its role in supporting criminal investigations though it remains essential to interpret results in light of the specific circumstances and inherent limitations of each case [23]. In addition to traditional identification methods based on morphological characteristics, molecular techniques have contributed to the advancement of insect species identification most notably through "DNA barcoding," which involves analysing specific DNA regions and comparing them against known reference sequences to identify an organism. Research has established the foundation for using a sequence from the (Cytochrome c Oxidase Subunit I, COI) gene as a common molecular marker for identifying animal species [24]. In the field of forensic entomology, molecular tools can be employed to assist in identifying insects associated with decomposed remains particularly when specimens are immature or damaged, or when different species share similar morphological traits [25, 26]. Subsequent studies have focused on the development and evaluation of molecular identification methods for insects of forensic significance. Among these is a study that presented a simplified method for DNA barcoding using the COI region to identify fly species associated with forensic investigations, including their larvae, pupae, and pupal casings. Such applications highlight the importance of employing molecular techniques alongside morphological examination to enhance the accuracy of insect species identification in forensic studies and practice [27].

Forensic entomotoxicology is an applied branch of forensic entomology that examines the impact of drugs, toxic substances, and chemical pollutants present in a corpse's tissues on the insects that feed upon or colonise it. Furthermore, these insects can serve as alternative specimens for detecting toxic substances, particularly in cases where obtaining suitable human tissue samples is not possible. Studies have shown that the presence of certain drugs or toxins in tissues can influence insect growth and development; if these effects are not accounted for, the accuracy of the estimated post-mortem interval (PMI) may be compromised. Early research in this field investigated the effect of heroin present in tissues on the growth rates of *Boettcherisca peregrina* larvae, demonstrating that the substance could alter developmental rates thereby affecting PMI estimates derived from insect age [28]. The potential for using insects as alternative specimens for toxicological analysis to detect pharmaceutical or toxic substances has also been discussed, alongside the challenges involved in interpreting such analytical results within a forensic context [29]. Subsequent research has expanded to encompass the effects of various chemical classes, including pesticides and heavy metals, on insect physiology, growth, and development, as well as the potential implications of these factors for patterns of insect succession and the estimation of the post-mortem interval [30]. Studies have shown that the succession patterns of insects and arthropods associated with cadavers can vary from one geographical region to another, depending on differences in climatic and environmental conditions, as well as the composition of local communities. These variations are not limited to the species that colonise the cadavers; they also encompass the timing of their arrival and the sequence in which they appear throughout the stages of decomposition. One study examined the succession patterns of arthropods associated with cadavers by analysing community composition and the sequence of appearance of its components, highlighting the importance of studying these patterns for interpreting entomological evidence in a forensic context [31].

Another study provided data on insect communities and other arthropods associated with animal remains in a Mediterranean environment in northern Spain, underscoring the necessity of documenting local communities and accounting for their specific characteristics when studying insect succession [32]. The composition of insect and arthropod communities associated with decomposing remains is not determined solely by climatic factors; it is also influenced by a range of ecological factors, such as the nature of the habitat, the distribution and availability of food resources, the spatial characteristics of the site, and the relationships and interactions among different species. In this context, the spatial aggregation of arthropods around ephemeral food resources such as cadavers plays a significant role in shaping the associated communities. Furthermore, ecological interactions between species can contribute to the structure of these communities [33]. One study specifically explored the interaction networks among insects and other groups of arthropods

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associated with animal remains [32]. On the other hand, research has demonstrated variations in decomposition stages and insect succession patterns within dry tropical climates, highlighting the importance of studying these communities in the context of local ecological conditions. Collectively, these studies indicate that the composition of communities associated with decomposing remains is shaped by the interplay of various environmental factors and inter-organismal relationships, rather than by any single factor [34].

Given the multitude of factors influencing communities associated with decomposing remains, interpreting insect succession patterns requires more than just identifying the species present; it necessitates tracking changes in community composition throughout the decomposition process and understanding how these changes relate to the surrounding environmental conditions. Studies have underscored the necessity of interpreting insect succession patterns within their specific ecological context, accounting for shifts in community composition and the timing of carcass colonisation when analysing entomological evidence for forensic applications [7, 10]. In this regard, local databases of forensically significant insects are essential for the advancement of forensic entomology. They facilitate the documentation of species present in specific geographic regions, while also defining their distribution patterns, seasonal abundance, life cycles, and growth rates. Such data are particularly critical for interpreting entomological evidence used to estimate the postmortem interval (PMI), as the composition of insect communities and the timing of carcass colonisation can vary significantly based on geographic location, as well as climatic and environmental conditions. In this context, a study has highlighted the importance of documenting data on the geographical distribution and abundance of insects associated with animal remains of forensic significance; such information is essential for understanding regional variations in insect communities. Consequently, the development of reference databases grounded in local field studies contributes to more accurate interpretation of entomological evidence and helps assess the applicability of data published in other regions to different environments [35].

Beyond environmental and ecological factors, the "mode of death" may also be linked to variations in decomposition patterns and associated insect activity. This is due to changes that may occur in tissues before or at the time of death, which subsequently alter the characteristics of the remains and their accessibility to insects. In this regard, one study [36] examined differences in decomposition rates and patterns among pig carcasses subjected to various modes of death including burning, asphyxiation, poisoning, and traumatic injury while monitoring insect colonisation and activity throughout the decomposition process. The findings revealed variations in both decomposition rates and insect activity across the different experimental conditions, underscoring the necessity of considering the mode of death when interpreting decomposition-related changes and entomological evidence. Therefore, evaluating the relationship between the mode of death, decomposition, and insect succession requires field studies that account for the specific nature of each case and the surrounding environmental conditions, while distinguishing between changes attributable to the mode of death and those resulting from other environmental factors.

Despite the advancements in forensic entomology and the growing body of research on carcass decomposition and insect succession, there remains a need for further field studies. Such research should document the insect communities associated with decomposing remains in local environments and investigate variations in decomposition patterns and insect succession linked to different modes of death, while accounting for the surrounding environmental conditions. This need is particularly significant in Libya, given the limited published data documenting these aspects within the local environment. Accordingly, the current study aimed to describe and compare the decomposition patterns and insect succession patterns associated with the carcasses of domestic chickens, *Gallus gallus domesticus* that underwent different treatments, including oral administration of an insecticide preparation before death, slaughter, and slaughter followed by burning with gasoline, at two sites in Tripoli, Libya. The study also sought to document the insect populations associated with different stages of decomposition and to track the timing of their emergence and compositional changes during the study period. These observations provide preliminary field data on decomposition patterns and insect succession in the local environment, which can contribute to enriching regional knowledge of forensic insects and support the careful interpretation of entomological evidence in forensic applications, including its use in estimating the postmortem interval (PMI.

Materials and Methods

Study Design

This field study was conducted using five carcasses of the domestic chicken *Gallus gallus domesticus*, with recorded weights ranging between 5 and 5.5 kg. The carcasses were placed at two different locations within the city of Tripoli, Libya, to study decomposition and insect succession in urban environments. The study took place from August 16 to September 5, 2024. The carcasses were placed directly on the soil at outdoor sites and covered with perforated plastic boxes (60 cm long, 40 cm wide, and 20 cm high, with 2 cm diameter holes). These boxes were designed to allow insect access to the carcasses while preventing access by scavenging animals. The study monitored the stages of decomposition and insect succession twice daily. Regular field observation of decomposition and insect succession, combined with the documentation of surrounding environmental conditions, constitutes a critical aspect of applied forensic entomology studies [15, 37].

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Study Area Description

The study was conducted at two locations within the city of Tripoli, Libya: Souq Al-Jumaa and Al-Hadba Al-Khadra. Both sites are situated in residential areas, though they differ in their proximity to the Mediterranean coast. Souq Al-Jumaa is located in the northeastern part of Tripoli, approximately 50 meters from the Mediterranean coast. The site coordinates were recorded as 32°54'17" N latitude and 13°15'31" E longitude. During the study period, temperatures ranged from 18°C to 42°C, while relative humidity ranged from 20% to 80%, according to NASA POWER data retrieved using the site coordinates. Al-Hadba Al-Khadra is located in the south-central part of Tripoli, approximately 6 km from the Mediterranean coast. The site coordinates were recorded as 32°50'57" N latitude and 13°11'36" E longitude. During the study period, temperatures ranged from 18°C to 40°C, while relative humidity ranged from 30% to 80%, according to NASA POWER data retrieved using the site coordinates. Temperature and relative humidity data were obtained from the NASA POWER (Prediction of Worldwide Energy Resources) database; data for each site were extracted individually using its respective geographical coordinates for the duration of the study period (NASA, n.d.) [38]. These data represent estimates of atmospheric conditions at the specified locations rather than direct field measurements taken at the exact spot of each carcass.

Killing Methods and Experimental Treatment

The study was conducted using five carcasses of the domestic chicken (*Gallus gallus domesticus*), distributed across two locations within the city of Tripoli, Libya: Souq Al-Juma'a and Al-Hadba Al-Khadra. The study involved three experimental treatments: oral administration of a liquid insecticide commercially known as "HALAC" to the bird to death; slaughtering with a sharp knife; and slaughtering followed by burning the carcass with gasoline. For the insecticide treatment, the commercial product HALAC was used, which contains 5% Cypermethrin and 1% Tetramethrin, along with Piperonyl Butoxide (PBO) as an insecticide synergist. A 60 ml portion of the commercial product was diluted in one litre of water, and each bird was administered 10 ml of this solution orally before death [39]. Three carcasses were placed at the Souq al-Juma'a site: one treated orally with the insecticide before death, one slaughtered, and one slaughtered and subsequently burned with gasoline. At the Al-Hadba al-Khadra site, two carcasses were placed: one treated orally with the insecticide before death, and the other slaughtered. Table (1) illustrates the distribution of the carcasses according to the study site and the experimental treatment. All carcasses were placed directly on the soil surface and covered with perforated plastic crates, which allowed insect access while preventing the approach of animals that might feed on the remains. Each carcass was documented according to its location and the treatment applied, in order to monitor changes associated with decomposition and insect succession throughout the study period.

Table 1. Distribution of chicken carcasses and experimental treatments according to the study site in the city of Tripoli, Libya.

Study location	Experimental treatment	Treatment description	Number of bodies
Souq Al-Jumaa	Treatment with an insecticide.	Administering the liquid preparation HALAC to the bird orally before death.	1
Souq Al-Jumaa	Slaughter the bird using a sharp knife.	Slaughter	1
Souq Al-Jumaa	The bird was slaughtered, and then its carcass was burned using gasoline.	Slaughter followed by burning	1
Al-Hadba Al-Khadra	Administering the liquid preparation—commercially known as HALAC to the bird before its death.	Treatment with an insecticide	1
Al-Hadba Al-Khadra	Slaughter the bird using a sharp knife.	Slaughter	1

Insect Collection, Processing, and Preservation

Adult insects and larvae found on the cadavers were collected daily using a soft brush, with care taken to handle the specimens gently to avoid damage. Adult insects were killed using acetone, left to dry, and then stored in dry containers designed for specimen preservation. Larvae were placed in hot (but not boiling) water until it cooled; they were then identified before being stored in glass vials containing 75% ethyl alcohol. The storage vials were labelled with data including the cadaver number, study site, and date of collection to ensure that each specimen could be linked to the specific cadaver, location, and date from which it was recovered. Environmental conditions and insect activity associated with each cadaver were recorded systematically. The stages of decomposition and insect activity were monitored twice daily, while insect specimens were collected once daily. The procedures for collecting, processing, and preserving entomological evidence were based on methods described in specialised literature on forensic entomology [7, 37]. Table 2 outlines the steps followed for the collection, processing, and preservation of specimens, as well as the recording of associated data.

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Table 2. Procedures for collecting, processing, and preserving insect specimens.

The step	Description
Sample collection	Adult insects and larvae were collected daily from the corpses using a soft brush.
Killing adult insects	The adult insects were killed using acetone, then left to dry after death.
Preserving adult insects	Dried adult insects were stored in boxes or containers designed for preserving specimens.
Larval treatment	The larvae were placed in hot—but not boiling—water until it cooled, and were then classified.
Preserving larvae	After being classified, the larvae were preserved in glass tubes containing 75% ethyl alcohol.
Sample labeling	The tubes were labelled with the body number, study site, and date of collection.
Data Recording and Follow-up	Environmental conditions and insect activity were recorded, and the stages of decomposition were monitored twice daily, while samples were collected once a day.

Insect Identification and Classification

Taxonomic examination of the insect specimens was conducted at the Forensic Entomology Laboratory, Faculty of Science, University of Tripoli, using microscopy and specialised taxonomic keys. Specimens were identified to the most precise taxonomic level possible; for most specimens of the order Diptera, identification was limited to the family level, specifically the families Calliphoridae, Muscidae, and Sarcophagidae. Specimens of the order Coleoptera were identified to the level of order in some instances, and to the family level in others, through the use of taxonomic keys and microscopic examination. The results of these identifications were used to document the insect assemblages associated with the cadavers and to track changes in their composition throughout the stages of decomposition, while accounting for local environmental conditions. Table 3 outlines the examination site, the tools used for identification, the insect orders, and the taxonomic levels covered in the study [3, 8].

Table 3. Criteria for the identification and classification of insects.

Element	the details
Analysis Site	Forensic Entomology Laboratory & Entomology Laboratory – Faculty of Science – University of Tripoli.
Inspection tools	Microscopic examination and specialised taxonomic keys.
The insect orders studied	Diptera (including the families Calliphoridae, Muscidae, and Sarcophagidae) and Coleoptera.
Taxonomic level	At the family level for most Diptera specimens, and at the order or family level for Coleoptera specimens, to the extent that verification was possible.
The purpose of the classification	Documenting insect assemblages associated with cadavers and monitoring changes in their composition throughout the stages of decomposition.
Scope of the study	Insects associated with corpses, taking into account local environmental conditions.

Monitoring Insect Succession and Decomposition

Throughout the study period, the stages of decomposition and the associated insect succession were monitored twice daily. This involved observing the visible changes in each carcass and classifying the decomposition process according to the stages established in specialised literature: the Fresh Stage, Bloat Stage, Active Decay Stage, Advanced Decay Stage, and Dry Remains Stage. Furthermore, the insect assemblages present on each carcass and the shifts in their composition throughout the various decomposition stages were recorded. Observations for each carcass were documented individually, noting the specific experimental treatment applied and the location, while correlating the observed changes with the environmental conditions recorded during the study period [1, 34, 40, 41].

Quality Assurance

Quality assurance procedures included the documentation of insect specimens collected from each carcass. Storage containers were labelled with identifying information such as the carcass number, study site, and date of collection—to ensure that all specimens could be traced back to their source. These procedures also encompassed the proper handling and preservation of adult insects and larvae, the systematic recording of field observations regarding decomposition stages and insect succession, and the documentation of environmental conditions at both study sites. To enhance the accuracy of taxonomic identification, the specimens were re-examined following their initial identification by comparing their morphological characteristics againstspecialised taxonomic keys, in order to verify that the taxonomic assignment aligned with the diagnostic traits used in classification [3].

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Results

Environmental Conditions

The study was conducted between August 16 and September 5, 2024, in the Souq Al-Juma'a and Al-Hadba Al-Khadra districts of Tripoli, Libya. According to data from the NASA POWER database retrieved for each location based on its specific geographic coordinates, temperatures in the Souq Al-Juma'a area ranged from 18°C to 42°C, with relative humidity levels between 20% and 80%. For the Al-Hadba Al-Khadra area, the data indicated a temperature range of 18°C to 40°C and relative humidity levels between 30% and 80%.

Decomposition Dynamics Under Different Causes of Death

Field observations revealed variations in the duration of the "fresh stage" of decomposition among the subjects which were subjected to different conditions as well as differences in the timing of insect arrival and their activity patterns on the remains. In cases where the subjects had been exposed to an insecticide treatment, the fresh stage of decomposition lasted for four days at both study sites, the longest duration recorded for this stage across all experimental conditions. During this period, the tissues were observed to shift incolour toward blue and green. Ants appeared within the first two days, followed by the presence of eggs and larvae of flies belonging to the families Calliphoridae and Muscidae during the third and fourth days. For the slaughtered carcasses, the fresh stage of decomposition lasted one day. Blood was observed on the carcasses, coinciding with the early onset of insect activity, including the presence of larvae. In the case of the carcass that was slaughtered and subsequently burned using gasoline, the fresh stage also lasted one day. Insect activity was observed to be concentrated in areas rich in fat, particularly the thigh region.

Insect Succession Across Decomposition Stages

During the "bloat" stage, the accumulation of gases within the carcasses was observed, accompanied by the release of odours resulting from the decomposition process. Flies and larvae from the families Calliphoridae, Sarcophagidae, and Muscidae were recorded, along with the appearance of beetles from the families Dermestidae and Histeridae; minor variations in the observed insect assemblages were noted between the two study sites. During the active decay stage, the rupture of certain parts of the carcasses was observed, coinciding with the buildup of gases. In carcasses treated with an insecticide, this rupture occurred in the lower portion, whereas in the slaughtered carcasses, it was observed in the upper portion. Intense activity by larvae and beetles, specifically those from the families Dermestidae, Histeridae, and Cleridae, was also recorded. This stage lasted two days for both the insecticide-treated and the slaughtered carcasses, compared to one day for the carcass that had been slaughtered and burned. The advanced decay stage was characterised by a significant reduction in the remaining muscle mass, with persistent insect activity. This stage lasted five to six days for carcasses treated with an insecticide, six days for those that had been slaughtered, and three days for the burned carcass. In the dry remains stage, skeletal elements persisted, and puparia appeared; the timing at which the carcasses reached this stage varied depending on the circumstances of death.

Overall Decomposition Pattern

In general, field observations indicated that carcasses subjected to slaughtering or burning reached advanced stages of decomposition earlier than the others, followed by the slaughtered carcasses, and finally those treated with an insecticide treatment. This sequence suggests variations in the decomposition pattern and the duration of its stages across the different experimental conditions.

Insect Succession Patterns

(Table 4) illustrates the patterns of insect succession and the distribution of insect families recorded throughout the various stages of decomposition, categorised by the circumstances of death and the study sites.

Table 4. Insect succession across decomposition stages under different death causes and study sites.

Death Treatment	Study Site	Fresh Stage	Bloat Stage	Active Decay Stage	Advanced Decay Stage	Dry Remains Stage	Recorded Insect Families
Insecticide-treated	Souq Al-Jumaa	+	+	+	+	–	Calliphoridae Muscidae Sarcophagidae Dermestidae Histeridae Cleridae Arachnidae
Insecticide-treated	Al-Hadba Al-Khadra	+	+	+	+	–	Calliphoridae Muscidae Sarcophagidae Dermestidae Histeridae Cleridae Arachnidae
Slaughtered	Souq Al-Jumaa	+	+	+	+	–	Calliphoridae Muscidae Sarcophagidae Dermestidae Histeridae Cleridae Staphylinidae

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Slaughtered	Al-Hadba Al-Khadra	+	+	+	+	–	Calliphoridae Muscidae Sarcophagidae Dermestidae Histeridae Cleridae
Slaughtered and burned	Souq Al-Jumaa	+	+	+	+	–	Calliphoridae Muscidae Sarcophagidae Dermestidae Labiduridae

Note. (+) indicates that the insect family was recorded during the corresponding decomposition stage, whereas (–) indicates that no insects were recorded during that stage. The absence of recorded insects does not necessarily indicate the absence of the decomposition stage.

Duration of Decomposition Stages

(Table 5) illustrates the duration of the various decomposition stages (in days) according to The death treatments, noting the time differences recorded during the study period.

Table 5. Duration of Decomposition Stages (in Days) According to Death Treatments.

Death Treatment	Fresh Stage	Bloat Stage	Active Decay Stage	Advanced Decay Stage	Dry Remains Stage
Insecticide-treated	4	2	2	5-6	Present
Slaughtered	1	2	2	6	Present
Slaughtered and burned	1	1-2	1	3	Present

Notes. Present: The stage was observed during the study period, & Duration values are based on field observations during the study period.

Discussion

Impact of Manner of Death on Decomposition Rate

Observations recorded in this study indicate a variation in the duration of the fresh stage of decomposition among carcasses subjected to different manners of death. This stage lasted longer in carcasses treated with an insecticide treatment compared to those that were slaughtered, or those that were slaughtered and subsequently burned. This difference may be linked to the potential impact of the insecticide treatment on insect activity or colonisation, or perhaps to its effects on the decomposition processes themselves. This observation is generally consistent with the findings of a previous study, which noted a delay in the complete decomposition of rabbit carcasses treated with an organophosphate insecticide compared to control carcasses, despite there being no difference in the insect species thatcolonised the remains [42]. Conversely, insect activity began earlier in the slaughtered carcasses, including the one that was slaughtered and then burned, than in the carcasses treated with an insecticide treatment. This discrepancy may be related to the characteristics of the wounds and the extent to which they exposed internal tissues to insects, as well as changes induced in the tissues by exposure to heat. However, based on the current data, it is not possible to independently determine the specific contribution of each of these factors. The significance of examining the relationship between the manner of death and insect succession is supported by a study conducted on domestic pig carcasses subjected to various manners of death [43]. In general, the observations indicate variations in the pattern of decomposition and the onset of insect activity across different modes of death. However, the limited number of cadavers and the lack of replicates for each mode of death make it impossible to definitively conclude that the mode of death was the direct cause of these variations, or to isolate its effect from other environmental factors. Therefore, these results should be interpreted as exploratory observations rather than conclusive evidence that, for instance, slaughter or burning necessarily accelerates decomposition or insect colonisation.

Environmental Influence. Temperature and Humidity

Temperature and relative humidity are environmental factors linked to rates of decomposition and the development of insects associated with cadavers. Variations in local conditions can contribute to differences in decomposition patterns and insect succession between sites. In the present study, environmental data collected for the Souq al-Juma'a and al-Hadba al-Khadra sites revealed differences in temperature and relative humidity ranges, which coincided with observed variations in decomposition and insect activity between the two locations. Nevertheless, it is not possible to determine the extent to which these environmental differences contributed to the recorded results, independently of the influence of the mode of death or other factors specific to each cadaver. The significance of environmental conditions in explaining decomposition and insect succession aligns with findings from previous research; studies have described the stages of decomposition and the sequential succession of arthropods on cadavers [1], while also demonstrating that the environment surrounding a cadaver can influence the rate of decomposition and the timing of insectcolonisation [15]. In the context of estimating the post-mortem interval, a previous study highlighted the importance of accounting for the effect of temperature on the development of *Lucilia sericata* larvae when using isomegalen diagrams a finding that underscores the significance of thermal data in interpreting forensic entomological evidence [20]. However, the differences observed between the two study sites should be interpreted with caution. Temperature and relative humidity data were derived from the NASA POWER database based on the geographic coordinates of each site, rather than being measured directly within the microenvironment surrounding each carcass. Furthermore, the study was limited to five carcasses—one for each

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experimental treatment with no experimental replicates, which hinders the ability to isolate the influence of environmental factors from that of other experimental variables. Consequently, while the results offer exploratory insights into the variation in decomposition and insect activity between the two sites, they do not allow for this variation to be attributed solely to temperature or humidity.

Insect Succession Dynamics Across Decomposition Stages

Observations from the current study revealed a pattern of insect succession linked to various stages of decomposition. Flies from the families Calliphoridae and Sarcophagidae were associated with the early stages, whereas beetles from the families Dermestidae and Histeridae were recorded during later stages of decomposition. This pattern is generally consistent with established descriptions of the ecological succession of arthropods associated with carcasses, where the specific insect assemblages present vary according to the stage of decomposition and the conditions surrounding the carcass [1]. Variations were also observed in the timing of the appearance of certain insect families across different carcasses and stages of decomposition, highlighting the importance of considering the temporal dimension when interpreting patterns of insect succession. This observation is particularly significant in the context of criminal investigations; it has been shown that combining evidence derived from insect development and insect succession can, in some cases, improve the estimation of the postmortem interval (PMI) [44]. However, the present study was limited to documenting the insect families associated with various stages of decomposition; it did not include an independent estimation of the postmortem interval based on insect development data, nor did it integrate different estimation methods. Consequently, the value of these findings lies in their contribution to documenting local patterns of insect succession, which may provide a foundation for more detailed future research on the applications of forensic entomology in Libya.

Effect of Toxins on Decomposition and Insect Activity

Observations concerning cadavers exposed to insecticides are significant from the perspective of forensic toxicology. The presence of toxic substances in a deceased body can influence the insects associated with it, affecting their development, physiological characteristics, orcolonisation patterns. In this context, a previous study discussed the potential impacts of toxic substances on insect growth, physiology, and successional patterns, noting how these factors might compromise the accuracy of estimating the postmortem interval (PMI). The study further highlighted that the nature of these effects varies depending on the specific toxin, the insect species, and the dosage; this necessitates caution when interpreting entomological evidence in cases where exposure to toxic substances is suspected [30]. In the present study, the commercial preparation used contained cypermethrin and tetramethrin, in addition to piperonyl butoxide (PBO). While the concentrations of the two active ingredients in the commercial product were known, the concentration of PBO was not specified. Furthermore, the actual level of exposure to each substance within the bird carcasses was not independently measured. Consequently, it is not possible to determine the specific contribution of each component of the preparation to the observed differences in decomposition or insect activity, nor can these differences be attributed to any single active ingredient. These observations highlight the importance of considering potential exposure to toxic substances when interpreting entomological evidence in criminal investigations; overlooking this factor could lead to an inaccurate interpretation of insect development or the timing ofcolonisation on a corpse. However, the current study did not include an independent assessment of the pesticide's impact on insect development or an estimation of the post-mortem interval (PMI) based on entomological evidence. Consequently, the practical implications of these findings warrant further research, specifically, studies that directly evaluate how toxic substances affect insect development and thecolonisation of remains, while accounting for actual exposure levels.

Regional and Mediterranean Context

The current findings underscore the importance of studying decomposition-associated insects within their specific regional context. They provide preliminary data on the insect families recorded on domestic chicken carcasses at the study sites in Tripoli, as well as the association between the appearance of these insects and various stages of decomposition. Such information is significant given the known geographical variation in insect communities associated with animal remains, which necessitates the documentation of local patterns before generalising findings from studies conducted in other geographical regions. In this context, a study [32] provided data on insect and mite communities associated with animal remains in a Mediterranean region of northern Spain, illustrating the value of examining these communities to understand their potential role in criminal investigations. This study underscores the importance of developing regional databases for insects associated with decomposition, while accounting for geographical and environmental variations when interpreting findings from previous research. However, the current study is exploratory and limited in terms of the number of cadavers and study sites; therefore, it cannot definitively establish an independent effect of the regional context on the rate of decomposition or the estimation of the postmortem interval (PMI). Instead, it provides preliminary data on insects associated with various stages of decomposition at two locations in the city of Tripoli. These data may serve as a foundation for broader future studies that encompass diverse geographical locations and seasons, larger sample sizes (number of cadavers), and more precise taxonomic identification of insect species.

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Comparison with Previous Studies

A comparison of the current findings with previous research reveals both similarities and differences regarding the timing of the appearance of certain beetle families during the decomposition process. One study reported the presence of beetles from the family Histeridae as early as the fresh stage, with some species persisting through the bloat and subsequent stages, while beetles from the family Dermestidae were observed during the "decay" stage [45]. The latter observation aligns with the results of the current study, in which the family Dermestidae was recorded during the advanced stages of decomposition. In contrast, the timing of the appearance of the family Histeridae differed between the two studies: it was observed during the advanced stages in the current study, whereas it appeared as early as the fresh stage in the other study [45]. This discrepancy may be attributed to variations in the experimental and environmental conditions between the two studies. While the previous study examined pork remains buried at a depth of 30 cm, the current study was conducted using chicken carcasses exposed to the environment and covered with perforated plastic boxes that allowed insect access. Nevertheless, this comparison does not make it possible to isolate the specific factor responsible for the difference in the timing of the appearance of the family Histeridae. These observations underscore the importance of interpreting patterns of insect succession in the context of the type of animal remains, the conditions of their exposure, and the surrounding environment, rather than assuming that the timing of the appearance of insect families remains constant across different conditions.

Forensic Implications

The current findings highlight the practical significance of forensic entomology in death investigations by documenting the insect families associated with various stages of decomposition, and by observing variations in the timing of their appearance across different postmortem conditions and study sites. When integrated with data on insect development, environmental conditions, and other forensic findings, this information can assist in interpreting entomological evidence to estimate the postmortem interval (PMI). Estimating this interval relies on analysing the age of the insects that have colonised a carcass or examining the species composition of the associated insect fauna, all while ensuring that such evidence is collected and preserved correctly [7]. Furthermore, the study underscores the importance of developing local reference databases for insects of forensic significance in Libya. These databases should include recorded species, their timing of appearance, the decomposition stages with which they are associated, and data on their development under local thermal conditions. Such databases are particularly crucial given the geographical and environmental variations that can influence patterns of insect succession. In this context, one study examined insect succession on rabbit carcasses in the Al-Jawf region of Saudi Arabia [46], while another review discussed recent developments and challenges associated with the application of forensic entomology [47]. Nevertheless, the results of the current study represent preliminary, exploratory data on the insect succession associated with the decomposition of domestic chicken carcasses at the study sites in Tripoli; they do not constitute a comprehensive local database or an actual estimate of the post-mortem interval. Therefore, expanding future research to encompass diverse geographical locations, various seasons, and a larger number of carcasses while ensuring precise identification of insect species and analysis of their growth rates would provide a more robust scientific foundation for developing local applications of forensic entomology.

Conclusion

The results of this study demonstrate variations in decomposition patterns, the duration of decomposition stages, and insect succession among carcasses subjected to different circumstances of death. Field observations indicate that the carcass subjected to both slaughter and burning reached advanced stages of decomposition more rapidly than the others. This was followed by the slaughtered carcasses, while the carcasses treated with an insecticide treatment exhibited a prolonged "fresh" stage of decomposition. The study also identified several insect families associated with various stages of decomposition, including fly families such as Calliphoridae, Sarcophagidae, and Muscidae, as well as beetle families such as Dermestidae, Histeridae, and Cleridae. The timing of the appearance and the distribution of these insect groups varied across the decomposition stages, underscoring the importance of monitoring insect succession to document post-mortem changes in carcasses. Furthermore, differences in atmospheric and environmental conditions were recorded between the two study sites, alongside variations in observations regarding decomposition and insect activity. These findings highlight the necessity of documenting local environmental conditions when studying decomposition and insect succession in diverse settings. Overall, this study sheds light on the relationship between the circumstances of death, the decomposition process, and insect succession within the Libyan environment. It provides preliminary field observations that can serve as a foundation for future research in the field of forensic entomology. There is a clear need for broader studies encompassing larger numbers of cadavers, diverse geographical locations, and various seasons to develop regional databases on insect succession and stages of decomposition. Such research would support the local application of forensic entomology, contribute to the refinement of methods for estimating the postmortem interval (PMI) in Libya, and provide a scientific basis for forensic education, professional training, and collaboration between academic institutions and investigative authorities.

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Author Contributions

All authors contributed to the study's conception and design. All authors are equal in material preparation, data collection, and analysis. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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