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Comparative analysis of the insect succession pattern and Stages of decomposition of Rabbit carcasses surrounding Palm and Olive trees

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Forensic entomology utilizes insects encountered on deceased bodies to aid in criminal investigations, particularly in estimating the post-mortem interval (PMI). This study was designed to identify the forensic insects in Aljouf region, and to study the insect succession pattern associated with rabbit carcasses under different conditions in Al-jouf, Saudi Arabia. To achieve our goals, twenty rabbits were subjected to decomposition under two types of death (natural and induced), and two tree placements (shady and exposed). Results determined five distinct decomposition stages of the rabbit carcasses (fresh, bloating, active decay, advanced decay, and dry stages). Each decomposition stage attracted varying insect colonizers and each stage lasted to varied durations. Herein we identified 13 insect species (seven dipterans, five coleopterans, and one hymenopteran), using both morphological and molecular identification methodologies. The variation in decomposition stage duration, and the insect colonizer species was affected by the closer plant trees, and/ or correlated to the difference in climatic conditions, especially, the temperature. Our results suggested that some colonizers are of special importance in PMI determination. *Sarcophaga dux* is the most significant colonizer in earlier decomposition stages and *Dermestes laniarius* is the most significant colonized in later decomposition stages.

Keywords: Forensic entomology; insects; decomposition stages; plantation; post-mortem interval.

INTRODUCTION

Forensic entomology is the study of arthropods, particularly insects that are present with cadavers to help solve crimes. According to Byrd and Tomberlin (2019), it can also include interpreting entomological data in order to help solve a criminal inquiry. It is also decreed to answer many other questions at the scene of crime. The concept of forensic entomology depends on the information that the arthropods can supply to ascertain the date and cause of death (Kotzé et al. 2021). Forensic scientists prefer to use insects found on the carrion to solve mysterious crime (Bonacci, 2016). Therefore, the colonization of corpses after death can be a good indicator for the relative post-mortem interval "PMI" by the insect development (Matuszewski, 2021).

Post-mortem interval is established due to the process of corpse decomposition by the sequence of post-mortem changes in soft tissues (Campobasso et al. 2001). When insects are present, additional data can be obtained to examine how the carcass breaks down using morphological scoring to determine the post-mortem interval (PMI). Early on in the postmortem process, this method might enable a trustworthy PMI estimation. Necrophagous insects develop on dead bodies in later phases of their life cycles, which can lead to morphological changes that can help establish minimum postmortem intervals (minPMI) (Pittner et al. 2020).

Most of forensic entomological research has carried as inferential to build a conclusion about the population of the entomofauna, depending on the area and the samples that are often used in the experiments (Moreau, 2021). Furthermore, the examination of succession in various geographic regions and habitats could yield a new taxonomic database (Matuszewski, 2021). Numerous variables can influence both insect succession and the decomposition of carrion. These include environment, temperature, season, humidity, time of day, corpse vulnerability, kind, size, location, vertebrate scavengers, abundance, and insect fauna biology. These elements could affect how long insects spend on the carrion and how long they can access it (Anderson, 2000).

The most common identification methods for both immature and mature insect specimens are dichotomous and pictographic keys. Moreover, the availability of molecular identification techniques, such as DNA barcoding, for arthropod identification aids in the differentiation of morphologically and behaviorally similar species (Kotzé et al. 2021).

The arthropods attracted to a cadaver had been investigated in various locations worldwide to define the structure of species and insect succession patterns. However, there are no species data about insects of forensic significance in Al-Jouf, Saudi Arabia. This study will investigate the entomofauna in Sakaka, Al-jouf region, to provide construction key identification and database for the important forensic insects that could be found.

MATERIALS AND METHODS

Study Site

The trials were carried out on a private farm in Sakaka, Al-jouf region, Saudi Arabia; the study site was outdoors. Al-Jouf region is situated in Saudi Arabia's northern continental desert, where the winters are typically cold and the summers are usually dry and hot. Olive and palm trees were planted on the field, which had sandy soil that were yellow to brown in color (Fig. 1 A&B).

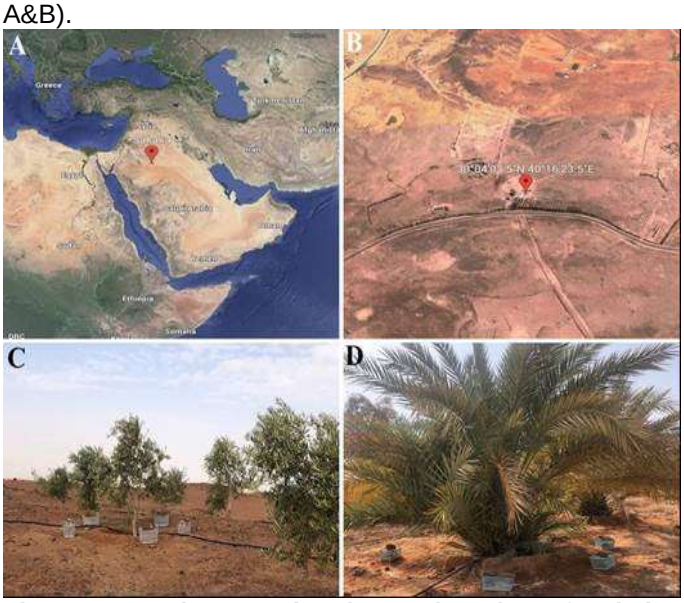

Figure 1: Study area showing A: location of Sakaka city; B: location of the farm in which experiments were done; C: olive tree surrounded by the boxes containing the rabbit corpses; and D: palm tree surrounded by the boxes containing the rabbit corpses.

The 32-day experimentation period spanned from November 20, 2021, to December 22, 2021. Using a weather application, the temperature and humidity were recorded throughout the experimentation period.

Experimental design

In this study, twenty rabbits with an average weight of 1.75 kg were supplied by a private rabbit farmer. Rabbits were sacrificed on the experimental site, and replicates were similarly oriented inside the experimental box. Rabbits were placed individually into a plastic box (33x 28x 26cm) with openings at all sides to allow insects to visit the carcass easily. Each box was provided with a moisten layer of soil and sawdust. Boxes were closed carefully to prevent carcass consumption by predators. These rabbits were divided into four group (2x 2x 5x). Group A was designated to the rabbits which were sacrificed with normal death and were placed 1-meter-distant around olive trees. Group B was designated to the rabbits which were sacrificed with a sharp-knife slaughter and were placed 1-meter-distant around olive trees. Group C was designated to the rabbits which were sacrificed with normal death and were placed 1-meter-distant around palm trees. Group D was designated to the rabbits which were sacrificed with a sharp-knife slaughter and were placed 1-meter-distant around palm trees (Fig. 1 C&D). To avoid odor interference, experimental groups were placed at least 20-to-30-meter distant from each other. Decomposition stage was observed, changes were recoded, and insect succession was reported daily for each rabbit carcass. Animal sacrificing procedure was approved by the ethical committee of Jouf University.

Sampling, Collecting and Observing

Sample collection from the cadavers was done once a day between 9.00 a.m. and 12.00 p.m. by using forceps. The collected larvae were preserved in 70% ethanol for further laboratory identification. Each sample was labeled with group, replicate and day of collection. Adult visitors and eggs were recorded at observance. Several larvae were collected from different body parts of the carcass. Adult flies and beetles were collected, manually, when possible and preserved in 70% ethanol. All collected samples were kept at 4 ˚C. The post-mortem changes and decomposing rates were observed from the first day of death.

Morphological Identification

Third larval instars were collected, dissected, and the spiracles looked at under a light microscope in order to identify the larvae by morphology. The provided identifying key served as the basis for identification (Szpila, 2012). To aid in the softening of the tissues, the larvae were cooked in a 15% potassium hydroxide solution for five minutes before dissection. The anterior and posterior spiracles, as well as the mouth parts (cephaloskeleton), were used to identify the larvae.

Molecular Identification

In order to extract DNA from insect tissue, the tissue must first be ground up in liquid nitrogen and then

reconstituted in a micro-centrifuge tube. After five minutes of incubation at 65°C, the sample is added to chloroform and centrifuged for two minutes at 10,000 rpm. Concentrated precipitation solution and sterile deionized water are combined to create a precipitation solution. After moving the top aqueous phase to a fresh tube, the DNA pellet dissolves in a solution of NaCl. After that, the DNA is dissolved in sterile deionized water and rinsed with cold ethanol. Following electrophoresis on an agarose gel, the concentration of DNA is determined either visually or by spectrophotometry. From 200 µl tissue, the recovered DNA typically has an average length of 2–10 µg. The DNA concentration and purity were checked and justified for further use. Specific primers (Table 1) are used in PCR reactions. The PCR procedures are carried out in a 25 µl total volume DNA thermal cycler (TC-3000). The automated thermal cycle (Model Techno 512) used for the DNA amplifications was set up to run 45 cycles of 1 minute at 94°C, 1 minute at 57°C, and 2 minutes at 72°C. The first cycle runs for 4 minutes at 94°C, and final extension step was run for 10 minutes at 72°C.

Table 1: A list of the primer names and nucleotide sequences that were employed in the dd-PCR process investigation

	Name	Sequence
1	COI Fwd	5´ TCG GGG ATA G 3`
2	COI Rev	5´ GAT GAC CGC C 3`

Data analysis

UVP-England program, an advanced software package from Gel Works, was used to create the similarity matrices. The SPSS Windows (version 20) application was used to analyze the associations between genotypes as shown by dendrograms. Plotting the phenogram among cultivars and calculating the pairwise difference matrix were done using the Dice computer software.

RESULTS

Insect succession

In order to track insect successions, the rate of carcass decomposition, and decomposition stages were observed. Additionally, the potential influences of carcass decomposition on insect succession patterns was carried out on twenty rabbit carcasses that were placed in various locations and scarified using various techniques. The insects that were gathered were identified; however, this study revealed that the insects belonged to three orders: Hymenoptera, Coleroptera, and Diptera. These orders were represented by nine families: Sarcopagidae, Calliphoridae, Muscidae, Drosophilidae, Tenebrionidae, Dermestidae, Coccinellidae, Histeridae, and Formicidae. Additionally, there are 13 species: *Wohlfahrtia nuba, Sarcophaga dux, Chrysomya albiceps, Lucilia cuprina, Drosophila melanogaster, Cataglyphis holgerseni, Coccinella undecimpunctata, Dermestes laniarius, Sprinus pseudocyaneus, Adesmia cancellata,* and *Pimelia granulicollis*

Decomposition stages

In this investigation, rabbit carcasses were seen at five different stages of decomposition: fresh, bloating, active decay, advanced decay, and dry stages (Fig. 2). To evaluate the post-mortem alterations of the corpses and insect succession pattern, the decomposition stage was observed for all experimental groups: A & B (Table 2), and C & D (Table 3).

Figure 2: Decomposition stages of rabbit corpses. A: fresh stage; B: bloating stage; C: active decay stage; D: advanced decay stage; and E: dry stage.

Table 2: **The time passed from death "postmortem interval" of the rabbits carcasses (group A, B) placed around the olive trees and insects succession.**

Stages of decay	Fresh stage	Bloating stage	Active decay stage	Advanced decay stage	Dry stage
Postmortem changes	soft and fresh look, No odor, cooling after death "Algor Mortis", stiffness "Rigor Mortis", larviparous	Abdomen bloating, Initialized odor, Oviposition, Discoloration of the abdomen "Livor Mortis"	Putrefaction, Strong odor, Loss of fluids "liquefaction", Tissue sloughing, Hair loss, Presence of larvae in different instars	Skin separation, Bone exposure, Less odor, Extreme hair loss, Pupation under the soil	Skeletonization, black outer skin, Decreased odor, Hatched adults, Dryness of the corpse
Time	1 2	3 4 5 6	7 8 9 10 11	12 13 14 15 16	17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Entomofauna families					
Sarcophagidae					
Wohlfahrtia nuba,	+	+ + + +	+ + + + +	+ + + + + +	+ + + + + +
Sarcophaga dux	+	+ + + +	+ + + + +	+ + + + + +	+ + + + + + + + + + + +
Calliphoridae					
Lucilia cuprina		+ + + +	+ + + + +	+ + + + + +	+ + + + + + + + + + +
Calliphora vicina		+ + + +	+ + + + +	+ + + + + +	+ + + + + + + + + + + +
Chrysomya albiceps		+ + + +	+ + + + +	+ + + + + +	+ + + + + + + + + + + + +
Muscidae					
Musca domestica		+ + + +	+ + + + +	+ + + + + +	+ + + + + + + + + + + +
Drosophilidae					
Drosophila melanogaster		+ + + +	+ + + + +	+ +	
Formicidae					
Cataglyphis holgerseni	+	+ + + +	+ + + + +	+ + + + + +	+ + + + + + + + + + + +
Dermestidae					
Dermestes laniarius		+ + +	+ + + + +		+ + + + + + + + + + +
Coccinellidae					
Coccinella undecimpunctata		+ + +	+ +		+ + + + + +
Histeridae					
Saprinus pseudocyaneus		+ + +	+ + + +	+ +	
Tenebrionidae					
Adesmia cancellata					+ + + + +
Pimelia granulicollis					+ + + + + +

Table 3: The time passed from death "postmortem interval" of the rabbits carcasses (group C, D) placed around the olive trees and insects' succession.

Stages of decay	Fresh stage	Bloating stage	Active decay stage	Advanced decay stage	Dry stage
Postmortem changes	soft and fresh look, No odor, cooling after death "Algor Mortis", stiffness "Rigor Mortis", No oviposition.	Abdomen bloating, Initialized odor, Oviposition, Discoloration of the abdomen "Livor Mortis", oviposition and Iraviposition	Putrefaction, Strong odor, Loss of fluids "liquefaction", Tissue sloughing, Hair loss, Presence of larvae in different instars	Skin separation, Bone exposure, Less odor, Extreme hair loss, Pupation under the soil	Skeletonization, black outer skin, Decreased odor, Hatched adults, Dryness of the corpse
Time	1 2 3	4 5 6 7 8	9 10 11 12 13 14	15 16 17 18 19	20 21 22 23 24 25 26 27 28 29 30 31 32
Entomofauna families					
Sarcophagidae *Wohlfahrtia nuba,* *Sarcophaga dux*		+ + + + + + + + + +	+ + + + + + + + + + + +	+ + + + + + + + + + + +	+ + + + + + + + +
Calliphoridae *Lucilia cuprina* *Calliphora vicina* *Chrysomya albiceps*		+ + + + + + + + + + + + + + +	+ + + + + + + + + + + + + + + + + +	+ + + + + + + + + + + + + + + + + +	+ +
Muscidae *Musca domestica*		+ + +	+ + + + + +		+ + + + + + +
Drosophilidae *Drosophila melanogaster*					
Formicidae *Cataglyphis holgerseni*		+ + +	+ + + + + +		+ + +
Dermestidae *Dermestes laniarius*		+	+ + + + + +		+ + + + + +
Coccinellidae *Coccinella undecimpunctata*					
Histeridae *Saprinus pseudocyaneus*			+ + + + + +		+ + + + + +
Tenebrionidae *Adesmia cancellata*					
Pimelia granulicollis					

This study was conducted on twenty rabbit carcasses divided into four groups (five rabbits/ group). To observe the factors that may affect insect succession patterns and decomposition stages of the carcass, rabbit carcasses were observed at the five stages. It was reported different duration of fresh and bloating stages in accordance with plantation in the site of the carcass. Carcasses which were placed around olive trees (group A and group B) spent two days at the fresh stage. Both actions will lead to the observed short fresh stage.

On the other hand, carcasses which were placed around palm trees (group C and group D) spent three days at the fresh stage. The extended fresh stage in our study may be due to the shadow effect of the palm trees which resulted in indirect sunlight exposure, and in turn, reduced temperature of the carcass leading to reduced decomposition rate.

Bloating stage which is characterized by great microbial activities leading to the accumulation of gases inside the carcass. Carcasses of the four groups (A, B, C, and D) lasted for 4 to 5 days in bloating stage.

Active decay stage was prolonged for approximately five days in carcasses placed around olive tree. However, the carcasses located around palm tree lasted for six days in active decay stage.

The advanced decay stage lasted for approximately

five days in carcasses of the four groups (A, B, C, and D).

Dry stage was shown at the seventeenth day for (A & B), while (C & D) at the twentieth day.

DISCUSSION

Investigators and entomologists utilize forensic insects to determine how long it has been since a person died. The entomofauna's succession varied depending on the climate and geographic areas. In addition, a carcass's state of decomposition is influenced by its surroundings, temperature, humidity, and insect activity. As a result, the corpse's time in each stage of decomposition may vary. A varied succession of insects may provide useful information for calculating the lowest and/or maximum post-mortem interval based on the insect's developmental stage. This study's primary goal was to ascertain the post-mortem interval by examining the entomofaunal succession of forensic insects and the various stages of rabbit carcass decomposition in Sakaka, Aljouf region.

This is the first study in Aljouf region that has been documented, as far as we know. Five decompositional stages were observed of rabbit carcasses in this study. Similar results were reported by Silahuddin et al. (2015). This work reported different duration of fresh and bloating stages in accordance with plantation in the site of carcass decomposition. Carcasses which were placed around olive trees (group A and group B) spent two days at the fresh stage. Similar results were indicated by El-Gawad et al. (2019) who studied the decomposition stages of rabbit carcasses, and by Ibrahim et al. (2013) who studied decomposition stages of guinea pig carcasses during spring season. The short fresh stage may be due to the close olive trees which contain many volatile oils that could affect carcass, attract insects or both. Short fresh stage may be also due to the exposure of carcasses to direct sunlight which raised the carcasses temperature and increased decomposition rate. Increasing decomposition rate by sunlight will lead to faster odor spread and increased insect colonization on the carcass. In addition, the attraction of insects by volatile oils will lead the insect colonizers to immediate visit, oviposition and/ or larviposition on the carcass. Both actions will lead to the observed short fresh stage.

On the other hand, carcasses which were placed around palm trees (Group C and Group D) spent three days at the fresh stage. Agreeable results were reported by Al-Dakhil and Alharbi (2020) who studied rabbit carcass placed around palm tree. In addition, Shaalan et al. (2017) reported that the rabbit carcass lasted for three days in winter. The extended fresh stage in our study may be due to the shadow effect of the palm trees which resulted in indirect sunlight exposure, and in turn, reduced temperature of the carcass leading to reduced decomposition rate.

Bloating stage of the four groups (A, B, C, and D) lasted for 4 to 5 days in bloating stage. Comparable results were reported by Aly et al. (2017) studied rabbit carcass decomposition outdoor in winter and found that duration of bloating stage was five days. In consent, Ibrahim et al. (2013) conducted his research in winter and spring seasons and reported the same duration of bloating stage. This may explain the effect of the light exposure, habitat, and temperature in fastening or retarding the decomposition changes.

Active decay stage was prolonged for approximately five days in carcasses placed around olive tree. These results were coincident with the results presented by Abouzied (2014) who investigated a rabbit carcass decomposition in different seasons and reported that the active decay stage continued for a period of five days in both summer and spring seasons.

However, we found that the carcasses located around palm tree lasted for six days in active decay stage. Similar results were presented by Mashaly et al. (2018), who observed that the active decay period was five days in autumn, and six days in spring.

The advanced decay stage lasted for approximately five days in carcasses of the four groups (A, B, C, and D). Similar duration was presented by Shaalan et al. (2017), who confirmed that the advanced decay in winter and autumn seasons was five days. Dry stage was shown at the seventeenth day for (A & B) carcasses while (C & D) carcasses at the twentieth day. Disagreeable results were presented by Mashaly et al. (2018) who documented that the rabbit carcass reached dry stage at the thirteenth day in the agricultural habitat. Combining our results to the previous results, it was concluded that different habitats, plantations, geographical locations, and seasonal climates play a crucial role in the decay process.

Our findings revealed that sarcophagid and calliphorid flies were the initial colonizers of rabbit carcasses, with calliphorids being more abundant. This aligns with previous research conducted in various regions (Smith, 1986; Malainey and Anderson, 2020; Anderson and Van Laerhoven, 1996; Pastula and Merritt, 2013).

Furthermore, *Chironomus albiceps*, a globally distributed species, was identified as a potential indicator of early-stage decomposition, corroborating observations from other studies (Zumpt, 1965; Laurence, 1981; Grassberger et al. 2003; Charabidze et al. 2014; Benecke, 2015; Moemenbellah-Fard et al. 2018).

Our findings suggest that flies exhibit a preference for oviposition on hairy body parts and body openings (mouth, nose, ears, and anus). This behavior might be driven by factors such as increased moisture content and reduced light intensity in these areas. Our observations align with previous research reporting similar oviposition preferences in flies (Norris, 1965).

CONCLUSIONS

This study reveals that three species of Calliphoridae; *Chrysomya albiceps, Luicilla cuprina*, and *Calliphora vincia*, and two species of Sarcophagidae; *Sarcophaga dux* and *Wholfortia nubi* can be used as indicators of PMI in Aljouf region, and may be of potential forensic significance. Since *Sarcophaga dux* was the most common and plentiful colonizer, it is of more significance. Even if their existence and colonization were influenced over time, other coleopteran taxa, such as those belonging to the Dermistidae, dominated in later phases of decay. It is also sparked that learning more and conducting more research in this area is recommended. The significance of forensic entomology in Sakaka, Aljouf, Saudi Arabia, is also highlighted by this study.

Supplementary materials

Not applicable.
All of the data is included in the article.

Author contributions

All authors were equally contributed in this work. All authors read and approved the final version.

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Institutional Review Board Statement

The study was approved by the Bioethical Committee of Jouf University.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable. All of the data is included in the article.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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