A preliminary investigation of the entomofauna composition of forensically important necrophagous insects in Al-Madinah Al-Munawwarah region, Kingdom of Saudi Arabia

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ABSTRACT
The key role in forensic entomology is the study of entomofauna composition through species identification, succession, colonization, and developmental rate. In this study, rabbit carcasses were used as a model to identify the forensic insect species in Al-Madinah Al-Munawwarah, Saudi Arabia. The appearance and succession of these insects can help in the estimation of the minimum postmortem interval (mPMI) and in many other applications in this region. During the decomposition process, the most important forensic insects observed were from the orders Diptera and Coleoptera. Seven species in five families of Diptera and four families of Coleoptera were identified. The most abundant species in the area that colonized the carcasses were Chrysomya albiceps Widemann (Diptera: Calliphoridae) and Dermestes maculatus De Geer, which can be used for mPMI estimations. This study was the first conducted in Al-Madinah Region to provide a qualitative assessment of forensically important necrophagous species in this region.

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1. Introduction
In forensic entomology, insects and related arthropods are often utilized in the system of justice [1]. The types of insects found on and associated with a corpse tend to be universal, but these insects differ at the species level. Insects locate a corpse immediately after death, sometimes within minutes, depending on the accessibility and environmental conditions [2]. The arrival of insects on a corpse in a specific order and pattern is called the entomofaunal succession [3]. Colonization occurs with the arrival of the first adults and oviposition or larviposition which can be used to estimate the time passed since death, called the minimum postmortem interval mPMI. The colonization process and arrival times are strongly influenced by the geographical region, habitats, environment, soil type, season, sun exposure and the concealment of a body [4–6].

The attraction of forensically important insects to a corpse varies from region to region and depends on many factors. Therefore, studies are needed to understand the behaviours of the primary colonizers of corpses. Many studies on forensic entomology have been concerned worldwide in past years, with some examining the succession pattern, colonization, and/or developmental rates of the forensic insects and, others identifying the species of these insects [6–9].

In the Kingdom of Saudi Arabia (KSA), studies in forensic entomology began in 2014. Only 11 studies have been conducted in 5 locations [10], which does not include the Al-Madinah Al-Munawwarah region. Most of these studies concern the molecular identification of forensic insects by DNA barcoding rather than their developmental rate or successional pattern [10]. Abouzied [11] described the necrophagous insects attracted to carcasses of the rabbits in the mountains of Al-Baha Province. Mashaly [12] studied the succession of carrion beetles during the decomposition process in three different habitats (agricultural, desert and urban) in Riyadh region and concluded that habitat type affected the decomposition process and beetle abundance. Al-Shareef and Al-Qurashi [13] studied the effect of temperature on the growth parameters of immature stages of Chrysomya albiceps Widemann (Diptera: Calliphoridae) in Jeddah Region. They demonstrated that with increasing temperatures, the duration of larval and pupal stages decreases, whereas with decreasing temperature, the body length of larvae of the same age increases. Shaalan et al. [14] examined the insect succession pattern at Al-Ahsaa Oasis in four different seasons and identified fourteen species from four orders and seven families. Mashaly [15] studied the entomofaunal succession patterns on partially burnt and

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unburnt rabbit carcasses and found significant difference between the carcass conditions.

Studies conducted on the forensically important insects in a given locality can help to establish a database that can aid crime scene investigators or entomologists in reaching their conclusions in medicolegal investigations [16]. Such localized studies not only provide clues about the successional pattern of the insects on a carcass in a particular region but also increase the knowledge of the species involved. The aim of this study was to follow the succession pattern, colonization and developmental rates of the forensically important insects during the decomposition process of rabbit carcasses in the Al-Madinah Al-Munawwarah region. These data will establish the first database of forensically important insects which can be used as forensic indicators to estimate minimal postmortem intervals in this region of Saudi Arabia.

2. Materials and methods

2.1. Study site

The study was conducted during January to February in the winter of 2019. It was carried out on a date palm tree farm in Al-Madinah Al-Munawwarah region of western KSA (24°36′12.6″N, 39°33′01.4″E, altitude = 568 m). Al-Madinah region has a desert climate in the winter which is rainy and cold. The ambient temperature and humidity were recorded daily using a USB Datalogger (BST-DL13). The soil surface of the farm is sandy with many grasses and mostly palm trees cover it. The daily soil temperature was recorded using a mercury thermometer.

2.2. Animal model

For this work, five rabbits with similar weights (1.250 kg ± 50 g) were used and they were treated in accordance with standard ethical procedures which approved by the Deanship of Scientific Research, Taibah University, KSA. The rabbits were killed in the field using a sharp knife. Each carcass was transferred into a cage with 30 × 30 × 30 cm dimensions which surrounded by wire mesh walls (1.5 × 1.5 cm) to prevent consumption by vertebrate scavengers while allowing insect entry (Figure 1). The distance between one cage and another was 50 m to prevent the individual fly from visiting the 5 boxes during the same day. The bottom of each cage was covered with a 5-cm-thick layer of soil from the study area. The cages were separated from each other by 50 m. All carcasses were put on their left side facing the same direction and were partially protected from the sun by date palm leaves. The decomposition stages of the carcasses were monitored and photographed day-to-day comparisons.

Figure 1. Photograph of a rabbit carcass inside a cage. The pan trap (T) is next to the cage and filled with insects.

2.3. Sampling procedure and preservation

A pan trap was placed beside each cage to collect the adult flies and they were replaced daily with new ones (Figure 1). The pan trap was a plastic tray filled with water, detergent, and salt. The insects from the traps were collected daily and frozen until identification. Additionally, larvae were collected daily from in, on, and underneath the carcasses using a small plastic spoon. To preserve the samples of larvae and prevent any possible changes, the larvae were killed immediately in boiling water for approximately 20 s before being stored in 80% ethanol [17]. Pupae were collected from the soil underneath the carcass using forceps and then transferred into separate cages to monitor their emergence times. All samples were labelled with the day of collection and the replicate number. During the first month of the study, insects were observed and collected once daily between 10:00 am until 3:00 pm. After a month, when the carcasses had reached an advanced stage of decomposition, Coleoptera samples were collected twice weekly, because the life cycle of beetles is long compared with that of Diptera.

The identifications were based on external morphological characteristics. A dissecting microscope (Binocular Stereo Microscope, AmScope, California, USA) and dichotomous keys were used to identify the adult insects [18–22]. For the larvae, the anterior and the posterior ends were dissected to observe the mouthparts and the posterior spiracles, respectively. They were used to determine the larval instar and for identification, using the dichotomous keys [23].

3. Results

3.1. Decomposition stages

During carcass decomposition, the daily average ambient temperature was 19.1 ± 4.6°C, and the average relative humidity was 66.6%. The mean soil temperature
in the field was 17.48 ± 2.7°C. The mean rectal temperature of the carcasses during the fresh stage was 17.75 ± 2°C, whereas the average temperature at the interface between the carcass and the soil during the fresh and bloat stages was 17.27 ± 2.7°C.

The pattern and duration of decomposition were the same for all replicates. The entire decomposition process was divided into four stages: fresh, bloat, decay and dry carcass (Figure 2). All carcasses reached the dry stage in 11 days. The fresh stage (Figure 2A) began immediately after death and continued for approximately four days; no changes were observed in their odours or bodies. In the bloat stage (Figure 2B), because of bacterial fermentation, the gases released inside the bodies, the carcasses became bloated appearance. During this stage, many insects were present. The carcasses remained in this stage for approximately three days. The decay stage was easily observed because of the disintegration of the body skin (Figure 2C). The larvae of Diptera were also easily observed in this stage, and the mean temperature larval mass was higher (22.6 ± 5.3°C) than the ambient temperature (19.1°C). The duration of the decay stage was approximately four days. Then, the carcasses progressed to the dry carcass stage and were reduced to hair, dry skin, and bones (Figure 2D). In this stage, pupae were observed under the carcasses.

### 3.2. Succession of necrophagous insects

A total of 11 families in three orders (Diptera, Coleoptera, and Hymenoptera) were identified (Table 1). The succession pattern of the carrion insects during the different stages of decomposition is presented in Table 2. *Pheidole megacephala* Fabricius (Hymenoptera: Formicidae) were observed on the corpses from the beginning to the end of the decomposition process. *Chrysomya albiceps* (Diptera: Calliphoridae), *Sarcophaga afera* Wiedemann (Diptera: Sarcophagidae) and *Fannia leucostica* Meigen (Diptera: Fanniidae) were the first dipterans to arrive, although they arrived on the last day of the fresh stage. On the second day of the bloat stage, *Chrysomya marginalis* Fabricius (Diptera: Calliphoridae) and *Musca domestica* L. (Diptera: Muscidae) arrived. *Stratiomyidae* sp. and *Liosarcophaga* sp. (Diptera: Sarcophagidae) arrived on the last day of the bloat stage in synchrony with the arrival of the first species of Coleoptera, *Euspiolus* (Hesperosaprinus) modestus Erichson (Histeridae). Another coleopteran, *Derastes maculatus* De Geer, was observed on the first day of the decay stage, which was followed by the coleopterans *Scarabaeidae* sp. and *Necrobia rufipes* De Geer (Cleridae), respectively. The yellow jacket, *Vespa crabro* L. (Hymenoptera: Vespidae), was collected when dipteran larvae were present from the end of bloat stage.
Table 1. Insects taxa associated with rabbit corpses.

| Order    | Family               | Species                                      |
|----------|----------------------|----------------------------------------------|
| Diptera  | Calliphoridae        | Chrysomya albiceps Widemann                  |
|          |                      | Chrysomya marginalis Fabricius              |
|          | Sarcoptagidae        | Sarcoptaga Africa Widemann                  |
|          | Muscidae             | Musca domestica L.                           |
|          | Fanniidae            | Fannia leucostica Meigen                    |
|          | Stratiomyidae        | Unidentified                                 |
| Coleoptera| Dermestidae          | Dermentes maculatus De Geer                 |
|          | Histeridae           | Euplotus modestus Erichson                  |
|          | Scarabaeidae         | Unidentified                                 |
|          | Cleridae             | Necrobia rufipes De Geer                    |
|          | Formicidae           | Pheidole megacephala Fabricius              |

3.3. Colonization and developmental rates

Chrysomya albiceps was the most abundant species of carrion insect and colonized all five carcasses at the end of the fresh stage. The egg batches of this species were observed on the eyes, teeth, and ears and around the anus of the corpses. After hatching, the first instar larvae could not be observed; because they were within the wounds and natural body openings. The first documentation of second instar larvae was on the seventh day of the decomposition process during the bloat stage. On the last day of the bloat stage, third instar larvae were observed which were followed daily until reaching the post feeding larval stage. The pupal stage was detected under the carcasses during the decay stage. After eight days of pupation, adult C. albiceps began to emerge.

Dermestidae sp. and Histeridae sp. were the most abundant coleopterans, arriving at the end of the bloat stage and remaining to the end of the decomposition process. However, only Dermentes maculatus colonized the completely dried corpses, with their larvae recorded on day 33 of decomposition. Pheidole megacephala were observed regularly on all carcasses throughout the experiment, although neither eggs nor larvae of this family were observed at any stage of the decomposition process.

4. Discussion

Forensic entomology has been used as a tool to estimate the minimum postmortem interval (mPMI) [24]. The decomposition stages of the corpse in relation to the entomofauna found on or around it can help in estimating the period of time passed since death through finding the period of insect activities on the corpse. The decomposition of a corpse is a continuous process that begins with the fresh stage immediately after death and ends when the body is reduced to a skeleton. The number of decomposition stages varies from one to nine, depending on the author and the geographic region [25].

In this study, four decomposition stages were identified on the basis of physical changes in the carcasses: fresh, bloat, decay and dry carcass. The fresh
stage, continued for approximately four days, whereas in another winter study conducted by Shaalan et al. [14], the fresh stage lasted for 3 days. The delay in decomposition in this study was likely the result of reduced temperatures which can lead to decreases in insect activities, causing the colonization of carcasses to be inhibited [25]. *C. albiceps*, *S. afric*a and *F. leucostica* were observed on the bodies at the end of the fresh stage, as shown in Table 2. The bloat stage was characterized by bacterial activity to breakdown the body. The balloon-like appearance of the carcasses was due to the accumulation of gasses resulting from the metabolism of nutrients by anaerobic bacteria, which produces a foul odour that attracts additional blowflies. At the end of this stage, the larval activity was notable, as also observed by Shaalan et al. [14]. However, this stage was shorter (3 days) than the 18 days reported by Shaalan et al. [14]. The decay stage was the third stage of the decomposition process, which was recognized by the breakup and sloughing off of the carrion skin. The gases began to escape from the bodies, and the inflation ceased gradually. At the end of this stage, the corpses were reduced to skin, cartilage, and bone with some flesh. The decay stage continued for approximately four days, although this stage was lasted in autumn for 9 days in Abouzied [11] study and persisted in winter for 18 days in Shaalan et al. [14] study. The variation in duration of some decomposition stages between this study and the studies of Abouzied [11] and Shaalan et al. [14] may be explained by the difference in the size and weight of the carcasses; approximately 1.5 and 2 kg in their works, respectively vs. 1.25 kg in this study. Simmons et al. [26] reported that the rate of decomposition can affected by the mass of carcass where decomposing more rapidly of smaller carcasses. Additionally, typical environmental conditions, such as the geographical location, ambient temperatures and altitude, which are different between mountains of Al-Baha, Al-Ahsaa Oasis and Al-Madinah Al-Munawwarah region, could also explain the differences between studies. The dry carcass stage was the longest in the decomposition process, and the abundant coleopteran families were Dermestidae, Histeridae and Scarabaeidae, which occurred when only hair, skin, and bones of the carrion remained as food sources.

The necrophagous species, particularly those of the dipteran families, are the most important in the estimation of the mPMI because of their association with a body immediately after death and because they are usually the most dominant species on a corpse [16]. Many factors may influence the insect succession and taxonomic diversity reported between present and previous studies such as, sampling frequency and number of animal models [27].

In the present work, the pattern of colonization observed for the species of Calliphoridae was consistent with that in other studies in different regions. *C. albiceps* was initially attracted, colonized and the most abundant species on the rabbit carcasses at the bloat stage, as also reported in other previous authors [11,14,28]. The larvae of *C. albiceps* were identified from the end of the bloat stage and were most active and recognizable during the decay stage because of the disintegration of the body wall, coinciding with the release of gases (deflation) and associated with a strong odour. Most pupae of this species were observed at the end of the decay stage in the soil under the carcasses. This blowfly species is also recorded in markets, garbage and slaughterhouses in Saudi Arabia [29]. For public health and veterinary concern, this blowfly is important because of its ability to cause myiasis and its involvement in the first wave of cadaver entomofauna succession, which can be used to determine the mPMI [13,30].

In the present work, adults of Sarcophagidae were associated with the carcasses for a longer period than the other dipteran families and was observed from the late fresh stage to the dry carcass stage, whereas they observed on the carcasses from bloat stage in the mountains of southwestern KSA [11]. However, flesh flies in this study did not colonize the carcasses which may be due to generally the lower temperature in the winter season. This result was also observed previously where the *Sarcophaga* spp. did not colonize the rabbit carcasses in autumn and winter seasons in Al-Ahsaa Oasis, KSA, while the immature stages were recorded only in the spring season [14].

Additionally, adults of *M. domestica*, *C. marginalis* and Stratiomyidae sp. were first observed during the bloat stage but did not colonize the carcasses; only their adult stages were collected, and no immature stages were found. Similarly, Abouzied [11] and Shaalan et al. [14] reported that no maggots of *M. domestica* were found on rabbit carcasses in the southwestern and eastern regions of KSA, respectively.

Adult *D. maculatus* were recorded in the present study from the early of the decay stage then they colonized the dried corpses. Similar findings were observed in the mountains of southwestern KSA where adults *D. maculatus* were common in the autumn but from the bloat stage, and their larvae were most active during the dry carcass stage [11]. However, Al-shareef and Zaki [31] recorded adults and larvae of Dermestidae, *Dermetes frischii*, in more later stages; between advanced decay and skeletal stage of an exposed human corpse in Jeddah region, KSA. In compare to our results, Shaalan et al. [14] recorded adults of *D. maculatus* in the winter and autumn, but they did not colonize the rabbit carcasses in Al-Ahsaa Oasis, KSA. Dermestidae beetles were known to feed on dry skin and hairs and other dry dead animal matter containing animal proteins [32].

Histeridae beetles, *Eupilus (Hesperosaprinus) modestus*, were the first coleopteran occurred on the studied carcasses which persisted from the bloat stage to some part of the dry stage as that was observed in the autumn by the study of Abouzied [11], and they also recorded in
the winter by Shaalan et al. [14]. Byrd and Castner [33] have indicated that Histeridae genera Saprinus, Margarinotus and Hister are predators of dipteran larvae and eggs associated with carrion.

In this study, adults of Necrobia rufipes (Cleridae) were observed only between the end of decay stage and the beginning of dry stage. This species of Cleridae was also collected from the human corpse in the later stages of decomposition by Al-shareef and Zaki [31]. Similar result was reported by Abouzied [11] in autumn experiment in the southwestern KSA for N. ruficollis which was collected from the active decay to the dry carcass stages of examined rabbit carcasses. Most of Cleridae are predators of larvae of flies, but the genus Necrobia has necrophagous habit, with a preference for corpses in dry stages of decomposition [34].

Adults of Scarabaeidae beetles were observed in the present study from the later decay to the dry stage. Scarabaeidae beetles were recorded previously from dog carcasses in South Africa [35] and from pig carcasses in Brazil [36]. In contrast to our results, these beetles did not record by the previous studies of Abouzied [11]; Shaalan et al. [14]; Al-shareef and Zaki [31] in KSA.

The ants P. megacephala were attracted to the carcasses throughout the duration of the study. This species apparently are predators of eggs and larvae of the flies [14]. In general, the highest number of forensic insect species occurred in the bloat stage, compared that recorded in the fresh and decay stages, whereas beetles were numerous in the decay stage, with a reduction in the number of flies. However, in the dry carcass stage, few insects were observed, consistent with a study conducted by Mashaly [15].

5. Conclusion

In this study, to determine the entomofauna associated with carcasses during the winter season in Al-Madinah Al-Munawwarah region, KSA, the basic patterns of succession, colonization and developmental rates of forensically important insect were examined using rabbit carcasses as a model. Because of the absence of local research in forensic entomology, the results of this study will be important in creating the baseline data needed to help in the medicolegal investigations of the region. However, Saudi Arabia has diverse climatic and geographic regions, and, therefore, further studies are important to characterize the forensic insect species and to understand aspects of their behaviour in each region.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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