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RESEARCH PAPER

# Diversity, succession pattern and colonization of forensic entomofauna on indoor rat carrions concerning the manner of death

**Mayada R. Farag[1] · Reham G. A. Anter[2] · Walaa M. Elhady[1] · Samah R. Khalil[1] · Shimaa M. Abou-Zeid[3] · Eman A. A. Hassanen[2]**

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**Abstract**
A carrion-related arthropod database is important for each locality especially temperate ecosystems. The present study aims to document baseline data about the decomposition process of indoor rat carrions and the diversity and succession pattern of the forensically important arthropods fauna that colonize these carrions. Twelve Sprague-Dawley rats were divided into four experimental rat models, killed by cervical dislocation, electric shock, drowning or slaughtering. The orders Diptera and Coleoptera were the main necrophagous arthropods that supported the decomposition process. Calliphoridae was the first insect family that colonized the carrions and lasted till the beginning of the dry decay stage. The succession pattern and the abundance of associated arthropods did not differ between control and drowning group. In the control and drowning groups, the insects of the order Diptera predominated the overall insect fauna (57.14%) followed by Coleoptera (42.85%), while their proportions were 50–50% in the electrocuted group. The slaughtered group showed 50, 42.85, and 7.14% for Diptera, Coleopteran, and Hymenoptera, respectively. This study submitted additional information in the context of forensic entomology, and provides a reference for the succession pattern and its influence on the decomposition process in the summer season in Egypt.

**Keywords** Forensic entomology · Body decomposition · Faunal succession · Manner of death · Diptera · Coleoptera

## 1 Introduction

Forensic entomology is an emerging field of forensic science in which the ecological and biological aspects of the colonizing insects and arthropods are investigated (Zar and Huang 2018). It gives scientific information about the invasion and pattern of succession of the insects with forensic importance through morphological identification and determining their stage of development found on the carcass and this is very useful in the estimation of postmortem interval (PMI) (Al Musawy et al. 2016). Insects recovered from the dead bodies are used in criminal and legal investigations as in case of suspicious and violent deaths since they do provide vital information on the cause of death (how, where, and when the death occurred?) (Mashaly and Al-Mekhlafi 2016).

After death, the carrions undergo various natural changes driven by the action of microorganisms (fungi and bacteria) and arthropods for which the carrions represent a changing and a temporary source of feeding (Putman 1983). In the absence of vertebrate scavengers, arthropods arrive and colonize the carrions of human or animals in predictable and definite pattern, and play a major role in combination with anaerobic microorganisms in carrion decomposition (Smith et al. 1973; Valdes-Perezgasga et al. 2010; Ortega-Hernández 2016).

The types, seasonal availability and succession pattern of carrion associated insects vary depending mainly on the geographic region including the habitats, type of soil, and vegetation in addition to the meteorological conditions of a particular area (Abd El-Bar et al. 2016). Moreover, the distributions of insects on the dead bodies are indicative of

✉ Mayada R. Farag
dr.mayadarf@gmail.com

[1] Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

[2] Parasitology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

[3] Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32897, Egypt

their decomposition stages, and time of death (Abajue et al. 2013). Therefore, it is important to know and understand the composition of the fauna associated with local carrions, the seasonal patterns of its succession and the time during which individual life stages of forensically important species are found on the carrion. To our knowledge, there are few studies about arthropods associated with carrions in Egypt, particularly in relation to the killing method. So far no investigations on carrion decomposition and arthropod succession have been carried out in Zagazig city.

The decomposition of the dead animal bodies is a natural process and it is necessary to return the organic matter of the dead bodies to the ecosystem (Ozdemir and Sert 2009). Decomposition characterized by a microsuccessive way in which various stages could be possibly distinguished by the corresponding changes of carrions and the proper associated necrophagous fauna (Kočárek 2003). The categorization of decomposition stages varied due to the differences in types of carrions and the period of decomposition. Bornemissza (1957) recorded five decomposition stages in guinea pig carrions during 450-day observation. Payne (1965) also reported five decomposition stages in piglets. Decomposition stages were found to be four in the study of Reed (1958) and only three stages as reported by Fuller (1934). On the other hand, Cornabay (1974) could not distinguish any stage of decomposition on the carrions of lizards and toads by visual observation. Therefore, the present study was designed for obtaining a preliminary data about the decay process and for documenting the forensically important insect species associated with indoor decomposed rat carrion killed by different methods (electrocution, drowning, and slaughtering) in the summer season and for establishing a relationship between the succession pattern of insects and the killing method.

## 2 Materials and methods

### 2.1 Study site and conditions

This study was conducted in the Department of Forensic Medicine and Toxicology and Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University. The faculty buildings are located in El-Zeraah Square, Zagazig City, capital of Al-Sharqia Governorate, Egypt, with Latitude: 30° 35′ 15″ N and Longitude: 31° 30′ 07″ E. The study site is a room with an area of 24 $m^2$ (8 m length × 3 m width) in the Department of Parasitology in the third floor (8 m from the ground). The room was subdivided into four equal compartments; each compartment has a window and a door and they both remained closed, except only a space of about 2 cm between the bottom of the door and the floor of the room, which allows entrance of the insects. This site was suitable to permit the daily observation of the corpses and to avoid the disturbances, which might be induced by scavengers or animals and was provided by a label with directions to restrict the human interferences. We choose to perform the study in a room to see whether different causes of death influence the necrophagous fauna. The experimental procedures were carried out during the summer season (July–September, 2019). The climate was dry with moderately constant temperatures (ranged from 22 to 36 °C with an average of 29 °C and the relative humidity (RH) ranged from 49 to 57% during the experimental period.

### 2.2 Experimental animals and procedure

A total of 12 healthy male Sprague-Dawley rats with an average weight (200 ± 50 g) were allocated for the present study. The rats were divided into 4 equal groups (3 rats each). Light anesthesia with diethyl-ether was applied to the rats before killing. Rats were killed by cervical dislocation (control group), electric shock, drowning, or slaughtering. The cervical dislocation was done by pulling the tail of rats after squeezing the neck with a hard subject (rats died immediately). In the electrocuted group, electric shock using 220 V was applied to left anterior extremities and tails (the rats died in 3–4 min). In the drowning group, rats were put into a container filled with water and pushed down to prevent them from coming up (rats died in 2–3 min). In the last group, rats were killed by slaughtering (slitting of the throat with a sterile blade) and rats died within 1–2 min.

Immediately after death (within 10 min), rats were transferred to the study room in labeled plastic cages (50 cm length, 30 width, and 30 height) covered with wire mesh (1 × 1 cm). Cages were placed approx. 1 m apart from each other to avoid the clash of the fauna. The day of killing and placement of corpses was designated as day 0. During the first 3 weeks, the carcasses were monitored daily at mid-day and then twice a week until the remains of rats completely dried and no live or active insects were found in any of the cages. The stages of decomposition were recorded. The detected insects and the decomposition of the remains were photographed using Canon digital camera. The monitoring of the carcasses was stopped on days 40, 39, 39, and 43 for control, electrocuted, slaughtered and drowning group, respectively. All the experimental procedures and methodology were approved by the Institutional Animal Care and Use Committee of Zagazig University (ZU-IAURC).

### 2.3 Collection of samples and identification

All over the monitoring period, the activities of insects were observed and some mature and immature samples were collected from the corpses (in, on, around, and beneath). The opportunistic sampling of specimens (non-random) was

done using sweep nets, forceps, and spoons (for the masses of larvae). The total captured specimens were approximately 25% of those found on each corpse.

Adult live insects were killed by placing in a jar containing a pad wet with chloroform. A fine needle was used to separate parts of the flies (wing and head), then the flies parts were cleared in lactophenol (10 g phenol + 10.6 ml glycerine + 8.2 ml lactic acid + 10 ml distilled water were mixed well until became homogenous) and mounted in Polyvol (6.3 g polyvinyl alcohol + 18 ml absolute ethyl alcohol + 35 ml distilled water + 45 ml lactophenol were mixed well and heated in a water path till became sticky clear solution) on clean slides, and dried in the hot air oven at 50 °C for at least 24 h.

Maggots were divided into two groups. The first group was killed immediately by immersing for 30 s in boiling water, then was preserved in 70% ethyl alcohol and a drop of glycerin was added if the larvae were preserved for a long time or 10% formalin for morphological examination (Soulsby 1982; Taylor et al. 2007), the second group was reserved in the laboratory for rearing.

### 2.4 Rearing of larvae

The collected larvae were reared for the complete life cycle and obtaining the adult flies following the technique described by John and Petri (2006). The collected larvae were put in a plastic container (12.5 cm in diameter and 10 cm depth) containing a piece of fresh meat placed on a layer of sand, then covered with a double layer of gauze and capped with a perforated plastic lid; the container was left in a fly-free area at room temperature for maturation and pupation. The newly emerged flies were collected for identification. The identification of the collected and reared flies, larvae and pupa was done according to the keys of Bowman (2009). Identification of Hymenoptera was done according to Mohammad et al. (2001) and Mullen and Durden (2009).

## 3 Results

### 3.1 The pattern of carcass decomposition

All groups showed the same decomposition pattern, which consisted of 5 remarkable stages including fresh, bloating, active decay, dry decay, and finally skeletal stage. The onset and duration of each stage in addition to the postmortem changes on carcasses from different experimental groups are summarized in Table 1. The post mortem picture of each stage of decomposition in all the experimental groups is described in Figs. 1, 2, 3, 4.

### 3.2 Insect diversity

The associated insect fauna recovered from carcasses of different groups were represented in 3 orders, 9 families, and 12 species (Table 2). The orders Diptera and Coleoptera were the main necrophagous arthropods that supported the carcasses decomposition. The first family of insects that colonized the carcasses from different groups was Calliphoridae and it lasted until the beginning of the dry decay stage.

### 3.3 The abundance of forensic insects

The proportions of the main orders that appeared on carcasses within each group and among different groups are represented in Fig. 5. In carcasses of control rats, the insects of the order Diptera predominated the overall insect fauna (57.14%) followed by Coleoptera (42.85%). The same proportion was found in the drowning group, while the proportions of Diptera and Coleoptera were 50–50% in carcasses of the electrocuted group. Slaughtered carcasses showed 50, 42.85, and 7.14% for Diptera, Coleoptera, and Hymenoptera, respectively.

## 4 The succession of insects on rat carrion

### 4.1 Fresh stage

The fresh stage takes one day in all groups and all carcasses showed discoloration of the skin. Dipterans, particularly adult flies of Calliphoridae (*Chrysomya bezziana* and *Chrysomya albiceps* adult) were the main insect fauna and they were found on the dead rats from all groups.

### 4.2 Bloating stage

The difference in the cause of death did not affect the onset of bloating which appear in the second day of killing in almost all carcasses in different groups and continued for one day before entering in the active decay (Table1). Necrophagous arthropods in this stage were mainly of Dipteran families (Calliphoridae, Sacrophagidae, and Fannidae) which were observed to be the first colonizers of almost all carcasses in different groups. Adult insects of *Chrysomya bezziana*, *Chrysomya albiceps* were the dominant species on all carcasses (Table 2) and constituted the primary factors in the process of decomposition. *Chrysomya bezziana*, *Chrysomya albiceps*, *Wohlfahrtia magnifica*, *Sarcophaga haemorrhoidalis* and *Fannia canicularis* adult were found on the carcasses of all groups except electrocuted group where *Sarcophaga haemorrhoidalis* adult was absent while *Saprinus chalcites* adult appears only in this group. *Musca domestica* adults appear on a slaughtered group only.

**Table 1** Onset, duration and postmortem changes in different stages of decomposition on rat carrions concerning the manner of death

| | Control | Electricity | Drowning | Slaughtering |
|---|---|---|---|---|
| Onset and duration (days) | | | | |
| Fresh | 0–1 | 0–1 | 0–1 | 0–1 |
| Bloating | 2-Jan | 2-Jan | 2-Jan | 2-Jan |
| Active decay | 8-Mar | 8-Mar | 9-Mar | 7-Mar |
| Advanced (dry) decay | 16-Sep | 19-Sep | 29-Sep | 26-Aug |
| Skeletal stage | 16 | 20 | 29 | 26 |
| Post mortem changes | | | | |
| Fresh stage | Carcass showed normal intact body with no change in the physical state. Just skin discoloration was observed<br>No morphological changes, putrid odors or swelling were seen | No swelling<br>No putrid odors<br>Discoloration of skin | No swelling<br>No putrid odors<br>Skin discoloration was more obvious than control | Discoloration of skin<br>No strong odors was noticed<br>No swelling |
| Bloating stage | Swelling of carcasses. Odor of decay became noticeable<br>Fluid and exudes of decomposition were observed beneath the carrion. This stage ended when carcass deflated | The body swelling and inflation were more obvious than control<br>Odor of putrefaction was stronger than control | The body swelling and inflation were more obvious than control<br>Odor of putrefaction was stronger than control | Gradual inflation then deflation<br>Strong odor of putrefaction<br>Fluid and exudes of decomposition oozed under the carrions |
| Active decay stage | Full decomposition, wandering of skin, Extensive fluid leakage, strong odors<br>Hair loss especially in areas with great maggot's activity | Odor of putrefaction stronger than control,<br>Skin wandering<br>Extensive fluid leakage | Odor of putrefaction stronger than control<br>Skin wandering<br>Extensive fluid leakage | This followed deflation<br>Strong odor of putrefactionSkin disintegration<br>Extensive fluid leakage were noticed |
| Advanced (dry) decay stage | Most of the fleshy tissue disappeared, odor were less<br>Dry skin started to separate from the body | Less flesh and fluids, less odor and dry tissues only presents (skin and bones) | Reduction in flesh and fluid. The odor started to fade. The carrions consisted of dry skin and bones only | Carrions were completely dehydrated with loss of weight<br>Less odor. dry skin and bone |
| Skeletal stage | Only dry skin, bones and cartilages were observed in addition to some scattered masses of remaining hair | Dry skin, bones, cartilages and hair remains | Dry skin, bones, cartilages and hair remains | Dry skin, bones, cartilages and hair remains |

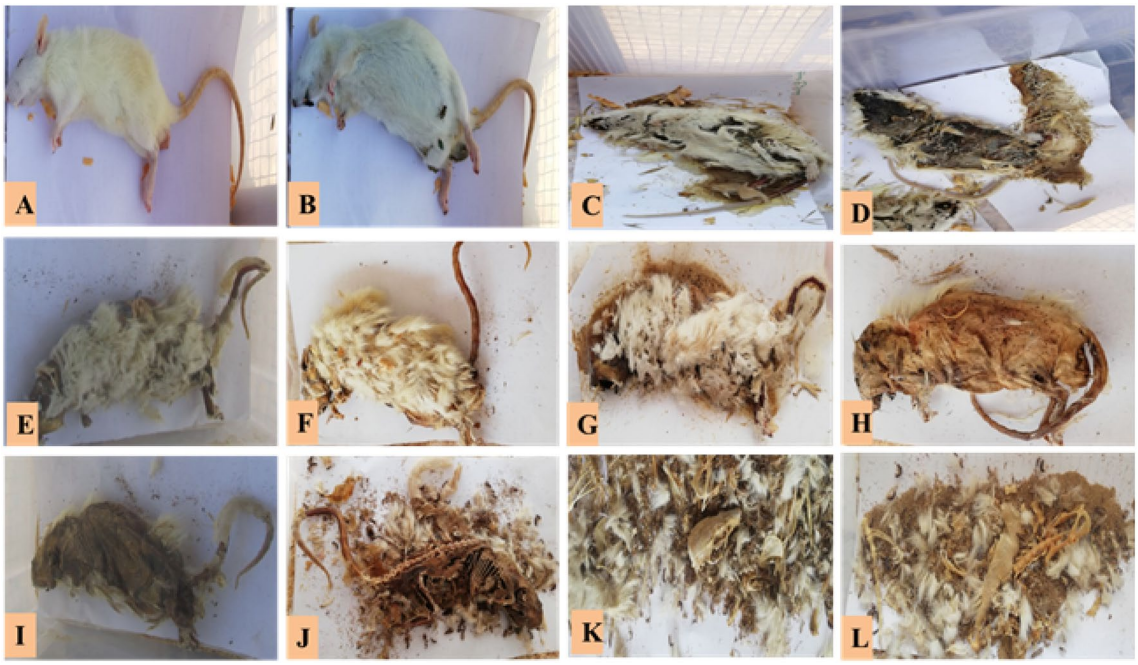


**Fig. 1** Decomposition stages of rat carrion from control group. **A** Fresh stage. **B** Bloating stage. **C–F** Active decay stage. **G–I** Dry decay stage. **J–L** Skeletal stage

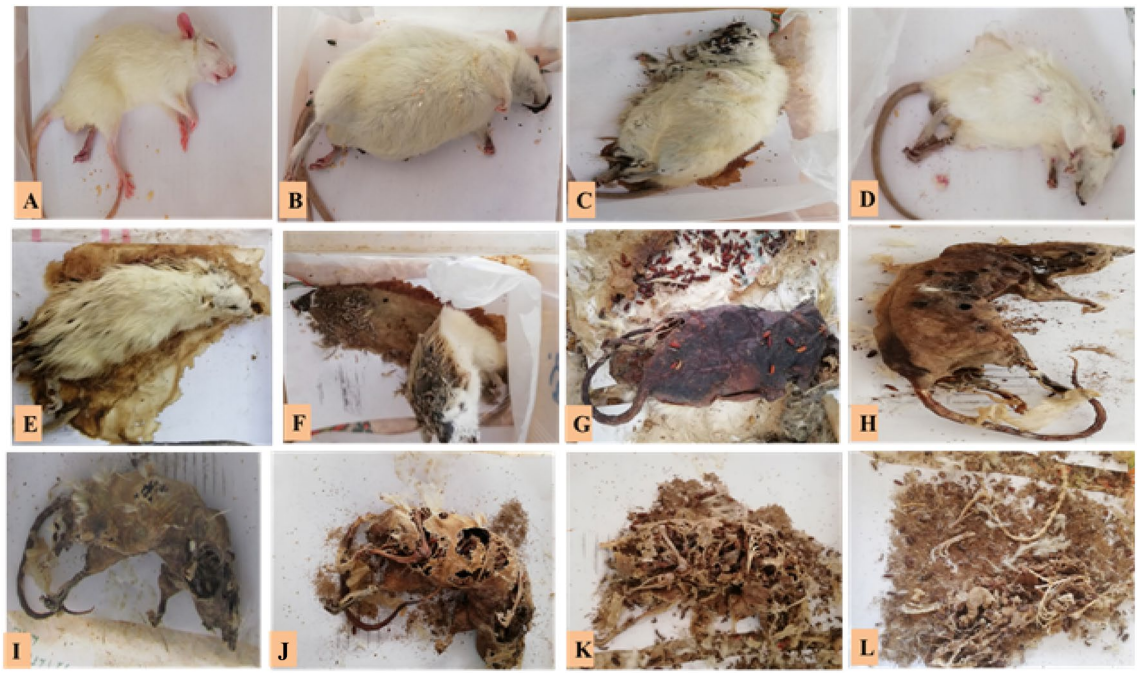


**Fig. 2** Decomposition stages of rat carrion from electrocuted group. **A** Fresh stage. **B** Bloating stage. **C–F** Active decay stage. **G–I** Dry decay stage. **J–L** Skeletal stage

### 4.3 Active decay stage

The active decay stage appeared after 2 days in all carcasses from different groups regardless of the cause of death which does not affect the onset of this stage. Active decay lasted for 5 days in the control and electrocuted group, while in the drowning group had a longer duration and lasted for 6 days.

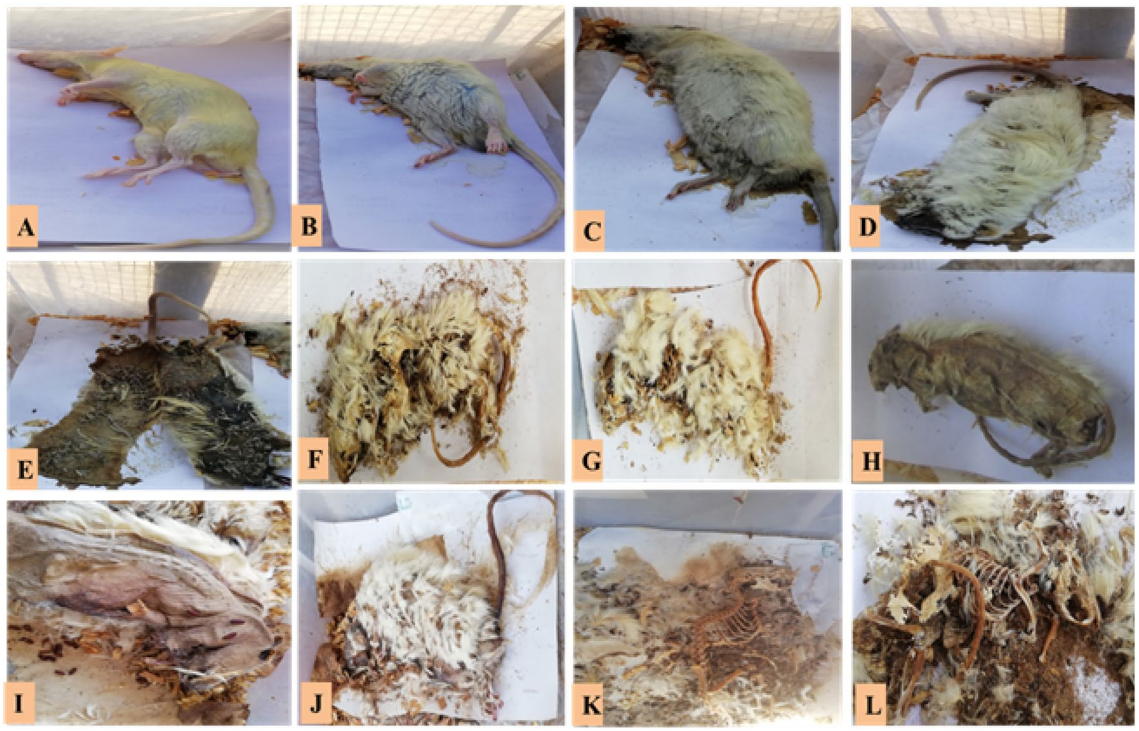


**Fig. 3** Decomposition stages of rat carrion from drowned group. **A** Fresh stage. **B** Bloating stage. **C–F** Active decay stage. **G–I** Dry decay stage. **J–L** Skeletal stage


**Fig. 4** Decomposition stages of rat carrion from slaughtered group. **A** Fresh stage. **B** Bloating stage. **C–F** Active decay stage. **G–I** Dry decay stage. **J–L** Skeletal stage

**Table 2** Insect diversity and day of arrival (succession day) on rat carrions in different stages of decomposition concerning the manner of death

| Stage | Insects | | | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Control | | Electricity | | Drowning | | Slaughtering | |
| | Order | Family | Species | *St | *AD | St | AD | St | AD | St | AD |
| Fresh stage | Diptera | Calliphoridae | *Chrysomya bezziana* | A | 0 | A | 0 | A | 0 | A | 0 |
| | | | *Chrysomya albiceps* | A | 0 | A | 0 | A | 0 | A | 0 |
| Bloating stage | Diptera | Calliphoridae | *Chrysomya bezziana* | A | 1 | A | 1 | A | 1 | A | 1 |
| | | | *Chrysomya albiceps* | A | 1 | A | 1 | A | 1 | A | 1 |
| | | Sarcophagidae | *Wohlfahrtia magnifica* | A | 1 | A | 1 | A | 1 | A | 1 |
| | | | *Sarcophaga haemorrhoidalis* | A | 1 | – | – | A | 1 | A | 1 |
| | | Fannidae | *Fannia canicularis* | A | 1 | A | 1 | A | 1 | | – |
| | | Muscidae | *Musca domestica* | – | | – | | – | – | A | 1 |
| | Coleoptera | Histeridae | *Saprinus chalcites* | – | | A | 1 | – | – | – | – |
| Active decay stage | Diptera | Calliphoridae | *Chrysomya bezziana* | L | 1st–4th | L | 1st | L | 1st-4th | L | 1st–4th |
| | | | *Chrysomya albiceps* | L | 1st–4th | L | 1st | L | 1st-4th | L | 1st–4th |
| | | | *Chrysomya* | P | 4th | P | 4th–6th | | | | |
| | | Sarcophagidae | *Wohlfahrtia magnifica* | L | 2nd | L | 1st | L | 3rd | L | 2nd–4th |
| | | | | P | 4th | – | – | – | | | |
| | | | | A | 4th | – | – | – | | | |
| | | | *Sarcophaga haemorrhoidalis* | L | 2nd | L | 1st | L | 3rd | L | 2nd–4th |
| | | | | P | 5th | – | – | | | | |
| | | Fannidae | *Fannia canicularis* | – | – | – | – | – | – | A | 2nd |
| | | Muscidae | *Musca domestica* | – | – | – | – | – | – | A | 3rd |
| | | Piophilidae | *Piophila casei* | – | – | A | 1st | A | 1st | – | – |
| | Coleoptera | Cleridae | *Necrobia rufipes* | A | 2nd–4th | A | 6th | A | 1st–3rd | A | 1st–4th |
| | | | | L | 5th | L | 6th | – | – | – | – |
| | | Dermestidae | *Dermestes maculatus* | A | 2nd–4th | A | 2nd–5th | A | 1st–3rd | – | – |
| | | Histeridae | *Saprinus chalcites* | A | 3rd | A | 5th | A | 4th | – | – |
| | | | *Hister sp* | – | – | A | 3rd, 4th | | | – | – |

**Table 2** (continued)

| Stage | Insects | | | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Control | | Electricity | | Drowning | | Slaughtering | |
| | Order | Family | Species | *St | *AD | St | AD | St | AD | St | AD |
| Advanced (dry) decay stage | Diptera | Calliphoridae | *Chrysomya* | P | 3rd | P | 1st–3rd | P | 1st | P | 1st–4th |
| | | | | – | – | – | – | – | – | – | – |
| | | Sarcophagidae | *Wohlfahrtia magnifica* | – | – | L | 1st–3rd | P | 1st | P | 1st–4th |
| | | | *Sarcophaga haemorrhoidalis* | – | – | L | 1st–3rd | P | 1st | P | 1st–4th |
| | | Piophilidae | *Piophila casei* | A | 1st | A | 1st–4th | – | – | A | 2nd |
| | Coleoptera | Cleridae | *Necrobia rufipes* | – | – | L | 2nd–17th | L | 2nd–20th | L | 4th–16th |
| | | | | – | – | A | 2nd -3rd | – | – | – | – |
| | | Dermestidae | *Dermestes maculates* | L | 1st–8th | L | 5th–8th | L | 2nd–20th | L | 2nd–16th |
| | | | *Dermestes maculates* | A | 3rd–6th | – | – | – | – | A | 1st–6th |
| | | Histeridae | *Saprinus chalcites* | | | A | 3rd | – | – | A | 2nd |
| | Hymenoptera | Formicidae | *Monomorium sp* | | | A | 6th | | | | |
| Skeletal stage | Coleoptera | Cleridae | *Necrobia rufipes* | L | 21th | L | 1st–16th | L | 1st–11th | L | 1st–10th |
| | | | | | | | | | | A | 10th |
| | | Dermestidae | *Dermestes maculates* | L | 1st–21th | L | 1st–16th | L | 1st–11th | L | 1st–10th |
| | | | | A | 21th | – | – | A | 1st–11th | A | 10th |
| | | Histeridae | *Hister sp* | A | 16th | – | – | – | – | A | 10th |

*St* stage of development, *AD* arrival date, *A* adult, *L* larva, *P* pupa

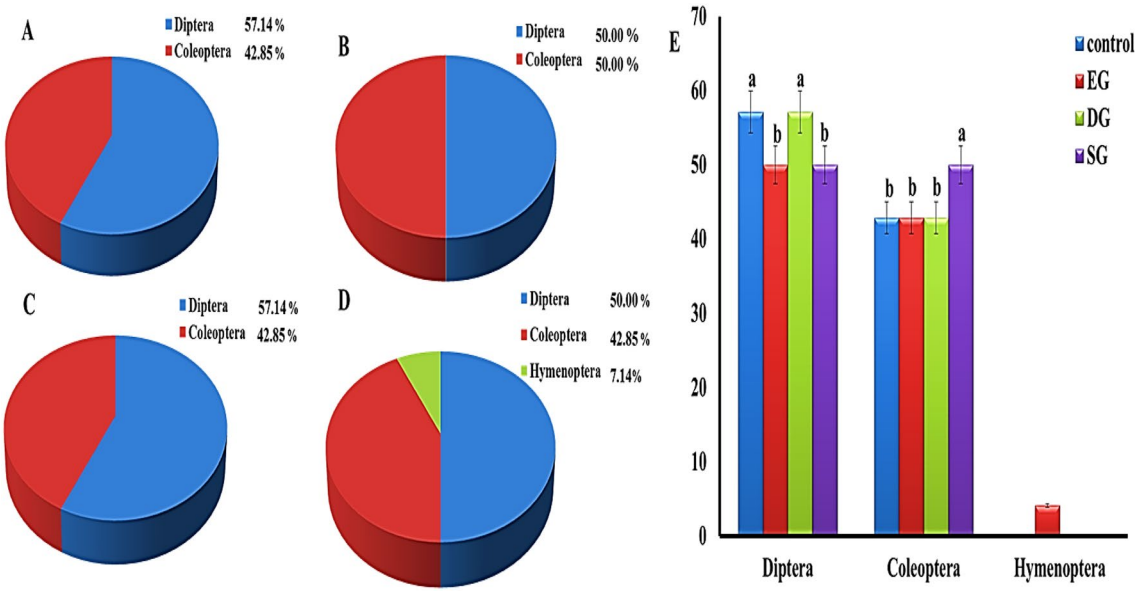


**Fig. 5** The proportions of the main orders of necrophagous arthropods on rat carrions within each group. **A** Control. **B** Electrocution. **C** Drowning. **D** Slaughtering. **E** the proportions of the main orders among different groups

On the other hand, it takes the shortest time (4 days) in the slaughtered group.

It was noted that the active decay in carcasses from different groups was driven by the larvae of Calliphoridae from the order Diptera (mainly *Chrysomya bezziana* and *Chrysomya albiceps* larvae) which appear on the carcasses on the first day of active decay (Table 2). We observed a large number of *Chrysomya albiceps* larvae in the electrocuted group compared to other groups. Chrysomya pupa was observed only in control and electrocuted groups on the 4th day of this stage.

Moreover, the larvae of Sarcophagidae (*Wohlfahrtia magnifica, Sarcophaga haemorrhoidalis*) were found on the carcasses of electrocuted group earlier than others (on the first day). While they were observed on second, third, from second to the fourth day in control, drowning and slaughtered groups, respectively (Table 2). The pupa of Sarcophagidae (*Wohlfahrtia magnifica, Sarcophaga haemorrhoidalis*) was observed only in the control carcasses on 4th and 5th day, respectively. Meanwhile, *Wohlfahrtia magnifica* adult appeared on the control group only.

Adult Fannidae and Muscidae (*Fannia canicularis*, *Musca domestica)* were found on the carcasses from the slaughtered group only on the 2nd and 3rd day of the active decay, respectively.

The only observed Piophilidae was adult *Piophila casei* which was found on the carcasses of electrocuted and drowning groups on the first day of active decay.

According to Coleoptera, the first observed Coleoptera in this stage was the adult *Necrobia rufipes* which appeared on the carcasses of drowning and slaughtered groups on the 1st day. It was observed on the 2nd day in the control group and delayed in appearance till the 6th day in electrocuted group. The larvae of *Necrobia rufipes* were observed in the control and electrocuted groups only on the 5th and 6th days, respectively.

Adult *Dermestes maculates* appeared first on the carcasses of the drowning group then on the second day in control and electrocuted groups, while it was absent in the slaughtered group.

Adult *Saprinus chalcites* was found on carcasses of control on the 3rd day, then it was observed on the 4th day in the drowning group then on 5th day in electrocuted group, while it was absent in the slaughtered group.

Adult Hister species was observed on the carcasses of electrocuted group on the 3rd and 4th days of active decay and was not observed on the other groups.

### 4.4 Dry decay stage

The onset and duration of the dry stage were varied in different groups as described in Table 1. The shortest and the longest duration were observed for control and drowning groups, respectively.

Chrysomya pupa appeared on all groups (1st day) earlier than control (3rd day) and they were observed in large numbers on the electrocuted group than others. Sarcophagidae (*Wohlfahrtia magnifica, Sarcophaga haemorrhoidalis*) larvae were observed on carcasses of the electrocuted group on the 1st day of this stage while their pupae were found on the drowning and slaughtered groups from the first day. On the

other hand, Sarcophagidae were not found on the carcasses from the control group.

*Piophila casei* adult was observed on carcasses from both control and electrocuted groups on the 1st day of dry stage and on the 2nd day in the slaughtered group while it was absent in the drowning group.

*Dermestes maculates* larvae were observed in the control group from the first day and on the second day in drowning group and were found all the period in the two groups. They also appeared on the second day in the slaughtered group till the 16th day, while they appeared for 3 days only (5th till the 8th day) on the electrocuted group. *Dermestes maculates* adult were observed on control and slaughtered groups only on 3rd to 6th and on 1st to 6th day, respectively.

Adult *Saprinus chalcites* were found on the carcasses from bleeding and electrocuted groups only on the 2nd and 3rd day, respectively. Moreover, there were a large number of ants on the carcasses of the electrocuted group on the 6th day of the dry decay stage.

### 4.5 Skeletal stage

The onset of the skeletal stage was varied among different groups. Control group began to undergo skeletonization earlier than other groups (16th day) followed by electrocution (20th day) and slaughtered group (on day 26), while the drowning group was the last one (29th day).

The predominant insect fauna was mainly Coleopteran. *Necrobia rufipes* larvae were found on the carcasses in the control group on the 21st of skeletonization. On the other hand, their larvae were observed from the 1st day of skeletonization in other groups, and continued to the 16th and 11th days in electrocuted and drowning groups. While in the slaughtered group, *Necrobia rufipes* larvae were found on the carcasses for 10 days after skeletonization.

Dermestidae larvae (*Dermestes maculates*) were found on the carcasses of the control rats on the 1st day of skeletonization till the day 21st, while their presence and staying period in other groups were similar to those of *Necrobia rufipes* larvae, while the adult *Dermestes maculates* appeared from the first day of skeletonization in the drowning group till the 11th day, while it was found on the day 10 and 21 in slaughtered and control groups, respectively. However, it was absent in electrocuted group.

The arrival of adult Hister sp took the same trend of adult *Dermestes maculates* in the control and slaughtered group, while it was absent in other groups. The sequence of insect succession on the rat carrions is represented in Fig. 6.

## 5 Morphological characterization of the obtained insect fauna

### 5.1 Flies belong to the order Diptera

Adult flies are composed of the head, thorax, and abdomen. Flies are easily distinguished from other insects by having fully developed front wings and two back wings modified into balancer (halters).

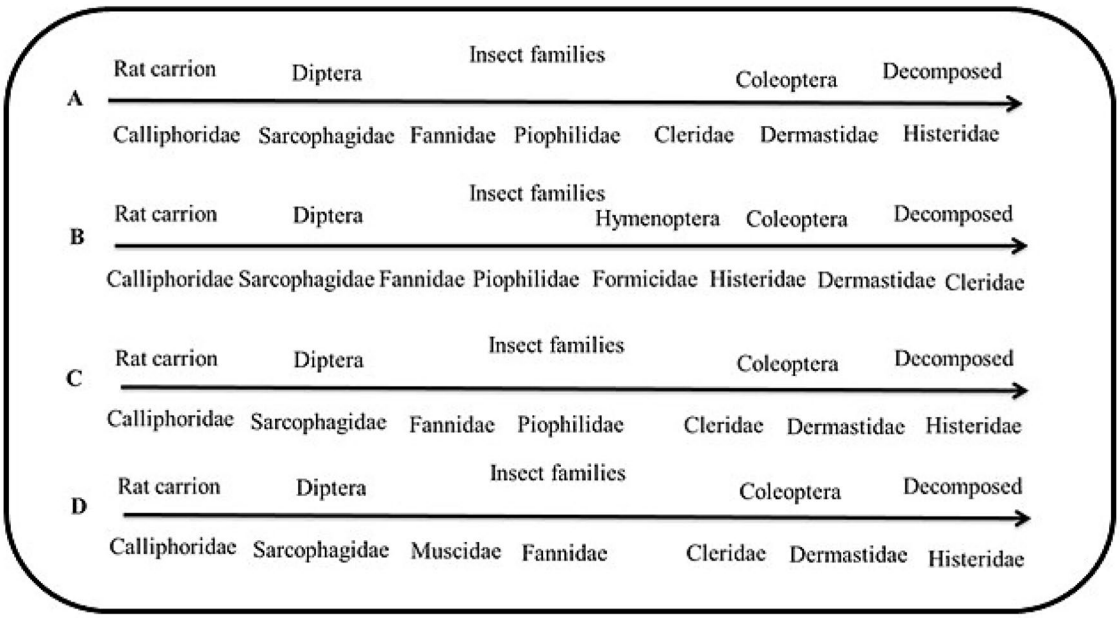


**Fig. 6** Sequence of insect succession on the rat carrions. **A** Control. **B** Electrocution. **C** Drowning. **D** Slaughtering

## 5.2 Family Calliphoridae, genus Chrysomya

### 5.2.1 Chrysomya bezziana

The adult's body is metallic green or blue with orange-brown eyes and medium size up to 8–12 mm in length. Abdominal segments with narrow dark bands along posterior margins. The frontal stripe of female head is parallel-sided. The wing is slightly darkened at the base, stem vein with a row of bristles dorsally and lower squamae waxy white which covered with long black hairs above. The flies have dark legs. Mature third stage larvae are about 18 mm in length and creamy white. Each segment carries a broad, encircling belt of strongly developed spines (Fig. 7).

### 5.2.2 Chrysomya albiceps

The adults are bluish-green in color with orange-brown eyes. It is 5–10 mm in length. The hind margins of the abdominal segments have blackish bands. There is no definite pattern on the thorax. The legs are red brown to blackish. Lower squamae covered with fine hairs above. Mature third stage larvae reach up to 18 mm length and white to dark yellow color. The larvae bear many thorn-like fleshy projections on most of the body segments which give these species their common name (hairy maggot blow flies) (Fig. 7).

## 5.3 Family Sarcophagidae

### 5.3.1 *Wohlfahrtia magnifica* (spotted flesh fly)

The adult was measured 11–14 mm in length. The thorax had 3 dark longitudinal stripes, while the abdomen had 3 dark, distinct, separate and rounded patches. The larva measured 10–12 mm in length. The body is pointed anteriorly, and rounded posteriorly. It is robust and extensively covered in black thorn-like spines (Fig. 8).

### 5.3.2 Sarcophaga haemorroidalis

The adult measured 11–15 mm in length. The thorax had 3 dark black longitudinal stripes; while the abdomen had

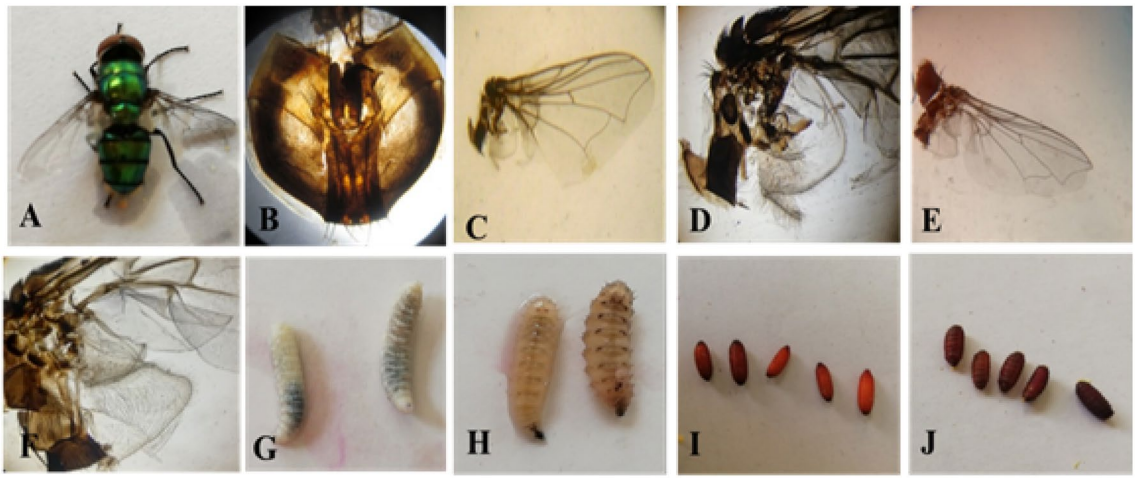


**Fig. 7** **A** *Chrysomya* species adult. **B** *Chrysomya bezziana* adult head region (X40). **C**, **D** *Chrysomya albiceps* wing (X5) and lower squamae (X40). **E**, **F** *Chrysomya bezziana* wing (X5) and lower squamae (X40). **G** *Chrysomya bezziana* 3rd larval stage. **H** *Chrysomya albiceps* 3rd larval stage. **I** *Chrysomya bezziana* pupa. **J** *Chrysomya albiceps* pupa

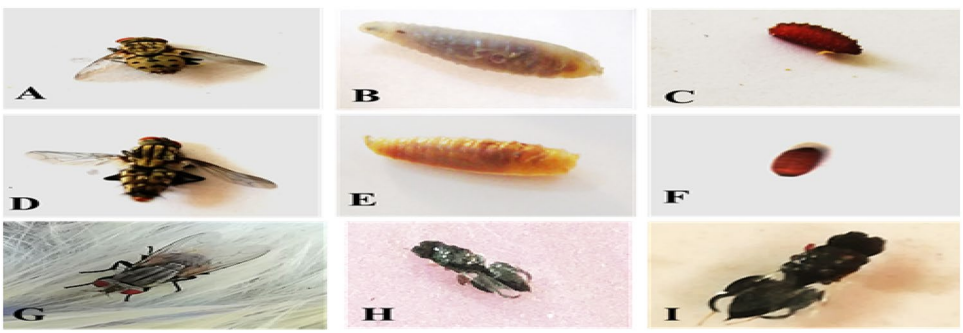


**Fig. 8** *Wohlfahrtia magnifica.* **A** adult, **B** 3rd stage larvae, **C** pupa. *Sarcophaga haemorroidalis;* **D** adult, **E** 3rd stage larvae, **F** pupa. **G** *Musca domestica* adult. **H**, **I** *Piophila casei* adult

black and grey patches like chessboard. The larva measured 11–13 mm in length. The body is pointed anteriorly and rounded posteriorly. It is less robust and without thorn-like spines (Fig. 8).

## 5.4 Family Fannidae

### 5.4.1 *Fannia canicularis* (lesser house fly)

It is dark grey with a yellow abdomen. It has three black stripes on the thorax. It is 4–6 mm in length. The wing is membranous with simple wing veins, 4th longitudinal vein is straight, and 6th longitudinal vein is short. The fourth longitudinal vein not bent as in *Musca* species.

## 5.5 Family Muscidae

### 5.5.1 Musca domestica

The adult is 6–8 mm in length, grey in color, thorax with 4 longitudinal stripes and the abdomen yellowish in color with black median longitudinal stripe. The wing is membranous with simple wing veins, and 4th longitudinal vein makes a sharp bent toward the 3rd one.

## 5.6 Family Piophilidae

These are small, shiny, black flies and measured 2.5–4.5 mm in length. The costal vein of the wing appears broken at one point in this family. *Piophila casei* is a small black fly 2.5–4 mm in length and it is commonly called the cheese skipper. It has prominent cheeks, which are more than half the eye height. These have yellow color on their legs, antennae on the jowls of their faces. Their ocellar bristles are fond opposite the simple eye (front ocellus), which are small and widely spaced (Fig. 8).

## 5.7 Beetles belong to the order Coleoptera

The beetles of the order coleoptera have biting mouthparts (mandibles), their antennae characteristically have 11 segments (although in some species may be fewer than this). Prothorax (1st segment of thoracic) is usually distinctive in shape and size and can be used as a means of identifying the beetle. The beetle exoskeleton is formed from hardened plates. Beetle adults are composed of a head, a thorax (three parts fused although the second and third parts are less visible dorsally), and an abdomen. They have two pairs of wings; the two forewings that are modified into hard wing cases (elytra) that cover and protect the hind membranous pair of wings and abdomen.

### 5.7.1 *Dermestes maculatus* (the hide beetle)

Adults are usually oval and range in size from 5.5 to 10.0 mm. Each side of the thorax has a band of white hairs. The underside of the abdomen is primarily white with black spots at the sides, and a large black patch on the last segment. The forewings (elytra) are dark brown or black, with hairs that are mostly black, yellow, or white. The antennae are short and segmented with a club at the tip. The edges of the abdominal end of the elytra are serrated and end in a small spine projecting straight out. Larvae: the bodies of the larvae are covered in rows of hairs of different lengths, called setae (called woolly bears). The underside of the abdomen is typically yellowish-brown while the dorsal surface is typically dark brown, usually with a central yellow line. Two long horn-like protrusions are located on the upper surface of the last segment, partially hidden by surrounding hairs. The protrusions, called urogomphi, curve upward and away from the tip of the abdomen (Fig. 9).

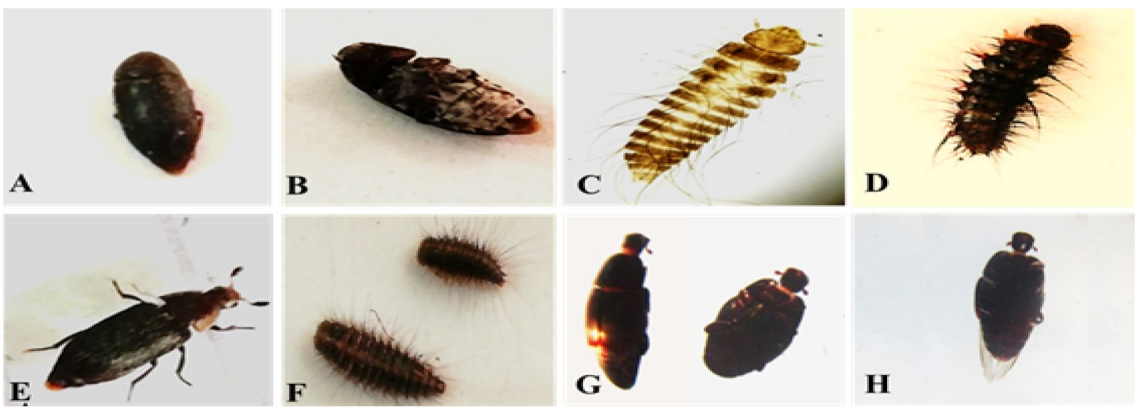


**Fig. 9** *Dermestes maculatus;* **A** adult dorsal view. **B** adult ventral view. **C** 1st stage larvae. **D** 3rd stage larvae. *Necrobia rufipes;* **E** adult, **F** 3rd stage larvae. *Saprinus chalcites* adult, **G** dorsal and ventral view. **H** *Hister* species adult

#### 5.7.2 *Necrobia rufipes* adult

Adults are 3.9–7 mm in length. They have an elongated oval shape with three clear-distinct body regions. They are blackish-blue in color with red legs. The antennae are 11-segmented with an expanded club region at the end. The wings are covered with 9 rows of little hairs. Larvae are 8-10 mm long. They have three pairs of legs in the middle of the body. The body is reddish to blackish, and the legs are a pale mustard color (Fig. 9).

#### 5.7.3 *Saprinus chalcites* adult

They have a length of about 4–6.8 mm. The body is oval, weakly convex and shiny. Pronotum is greenish-black, while elytra may be dark blue. Elytra show 3–4 external dorsal striae and marginal sulcus of pygidium extending to the base.

### 5.8 *Hister* species (clown beetles)

*Hister* species are flat in shape. The head has compound eyes and can sink into their prothorax and two antennae. A main characteristic feature is that the antennae are elbowed and form a club at the end. They have shiny elytra, which are typically shiny black or metallic green. The elytra are shorter than the abdomen with typically two of the seven tergites exposed (Fig. 9).

## 6 Discussion

In the present study, five remarkable stages of decomposition including fresh, bloating, active decay, dry decay, and finally skeletal stage were recognized in all studied groups. Similar observations were reported by on rat carcasses in Egypt (Aly et al. 2013; El-Aziz and El Shehaby 2019) and in other countries such as Mexico (Valdes-Perezgasga et al. 2010). Herein, the fresh stage on rat carrion took one day at temperature (22–36 °C) and R.H (49–57%), while the fresh stage took only 0.5 day on rabbit carrion at 56% RH and 22–33 °C in Egypt as reported by Zeariya et al. (2015). Al-Shareef and Al-Mazyad (2016) stated that, the fresh stage on rabbit carcasses took 2 days at 26–32.8 °C, and 53.5% RH, while Bharti (2003) recorded 1.5 days for fresh stages on rabbit carcasses in urban areas in India at 16 ± 1.8–31.3 ± 1.4 °C and 59.6% RH).

Here, the bloating stage lasted for one day (temperature; 22–36 °C, RH; 49–57%), while it took from 1 to 2 days in another study conducted in Egypt at 60% R.H and 22 °C, 32 °C (Zeariya et al. 2015) and from 1.29–3.63 days (31–87 h) in India at 59.6% RH and 16 ± 1.8$^0$C, 31.3 ± 1.4 °C (Bharti 2003). Additionally, Ojianwuna et al. (2019) reported that fresh and bloating stages took 2 days each in carcasses from strangulated and slaughtered carcasses in Nigeria at 30 °C and 70% RH. The differences in duration of the fresh and bloat stage in our study than others could be attributed to that the carcasses here were placed indoor and were not expose directly to the sun which might enable them from retaining higher levels of moisture (Sharanowski et al. 2008).

The active decay stage in the present study had equal duration in control and electricity group (5 days), while active decay in the drowning group had a longer duration and lasted for 6 days while it recorded the shortest duration in the slaughtered group (4 days). This variation could be attributed to the difference in the moisture content of each type of carcasses where drowned body may contain higher moisture than others while that of the slaughtered rats were devoid of blood which is required for microbial development. In Egypt, El-Aziz and El Shehaby (2019) reported that the active decay stage on rat carrion was 4–6 days, while it ranged from 5 to 6 days at 56.66% RH and 23.76 °C, 32.73 °C (Al-Shareef 2016), and 3.67–6.916 days (88–166 h) in India at 59.6% R.H and 16 ± 1.8$^0$C, 31.3 ± 1.4 °C (Bharti 2003), while Ojianwuna et al. (2019) recorded 5–6 days for active decay in strangled and slaughtered carcasses, respectively.

The dry decomposition stage in this study took 7, 10, 18 and 20 days in control, electricity, slaughtered, and drowning groups, respectively. As shown, the longest duration was recorded for drowning group (20 days). Water may provide a thermal buffer which could slow down the putrefaction of organic matters, inhibit the proliferation of bacterial and preserve the tissues of corpse longer (Papp 2002). The dry decomposition stage took 19–30 days at 53% RH and 23 °C, 37 °C, 6.96 days at 59.6% RH and 16 ± 1.8 °C, 31.3 ± 1.4 °C and 3–4 days at 62.5% RH and 23.6 °C, 31.55 °C (Bharti, 2003; Al-Shareef & Al-Mazyad, 2016). Al-Mesbah (2010) reported that rabbit carcasses at the urban area in Kuwait lasted 14 days to reach dry stage at 17.40 °C, 20.55 °C and 73.43% RH. Azmi and Lim (2013) recorded the duration of dry decomposition as 10 days at 27.5 $^0$C and 28.2 $^0$C on the carcasses in 2 different sites in Malaysia. The differences between us could be attributed to the non-exposure to the sun and may be due to the difference in the carcass type. Okiwelu et al. (2008) confirmed that the sun-exposed carcasses decomposed faster than the shaded carcasses.

Deterioration of dead animal begins within minutes from death because of the different physiological alterations which lead to putrefaction. Insects like different fly species are considered as evidence of such deterioration because they detect these alterations and arrive on the carrions (Abajue et al. 2015). In the present study, different insect species were recovered from different carcasses. They were few fairly 3 orders, 9 families, and 12 species represented in 7 species of Diptera belong to 5 families, 4

species of Coleoptera belong to 3 families and one species of Hymenoptera. Similarly, Al-Shareef and Al-Mazyad (2016) reported a few insect species on rabbit carcass (6 species of Diptera belonging to 3 families, 2 species of Coleoptera belong to 2 families and 1 species of Isoptera). Abd El-bar and Sawaby (2011) stated that only 16 species of arthropods were recovered from all tested rabbit carrions. This observation could be explained based on the small size of the dead carcasses, which may be rapidly consumed by the early invader arthropods giving no chances to later colonizer species and also lead to faster decomposition and shorter postmortem duration (Abouzied, 2014). Moreover, smell and odor were much more pronounced on the sun-exposed carcasses (Mabika et al. 2014). The low insect diversity in relation to small carrion size was reported by Tomberlin and Adler (1988). Similarly, a small number of insect species was collected from guinea pig (Abd El-Bar et al. 2016). El-Aziz and El Shehaby (2019) collected 13 arthropod species and 10 families from rat carrions, while Aly et al. (2017) indicated 18 species from the dipterous insects on rabbits, Aly et al. (2013) listed 10 arthropod species and 7 families from rabbit and rat, Yassa et al. (2014) observed 6 species of insect and Valdes-Perezgasga et al. (2010) recorded 19 species of arthropods on pig. Large animal carcass usually attracts many more species, such as the 47 species in the study of Wang et al. (2008).

The succession of insects on dead bodies depends on the stage of decomposition (Kyerematen et al. 2013). Herein, the first insect colonizers were Diptera (blow flies). Blowflies have been reported to be attracted to the carrions within few minutes following death, they could detect the carcass primarily by odor, however the attractiveness varies based on the decomposition degree and they could travel for great distances to reach the places for their lay down (Erzinclioglu 1996). Calliphorids (blowflies) were observed in all stages of decomposition except the skeletal stage in all carcasses. This correlated with previously reported findings (Centeno et al. 2002; Bharti and Singh 2003; Ekanem and Dike 2010). Necrophagous Diptera can aid in the cycling of nutrients in the terrestrial ecosystem resulting in acceleration of animal tissue disintegration and can facilitate the actions of microorganisms (Savage 2002). Because Calliphorids are always found on decaying carrions, they could be used in the study investigating the suspicious deaths. Then Sacrophagidae arrived next to Calliphoridae in the bloating stage in all groups. Similarly, Whitworth (2006) found that Sacrophagidae generally arrived to carrion next to Calliphoridae. On the other hand, El-Aziz and El Shehaby (2019) stated that Calliphoridae, Sarcophagidae, and Muscidae were observed flying around carrion.

Calliphoridae and Sarcophagidae have been reported as most insect colonizers of dead bodies (Watson and Carlton 2003). The common existence of these two families and also their abundance of human cadavers indicate their important role in forensic investigation (Cruz et al. 2013). The dominant Calliphoridae species on all carcasses were *Chrysomya albiceps,* which constituted the primary factors in the process of decomposition and it exhibited an earlier succession and is considered as one of the most important corpse breeding flies in different geographical regions as reported in Egypt (Tantawi et al. 1996; Adham et al. 2001). It represented the first waves and the most species of fly found on rabbit carcasses (Al-Mesbah et al. 2012). It is an aggressive species and may feed on larvae of other species explaining its predominance over other calliphorids. On the other hand, Grassberger and Frank (2004) and Arnaldos et al. (2015) found *Chrysomya albiceps* in the bloated stage only. Since *Chrysomya albiceps* reached corpse a few minutes after death it could be of importance in the estimation of postmortem intervals (Carvalho et al. 2004; Niederegger and Spiess 2012).

The second type of insect that was observed on the carcasses following to Calliphoridae is Sacrophagidae (flesh flies). Sarcophagids have been reported to be primary invaders of corpses in subtropical and tropical regions (Early and Goff 1986). Sarcophagids found to be reached and colonized the carcasses during the first 2 days in all the different seasons where they observed four Sacrophagidae species in autumn and recorded the presence of *Sarcophaga hirtipes* on rabbit carrions all over the year in India (Bharti 2003). Sacrophagidae were collected from pig carcasses in Australia (Voss et al. 2008), rabbit carcass in Malaysia (Azmi and Lim 2013), and Egypt (Zeariya et al. 2015). Abd El-Bar et al. (2016) found that Sarcophagidae colonized the carcasses on the same day of Calliphoridae colonization.

In the present study, it was observed that the abundance of Sacrophagidae was lower than that of Calliphoridae during the day. This could be attributed to that Sarcophagidae like cooler period and they oviposit or larviposit at night only (Wang et al. 2008). The present study was conducted in warm season, the carcasses were visited during the day, and this could be a reason. Fannidae appeared after Sarcophagids during the bloating stage in all groups except slaughtered carcasses and the opposite occur in the active decay stage, may be due to putrefaction odor. Fannidae has been reported to be useful in forensic investigations especially for estimating the postmortem intervals (Grisales et al. 2016).

*Musca domestica* was the only observed species of the family Muscidae, and it was shown only on slaughtered carcasses during the bloat and active decay stages. This could be probably due to the presence of blood on the carcasses. Muscid species have been recorded on animal corpses by several authors in different regions. *Musca domestica* was found on pig carcasses in Brazil (Barbosa et al. 2009) and Australia (Voss et al. 2008). It was also observed on rabbit carcasses in India, Malaysia, Kuwait, Saudi Arabia and

Egypt. *Musca domestica* was collected from rat carcasses following different methods of killing in Egypt, and Nigeria. Other muscids such as *M. sorbens* were found on carcasses of monkey in Malaysia (Emerson et al. 2001), decomposed vertebrate tissues in Iran (Tüzün et al. 2010), reflecting their forensic importance.

Adult species of Piophilidae are usually attracted to proteinaceous substances such as fish, meat and cheese and they are major pests in food industries and considered as myiasis agents (Castro et al. 2012). Piophilidae is frequently cited for its common presence on bodies of dead animals (Martin-Vega 2011). The succession of piophilidae on the carcasses differed according to the method of killing. Adult flies were seen first during the active decay stage on the carcasses from electrocuted and drowning groups only, while their appearance was delayed till the dry stage in other groups may be due to the stronger odor of putrefaction in electrocuted and drowning groups. Piophila casei was observed on 1st week and last to the 14th day after death (Islam et al. 2016).

The dipterous maggots that observed during the active decay stage are primarily of the Calliphoridae and Sarcophagidae. Dipterans lay their eggs on decomposed flesh then the eggs developed till the insects become adults. However, the larvae of calliphorids were observed on the 1st day of active decay in all groups while the larvae of Sarcophagidae were found on the first day on the electrocution group only and delayed in other groups. The succession of the same Dipteran families from the onset of decay till the active stage in carcasses from different groups showed the same pattern. Succession patterns showed that the maggots of necrophagous blowflies are active in the active decay and dry stages of decomposition, while Abd El-bar and Sawaby (2011) showed that these maggots are active in the first 3 stages of decomposition but not in the dry stage decomposition. Additionally, their activity was continuous all over the decomposition period (83 days) on pig carcass (Martinez et al. 2007).

In the present study, the coleopteran insect's succession differs according to the killing method. They were first observed during the bloat stage on the carcasses from electrocuted group only (one adult species of coleopteran Histeridae; Saprinus chalcites). Then the number of coleopteran species reached a maximum during active decay stage and the adult and larvae of them were observed till the dry and skeletal stages in all studied groups. Similarly, Abd El-bar and Sawaby (2011) found that the activity of Coleoptera was completed during all stages of decay. This observation was supported by the results of El-Aziz and El Shehaby (2019) and Midgley et al. (2010); they reported that beetles are highly adaptive and may be present in almost all types of environment. Beetles preferred the corpse when it is more decomposed or in the advanced decay stage. On the other hand, Abd El-Bar et al. (2016) indicated that coleopteran species were observed during the active decay stage in intoxicated and control rabbits. Beetles are one of the most diverse macroinvertebrates and could be found in all continents of the world except Antarctica (Bilton et al. 2019). Coleopteran is predators and had been observed in this study during the presence of dipterous larvae similar to the observations of Ozdemir and Sert (2009). The presence of predator beetles on carcasses associated with the abundance of the basic prey. Because the maggots of blowflies as the most predominant prey are typical to the stage of decay, the activities of predator beetles culminated at this decay stage (Kočárek 2003). In later stages, when less flesh was still found, the presence of larvae and adults of dermestes beetles was observed. Kulshrestha and Satpathy (2001) reported that the presence of larvae of Dermestidae could be considered as an evidence for the actual species infestations. Dermestes species prefer dried corpses, feathers, hairs and skins and they could fed directly on the corpse in contrast to the rove beetles and may accelerate the process of decaying (Shroeder et al. 2002).

In the present study, the presence of Cleridae was recorded where a single Clerid species, (*Necrobia rufipes*) was recovered from the carcasses in different groups. They are predators on some Piophilidae flies and larvae of Dermestidae. Adult Cleridae were noticed to feed on larvae of the order Diptera and on carrions as well. Similar actions have been observed where the Dermestidae (Dermestes frischii) and Cleridae (Necrobia violacea) were noted in advanced decay and dry decay decomposition of pig carcasses and bovine internal organs in Turkey (Bana and Beyarslan 2012). The beetle families observed on the carcasses differed, Histeridae was found on the carcasses from electrocuted group only. Clerids was found in all groups, while Dermestids and Histeridae were found on all groups except slaughtered carcasses during active decay stage. These differences in the number of species on the carcasses from different groups could be correlated to the differences in the duration of different stages of decomposition. Moreover, the abundance of coleopteran species in the active decay stage in control, electrocuted and drowning groups may be associated with the decreased or absence of some maggot of some dipteran species in these groups.

In the present study, there was a large amount of Formicidae (ants) just on the dried electrocuted carcasses and it was the only observed Hymenoptera in the present study. On the other hand, Ojianwuna et al. (2019) noticed the presence of ants around the slaughtered and strangulated rat carcasses from the fresh stage to the stage of active decay without a particular pattern. Another study on rabbit and Guinea pig conducted by Abd El-bar and Sawaby (2015) showed that ants were mostly found in the early decomposition stages on carcasses of both animals and represented about 17.3% from the all observed insects. Arnaldos et al. (2005) stated that

Hymenoptera represented 15.39% of collected insects and was not associated to a particular stage of decomposition. Mabika et al. (2019) found that Hymenoptera (Formicidae) were present throughout the decomposition period, however they showed no impacts on the process of decomposition. This is disagreed with Morreti et al. (2013) where ants fed on carcass and the present maggots so that they were categorized as important components of the Sarcophagidae communities.

Formicidae can be considered as omnivorous predators and can use the dead bodies as refuges to get food and humidity. Ekanem and Dike (2010) reported that Formicidae species have strong mandibles enable them to take off pieces of decayed bodies and could also prey on eggs and larvae of dipterous insects and other arthropods they can reach on the carcass. Their coming in the form of colonies may affect the level of decomposition by predating on immature diptera flies or by taking portions of the carcass. The Hymenoptera observed in the present study did not necessarily breed on the rat carcass or influence the decay process as it appeared in the dry stage which makes them not one of the most important forensic insects (Ekanem and Dike 2010).

The necrophagous insects mainly the Calliphorids and Sarcophagids predominated on the corpses. The predators of such species like Histeridae and Formicidae arrived in a sequence in responses to the abundance of large numbers of larvae, eggs, and also adult flies (Gill 2005). By the progression of carrion decomposition, they became more favorable to the species invade the carcass at the later decomposition stages as Dermestidae and Cleridae. Electrocuted carcasses showed a large number of immature stages, it might explain the abundance of Histeridae and Formicidae in such group than others.

## 7 Conclusion

There is an infinite possibility for the application of entomofauna in forensic investigations, as each insect can act as a witness on the conditions and the places that the dead bodies have passed through. Moreover, during the investigation of suspicious death, it is important to know the method of killing. The present study showed that the killing method influenced the diversity, abundance and succession pattern of the forensically important arthropods fauna that colonize the rat carrions and consequently the decomposition process. Therefore, this preliminary study could be a base for further investigations using more killing methods at different sites and different climatic conditions throughout the year. This could be helpful for the forensic science in the future to make the complicated death cases simpler.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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