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Death-Leading Envenomization of Rabbits with Snake Versus Scorpion Venoms: A Comparative Forensic Investigation of the Postmortem Decomposition and Beetle Succession

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Simple Summary: This study investigates the impact of antemortem envenomization of rabbits by snake versus scorpion venom on the postmortem decomposition process and the succession pattern of the associated beetles. Results revealed a venom-dependent impact on the decomposition process and beetle succession. The decomposition duration was prolonged for up to one day. The succession pattern and quantitative and qualitative analysis of the beetle community varied significantly between the treated corpses and the decomposition stages. This could be of forensic importance since envenomation with venomous animals is a considerable death-leading cause worldwide.

Abstract: Background: Envenomation by poisonous creatures is a major global cause of mortality. Its concomitant impact on the postmortem corpse decomposition and associated insect succession pattern is yet poorly understood. **Purpose of the study:** This study comparatively investigates the impact of envenomization with the snake, *Walterinnesia aegyptia* L., versus the scorpion, *Androctonus crassicauda* L., venoms on rabbit corpse decomposition and beetle succession. **Methods:** Three groups of rabbits (5 animals each) were injected with the snake venom, the scorpion venom, or 0.9% saline (control) prior to euthanasia with CO_2. Corpse decomposition stages and beetle succession were monitored over 11 days. **Results:** Four stages of decomposition, with venom-dependent duration variation, were observed. The scorpion-envenomized corpses showed a longer decay stage and a delayed dry stage. A total of 1094 beetles belonging to 27 species of 14 families were reported. Histeridae, Dermestidae, Scarabaeidae, and Tenebrionidae were the most diversified and prevalent families. Chrysomelidae, Elateridae, Hybosoridae, and Ptinidae were distinctively attracted to control corpses, while Nitidulidae and Zopheridae were only found on control and snake-envenomized ones. Four species belonging to the families Anthicidae, Histeridae, Scarabaeidae, and Tenebrionidae were predominant on all corpses. While four species belonging to the families Chrysomelidae, Curculionidae, Elateridae, and Hybosoridae were distinctively associated with the control corpses. **Conclusion:** These findings provided evidence that envenomation impacted the decomposition process and beetle succession in a venom-dependent manner, which could be significant for forensic investigations.

Keywords: beetles; corpse; decomposition; envenomization; forensic; snake; scorpion; venoms

1. Introduction

Forensic Entomology is a rapidly growing field of study that involves the use of insects to aid in criminal investigations. In this field, necrophagous insects and other arthropods are used as tools of forensic investigation to detect, elucidate, and establish evidence during forensic investigation [1].

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This is due to the capability of these insects to reach a corpse within minutes post-death [2]. The diversity of these corpse-seeking insects, their successional behavior towards the corpse, the relationship between their arrival time and the developmental process of their immature stages, and their feeding manner on the corpse all provide valuable data to forensic entomologists that help them solve crime secrets [3–5] and estimate the post-mortem interval (PMI) [6].

There are four groups of corpse-seeking insects, which are categorized based on their feeding manner [7]. The first group is the sarcosaprophages, which include dipteran flies from Calliphoridae, Muscidae, and Sarcophagidae, as well as coleopteran beetles from the Dermestidae, which feed on decomposing corpses. The second group is the coprophages, which include beetles from Scarabaeidae and flies from Muscidae, which are attracted to the herbivores' rumen contents. The third group is the dermatophages, which include beetles from Dermestidae, which feed on the cadaver remnants such as bones, hair, and dried skin. The fourth group is the predaceous insects, such as Staphylinid and Histerid beetles and Formicid ants, which feed mainly on corpse-colonizers like dipteran larvae. These insects colonize the corpse along with bacteria, where they work to decompose the corpse [8]. During decomposition, the corpse goes through five stages (fresh, bloating, active decay, advanced decay, and remain), where each stage is characterized by a specific attraction of insects [7].

Necrophagous beetles constitute a forensically determinant factor due to their ability to inhabit the majority of the corpse environment, offering valuable data for forensic investigation [9]. They belong to Coleoptera, the largest Order comprising over one-third of all known insect species [10]. These corpse-associated insects provide valuable data that help in estimating PMI of dried corpses, as well as evaluating the damage and variations that may have occurred to the corpse status [7]. The most forensically important beetle families include Cleridae, Dermestidae, Histeridae, Staphylinidae, and Scarabaeidae [7]. Nevertheless, much less research on corpse-seeking insects has given attention to these beetles compared to flies [11–14]. Therefore, the present study was implemented to participate in compensating for this deficiency.

Envenomation-related death cases constitute a vital and considerable aspect from the forensic point of view. This is based on the fact that venomous snakes and scorpions are distributed all over the world [15–24] and affect millions of people [24–33] and, consequently, envenomation is considered a significant cause of death worldwide [16,24,34–37]. In this regard, the majority of envenomation-related deaths are caused by arthropods [36,38], followed by snakes and spiders [24,26,35]. In regions like Saudi Arabia, where snakebites [33,39,40] and scorpion stings [39,41–43] are prevalent, understanding the impact of envenomization on decomposition is crucial for forensic science. The desert black snake, *Walterinnesia aegyptia* L., and the fat-tail scorpion, *Androctonus crassicauda* L., are significant contributors to envenomation-related deaths, yet there is a lack of research on how their venoms affect the decomposition process and beetle succession. Thus, this study aims to bridge this gap by comparing the effects of *W. aegyptia* and *A. crassicauda* venoms on rabbit corpses, focusing on the decomposition stages and beetle succession patterns. By addressing this knowledge gap, this research seeks to enhance the PMI estimation and provide valuable insights into differentiating between causes of death involving venomous animals, ultimately contributing to the advancement of forensic science in regions with high envenomation rates.

2. Materials and Methods

2.1. Meteorological Parameters

Experiments of this study ran for 11 days in the summer (from 6th to 17th of June, 2023). The atmospheric parameters were determined over the study period via the Saudi National Center for Meteorology [44]. In addition, the on-site atmospheric parameters were also reported manually at the experimental site in a daily manner (at midday) during the study period following [45,46]. The relative humidity (RH) and ambient temperature (°C) were measured using a hygrometer and digital thermometer devices (Elitch, China) following their instruction manuals. The Skywatch Wind Meter

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device (Skywatch®, Switzerland) was used for monitoring wind speed following its instruction manual.

2.2. Experimental Site

Experiments were carried out in the botanical garden of the Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia (N 24°43.174', E 46°36.954') (Figure 1A). This garden covers an area of about 10,000 m² with a clayey sandy soil with abundant grasses and herbs. It houses a significant number of plant species, ranging from wild, medicinal, and endangered plants to economically important plants [47].

2.3. Experimental Animals

2.3.1. Rabbits and Mice

Swiss albino mice (≈ 18-20 g each) were obtained from the Animal House, Zoology Department, College of Science, King Saud University for carrying out the lethality of snake and scorpion venoms following [48]. Male domestic rabbits, *Oryctolagus cuniculus domesticus* (Linnaeus, 1758) (≈ 2.8-3.0 kg each) were purchased at a specialized local farmer's market in Riyadh City, Saudi Arabia. They were used as experimental models for conducting the forensic experiments in this study according to [49] and following other studies [46,50–53]. Prior to use for experiments, rabbits were first kept for acclimatization for 2 days in the animal house. They were housed in standard rearing conditions with suitable feed and aeration by skillful specialists in accordance with the Research Ethical Committee at King Saud University (approval code: KSU-SE-23-83).

2.3.2. Snakes and Scorpions

The Saudi desert black cobra snake, *W. aegyptia* Lataste, 1887 (Squamata, Elapidae) [18,54–56] (Figure 1B; from [57]) and the fat-tail scorpion, *A. crassicauda* (Olivier, 1807) (Scorpiones, Buthidae) [21,58–60] (Figure 1 C; from [61]) were used from the Animal House, Zoology Department, College of Science, King Saud University for carrying out this study. Snakes and scorpions are being housed in standard laboratory conditions in the animal house with feed and aeration, according to [62] and [63], respectively. Dealing with snakes and scorpions took place by a qualified specialist from the Herpetology laboratory in Zoology Department and in accordance with the Research Ethical Committee at King Saud University (approval code: KSU-SE-23-83).

2.4. Collection of Venoms

The snake crude venom was milked from five adult snakes, according to [62,64]. While scorpion crude venom was collected from 100 scorpions by inducing electric shock according to [65] and as detailed in [63]. Collected samples of each venom were pooled, lyophilized, and stored at -20°C until used. Prior to experimental use, an aliquot from each venom was freshly prepared in 0.9% saline (pH 7.2), according to [66] in a final concentration of 1.0 mg/ml (w/v). Carrying out venom collection took place by a qualified specialist from the Herpetology Laboratory in Zoology Department and in accordance with the Research Ethical Committee at King Saud University (approval code: KSU-SE-23-83).

2.5. Lethality Assay

The lethality, in terms of both LD_{50} and LD_{95}, for each venom was determined following the World Health Organization guidelines [67] and as per detailed in our previously published study [48]. Briefly, a preliminary dose-finding experiment was first carried out using Swiss albino mice. The preliminary screening was carried out using a wide range of 8 descending dilutions from each venom aliquot (1,000, 500, 250, 125, 62, 31, 15, and 7 μg/ml). Then, an amount of 0.2 ml from each concentration was subcutaneously injected into each of eight mice (one mouse for each dilution).

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Mortalities were then recorded at 24 hours post-envenomization. The range of each venom concentration was then narrowed to the required concentration for subsequently conducting the main full lethality assays.

Based on the preliminary screening of each venom lethality, the 4 actively effective concentrations of each venom (data not shown) that caused mice mortality ranging from 0.0 to 100% were used to carry out a full lethality test to determine the LD_{50} and LD_{95}. In this experiment, 5 groups (one group for each concentration) of mice (5 mice/group; $n = 5$) were subcutaneously injected with the venom. Mortalities were recorded at 24h post-envenomization, and both LD_{50} and LD_{95} were calculated by the Probit analysis according to [68] and following [69].

2.6. Envenomization of Rabbits

Upon determining the LD_{95} of each venom in mice, it was converted into the equivalent doses for rabbits according to [70] and following [48]. Fifteen rabbits were divided into three groups: A, B, and C (5 rabbits each; $n = 5$). Each animal in groups A and B was injected with 0.5 ml venom aliquot that contained the equivalent LD_{95} of the *W. aegyptia* snake (0.264 mg/rabbit) and *A. crassicauda* scorpion (10.064 mg/rabbit), respectively. Venoms were intravenously injected into rabbits via the ear vein (Figure 1D) following [71]. The use of intravenous injection was chosen for its precision in delivering a controlled dose, although it does not perfectly mimic natural bites or stings. Envenomized rabbits were deceased within 10 to 20 min post-envenomization. In parallel, the control group (group C) was injected with 0.9% saline prior to euthanasia with CO_2 according to [72] and following [46]. Dealing with and killing animals took place in accordance with the Research Ethical Committee at King Saud University (approval code: KSU-SE-23-83).

2.7. Experimental Design

Within a maximum of 1 h from confirmed death, rabbit corpses were immediately translocated to the experimental site. Corpses were individually placed in a metal cage (50 × 40 × 25 cm each) (Figs. 1E) to protect them against predation, as detailed in [45,46]. Cages were placed a minimum of 10 meters away from one another to provide isolated resources for corpse-seeking insects, as recommended by [73] and to reduce the effect of odor interference, which could affect insect attraction [48,74]. Three pitfall traps (each of 10 cm in diameter, 5 cm in depth) were placed adjacent to each corpse (Figure 1E) to collect the attracted insects beyond the collection times [75]. Each trap was containing a solution composed of 250 ml of water, 5% soap powder, and 5% NaCl according to [45]. Since direct sunlight impacts insect succession compared to shadow [76], all experimental corpses were standardized by placing them in shady places underneath trees.

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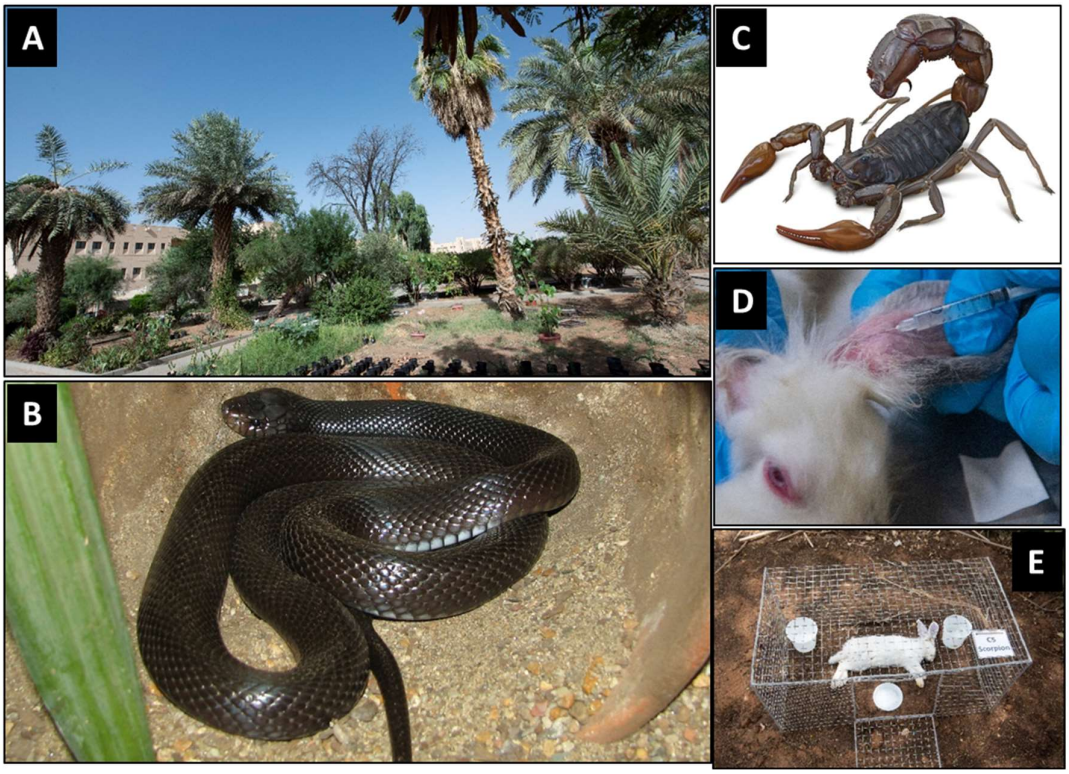

Figure 1. A displaying plate shows A: part of the botanical garden targeted by the study, B: the desert black snake, *W. aegyptia* (from [57]), C: the fat-tail scorpion, *A. crassicauda* (from [61]), D: the ear intravenous injection of rabbits, and E: an example of rabbit corpses in its protective metal cage along with the pitfall traps.

2.8. Decomposition Process

Each corpse was examined and investigated for 5 minutes in a daily manner, from the first day of the exposure until complete skeletonization, according to [48,77]. The four known decomposition stages: fresh, bloating, decayed, and dry (Figure 3) were observed and described following [45,46,48]. The duration of each stage (in days) was reported until complete skeletonization (dryness).

2.9. Beetles Collection and Identification

Corpse-attracted beetles were collected from corpses on an hourly basis (2 minutes for each corpse) from 6 a.m. to 4 p.m. over the first three days of the experiment. Then, insects were collected once a day at 6 a.m. until the end of the experiment according to [78] and following [45,48]. Only adult were included in the counting of collected beetles during this study. Beetles were collected from and underneath corpses using soft forceps and a spatula (3 cm in width and 10 cm in length) following [79]. Pitfall traps were also used not only to maintain monitoring beyond the time of collection but also to reduce the disturbance of carcasses-inhabitant insects for later sampling. Collected beetles were preserved in 70% ethanol and stored at 4°C until used for counting and identification as detailed in [79]. Beetles were morphologically identified to the species level at the King Saud University Museum of Arthropods (https://cfas.ksu.edu.sa/en/node/3075) by the 3rd and 4th authors, H. Al-Dhafer and M. Abdel-Dayem, respictively.

2.10. Statistical Analysis

Mice-corrected mean lethality values were used to calculate the LD_{50} and LD_{95} of each venom using Probit Analysis following [68]. The resulting mean values from Probit analysis are considered significant ($P < 0.5$) if their 95% confidence limits (Upper to Lower) are not overlapped, according to [80,81]. The Minitab software (MINITAB, State College, PA, version 18.1, 2018, UK) was used to

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statistically analyze the results of total counts of beetles. Prior to any further analysis, the normality of insects' counts was tested using the Anderson–Darling Normality Test, according to [82]. Because the overall data of beetle counts were normally distributed, the One-way ANOVA was used for the comparisons between the treated groups, and the Multiple Tukey's Pairwise Comparison test was used to analyze the differences between means, according to [82]. However data sets pertaining to the coleopteran families and species counts were not normally distributed and, thus, were analyzed using the non-parametric Mann–Whitney *U*-test. Finally, singleton or doubleton is considered when only one or two individuals of a particular beetle species are reported, respectively, according to [83] and following [45,46,48,76]. All results are presented as means of 5 replicates using five different individual rabbit corpses ($n = 5$) ± standard errors (SE), as determined by the Basic Statistical Analyses

3. Results

3.1. Venoms Lethality

As shown in Table (1), Probit analysis revealed LD_{50} of the *W. aegyptia* snake venom was 26.7 times higher than that of the *A. crassicauda* scorpion ($P < 0.05$), with non-overlapping confidence limits. In addition, the LD_{95} of *W. aegyptia* venom was 38.12 times higher than that of *A. crassicauda* ($P < 0.05$), with non-overlapping confidence limits. These LD_{95} values were converted into the equivalent (4×) for rabbits (0.264 and 10.064 mg/rabbit, respectively) and were considered the lethal doses used for rabbit envenomization.

Table 1. Results of Probit analysis showing LD_{50} and LD_{95} of snake and scorpion venoms in mice at 24h post-envenomization.

Venom types	LD_{50} (mg/kg) (lower-upper)	LD_{95} (mg/kg) (lower-upper)	Slope ± SE
W. aegyptia	0.053* (0.052-0.054)	0.066** (0.063-0.069)	18.29 ± 3.22
A. crassicauda	1.416* (1.288-1.558)	2.516** (2.251-2.813)	6.59 ± 0.91

Values marked by * and by ** are significantly different ($P < 0.5$) as their 95% confidence limits (lower to upper) are not overlapped, according to [80,84].

3.2. Meteorological Parameters

3.2.1. Atmospheric Parameters

The atmospheric parameters over the course of the 11-day experiment in Riyadh City are shown graphically in Figure (2). The maximum, minimum, and mean temperatures were constant (Figure 2A), with averages of 41.0, 29.27, and 35.14°C, respectively (Figure 2B). The relative humidity (RH) was fluctuating from 5% during the first day to 13% during the last day of the experiment (Figure 2A), with an average of 7.8% (Figure 2B). Wind temperature was constant with an average of 32.03°C, while wind speed was fluctuating from 5 to 31 km/h during days 7 and 9, respectively (Figure 2A), with an average of 13.15 km/h (Figure 2B). These atmospheric parameters are within the natural range during this time of the year in Riyadh city [44].

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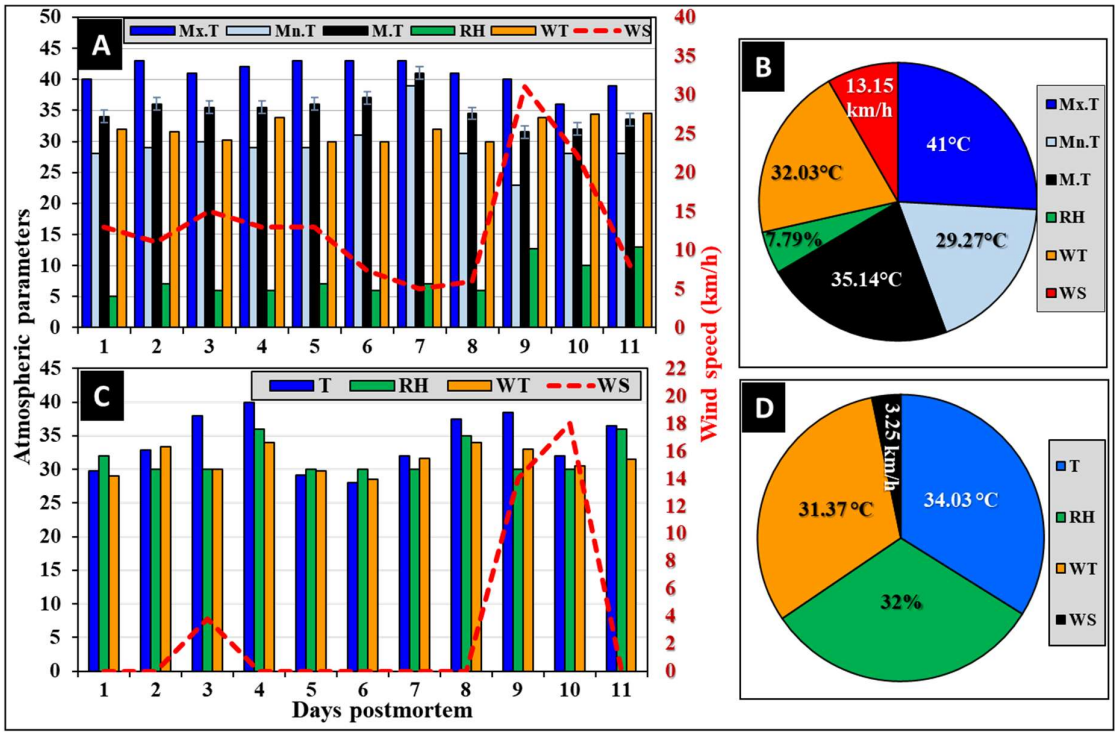

Figure 2. The meteorological parameters over the experimental period show the atmospheric parameters (A) and their overall averages (B) in Riyadh city, and the on-site manually recorded daily weather parameters (C) and their overall averages (D) at the experimental sites. Max.T: maximum temperature, Min.T: minimum temperature, M.T: the mean of maximum and minimum temperatures (n = 11), RH: relative humidity, WT: wind temperature, and WS: wind speed.

3.2.2. On-site Recorded Weather Parameters

The on-site manually recorded weather parameters (Figure 2C and D) were fluctuating over the experimental period compared to the officially recorded ones by the Saudi National Center for Meteorology for Riyadh City (Figure 2A and B). The temperature recorded a minimum of 29°C on day 6 and a maximum of 38°C on day 4 (Figure 2C), with an average of 34°C (Figure 2D). Relative humidity recorded a minimum of 30% and a maximum of 36% (Figure 2C), with an average of 32% (Figure 2D). Wind temperature recorded a minimum of 29°C and a maximum of 34°C (Figure 2C), with an average of 31.37°C (Figure 2D). Fluctuations were noticeable in the on-site recorded temperature, RH, and wind temperature. The maximum fluctuation was recorded in wind speed, as most days recorded 0.0 km/h, while the maximum speed was 18 km/h on day 10 (Figure 2C), with an average of 3.25 km/h (Figure 2D).

3.3. Decomposition Process

The four reported decomposition stages over the study period are the fresh, bloating, decay, and dry (Figure 3). In all three treatments, rabbit corpses appeared in the fresh stage as if they were alive in terms of softness and flexibility at the beginning of death (Figure 3A). This stage lasted for 31 hours in the control group, whereas it lasted for 21 hours in both envenomized corpses (Table 2). It was noticeable that snake- and scorpion-envenomized corpses became stiffened and somewhat rigid by 30 minutes and 6 hours postmortem, respectively, and both began to emit slight unpleasant odors. This may indicate that envenomization have shortened the duration of the fresh stage as the bloating stage started earlier compared to the control (Table 2).

Table 2. Duration of the decomposition stages of envenomized corpses post-envenomization with the snake and scorpion venoms.

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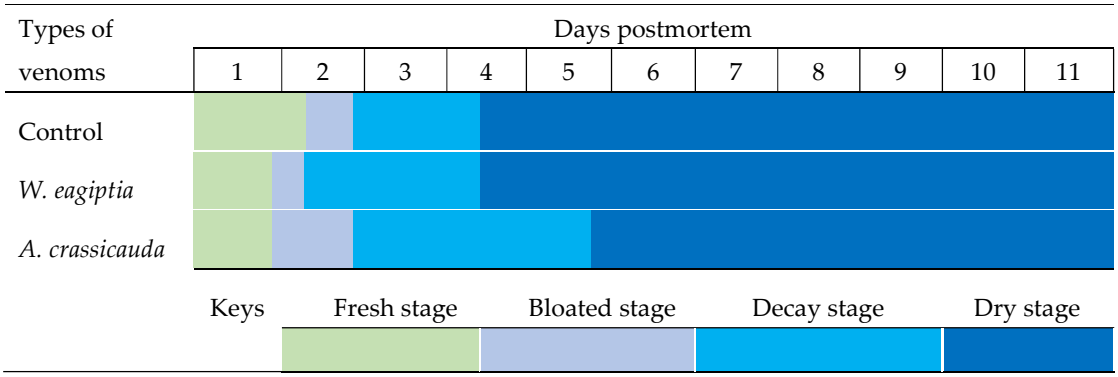

In the bloating stage (Figure 3B), corpses started to swell from the abdominal area, progressing to the chest and neck, releasing offensive odor, but with different durations between treatments. This stage lasted for 10 and 24 hours in snake- and scorpion-envenomized corpses, respectively, compared to 14 hours in the control ones (Table 2). It was noticeable that scorpion-envenomized corpses were releasing stronger offensive odor in this stage compared to those of snake-envenomized and control ones. This may indicate that envenomization with snake venom shortened the bloating stage as the decay stage started earlier compared to those of control and scorpion-envenomized ones.

The decay stage also varied among the three treatments (Figure 3C1). The control corpses showed distributed decomposition across body areas, partial appearance of larvae from body openings, strong offensive odors, fluid exudation, and lasted for about 48 hours (Table 2). Snake-envenomized corpses started decomposition earlier and was observed in the chest and neck areas, the abdominal contents were noticeably spreading around the corpse, significant fluid exudation, and lasted for approximately 62 hours (Table 2). While scorpion-envenomized corpses started the decomposition with the control ones that was evident in the neck, chest, and forelimbs, with a stronger offensive odor. There weren't as many corpse-colonizing larvae as in the control and snake-envenomized ones (Figure 3C2). The smell was also worse and stronger than in the other groups, and lasted for about 72 hours (Table 2). This may indicate that envenomization prolonged the duration of the decay stage compared to that of the control.

In the dry stage, all corpse groups were characterized by complete dryness and rigidity (Figure 3D). This stage started at approximately 117 hours (4.9 days) postmortem in scorpion-envenomized corpses compared to 93 hours (3.9 days) in both control and snake-envenomized ones (Table 2). This may indicate that envenomization with scorpion venom delayed the start of the dry stage (up to 1 day) since the durations of the preceding stages have increased.

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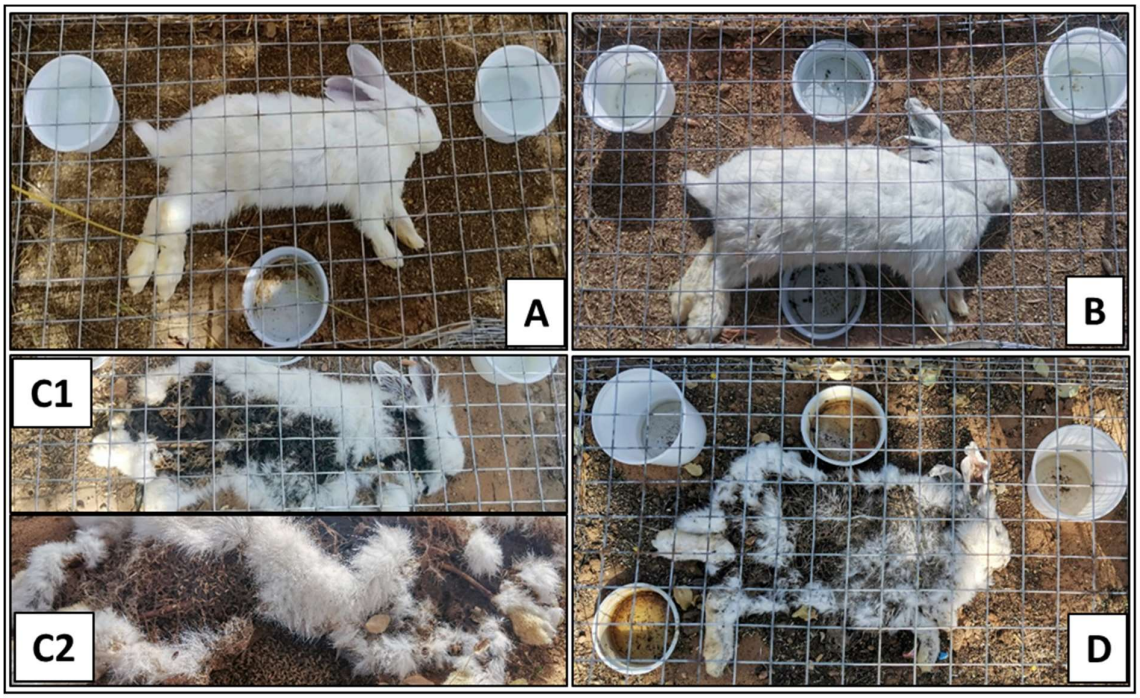

Figure 3. The postmortem characteristic features of the reported four decomposition stages of the rabbit corpses inside their metal cages. A: Fresh stage, B: bloating stage, C1 & C2: decayed stage & the associated colonizing larvae, respectively, and D: dry stage.

3.4. Abundance of Corpse Associated Beetles

A total of 1094 corpse-attracted beetles were collected from all the experimental corpses during the entire experimental period of this study. Out of them, 36.2% (396) and 29.1% (319) beetles were collected from snake- and scorpion-envenomized corpses, respectively, compared to 34.7% (379) collected from the control ones. As shown in Figure (4A), beetles attracted to all corpses from the first day of exposure in fewer numbers increased to the maximum during days 4 and 5, after which it reduced again to the minimum at the end of the experiment. The Interval Plot of beetles succession in each treatment *versus* days postmortem over the experimental period (from day 1 to day 11) was calculated and created by the Pooled Standard Deviation, which revealed four major succession peaks (waves) of attracted beetles to corpses during the period from day 2 to day 7, with the highest peak on day 4 (Figure 4B)

When comparing the envenomized corpses over the 11 day-experimental period, the one way ANOVA revealed significant more beetles attracted to the snake-envenomized corpses on days 2 and 3, about the same number on day 4, and significantly fewer on day 5 compared to the scorpion-envenomized ones ($F_{32,132}$ = 12.36, P < 0.05, n = 5) (Figure 4A). However, scorpion-envenomized corpses attracted significant more beetles later on day 5 (Figure 4A). These data may indicate that snake-envenomized corpses attracted more beetles during the first 4 days of exposure compared to the scorpion-envenomized ones, which attracted more beetles later. This may suggest a distinctive difference in beetles' succession in a venom-dependent manner.

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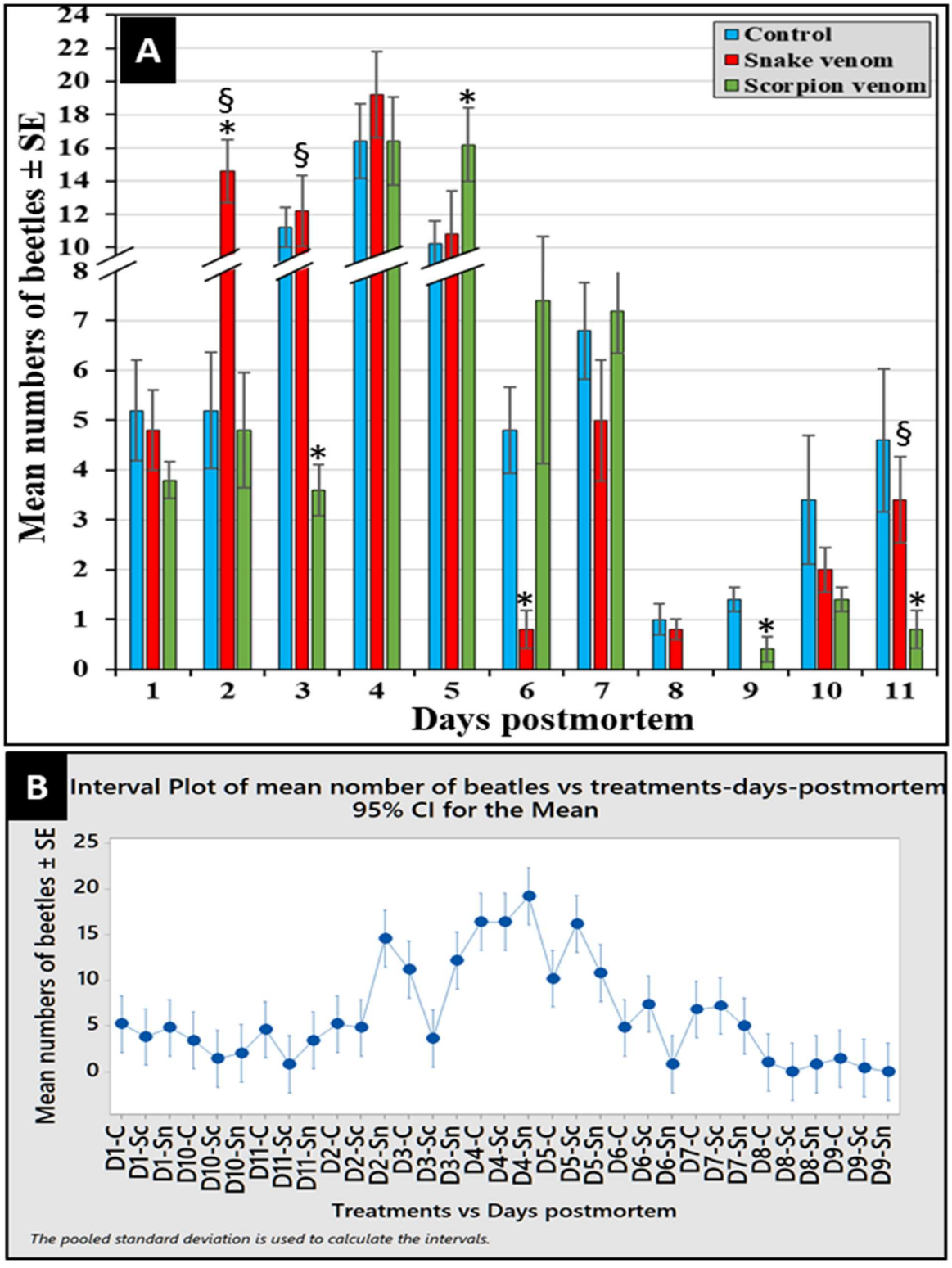

Figure 4. The abundance of corpse-attracted beetles post-envenomization (A), and the Interval Plot of mean numbers of beetles (B) in each treatment versus days over the experimental period (from D1 to D11). C: control, Sn: snake-envenomized, and Sc: scorpion-envenomized corpses. Error bars represent standard errors of means of 5 replicates (n = 5). The sign (*) indicates significant differences compared to the control, while sign (§) indicates significant differences between envenomized corpses ($P < 0.05$, $n = 5$).

3.5. Differential Abundance of Beetles

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The impact of venom type on the differential abundance of attracted coleopteran families is represented in Figures (5A). Overall, the most abundant families were Histeridae followed by Dermestidae, Scarabaeidae, and Tenebrionidae, while the least abundant ones were Zopheridae, followed by Ptinidae, Hybosoridae, Chrysomelidae, Nitidulidae, and Elateridae (Figure 5A). As shown in Figure (5B), the Interval Plot of the pooled standard deviations revealed three highest waves of beetles' succession distinctively to the snake-envenomized corpses represented by (Histeridae, followed by Dermestidae and Scarabaeidae), followed by another five waves to the control ones by (Dermestidae followed by Histeridae, Scarabaeidae, Curculionidae, and Tenebrionidae). While only three moderate waves, represented by (Histeridae followed by Dermestidae and Scarabaeidae), were attracted to the scorpion-envenomized corpses. These data clearly show that Dermestidae, Scarabaeidae, and Histeridae are the 3 prevalent families attracted to all corpses, while Curculionidae and Tenebrionidae were distinctively attracted to control corpses only. These data may indicate that beetles are less attracted to envenomized corpses compared to control ones, the intensity of attracted families varies based on the type of venom; in a venom-dependent succession manner, and that scorpion-envenomized corpses attracted less families compared to the snake-envenomized ones (see also Figure 4).

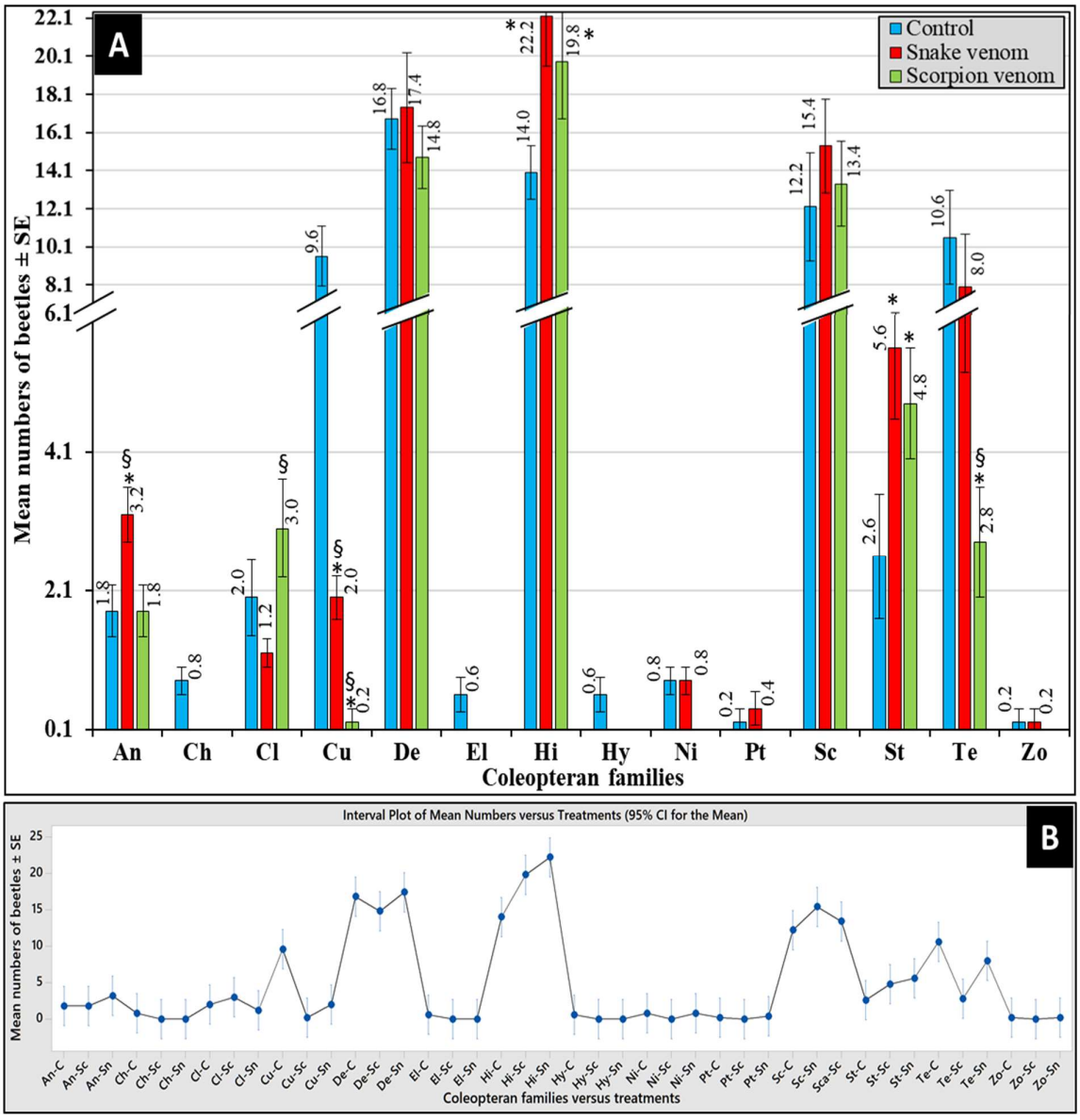

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Figure 5. The abundance of reported corpse-attracted coleopteran families post-envenomization over the experimental period (A). Error bars represent the standard errors (SE) of means of 5 replicates ($n = 5$). The Interval Plot of the pooled standard deviations (B) shows various succession waves of coleopteran families versus envenomization with snake- (Sn), scorpion-envenomized (Sc), and control (C). An: Anthicidae, Ch: Chrysomelidae, Cl: Cleridae, Cu: Curculionidae, De: Dermestidae, El: Elateridae, Hi: Histeridae, Hy: Hybosoridae, Ni: Nitidulidae, Pt: Ptinidae, Sc: Scarabaeidae St: Staphylinidae, Te: Tenebrionidae, and Zo: Zopheridae. The sign (*) indicates significant differences compared to the control, while sign (§) indicates significant differences between envenomized corpses ($P < 0.05$).

The overall Kruskal–Wallis test revealed significant differences in the mean number of attracted beetles within treatments ($\alpha < 0.05$, H = 182.74, DF = 41, $P < 0.05$). As shown in Figure (5A), Mann-Whitney *U*-test revealed that snake-envenomized corpses attracted significant higher numbers of beetles from Anthicidae and Staphylinidae but significant lower of Cleridae and Curculionidae, compared to control ones ($P < 0.05$, $n = 5$). However, scorpion-envenomized corpses attracted significantly higher numbers of beetles of Histeridae and Staphylinidae, but lower of Curculionidae and Tenebrionidae compared to control ones. When comparing the envenomized corpses, the snake-envenomized ones attracted significantly higher numbers of Anthicidae, Curculionidae, and Tenebrionidae but significant lower of Cleridae compared to scorpion-envenomized ones ($P < 0.05$, $n = 5$). While control corpses distinctively attracted Chrysomelidae, Elateridae, and Hybosoridae. These data may indicate that the recorded families are distinctively attracted to corpses in a venom-dependent succession manner and that more beetles were attracted to snake-envenomized corpses compared to scorpion-envenomized ones.

3.6. Differential Succession of Beetles

A total of 27 species of beetles belonging to 14 families were reported and identified in this study (Table 3). Of them, Anthicidae is represented by 38 specimens of *Omonadus formicarius* (Goeze, 1777); Chrysomelidae is represented by 4 *Caryedon acaciae* (Gyllenhal, 1833); Cleridae is represented by 23 *Necrobia rufipes* (Fabricius, 1781) and 8 *Necrobia sp.*; Curculionidae is represented by 2 *Coccotrypes rhizophorae* (Hopkins 1915) and 57 *Dinoderus sp.*; Dermestidae is represented by 9 *Attagenus posticalis* Fairmaire, 1879, 127 *Dermestes maculatus* De Geer, 1774, and 114 *Dermestes frischi* Kugelman, 1792; Elateridae is represented by 3 *Aeoloides grisescens* (Germar, 1844); Histeridae is represented by 294 *Saprinus chalcites* (Illiger, 1807) and 4 *Saprinus caerulescens* (Hoffmann, 1803); Hybosoridae is represented by 3 *Hybosorus illigeri* Reiche, 1853; Nitidulidae is represented by 8 *Carpophilus hemipterus* (Linnaeus, 1792) and a singleton (1 individual beetle) of *Urophorus humeralis* (Fabricius, 1798); Ptinidae is represented by 3 *Stegobium paniceum* (Linnaeus, 1758); Scarabaeidae is represented by 181 *Aphodius adustus* Klug, 1855, 27 *Malader insanabilis* (Brenske, 1894), and 10 *Rhyssemus saoudi* Pittino, 1984; Staphylinidae is represented by 4 *Leptacinus* sp. and 62 *Philonthus* sp.; Tenebrionidae is represented by a singleton of *Adesmia cancellate* Klug,1830, 11 *Alphitobius diapernius* (Pancer, 1797), 22 *Mesostena pincticollis* Solier, 1835, 45 *Opatroides punctulatus* Brulle, 1832, and 31 *Thriptera crinite* (Klug,1830); and Zopheridae is represented by a doubleton (2 individual beetles) of *Synchita* sp (Table 3).

Table 3. Recorded coleopteran families and species at different stages of decomposition of the experimental copses.

Coleopteran Families	**Beetle Species**	**Control**				***W. aegyptia***				***A. crassicauda***			
(Total number)	**(Total number)**	**Fr**	**Bl**	**De**	**Dr**	**Fr**	**Bl**	**De**	**Dr**	**Fr**	**Bl**	**De**	**Dr**
Anthicidae (38)	*O. formicarius* (38)	+	–	+	+	+	–	+	+	+	+	+	+
Chrysomelidae (4)	*C. acaciae* (4)	+	–	+	+	–	–	–	–	–	–	–	–
Cleridae (31)	*N. rufipes* (23)	–	–	+	+	–	–	+	+	–	–	+	+

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	Necrobia sp. (8)	–	–	+	–	–	–	–	+	+	+	–	–
Curculionidae (59)	*Dinoderus* sp. (57)	+	–	+	+	+	–	+	+	–	–	+	–
	C. rhizophorae (2)	+	–	–	–	–	–	–	–	–	–	–	–
Dermestidae (250)	*D. maculatus* (127)	–	–	+	+	–	+	+	+	–	+	+	+
	D. frischi (114)	–	–	+	+	–	–	+	+	–	+	+	+
	A. posticalis (9)	–	–	+	+	+	–	+	–	–	–	+	–
Elateridae (3)	*A. grisescens* (3)	–	+	+	–	–	–	–	–	–	–	–	–
Histeridae (298)	*S. chalcites* (294)	–	+	+	+	+	+	+	+	–	+	+	+
	S. caerulescens (4)	–	–	–	–	–	–	+	–	–	–	+	+
Hybosoridae (3)	*H. illigeri* (3)	+	–	+	–	–	–	–	–	–	–	–	–
Nitidulidae (9)	*C. hemipterus* (8)	–	–	+	+	–	–	+	+	–	–	–	–
	U. humeralis (1)	–	–	–	–	–	–	+	–	–	–	–	–
Ptinidae (3)	*S. paniceum* (3)	+	–	+	–	–	–	–	–	–	–	–	–
Scarabaeidae (218)	*A. adustus* (181)	+	–	+	+	+	–	+	+	–	+	+	+
	R. saoudi (10)	–	–	+	+	+	–	+	+	–	+	+	+
	M. insanabilis (27)	–	–	+	+	+	–	+	+	+	+	+	+
Staphylinidae (66)	*Philonthus sp.* (62)	–	–	+	+	–	–	+	+	–	–	+	+
	Leptacinus sp. (4)	–	–	–	–	–	–	+	–	–	–	–	–
Tenebrionidae (110)	*M. pincticollis* (22)	+	–	+	+	+	–	–	+	+	–	–	–
	T. crinite (31)	+	–	+	+	+	–	–	+	+	–	–	+
	A. diapernius (11)	–	–	–	+	–	–	–	–	–	–	–	–
	O. punctulatus (45)	+	–	+	+	+	–	+	+	+	+	–	+
	A. cancellate (1)	–	–	–	–	+	–	–	–	–	–	–	–
Zopheridae (2)	*Synchita* sp.(2)	–	–	+	–	–	–	+	–	–	–	–	–

Fr: fresh stage, Bl: bloating stage, De: decayed stage, Dr: dry stage. The positive sign (+) indicates recorded beetle while negative sign (-) indicates no record.

Figure 6 represents the differential succession of the 14 coleopteran families during the four decomposition stages. Out of them, 8 families were attracted to all corpses in various succession manners (Figure 6A, C, D, E, G, K, L, and M). The non-parametric Mann-Whitney *U*-test revealed that control corpses attracted significant more beetles from Curculionidae, Histeridae, Nitidulidae, and Staphylinidae during the decay and/or dry stages comparted to the envenomized ones ($P < 0.05$, $n = 5$) (Figure 6C, D, K, and M). For comparing the envenomized corpses, the snake-envenomized ones attracted significant more beetles from Anthicidae, Dermestidae, and Histeridae during the decay stages compared to the scorpion envenomized ones ($P < 0.05$, $n = 5$) (Figure 6A, E, and G). While scorpion-envenomized corpses attracted significant more beetles from Cleridae and Histeridae during the dry stages compared to the snake-envenomized ones ($P < 0.05$, $n = 5$) (Figure 6C and G). This may indicate a differential succession to corpses in a distinctive venom-dependent manner.

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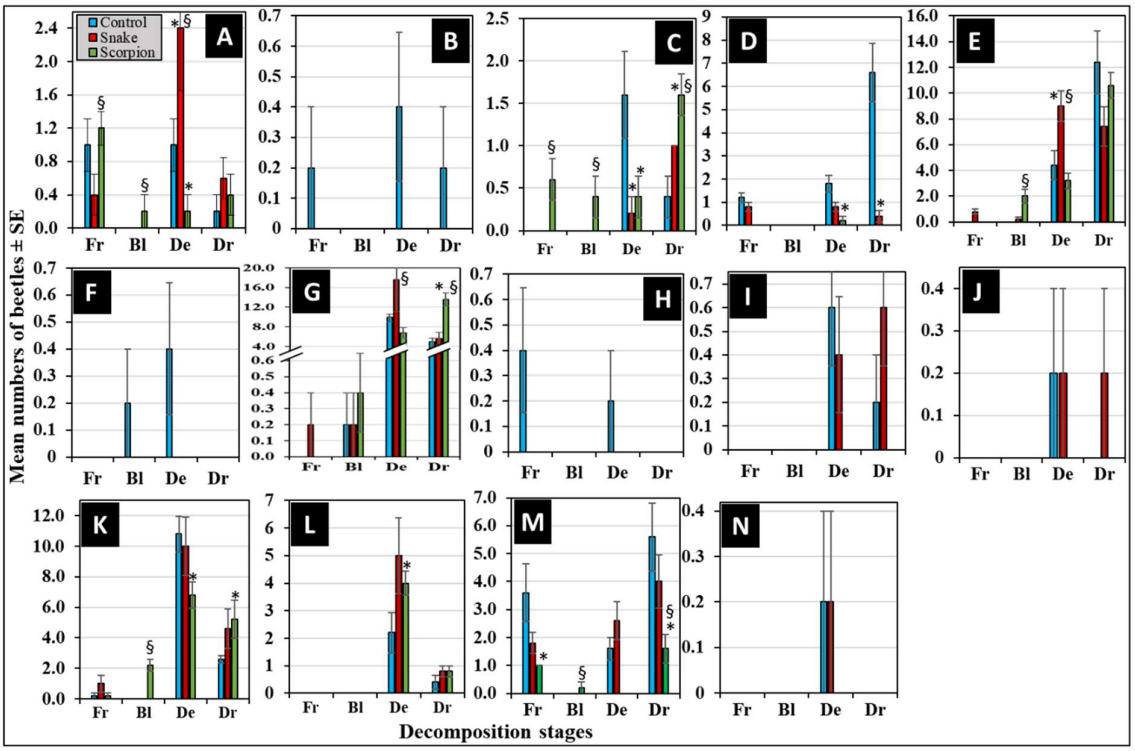

Figure 6. Differential abundance of corpse-attracted coleopteran families during the decomposition stages: fresh (Fr), bloating (Bl), decayed (De), and dry (Dr). Individual figures represent families Anthicidae (A), Chrysomelidae (B), Cleridae (C), Curculionidae (D), Dermestidae (E), Elateridae (F), Histeridae (G), Hybosoridae (H), Nitidulidae (I), Ptinidae (J), Scarabaeidae (K), taphylinidae (L), Tenebrionidae (M), and Zopheridae (N). The sign (*) indicates significant difference compared to the control, while (§) indicates significant difference between envenomized corpses ($P < 0.05$, $n = 5$).

The other 6 families were attracted to corpses in a selective manner. This was evidenced by Chrysomelidae, Elateridae, and Hybosoridae that were distinctively attracted to the control corpses only during most of the decomposition stages (Figure 6B, F and H). While Nitidulidae, Ptinidae, and Zopheridae were distinctively attracted to the control and snake-envenomized corpses only during the decay and dry stages (Figure 6I, J, and N). While, none was distinctively attracted to scorpion-envenomized corpses. This may indicate that these particular families are selectively attracted to corpses in a venom-dependent manner during definitive decomposition stages.

At the species level, data in Table 3 reveal that *C. acaciae* (Chrysomelidae) and *A. grisescens* (Elateridae), followed by a doubleton of *C. rhizophorae* (Curculionidae) and *H. illigeri* (Hybosoridae) were distinctively associated with control corpses only. While *O. formicarius* (Anthicidae), *S. chalcites* (Histeridae), *A. adustus* (Scarabaeidae), and *O. punctulatus* (Tenebrionidae) were predominantly associated with all corpses during most decomposition stages (Table 3). There was a singleton of *U. humeralis* (Nitidulidae) and *A. cancellate* (Tenebrionidae) reported distinctively associated with snake-envenomized corpses. While a double ton of *Synchita* sp. (Zopheridae) was distinctively associated with control and snake-envenomized corpses during the decay stages (Table 3). These data may suggest a possible definitive relationship between the occurrence of a single species and a particular stage of decomposition in a venom-dependent manner.

4. Discussion

The present study proposed a forensic scenario for the envenomation-related death and its impact on the postmortem decomposition process and the succession pattern of the corpse-attracted

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beetles. It is important to clarify three points: a) neurotoxic venoms from two different local venomous animals, the *W. aegyptia* snake and the *A. crassicauda* scorpion, have been targeted to achieve the goal of this study, b) venoms were freshly extracted from the animals and their lethality were determined prior to carrying out this study, and c) the experimental rabbits have been envenomized by intravenous-injection with the lethal doses of venoms aliquots rather than being directly bitten or stung with the snake or scorpion, respectively.

It is well-known that the process of corpses decomposition constitutes a crucial step in the recirculation of organic materials throughout the food-chain in the nutrition cycle [77,85]. From the forensic point of view, this process provide vital evidence in the legal investigations as it has unique spatial- and temporal-dependent successive stages that aid the investigators in determining the PMI [86,87]. This process usually takes place in 4-5 stages [7] depending on many interacting environmental and non-environmental factors. The environmental factors include the atmospheric temperature and humidity [88,89], the habitat [79,90,91], and the type of soil in scene [92]. While the non-environmental factors include the corpse's chemical composition [8], microbial composition and activity [93,94], the activity of colonizing insects [95], the barriers such as clothes and coverages [96,97], and the cause of death [48,98–100].

Based on the aforementioned parameters, and in order to avoid any possible discrepancies, the current study was carried out in a botanical garden with a homogeneous clayey sandy soil, and all experimental groups of corpses were placed in identical metal cages in similar shady places away from the direct sunlight. Further, this study was conducted during June, which is a hot and dry month and characterized by stable climatic conditions with the normal atmospheric averages of temperature, humidity, and wind speed of this time of the year in Riyadh city [44]. Thus, through the 11-day experimental duration, .corpses reached the dry stage within 4 to 5 days in these atmospheric conditions. Therefor, we would expect that there was no external climatic impact on the decomposition process and beetle succession in this study, and hypothesize that the reported variation of the abundance and succession of beetles between the treated corpses may be attributed to the antemortem envenomization. Consequently, the current study reported four main successive decomposition stages: fresh, bloating, decaying, and dried, as per reported in our previously published works [45,46,48].

Envenomation by venomous animals is a considerable global death-leading cause [28,29,33,101,102]. In the Middle East and Africa, thousands of snakebites as well as scorpion and spider stings were documented [16,30,33,103,104]. Yet, few studies in some countries have investigated the decomposition process and succession of forensic insects on corpses upon death by envenomation with scorpion venoms [100,105] and snakes [48,51,106]. To the best of our knowledge, no such studies have been conducted in Saudi Arabia, and hence, the present study was undertaken to address this gap. Our data showed clearly that envenomization with the snake and scorpion venoms both reduced the duration of the fresh stage and elongated that of the decay stage. The reported significant variation in lethality between the *W. aegyptia* snake and the *A. crassicauda* scorpion has been reflected as a variation in the rate of decomposition. The prolonged decomposition rate was more pronounced in the scorpion-envenomized corpses in terms of longer duration in the bloating and decay stages. Consequently, the overall duration of the decomposition process was elongated in terms of a delay (up to one day) at the start of the dry stage compared to that of the snake-envenomized ones. In contrary, our per published work [48] reported an acceleration in the decomposition rate of rabbit corpses upon envenomization with venoms from the Egyption *Naja haje* and *Cerastes cerastes* snakes. These findings may indicate that the postmortem decomposition rate is venom-dependent and, hence, could lead to bias in the estimation of PMI. There are evidences for this conclusion provided by other studies that investigated intoxication with different types of toxic substances have differently impacted the postmortem carcass decomposition and insect succession [53,107–109]. Comprehending these venom-dependent phenomena is essential for forensic entomologists, as it facilitates the enhancement of PMI approximations by considering the particular venom variant implicated.

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The lethality has been estimated differently in different studies for the *W. aegyptia* snake (from 0.170 to 0.180 mg/kg) [62,110] and the *A. crassicauda* scorpion (from 1.1 to 1.7 mg/kg) [63,69]. These variations could be attributed to the variation of the laboratory and experimental conditions between investigators and to many other factors [111,112]. Our data revealed similar lethality (1.4 mg/kg) to that previously reported. While it was around 3.3 times greater than that determined by Al-Sadoon's group for the snake venom. This difference in lethality could be attributed to the variation in the injection routes and in both the type and body weight of the experimental animals [63,113]. In this regard, Al-Sadoon and his colleagues used the intraperitoneal route in rats (weighing 200–250 g each), while we used the subcutaneous route in mice (weighing 18-20 g each), as well as for other factors [111]. Our data also determined the snake venom lethality of 26.7 times that of the scorpion, which could be attributed to its chemical components as it contains many different kinds of enzymes [114,115] and non-enzymatic proteins [116]. Consequently, it has multiple modes of action, mainly cardio and neurotoxicity, interruption of many vital systemic functions [62,110,117–119], and finally systemic dysfunction leading to death [120]. While the *A. crassicauda* scorpion venom contains smaller neurotoxin polypeptides of low molecular weight simple proteins with lethal neurotoxic and paralytic effects [69] resulting in cardio-respiratory failure and finally death [120,121]. This variation in the chemical composition and mode of action, may explain the concomitant variation of the decomposition process, as scorpion-envenomized corpses decomposed slower than the control and snake-envenomed ones. In contrary, other causes of death, like antemortem heroin-injection, was found to accelerate the decomposition of rabbit corpses [71]. These findings may suggest that the antemortem cause of death could potentially impact the postmortem decomposition process and that envenomation impacts differently the decomposition process based on the type of venom.

It is well known that dipteran flies are the first to attract to corpses during the very early decomposition stages, while others, like coleopteran beetles, are usually attracted to corpses during the latter stages [7]. Our data showed a maximum abundance of corpse-attracted beetles between days 4 and 5 (during the decay stage). This unique pattern of insect succession could be due to the effect of unique cadaveric volatile organic components which give cadavers their unique smell and attract a wide range of cadaver-seeking insects. In this context, up to 104 cadaveric volatile chemical compounds were identified during the decomposition process [122]. Therefore, the stronger and more unpleasant smell that we smelled coming from scorpion-envenomized corpses, compared to those of the other groups, may be attributed to the effect of certain chemicals in the scorpion venom [63,65]. Evidence for this is provided by [122], who reported cadaveric volatile components differently in different biotopes. This may explain the attraction of few beetles to the scorpion-envenomized corpses compared to those of the control and snake-envenomized ones. Moreover, the delay in the abundance of attracted beetles on the scorpion-envenomized corpses (up to day 5), compared to the snake-envenomed ones, may be attributed to the difference in the chemical components and the mode of action of venoms. A recent study conducted by [51] reported fewer number of attracted beetles to envenomed carcasses compared to controls, and fewer attracted beetles to *N. haje* snake envenomed carcasses compared to *C. cerastes* snake-envenomed ones. However, other causes of death, like heroin injection, showed no impact on the postmortem beetles succession patterns [71].

Data of differential abundance revealed variation in the succession of the coleopteran families. The most predominant corpses-attracted families were Histeridae (298 beetles) during all decomposition stages except the fresh stage. The second was Dermestidae (250 beetle) followed by Scarabaeidae (218 beetles) both during all stages except fresh and bloating ones. The third was Tenebrionidae (110 beetles) during selective decomposition stages. The least was Zopheridae (2 beetles) during the decay stage. In agreement with our results, Dermestidae, Histeridae, and Scarabaeidae were also reported as predominant families associated with rabbit corpses [79,123] and with pig corpses [124]. These data suggest two differential succession manners: the first is a differential predominant manner shown by four families (Curculionidae, Histeridae, Nitidulidae, and Staphylinidae), three families (Anthicidae, Dermestidae, and Histeridae), and two families

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(Cleridae and Histeridae) that were particularly predominant on control, snake-envenomized, and scorpion-envenomized corpses, respectively, during the decay and/or dry stages. Histeridae was the only predominant family on all corpses, regardless of the type of treatment. The second manner is a distinctive predominant manner showed by three families (Chrysomelidae, Elateridae, and Hybosoridae) that were distinctively attracted to control corpses only, and three families (Nitidulidae, Ptinidae, and Zopheridae) that were attracted to both control and snake-envenomized corpses only. While no definitive families were distinctively attracted to scorpion-envenomized corpses. These differentially attracted beetles could serve as potential indicators for differentiating between the types of venoms and, consequently, suggest a venom-dependent succession manner. On the other hand, the unexpected presence of Chrysomelidae, Curculionidae, Elateridae, and Zopheridae on corpses provides insight into the intricate ecological interactions surrounding the corpses. These particular families are known primarily for their plant-feeding or wood-boring behaviors rather than being necrophagous [125]. This may be attributed to either the surrounding vegetation of the experimental site or their opportunistic feeding on fungi growing on corpses. This, in fact, may suggest a broader ecological context for the decomposition process, a potential impact on the succession pattern of more conventional necrophagous insects, and ecological indication of the crime scene, which could be of forensic relevance. In forensic investigations, the accurate identification of particular species of beetles may assist in ascertaining whether a fatality resulted from envenomation by a snake or a scorpion, thereby offering critical evidence in circumstances where the etiological factors of death remains ambiguous.

At the species level, *C. acaciae* (Chrysomelidae) was distinctively associated with control corpses only during all decomposition stages except the bloating stage. While *O. formicarius* (Anthicidae), *S. chalcites* (Histeridae), *A. adustus* (Scarabaeidae), and *O. punctulatus* (Tenebrionidae) were predominantly associated with all corpses during most decomposition stages. These data may indicate an association of a certain beetle species with the treatment but not with the decomposition stages. Further, the reported singleton and doubleton were associated with the fresh and decay stages, which may suggest an association of the singleton or doubleton with particular decomposition stages of a particular treatment. This is in contrary to the findings of [79], who reported no association between the attraction of a single species and a particular stage of decomposition. This may be due to the difference in the experimental sites, as they were comparing between different habitats (agricultural, desert, and urban). No definitive species was distinctively reported as associated with the scorpion envenomized corpses. Reporting certain corpse-associated beetles during the early stages of decomposition, like fresh and bloating stages, might be due to their seasonal appearance rather than linking to the decomposition stage [126].

5. Conclusions

This study revealed that antemortem envenomization of rabbits with snake and scorpion venoms has significantly affected the corpses' decomposition rate, with the impact being more pronounced in scorpion-envenomized ones. This impact was evidenced by the elongation of the bloating and decay stages, as well as a delay of up to one day in reaching the dry stage compared to the control corpses. The differential abundance and succession pattern of corpse-associated beetles varied significantly between the envenomized and control corpses, suggesting a venom-dependent succession. Notably, families such as Histeridae, Dermestidae, Scarabaeidae, and Tenebrionidae were predominant in all corpses. While others showed distinctive associations with specific treatments or decomposition stages. At the species level, there were unique associations between certain beetle species and specific treatments or decomposition stages. For instance, the singleton of *U. humeralis* (Nitidulidae) and *A. cancellate* (Tenebrionidae) were distinctively associated with snake-envenomized corpses. The doubleton of *Synchita* sp. (Zopheridae) was distinctively associated with the control and snake-envenomized corpses. While, *C. acaciae* (Chrysomelidae) and *A. grisescens* (Elateridae), followed by a doubleton of *C. rhizophorae* (Curculionidae) and *H. illigeri* (Hybosoridae), were distinctively associated with control corpses solely. These findings may indicate that the

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succession of the reported beetles is venom-dependent, which could be helpful as envenomation markers. This may aid in understanding the influence of various venom types of various lethality from various venomous animals on both corpse decomposition and insect succession, as well as identifying which venom type has the most significant postmortem impact. Finally, the herbivorous (non-necrophagous) families, Chrysomelidae, Curculionidae, Elateridae, and Zopheridae, were unexpectedly reported associated with corpses, which could be potential ecological markers of the crime scene. This, in fact, may provide additional valuable forensic evidence in forensic investigation and updating the database of the envenomation-related corpse decomposition process and the associated beetle taxa in Saudi Arabia. These findings can enhance PMI estimation and aid in differentiating between causes of death involving venomous animals, ultimately contributing to more accurate crime scene reconstructions and legal investigations.

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Data Availability Statement: All data pertinent to this work are presented in this article. Further requests should be directed to the corresponding author.

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