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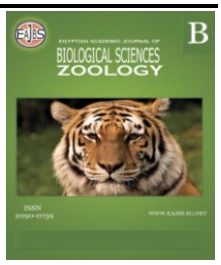

Influence of Scorpion Venom on Decomposition and Arthropod Succession Using Rabbits' Carrions

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ABSTRACT

Background: The contributions of forensic entomology and arthropod in legal investigations have been known for centuries. There are many ways that insects can be used to help in solving a crime, but the primary purpose of forensic entomology is the determination of postmortem interval. The aim of this work is to evaluate the effect of scorpion venom on the decomposition of rabbits' carcasses and on arthropods' colonization of rabbits' carcasses in indoor and outdoor environments. Methods: Twenty male rabbits were included in the study, divided into four groups, with 5 rabbits in each group. two groups were killed by spinal cord separation; group (1) kept in an indoor environment and group (3) in an outdoor environment. The other two groups were killed by scorpion stings (groups 2 and 4), kept indoors, and outdoor respectively. The carcasses were allowed to decompose, and arthropods were collected daily and examined morphologically. Results: The decomposition findings were less prominent in groups 2 and 4 with less maggot mass compared to the control groups. Furthermore, arthropod species were different and showed morphological changes in the form of dryness with the formation of ulcers in the larvae, appearance of irregular groves and tunnels in insects and dryness of the outer layer with damage to the ends of beetles. Conclusion: Scorpion venom affected the decomposition rate of rabbits' carcasses and arthropods' colonization with the difference in morphological appearance of arthropods between the control groups and scorpion stinging groups, which may be of forensic importance.

INTRODUCTION

Forensic arthropodology is the science that deals with arthropods and insects and its application with other forensic sciences in medicolegal investigations (Abdelaziz *et al.*, 2022). The identification of different species and developmental stages of arthropods on corpses helps in estimating the post-mortem interval. This can be achieved successfully by using other crucial data such as the season of death, the scene of death, and the movement of the victim's remains after death (Sharma *et al.*, 2015). Forensic anthropology also could determine the presence of toxins, in addition to the

identification of the victims, the geographical area, and the season according to the stage of decomposition of the body (Jaf *et al.,* 2021).

Arthropods are a very large and diverse group of invertebrates, found in all types of environments (Tiemeyer *et al.,* 2017 and Abdelaziz, 2018). Two important groups of insects are attracted to human cadavers and animal carriers and yield beneficial evidence in the medicolegal investigation: the flies and the beetles (Catts and Goff 1992). In general, Diptera is the main fly inhabiting decomposing cadavers and carrion especially Calliphoridae, Sarcophagidae, Muscidae and Piophilidae families (Smith, 1986). Beetles are commonly detected in the advanced stages of decomposition (Byrd and Castner 2009). So far, limited data is obtainable on beetles' colonization and decomposition process in temperate regions (Dekeirsschieter *et al.,* 2011).

Scorpions belong to the phylum Arthropoda found in every place in the world except Antarctica. Scorpions sting by using their long, flexible tails causing painful sensations associated with the envenomation (Cao *et al.,* 2014). Scorpion envenomation is a life-threatening condition that causes significant morbidity and mortality. The number of scorpion stings is estimated to be more than 1.2 million per year, the largest number of the lethal species are in the family Buthidae including Leiurusquin questriatus. (Erickson and Cheema, 2017, Abd El-Aziz *et al.,* 2022). It is important for forensic entomologists to know the time between sting and death, the aim of the present work is to assess the effect of scorpion venom on the decomposition of rabbits' carcasses and on a succession of arthropods in the indoor and outdoor environment.

MATERIALS AND METHODS

Experimental Design:

The present experiment included twenty male rabbits aged (2-3 weeks) with (2.5- 2.76 Kg.) average body weight. Animals had been purchased from the Animal House, Faculty of Science Assiut University, Egypt. All ethical guidelines for animal handling and treatment were followed. The experiment was conducted in the faculty of medicine, Assiut university during the period from 5th September to 25th November 2020 in a ~ 20,000- m² open field (outdoor) and indoor (27º 14¯ N and 31º 11¯ E) and indoor in a ~ 14 m².

Field Protocols:

The studied rabbits were divided into four groups (five rabbits uncovered in each group). Group 1: Control (indoor), Group 2: died from scorpion Sting (indoor), Group 3: Control (outdoor) and Group 4: died from scorpion Sting (outdoor). Group (2 and 4) rabbits were kept in a cage with 30 scorpions (*Leiurus quinquestriatus*) for half an hour.

Arthropods Handling and Preservation:

Through the first month after death, the rabbits' carrions were inspected daily, arthropods' samples were collected from each group and transferred to Zoology Laboratory, Faculty of Science, Assiut University for examination and identification, for permanent preservation arthropods were placed in ethanol (72%) or 10% neutral formalin**.**

Observation of Decomposition Process:

In this section of our work, the decomposition process of rabbits' carrions is described for each group separately and compared between them.

Statistical Analysis:

Statistical analysis was conducted by using Open Epi version 2.3.1 (Dean, 2010) and PAST software version 3.24 (Hammer *et al.,* 2001)**.** Data were presented as

frequencies and proportions. Chi-squared test was utilized to compare different groups. A P-value of <0.05 was accepted as statistically significant. The Simpson and Equitability index was calculated using the PAST software. The equitability index varies between 0 and 1, and tends to 0 when almost all the numbers are concentrated on one species; it is 1 when all species have even abundance.

RESULTS

Forensic Arthropods:

During the present work, fourteen species belonging to eleven families of arthropods were collected and identified during the period from 20 October to 20 December 2020. At the end of the study, some species were found to have different distributions across the studied groups as shown in Tables (1 & 3) & Figure (1). *Musca domestica, Dermestes maculates* and *Dermestes frischii* were significantly prominent in group (1). *Dermestes maculates* and *Dermestes frischii* were also prominent in the group (2) in addition to *Dermatophagoides* sp., *Cimex lectularis,* and *Sarcophaga* sp. and *Saprinus* sp. were also significantly prominent. Regarding group (3); *Dermestes frischii, Musca domestica*, *Dermestes maculates*, *Chrysomya albiceps*, *Sarcophaga argyrostoma* and *Wohlfahrtia magnifica* were significantly prominent. While *Chrysomya albiceps* and *Sarcophaga* sp. were significantly prominent in group (4).

Morphological characterization of arthropods in different groups showed the difference between the control groups and scorpions stinging groups. Figure (2) shows adults of *Chrysomya albiceps*, *Sarcophaga* sp., *Musca domestica*, *Saprinus* sp. and *Dermestes* sp. collected from the control and envenomation group. Different changes were noticed in adults collected from envenomated carrions, those changes included dryness of the skin with the formation of ulcers in some parts, irregular grooves, and tunnels with dryness of the outer layer with mild damage to the wings ends.

Figure (3) shows larvae of beetles, *Dermestes maculates*, *Nasonia* sp., *Sarcophaga* sp. and *Musca domestica* from control and envenomation groups. In Larval stages collected from envenomation groups, we observed dryness and erosions as well broken ends. Moreover, severe ulcers with complete necrosis and desquamation of epidermis, deeply separated grooves and crusts formation on the body (shrinks and darkness) were also recorded.

Table (2) showed that;

At 2 – 4 Days Postmortem:

- Group (2) had significantly more eggs and first stage larvae, and less third stage larvae compared to group (1).
- Group (3) had significantly more adults, and less first and third stage larvae compared to group (1).
- Group (4)had significantly more eggs, first and second stage larvae, while less third stage larva and adults compared to group (1).

At 5 – 7 Days Postmortem:

- Group (2) had significantly more first stage larva, and less third stage larvae compared to group (1).
- Group (3) had significantly more adults, and less first and third stage larvae compared to group (1).
- Group (4) had significantly more eggs and pupa, and less third stage larva and adults compared to group (1).

As shown in Table (4); all groups had high Diversity Indices which indicates high diversity of species. Groups 2 and 3 had the highest diversity. Groups 1 and 4 had

less diversity. In group 4, Sacrophaga sp., *Wolfahrtia magnifica* and *Parasarcophaga orgyrostama* were abundant. In group 1, *Dermestes frischi*, *Musca domestics*, and *Procellionides pruinosus* were abundant. The value of diversity indices ranges between 0 and 1. With this index, 1 represents infinite diversity and 0, no diversity.

Table 1: The collected forensic arthropods from the indoor and outdoor rabbit carrions during the different four groups.

Family	Species	Days postmortem	Groups (adult Arthropoda no.)			
			Group1	Group 2	Group 3	Group 4
Calliphoridae	***Chrysomya albiceps*** (Blowfly)	(0–1), (2–4), (5–7) Days	**50**	**2**	**45**	**4**
Apidae	***Apis sp.***	(0–1), (2–4), (5–7) Days	**1**	-	**1**	-
Muscidae	***Musca domestica*** (Housefly)	(0–1), (2–4), (5–7) Days	**860**	**13**	**120**	**50**
Sarcophagidae	***Sarcophaga* sp.** (Flesh fly)	(0–1), (2–4), (5–7) Days	**65**	**30**	**34**	**21**
	Wohlfahrtia magnifica	(0–1), (2–4), (5–7) Days	**54**	**6**	**33**	**11**
	Parasarcophaga orgyrostama	(0–1), (2–4), (5–7) Days	**7**	**1**	**15**	**7**
Dermestidae	***Dermestes maculates*** (Hide beetle)	(2–4), (5–7) Days	**346**	**54**	**111**	**15**
	Dermestes frischi	(2–4), (5–7) Days	**320**	**98**	**176**	**11**
Histeridae	***Saprinus* sp.** (Clown beetles)	(2–4), (5–7) Days	**13**	**9**	**3**	**3**
Pteromalidae	***Nasonia* sp.**	(2–4), (5–7) Days	**9**	-	**7**	**2**
Lycosidae	**Spider**	(2–4), (5–7) Days	**63**	**23**	**10**	**2**
Pyroglyphidae	***Dermatophagoides* sp.** (Dust mites)	(2–4), (5–7) Days	**11**	**52**	**2**	**2**
Cimicidae	***Cimex lectularis*** (Bed bugs)	(2–4), (5–7) Days	**45**	**41**	**3**	**1**
Porcellionidae	***Porcellionides pruinosus*** (woodlouse)	(2–4), (5–7) Days	**13**	**3**	**1**	**1**

Table 2: Comparison between the studied groups in Arthropod of Forensic Rabbit carrions:

Variables		Group 1		Group 2		Group 3		Group 4	
		No.	%	No.	%	No.	%	No.	%
0 – 1	Eggs	27	100	26	100	18	100	73	100
	Total	27	100	26	100	18	100	73	100
	P-value	NA		NA		NA		NA	
2 – 4	Eggs	27	1.9	26	9.7	19	3.6	73	23.1
	Larva 1	336	23.7	109	40.8	17	3.2	52	16.5
	Larva 2	121	8.5	17	6.4	81	15.3	74	23.4
	Larva 3	460	32.4	17	6.4	11	2.1	39	12.3
	Adult	477	33.5	98	36.7	403	75.8	78	24.7
	Total	1421	100	267	100	531	100	316	100
	P-value	Reference		**<0.001***		**<0.001***		**<0.001***	
5 – 7	Eggs	27	0.9	26	4.9	19	2.7	73	16.6
	Larva 1	336	11.6	109	20.8	17	2.4	52	11.8
	Larva 2	121	4.3	17	3.2	81	11.3	74	16.7
	Larva 3	460	16.0	17	3.2	11	1.6	39	8.8
	Pupa	92	3.2	26	4.9	21	3.0	73	16.6
	Adult	1843	64.0	332	63.0	560	79.0	130	29.5
	Total	2879	100	527	100	709	100	441	100
	P-value	Reference		**<0.001***		**<0.001***		**<0.001***	

Table 3: Comparison between isolated species of Arthropod in the studied groups:

Species	Total	Group 1		Group 2		Group 3		Group 4		P
		No.	%	No.	%	No.	%	No.	%	
Musca domestica	1309	964	73.6	54	4.1	145	11.1	146	11.2	Reference
Dermestes frischi	1254	961	76.6	99	7.9	178	14.2	16	1.3	**<0.001***
Dermestes maculates	856	543	63.4	142	16.6	146	17.1	25	2.9	**<0.001***
Sarcophaga sp.	395	119	30.1	60	15.2	58	14.7	158	40.0	**<0.001***
Chrysomya albiceps	202	72	35.6	17	8.4	88	43.6	25	12.4	**<0.001***
Spider	107	69	64.5	25	23.4	11	10.3	2	1.9	**<0.001***
Wohlfahrtia magnifica	160	63	39.4	16	10.0	39	24.4	42	26.3	**<0.001***
Cimex lectularis	90	45	50.0	41	45.6	3	3.3	1	1.1	**<0.001***
Dermatophagoides sp.	65	11	16.4	52	77.6	2	3.0	2	3.0	**<0.001***
Saprinus sp.	44	15	34.1	11	25.0	9	20.5	9	20.5	**<0.001***
Parasarcophaga orgyrostama	34	7	20.6	2	5.9	16	47.1	9	26.5	**<0.001***
Nasonia sp.	33	10	30.3	5	15.2	13	39.4	5	15.2	**<0.001***
Porcellionides pruinosus	18	13	72.2	3	16.7	1	5.6	1	5.6	0.06

Table (4): Diversity indices:

Groups	Simpson's Index (Is)		Equitability (E)	
	Value	95% CI	Value	95% CI
Group 1	0.74	0.73 – 0.75	0.63	0.61 – 0.64
Group 2	0.85	0.84 – 0.86	0.82	0.79 – 0.85
Group 3	0.83	0.82 – 0.84	0.76	0.73 – 0.78
Group 4	0.75	0.72 – 0.77	0.65	0.62 – 0.69
All groups	0.80	0.79 – 0.80	0.72	0.71 – 0.73

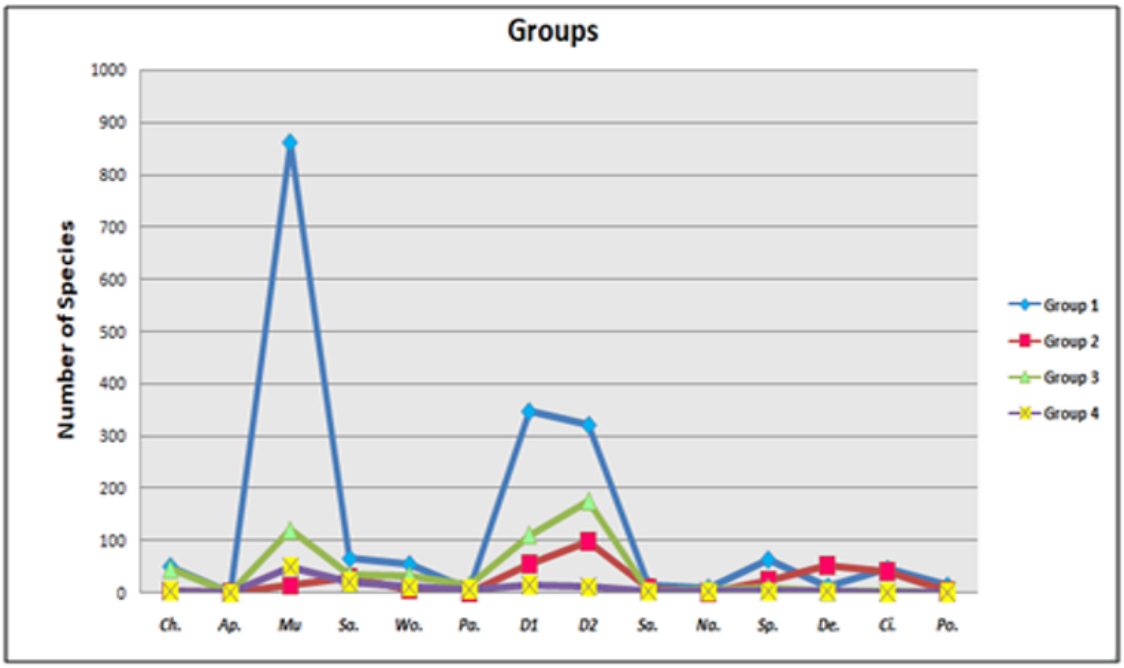

Fig.1 : Number of arthropod from Rabbit carrions in all four groups.

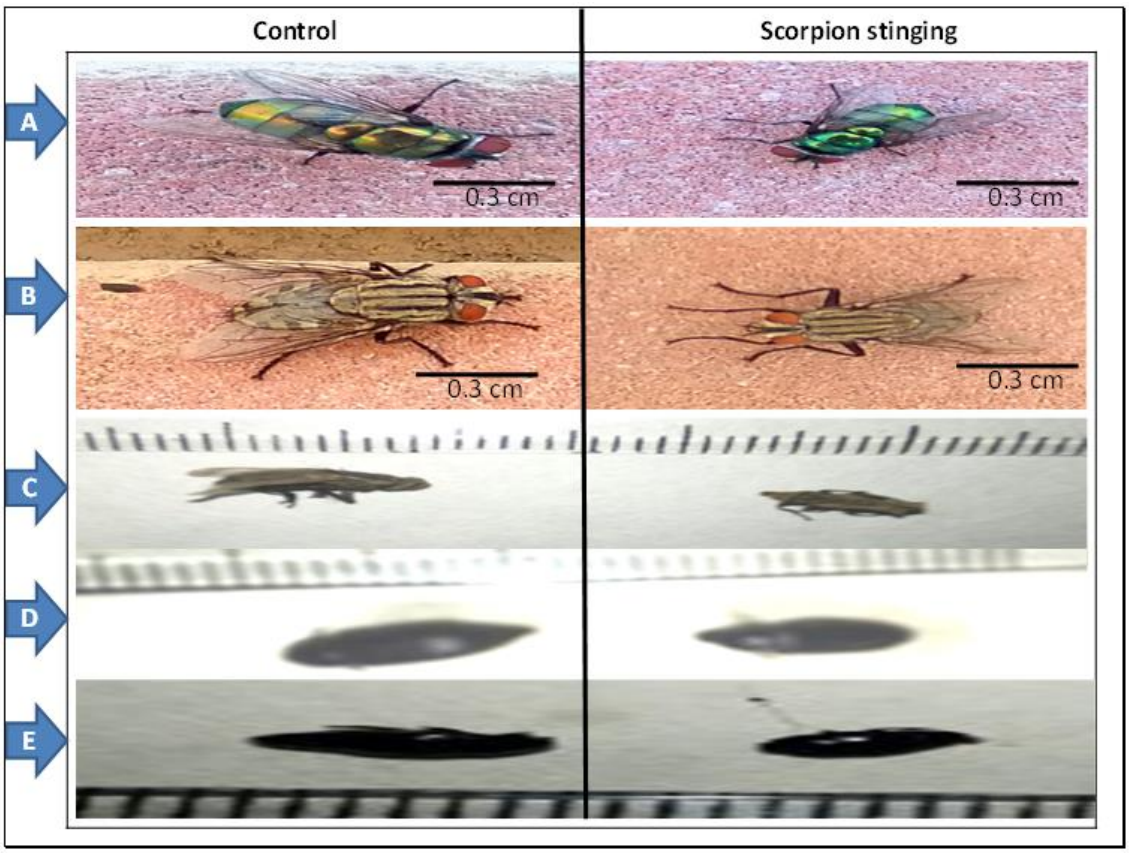

Fig. 2: (A) *Chrysomya albiceps*, (B) *Sarcophaga* sp., (C) *Musca domestica*, (D) *Saprinus* sp. And (E) *Dermestes* sp.

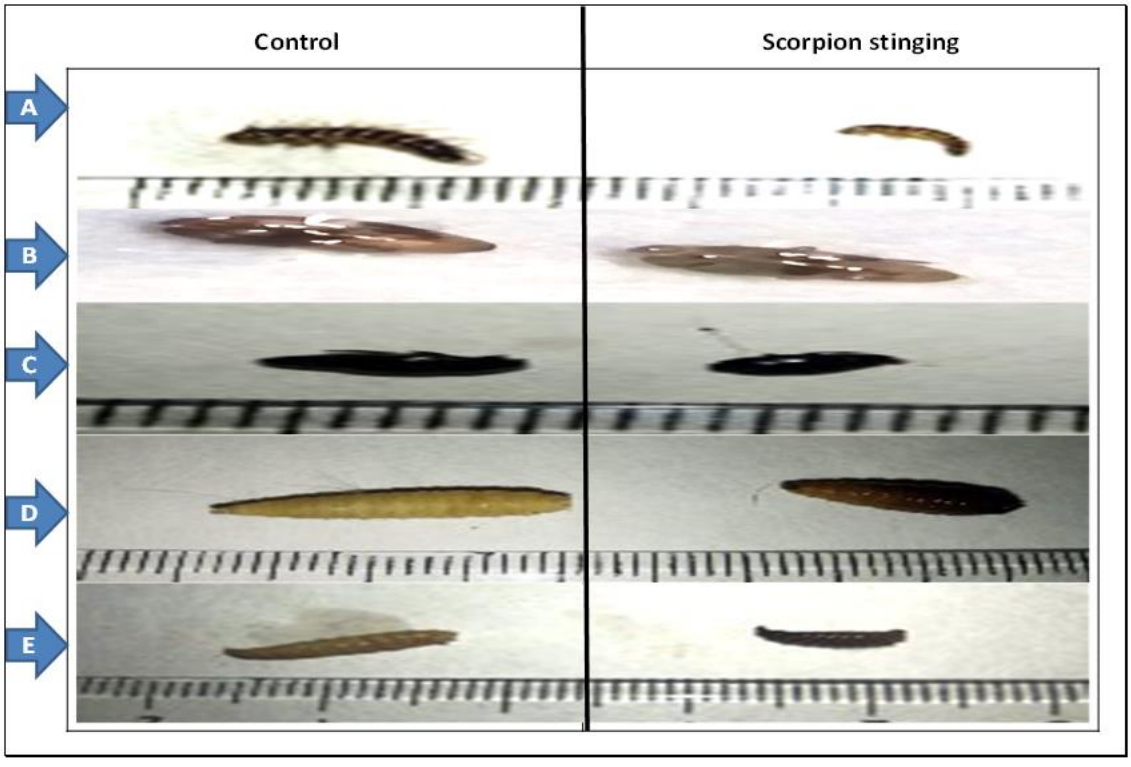

Fig. 3: (A) Beetles larvae, (B) *Dermestes maculates*,(C) *Nasonia* sp., (D) *Sarcophaga* sp. larvae,(E) *Musca domestica* larvae**.**

Postmortem Interval and Decomposition Stages:

Table (5) describes the decomposition stages of rabbits' carcasses in different groups. At 0-12h after death (Fresh Stage), no putrefactive alterations in control (1 and 3) carcasses while local findings such as red discoloration and swelling at the sting site were noticed in groups (2) and (4). From 12 hours to 3 days after death (Bloat Stage), carcasses of the control groups were bloated, internal organs and gut region were burst open; in addition to the formation of fluid blisters and purple to green discoloration. Furthermore, the presence of immature blowflies in the orifices was noticed. While fewer arthropods were found in groups (2) and (4) compared to the control groups with the same decomposition stage. From 4 to 6 days (Active Decay Stage), we noticed large populations of maggots feeding on the carcasses of the control group with the presence of Immature blowflies. The majority of tissue was remaining. Fewer populations of maggots were observed in groups (2) and (4). From 7 to 29 days (Advanced Decay Stage) we observed maggot masses surrounding carcasses of the control groups; we observed also decomposition fluid covering the carrions with exposure of bones. Those changes were less advanced in groups (2) and (4) with fewer arthropods compared to the control. From 30 to 70 days (Dry remains sage), there is an appearance of bones with small parts of decomposed tissue in the control groups. The same findings were noticed in groups (2) and (4) with the presence of dead arthropods.

Table 5: Description of decomposition stages of the postmortem interval (PMI) of Rabbits carcasses in different groups.

Postmortem interval (PMI)	Decomposition stage/ description	(Groups 1&3) Control	(Groups 2&4) Scorpion stinging
(0-12h)	Fresh Stage	There are no putrefactive alterations in these **carcasses** (no discoloration	Local effects such as redness, pain, burning, and swelling.
(12h-3 days)	Bloat Stage	Internal organs and gut region break open; fluid blisters; bloated; gray purple to green discoloration; inflation of the abdomen and elevated limbs; internal organs and gut region burst open; The presence of immature blowflies in the orifices and the presence of immature blowflies in the orifices between the two poles	The Arthropods were less than those found in control groups with the same pathological forensic.
(4-6 days):	Active Decay Stage	Large populations of maggots eat on the whole carcass; skin discoloration; foaming of purged fluids; the majority of tissue remaining Immature blowflies are present.	Less populations of maggots eat on the whole carcass. there are no signs of scorpions bits.
(7-29 days)	Advanced Decay Stage	Maggot masses inside the container; exposure of bones; brown, greasy body fluids; frothing of decomposition fluid covering the carrions; Presence of immature blowflies	Forensic pathological less than control and arthropods less than too
(30-70 days)	Dry (remains) Stage	The appearance of bones with small parts of decomposed tissue, greasy grey, brown fluids; Presence of a few immature blowflies	The appearance of bones with small parts of decomposed tissue, with the presence of dead arthropods.

DISCUSSION

Forensic entomology is a valuable tool for estimating postmortem intervals. Meticulous analysis of entomological data is a key in legal investigations (Sardar *et al.*, 2021). According to Campobasso and coauthors (2001), insects are very vital factors concerning the speed of body decomposition. The present work investigated the impact of scorpion envenomation on arthropods colonization and decomposition of rabbits' carcasses in the indoor and outdoor environment. In the current investigation, the factors measured which may affect the distribution of arthropods taxa in the carrions were: temperature, scorpion venom, outdoor and indoor. According to Mann *et al.* (1990) the most important factor affecting the decomposition of a body is the temperature, for this work the investigation was done in the Autumn.

Carrions progress through a series of decomposing stages, from fresh to skeletal, over a length of time. The arthropods arise in predictable series depending on the degree of decay (Turchetto *et al.,* 2004). Five stages of rabbits' decomposition have been identified in the present study. The decomposition changes were less advanced in envenomated carrions than control carrions in each decomposition stage. Furthermore, our investigation showed that the number of species in carrions varied greatly in different groups. Arthropods found on the control carrions were more than arthropods found on carrions exposed to scorpion stings before death.

The collected arthropods from the present investigation were identified morphologically. fourteen species were identified including *Musca domestica, Dermestes maculates, Dermestes frischi,* Sarcophagae, and *Chrysomya albiceps.* Other rare species have been introduced to forensic as reported by Abdelaziz and El Shehaby (2019) and Abdelaziz *et al.* (2022), for example, spiders, Apis sp., Cimex lectularis and Porcellionides Pruinosus. In the present work, all the above-mentioned species were recorded too.

Chrysomya albiceps flies (green blowfly), were the first species to colonize the carrions. In agreement with the present results, Yassin and Mohamed 2015) reported that *C. albiceps* is one of the first insects to inhabit corpses and carcasses. *Chrysomya albiceps* belong to the Calliphoridae family, which feed on corpses, carcasses, garbage and feces (Whitworth, 2006). The family Calliphoridae (blowflies) is a diverse group of calyptrate flies with a global distribution that includes species with medicolegal significance (Nasser *et al.,* 2021). The Sarcophagidae family (Flesh flies) commonly inhabit cadavers and carrions slightly later than *Chrysomya albiceps* from and are viewed as the second significant species for forensic investigations (Szpila *et al.,* 2015).
Musca domestica flies (muscidae family) were the most abundant arthropod on control carrions and from the first species to appear on carrions. Muscidae can be detected on cadavers and carrions in the early and advanced stages of decomposition. Few Muscidae flies are known to colonize carrions and cadavers than other families. However, muscids can colonize carrions under diverse environmental conditions (in outdoor or indoor environments, in sunny or shaded sites, in damp or dry conditions, in exposed or concealed areas) (Matuszewski *et al.,* 2008 & Grzywacz *et al.*, 2017).

Dermestidae beetles (*Dermestes maculatus* and frischii) were identified on carrions of all groups. Beetles have an innate tendency to devour tissues throughout all stages of decomposition (Sanger *et al.,* 2020). Dermestidae and other beetles may help in the determination of postmortem interval, particularly when decomposition is advanced (Bonacci *et al.,* 2017).

Biological diversity can be measured by various methods. Richness and everness are important items to be considered when measuring diversity.Simpson's Diversity

Index is a measure of diversity which takes into account both richness and evenness (Morriset al., 2014). While, equitability (also known as Pielou's evenness) measures the abundance of species relative to other species in a given community (Su, 2018). In our sample, all groups had high Diversity Indices which indicates high diversity of species. Groups 2 and 3 had the highest diversity. Groups 1 and 4 had less diversity. In group 4, Sacrophaga sp., Wolfahrtia magnifica and Parasarcophaga orgyrostama were abundant. In group 1, Dermestes frischi, Musca domestics, and Procellionides pruinosus were abundant.

In the present work, the results corroborate previous data on the influence of drugs and toxins in the decomposing body on the rate of insect colonization of that body (Hamdy *et al.*, 2022). The present work also gives new evidence by the description of the morphological characterization of different arthropods species collected from rabbits 'carrions exposed to a scorpion sting. The morphological changes included dryness of the skin with the formation of ulcers in the larvae, irregular grooves and tunnels in insects and dryness of the outer layer with damage to the ends of beetles.

Postmortem diagnosis of scorpion sting is a very difficult task for any forensic pathologist. Scorpion venom is complex from proteins and enzymes so, the forensic laboratories Cannot identify the venom, furthermore the mark is rapidly obscured by decomposition (Kumar *et al.*, 2012). In the present work, we noticed the presence of local findings such as red discoloration and swelling in the fresh stage of decomposition that disappeared in the bloat stage.

Conclusion: Scorpion venom delayed the decomposition of rabbits' carrions and also caused differences in the morphological appearance of arthropods colonized the envenomated carrions in addition to fewer arthropods colonization compared to the control carrions.

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