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A preliminary identification of insect successive wave in Egypt on control and zinc phosphide-intoxicated animals in different seasons

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Abstract The presented study aimed primarily to document a baseline data of the decay process of rabbits and guinea pigs and their associated arthropod fauna, which are placed in an urban city: El Abbassyia, Cairo Governorate, Egypt, during winter and summer seasons, and to compare these data with the corresponding figure for zinc phosphide-intoxicated carrions. Generally, control rabbits and control guinea pigs were faster in their decay comparing the corresponding figure of the zinc phosphide–intoxicated group. A delay in colonization of insects was noticed either in the winter season for both groups, or additionally for the zinc phosphide groups. The associated insect fauna was represented in 6 orders, 20 families, and 36 genera and species. Necrophagous arthropods that supported decomposition of carcasses were mainly of orders Diptera and Coleoptera. Calliphoridae was the first insect family that colonized the different carcasses. The mean numbers of control immature dipterous maggots and similarly, the control coleopteran larvae significantly exceeded the corresponding mean numbers for the zinc phosphide-intoxicated groups in both winter and summer seasons in either rabbits or guinea pig groups. Moreover, the mean numbers of dipterous maggots or coleopteran larvae of rabbits significantly surpassed the corresponding figures for guinea pigs in both seasons. This study may add as a reference for the succession wave arthropod fauna in Cairo Governorate in winter and summer seasons. Moreover, it is the first record of the arthropod successive wave on zinc phosphide–intoxicated remains.

1. Introduction

Forensic entomology is the science in which the biological and ecological aspects of the colonizing arthropod fauna are studied. It is a branch of forensic science.[1] In the absence of vertebrate scavengers, animal carcasses provide us with a rich

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complex of insects that aid, in combination with anaerobic bacteria in driving the decomposition process.[2] This branch of study is a useful tool to estimate the time interval between death and the discovery of body, and hence, in determining the post mortem interval (PMI). For intervals more than 72 h, forensic entomology would be more precise (if not the only tool) in determining PMI than traditional methods.[3]

Insects are attracted in a succession manner by chemicals released during the decay process and /or other organisms visiting the remains.[4,5] The dead remains are an ephemeral habitat; a source of food and a shelter as well to large types of insects. The usual classification of arthropod fauna separates them into: necrophagous; necrophilous; omnivorous; opportunists; and accidentals. Diptera and Coleoptera are considered the most important orders in the process of decomposition as their immature stages use carrions to develop. The presence of eggs and larvae of these insects subsequently attracts other insect predator species.[6,7] Decay process, faunal composition and succession, development time of insects, are affected by some factors such as temperature, wind, rainfall, and geographical locality. Hence, when local climatological data are available, the sequence of colonizing fauna can be used to detect the PMI.[8] A carrion-related arthropod database is important for each locality especially temperate ecosystems.[9]

After certain elapsed time and when the traditional specimens (blood, urine, organs) are not available for the corpses, the carrion-feeding insects may provide a potential alternative for toxicological specimens. Moreover, recent studies had proven that the presence of drugs/toxins in decomposing tissues may alter the colonization pattern and rate, a branch of investigation; entomotoxicology.[10]

Zinc phosphide is a commonly used pesticide and an effective rodenticide, which has a non-specific toxicity.[11,12] It is a gray-black finely ground crystalline that is non-soluble in water and alcohol. It was first synthesized in 1740 and was used for the first time as a rodenticide by the Italians in 1911. Zinc phosphide manifests its immediate toxical symptoms through production of phosphine gas. It is rapidly absorbed throughout the gastrointestinal tract and is loaded to the liver by the portal vein.[12] Upon ingestion, it reacts with the diluted acids in the gastrointestinal tract and produces phosphine which is carried to the blood stream. Chronic exposure to phosphine causes nausea; vomiting; diarrhea; cough; headache; and feeling cold. Its treatment is symptomatic by evacuating the intestinal tract and no antidote is known.[11] Deaths are directly related to cardiotoxicity.[13]

Zinc phosphide poisoning was a matter of study worldwide.[12,14–18] In Egypt, Shreed et al. 19 recorded that 3.9% of parasuicide in the Governorate of Damietta was of zinc phosphide poisoning. While another study in Dakahlia[20] had shown that 5.56% of elderly unnatural deaths were of zinc phosphide poisoning. El Naggar and El Mahdy[21] recorded that among 11.8% of poisoned cases that were admitted to the National Center of Clinical and Environmental Toxicological Research (NECTR), 15% of this percentage was because of zinc phosphide poisoning.

The presented study aimed primarily to document a baseline data of the decay process of two types of animals and their associated arthropod fauna, which are placed in an urban city: El Abbassyia, Cairo Governorate, Egypt, during winter and summer seasons, and to compare these data with the corresponding figure for zinc phosphide-intoxicated carrions. The study aimed also to compare the insect wave succession pattern for both types of animals which differ in size.

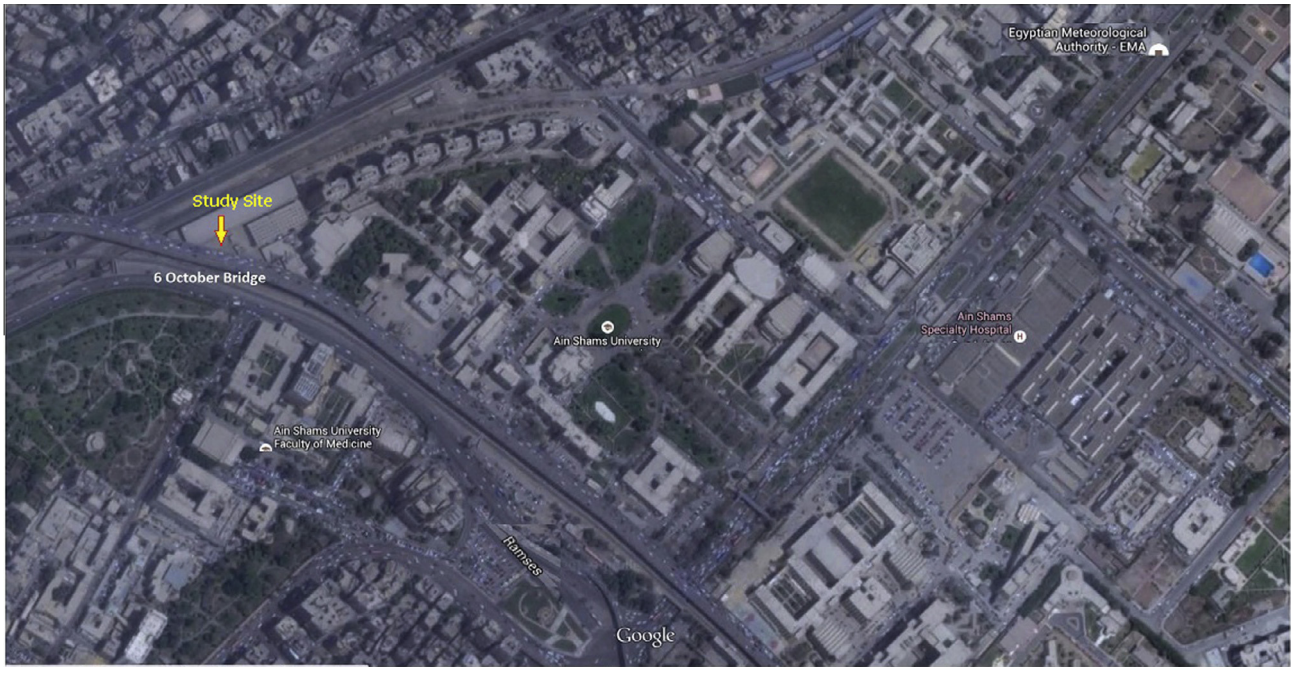

Figure 1 Map showing the study site in Abbassyia Area Cairo Governorate Egypt.

Table 1 Maximum and minimum ambient temperature, relative humidity, and numbers of rainfalls during the period of (Jan.2 – Mars 30, 2012) and summer season (July 14 – July 30, 2012).

	Maximum temp.	Minimum temp.	Maximum humidity	Minimum humidity	Rainfall
Winter	15–37	7–23	65–95	20–60	3 times in 3 separate days each 0.2 mm
Summer	36–40	25–26	80–90	25–40	–

2. Materials and methods

2.1. The study area

This study was conducted during winter season (January 2–April 6, 2012) and summer season (July 14–July 30, 2012) in the campus of Ain Shams University that is located in Abbassyia region in Cairo Governorate, Egypt (Fig. 1) (30^0 04′38. 0 N, 31016′57.0bE). Elevation of the study area is 30.5 m above the sea level. The investigation site selected was convenient for permitting the daily observation of the carrions and minimizing the disturbance that might be caused by humans and other animals. The selected experiment site is a floor of tile area covered with sand under each cage to be suitable for developing larvae pupation. Vegetation of the site was composed of the grass *Launaea nudicaulis* and *Alhagi maurorum* (identification had been made at the Herbarium, Botany Department, Faculty of Science, Ain Shams University). The place has a space area of approx. 25 m^2 and is surrounded by a wire dimension of 1 × 1 cm fixed in long wood wedges. Temperature ranged from 8 to 37 °C and 25 to 40 °C in winter and summer seasons respectively. The mean relative humidity was between 25–95% and 30–90% in wet and dry seasons respectively (Table 1). It rained 3 times during the experiment: on the 16th, 20th and 21th days from the beginning of the winter experiment, each with 0.2 mm precipitation.

Physical environmental conditions at the study area were obtained daily from the Egyptian Meteorological Authority located just 943.6 m away from the study location covering an area of diameter 100 km around.

2.2. Experimental animals and procedure

During our studies, pigs were not allowed in Egypt due to spread of H1N1 virus in the country. Thus, rabbits and guinea pigs were used instead.

The experiments were carried out on six healthy domestic male rabbits (*Oryctolagus cunicullus* L.) weighting approx.

Table 2 Control and Zinc Phosphide-intoxicated rabbit carcass decomposition duration and the associated insect succession wave in winter season.

Stage of decay	Control carcasses		Zinc Phosphide-intoxicated carcasses	
	Duration (days post killing)	Decay aspects	Duration (days post killing)	Decay aspects
Fresh	0–1	Discoloration of skin	0–1	Discoloration of skin
Bloat	1–15	Swelling of carcasses; hardening of the extremities; appearance of Dipterous flies from the 11th day; putrefaction of neck and then abdominal regions; leakage of fluids with awful putrefying odors	1–20	Swelling of carcasses; hardening of the extremities; Putrefaction and degeneration of the eyes; beginning of Formicidae appearance and leakage of fluids starting from mouth and nose regions; putrefaction of mouths; appearance of dipterous flies from the 14th day
Active decay (wet decomposition)	16–87	By the beginning of the stage:Body still ballon-like; penetration of decomposing maggots starting from 16th day post-killing colonizing mouth, ears and nose regions; then: the skin is cracked and ruptured in some regions Appearance of Coleopteran larvae from 17th day post-killing. By the end of the stage: maggot departure of the carcasses; and beginning of pupation in the soil-	21–87	Body still swollen; small number of decomposing maggots; appearance of Coleopteran larvae starting from 25th day post-killing. Putrefaction of neck region; foams in its mouths; Beginning of rupture from the 19th day; By the end of the stage: maggot departure of the carcasses; and beginning of pupation in the soil
Advanced decay (Dry decomposition)	88–90	Compressed body; then reduction in flesh; hardening of the bodies; departure of all maggots and pupation in the soil; many scattered pupae around carcasses in the soil and only Coleopteran insects and Formicidae on carcasses	88–92	Compressed body; then reduction in flesh except that facing the soil; no maggots; only Coleopteran and Formicidae insects; scattered pupae in the soil around carcasses; cracked bodies
Skeletal	91-	Only dry skin, cartilages, scattered masses of fur	93-	Only dry skin, cartilages, scattered masses of fur

1500 kg and other six guinea pigs (*Cavia porcellus* L.) of approx. 400 g. Rabbits were purchased locally, while guinea pigs were purchased from an Animal-house (Abo-Rawash, Giza Governorate, Egypt).

Two experiments were carried out during this study, one during winter and the other during the summer season, 2012. In each experiment: either rabbits or guinea pigs were divided into two groups, each group included three replicates. In the control group animals were killed by Asphyxia, and in the test group animals were killed by administration of zinc phosphide. Effective gradient in zinc phosphide was 80% which is equivalent to 19% effective phosphorus. Each kg contains 800 gm effective material produced by El-Nasr for chemicals (Industrial area at Abo-Rawash, Giza Gov., Egypt, under the national registration/103.

Each test rabbit was administered 3 g of substance dissolved in 12 ml H_2O. While each guinea pig was injected with 2.5 g of substance dissolved in 7.5 ml water. Injection was carried out orally using a gastric tube according to ethical criteria as this method simulates death occurring from poisoning by this substance. Immediately after killing, carcasses were transferred to the study site to labeled sealed cubic (50 cm length, width, and height) cages which were wired mesh (1 × 1 cm) that prevent scavengers. Cages were in full contact with the ground and sand was placed under each cage to facilitate collection of larvae and to help maggots to pupate. Cages were placed approx. 1 meter apart from one another at the study site. The day of killing and placement of carcasses was designated as day 0.

The carcasses were visited daily from the beginning of the experiment in summer, and in winter: they were visited daily and then once each five days until animal remains became completely dry and no active or live insects were detected in any of the cages. We stopped visiting the carcasses on days 91 and 93 for control and zinc phosphide-intoxicated rabbit carcasses respectively, on days 90 and 92 for control and zinc phosphide-intoxicated pig carcasses in winter, while in summer, we stopped visiting control and zinc phosphide-intoxicated rabbit carcasses on the 16th and 18th days respectively. And for pig carcasses we stopped visiting them on the 16th and 19th days for control and zinc phosphide carcasses respectively. During each visit, approx. 25% of the arthropod samples were collected from/in/on/around the carcasses and the decomposition stages were recorded. Collection tools (forceps, spoons, entomological nets) were used.

2.3. Collection of samples and Identification

Collection was non-randomized. The different collected samples were placed in plastic labeled vials. Adult flies collected were transferred to a killing jar containing ethyl acetate, some of them were preserved dry in freezer and the others either kept in 70% alc. or pinned. Dead samples (if any) were removed and maintained in a domestic freezer. Maggots were separated into two groups. The first group was killed immediately by immersing in a beaker of boiling water for 30 s, and then preserved in 70% ethyl alcohol for preservation and identification (according to the view of the board and the members of the European Association for Forensic Entomology,[22] while the second group of maggots was reserved in lab for rearing in plastic cups covered with a net mesh and larvae were fed rotten minced meat at room temp. for confirmatory identification.

Insects and decomposing remains were photographed. Identification of samples was carried out in the Museum of Entomology Department, Faculty of Science, Ain Shams University.[23–27]

Table 3 Control and Zinc Phosphide-intoxicated guinea pig carcass decomposition duration and the associated insect succession wave in winter season.

Stage of decay	Control carcasses		Zinc phosphide-intoxicated carcasses	
	Duration (days post killing)	Decay aspects	Duration (days post killing)	Decay aspects
Fresh	0–3	Discoloration of skin	0–3	Discoloration of skin
Bloat	3–16	Swelling of carcasses; more much awful putrefaction odor than rabbits; hardening of the extremities; appearance of Dipterous fliesputrefaction of neck and then abdominal regions; leakage of fluids with awful putrefying odors	3–36	Swelling of carcasses; more much awful putrefaction odor than rabbits; hardening of the extremities; appearance; degeneration of the eyes; putrefaction fluids; appearance of Dipterous flies with very tiny number
Active decay (wet decomposition)	17–87	Putrefaction; penetration of decomposing maggots from 17th day post-killing; Formicidae are in mouths and noses; Coleopterous larvae also started to appear from the 17th day post-killing. Maggots presented till day 87 but with very tiny numbers	37–87	Much more swellings; Formicidae; Penetration of maggots by the 37th day post-mortem with small numbers; much more internal putrefaction; Coleopteran larvae appeared by the 25th day post-killing
Advanced decay (Dry decomposition)	88–89	Dead dried dipterous eggs; just Coleopterous insects; Compressed bodies; Scattered fur masses	88–91	Absolutely No dipterous insects had been seen in/on the corpses, just under them; Compressed bodies; Scattered fur masses; coleopteran insects between skin and muscles
Skeletal	90-	Only dry skin, cartilages, separated bones, scattered masses of fur	92-	Only dry skin, cartilages, separated bones, scattered masses of fur

2.4. Statistical analysis

SPSS V.20 software was used to analyze the data.

3. Results

Five remarked stages were reported for the two groups of each animal type of carcasses during the winter and summer seasons (Tables 2–5 and Figs. 2–5). Generally, control rabbits and control guinea pigs were faster in their decay comparing the corresponding figure of the zinc phosphide–intoxicated group. In the winter season, a delay of 5 and 20 days post-killing was noticed in maggots' colonization of intoxicated rabbits and guinea pigs respectively, than the corresponding control groups (Tables 2 and 3), while in the summer experiment, a delay of 2 days was noticed in colonization intoxicated animals comparing the control animals (Tables 4 and 5). The process of decomposing started from head regions in most animals and was noticed for all the body parts during the experiments. Decomposition of all carcasses in the summer experiment was obviously more rapid than the corresponding winter carcasses. The decay process of guinea pigs ended with clear skeletonization than that of rabbits (Figs. 2–5).

The associated insect fauna recovered from different carcasses was represented in 6 orders, 20 families, and 36 genera and species (Table 6). Necrophagous arthropods that supported decomposition of carcasses were mainly of orders Diptera and Coleoptera. Calliphoridae was the first insect family that colonized the different carcasses and lasted till the end of the active decay stage.

In the control carcasses, and during winter season, dipterous insects predominated the overall insect fauna (56.8%), followed by coleopterans (41.6%). In the summer season for the same group, the proportion of dipterous colonizing insects exceeded 83% followed by coleopteran insects (10.2%). The proportion of coleopteran insects doubled the collected dipterous flies and maggots in the winter season for the zinc phosphide-intoxicated group (62.6% and 31.7%, respectively). In the summer season however, the dipterous insects overcame other orders (72.6%) (Fig. 6a and b).

Applying the non-parametric Mann–Whitney U test for two independent samples, the control and zinc phosphide-intoxicated animals were compared for the mean number of immature dipterous insects that were collected from the rabbit and guinea pig carcasses during both winter and summer seasons (Tables 7 and 8). For the rabbits, the mean number of immature Diptera stages significantly exceeded the corresponding mean number of the intoxicated group in both winter ($U = 52751.000$, $P < 0.05$) and summer seasons ($U = 1228365.000$, $P < 0.05$). The situation was the same for guinea pigs for the two groups in both winter ($U = 308.000$, $P < 0.05$) and summer ($U = 8646.000$, $P < 0.05$) seasons as well.

The non-parametric test was applied also to compare the mean numbers of immature dipterous insects collected from, and compared for the both types of animals for control and also zinc phosphide groups in both winter and summer sea-

Table 4 Control and Zinc Phosphide-intoxicated rabbit carcass decomposition duration and the associated insect succession wave in summer season.

Stage of decay	Control carcasses		Zinc Phosphide-intoxicated carcasses	
	Duration (days post killing)	Decay aspects	Duration (days post killing)	Decay aspects
Fresh	0	Discoloration of skin	0	Discoloration of skin
Bloat	1	Swelling of carcasses; appearance of Dipterous flies with high numbers; leakage of body fluids with awful putrefying odors. Beginning of appearance of dipterous maggots. Formicidae in and on the carcasses	1–2	Swelling of carcasses; Putrefaction degeneration of the eyes; beginning of Formicidae appearance and leakage of putrefying fluids form the notably worn bodies; appearance of dipterous flies
Active decay (wet decomposition)	1–14	At the end of first day post-killing, penetration of carcasses by dipterous maggots started even in the first day post killing; which were much concentrated in head and between legs regions. Maggots were smaller in sizes than in winter season. The skin is cracked and ruptured in some regions. Appearance of coleopteran larvae from the 2nd day post-killing. By the end of the stage: maggot departure of the carcasses; and beginning of pupation in the soil	3–16	Carcasses deflated; apparently smaller number of decomposing maggots than control rabbits; Maggots are smaller in size comparing winter corresponding figure; Maggots were much concentrated in head and between legs regions; Appearance of Coleopterous larvae form the 5th day post-killing. At the end of this stage, beginning of maggots pupation in the soil
Advanced decay (Dry decomposition)	15	Little flesh presented; Many scattered pupae around carcasses in the soil Formicidae on carcasses; hardening of the dead bodies. Cartilages appeared. At the end of the 14th day post killing: absence of dipterous maggots and coleopteran larvae	17	Rupture of the bodies especially lower parts; scattered pupae in the soil around carcasses; some Coleopteran insects and dead flies
Skeletal	16-	Only dry skin, cartilages. Scattered masses of fur	18-	Only dry skin, cartilages. Scattered

Table 5 Control and Zinc Phosphide-treated guinea pig carcass decomposition duration and the associated insect succession wave in summer season.

Stage of decay	Control carcasses		Zinc Phosphide-intoxicated carcasses	
	Duration (days post killing)	Decay aspects	Duration (days post killing)	Decay aspects
Fresh	0	Discoloration of skin	0	Discoloration of skin
Bloat	1	Swelling of carcasses; appearance of Dipterous flies; leakage of body fluids with awful putrefying odors; Formicidae in and on the carcasses	1	Too much swelling of smoothen carcasses; Putrefaction degeneration of the eyes; Formicidae appearance and leakage of putrefying fluids form its ears; appearance of dipterous flies
Active decay (wet decomposition)	1–14	Carcasses deflated. Penetration of carcasses by dipterous maggots, by the end of the first day post-killing, which were much concentrated in head and between legs regions; Maggots smaller in sizes than in winter season then: the skin is cracked and ruptured in some regions. Appearance of Coleopteran insects; By the end of the stage: maggot departure of the carcasses; and beginning of pupation in the soil	3–16	Carcasses started to deflate; apparently smaller number of decomposing maggots than control rabbits; Maggots are smaller in size comparing winter corresponding figure; at the end of this stage, beginning of pupation in the soil. Appearance of Coleopteran larvae from 6th day post killing
Advanced decay (Dry decomposition)	15	Little flesh presented; Parts of the carcasses were separated. Many scattered pupae and pupariae around carcasses in the soil and only Coleopteran insects and Formicidae on carcasses; hardening of the dead bodies	17–18	Skull of one animal became uncovered; but some flesh still presented. Absolutely no maggots inside and many dead flies were scattered around them; some Coleopterous insects presented
Skeletal	16-	Only dry skin, cartilages, bones appeared, scattered masses of fur	19	Only dry skin, cartilages, bones appeared, scattered masses of fur

sons. The mean number of immature (larvae and pupae) dipterous insects collected from control rabbits surpassed that collected from guinea pig carcasses, either in winter ($U = 27608.000$, $P < 0.05$) or in summer ($U = 397323.000$, $P < 0.05$). The same results were applied also for zinc phosphide killed rabbits comparing guinea pigs in both winter ($U = 588.500$, $P < 0.05$) and summer ($U = 26730.000$, $P < 0.05$) seasons.

The mean numbers of coleopteran larvae (Tables 9 and 10) were tested also for the significance. The mean number of control coleopteran larvae that was collected from control rabbits was significantly higher either in winter ($U = 7572.500$, $P < 0.05$) or in summer ($U = 9796.000$, $P < 0.05$) season comparing that of zinc phosphide group. In addition, the number of larvae collected from control guinea pigs was also higher

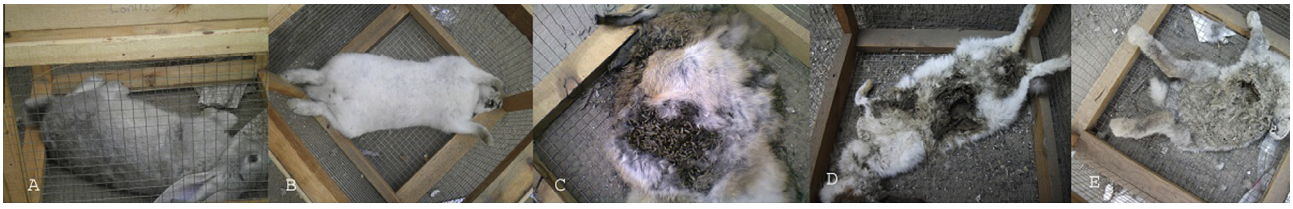

Figure 2 Decomposition stages of carcasses of control rabbits. A. Fresh stage. B. Bloat stage. C. Active decay stage. D. Advanced decay stage. E. Skeletal stage.

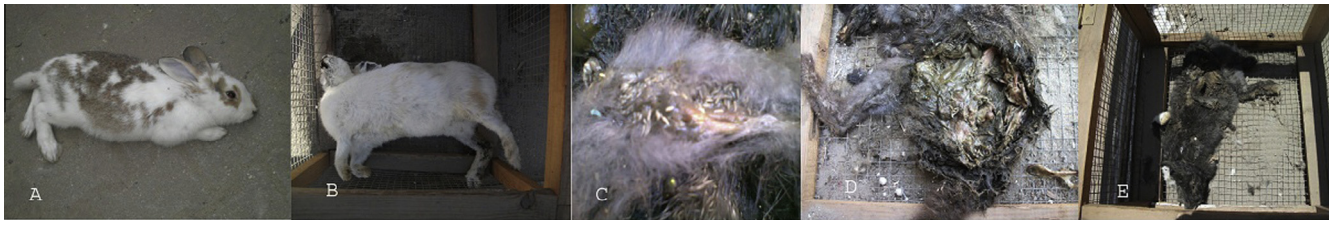

Figure 3 Decomposition stages of zinc Phosphide intoxicated rabbit carcasses. A. Fresh stage. B. Bloat stage. C. Active decay stage. D. Advanced decay stage. E. Skeletal stage.

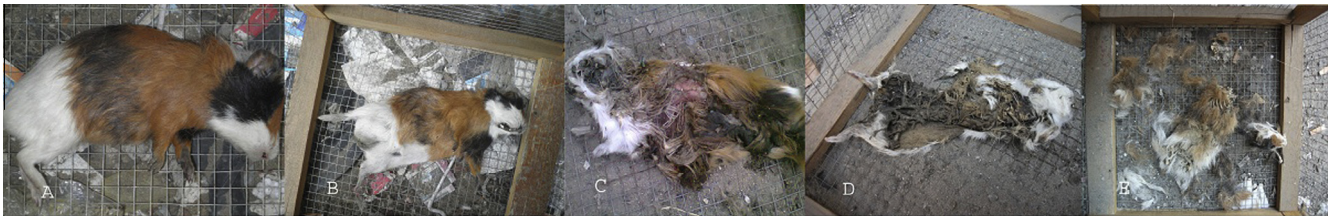

Figure 4 Decomposition stages of carcasses of control guinea pigs. A. Fresh stage. B. Bloat stage. C. Active decay stage. D. Advanced decay stage. E. Skeletal stage.

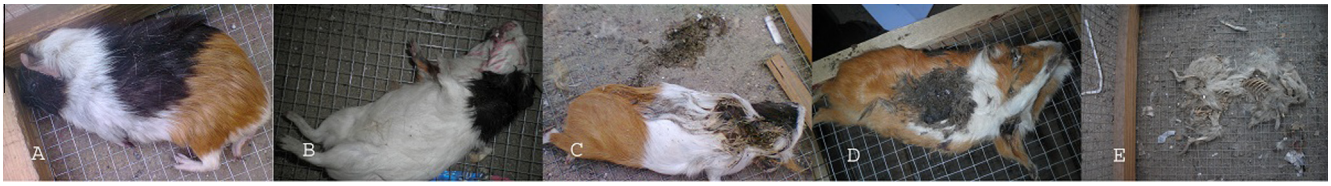

Figure 5 Decomposition stages of zinc phosphide intoxicated Guinea pig carcasses. A. Fresh stage. B. Bloat stage. C. Active decay stage. D. Advanced decay stage. E. Skeletal stage.

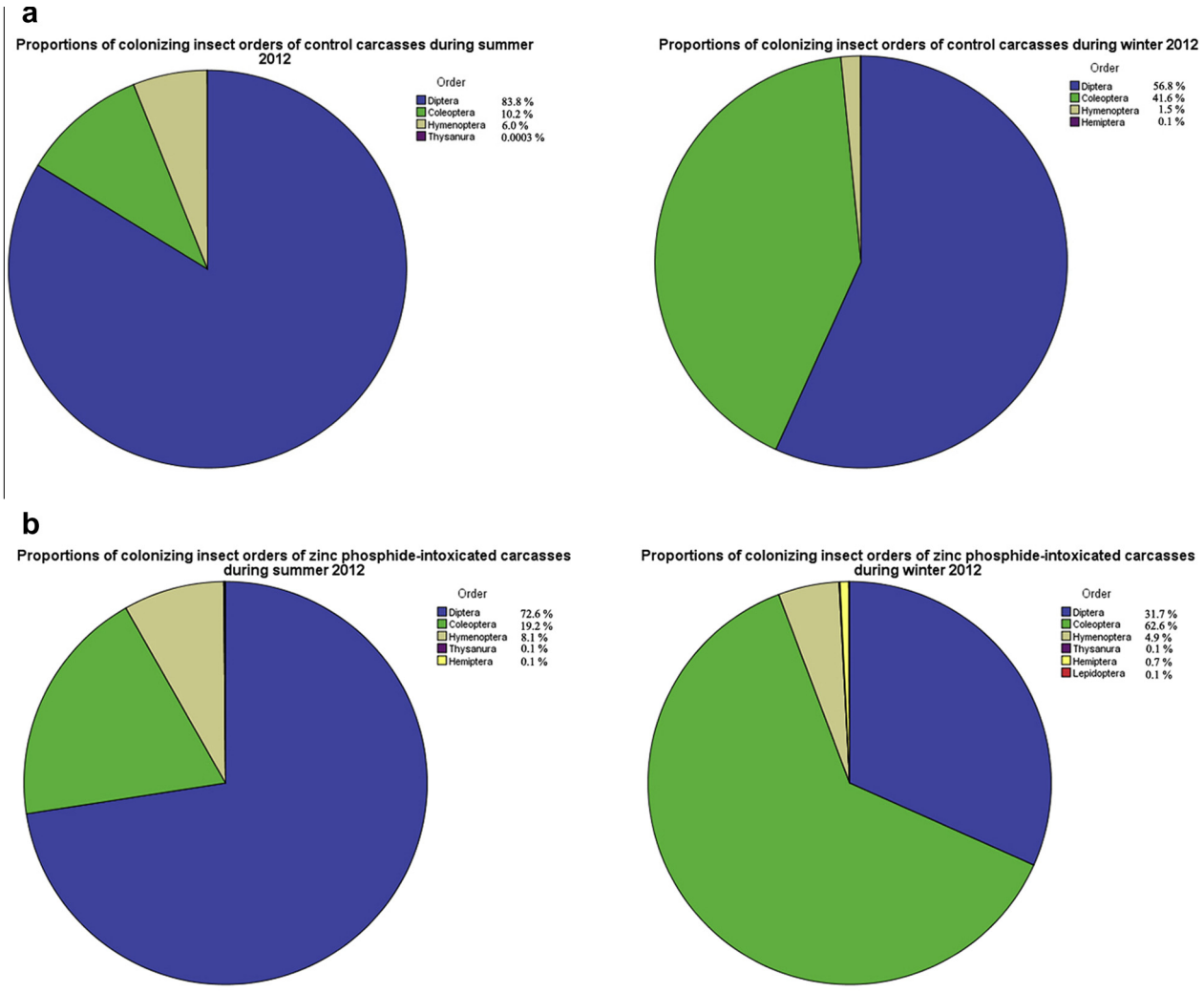

Figure 6 (a) Proportions of colonizing insect orders of control carcasses (b) Proportions of colonizing insect orders of zinc phosphide-intoxicated carcasses.

Table 6 Arthropods associated with control and zinc phosphide-intoxicated animals in Abbassyia, Cairo Governorate, Egypt, during winter and summer seasons, 2012.

Class	Order	Family	Genus/species
Insecta	Diptera		
		Phoridae	
		Calliphoridae	
			Chrysomya albiceps
			Chrysomya rufifacies
			Chrysomya megacephala
			Lucilia sericata
			Lucilia cuprina
		Muscidae	
			Musca domestica
			Musca sorbens
			Muscina stabulans
			Synthesiomya nudesta
		Sarcophagidae	
			Sarcophaga argyrostoma
			Sarcophaga hertipes
			Wolfhartia spp.
		Ulidiidae	
			Physiphora alceae
	Coleoptera		
		Dermestidae	
			Dermestes maculatus
			Dermestes frichii
			Dermestes ater
			Attagenus fasciatus
		Histeridae	
			Saprinus chalcites
			Saprinus furvus
			Saprinus caerulescens
			Saprinus semistriatus
		Cleridae	
			Necrobia rufipes
		Pteromalidae	
			Nasonia spp.
		Nitidulidae	
			Carpophilus hemipterus
		Staphylinidae	
			Philonthus stragulatus
			Philonthus longicornis
		Tenebrionidae	
			Trachyderma hispidae
			Zophosis abbreviata
			Creophilus maxillosus
			Mesostina puncticollis
		Anobiidae	
			Lasioderma spp.
	Hymenoptera		
		Formicidae	
		Chalcididae	
			Brachymeria spp.
		Chrysididae	
			Chrysis spp.
		Psychodidae	
	Hemiptera		
		Aphididae	
			Aphids spp.
		Cicadellidae	
			Empoasca spp.
	Thysanura		
		Lepismatidae	
			Lepisma saccharina
	Lepidoptera		
Arachnidae			

Table 7 Mean numbers of dipterous immature stages collected from control and zinc phosphide–intoxicated groups for rabbit carcasses in winter and summer seasons.

Season	Group	Replicates	Mean number ± SD
Winter[a]	Control	R1: 329	18.3 ± 13.0
		R2: 336	18.7 ± 14.0
		R3: 321	17.8 ± 14.1
		Total:986	
	Zinc phosphide–intoxicated	R1: 36	2.3 ± 1.2
		R2: 44	2.8 ± 1.5
		R3: 27	1.8 ± 0.9
		Total: 107	
Summer[b]	Control	R1: 1050	75.0 ± 142.3
		R2: 1019	72.8 ± 138.5
		R3: 964	68.9 ± 136.7
		Total: 3033	
	Zinc phosphide–intoxicated	R1: 280	20.0 ± 14.0
		R2: 419	29.9 ± 23.4
		R3: 111	7.9 ± 4.4
		Total: 810	

[a] No. rabbit-visit = 23 days post-killing for both control and zinc phosphide intoxicated carcass groups.
[b] No. rabbit-visit = 14 days post-killing for control carcass group and 16 days for zinc phosphide intoxicated carcass group.

Table 8 Mean numbers of dipterous immature stages collected from/and compared for control and zinc phosphide–intoxicated groups for guinea pig carcasses in winter and summer seasons.

Season	Group	Replicates	Mean number ± SD
Winter[a]	Control	R1: 18	1.4 ± 0.8
		R2: 18	1.4 ± 0.7
		R3: 20	1.3 ± 0.5
		Total: 56	
	Zinc phosphide–intoxicated	R1: 3	1.0 ± 0.0
		R2:5	1.0 ± 0.0
		R3: 3	1.0 ± 0.0
		Total: 11	
Summer[b]	Control	R1: 90	6.4 ± 5.0
		R2: 85	6.1 ± 4.8
		R3: 87	6.2 ± 5.3
		Total: 262	
	Zinc phosphide–intoxicated	R1: 16	1.1 ± 0.4
		R2: 20	1.4 ± 1.1
		R3: 30	2.1 ± 1.0
		Total: 66	

[a] No. guinea pig-visit = 23 days post-killing for both control and zinc phosphide intoxicated carcass groups.
[b] No. guinea pig-visit = 14 days post-killing for control carcass group and 16 days for zinc phosphide intoxicated carcass group.

Table 9 Mean numbers of coleopteran immature stages collected from control and zinc phosphide–intoxicated groups for rabbit carcasses in winter and summer seasons.

Season	Group	Replicates	Mean number ± SD
Winter[a]	Control	R1: 76	5.1 ± 4.3
		R2: 77	4.8 ± 4.3
		R3: 80	5.3 ± 4.9
		Total: 233	
	Zinc Phosphide–intoxicated	R1: 21	1.5 ± 1.2
		R2: 28	2.3 ± 1.3
		R3: 16	1.2 ± 0.4
		Total: 65	
Summer[b]	Control	R1: 144	12.0 ± 8.7
		R2: 42	4.7 ± 3.4
		R3: 62	5.2 ± 4.4
		Total: 248	
	Zinc Phosphide–intoxicated	R1: 23	2.3 ± 2.0
		R2: 30	3.0 ± 1.9
		R3: 26	2.6 ± 2.3
		Total: 79	

[a] No. rabbit-visit = 23 days post-killing for both control and zinc phosphide intoxicated carcass groups.
[b] No. rabbit-visit = 14 days post-killing for control carcass group and 16 days for zinc phosphide intoxicated carcass group.

Table 10 Mean numbers of coleopteran immature stages collected from/and compared for control and zinc phosphide–intoxicated groups for guinea pig carcasses in winter and summer seasons.

Season	Group	Replicates	Mean number ± SD
Winter[a]	Control	R1: 16	1.1 ± 0.4
		R2: 20	1.3 ± 0.7
		R3: 11	1.0 ± 0.0
		Total: 47	
	Zinc Phosphide–intoxicated	R1: –	
		R2: 3	1.0 ± 0.0
		R3: 1	1.1 ± .
		Total: 4	
Summer[b]	Control	R1: 21	2.1 ± 1.6
		R2: 27	2.5 ± 1.2
		R3: 31	2.6 ± 1.4
		Total: 79	
	Zinc Phosphide–intoxicated	R1: 8	1.1 ± 0.4
		R2: 6	1.0 ± 0.0
		R3: 10	1.1 ± 0.3
		Total: 24	

[a] No. guinea pig visit = 23 days post-killing for both control and zinc phosphide intoxicated carcass groups.
[b] No. guinea pig-visit = 14 days post-killing for control carcass group and 16 days for zinc phosphide intoxicated carcass group.

in the winter ($U = 94.000$, $P < 0.05$) and summer seasons ($U = 984.000$, $P < 0.05$) as well.

Comparing the mean number of coleopteran larvae collected from rabbits and guinea pigs, those collected from control rabbits were significantly higher in winter ($U = 5475.000$, $P < 0.05$) and in summer ($U = 9796.000$, $P < 0.05$). Furthermore, comparing intoxicated rabbits and

guinea pigs, the mean number collected from rabbits was significantly higher in winter ($U = 130.000$, $P < 0.05$), as well as in summer ($U = 948.000$, $P < 0.05$).

4. Discussion

Many studies had been conducted in several parts of the world to detect species composition and the successive arthropod waves on carrions.[28–36] Decay process is a natural and necessary way which is responsible for the return of the organic material to the ecosystem.[37] In our study, the duration of different stages of decomposition was obviously proportional to the temperature, which is in agreement with the study of Ozdemi and Sert .[37] The carcasses exposed to sun decayed faster in the presence of higher temperature than cooler darker conditions of winter, which was also recorded by Joy et al. [38] In general, carcasses decomposed faster during the summer seasons because of the large attraction of insects to bodies in warmer climates.[39] Furthermore, we reported rainfall three times during winter season. Rain may contribute to delayed oviposition and delayed pupation.[40]

All animal control carcasses decayed faster than the groups of zinc phosphate-intoxicated bodies. In our previous study[41], the decomposition was restricted to the lower parts of the rabbits, this was not the case in the present study, since the decay process included all the body parts. This may attribute to the fact that in the previous study, organophosphorus injection was directed to the heart, while in this study, the toxin was directed to the stomach (using a gastric tube for ethical criteria). Oxidative stress is defined as the "imbalance between the production of free radicals that cause peroxidation of the lipid layer and the body's antioxidant defense".[42] The mode of action of zinc phosphide is argued that it inhibits cytochrome C oxidation but however, inhibition was recorded less in virto.[17] It is more likely that zinc phosphide perturbs mitochondrial morphology and so, inhibits the oxidative respiration. Failure of cellular respiration is likely contributed to a mechanism other than oxidative inhibition, phosphine and hydrogen peroxide can react to form hydroxyl radicals which are highly reactive and inhibit catalase and peroxidase which lead consequently to lipid peroxidation. There is usually a short time interval between the appearance of symptoms of toxicity and the phosphine ingestion, the clinical features include myocardial contractility impairment; pulmonary edema; and fluid loss; which subsequently lead to circulatory failure. This may explain to some extent the cause of decomposition of all body parts of intoxicated animals in our experiments.

However, for the intoxicated rabbit and guinea pig groups, it was noticed that the toxin did not mask the odor of the carcasses, just as Voss et al. [43] and Abd El-Bar and Sawaby [41] had reported for other types of toxins, and in contrast to El Kady et al. [44] In the later study, neither decomposition occurred nor arthropods were captured from the arsenic oxide poisoned rabbits.

Delay in decomposition of a carcass by arthropods might be attributed to several factors; these factors are rain, lower temperature, and hampered accessibility. Determination of these factors would certainly make better the interpretation of entomological evidences.[45] In our study, the beginning of insect colonization had been delayed in the phosphide intoxicated bodies. Drugs and toxins may alter the rate of insect invasion and development of the immature stages.[41,44] The mean numbers of immature dipterous and coleopteran insects were significantly lower comparing them to control and intoxicated groups. Small numbers of immature stages undoubtedly delayed the decomposition processes.

Slone et al. [46] had reported that larger sample sizes lead to more precision for the models. Indeed, we recorded a significant difference in the mean number of collected dipterous maggots and pupae and also in coleopteran immature stages captured from rabbits versus those collected from guinea pigs, either in control or intoxicated groups or during winter and summer seasons. The small number of specimens captured from guinea pigs might be due to the small sizes of carcasses.[39]

Neither obvious differences in community composition of our detected insect fauna between winter and summer seasons (as the findings of Wang et al. [32]) nor between the control and intoxicated groups.

In forensic entomology, necrophagous insects are useful for determination of the PMI, post mortem transfer, and presence of toxins.[6] In the present study, blow flies were the first insect colonizers. The dipteran assemblage in early stages of decay was conservative, just like the findings of Al-Mesbah et al. [34] who studied rabbit decomposition in Kuwait. *Chrysomya albiceps* consistently presented early in succession as it is in other global regions. This species is aggressive and may feed also on other larvae, which can explain its dominance over the other calliphorid larvae.[34] *Chrysomia megacephala* and *Chrysomia rufifacies* are the two most forensically important species in many regions worldwide[47] *Chrysomya albiceps* and *Lucilia cuprina* are useful in determining the minimum PMI.[39]

Family Phoridae was identified in our study. The records of species involved in forensic cases in warm climates are almost restricted to those involved *Megaselia scalaris*. The first records of two species of oriental scuttle flies on real human case was done by Thevan et al. [48]

The development rate of fly larvae depends primarily upon the environment temperature, the higher the temperature the faster the development and vice versa.[49] Furthermore, larvae can benefit from increasing maggot mass temperature and speed up its development,[50] which may explains the faster termination of decay process in warmer seasons.

We noticed that in the morning, a few blow fly adults were captured. Indeed, blow flies are considered inactive at night although some studies are contradictory.[45,51] Amendt et al. [45] reported that the blow fly vision is less sensitive to light during night time. Wooldridge et al[51] suggested that flight orientation is relatively low in the dark.

As to family Sarcophagidae (flesh flies), in our study, they colonized the corpses on same day as did the Calliphoridae, which is consistent with Cherix et al. [52] Out of the collected dipterous flies, *Piophila casei* (F. Piophilidae, Diptera) was identified. Adults of this species are known to attract to proteinaceous materials such as meat, fish, and cheese[53] and is a major pest for food industry and is a myiasis agent.[54] This family is frequently cited for its common presence on corpses.

In the present study, appearance of coleopteran insects was remarked during the active decay stages for both groups for both types of animals. Order Coleoptera constitutes a main entomological evidence for the PMI determination[55] which is based upon succession pattern. Among Coleoptera, Staphylinidae and Histeridae are considered as predators and had been

seen in our experiments during the presence of dipterous flies decomposing larvae exactly like the findings of Ozdemir and Sert. 37 In later stages of active decay, we noticed the presence of dermestes beetles larvae and adults while less flesh still presented, Kulshrestha and Satpathy[55] recorded that only the presence of *Dermestes* larvae is an evident for actual species infestation. Indeed, larder beetles (Dermestidae) prefer dried carrions[56] and they can accelerate decaying process. Dermestidae, in contrast to rove beetles, fed directly on the corpse itself.[37] We had recorded also the presence of Nitidulidae and Cleridae. Nitidulidae is saprophilic and necrophilic while Cleridae are predators on some members of Piophilae flies and dermestis larvae.[37] By calculating the proportion of different orders during different seasons in both control and zinc phosphide intoxicated groups, we noticed that the Coleopteran insects (adults and larvae) predominate and double the proportion of dipterous insects (adults and maggots) for zinc phosphide intoxicated groups in the winter season. This may be due to the small number of dipterous larvae on intoxicated carcasses and especially in winter season, putting in mind the fact that coleopterous insects are predators beside some members feed on the carrions themselves.

5. Conclusion

Faunal composition can be predicted for any given area under specific conditions 57. The fields of forensic entomology and entomotoxicology research are still scanty in Egypt except for some studies.[41,44,58–61] This study may add as a reference for the successive wave arthropod fauna in Cairo Governorate in winter and summer seasons. Moreover, it is the first record of the arthropod successive wave on zinc phosphide–intoxicated remains. Further biochemical and molecular research upon insects that are collected from corpses may be needed.

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Ethical approval

Necessary ethical approval was obtained from the institute ethics committee.

Conflict of interest

None declared.

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