Forensically Important Coleopteran Insects and Other Orders' Species Attracted to Control and Warfarin-Intoxicated Rabbit Carrions

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ABSTRACT

Forensic entomology is an expanding discipline of entomology that combines an entomologist's knowledge of insect species, its life cycles, and habitats with aspects of law enforcement. Orders Diptera and Coleoptera constitute major entomological evidence for PMI determination. This study was designated to examine the decomposition process and beetles' succession (Order: Coleoptera) on exposed rabbit carrions. Six rabbits were divided into two groups: the first group was intoxicated with Warfarin rodenticide (Warfarin-intoxicated, WI), and the second group was asphyxiated via hanging as the control (C). Five decomposition stages were observed in all carcasses. Four coleopteran families were represented by seven species: *Attagenus faciatus, Dermestes maculates, Dermestes frischii* (Dermestidae), *Creophilus maxillosus* (Staphylinidae), *Saprinus chalcites, Saprinus furvus* (Histeridae) and *Necrobia rufipes* (Cleridae). Of these, *Attagenus faciatus* (29.9 % off C) and *Saprinus furvus* (20.9% off WI) were the most common species. In general, Dermestidae was the first attracted group and more common (66. 8% off C; 35.8% off WI) followed by Histeridae (17.3% off C; 34.1% off WI), Staphylinidae (8.5% off C; 16.8% off WI) and Cleridae (7.5% off C; 13.3% off WI). Eleven species of other orders were collected off both carcasses. *Monomorium pharoanis* (Hymenoptera: Formicidae) was the predominant species collected off both control and intoxicated carcasses (50:57.5% and 90: 57.3%, respectively). This study provides information on the diversity, succession, relative abundance, and occurrence of forensically important coleopterans in Cairo, Egypt. Moreover, it is the first record of coleopteran insects on Warfarin-intoxicated remains.

Keywords: Forensic entomology, Entomotoxicology, Warfarin rodenticide, Rabbit carcasses, Coleopteran succession, Egypt.

INTRODUCTION

Forensic entomology (FE) is an expanding discipline of entomology that combines an entomologist's knowledge of insect species, its life cycles, and habitats with aspects of law enforcement (Varatharajan, 2000; Dekeirsschieter and Verheggen, 2022; Mohr and Tomberlin, 2023). This specialized discipline plays a pivotal role in diverse aspects of death investigations, ranging from the estimation of post-mortem interval (PMI) to the determination of body movement post-mortem. Estimating PMI is a critical objective in FE, as it provides valuable information about the time since death, aiding investigators in criminal cases. The utilization of insects as forensic evidence dates back to ancient civilizations when various cultures recognized the potential value of insect observations at crime scenes (Alam et al., 2024).

Today, FE is defined as "The legal application of the science of entomology" or "The use of the insects and their arthropod relatives that inhabit decomposing remains to aid legal investigations", *i.e*. help solve crimes (Catts and Goff, 1992).

Medico-legal forensic entomology (MLFE) focuses on crimes involving violence and deals with the necrophagous-feeding insects that infest body remains (Anderson, 1997; Dadour et al., 2001; Amendt et al., 2004) and feed only on decomposing tissues. MLFE relates to death investigations principally the determination of the time interval between death and body discovery which is the primary purpose of FE today.

Carrion decomposition begins at the moment of death, caused by three recognizable processes: autolysis (the breaking down of tissues by the body's internal chemicals and enzymes), putrefaction (the breakdown of tissues by bacteria resulting in the release of gases

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that are the chief source of the undesirable putrid odor of decaying tissues) and skeletal parts decomposition (Gennard, 2012).

A diverse range of insect species are drawn to decaying remains and contribute to decomposition (Campobasso et al., 2001; Dekeirsschieter and Verheggen, 2022). However, two primary groups: flies (Diptera) and beetles (Coleoptera), are typically the most significant. Diptera, whose larvae thrive in semi-liquid environments, are among the first to be attracted to and colonize decomposing remains. These fly larvae are primarily responsible for the rapid breakdown of the body's tissues. Most beetle species (Histeridae, Staphylinidae, Cleridae, Carabidae, and Tenebrionidae) act as predators on eggs, maggots, and pupae of flies and larvae of other beetles. Later, when the corpse has largely dried out, other beetles (Dermestidae and Scarabidae) feed on dried skin and tissues during the latter stage of decomposition.

Studies by (Hegazi et al., 1991; EL-Kady et al., 1994; Tantawi et al., 1996; Shalaby et al., 2000; EL-Ghaffar et al., 2008; Abd El-Bar and Sawaby, 2011; Aly et al. 2013; Ibrahim et al., 2013; Zeariya et al., 2015; Abd El-Bar et al., 2016; Aly et al., 2017; Zeariya et al., 2018; Abd El-Gawad et al., 2019; Zeariya and Kabadaia 2019; Farag et al., 2021) were conducted in various regions of Egypt to investigate the composition of entomofauna attracted to different stages of decomposition in animal carcasses, including rabbits (*Oryctolagus cuniculus* and *Lepus cuniculus*), guinea pigs (*Cavia porcellus*), and dogs (*Canis lupus familiaris*). The reported insect orders are: Diptera (15 Family, 43 species.), Coleoptera (8 F, 24 sp.), Hymenoptera (8 F, 12 sp.), Dermaptera (5 F, 3 sp.), Zygentoma and Blattodea (1 F and 1 sp. each), and Hemiptera (2 F and 2 sp.).

The presented study was planned to examine the decomposition process and succession waves of beetles (Order: Coleoptera), and other orders' species attracted to rabbit carrions killed with the rodenticide Warfarin which was initially used as an oral anticoagulant in 1948 and then approved in Egypt in 1954 (Holbrook et al., 2005).

MATERIAL AND METHODS

The Study Site

The study was conducted during the spring of 2016 on the roof of the Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt (Fig. 1). The location and description of the study site were previously presented by Abd El-Gawad et al., 2019.

Experimental Animals and Toxin

According to Abd El-Gawad et al., 2019, six healthy mature rabbits (*Oryctolagus curicullus* Linnaeus, 1758) each weighing 1.5 kg were used as a surrogate model to mimic the human body decomposition. The rabbits were divided into two groups of three: the control group (C) was asphyxiated by hanging, while the second group (WI) was poisoned with an oral dose (50 mg/kg) of Warfarin rodenticide. After death, the rabbit carcasses were transported to the study site and kept separately in labeled wooden framed cages (50 cm^3) covered with stainless steel wire mesh (1*1cm). Each cage was placed almost 1 meter apart to simulate an isolated source for insects dwelling in each carcass. The animals were held and treated according to the guiding principles of the Research Ethics Committee (REC) for animal subject research at the Faculty of Science, Ain Shams University, Cairo, Egypt, which approved the experiments.

Collection and Processing of Specimens

All stages of insects were daily collected from different parts of the rabbit body and the soil by spoons for immatures and forceps for adults. The collected adults and immatures were kept separately in labeled plastic vials and transferred to the laboratory for counting and identification according to the morphological keys (Majka, 2011; Sawaby et al., 2016).

Statistical Analysis

Means and Standard Deviations (SDs) of the examined attributes were calculated and compared with one-way ANOVA using the SSP (Smith's Statistical Package) software (Smith, 2004).

RESULTS AND DISCUSSION

RESULTS:

Decomposition Stages and Succession Pattern of Coleopteran Insects

Five stages of decomposition were observed: fresh, bloated, active decay, advanced decay, and dry or skeletonization. The adult and immature appearance intervals on various cadavers demonstrated that they first emerged on days 3 or 4 (the bloated stage) on both the control (C) and Warfarin-intoxicated (WI) carcasses, and they remained until the start of the dry stage (days 9 to 11).

Collected Coleopteran Adults and Immatures

Based on the average number of adults and immatures collected per rabbit that was calculated and compared between the C and WI groups. The results (Table 1) indicated that more adults and immatures were attracted to intoxicated carcasses ($P < 0.05$) than to control ones.

Table 1. Means of collected coleopteran adults and immatures per rabbit on control (C) and Warfarin-intoxicated (WI) rabbit carcasses (n=3)

Stage	Carcasses	Range	Total	Mean± SD
Immatures	C	93-333	664	175.7±136.3
	WI	168-339	680	283.0±99.6
Adults	C	88-107	290	108.3±21.0
	WI	130-153	505	138.3±12.7

Fig. 1. Map showing the location of the study site at Abbassia in the north of Cairo

Relative Abundance of Coleopteran Families per Decomposition Stage:

No individuals were reported during the fresh stage of C and WI carcasses. Four families were reported of which, Dermestidae was the most common during the subsequent 4 decomposition stages of C carcasses (40.0-79.9%) than the other families (Fig. 2). Additionally, during the four stages of WI carcasses, Dermestidae was more abundant (32.7–46.5%), with a possible exception of the active decay stage, when Histeridae was more common (43.6%) than the other families. In general, in all stages of tested families, Dermestidae was the most common (66. 8% off C; 35.8% off WI) followed by Histeridae (17.3% off C; 34.1% off WI), Staphylinidae (8.5% off C; 16.8% off WI) and Cleridae (7.5% off C; 13.3% off WI).

Identified Coleopteran Species

Table (2) showed the total of 7 species of the 4 families was collected on both C and WI carcasses. The most common species were: *Attagenus faciatus* (Dermestidae) on C (29.9%) and *Saprinus furvus* (Histeridae) on WI (20.9%) carcasses. The least collected species were *Necrobia rufipes* on C (5.8%) and *Attagenus faciatus* on WI (8.6%) carcasses.

Table 2. The percentages (%) of collected coleopteran species off control (C) and Warfarin- intoxicated (WI) rabbit carcasses

Family	Species	C	WI	Common names
Dermestidae	*Attagenus faciatus* (Thunberg, 1795)	29.9	8.6	The banded black carpet beetle
	Dermestes maculatus (De Geer, 1774)	21.7	10.3	The hide beetle
	Dermestes frischii (Kugelann, 1792)	15.2	16.9	The hairy beetle
Staphylinidae	*Creophilus maxillosus* (Linnaeus, 1758)	8. 5	16.8	The hairy rove beetle
Histeridae	*Saprinus chalcites* (Illiger, 1807)	11.5	13.2	The Clown beetles
	Saprinus furvus (Erichson, 1834)	5. 8	20.9	
Cleridae	*Necrobia rufipes* (De Geer, 1775)	7.5	13.3	The red-legged ham beetle

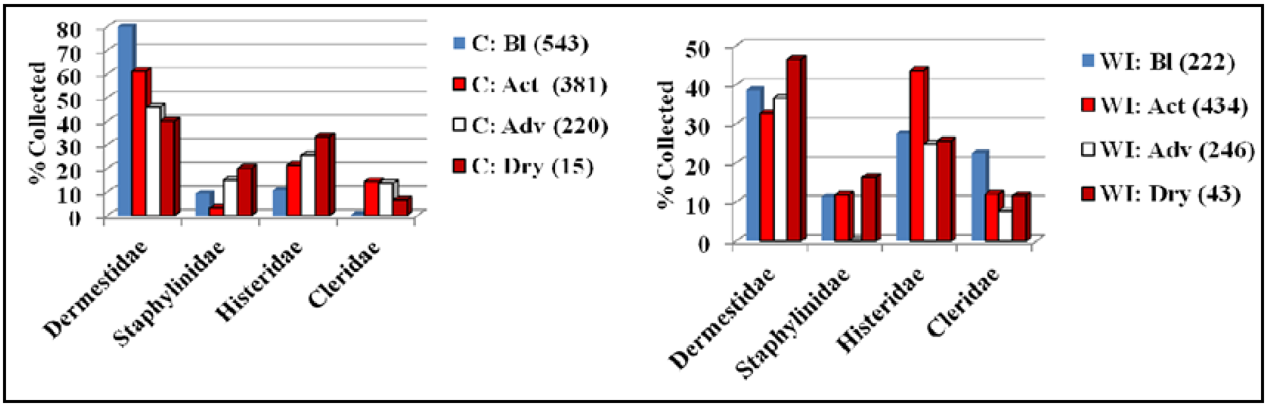

Fig. 2. The percentages of collected coleopteran families per decomposition stage (Bl: bloated, Act: active decay, Adv: advanced decay and dry stages) of control (C) and Warfarin-intoxicated (WI) rabbit carcasses

Table 3. Numbers of individuals of other orders collected off control (C) and warfarin-intoxicated (WI) rabbit carcasses

Order	Family	Species	C (87)	WI (157)	Common names
Hymenoptera	Vespidae	*Vespa orientalis* (Linnaeus, 1771)	3	5	Oriental hornet
	Apidae	*Apis mellifer*a (Linnaeus, 1758)	1	2	Honeybee
	Formicidae	*Monomorium pharoanis* (Linnaeus, 1758)	50	90	Pharaoh ant
	Braconidae	*Microplitis rufiventris* (Kokujev, 1914)	6	30	Braconid wasp
	Pteromalidae	*Nasonia vitripennis* (Ashmead, 1904)	4	6	Jewel wasp
	Evaniidae	*Evania appendigaster* (Linnaeus, 1758)	5	4	Ensign wasp
Dermaptera	Labiduridae	*Forficula auricularia* (Linnaeus, 1758)	3	3	Common/European earwig
Zygentoma	Lepismatidae	*Thermobia aegyptiaca* (Lucas,1840)	9	13	Silver fish
Blattodea	Blatellidae	*Blatella germanica* (Linnaeus, 1767)	1	1	German cockroach
Hemiptera	Cydnidae	*Cydnus aterrimus* (Forster, 1771)	2	2	Burrowing bug
	Cicadellidae	*Empoasca decipiens* (Paoli, 1930)	3	1	Leafhoppers

Collected Adults of Other Orders

Table (3) showed a few individuals of 11 species belonging to 11 families of 5 orders were collected off C and WI rabbit carcasses of which *Monomorium pharoanis* (Hymenoptera: Formicidae) was the most common species (50% off C; 90% off WI).

DISCUSSION:

During this study, five stages of decomposition of rabbit carcasses were observed: fresh, bloated, active decay, advanced decay, and skeletonization. Previous authors (Tantawi et al., 1996; Galal et al., 2009; Albushabaa and Almousawy, 2016) detected only four stages (fresh, bloated, active decay, and dry) while Payne (1965) reported six decay stages (fresh, bloated, active decay, advanced decay, dry, and remains).

Family Dermestidae is usually encountered on dried corpses and carcasses, particularly those that have been skeletonized or mummified. They are forensically important insect species for estimating longer PMI (Wang et al., 2021). The majority of reported beetle species (Histeridae, Staphylinidae, and Cleridae) act as predators on eggs, maggots, and pupae of flies and larvae of other beetles while others (Dermestidae) feed on dried skin and tissues during the latter stage of decomposition. Moreover, several coleopteran insects consume corpses in the advanced decomposition stage and can be used to estimate the minimum post-mortem interval (PMI_{min}) by analyzing the community succession present on a corpse. In this case, many fly larvae have already left the corpse, leaving mostly Coleoptera, by which such an estimate can be made.

The results indicated that Dermestidae was the first group attracted during the present study. Abd El-Bar et al. (2016) had similar observations, while Polat and Kökdener (2014) reported Staphylinidae as the first invaders. During this study in spring, *Dermestes maculatus*, *Dermestes frischii*, and *Attagenus faciatus* were reported. These species were reported previously only during summer (Polat and Kökdener**,** 2014) and *Dermestes ater* were observed invading carcasses during summer and winter (Tantawi et al., 1996; Abd El-Bar et al., 2016). Furthermore, similar to earlier studies, Dermestids were observed early as immatures and adults at the bloated stage, and they progressed through active decay, advanced decay, and skeletonization (Abajue et al., 2013; Aly et al., 2013; Abouzied, 2014; Abd El-Bar et al., 2016) while they were conspicuously absent in the fresh, bloated, and early decay stages of decomposition (Goyal, 2012).

In the family Histeridae, only *Saprinus chalcites* and *Saprinus furvus* were observed on carcasses while four species: *Saprinus furvus*, *Saprinus chalcites*, *Saprinus semistaiatus*, and *Saprinus caerulescens* were reported in summer and winter seasons (Tantawi et al., 1996; Abd El-Bar et al., 2016), and only *Saprinus semistriatus* was reported by Giordani et al., (2018), while no collection of any representatives of this genus was obtained by Zeariya et al., (2015). Our results documented the presence of Histeridae beetles from the bloated stage to skeletonization. These beetles were found invading the cadavers from the fresh stage to the active decay (Abd El-Bar et al., 2016) and just during the active decay stage (Abouzied, 2014).

Among Staphylinidae**,** only one species; *Creophilus maxillosus* was reported. Zeariya et al. (2015) had similar observations, while four species; *Creophilus maxillosus*, *Trachyderma hispidae, Zophosis abbreviate*, and *Mesostina puncticollis* were previously reported (Abd El-Bar et al., 2016). This species occurred in all decomposition stages except the fresh stage. Other authors reported different stages namely: active, advanced, and dry stages (Wolff et al., 2001), bloated stage (Voss et al., 2008), and bloated and active decay (Kyerematen et al., 2013).

Anderson and VanLaerhoven (1996) reported that Cleridae is one of the most important families in forensic entomology and is classified as omnivorous insects that feed on carcass hair and keratin. This family was represented by one species, *Necrobia rufipes*, which agreed with the previous observations (Tantawi et al., 1996; Abd El-Bar et al., 2016). Our study revealed that this species was observed during the bloated, active, advanced decay, and skeletonization stages. Abajue et al. (2013) had similar findings while other authors reported different stages for example, the advanced and dry stages (Wolff et al., 2001; Polat and Kökdener, 2014), active decay (Dupont et al., 2011), and active and advanced decay (Abd El-Bar et al., 2016).

Abd El-Gawad et al. (2018) reported that Warfarin rodenticide had effects in producing abnormal and malformed fly adults, but this effect was not observed with coleopteran adults.

On the other orders; *Vespa orientalis* and *Apis mellifera* (Hymenoptera) were observed hovering over carcasses feeding on the carcasses' fluid, *Nasonia vitripennis*(Hymenoptera):(asmall pteromalid parasitoid wasps that sting and lay eggs in the pupae of numerous flies usually blow flies and flesh flies) and this parasitoid wasp was observed throughout the bloated, active decay, and advanced decay stages of decomposition and *Thermobia aegyptiaca* (Zygentoma) is considered to be opportunistic species that use carcasses as shelters and found at the dry stages of decomposition. This result was in agreement with other studies (Tantawi et al., 1996; Abd El-Bar et al., 2016). *Microplitis rujiventris* (Hymenoptera) was previously collected during active decay, advanced decay, and dry stages (Tantawi et al., 1996), while it was observed throughout the active decay and advanced stages of decomposition in our study. In other species, *Evania appendigaster* (Hymenoptera) was reported which is a parasitoid wasp on cockroach eggs, Moreover, *Forficula auricularia* (Dermaptera) (an omnivorous insect) and *Monomorium pharoanis* were also collected, as they feed on fly eggs.

Overall, the fact that this study was carried out in the spring may have had a major impact on the variations in the quantity and variety of species reported between this study and certain other studies. It was reported that spring-placed carcasses attracted a more diverse assemblage of insects than summer-placed ones (Albushabaa, and Almousawy, 2016). Based on the results of the present study and previous reports, lists of forensically important coleopteran species and other species with minor importance collected off human and animal carcasses were prepared and presented in **Tables 4** and **5**.

Table 4. Reported forensic coleopteran species in Egypt

Family/ Species	Family/ Species	Family/ Species
Histeridae	Dermestidae	Staphylinidae
Saprinus chalcites	*Attagenus faciatus*	*Creophilus maxillosus*
Saprinus furvus	*Dermestes maculatus*	*Trachyderma hispidae*
Saprinus caerulescens	*Dermestes frischii*	*Philonthus stragulatus*
Saprinus semistaiatus	*Dermestes ater*	*Philonthus longicornis*
Saprinus lugens	*Dermestes vulpinus*	*Zophosis abbreviate*
Saprinus gilvicornis	*Dermestes* sp.	*Mesostina puncticollis*
Saprinus semipunctatus	Tenebrionidae	*Atheta* sp
Hister sp.	*Trachyderma hispidae*	Anobiidae
Cleridae	*Zophosis abbreviata*	*Lasioderma* sp.
Necrobia rufipes	*Creophilus maxillosus*	Nitidulidae
Pteromalidae	*Mesostina puncticollis*	*Carpophilus hemipterus*
Nasonia sp.		

Table 5. Additional reported insect species in the study (with minor forensic importance in Egypt)

Order / Family/ Species	Order / Family/ Species	Order / Family/ Species
Hymenoptera	Hymenoptera	Dermaptera
Vespidae	Evaniidae	Eulophidae
Vespa orientalis	*Evania appendigaster*	*Tetrastichus* sp
Dolichovespula sp	Chalcididae	Zygentoma
Dolichovespula sp	*Brachymeria* sp.	Lepismatidae
Apidae	Chrysididae	*Thermobia aegyptiaca*
*Apis mellifer*a	*Chrysis* sp.	Blattodea
Formicidae	Dermaptera	Blatellidae
Monomorium pharoanis	Labiduridae	*Blatella germanica*
Cataglyphis bicolor	*Forficula auricularia*	Hemiptera
Pheidola megacephala	Carcinophoridae	Cydnidae
Solenopsis geminata	*Euborellia annulipes*	*Cydnus aterrimus*
Paratrechina longicornis	Chalcididae	Cicadellidae
Camponotus maculatus	*Brachymeria fonscolombei*	*Empoasca decipiens*
Unidentified sp.	*Euchalcida* sp	
Braconidae	*Chalcis* sp	
Microplitis rufiventris	*Brachymeria fonscolombei*	
Pteromalidae	Pteromalidae	
Nasonia vitripennis	*Pteromalus* sp	

CONCLUSION

The fields of forensic entomology research are still scanty in Egypt except for limited studies. This study may add information on coleopteran insects' diversity, relative abundance, and occurrence of forensic coleopterans in Cairo, Egypt during the spring season. Moreover, it is the first record of the coleopteran insects on Warfarin-intoxicated rabbit remains.

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الملخص العربي

الحشرات غمدية الأجنحة ذات الأهمية الجنائية ورتب أخرى من الحشرات التي انجذبت إلى جيف أرانب ضابطة وأخرى مسممة بالوارفارين

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علم الحشرات الجنائي هو تخصص متوسع في علم الحشرات يجمع بين معرفة عالم الحشرات بأنواع الحشرات، ودورات حياتها، وموائلها مع جوانب من إنفاذ القانون. تشكل رتبتا ذوات الجناحين (Diptera) وغمدية الأجنحة (Coleoptera)الأدلة الحشرية الرئيسية لتحديد فترة ما بعد الوفاة . (PMI) تم تصميم هذه الدراسة لفحص عملية التحلل وتعاقب الحشرات غمدية الأجنحة (رتبة: Coleoptera) على جثث الأرانب المكشوفة. تم تقسيم ستة أرانب إلى مجموعتين: تم تسميم المجموعة الأولى بمبيد القوارض الوارفارين المسممة بالوارفارين،(WI)، وتم خنق المجموعة الثانية عن طريق الشنق كمجموعة ضابطة (.Cتم ملاحظة خمس مراحل من التحلل في جميع الجثث. تم تمثيل أربع عائلات من الحشرات غمدية الأجنحة بسبعة أنواع: Attagenus faciatus، Dermestes frischii، Dermestes maculates، (Dermestidae)، Creophilus maxillosus (Staphylinidae)، Saprinus chalcites، Saprinus furvus (Histeridae)، Necrobia rufipes (Cleridae).من بين هذه الأنواع، كان Attagenus faciatus (29.9% من المجموعة الضابطة) و20.9% Saprinus furvus (من المجموعة المسممة) هما النوعان الأكثر شيوعاً. بشكل عام، كانت عائلة Dermestidae هي المجموعة الأولى التي انجذبت وكانت الأكثر شيوعًا (٦٦,٨% من المجموعة الضابطة؛ ٣٥,٨% من المجموعة المسممة)، تلتها 17.3% Histeridae من المجموعة الضابطة؛ ٣٤,١% من المجموعة المسممة)، 8.5% Staphylinidae من المجموعة الضابطة؛ ١٦,٨% من المجموعة المسممة) و7.5% Cleridae من المجموعة الضابطة؛ ١٣,٣% من المجموعة المسممة). تم جمع أحد عشر نوعاً من رتب أخرى من كلا الجثتين. كان Monomorium pharaonis (Hymenoptera: Formicidae) هو النوع السائد الذي تم جمعه من كلا الجثتين الضابطتين والمسممتين (٥٠:٥٧,٥% و٩٠:٥٧,٣% على التوالي). تقدم هذه الدراسة معلومات عن التنوع والتعاقب والكثافة النسبية وظهور الحشرات غمدية الأجنحة ذات الأهمية الجنائية في القاهرة، مصر. علاوة على ذلك، تعتبر هذه الدراسة أول تسجيل لوجود الحشرات غمدية الأجنحة على جثث مسممة بالوارفارين.