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Comparative Study on Forensic Insects encountered on Rabbit Corpses killed with different methods in Upper Egypt

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Abstract

This work aims to compare between the forensic insect species, their succession patterns and decomposition stages of rabbit corpses killed with different methods in Qena city, Egypt. Four experiments were carried out during this study for four successive seasons. Four rabbits were placed in each experiment, the first two rabbits received a double LD_{50} of tramadol hydrochloride and cypermethrin via ear vein injection. The third rabbit was slaughtered while the fourth rabbit was euthanized with air injection (control). The LD_{50} of tramadol and cypermethrin in male rabbit was calculated from logarithmic scale and found to be 132 mg/kg and 126 µl/kg body weight, respectively.Currently, a total of 14271 insect individuals were collected from the screened bodies belonging to three orders, thirteen families and seventeen species. Moreover, corpses were colonized by different insect life stages (11689, 352 and 2230 individuals) included dipteran flies, Coleopteran and Hymenopteran insects, respectively The total number of species collected in summer was lower than in other seasons. Tramadol-treated rabbits decayed with similar rate to the control corpses during all seasons except in winter. As well as, decomposition process was significantly prolonged in cypermethrin killed rabbits during all seasons. However, slaughtered carrions decayed faster than control in winter and spring. Maggots deposited on cypermethrin-intoxicated carrions were very few and have been dead after few days from oviposition. Density of maggots collected from control corpse was loweras compared to the slaughtered one and higher than that on tramadol treated corpse. Therefore, these results are recommended to be taken in criminal investigations.

Keywords: Forensic insects; Corpse decomposition; Entomotoxicology; Tramadol; Cypermethrin.

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Introduction

Forensic entomology is the science interested with studying insects and other arthropods associated with cadavers which uses the data about their development or succession pattern to estimate the post mortem interval and hence, assistance in solving criminal cases (Aly et al., 2017; Kenawy and Abdel-Hamid, 2019; Byrd and Sutton, 2020 and El-Samad et al*.,* 2020). It is divided into three components: urban, stored products and medico-legal (criminal) forensic entomology (Tuccia et al., 2018 and El-Samad et al., 2021).

Insects are the first colonizers to detect and colonize the corpses after death. For this reason the entomologists often use the insects and other arthropods associated with carcasses in solving the violent crimes (Bonacci, 2016). Arthropods are divided according to their attraction to the cadaver into four categories: Necrophagous; Necrophalous; Omnivores and Opportunists (Aly et al., 2017 and Kreitlow, 2009).

In death-related investigations, the Post Mortem Interval (PMI) of a person is very important to reconstruct the events of the crime and to support or refute the alibi. However, as time goes on, it becomes more challenging to estimate PMI using various medical indicators. The most accurate and possibly approach to estimate PMI after 72 hours after death is the forensic entomology (Sharma et al., 2022). Forensic entomology can be used not only to determine the PMI but also, in detect the cause of death as drug abuse or poisoning by chemicals (Hamdy et al. 2022).

The presence of drugs or other toxins in dead bodies affects the developmental rate of forensic insects via food eating or food shackles transmitted by necrophagous insects, and consequently, this in turn impairs the accuracy of postmortem interval (PMI) estimation (Abd Al Galil et al., 2020). So, Entomotoxicology is a new branch of forensic entomology which uses the forensic insects in detecting the presence of drugs or other toxins ingested by victim before death via toxicological analysis (Campobasso et al., 2019 and Samnol et al., 2020).

Currently, the rate of slaughter or suicide crimes in our society is alarming and the police may in times lack the scientific means or evidence of investigating the proper cause of death as a cadaver can neither talk nor give proper information that could enhance investigations. Consequently, many innocent people therefore fall victim to such allegations. In addition, often frustrated peoples may suicide by drinking any of the common chemicals as cypermethrin or intake overdoses of drugs as tramadol and these kinds of crimes either intentional or by mistake are fast growing in our society and have necessitated this research to assist the law enforcement agents in criminal investigations using the insect evidences.

Therefore, the present study aims for obtaining a preliminary data about the decomposition process and documenting the forensically important insect species that colonize rabbit carrions killed by different methods (slaughtering and poisoning with tramadol and cypermethrin) and compare it with the control carcasses during the four seasons in Qena city, Egypt.

Material and Methods

The Study Site

The study site was located at the south valley university (roof of science faculty) (26.19192N, 32.74391E) in Qena city, Egypt. Study was conducted for one year from January 2019 to December 2019.

Climatic conditions in Qena differ according to seasons where mean temperature and humidity readings recorded by digital thermometer in winter (17.8-28.9°C and 16.6-48.6%), spring (28.2-39.5°C and 10.1-28.7%), summer (40.3-44.1°C and 4.1-23.6%) and autumn (26.9-35.6°C and 15.8-41.1%), respectively.

Experimental animals

Sixteen healthy male rabbits, *Oryctolagus cuniculus*, were chosen to simulate the soft skin of a new baby and it was characterized by a relatively uniform size (≈ 600 ± 50 gm). The fore-mentioned rabbits were purchased locally with different colors.

LD_{50} Determination of Tramadol Hydrochloride and Cypermethrin

A total number of 32 male rabbits was used for LD_{50} determination of both of tramadol and cypermethrin. Animals were divided into eight groups (4 rabbits/ group). The first seven groups were injected via ear vein with different single doses ranging from (100 to 150 µl/kg) and (31.5 to 200 mg/kg) of cypermethrin and tramadol, respectively. The eighth group was served as control group and injected with distilled water. The animals were observed for mortality during the 24 hour observation period. The LD_{50} was determined by graphical method. The injection doses in each group were illustrated in table 1 and 2.

The killing methods and the experimental layout

Sixteen rabbits were used during this study for one year. One experiment was conducted in each season. Four rabbit carcasses were placed in each experiment, the first two rabbits received toxins of a double LD_{50} of tramadol hydrochloride and cypermethrin via ear vein injection. The third rabbit was slaughtered by knife at the neck while the last fourth rabbit was euthanized with air injection to mimic the normal death case without any chemicals or drugs and used as control. After death, the animals were immediately delivered post-mortem to the research site. A tray containing sawdust was placed under each cage to facilitate the collection of larvae leaving carrions for pupation. All efforts were made to keep cadaver disturbance to a minimum during taking samples. The temperatures, humidity and wind velocity readings were taken with digital thermometer.

Sample handling and preservation

During the collection days, representative samples of immature and adult insects were collected from and around the carrions. Adult flies catched in the upper part of dipterous trap were collected by separating this part and killed them by using chloroform or freezing, then preserved as it is in a glass vials or in 70% alcohol for identification. In addition, air insects hoving around carrions were collected using sweeping net whilst eggs and first larval instars were collected by brush. Adult beetles, second and third larval instars, and other hard-bodies crawling insects were collected by hand or with forceps and also immersed in 70% alcohol. Collected samples were placed in plastic vials labeled with the date and method of killing. For each carrion, approximately 50 larvae were collected from natural body openings (mouth, nose and anus) and from wounds if any on the body. The collected Maggots specimens were kept alive in laboratory for rearing in glass jars covered with a net mesh and containing fresh beef liver for feeding at room temperature for identification.

Identification

The collected insects were identified in the entomology laboratory, faculty of science, south valley university using several entomological keys: Marshall et al. (2011); Irish et al. (2014) and Al-Shareef (2016). Finally, the samples were sent to agricultural research center, plant protection research institute and insect identification unit for confirmation of the identified species. Most of the collected insects were identified to the species level. Larvae were identified by the rearing inside the laboratory of entomology.

Data and statistical analysis

The Student's excel sheet was employed to compare between the temperature, humidity and wind velocity readings for each season separately and also for determining of LD_{50} of tramadol and cypermethrin. Analysis of Variance on SPSS software package (Norusis, 2005), (version 16) (SYSTAT statistical program) was used to test the present data. Pearson correlation coefficients and multiple regressions were applied in the present data. Stepwise multiple regressions were used to select the affected variable.

Ethical approval:

The research protocol of the present study was performed in accordance with the Ethical Research Committee of the Faculty of Science, South Valley University, Qena governorate, Egypt. (Approval No. 014/11/22).

Results

Intraveinously LD_{50} of Cypermethrin:

The experimental trials for intraveinously LD_{50} injection of cypermethrin after 24hr of administration in male domestic rabbits revealed that the mortality commenced at 115 µl kg^{-1} body weight, recording mortality percentage of 25% (Table 1). Increasing cypermethrin dose to 120, 125, 130 and 140 µl kg^{-1} resulted in mortality percentages of 25, 50, 75 and 75%, respectively. The mortality rate was a function of dose increase. The maximum concentration of cypermethrin which killed all animals in the group was found to be 150 µl kg^{-1} body weight. The calculated intraveinously LD_{50} of cypermethrin in male domestic rabbits from the linear regression was found to be 126 µl kg^{-1} body weight (Fig. 1).

Table 1: Mortality percentage of male domestic rabbits injected with different doses of Cypermethrin after 24hr.

Group	Cypermethrin Dose (µl/kg body weight)	no. of animals died/total	% mortality
Group 1	100	0/4	0
Group 2	115	1/4	25
Group 3	120	1/4	25
Group 4	125	2/4	50
Group 5	130	3/4	75
Group 6	140	3/4	75
Group 7	150	4/4	100
Group 8	control	0/4	0
LD_{50}	126 µl/kg		

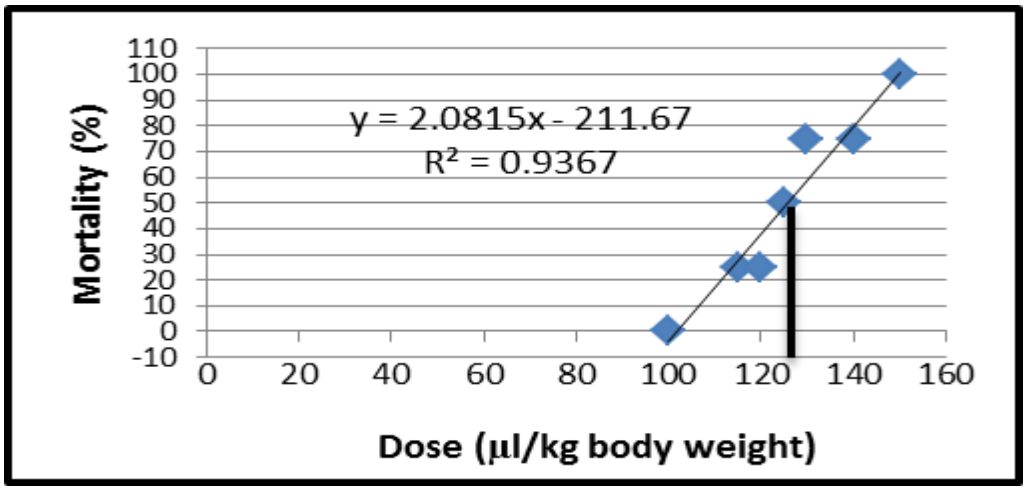

Fig. (1): Logarithmic scale of LD_{50} of Cypermethrin in male domestic rabbits (LD_{50} = 126 µl/kg body weight)

Intraveinously LD_{50} of Tramadol hydrochloride

The experimental trials for intraveinously LD_{50} injection of tramadol hydrochloride after 24hr of administration in male domestic rabbits revealed that the mortality commenced at 63 mg kg^{-1} body weight, recording mortality percentage of 25% (Table 2). Increasing tramadol hydrochloride dose to 100, 126, 150 and 163 mg kg^{-1} resulted in mortality percentages of 25, 25, 50.0 and 75%, respectively. The mortality rate was a function of dose increase. The maximum concentration of tramadol hydrochloride which killed all animals in the group was found to be 200 mg kg^{-1} body weight. The calculated intraveinously LD_{50} of tramadol in male domestic rabbits from the linear regression was found to be 132 mg kg^{-1} body weight (Fig. 2).

Table 2: Mortality percentage of male domestic rabbits injected with different doses of Tramadol after 24hr.

Group	Tramadol hydrochloride Dose (mg/kg body weight)	no. of animals died/total	% mortality
Group 1	31.5	0/4	0
Group 2	63	1/4	25
Group 3	100	1/4	25
Group 4	126	1/4	25
Group 5	150	2/4	50
Group 6	163	3/4	75
Group 7	200	4/4	100
Group 8	control	0/4	0
LD_{50}	**132 mg/kg**		

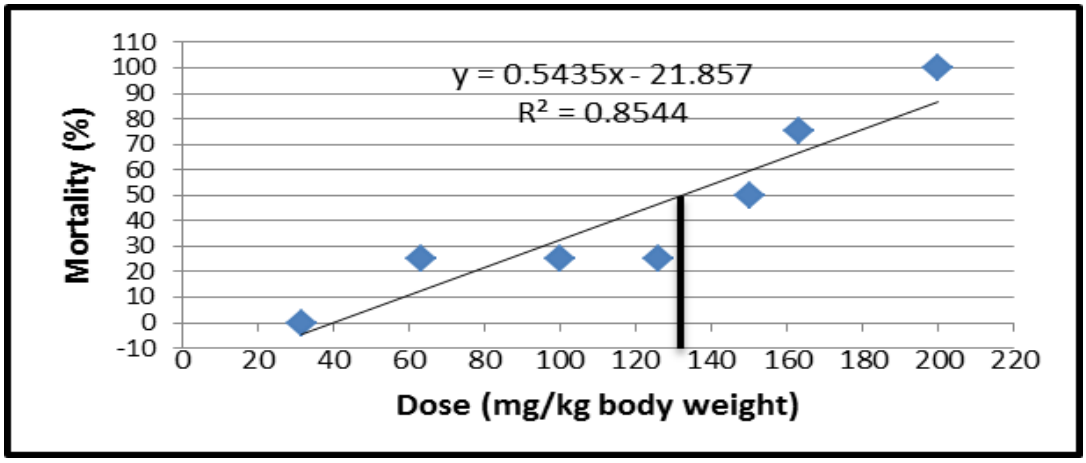

Fig. (2): Logarithmic scale of LD_{50} of Tramadol in male domestic rabbits (LD_{50} = 132 mg/kg body weight)

Seasonal Corpse Decomposition Stages

Decay rates of all rabbit corpses in different seasons from January 2019 to December 2019 in Qena governorate, Egypt are shown in table (3) and fig. (3). All decomposition stages prolonged the highest period in winter and the lowest period in summer. The process of corpse decomposition was divided into the following stages:

Fresh stage: Began with the moment of death and continued until the first signs of bloating. No odor or swelling. During this stage, flies of Calliphoridae and Sarcophagidae began arrive to the corpses and lay their eggs or larvae. Fresh stage was observed on the 0 day only for all corpses in all seasons except in winter it lasted from (0-5) days for all carrions (Fig. 3).

Bloating stage: This stage marked the beginning of putrefaction, began when the abdomen bloated due to the autolysis of tissues and the activity of fungi and bacteria and ended when abdomen deflated due to the releasing of gases. Bloating stage began on day 1 postmortem for all carrions in all seasons except in winter; it lasted from (6-10) days postmortem for all carcasses (Fig. 3).

Active decay stage: This stage began when gasses released, liquefaction occurred and the abdomen deflated and during it the decay odor became very strong. Liquefaction first occurred on the 2nd day for all carrions in summer and on day 3 postmortem for all cadavers in autumn and spring. However, liquefaction first occurred on day 11 postmortem for all carrions in winter season (Fig. 3).

Advanced decay stage: During this stage, odor began to fade, most of the flesh has disappeared, some soft tissue still found in the abdomen and the coleopteran species are the predominant. This stage was arrived on the 3rd day for all corpses in summer except cypermethrin-intoxicated corpse was on day 4 postmortem and on the 4th day for all carrions in autumn and spring except cypermethrin-treated corpse was arrived on the 6th day. Whilst, in winter advanced decay began on days 15, 14, 16 and 20 postmortem for control, slaughtering, tramadol and cypermethrin-killed rabbits, respectively (Fig. 3).

Dry stage: In this stage, odor disappears and the remains become consistent only of

bones and hairs. All carrions in summer reached dry stage on the 4th day except cypermethrin-treated carrion on the 7th day. In autumn, all carcasses arrived to dry stage on the 6th day except cypermethrin-treated carrion on the 9th day. However, dry stage began on days 8, 7, 8, 13 and on 21, 19, 24, 29 postmortem for control, slaughtering, tramadol and cypermethrin-treated carrions in spring and winter, respectively (Fig. 3).

In general, decomposition process was observed to begin from the head region in all carrion types and then prolonged for all the body parts of dead animal. In this study was observed that decomposition process was slower in winter season and varied between the four carrion types compared to the other seasons. However in summer season, the decomposition process not varied greatly between the four studied cases due to the action of high temperatures. Consequently, the climatic condition data revealed that there was a direct relation between the decomposition process and temperature which the high temperature contributed to fast the decomposition process whereas the low temperature prolonged it.

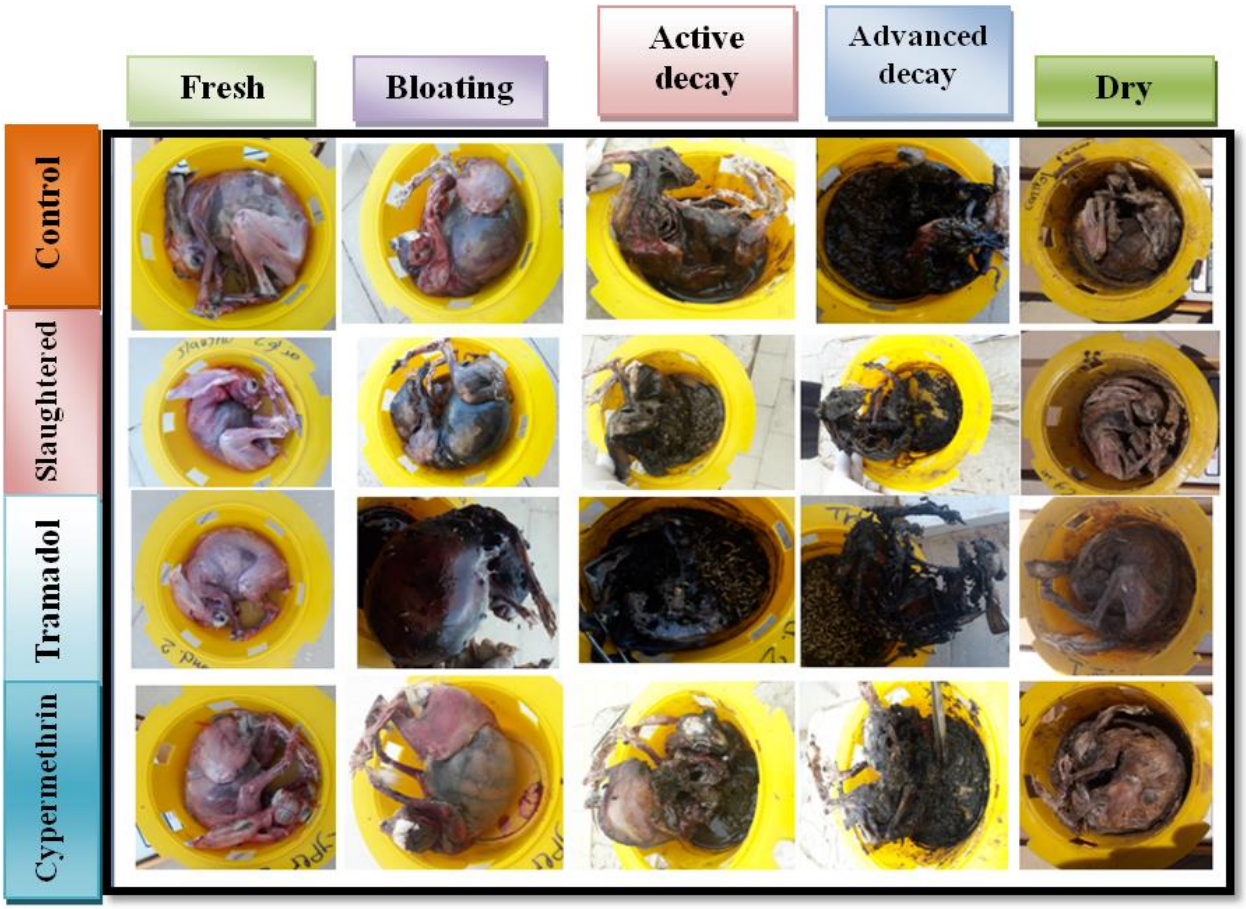

Fig. (3): Decomposition of the postmortem interval (PMI) for rabbit corpses showing the decomposition stages of different carrions: Control; Slaughtered; Tramadol and Cypermethrin.

Table (3): Decay rates of rabbit corpses killed by different methods in different seasons from January 2019 to December 2019.

		Days postmortem				
Season	Carrion	Fresh	Bloating	Active decay	Advanced decay	Dry
Summer	Control	0	1	2	3	4-15
	Slaughtering	0	1	2	3	4-15
	Tramadol	0	1	2	3	4-15
	Cypermethrin	0	1	2-3	4-6	7-20
Autumn	Control	0	1-2	3	4-5	6-21
	Slaughtering	0	1-2	3	4-5	6-21
	Tramadol	0	1-2	3	4-5	6-21
	Cypermethrin	0	1-2	3-5	6-8	9-27
Winter	Control	0-5	6-10	11-14	15-20	21-45
	Slaughtering	0-5	6-10	11-13	14-18	19-42
	Tramadol	0-5	6-10	11-15	16-23	24-49
	Cypermethrin	0-5	6-10	11-19	20-28	29-57
Spring	Control	0	1-2	3	4-7	8-22
	Slaughtering	0	1-2	3	4-6	7-21
	Tramadol	0	1-2	3	4-7	8-24
	Cypermethrin	0	1-2	3-5	6-12	13-31

Forensic Catches:

A total of 14271 individuals of adult insects was collected during this study from the four corpse types belonging to three orders, thirteen families and seventeen species as shown in table (5 and 6) and fig. (5-8). The order of Diptera comprised (11689) individuals (81.9%), However the order of Coleoptera and Hymenoptera comprised (352 and 2230) individuals (2.5% and 15.6%) respectively (Fig. 4). The most diverse and abundant order during this study was Diptera as several individuals recorded belonged to seven families of this order with eleven species.

The most dominant forensic species observed during this study were mainly from order Diptera which were collected from carrions as adults and maggots {*Sarcophaga ruficornis* (1178+2510), *Wohlfahrtia magnifica* (338+3444), *Chrysomya albiceps* (2141+510) *and Lucilia cuprina* (514+135)}, respectively.

It was observed that the insect species that invading and colonizing the decomposed corpses were highly affected by the climatic condition changes in various seasons and also by the killing methods.

For adult species, it was noticed that the house fly (*Musca domestica*) was the predominant species during all seasons in all study cases except in winter where the number of adult collected from *Nasonia* sp. made it the most predominant species. House fly was collected as adult only although it was found during all decomposition stages means that this species fed only on the corpses but did not breed on it. However, *Nasonia* was collected as adult and maggots from control corpse in winter only where this species parasiting on fly pupae and laid its eggs on it. So Nasonia parasite is considered of forensic importance in case of absence from dipteran flies or the dead body was more decomposed.

Focusing on maggots collected from all corpses (Table 4), it was observed that *Sarcophaga* maggots were the most predominant immature stages during all

seasons and all corpses except in summer where *Wohlfartia* maggots were the most predomint. This may be attributed to the fact that *Sarcophaga* species not prefered the hot seasons and vice versa for *Wohlfartia* species. Acoording to killing method, the slaughtered carrions were colonized with the highest numbers of maggots whilst the cypermethrin treated carrions were colonized with the very little numbers of maggots and all maggots have been dead after few days from oviposition. So, decomposition process was prolonged in cypermethrin treated corpses however was shorten in slaughtered corpses and this indicated the important role of the presence of maggots on dead bodies.

According to killing methods, there was observed that control carrions have the highest abundance and diversity of insect species compared with the other types of carrions. This may be attributed to that the control carrions passed with natural decomposition process without any effect of chemical materials or wounds that can changes the colonization of insects. However, in regard to seasons, it was noticed that the highest number of individuals was collected in spring and the lowest number in summer.

Waves of Seasonal insect Succession patterns

According to seasonal climatic conditions recorded in the current study, it was noticed that the warm seasons especially summer season caused most succession insects to colonize earlier via accelerating the activity of the immature stages of insects, and as a result, the degradation of carrion accelerated through only 15-20 days post killing. In contrast, the cooler winter season slowed the rate of insect colonisation by slowing the development of the immature stages, and as a result, the degradation of carrion prolonged up to 57 days post killing. On the same context, there was retardation in succession of insects on all carrion types in winter season where began the influx of insects on corpses at the 4th day postmortem. Whilst insect succession in the other seasons occurred early on the zero-day postmortem (Day of carrion exposure).

The present results shown that the waves of insect succession on all study carrions were minimal at the fresh stage of decomposition process before rising to a maximum during the bloating and active decay stages whereas in advanced decay and dry stages, the succession was observed decreased gradually once more to fade completely.

dipteran flies (Calliphorid and sarcophagid) flies were observed the first colonizers to arrive and breed on rabbit carcasses in the early stages of decomposition. However, sarcophagid species (maggots and adult) were predominant than other fly species during all seasons. The common existence of this two species and also their abundance on all carrions reveled their important role in forensic investigation.

According to the succession of insects on corpses, it was observed three different waves of succession, the first wave was represented mainly by dipteran flies and some species of coleopteran and hymenpteran (ants) and during this wave, dipteran flies were observed to laid their eggs/larvae on the natural openings of both control and tramadol treated carrions as mouth, nose and anus however in slaughtered carrions, the atificial wounds in neck area were the first positions for fly oviposition followed by the natural body orifices. On the other hand, in cypermethrin treated carrions, very little maggots were

found that were spreaded over different parts of body away about the natural openings. Once the appearance of dipteran maggots and increased its feeding activity, the second wave of succession was initiated and was marked also by the continuation of flocking from adult flies, beetles and ants where the insect variety reached its peak during this wave causing in rapid rate of degradation, then fell gradually again with advancing the decomposition and dipteran maggots departured apart away the cadavers. The third wave of succession then started and was marked by little adult numbers of beetles, ants and wasps where cadavers became completely decomposed.

Table (4): Mean numbers of dipteran maggots collected from rabbit corpses killed by different methods during various seasons from January 2019 to December 2019.

Season	Carrion type	Mean numbers of collected maggots				Total	
		Control	**Slaughtered**	**Tramadol**	**Cypermethrin**		
Winter	***Sarcophaga carnaria***	387	469	352	0	1208	**1658**
	Wohlfartia magnifica	0	0	0	0	0	
	Chrysomyia albiceps	93	138	80	4	315	
	Lucilia cuprina	44	59	32	0	135	
Spring	***Sarcophaga carnaria***	573	0	398	0	971	**2026**
	Wohlfartia magnifica	323	387	239	0	949	
	Chrysomyia albiceps	0	106	0	0	106	
	Lucilia cuprina	0	0	0	0	0	
Summer	***Sarcophaga carnaria***	0	0	60	0	60	**2402**
	Wohlfartia magnifica	787	925	620	10	2342	
	Chrysomyia albiceps	0	0	0	0	0	
	Lucilia cuprina	0	0	0	0	0	
Autumn	***Sarcophaga carnaria***	87	122	62	0	271	**513**
	Wohlfartia magnifica	49	70	34	0	153	
	Chrysomyia albiceps	0	86	0	3	89	
	Lucilia cuprina	0	0	0	0	0	
	Total	2343	2362	1877	17	**6599**	

Table (5): The collected forensic insects from the different rabbit corpses during autumn and winter seasons from January 2019 to December 2019

Order	Family and Species	Autumn carrions					Winter carrions				
		Control	Slaughtering	Tramadol	Cypermethrin	Total	Control	Slaughtering	Tramadol	Cypermethrin	Total
Diptera	**Family: Sarcophagidae**										
	Sarcophaga ruficornis	53	45	43	29	170	28	31	15	27	101
	Wohlfartia magnifica	33	35	42	28	138	3	1	1	2	7
	Family: Calliphoridae										
	Chrysomyia albiceps	175	162	153	115	605	64	46	73	97	280
	Lucilia cuprina	43	25	59	25	152	33	26	14	59	132
	Family: Muscidae										
	Musca domestica	290	450	315	167	1222	116	51	41	118	326
	Muscina stabulans	0	0	0	0	0	1	0	0	2	3
	Atherigona varia	35	71	57	20	183	0	0	0	0	0
	Family: Phoridae										
	Megaselia scalaris	0	0	0	0	0	3	1	2	6	9
	Family: Culicidae										
	Culex pipiens	6	0	1	6	13	0	3	1	1	5
	Family: Otitidae										
	Physiphora demandata	58	75	64	19	216	10	1	3	12	26
	Family: Surphidae										
	Syrphus corollae	0	2	1	0	3	0	0	0	0	0
Coleoptera	**Family: Dermestidae**										
	Dermestes vulpinus	20	15	0	0	35	18	12	15	25	70
	Family: Cleridae										
	Necrobia rufipes	0	0	0	15	15	0	1	2	3	6
	Family: Histeridae										
	Saprinus gilvicornis	15	11	7	0	33	0	0	2	5	7
Hymenoptera	**Family: Formecidae**										
	Camponotus maculatus	80	0	0	366	446	0	0	0	0	0
	Family: Chalcididae										
	***Chalcis* sp.**	0	0	0	0	0	15	0	0	0	15
	Family: Pteromalidae										
	***Nasonia* sp.**	38	0	0	0	38	1379	3	85	75	1542
Total		846	891	742	790	3269	1667	176	254	432	2529

Table (6): The collected forensic insects from the different rabbit corpses during spring and summer seasons from January 2019 to December 2019.

Order	Family and Species	Spring carrions					Summer carrions				
		Control	Slaughtering	Tramadol	Cypermethrin	Total	Control	Slaughtering	Tramadol	Cypermethrin	Total
Diptera	Family: Sarcophagidae										
	Sarcophaga ruficornis	222	243	251	172	888	5	3	7	4	19
	Wohlfartia magnifica	24	22	31	17	94	36	22	21	20	99
	Family: Calliphoridae										
	Chrysomyia albiceps	328	293	347	267	1235	2	9	5	5	21
	Lucilia cuprina	68	39	79	44	230	0	0	0	0	0
	Family: Muscidae										
	Musca domestica *Muscina stabulans* *Atherigona varia*	12953 300	83311 233	11386 249	6654 175	3931 24 957	520 0	830 0	550 6	610 3	2510 9
	Family: Phoridae										
	Megaselia scalaris	0	0	0	0	0	0	0	0	0	0
	Family: Culicidae										
	Culex pipiens	2	0	0	0	2	8	23	12	6	49
	Family: Otitidae										
	Physiphora demandata	45	41	52	55	193	14	22	28	30	94
	Family: Surphidae *Syrphus corollae*	1	0	1	0	2	0	0	0	0	0
Coleoptera	Family: Dermestidae										
	Dermestes vulpinus	29	17	39	43	128	0	0	0	0	0
	Family: Cleridae										
	Necrobia rufipes	8	5	0	5	18	0	0	0	0	0
	Family: Histeridae										
	Saprinus gilvicornis	13	12	15	0	40	0	0	0	0	0
Hymenoptera	Family: Formecidae										
	Camponotus maculates	0	84	0	0	84	0	0	0	0	0
	Family: Chalcididae										
	Chalcis sp.	0	0	0	0	0	0	0	0	0	0
	Family: Pteromalidae										
	Nasonia sp.	50	5	29	21	105	0	0	0	0	0
Total		2388	1838	2237	1468	7931	117	162	134	129	542

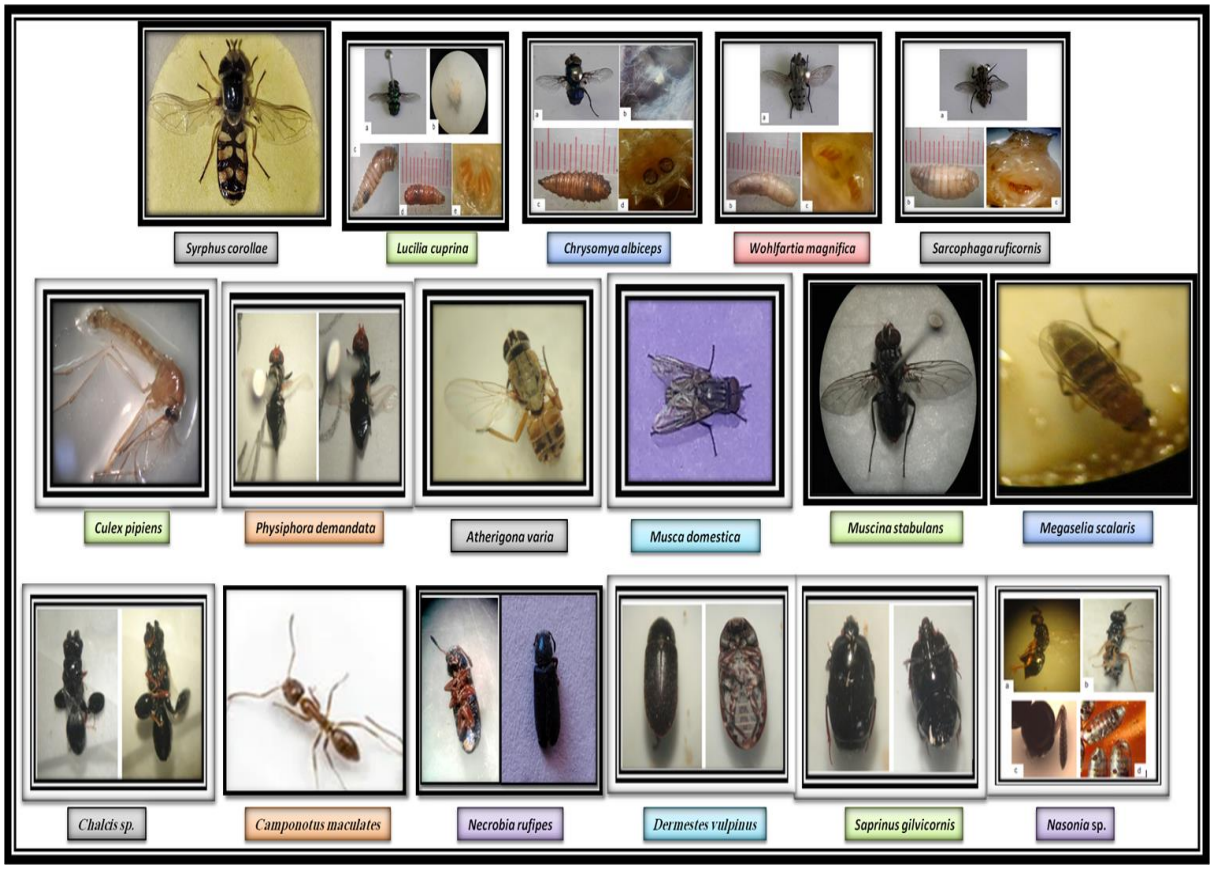

Fig. (4): plate of insect species collected from rabbit corpses killed with different methods in Qena governorate, Egypt

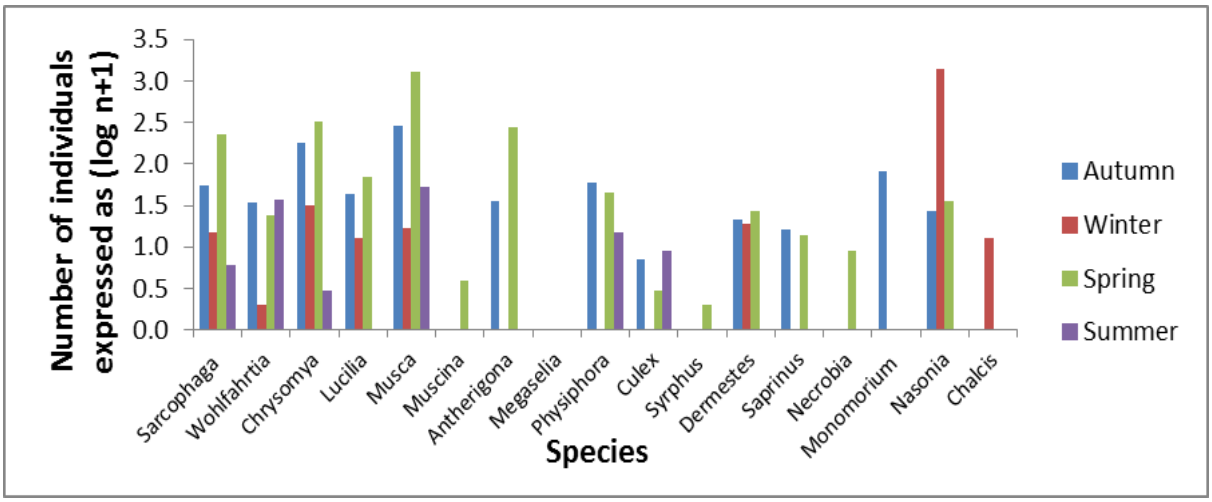

Fig. (5): The forensic insect species recorded on control corpses during different seasons from January 2019 to December 2019

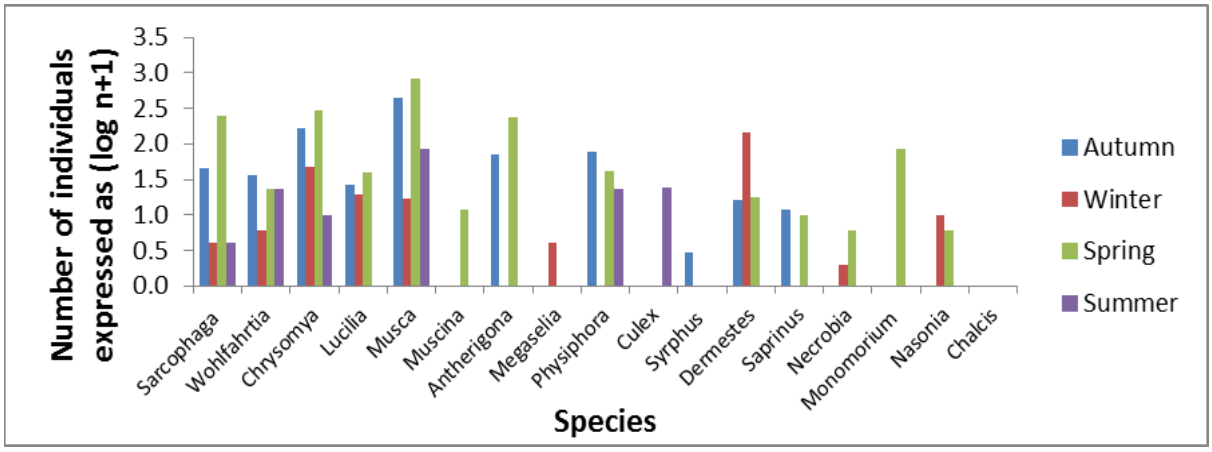

Fig. (6): The forensic insect species recorded on slaughtered corpses during different seasons from January 2019 to December 2019.

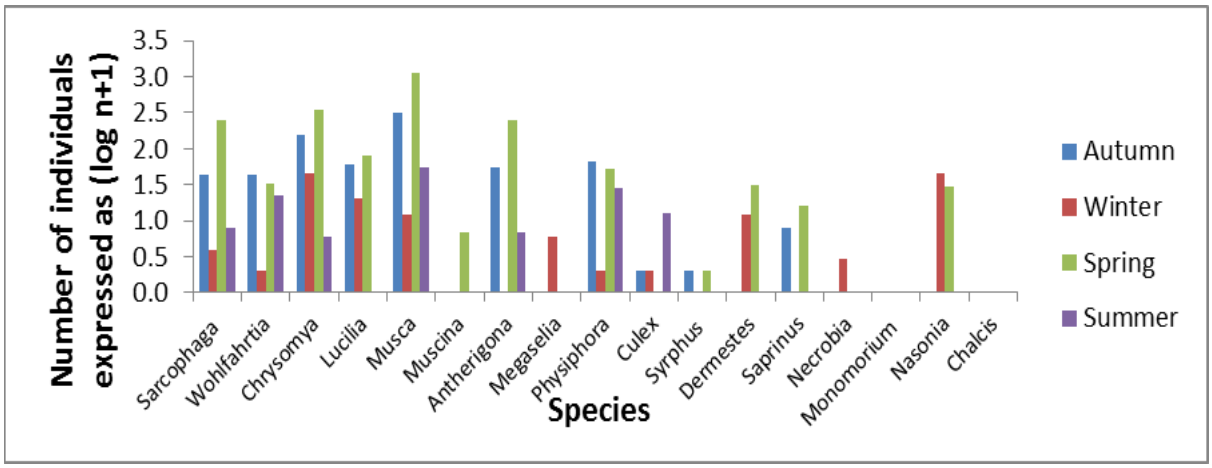

Fig. (7): The forensic insect species recorded on Tramadol treated corpse during different seasons from January 2019 to December 2019

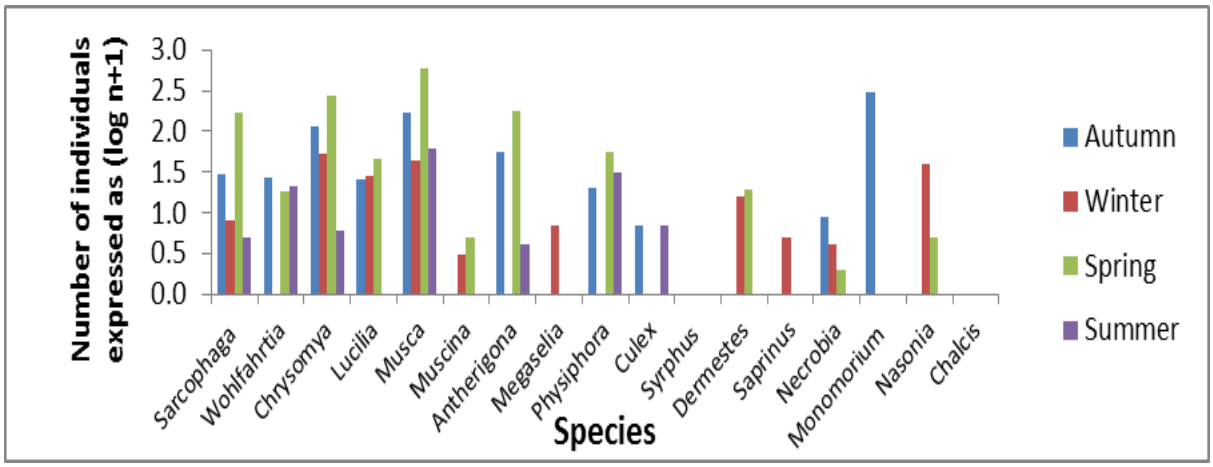

Fig. (8): The forensic insect species recorded on Cypermethrin treated corpse during different seasons from January 2019 to December 2019

Effect of the environmental factors

Correlation between the environmental factors and the numbers of recorded species

By applying the correlation analysis between the physical factors recorded during the period of experiment and its effects on the recorded numbers of forensically important species (Table 7), it was concluded that the numbers of *Sarcophaga ruficornis* maggots were negatively correlated with temperature and positively correlated with humidity. However, the numbers of *Wohlfahrtia magnifica* maggots were positively correlated with temperature and wind velocity and negatively correlated with humidity. The numbers of *Lucilia cuprina* maggots were negatively correlated with temperature and positively correlated with humidity.

Table (7): Correlation coefficients for association between the different species and physical factors during the period of experiment for the different corpses.

Species	Carrion type		Temperature	Humidity	Wind velocity
Sarcophaga ruficornis	Control	R Sig.	-0.216 *	0.092 NS	-0.161 NS
	Slaughtered	R Sig.	-0.255 **	0.257 **	-0. 097 NS
	Tramadol	R Sig.	-0.240 *	0.120 NS	-0.189 NS
	Cypermethrin	R Sig.	-	-	-
Wohlfahrtia magnifica	Control	R Sig.	0.269 **	-0.265 **	0.185 NS
	Slaughtered	R Sig.	0.276 **	-0.265 **	0.179 NS
	Tramadol	R Sig.	0.277 **	-0.272 **	0.177 NS
	Cypermethrin	R Sig.	0.163 NS	-0.095 NS	0.425 **
Lucilia cuprina	Control	R Sig.	-0.235 *	0.235 *	-0.097 NS
	Slaughtered	R Sig.	-0.192 *	0.232 *	-0.041 NS
	Tramadol	R Sig.	-0.234 *	0.234 *	-0.106 NS
	Cypermethrin	R Sig.	-	-	-

***. Correlation was significant at the 0.05 level.**
NS correlation is not significant.
R Pearson correlation
Sig. Significance

Stepwise multiple regression between the numbers of recorded species with the physical factors:

Stepwise multiple regression was applied to select a model in which all variables are significant (Table 8), it was concluded that the numbers of *Sarcophaga ruficornis* maggots were affected by

temperature for control and tramadol treated cadavers and by humidity for slaughtered cadaver. However, the numbers of *Wohlfahrtia magnifica* maggots were affected by temperature for all corpses except cypermethrin treated carrion was affected by wind velocity. The numbers of *Lucilia cuprina* maggots were affected by humidity for control and slaughtered carcasses and by temperature for tramadol treated carcasses.

Table (8): Stepwise multiple regression between the numbers of different species with the physical factors for the different corpses during the period of experiment

Dependent variable	Carrion type	Selected variable	R	R^2	Std. error Of the Estimate	Unstandardized Coefficients		Standardized coefficients	t	Sig.
						B	Std. error	Beta		
Sarcophaga ruficornis	Control	Constant	0.216	0.047	187.21627	216.204	71.016		3.044	0.003
		Temperature				-5.308	2.337	0.216	2.272	0.025
	Slaughtered	Constant	0.257	0.066	200.23722	-74.959	52.780		-1.420	0.159
		Humidity				5.038	1.902	0.257	2.649	0.009
	Tramadol	Constant	0.237	0.056	179.76561	231.866	68.354		3.392	0.001
		Temperature				-5.611	2.257	-0.237	-2.486	0.014
	Cypermethrin	-	-	-	-	-	-	-	-	-
Wahlfahrtia magnifica	Control	Constant	0.269	0.072	221.36689	-163.186	83.970		-1.943	0.055
		Temperature				-7.904	2.763	0.269	2.860	0.005
	Slaughtered	Constant	0.276	0.076	254.56603	-192.762	96.563		1.996-	0.048
		Temperature				9.345	3.178	0.276	2.941	0.004
	Tramadol	Constant	0.277	0.077	203.28974	156.437-	77.113		-2.029	0.045
		Temperature				7.508	2.538	0.277	2.959	0.004
	Cypermethrin	Constant	0.425	0.181	0.87917	-0.379	0.130		-2.919	0.004
		Wind velocity				0.279	0.058	0.425	4.813	0.000
Lucilia cuprina	Control	Constant	0.235	0.055	12.05266	-4.479	3.026		-1.480	0.142
		Humidity				0.273	0.110	0.235	2.477	0.015
	Slaughtered	Constant	0.232	0.054	16.42924	-6.026	4.331		-1.391	0.167
		Humidity				0.370	0.156	0.232	2.369	0.020
	Tramadol	Constant	0.234	0.055	10.52278	11.606	4.001		2.901	0.005
		Temperature				-0.324	0.132	-0.234	-2.454	0.016
	Cypermethrin	-	-	-	-	-	-	-	-	-

R Correlation coefficient **B Regression coefficient** **Sig. Significance**
R^2 Determination coefficient **t Test** **Std. Standard**

Discussion

In the present study, the maximum number of forensic species was represented by the dipteran insects (11 species belong to 7 families). However, the second and third important forensic orders were represented by Coleoptera (3 species belong to 3 families) and Hymenoptera (3 species belong to 3 families), respectively. Likewise, Abd El-bar and Sawaby (2011) recorded only 16 species, Al-Shareef and Al-Mazyad (2016) reported fewer forensic insects (9 species), Aly et al. (2017) recorded 18 species and El-Samad et al. (2021) listed 17 species. However, some other studies collected more insect species such as Wang et al. (2008), Abd El-Bar et al. (2016) and Hamdy et al. (2022) collected 47 species, 36 species and 67 species, respectively. This difference in number of the collected species may be attributed to the used carrion size, climatic conditions, the geographic region, the killing methods and antemortem ingestion of drugs or toxins (Hamdy et al., 2022).

Herein, it was observed that the number of cadaver colonized insects particularly calliphorids and sarcophgids was very low at the fresh stage in all study cases during all seasons then gradually increased in both bloating and active decay stages which reached to the maximum number because the odor become obvious and the cadavers have been already occupied by eggs and larvae and then decreased again at both of the advanced and dry stages. This result is agreed with Hamdy et al. (2022).

Calliphorid and sarcophagid flies were the first colonizers to arrive and breed on rabbit carcasses. This was consistent with Watson and Carlton (2003). However, sarcophagid flies (maggots and adult) were predominant than other fly species during all seasons. This was consistent with Ibrahim et al. (2013). The common existence of these two species and also their abundance on all carrion types reveled their important role in forensic investigation (Farag et al., 2021).

The present study showed that *S. ruficornis* was the most important sarcophagid of insect succession on all rabbit carcasses during all seasons. Agreeable results were presented by Aly et al. (2017). In addition, larvae of *Sarcophaga* were collected during the four seasons from all corpses except cypermethrin-intoxicated corpse.

Family Calliphoridae was represented by two species (*C. albiceps* and *L. cuprina*) in this study. However, *C. albiceps* was the numerically predominant calliphorid species in our study as noticed in the other studies of Abd El-bar and Sawaby (2011) and Keshavarzi et al. (2015), since this species was considered a very good competitive with other species in concern with its feeding on the carcass as well as its predatory behavior on the other maggots infesting the cadavers as stated by Silva et al. (2014).This explains the occurrence of dead maggots of *S. ruficornis* and *W. magnifica* near the cadavers throughout the experiments and also explains its dominance over the other calliphorid maggots as stated by Al-Mesbah et al. (2012).

In this study, it is noticeable that Muscidae (Order: Diptera) was the numerically predominant family in all seasons except in winter, family Pteromalidae (Order: Hymenoptera) was the most predominant one. This is not compatible with Abd El-bar and Sawaby (2011) in Qalyubiya Governorate in Egypt, who carried their study with organophosphate insecticide in summer

season which found Calliphoridae was the predominant family in their work. Also, Hamdy et al. (2022) in Cairo, Egypt, conducted their study with tramadol hydrochloride in winter and summer seasons where stated that Dermestidae (Order: Coleoptera) was the predominant family in winter, whilst Formicidae (Order: Hymenoptera) was the most predominant family in summer.

In the present study, Muscidae was observed to be the most diverse dipterous family represented by 3 species; this is in agreement with Silva et al. (2014) and Mabika et al. (2014), however in contrast with Hamdy et al. (2022) where Calliphoridae was the most diverse family represented by 6 species in their study.

It is worthy to mention that not all species of flies visited the cadavers to put eggs or larvae where *Musca domestica* was found visiting, copulating, and feeding on the substrate or using it as an extension of its habitat. This observation was convenient with Eze (2017). Similarly, *Atherigona varia*, *Physiphora demandata*, *Culex pipiens* and *Syrphus corollae* were collected as adult only as they visited the carrion to feed not to breed (Aly et al., 2017).

The scuttle fly (*Megaselia scalaris*) was observed as only adult individuals in winter on all corpses and also it was observed not breed on the corpses since *Megaselia* was a common cosmopolitan species in cooler seasons and its breeding was more recorded in indoor habitats as mentioned by Thevan et al. (2010). It could coexist with other sarcosaprophagous dipterans on the same corpse (Kumara et al., 2012 and Bugelli et al., 2015). Hence, in the absence of other sarcosaprophagous species, it can be a sole indicator to help in solving the criminal cases (zuha et al., 2016).

The emanating results clarified that flies prefer an oviposition or larviposition at natural body openings and also hairy areas of the dead body. This may be due to the high moisture and lower intensity of light as observed by Norris (1965). Density of dipterous maggots deposited on the slaughtered corpse was more than that on the control one. This was due to that artificial cut in neck and bleeding blood from the slaughtered cadaver were more attractive sites and preference for fly colonization than the natural openings in control corpse. This observation is convenient with (Rodriguez and Bass, 1983).

Coleopteran species were observed preferred the cadaver when it is more decomposed or in the advanced stage which less flesh was still found as recorded by Midgley et al. (2010) and El-Aziz and El Shehaby (2019). In addition to dipterous flies, species of order Coleoptera constitutes a main entomological evidence for the PMI determination which is based upon succession pattern as demonstrated by Abd El-Bar et al. (2016).

Saprinus gilvicornis frequented corpses in decay and dry stages and its feeding was confined to dipterous larvae which this may be significant in reducing the maggots on corpses and this explain its appearance on all corpses except cypermethrin-intoxicated one which no maggots. This is consistent with (Özdemir and Sert, 2009).

Dermestes vulpinus visited the corpses at bloating, decay and dry stages and it was observed fed on carrions as stated by Tantawi et al. (1996) who found that these beetles fed on corpses earlier during the bloated stage. However, in contrary with (Reed, 1958) who reported that *Dermestes* visited corpses at later stages of

decomposition only. Dermestids prefer dried cadavers, feathers, hairs and skins and they could feed directly on the corpse and consequently may accelerate the process of decay (Shroeder et al., 2002).

Single Clerid species (*Necrobia rufipes*) collected from the cadavers in different seasons. *Necrobia* adults were observed as predators feed on dipterous maggots found on carrion in addition to the carrion itself. This is agreeable with the result of (Bana and Beyarslan, 2012 and Farag et al., 2021).

Pteromalid (*Nasonia* sp.) is a small polyphagous parasitic species, living in different habitats, and may parasitize on several dipterans laying its eggs inside their pupae, it considered as secondery forensic agent in case of absence from dipteran flies (Aly et al., 2017).

In our study, a large amount of ants was collected in autumn from cypermethrin-intoxicated carcass compared to the control one throughout the decay period except fresh stage while a low amount collected in spring from the slaughtered corpse in dry stage only. Abd El-bar and Sawaby (2011) agreed with our results which observed that ants were mostly found in early decomposition stages of organophosphate treated rabbit. However, reverse our results for slaughtered carrion; Ojianwuna et al. (2019) recorded the appearance of ants around the slaughtered rat cadavers starting from the fresh stage to the active decay stage without a particular pattern. Consequently, we concluded that ants did not necessarily breed on the carcasses or influence the decomposition process which makes them not one of the most important forensic insects (Mabika et al., 2014). Whilst, this is disagreed with Morreti et al. (2013) where observed ants fed on carcass and dipterous maggots so that they were categorized as important species of the necrophagous insects.

Decay process of dead bodies is a natural and necessary process responsible for the return of the organic materials to the ecosystem (Abd El-Bar et al., 2016). According to the morphological changes of corpses, just five stages of decomposition were noticed in the present study namely: fresh, bloated, active decay, advanced decay and dry or skeletonization stages similar to the results of previous studies (El-Gawad et al., 2019 and Hamdy et al., 2022). However, some researches recorded only four stages of decomposition such as (Galal et al., 2009 and Albushabaa and Almousawy, 2016). In the present study, decomposition process was observed starting from the oviposition time of dipterous flies and ended when the dead body remnants were completely dried and no live or active insects were discovered in any of the cages. This observation is in agreement with Zeariya et al. (2015) and Hore et al. (2017).

Herein, decomposition began from the head region in most dead animals and then spreaded later in the remaining body parts. This was attributed to the preference of dipterous flies for oviposition firstly at the natural body orifices like mouth, ear, nose and eyes. The same observation was noticed by Hamdy et al. (2022).

Fluctuations in the climatic factors between seasons as temperature, relative humidity, wind velocity and rainfall affected greatly the decomposition rate of corpses (Matoba and Terazawa, 2008). In the current study, there were no significant differences in humidity between seasons, unlike the temperature between seasons. The decomposition rate required longer time in seasons with lower temperatures.

Consequently, we could deduce that humidity affected the decomposition rate less than temperature. This is convenient with (Kočárek, 2003). Temperature also affects the insect population dynamics and activity due to its control of growth and reproduction (Elba et al., 2006). In Egypt especially in Upper Egypt, temperature during summer season is usually very high and vice versa in winter; therefore the duration of cadaver decomposition process was shorter than that in winter season, this was due to faster chemical and microbial reactions and also the increased activity of the cadaver insects in summer. This result was obvious in the present study where decomposition in summer lasted only for 20 days postmortem whilst decaying process in winter was prolonged to 57 days. This is the same result with the previous studies of Wang et al. (2008).

In our study, tramadol treated carcasses decayed with the same rate as the control carcass except in winter season which slightly delayed. This result was agreeable with El-Samad et al. (2011), Ekrakene and Odo (2017) and Hamdy et al. (2022). However, in contrast with our result, AbouZied (2016) found that the decomposition process of tramadol treated rabbits was slightly faster than that of control group; however he observed also that the total decomposition rate of the tramadol-treated corpses was not significantly faster than the control ones during his study period in spring season.

In the present study, the decomposition process reveled that except for the fresh and bloating stages, the degradation rate was significantly prolonged in cypermethrin-intoxicated carrions relative to the control one in all seasons. This is similar to the result by Ekrakene and Odo (2017) reported that the degradation rate was prolonged in cypermethrin-treated carrions compared with the control and Abd El-bar and Sawaby (2011) who recorded lagging in decomposition process of rabbit carcasses killed by organophosphate pesticide when compared with control ones. In contrary, El-Gawad et al. (2019) reported that except for the fresh stage, the warfarin-intoxicated carcasses decayed significantly faster than control carcasses, this may due to the action mode of each toxin.

On the other hand, the decomposition rate of the slaughtered carrions was faster than control carrions except for the fresh and bloating stages in winter and spring seasons only. This may be due to that the artificial cut in the neck of rabbits by slaughtering had revealed the internal organs of the corpses and consequently the flies invaded these corpses much earlier than that in control one. Also the abundance of maggots on the slaughtered carrions was more than that on control carrions so the slaughtered corpse consumed and decayed faster. This result is in agreement with Ojianwuna et al. (2019) and Farag et al. (2021).

Regarding cadaver decomposition with fly activity, calliphorid and sarcophagid flies (first colonizers) played a fundamental role in cadaver decomposition. In cypermethrin-intoxicated corpse, insects were observed arrived to the carrion however without breeding on it except two dipterous flies (*Chrysomyia* and *Wohlfartia*) resulting in occurring prolongation of decomposition rate of these corpses. This observation is agreement with Utsumi et al. (1958) who stated that the toxins as parathion or arsenic acid prolonged the decomposition rate of corpses and the carrion insect fauna though rich at first of decay, it had declined later. Moreover, El

Kady et al. (1994) noted that neither decomposition occurred nor arthropods were collected from the arsenic oxide poisoned rabbits.

Number of dipteran maggots deposited on the tramadol-intoxicated carrion was slightly lower than that on the control carrion during all seasons. This is agreed with Hamdy et al. (2022). Furthermore, a very low number of definite dipterous species maggots was deposited on the cypermethrin-intoxicated cadavers compared with the control one at all seasons and these maggots dead after few days and not entered in pupation. This may be a reason of prolongation of their decomposition period. This is agreed with El-Kady et al. (1994) in the gas killed carrions, Abd El-bar and Sawaby (2011) in the organophsphate intoxicated carcasses and with Abd El-Bar et al. (2016) who demonstrated lower numbers of dipteran and coleopteran maggots on the zinc phosphide-intoxicated animals compared with control group in summer and winter where small number of immature stages undoubtedly delayed the decomposition process. However, this result is contrary to the result of El-Gawad et al. (2019) who found that, unexpectedly, significantly more adults and immature stages were attracted to warfarin than to control carcasses and consequently, they found that warfarin accelerated the larval development. Drugs and toxins may alter the rate of insect invasion and development of maggots influencing the carrion decomposition process (Abd El-Bar and Sawaby, 2011).

Delaying of insect infestation to corpses resulted in significantly retarded and incomplete cadaver decomposition, consequently, there is inter-dependence between insect colonization and the decomposition rate. This observation was recorded also by Simmons et al. (2010) and this could explain why the cadaver took more time for decomposition in winter rather than other seasons.

In the present study, the total number of insect species collected in summer was less than in other seasons for all carrions. The same result as the results from Aly et al. (2017). This is due to succession in warmer seasons was driven by the rapid resource depletion because the corpses decomposed faster due to the higher temperatures. However, succession in cooler seasons was much influenced by the cold temperatures and rainfall which retarded the corpse decomposition and also the insect succession. Hence, larger numbers of insect species were attracted to cadaver. However, in contrast with our results, Rodriguez and Bass (1983) found that the corpse fauna was richer in warmer seasons than in cooler seasons. In summer season, insect invasion occurred early at the day of cadavers exposure (day o), while retarded to some days in winter. This agreed with Aly et al. (2017) and Hamdy et al. (2022).

According to the killing method, it was found that the presences of tramadol or cypermethrin in the treated cadavers not cover or mask the odors of corpses and not prevent the invasion or attraction of insects. This observation is agreed with Wolff et al. (2004) who worked on parathion and malathion intoxicated rabbit cadavers, Voss et al. (2008) in case of the carbon-dioxide poisoned carcasses and with Hamdy et al. (2022) in case of tramadol treated rabbits. However, cypermethrin was noticed prevented the oviposition of many dipterous species and killed several adult and immature stages as recorded by El-Kady et al. (1994) who stated that no decomposition observed nor arthropods

were recovered from the arsenic oxide poisoned rabbits.

Statistical analysis of the present results clarified that *Sarcophaga* maggots were affected negatively by temperature and positively by humidity. In this study, we found that *Sarcophaga* maggots have been dead when the exposure to high temperatures in summer up to 47.4°C and also at the exposure to low temperatures and high humidity up to 50%. Also, Sam (2006) found that *Sarcophaga* sp. larvae that were exposed to the outside environment's cold temperature (low of 4ºC) all died the first night of initial exposure because no movement was detected the next day. He suggested that *Sarcophaga* sp. cannot survive in a cold temperature climate unless there was some source to provide them heat or incubation, such as a dead body or animal carcass.

From the present study, we found that *Wohlfahrtia* maggots were affected positively by temperature which the speed of development increased with the increasing of temperatures, thus, the developmental time decreased. This is consistent with (Tantawi et al., 1996) who stated that *Wohlfahrtia* larvae in summer which temperature is high, developed rapidly and pupariated earlier. Also *Lucilia* maggots were recorded negatively correlated with temperature and positively with humidity. This result is disagreed with Bansode et al., (2016) who found that the development rate of the *L. cuprina* was slow at low temperature and fast at high temperature.

Furthermore, studies on the succession of insects associated with dead bodies in special microenvironments are significant for their contribution in the development of forensic science, as well as their acting as a potential forensic tool in cases of human carrions killed by different methods.

VII. Conclusions

The results of the current study demonstrated that the killing techniques had an impact on the variety, number, and succession pattern of the forensically significant insect species that colonized rabbit cadavers and therefore the decomposition process. Furthermore, this study represents the first step to throw a spotlight on the important role of the entomologist and the value of entomological information in legal investigations related to the killing methods in Qena city, Egypt.

We anticipate that the results obtained in this paper will instigate further research on the applications of entomological evidence for forensic purposes in Qena city and finally, we hope that the Egyptian government provides forensic entomologist with the chance to work with law enforcement officials in the criminal justice system.

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