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Identification of insects colonizing carrions of tramadol-intoxicated rabbits and guinea pigs in relation to seasonal variances in Cairo, Egypt

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ABSTRACT

This study gives out the identification of insect faunal waves colonizing decomposing carrions of rabbits and guinea pigs treated with certain drug agent (tramadol) in Cairo, Egypt. Each of six rabbits and six guinea pigs is divided into 2 groups, where the first was killed by tramadol and the second group was killed by asphyxia as a control experiment. Generally, decomposition process was observed to have a slower rate in winter. Reaching to the skeletal stage was delayed in tramadol-intoxicated carcasses in comparing to the control carcasses. A total of 12966 arthropod individuals (Immature stages and adults) were collected. In concern of class Insecta, 67 species belonging to 6 Insecta orders and 2 Arachnida orders, 37 families and 51 genera were identified from this study during winter and summer seasons. Significantly lower numbers of immatures were observed and collected in the present study from the treated carcasses. The proportions of adult insects were also significantly different in control carcasses when compared with tramadol intoxicated ones in both winter and summer season for both rabbits and guinea pigs. This study provides background for the influence of tramadol on the colonizing wave of arthropod fauna which is of relevance to forensic science. We can conclude that drugs and toxins present in a decomposing body may alter the rate of insect invasion of that body.

1. Introduction

Forensic entomology is a branch of forensic science that uses corpses- colonizing insects' information to determine the post mortem interval (PMI) (the time between death and corpse discovery) in legal criminal cases involving people [1]. Insects are known to have been used in the detection of crimes for a long time and a number of researchers have written about the history of forensic entomology [2,3]. Academics and practitioners collaborated with police and legal authorities to refine and enhance forensic entomology as a scientific tool in the late twentieth and early twenty-first centuries, which succeeded in achieving acceptability. The five stages of body decomposition have been of prime interest for scientists over a long period of time. Necrophagous, necrophilous, omnivorous, opportunists, and accidentals are the five ecological groupings that sarcosaprophagous arthropod fauna are classified into. In general, necrophagous, necrophilous and omnivorous insects are the most important for forensic determinations [4,5]. There are some examples for the application of forensic entomology in criminal investigation all over the world [6-14].

Drug-related deaths have been increased worldwide. 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 [mainly Diptera (flies) and Coleoptera (beetles)] [8,15]. 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 [15-19]. In Egypt, few studies have been done on forensic entomology and entomotoxicology [16, 18-26].

In the Egyptian community, Tramadol drug misuse has become an increasingly alarming phenomenon [27] especially among students, workers and drivers [27,28]. For example, **Abd-Elkader *et al.*** [28] reported that "Numerous studies involving dead or injured drivers of road traffic accidents confirmed that they were under the effect of drugs: and these drugs as tramadol significantly reduce driving ability, leading to reduced perception". Tramadol-related deaths in 2008 in Egypt were 32.5 times more common than in 2005 [27]. WHO has highlighted the dangers of Tramadol abuse and dependence [27].

Thus, the present study aims to identify insect fauna that colonize Tramadol-intoxicated carcasses and compare it with the corresponding figure of control carcasses in both winter and summer seasons in Cairo, an urban city in Egypt.

2. Materials ad Methods

2.1 Study location

The study was conducted during the winter season (Jan.2 – April 6, 2012) and summer season (July 14 – July 30, 2012) in the campus of Ain-shams University Fig. 1, that's located in Abbassyia region, Cairo Governorate, Egypt. The experimental site selected is an area of a floor of tile covered with sand under each cage to be suitable for pupation of the developing larvae. The place having a space area of approximately 25 m^2 and surrounded by a wire of dimintions 1×1 cm fixed in long wood wedges to prevent entrance of any person or vertebrates to the study area. Physical environmental conditions, (temperature, humidity and rainfall) at the study area were obtained daily from the Egyptian meteorological authority located just 943.6 m away from the study location and covering an area of diameter 100 km around. Rainfall was six times during this period of the experiment.

2.2 Experimental animals

Six healthy domestic rabbits (*Oryctolagus cuniculus* L.) weighting approximately1.5 kg each and six guinea purchased locally, while guinea pigs purchased from an animal house in Abo-Rawash, Giza Governorate, Egypt.

2.3 Experimental procedure

Two experiments were carried out during this study, one during winter and other during summer seasons through the same year. Test animals were poisoned by the administration of tramadol.

Tamol® tablets produced by Hikmapharma S.A.E, 6th October city-Egypt under M.O.H. Reg. No.(21750/2002). Each tablet contains tramadol Hcl 200 mg. Tramadol Fig. 2 is a novel centrally acting analgesic used for the treatment of mild to severe pain and became the most prescribed opioid worldwide. Tramadol overdose appears to be attributable to the monoamine uptake inhibition. Each rabbit was given 2 tablets (400 mg) dissolved in 10 ml of water. Each guinea pig given 0.5 tablet (100 mg) dissolved in 2.5 ml of water. Ethically, animals had been administrated the drug orally via gastric tube. Death occurs immediately but with muscle tremors occurrence just before death.

Immediately after killing and death was confirmed, the carcasses were transferred without delay to the study site in labeled sealed plastic bags immediately to prevent arthropod colonization. The animal carcasses were guarded against vertebrate scavengers with stainless steel wire mesh (1 x 1 cm) that permits entrance of all insects and other arthropods. The wire mesh was used to form cubic cages (50 cm length, width and height) supported with a frame of wood. Each animal was placed inside the screened labeled cage. Cages design ensured that the carcasses would be in full contact with the ground Fig. **1.** Sand was placed under each cage to facilitate collection of larvae and to help it to pupate. Cages were then placed at approximately 1 meter apart from one another.

2.4 Observation of animals and collection of samples

The day of killing and placement of carcasses were designated as day 0. Each experiment continued until the animal remains were completely dry and no active or alive insects were detected in any of the cages. The carcasses were visited daily at the beginning of the experiment until the end of active decay stage of decomposition and then once every two days during the advanced decay stage and then twice a week until complete decomposition of the carcasses. Observations were recorded and photographed by using Canon HD 16M Pix. Lens 5.0-25.0 mm digital camera.

Fig. 1 The study area and the experimental cages

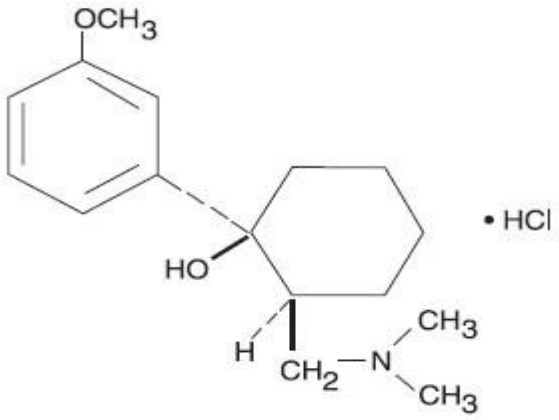


Fig.2 Molecular structure of Tramadol

During the sampling time, insect activity was observed and representative samples of adult and immature stages (from natural apertures -eyes, mouth, nose, and ears-) were collected, and during advanced stages of decomposition, samples of soil were taken, both from under the body and around it to capture the insects that flew near the carcasses. Catching devices included forceps (for beetles), spoons (for the larvae masses), entomological nets (for flying insects) to ensure as many different species as possible were collected. Collected samples were placed in plastic vials labeled with the date, method of killing, type of animal. Adult flies collected were transferred to a 'killing' jar containing ethyl acetate. Approximately 25% of immature stages were captured from each case. Sampling stopped when the carcasses became only bones.

2.5 Identification of specimens

The captured immature samples were separated into two groups. The first one was killed immediately by immersing in a beaker of hot water for 30 seconds and then preserved in 70% alcohol for preservation and identification to the family level by using the taxonomic keys of **Smith** [29]**; Szpila** [30] and **Thyssen** [31]. The second group of maggots was reserved in lab for rearing in plastic jars covered with a net mesh (larvae were fed rotten minced meat) at room temperature for confirmatory identification purposes. Dead samples (if any) were also removed and maintained in a domestic freezer. Adults were observed and collected from the carcasses from day 0 to the end of the study during both seasons. All collected adult insects were prepared for identification; they were killed either by freezing or by ethyl acetate. Some adults were preserved in 70%

alcohol, some were preserved dry in freezer, while others were mounted on pins of different thicknesses according to their sizes and provided with labels indicating the date, kind of animal and the type of killing method, then they were kept in preservative boxes supplied with naphthalene.

Prepared specimens were examined under LABOMED, CZM4 dissecting, light binocular microscope for identification. All these collected insects were examined and identified accurately to family, genus and species level according to all keys and available descriptions and by comparing the specimens in the different

Egyptian collections. Insects and decomposing remains were photographed.

Identification of samples was carried out in the Museum of Entomology Department, Faculty of Science, Ain Shams University [32,33].

2.6 Statistical Analyses

SPSS V.25 software was used to analyze data. Non-parametric Mann- Whitney U test for two independent samples was used to analyze data of immature stages, while data of adults was analyzed by using Pearson chi-square test.

3. Results

The present work carried out on 6 exposed rabbit and 6 exposed guinea pig carrions, each group animal is divided into two subgroups: the first subgroup is treated by tramadol and the second control subgroup is killed by asphyxia during winter and summer seasons (2012) in Cairo, Egypt. Results yielded significant information about the decomposition process and the associated insect wave patterns.

3.1 Decomposition stages

Five stages of decomposition were noticed during the present study for all carcasses (12 cases): fresh, bloat, active decay, advanced decay and skeletonization Figs. 3-6**.**

3.1.1 Fresh stage

This stage began from the instant of death to the onset of bloating signs. During this stage, the decomposition occurred internally: attributed to the activity of micro-organisms present in the body before death, while no odors and no morphological changes were observed externally.

Carcasses were described by normal flexible appearance of body and extremities. The internal temperature of carcass gradually decreased immediately after death (*algor mortis*) and this is accompanied by time with the stiffness of the body (*rigor mortis*). The fresh stage was also characterized by the presence of adult flies hovering around carcasses for oviposition.

3.1.2 Bloat stage

It began by the appearance of the first signs of body inflation and ended when the carcass deflated. Bloat stage was also marked by presence of eggs and the first instars of maggots in the body openings with the continuous presence of the adult flies. Putrefaction started during this stage; abdomen distention occurred by the accumulation of gases, and this resulted in the appearance of the noticeable offensive odor. The body became balloon-like reaching the maximum degree of rigidity. The carcass internal temperature began to rise again.

3.1.3 Active decay stage

This stage started when carcass deflated as gases escaped and ended when the remaining tissues become relatively dry and the majority of maggots left for pupation. The body became wet with creamy consistence and the putrefaction odor became very offensive. Carrion fluids began seeping from the carcass orifices (mouth, nose, and anus) into the soil forming Carrion decomposition island. During this stage, the body started to lose its original shape and became infested with maggots all over it. Carrion skin is ultimately ruptured by putrefactive-bloating pressure and maggots which consumed the most of body mass. The end of active decay stage and starting of advanced decay stages was characterized by the stopping of maggot feeding and their departure beneath and around carcasses for pupation.

3.1.4 Advanced decay stage

It is designated by great reduction of body flesh and fluids with the most of carrion remains were represented by relatively dry skin, cartilages, some muscular tissue and bones. Some decayed internal tissues of the carrion with insect materials and other products of decomposition were forming viscous putrefying adipocere-like substance (by-product of decomposition) (BOD) which was found on the carrion

and around it. Carrion undergone slow decaying rate with the decomposition odor diminishes away; weight loss became much slower and rapid dropping in the carrion temperature occurred. The mainstream of the larval masses departs the carrion and accumulated in the muddy ground under the body and in the immediate vicinity for pupation. Post feeding larvae; and the pupae and puparia were scattered around the body. The end of this stage was defined by absence of any flesh and when the activity of the species present becomes the lowest.

Fig.3. Decomposition of control rabbit carcass

A. fresh stage, B. bloat stage, C. active decay stage, D. advanced decay stage and E. skeletonization stage

Fig.4. Decomposition of Tramadol-intoxicated rabbit carcass

A. fresh stage, B. bloat stage, C. active decay stage, D. advanced decay stage and E. skeletonization stage

Fig.5. Decomposition of control guinea pig carcass

A. fresh stage, B. bloat stage, C. active decay stage, D. advanced decay stage and E. skeletonization stage

Fig.6. Decomposition of Tramadol-intoxicated guinea pig carcass

A. fresh stage, B. bloat stage, C. active decay stage, D. advanced decay stage and E. skeletonization stage

3.1.5 Dry stage (Skeletonization)

In this stage carcass constituents were only dry skin, fur, nails, teeth and dislocated bones; the BOD dried up and began mixing with the soil composition. Skeletonizaion ended by absence of the associated carrion taxa whereas, only empty puparia were found. In the present study, it became difficult to determine the end of this stage where the carcass remained occupied by different the Arachnida [Order: Acariformes (mites) and Order: Araneae (spiders)] over long duration.

In general, decomposition process started from head regions in most animals and was noticed for all the body parts during the experiments. It is also found that decomposition process was faster in guinea pig carcasses than in rabbit ones in both winter and summer seasons.

For rabbit carcasses, Table 1 showed the duration of each decomposition stage of control and Tramadol-intoxicated carrions during winter and summer seasons respectively.

From the view point of seasons, decomposition process observed to have a slower rate in winter; was delayed by 64 and by 68 days in comparing with summer season for control and Tramadol-intoxicated carcasses, respectively. During the winter season, reaching to the skeletal stage was delayed by 6 days in tramadol carcasses with regard to control carcass when the daily averaged temperature ranged from 10º C to 28º C. While in the summer season, the duration of each decaying stage was not varied greatly among the two studied cases due to rapid decomposition under the effect of high temperature. The reaching to the skeletal stage was delayed by 2 days in tramadol intoxicated carcasses comparing with control carcass when the daily averaged temperature ranged from 29º C to 32º C.

For guinea pig carcasses, Table 1 showed the duration of each decomposition stage of control and tramadol intoxicated cases during winter and summer seasons respectively.

Decomposition process was observed to have a slower rate in winter season, it was delayed by 48 and by 55 days in comparing with summer season for control and Tramadol-intoxicated carcasses, respectively. During winter season, reaching to the skeletal stage was delayed by 7 days in tramadol carcasses with regard to control carcass when the daily averaged temperature ranged from 10º C to 28º C. Whereas in the summer season, tramadol intoxicated carcass reached to skeletal stage at the same time of control one when the daily averaged temperature ranged from 29º C to 32º C.

The climatic data revealed the direct relation between the decomposition process and temperature; the high temperatures contributed to fast decomposition whereas low temperatures prolonged it. However, the inverse relationship was observed between the decomposition process and rain where it retarded the decay process.

Except for Active decay stage in winter where rabbit Tramadol-Intoxicated was lower (16-35 days) than that of guinea pig (10-48 days), ending with clear skeletonization and also their odor were noticeable to be more offensive than that of rabbit carcasses.

Table 1 Duration of each decomposition stage for control and Tramadol-intoxicated rabbit and guinea pig carcasses in winter and summer seasons 2012.

Stage of decay	(Days post-mortem)							
	Rabbit carcasses				Guinea pig carcasses			
	Winter		Summer		Winter		Summer	
	Control	Tramadol-Intoxicated	Control	Tramadol-Intoxicated	Control	Tramadol-Intoxicated	Control	Tramadol-Intoxicated
Fresh	0-4	0-2	0-12 hours	0-12 hours	0-2	0 -2	0-12 hours	0-12 hours
Bloat	5- 11	3-15	12-24 hours	12-24 hour	3-9	3-9	12-24 hours	12-24 hours
Active decay (wet decomposition)	12-38	16-35	1-4	1-5	10-34	10-48	1-2	1-3
Advanced decay (Dry decomposition)	39- 70	36-76	5-6	6-8	35-53	49-60	3-5	4- 5
Skeletonization	71-	77-	7-	9-	54-	61 -	6-	6-

3.2 Associated insect fauna

A total of 12966 arthropod individuals (Immature stages and adults) were collected. In concern of class Insecta, 67 species belonging to 6 insect orders, 37 families and 51 genera were identified from this study during winter and summer seasons Table 2. In the present study orders Diptera, Coleoptera and Hymenoptera were dominated the carrion community, while necrophagous insects that supported decomposition of carcasses were mainly of orders Diptera and Coleoptera. The insect fauna invading decaying carrions were greatly affected by climatic changes in different seasons as well as by manner of killing.

For adult insects, it was noticeable that, percentage of collected adults of family Dermestidae (skin beetles) (Order: Coleoptera) made it the predominant family during winter season in all cases of the study [Control rabbit and guinea pig carcasses, and tramadol intoxicated rabbit and guinea pig carcasses respectively] Fig. 7: A-D, while during summer season, percentage of adults of family Formicidae (ants) (Order: Hymenoptera) was observed to be the highest one in all study cases except in tramadol treated guinea pig carcasses where the percentage of adult flies of family Calliphoridae (blow flies) (Order: Diptera) made it the most predominant family Fig. 7: E-H**.** Focusing on order Diptera, in winter season, Phoridae (hump-backed flies) was found to be the most dominant family followed by family Calliphoridae in all study cases except in tramadol intoxicated rabbit carcasses where family Phoridae was followed by family Sarcophagidae (flesh flies) Fig. 7: A-D**.** During summer season, family Challiphoridae was the most predominant one, while the second most abundant family was Muscidae (house flies) in all studied cases Fig. 7: E-H. Calliphoridae was observed to be the most diverse family represented by six species with *Chrysomya albiceps* (blow fly) was numerically dominant species among the family in this study.

Table 2 Arthropods associated with rabbit and guinea pig carcasses in Abbassyia, Cairo Governorate, Egypt, during winter and summer seasons 2012

Class	Order	Family	Genus/species
Insecta	Diptera	Agromyzidae	*Phytomyza sp.*
		Hippoboscidae	Unidentified
		Calliphoridae	*Chrysomia albiceps*
			Chrysomia rufifacies
			Chrysomia megacephala
			Lucilia sericata
			Lucilia cuprina
			Calliphora vicina
		Chironomidae	Unidentified
		Dolichopodidae	*Medetera sp.*
		Drosophilidae	*Drosophila repleta*
		Ephydridae	*Chlorichaeta albipennis*
		Fanniidae	*Fannia sp.*
		Muscidae	*Musca domestica*
			Musca sorbens
			Muscina stabulans
			Synthesiomyia nudiseta
			Megaselia scalaris
		Phoridae	Unidentified
		Piophilidae	*Piophila casei*
		Psychodidae	*Tinearia alternata*
		Sarcophagidae	*Sarcophaga argyrostoma*
			Sarcophaga hertipes
			Wolfhartia nuba
		Sphaeroceridae	*Pullimosina heteroneura*
		Tipulidae	Unidentfined
		Ulidiidae	*Physiophora alceae*
		Anobiidae	*Lasioderma serricorne*
			Ptinus variegates
			Stegobium paniceum
		Anthicidae	*Anthicus floralis*
		Cleridae	*Necrobia rufipes*
		Curculionidae	Unidentified

		Dermestidae	*Attagenus fasciatus*
			Dermestes ater
			Dermestes frichii
			Dermestes maculatus
		Histeridae	*Saprinus caerulescens*
			Saprinus chalcites
			Saprinus furvus
			Saprinus semistriatus
		Latridiidae	*Corticaria sp.*
		Nitidulidae	*Carpophilus hemipterous*
		Staphylinidae	*Aleochara moesta*
			Aleochara tristis
			Atheta sp.
			Creophilus maxillosus
	Coleoptera		*Gabronthus maritimus*
			Philonthus quisquiliarius
			Philonthus sordidus
			Platystethus cornatus
			Platystethus nitens
			Scopaeus debilis
			Unidentified species
		Tenebrionidae	*Mesostina puncticolli*
			Scelosodis castaneus
			Trachyderma hispida
Insecta			*Zophosis abbreviata*
		Apidae	*Apis mellifera*
		Chalcididae	*Brachymeria femorata*
			Dirhinus excavates
		Chrysididae	*Chrysis sp.*
		Formicidae	*Cardiocondyla minutor*
			Monomorium carbonarium
	Hymenoptera		*Monomorium lipenyi*
			Monomorium niloticus
			Monomorium salmonmis sommieri
			Plagioleps maura
		Pteromalidae	*Nasonia vitripennis*
		Vespidae	*Vespa orienalis*
		Aphididae	*Aphids sp.*
	Hemiptera	Cicadellidae	*Empoasca sp.*
	Lepidoptera	Tineidae	Unidefined
	Psocoptera	Liposcelididae	*Liposcelis sp.*
	Zygentoma	Lepismatidae	Unidentified species
Arachnida (comprising spiders and mites)			

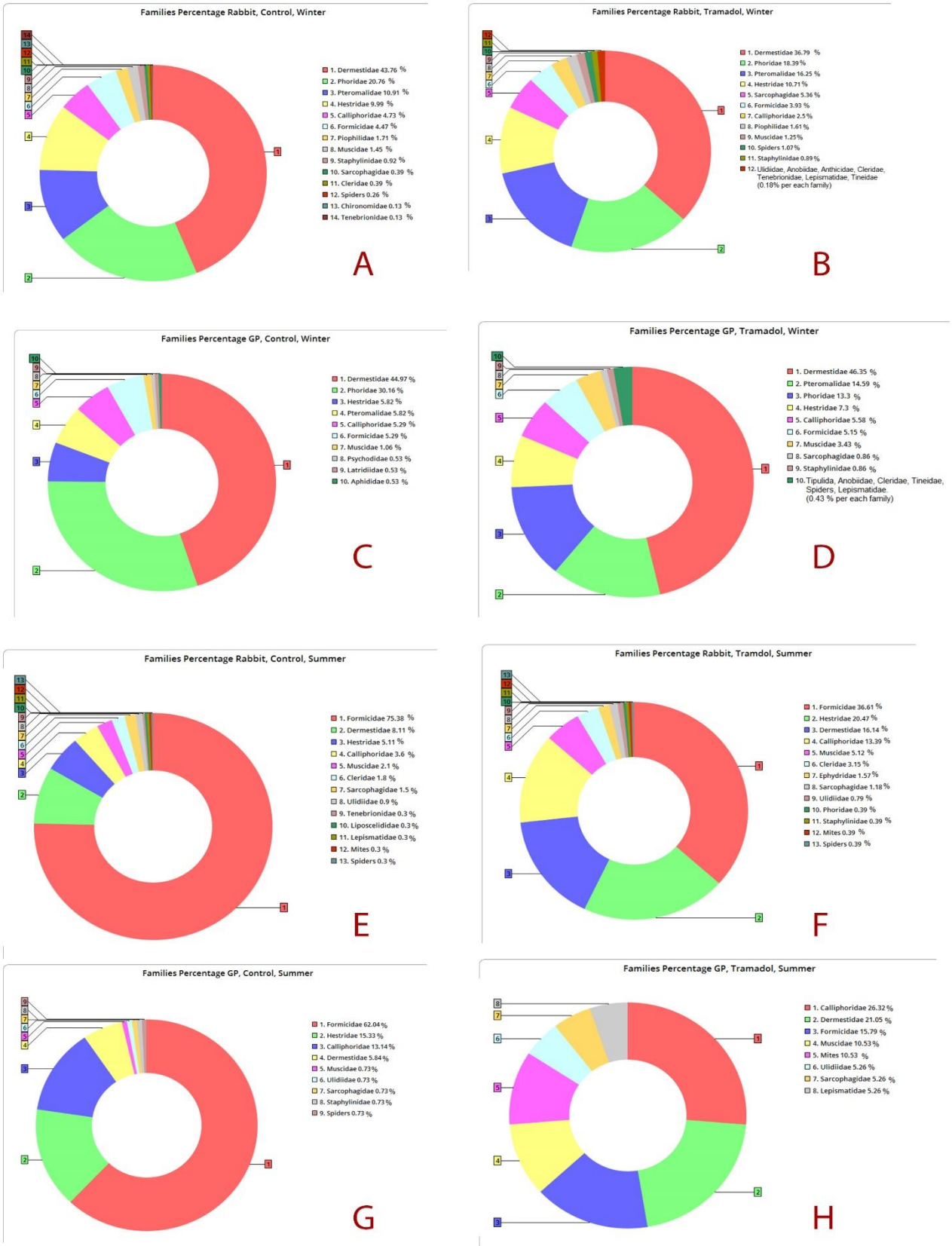


Fig. 7 Percentages of collected insect adult families from rabbit and guinea pig carcasses during winter (A-D) and summer (E-H)

3.2.1 For rabbit carcasses

From the view point of killing method; one unidentified dipterous, five coleopterous, one hymenopterous species and one species belonging to order Psocoptera were not collected from carcasses intoxicated by tramadol and limited only to control carcasses. These species were; unidentified species of Chironomidae (nonbiting midges), *Gabronthus maritimus*, *Philonthus sordidus* and *Creophilus maxillosus* (Family: Staphylinidae) (rove beetles), *Mesostena puncticollis* and *Zophosis abbreviata* (Family: Tenebrionidae) (Darkling beetles), *Plagioleps maura* (Family: Formicidae), *Liposcelis sp.* (family: Liposcelidae). Whereas, 2 dipterous and 5 coleopterous species restricted only to tramadol intoxicated carcasses, these were: *Musca sorbens* (bazaar fly or eye-seeking fly) (Family: Muscidae), *Calliphora vicina* (family: Calliphoridae), *Ptinus variegates* (Family: Anobiidae) (death-watch beetles), *Anthicus floralis* (Family:Anthicidae) (ant-like beetles), *Aleochara moesta* and *Platystethus cornatus* (Family: Staphylinidae) (rove beetles) and *Scelosodis castaneus* (Family: Tenebrionidae) (Darkling beetles).

3.2.2 For guinea pig carcasses

With regard to methods of killing, 2 dipterous, 4 coleopterous and one hymenopterous species were absent in the intoxicated animals and restricted only to control carcasses which were *Synthesiomyia nudiseta* (Family: Muscidae), *Tinearia alternate* (Family: Psychodidae) (sewer flies), *Attagenus fasciatus* and *Dermestes ater* (Family: Dermestidae) (skin and leather beetles), *Saprinus semistriatus* (Family: Histeridae) (clown beetles), *Corticaria sp.* (Family: Latridiidae) (minute brown scavenger beetles or fungus beetle) and *Cardiocondyla minutor* (Family: Formicidae) (ants). For only tramadol intoxicated carcasses the following species were attracted; *Muscina stabulans* (stable fly)(Family: Muscidae) (house and stable flies), *Calliphora vicina* and *Chrysomya megacephala* (family: Calliphoridae) (blow flies), unidentified species belonging to family Tipulidae (Crane flies), *Stegobium paniceum* (Family: Anobiidae) (death-watch beetles), *Creophilus maxillosus* (family: Staphylinidae) (rove beetles), *Plagioleps maura* (Family: Formicidae) (ants), unidentified species belonging to family Tineidae (tineid moths) and family Lepismatidae (silverfish) and mites, *Tinearia alternata* (Family: Psychodidae) (sewer flies), *Attagenus fasciatus* and *Dermestes ater* (Family: Dermestidae) (skin and leaher beetles), *Saprinus semistriatus* (Family: Histeridae) (clown beetles), *Corticaria sp.* (Family: Latridiidae) (minute brown scavenger beetles or fungus beetle) and *Cardiocondyla minutor* (Family: Formicidae) (ants).

3.3 Waves of arthropod fauna patterns

The patterns of arthropod wave observed in this study for control and tramadol intoxicated rabbit and guinea pig carcasses during winter and summer seasons were summarized in Gantt charts Tables 3-10 respectively.

It was observed that the cooler climate of winter season slowed the rate of insect colonization by slowing the development of the immature stages and therefore the degradation of carrion prolonged to 96 days post killing, whereas warmer climate of the summer season made the colonization by most succession insects occurr earlier by accelerating the activity of these immature stages of insects so the degradation of carrion accelerated through only 17 days post killing. During winter season, insect succession began at day 1 post killing in all study cases, results showed that *Megaselia scalaris* with other phorid flies and members of family Dermestidae were the first invaders in cases of control, and tramadol-intoxicated rabbit and guinea pig carcasses followed by other dipterous flies with noticeable retardation in colonization of calliphorids in the treated carrions.

In regard to summer season, insect invasion occurred early at the day of carcasses exposure (day o) except in cases of tramadol intoxicated guinea pig carcasses where invasion lagged to day 1 post killing. For rabbit carcasses, calliphorid flies especially *Chrysomya albiceps* were the first invaders (day 0) in case of control and tramadol intoxicated carrions. For guinea pig carrions, control carcasses were firstly invaded by *Chrysomya albiceps* (Family: Calliphoridae) as in rabbit ones at the day of deposition. Tramadol treated carcasses were invaded firstly by mites at day one post killing followed by dipterous calliphorids and muscids at the second day post mortem.

The wave patterns of carrion insects was observed to be low at the fresh stage of decomposition in all experimental carcasses then increased reaching to the maximum during bloat and active decay stages. The succession declined again at advanced decay and dry stages.

The insect's succession divided into different waves over the stages of decomposition according to the attractiveness of the decayed carcasses to the different visiting insects. The first invasion wave was exploited by dipteran adults especially blow flies also with some members of coleopterans and ants during as the first, second and third stages of decomposition. Within this wave, the flies deposited on the carrions and laid their eggs. The second wave started by the appearance of dipterous maggots and continuation of coleopteran adults and ants, the diversity reaches its maximum during this wave resulting in fast rate of carrion decomposition, then marked decrease in their populations observed and insect's departure from the carcasses at the late stages of decomposition Occurred. The third identified insect wave was dominanted by coleopterous insects with their larvae as well as ants.

The first record of blow fly maggots was observed on the day of deposition (day 0) for control rabbit carcasses in summer, while in winter season the first record was occured on the 3rd day for control guinea pig carcasses. Lagging - by a different timing - in the appearance of maggots and their prolonged presence were noticeable in the other treated rabbit and guinea pig carcasses during both seasons Tables **3-10.** Several arthropod and insect species were attracted to carrions for many purposes, while the main insect colonizing carcasses were those belonging to orders Diptera, Coleoptera and Hymenoptera.

Table 3 Temporal patterns of arthropod succession of control rabbit carcasses during winter season 2012

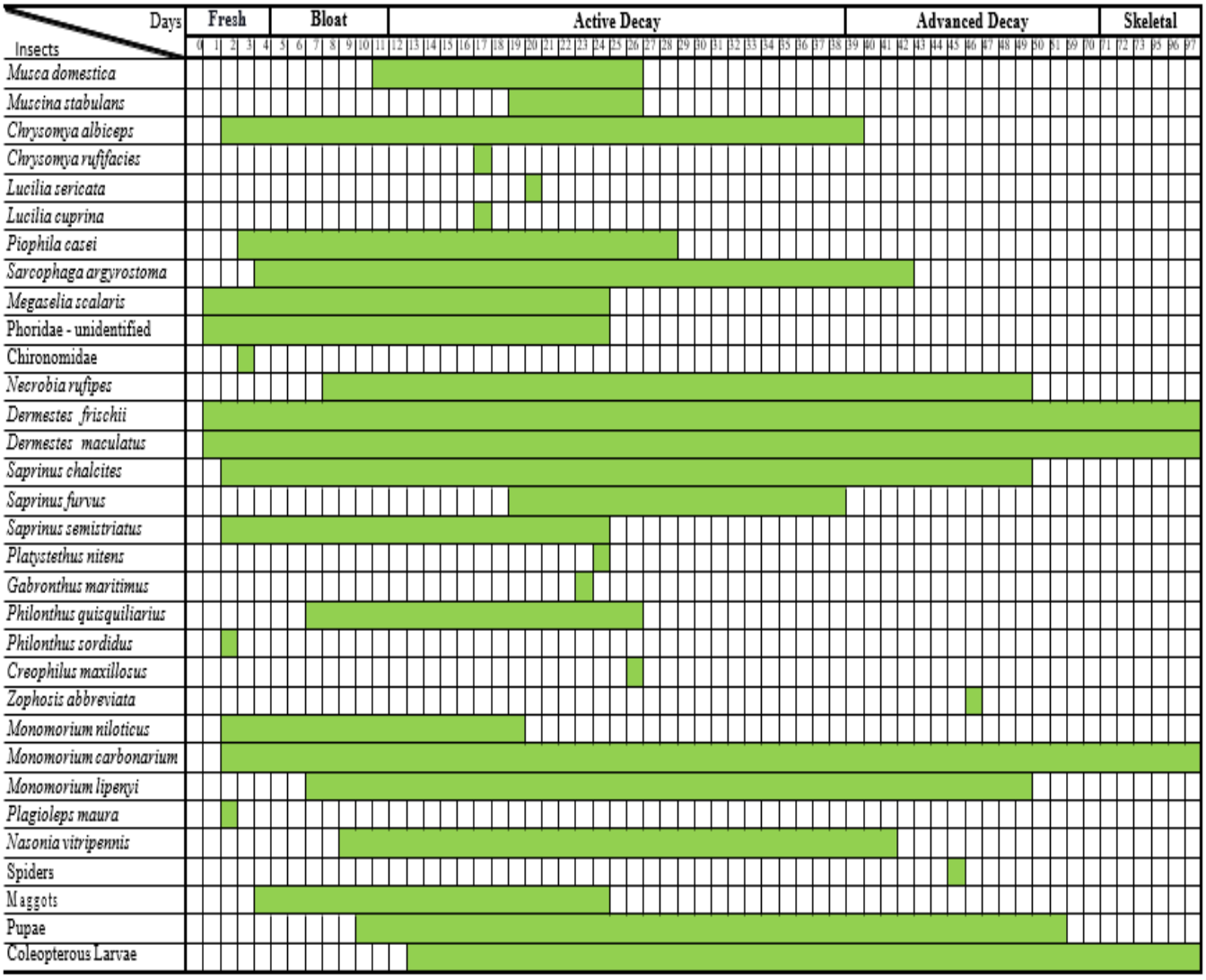


Table 4 Temporal patterns of arthropod succession of tramadol intoxicated rabbit carcasses during winter season 2012

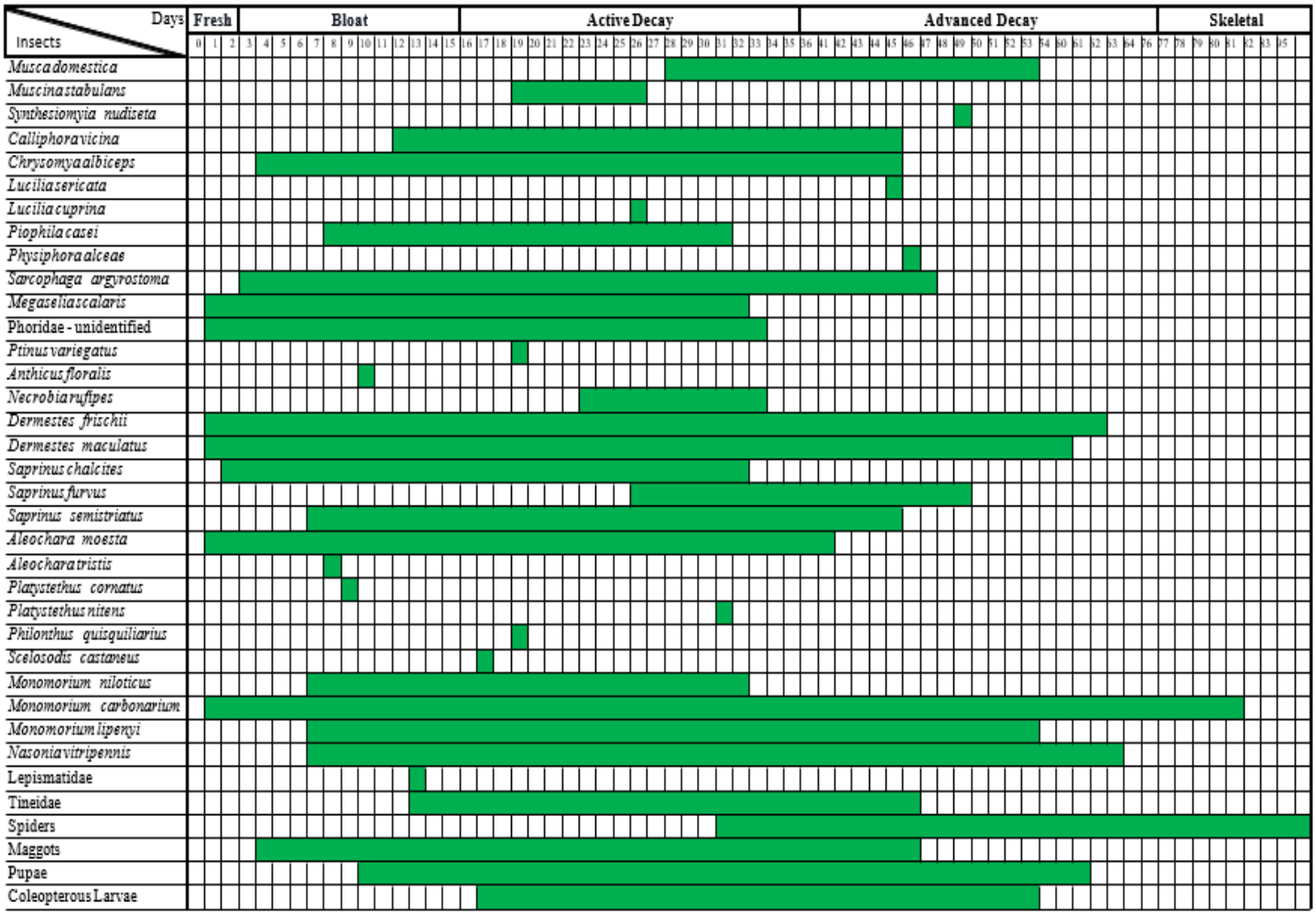


Table 5 Temporal patterns of arthropod succession of control guinea pig carcasses during winter season 2012

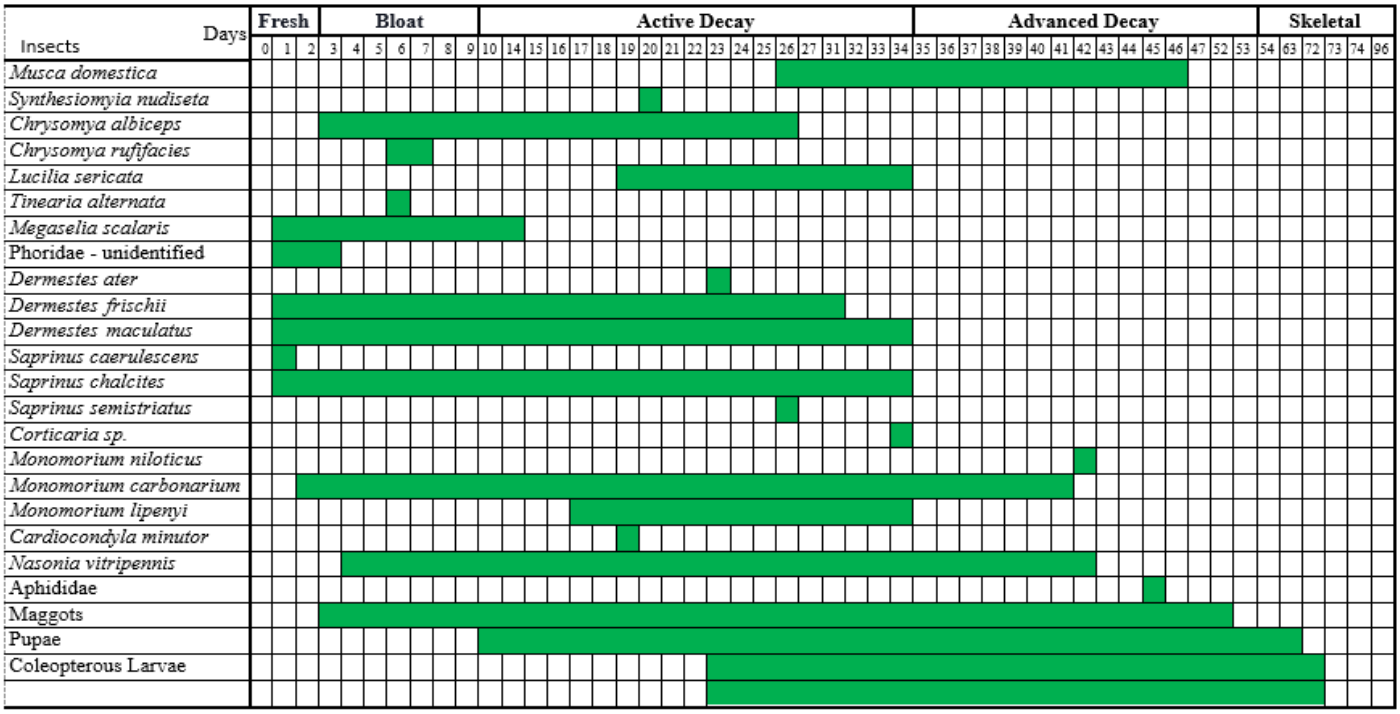


Table 6 Temporal patterns of arthropod succession of tramadol intoxicated Guinea Pig carcasses during winter season 2012

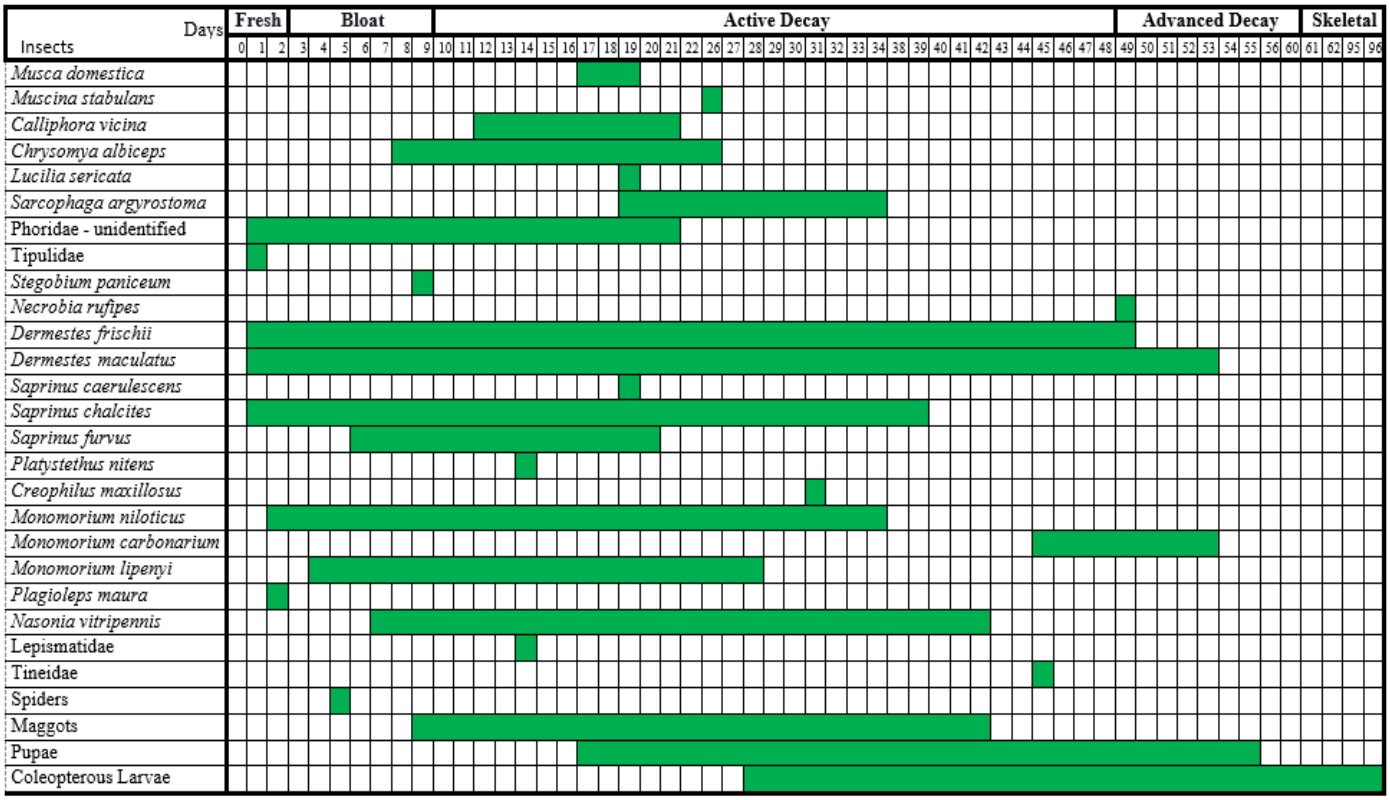


Table 7 Temporal patterns of arthropod succession of control rabbit carcasses during summer season 2012

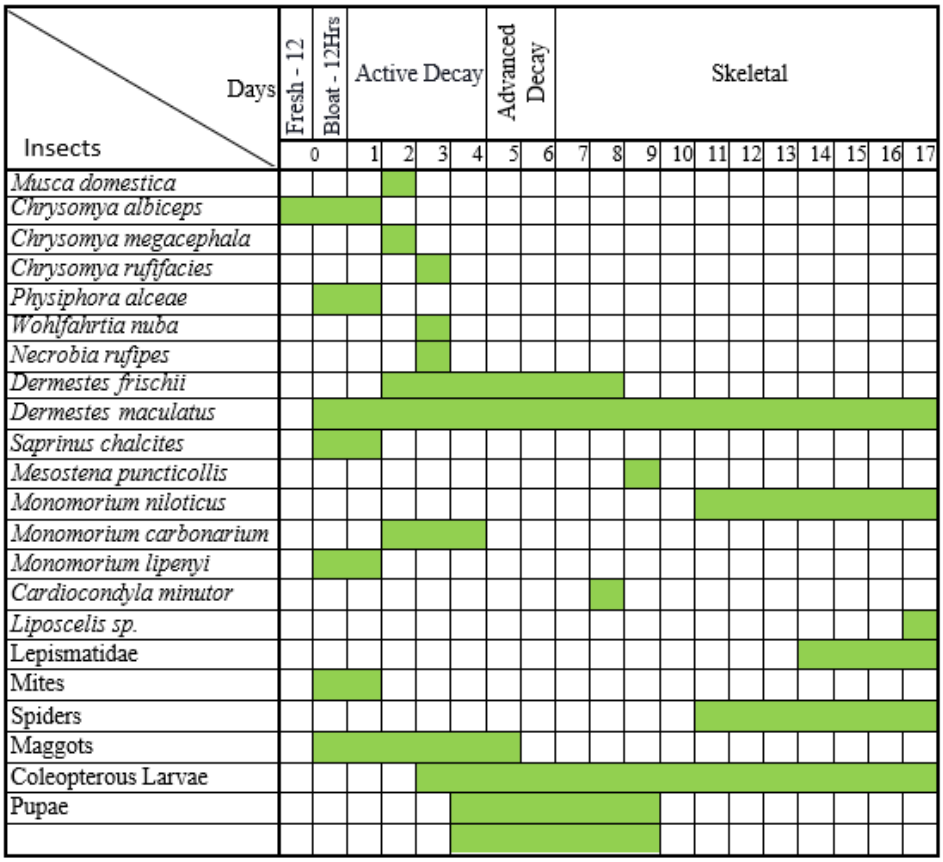


Table 8 Temporal patterns of arthropod succession of tramadol intoxicated rabbit carcasses during summer season 2012

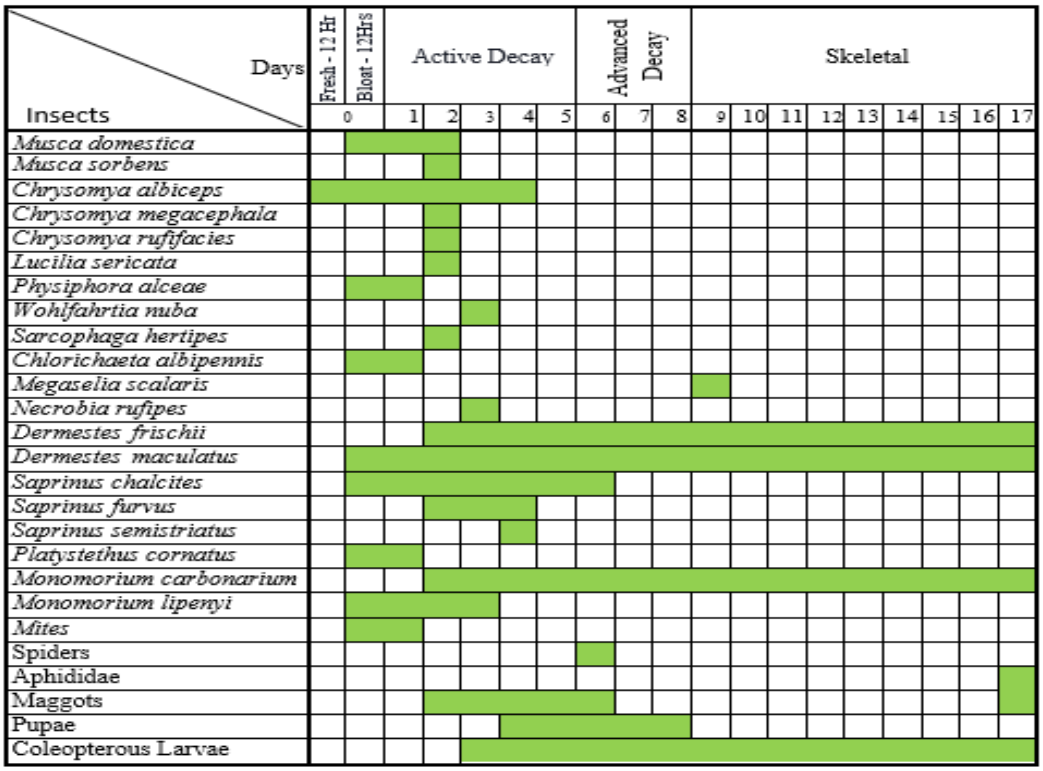


Table 9 Temporal patterns of arthropod succession of control Guinea Pig carcasses during summer season 2012

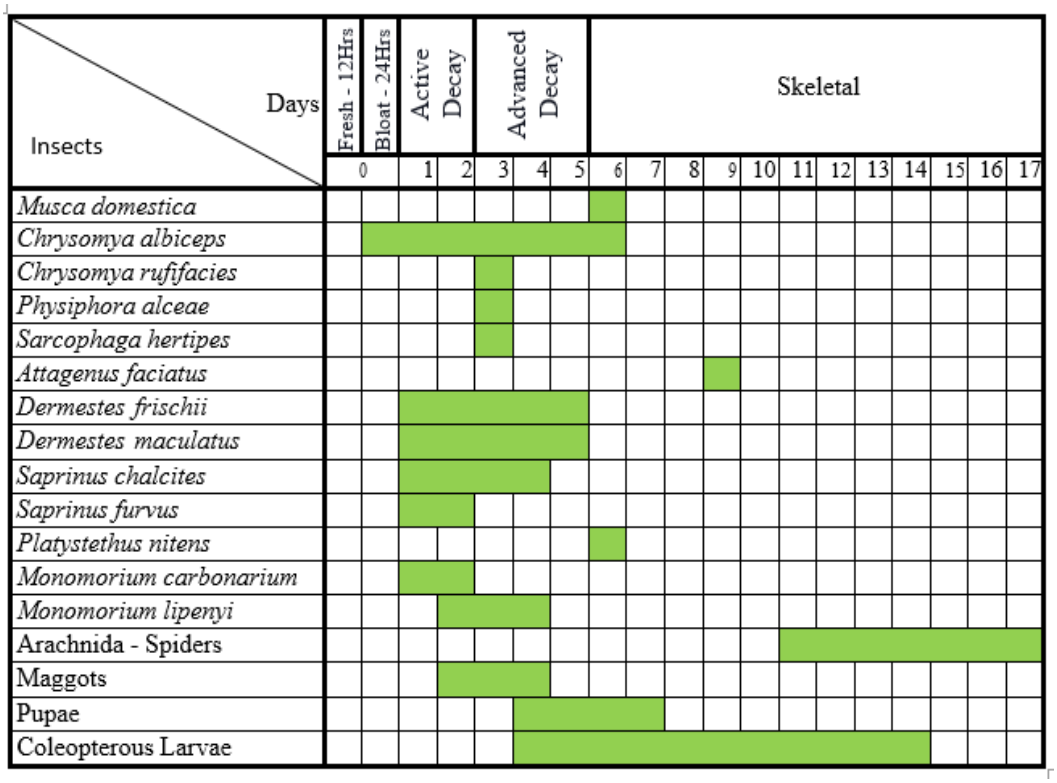
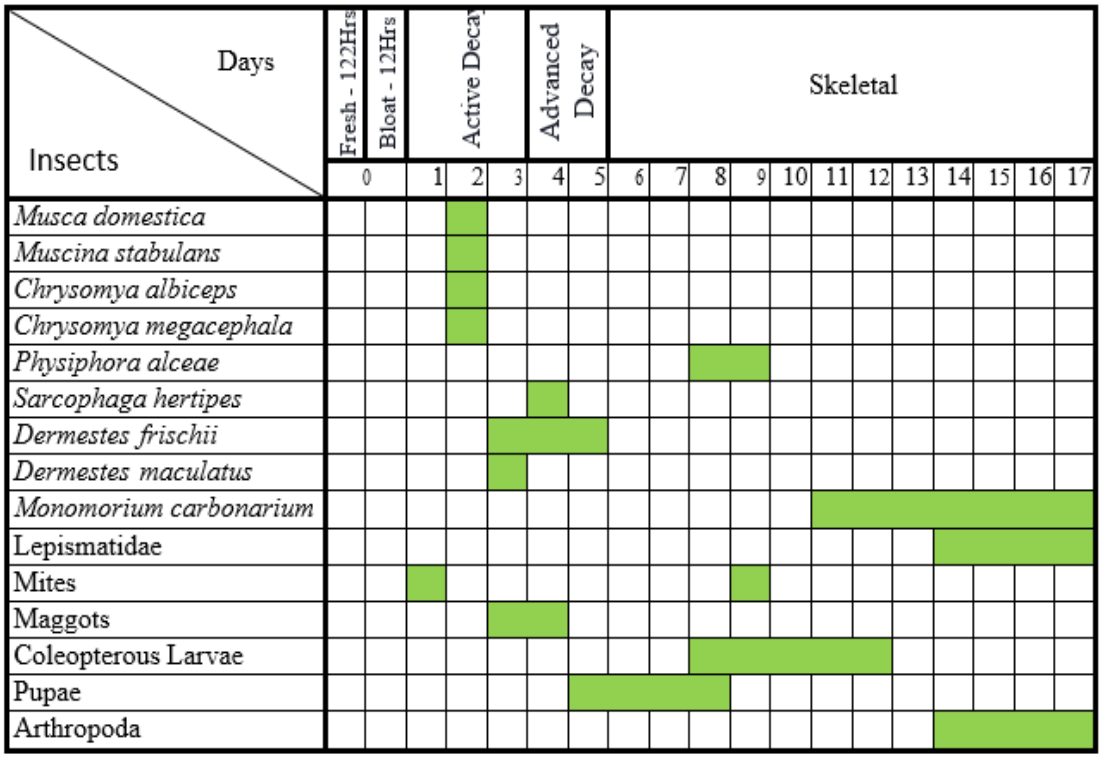


Table 10 Temporal patterns of arthropod succession of tramadol-intoxicated Guinea Pig carcasses during summer season 2012



3.4 Statistical analyses

3.4.1 Immature stages

3..4.1.1 Dipteran larvae

The mean numbers of the collected dipteran immatures of rabbit and guinea pig carcasses in both winter and summer seasons were listed in Tables 11 & 12**.** By applying Non- Parametric Mann-Whitney U test for two independent samples, the mean number of collected immature dipterous larvae and pupae were tested to determine their significance in the two seasons (winter and summer), in the two study cases (control and tramadol-intoxicated carcasses) and in the two sized animals (rabbits and guinea pigs).

For comparing the mean numbers in the different seasons, it was found that, the mean numbers collected from control and tramadol intoxicated rabbit carcasses in winter were significantly lower than the corresponding ones in summer [control: (328.7 ± 7.5 and 1011 ± 43.6), Tramadol: 46.7 ± 5.9 and 701 ± 105.5)] (U= 1495269.000, P < 0.05), (U= 147210.000, P < 0.05) respectively (**Table 11**). The situation was the same for the mean numbers of the collected maggots from the two guinea pig cases in winter as it was found to be significantly lower than the corresponding ones in summer) [control: (18.7 ± 1.2 and 87.3 ± 2.5), Tramadol: (11.7 ± 1.2 and 32 ± 1)] (U= 7336.000, P < 0.05) and (U=1680.000, P <0,05) respectively **(Table 12).**

In regard with comparing the mean numbers for each killing method, results showed that, the maggots mean numbers of control rabbit carcasses were significantly exceeding the corresponding mean numbers of tramadol-intoxicated rabbit groups in both winter (328.7 ± 7.5 and 46.7 ± 5.9) (U= 69020.000, P < 0.05) and summer season(1011 ± 43.6 and 701 ± 105.5) (U= 3189199.500, P < 0.05) respectively (**Table 11)**. Also for guinea pig carcasses the mean numbers of maggots collected from control cases found to be exceeding those of tramadol-intoxicated groups in winter (18.7 ± 1.2 and 11.7 ± 1.2) (U= 980.000, P < 0.05) and summer (87.3 ± 2.5 and 32 ± 1) (U= 12576.000, P < 0.05) respectively **(Table 12).** .

For comparing the mean numbers of the collected maggots in the tested animals, results revealed that, the mean numbers of dipterous larvae of tramadol rabbit carcasses were significantly surpassing the corresponding ones of guinea pig carrions in both winter (46.7 ± 5.9 and 11.7 ± 1.2) (U= 2450.000, P < 0.05) and summer season (701 ± 105.5 and 32 ± 1) (U=100944.000, P < 0.05) respectively **(Tables 11 and 12).**

Table 11 Mean numbers of dipteran immature stages of control and tramadol-intoxicated rabbit carcasses in winter and summer seasons 2012.

Season	Group	Replicates		Mean ± SD
		Replicates	Total	
Winter[a]	Control	R1	329	
		R2	336	328.7 ± 7.5
		R3	321	
	Tramadol	R1	51	
		R2	49	46.7 ± 5.9
		R3	40	
Summer[b]	Control	R1	1050	
		R2	1019	1011 ± 43.6
		R3	964	
	Tramadol	R1	730	
		R2	789	701 ± 105.5
		R3	584	

a: No. rabbit carcasses-visits = 68 days post-killing for control and 61 days for tramadol-intoxicated carcasses
b: No. rabbit carcasses -visits = 6 days post-killing for control and 8 days for tramadol-intoxicated carcasses

Table 12 Mean numbers of dipteran immature stages of control and tramadol-intoxicated guinea pig carcasses in both winter and summer seasons 2012

Season	Group	Replicates		Mean ± SD
		Replicates	Total	
Winter[a]	Control	R1	18	
		R2	18	18.7 ± 1.2
		R3	20	
	Tramadol	R1	13	11.7 ± 1.2
		R2	11	
		R3	11	
Summer[b]	Control	R1	90	
		R2	85	87.3 ± 2.5
		R3	87	
	Tramadol	R1	31	
		R2	33	32 ± 1
		R3	32	

a: No. of guinea pig carcasses-visits = 63 days post-killing for control and 59 days for tramadol-intoxicated carcasses
b: No. of guinea pig carcasses-visits = 7 days post-killing for control and 8 days for tramadol-intoxicated carcasses

3.4.1.2 Coleopteran larvae

The mean numbers of the coleopteran larvae collected from rabbit and guinea pig carcasses in both winter and summer seasons were listed in Tables 13 & 14. By applying the mentioned statistical test, the mean numbers of collected immature coleopterons larvae were compared to determine their significance in the two seasons, in the two study cases and in the two sized animals.

For comparing the mean numbers of coleopteran larvae in the different seasons, results showed that, the mean numbers collected from control and tramadol intoxicated rabbit carcasses in winter were found to be significantly lower than the corresponding ones in summer [Control: (77.7 ± 2.1 and 82.7 ± 54), Tramadol: (35.7 ± 1.5 and 39 ± 5)] ($U= 28892.000$, $P < 0.05$), ($U= 6259.500$, $P < 0.05$) respectively Table 13.

Also the mean numbers of the Coleopteron larvae collected from the two guinea pig cases in winter were found to be significantly lower than the corresponding ones in summer [Control: (15.7 ± 4.5 and 26.3 ± 5), Tramadol: (9.7 ± 1.5 and 16.7 ± 1.2)] (U= 1856.500, P < 0.05), (U= 725.000, P < 0.05) respectively Table **14**.

In regard with comparing the mean numbers of coleopteran larvae for each killing method, as expected, the mean numbers of control rabbit carcasses significantly exceeded the corresponding mean numbers of tramadol-intoxicated rabbit groups in winter (77.7 ± 2.1 and 35.7 ± 1.5) (U= 12465.500, P < 0.05) and summer season (82.7 ± 54 and 39 ± 5) (U=14508.000, P < 0.05) respectively Table **13**. Also for the guinea pig carcasses the mean numbers collected from control cases found to be exceeding those of tramadol-intoxicated groups in both winter (15.7 ± 4.5 and 9.7 ± 1.5) (U= 681.500, P < 0.05) and summer seasons (26.3 ± 5 and 16.7 ± 1.2) (U= 1975.000, P < 0.05) Table **14**.

For comparing the mean numbers of the collected coleopteran larvae in the two experimental animals, results revealed that, the mean numbers collected from tramadol rabbit carcasses were significantly superior than the corresponding ones of guinea pig carrions in winter (35.7 ± 1.5 and 9.7 ± 1.5) (U= 1551.500, P < 0.05) and in summer season (39 ± 5 and 16.7 ± 1.2) (U=2925.000, P < 0.05) respectively Tables **13 & 14**.

Table 13 Mean numbers of immature coleopteran larvae of control and tramadol-intoxicated rabbit carcasses in winter and summer seasons 2012

Season	Group	Replicates		Mean ± SD
		Replicates	Total	
Winter[a]	Control	R1	76	77.7 ± 2.1
		R2	77	
		R3	80	
	Tramadol	R1	37	35.7 ± 1.5
		R2	36	
		R3	34	
Summer[b]	Control	R1	144	82.7 ± 54
		R2	42	
		R3	62	
	Tramadol	R1	44	39 ± 5
		R2	34	
		R3	39	

a: No. of rabbit-visit = 63 days post-killing for control and 96 days for tramadol-intoxicated carcasses
b: No. of rabbit carcasses-visits = 17 days post-killing for control and tramadol-intoxicated carcasses

Table 14 Mean numbers of immature coleopteran larvae of control and tramadol-intoxicated guinea pig carcasses in winter and summer seasons 2012

Season	Group	Replicates		Mean ± SD
		Replicates	Total	
Winter[a]	Control	R1	16	15.7 ± 4.5
		R2	20	
		R3	11	
	Tramadol	R1	10	9.7 ± 1.5
		R2	11	
		R3	8	
Summer[b]	Control	R1	21	26.3 ± 5
		R2	27	
		R3	31	
	Tramadol	R1	18	16.7 ± 1.2
		R2	16	
		R3	16	

a: No. of guinea pig carcasses-visits = 63 days post-killing for control and 96 days for tramadol-intoxicated carcasses
b: No. of guinea pig carcasses-visits = 14 days post-killing for control and, 12 days for tramadol-intoxicated carcasses

3.4.2. Adults

Proportions of adults collected from control, and Tramadol-treated rabbit and guinea pig carcasses in both winter and summer seasons were listed in Table 15 Pearson Chi-square-test was used for comparing the proportions of adults for significance in different seasons, different killing methods and in the two tested animals.

Results cleared that the relative proportions of adults collected from control and tramadol intoxicated rabbit carcasses in winter were significantly different from the corresponding ones in summer ($X2$ = 6.887E2, df = 32, P< 0.05) ($X2$ = 4.119E2, df = 37, P < 0.05) respectively. The same situation occurred for control, and tramadol- intoxicated guinea pig carcasses, ($X2$ = 1.729E2, df = 20, P < 0.05), ($X2$ = 0.027, df = 1, P < 0.05) respectively.

For comparing the proportions of adults for the different killing methods, results revealed that, the proportions were significantly different in control rabbit carcasses when compared to tramadol-intoxicated ones in both winter ($X2$ = 4.847, df = 1, P < 0.05) and summer season ($X2$ = 1.187E2, df = 24, P < 0.05) respectively. The same results were obtained for guinea pigs, where the proportions of adults collected from control carcasses was found to be significantly different comparing to tramadol treated ones in winter ($X2$= 58.825, df = 27, P < 0.05) and in summer season ($X2$ = 64.301, df = 14, p < 0.05) respectively.

By comparing the proportions in the two animals, data showed a significance of proportions in control, and tramadol rabbit carcasses when compared to the corresponding ones of guinea pigs in winter ($X2$ = 74.849, df = 30, P < 0.05), ($X2$ = 80.392, df = 34, P < 0.05) and summer season ($X2$ = 66.681, df = 18, P < 0.05), ($X2$ = 53, df = 22, P < 0.05) respectively.

Table 15 Proportions of adults collected from control and tramadol-treated rabbit and guinea pig carcasses in winter and summer

Insect Family	Insect Species	Seasons							
		Rabbit				Guinea pig			
		Winter		Summer		Winter		Summer	
		Control	Tramadol	Control	Tramadol	Control	Tramadol	Control	Tramadol
Muscidae	*Musca domestica*	1.05	0.18	2.10	4.72	0.53	3.00	0.73	5.26
	Musca sorbens	-	-	-	0.39	-	-	-	-
	Muscina stabulans	0.39	0.36	-	-	-	0.43	-	5.26
	Synthesiomyia nudiseta	-	0.71	-	-	0.53	-	-	-
Calliphoridae	*Calliphora vicina*	-	0.54	-	-	-	1.29	-	-
	Chrysomya albiceps	4.20	1.61	2.40	9.84	3.17	3.43	12.41	21.05
	Chrysomya megacephala	-	-	0.30	1.97	-	-	-	5.26
	Chrysomya rufifacies	0.13	-	0.90	0.79	0.53	-	0.73	-
	Lucilia sericata	0.26	0.18	-	0.79	1.59	0.86	-	-
	Lucilia cuprina	0.13	0.18	-	-	-	-	-	-
Piophilidae	*Piophila casei*	1.71	1.61	-	-	-	-	-	-
Ulidiidae	*Physiphora alceae*	-	0.18	0.90	0.79	-	-	0.73	5.26
Psychodidae	*Tinearia alternata*	-	-	-	-	0.53	-	-	-
Sarcophagidae	*Wohlfahrtia nuba*	-	-	1.50	0.39	-	-	-	-
	Sarcophaga argyrostoma	0.39	5.36	-	-	-	0.86	-	-
	Sarcophaga hertipes	-	-	-	0.79	-	-	0.73	5.26
Sphaeroceridae	*Pullimosina heteroneura*	-	-	-	-	-	-	-	-
Phoridae	*Megaselia scalaris*	19.84	18.04	-	0.39	24.34	-	-	-
	Phoridae - unidentified	0.92	0.36	-	-	5.82	13.30	-	-
Tipulidae	Tipulidae- unidentified	-	-	-	-	-	0.43	-	-
Chironomidae	Chironomidae- unidentified	0.13	-	-	-	-	-	-	-
Anobiidae	*Stegobium paniceum*	-	-	-	-	-	0.43	-	-
	Ptinus variegatus	-	0.18	-	-	-	-	-	-
Anthicidae	*Anthicus floralis*	-	0.18	-	-	-	-	-	-
Cleridae	*Necrobia rufipes*	0.39	0.18	1.80	3.15	-	0.43	-	-
Dermestidae	*Attagenus faciatus*	-	-	-	-	-	-	0.73	-
	Dermestes ater	-	-	-	-	0.53	-	-	-

	Dermestes frischii	27.20	16.61	4.20	9.06	17.99	33.48	0.73	15.79
	Dermestes maculatus	16.56	20.18	3.90	7.09	26.46	12.88	4.38	5.26
Histeridae	*Saprinus caerulescens*	-	-	-	-	0.53	0.43	-	-
	Saprinus chalcites	8.94	8.75	5.11	17.72	4.76	6.01	12.41	-
	Saprinus furvus	0.79	1.61	-	2.36	-	0.86	2.92	-
	Saprinus semistriatus	0.26	0.36	-	0.39	0.53	-	-	-
Latridiidae	*Corticaria sp.*	-	-	-	-	0.53	-	-	-
Staphylinidae	*Aleochara moesta*	-	0.18	-	-	-	-	-	-
	Aleochara tristis	-	0.18	-	-	-	-	-	-
	Atheta sp.	-	-	-	-	-	-	-	-
	Platystethus cornatus	-	0.18	-	0.39	-	-	-	-
	Platystethus nitens	0.13	0.18	-	-	-	0.43	0.73	-
	Scopaeus debilis	-	-	-	-	-	-	-	-
	Gabronthus maritimus	0.13	-	-	-	-	-	-	-
	Philonthus quisquiliarius	0.39	0.18	-	-	-	-	-	-
	Philonthus sordidus	0.13	-	-	-	-	-	-	-
	Creophilus maxillosus	0.13	-	-	-	-	0.43	-	-
Tenebrionidae	*Mesostena puncticollis*	-	-	0.30	-	-	-	-	-
	Scelosodis castaneus	-	0.18	-	-	-	-	-	-
	Zophosis abbreviata	0.13	-	-	-	-	-	-	-
Formicidae	*Monomorium niloticus*	0.66	0.36	0.60	-	0.53	0.43	-	-
	Monomorium carbonarium	2.23	3.04	19.52	8.66	1.59	2.15	24.82	15.79
	Monomorium lipenyi	1.45	0.54	54.05	27.95	2.12	2.15	37.23	-
	Plagioleps maura	0.13	-	-	-	-	0.43	-	-
	Cardiocondyla minutor	-	-	1.20	-	1.06	-	-	-
Chalcididae	*Brachymeria femorata*	-	-	-	-	-	-	-	-
Pteromalidae	*Nasonia Vitripennis*	10.91	16.25	-	-	5.82	14.59	-	-
Vespidae	*Vespa orientalis*	-	-	-	-	-	-	-	-
Liposcelididae	*Liposcelis sp.*	-	-	0.30	-	-	-	-	-
Lepismatidae	silverfish	-	0.18	0.30	-	-	0.43	-	5.26
Cicadellidae	*Empoasca sp.*	-	-	-	-	-	-	-	-
Tineidae	Tineidae-unidentified	-	0.18	-	-	-	0.43	-	-
	Mites	-	-	0.30	0.39	-	-	-	10.53
	Spiders	0.26	1.07	0.30	0.39	-	0.43	0.73	-
	Total	100%	100%	100%	100%	100%	100%	100%	100%

4. Discussion

4.1 Decomposition stages

According to the morphological changes of carrions, just five stages of decomposition were observed in the present study which were: fresh, bloated, active decay, advanced decay and skeletonization as stated by **Voss *et al.*** [34], **Abd El-bar & Sawaby** [16], **Zeariya *et al.*** [24]. It was difficult to determine the end of the last stage of decomposition due to its long duration time as occurred with **Tantawi *et al.*** [21]. In general, decomposition process in this work started from head regions in most animals and was noticed later in the all body parts during the experiments. This occurred because the oviposition of dipterous flies began firstly at body orifices including mouth, nose, ears and eyes. The same observation was obtained by **Tantawi *et al.*** [21].

Few studies were systematically conducted about the influence of body size on the rate of cadaver decomposition [35]. In the present study, the decaying process was found to be affected by the carcasses size where the smaller guinea pig carcasses observed to be decayed more rapid than larger rabbit ones. This may be due to a more challenging environment which was created by the carcasses of small size for colonization and development by the fly larvae. This limited food resource results in more intense inter- and intra-specific competition and so fasten the decomposition process as illustrated by **Gill** [36].

Our conclusions about the effect of size of animal on the decaying process are also agreed with that of **Zeariya *et al.*** [24] in their spring, summer and autumn experiments on rabbit and dog carrions.

Fluctuations in climatic factors such as temperature, relative humidity and rainfall affected greatly the rates of carrion decomposition. During summer season, temperature is usually high; therefore the duration of the decaying process of carrions was shorter than that of winter season due to faster chemical and microbial reactions and also the increased activity of the carrion insects. This result was obvious in the present study where decomposition in summer lasted only for 17 days post mortem while in winter decomposition was prolonged to 96 days. This is agreeable with the previous studies of **Tantawi *et al.*** [21], **Wang *et al.*** [37], and **Polat and Kökdener** [38]**.**

In the present study, the climatic data revealed the inverse relationship between the decomposition process and rainfall in winter season. Rains and low temperature together retarded the carcasses degradation by prolongation of the active decay stage as a result of increasing maggots feeding period due to decreasing their activity. Hence, rains making the carcasses suitable for flies re-invasion and re-oviposition. This agreed with **Tantawi *et al.*** [21]**, Gill** [36]**,** and **Ahmad *et al.*** [39].

In our study the decomposition process showed that, degradation rate was distinctly prolonged in tramadol-treated carcasses relative to the control, regardless of season and type of the experimental animals. This result was agreeable with that of **Abd El-bar and Sawaby** [16] who observed lagging in decomposition process of rabbit carcasses killed by Organophosphate (OP) pesticide when compared with control ones. In contrary, **Abd El-Gawad *et al.*** [25] reported that except for the fresh stage, the Warfarin-intoxicated carcasses decayed significantly faster than control carcasses, this may due to the mode of action of each toxin.

4.2 Associated insect fauna and waves of insect succession patterns

Insects attracted to the dead bodies immediately after death, often within minutes, so data about development and successional waves of insect species colonized carcasses can be used for estimation of PMI of homicide and suicide cases especially in the late stages of decomposition when the earliest invaders departed.

Insects colonized the decomposed carcasses in a somewhat predictable sequence depending upon the geographical region and the environmental climatic conditions under which the corpses were discovered**.**

Several insect species invaded the different decomposed carrions during the study period. A total of 67 species, 37 families and 6 orders of insects were collected from this study. Generally, richness and numbers of collected species differed according to the climatic conditions, the geographic region, the killing methods and the antemortem ingestion of drugs or toxins as demonstrated by many authors [16, 18, 21,25,40-44].

The carrion invading insects in the present study were observed to be low at the fresh stage of decomposition in all study cases in both seasons then increased reaching to the maximum during bloat and active decay stages because the carcasses were more attractive to a wider range of species than the fresh stage. The succession declined again at advanced decay and dry stages. These results agreed with **Abajue *et al.*** [45]**.**

In this study, it is noticeable that Dermestidae (Order: Coleoptera) was the predominant family in winter, while in summer family Formicidae (Order: Hymenoptera) was the most predominant one. This is incompatible with **Abd El-bar & Sawaby** [16] in Qalyubiya Governorate in Egypt, who conducted their study with pirimiphos-methyl in summer season where Challiphoridae (Order: Diptera) was the predominant family and Formicidae represented only low percentage of the collected insects in their work.

In the present study, Calliphoridae was observed to be the most diverse dipterous family represented by 6 species; this is contrary to **Silva *et al.*** [46] and **Mabika *et al.*** [47] where Muscidae was the most diverse family in their study. Among Calliphoridae, *Chrysomya albiceps* was numerically dominant one as observed in the studies of **Abd El-bar & Sawaby** [16]**, Polat & Kökdener** [38], **and Keshavarzi *et al.*** [48], since this species was considered a very good competitor with others in regard with its feeding on the corpse as well as its predatory behavior on the other larvae infesting the carrions as mentioned by **Tantawi *et al.*** [21]**, Ibrahim *et al.*** [23] and **Silva *et al.*** [46].

In this work during winter season, insect succession began by invasion of *Megaselia scalaris* (Family: Phoridae) with other Phorids and members of family Dermestidae followed by other dipterous flies, these results differed from other authors as **Tantawi *et al.*** [21], **Eberhardt & Elliot** [49]**, Sert *et al.*** [50]**, Mabika *et al.*** [47] and **Calzolari *et al.*** [51] who recorded calliphorids, sarcophagids or muscids as the first colonizers, while

Ekanem & Dike [44] mentioned Phorids among the initial invaders in their research with calliphorids, sarcophagids and hymenopterans.

In the current study, we noticed that the appearance of coleopterans was at the early stages of decomposition. In concern with summer season, invasion occurred firstly by *Chrysomya albiceps* (Family: Calliphoridae) in control carcasses and these results agreed with Abd El-Bar and Sawaby [16]. In other studies, as **Ahmad *et al.*** [39], they found ants the first visitors followed by blowflies.

For control carcasses, in regard to summer season, insect invasion occurred early at the day of carcasses exposure (day o), while retarded to the second day in winter. This agreed with **Centeno *et al.*** [52].

In the current study, it was apparent that, greater diversity was observed in association with the carcasses of winter season than those of summer and this may be due to the high temperature in summer which accelerated the decaying process leading to rapid depletion of food resource and decrease of arthropod colonization time as mentioned by **Ibrahim *et al.*** [23]. Our results are differed from that of **Kyerematen *et al.*** [53] as they mentioned that the warmer temperatures increased the number and diversity of insects colonized the carcasses, which in turn accelerated the development of maggots and this illustrated the observed faster rate of decomposition.

In some cases, the flesh from the corpse can retain some kinds of drugs or toxins that the victim had consumed before he or she died, and which may have even been the cause of death; these substances can be recovered by examining the necrophagous insects [19,54]. Thus, entomotoxicology trends have been aimed at understanding the consequences of drugs of abuse and use on development of fly larvae (mainly blow flies) that can be large enough to significantly bias PMI estimates.

According to killing method, it was found that presence of tramadol in the treated carcasses didn't prevent the invasion of insects just as mentioned by Voss *et al* [34]] who worked on the carbon-dioxide poisoned carcasses and by **Wolff *et al.*** [55] in case of parathion and malathion intoxicated rabbit carcasses and **Wolff *et al.*** [55] in case of parathion and malathion intoxicated rabbit carcasses.

In contrary, **El- Kady *et al.*** [20] didn't find any invading insects on the arsenic oxide poisoned carrions. In the tramadol-treated carrions, the insect invasion was retarded than that of control as occurred in the studies of **Wolff *et al.*** [55] in case of malathion treated carrions, **Voss *et al.*** [34] in case of Co_2 poisoning and **Abd El-bar & Sawaby** [16] in case of organophosphate insecticide poisoning.

Concerning the immature stages, significantly lower numbers were observed and collected in the present study from the Tramadol-treated carcasses; this may be a reason of prolongation of their decomposition period as occurred with **Abd El-bar & Sawaby** [16] in the organophsphate intoxicated carcasses and with **El-Kady *et al.*** [20] in the gas-killed carrions. However, this situation is contrary to the study of **Abd El-Gawad *et al.*** [25] who revealed that, unexpectedly, significantly more adults and immatures were attracted to Warfarin than to control carcasses. Moreover, they found that Warfarin accelerated the larval development.

Tramadol is centrally performing opioid analgesics consumed for the managing of mild to severe pain. The analgesic effects of tramadol result from binary mechanism of action by agonist actions of the drug at the μ-opioid receptor causing analgesia and sedation by inhibiting serotonin and norepinephrine reuptake reducing pain perception [56]. Tramadol differs from other traditional opioid medications in that it doesn't just act as a μ-opioid agonist, but also affects monoamines by modulating the effects of neurotransmitters involved in the modulation of pain such as serotonin and norepinpehrine which activate descending pain inhibitory pathways [57]. This effect may make the body non-attractive for the colonizing insects after death and this may be the cause of lagging of insect colonization and the significantly lower numbers of recovered immatures presented on the Tramadol-intoxicated carcasses.

5. Conclusion

Outlining of decomposing-insect fauna data base for different localities, climatic conditions, and different toxins is crucial in the determination of post-mortem interval. Generally in this study, decomposition process observed to have a slower rate in winter. Tramadol had an effect on prolongation of the development of decomposing insects which is an important indication of cause of death and may be a useful tool for PMI estimation based on entomological evidences.

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