



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: [www.sciencedirect.com](http://www.sciencedirect.com)

Original article

# Impacts of antemortem ingestion of alcoholic beverages on insect successional patterns

 Mohammed Al-Khalifa<sup>a</sup>, Ashraf Mashaly<sup>b,\*</sup>, Abdullah Al-Qahtni<sup>a</sup>
<sup>a</sup> Department of Zoology, College of Sciences, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia

<sup>b</sup> Department of Zoology, Faculty of Science, Minia University, El Minia 61519, Egypt


## ARTICLE INFO

## Article history:

Received 20 September 2020

Revised 25 October 2020

Accepted 26 October 2020

Available online 5 November 2020

## Keywords:

Entomotoxicology

Forensic entomology

Insects

Successional pattern

## ABSTRACT

This paper studies the impacts of antemortem ingestion of alcoholic beverages by the domestic rabbit, *Oryctolagus cuniculus* L., on postmortem successional patterns of insects during winter and summer 2018 in Riyadh, Saudi Arabia. Insect samples were collected from the carcasses of rabbits fed alcoholic beverages as well as untreated rabbits for 15 days postmortem during two successional studies in each season. The results showed that, during both seasons, the decomposition process for the carcasses of rabbits fed alcoholic beverages antemortem was one to two days longer. The results also showed, however, that alcoholic beverages did not affect insect succession patterns in either season. In fact, the number of insects appeared to be influenced by the ambient temperature during the two seasons, with 4415 insects in the winter compared to 1033 insects in the summer. In total, 30 insect taxa were collected during the winter study from the carcasses of rabbits fed alcoholic beverages antemortem; while 26 of these same taxa were collected from the carcasses of the untreated rabbits. Among the treated rabbits, those fed 25 ml alcoholic beverages treated attracted the highest number of insect taxa (24 taxa). In the summer study, 21 insect taxa were collected in total, 19 from the carcasses of the alcohol-treated rabbits and 13 from untreated rabbits. Among the treated rabbits, those fed 50 ml alcoholic beverages attracted the highest number of insect taxa (14 taxa). These results contribute to the understanding of the factors affecting the use of insects in medical investigations, given that alcoholic beverages are a common addictive agent.

© 2020 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

In the forensic sense, succession is the timed, predictable progression of faunal specimens accessing the cadaver. The type and composition of fauna present on a cadaver is descriptive of its decay stage (Reed, 1958; Payne, 1965). Succession patterns may be influenced by a number of factors, including temperature, relative humidity, precipitation, exposure to sunlight and whether or not the body is shielded by a structure. Details on species diversity, the individual numbers of each species, the life stages present, and the number of individuals of each life stage can be determined

from succession studies. This information can be used to estimate PMI, particularly if the person has been dead for only a few weeks (Keh, 1985). Although insect succession usually follows a similar pattern at the family level, there appears to be a lot of variation between locations in the genus and species. Nevertheless, each location and type of environment appears to have a consistent and representative pattern of arthropod succession (Anderson, 2001).

Chick et al. (2008) stated that if carrion is to be considered an ecosystem, chemicals found in the body prior to death can be considered a form of pollution. Introna et al. (2001) refer to the study of these toxins and their interactions with insects as entomotoxicology. This “cadaver pollution” can take the form of many different toxins, which in turn can have many effects on the carrion ecosystem. One category of toxin that is of particular interest to investigators is commonly-abused drugs (including alcohol). Entomotoxicology incorporates entomological and toxicological evidence for the purpose of assessing the manner or cause of death and estimating the postmortem interval (PMI) (Goff and Lord, 2001). Drug/toxin use can contribute to, or indicate, the manner

\* Corresponding author.

E-mail address: [mashaly69@gmail.com](mailto:mashaly69@gmail.com) (A. Mashaly).

Peer review under responsibility of King Saud University.



of death; therefore, it is important to know whether antemortem use of these substances occurred (Beyer et al., 1980). Goff (2009) indicated that while larvae of forensic insects are actively feeding on cadaveric tissue, drugs and other toxic substances that are present in the tissues are transferred to the metabolic larvae system. In addition, these drugs are transferred through the food chain to other arthropods that predate larvae (Introna et al., 2001).

Alcohol use and alcohol-related disorders differ greatly across the globe, but the risk of addiction and mortality is high in most countries (Ramchandani et al., 2001). In many developing countries, various types of home-made or locally-produced alcoholic beverages, such as sorghum beer, palm wine or sugarcane spirits, continue to be the main available alcoholic beverages. Ethanol, or ethyl alcohol, is the alcohol contained in beverages such as wine, beer and spirits. It is also termed drinking alcohol or grain alcohol.

Ethanol may be found in the bodies of persons that have died, especially in cases of violent death (Baselt and Cravey, 1980). Ethanol is also one of the main causes of death by poisoning (Caplan et al., 1985). In that context, there appear to be two points of interest: (1) the analysis of insect/larval fauna in order to determine whether a cadaver contains alcohol (as possible relevant information in determining cause of death); (2) analysis of insect succession patterns to provide background data for forensic scientists/pathologists to factor in to their calculations of PMI in circumstances where they have already determined that a cadaver contains alcohol. Most of the published data concentrated on the effect of alcohol on the fly maggot's development (Tabor et al., 2005; Monthei, 2009; King and Vanin, 2016). On the other hand, no studies on the effect of alcohol on insect succession, Tabor et al. (2005) argued that antemortem consumption of ethanol did not seem to have an impact on the succession patterns of insects on domestic pigs. The aim of this study was to establish whether the antemortem consumption of alcoholic beverages influences post-mortem insect successional patterns on rabbit carcasses in two separate seasons, winter and summer, in Riyadh, Saudi Arabia.

## 2. Materials and methods

Studies were conducted during winter and summer, 2018 in an urban habitat in Riyadh, Saudi Arabia to investigate the impact of antemortem ingestion of alcoholic beverages on insect successional patterns on rabbit carcasses. Riyadh is situated in the middle of Saudi Arabia; average temperatures in the city are 20.3 °C during the winter and 44.5 °C during the summer. Two successional studies were performed in each season.

### 2.1. Animals and alcoholic beverages dosing

A pilot experiment was conducted to determine the concentration that induces alcohol addiction, and it was found that the amount of 25 ml leads to the appearance of the effect of alcohol on rabbits, with the 50 ml dose representing the overdose, and the 75 ml dose leading to the death of rabbits. Alcoholic beverages used in the experiments are produced illegally by primitive methods and are obtained by the anti-drug police. For each successional study, four live mature rabbits (*Oryctolagus cuniculus* L.) were obtained from the animal house of the College of Pharmacy. The animals ranged in weight from 2.3 to 2.5 kg. Three of the animals were administered different amounts of alcoholic beverages (25, 50 and 75 ml) before euthanasia, and the remaining animal was left untreated. Alcohol was administered orally to achieve a blood alcohol concentration. Rabbits used as the control were designated as (C), and the other three rabbits were designated as A1, A2 and A3.

### 2.2. Insect succession

Pilot experiment indicated that an hour is an appropriate time to achieve a blood alcohol concentration in rabbits for the doses administered. So, one hour after alcoholic beverages was administered, the rabbits were killed in a carbon dioxide chamber. Upon evidence of death, the carcasses were transported immediately to the decomposition site. The carcasses were individually placed under open-bottomed cages, which were located 10 m apart and were allowed to decompose for 15 days. Each cage measured 50 by 40 by 25 cm and was made of iron and lined with a stainless steel wire mesh. Cages were pushed into the ground to keep the carcasses from being destroyed by scavengers. Collections were taken twice a day at 9.00 a.m. and 5.00p.m. during the sampling period, with the day on which the rabbits were killed and exposed was defined as day 0. Samples of adult insects were obtained for qualitative evaluation from ten aerial net sweeps (five at each collection in the day) over and around the carcass, as well as from pit-fall traps, and directly off the carcass. In addition, we gathered maggots and pupae, when they were present, using soft-touch forceps. The collected samples contained specimens from all stages and were stored separately in plastic vials (marked by date and carcass number) and then moved to the counting and identification laboratory. Live adult specimens were killed and put in a vial that was then stored in a freezer (−20 °C). Larval specimens, meanwhile, were immersed in near-boiling water and then transferred to alcohol at 80% (Brown et al., 2012). The identification of the insects was carried out on the basis of suitable keys, for flies: Büttiker et al. (1979), Pont (1991) and Akbarzadeh et al. (2015); for beetles: Borror et al. (1989) and Catts and Haskell (1990) and for ants: Collingwood (1985), Bolton (1994) and Collingwood and Agosti (1996).

### 2.3. Data and statistical analysis

We followed Tabor et al.'s (2004) method in respect to ethanol-treated and untreated rabbit carcasses to classify the successional patterns of insect taxa over the 15 day sampling duration, and to assess if the patterns were identical. First, we merged the data on the presence of insects on ethanol-treated and untreated rabbits from the two succession studies in each season to create a single succession table. Means and standard deviations (SDs) were calculated for all the parameters evaluated and applied to the one-way ANOVA. Normality was assessed using the Anderson-Darling test. Next, ANOVA for block design was used in all analyses including season decomposition stage, and alcoholic beverages concentration as factors. A 5% level of significance was accepted in all analyses. Calculations were conducted using Minitab 17 (Minitab, LLC).

## 3. Results

Four recognisable stages of decomposition were described in all carcasses (Table 1). Results showed that, in general, treated carcasses took slightly longer to decompose than control carcasses. In winter, the process of decay in the control carcasses entered the dry stage 13 days after the rabbits were killed, whereas the A1 treated carcasses entered the dry stage after 14 days and the A2 and A3 treated carcasses after 15 days. In summer, decay was more rapid, with control carcasses entering the dry stage after nine days, and A2 and A3 carcasses after ten days. In contrast to the winter season, the A1 carcasses decomposed slightly more rapidly than the control in summer (eight days).

Over the two seasons, insects were present at all stages of decay, with the number of insects present on carcasses varying depending on stage of decay and season, but not on the concentra-

**Table 1**  
Duration of the post-mortem interval in the two seasons, indicating the stage of decomposition.

Stage of decomposition	Duration (days post-death)							
	Winter				Summer			
	C	A1	A2	A3	C	A1	A2	A3
Fresh	0–1	0–2	0–3	0–4	0–1	0–1	0–1	0–1
Bloated	1–3	2–5	3–5	4–7	1–2	1–2	1–2	1–3
Decayed	3–13	5–14	5–15	7–15	2–9	2–8	2–10	3–10
Dry	13–	14–	15–	15–	9–	8–	10–	10–

tion of alcohol as indicated by Kruskal-Wallis test (Table 2). For all types of carcass, the highest number of adult insects was recorded in the decay stage, followed by the dry stage. The fresh stage attracted the lowest number of adult insects, followed by the bloating stage. Again, the number of insects was higher in the winter than in the summer.

The insects attracted to the carcasses included flies, beetles and ants, with the mean number of all insects collected from all types of carcasses being higher in the winter (4415 insects) than the summer (1033 insects). Dipterans were the dominant insects collected, representing 87.7% and 91.38% of all specimens collected during winter and summer, respectively; and also the first to arrive at both types of carcasses. Eight families of this group were identified in the winter compared to six in the summer (Table 3). The Muscidae family alone accounted for 57.55% of all insects collected in winter, followed by Calliphoridae with 24.92%. In summer, however, the Calliphorid family was the dominant one, with 41.63% of all insects collected followed by the Muscidae family with 30.01%. Two fly families were reported only in the winter (Heleomyzidae and Chloropidae). Beetles were the least common group of insects collected, representing 3.99% and 3.2% of the total number of specimens, in the winter and summer, respectively. Nine families of this group were identified in the winter compared to four families in the summer. Dermestidae was the dominant family of beetles, comprising 1.95% and 0.97% of all insect specimens, in the winter and summer, respectively. Melyridae, Tenebrionidae, Carabidae, Coccinellidae and Staphylinidae were represented only in the winter. The Formicidae family of ants, meanwhile, constituted 8.31% of the winter samples and 5.42% of the summer ones.

At the species level, in general, there was more species diversity among the colonisers of the untreated rabbits compared to the treated ones. In total, insects from 30 taxa were collected during the winter study (Table 4). The carcasses of the untreated rabbits were colonised by 26 taxa. Among the treated rabbits, the A1 carcasses attracted the highest number of insect taxa (24), the A3 carcasses attracted 23 taxa and the A2 carcasses 18. In the summer, 21 separate insect taxa were collected in total: 13 from the carcasses of the untreated rabbits, respectively (Table 4). Among the treated rabbits, the A1 carcasses attracted the lowest number of insect taxa (8), the A2 carcasses attracted 14 taxa and the A3 carcasses 11.

Table 5 details individual and total insect species abundance across the decomposition process in the winter. Thirteen fly species were recorded in the winter, with 13 and 12 species on the carcasses of treated and untreated rabbits, respectively. Eleven

fly species were recorded from the A1 carcasses, with *Paralucilia paraensis* and *Sepsis dissimilis* being the only fly species not reported from this group of carcasses. Eight species of flies were recorded from the A2 carcasses. Eleven fly species were also recorded on the A3 carcasses; in this case *Trixoscelis fumipennis* and *Chloropidae* sp. were the species not recorded. On the control carcasses, meanwhile, 12 fly species were recorded, with *S. dissimilis* being the only species not found. Number of fly species present at the fresh stage varies from 1 to 4 species. This number increased to be from 4 to 7 species in the bloat stage. Dipteran abundance peaked at the decay stage, where species number varied from 8 to 12 species. Following this, the number of individual and species (ranging from 2 to 5 species) continuously decreased during the dry stage. Calliphorids and Muscids were the most abundant group of flies found on the rabbits. In the fresh stage, *Chrysomya albiceps* was present on all carcass types, while *Musca domestica* was present only on the A2 and A3. Also, *Ch. albiceps* was the only fly present at the fresh stage of the control carcasses. During, the decay stage *S. dissimilis* reported only on the A3 carcasses, while *T. fumipennis* appeared on the A1 and control carcasses. Different fly species were trapped on the rabbits on the bloat stage, with Muscids and Calliphorids being the most abundant. In the dry stage, *M. domestica* and *Coenosia attenuate* were the only fly presented on all rabbits, but in extremely low numbers. Unlike the Muscid and Calliphorid flies, sarcophagids and other fly families were low in abundance throughout the decomposition process and concentrated mainly on the decay stage.

Eleven beetle species appeared in winter, of which *Saprinus chalcites* and *Dermestes frischii* were the only beetles identified on all carcass types (Table 5). Also, beetle's abundance was lower than that of fly abundance. Eight beetle species were abundant on control carcasses, while the treated carcasses attracted seven, five and seven species in A1, A2 and A3, respectively. *Dermestes frischii* was the most abundant beetle, while *N. reunion* was the lowest. Some beetle species appeared on specific carcass type, such as *Harpalus reversus* on A1, *Nephus reunion* on A3, *Microdera kraatzi alashanica* on A1 and A2, *Philonthus longicornis* on A2 and the control, and *Sa. splendens* on A3 and control. *Aplocnemus virens* and *D. maculatus* appeared on all carcass types except A2, while *Ph. longicornis* and *Necrobia rufipes* appeared on all carcass types except A3. Beetle's activity began on day 12, an eleven days later than the first fly arrival, and progressed through the dry stage. Beetles arrived after maggots were present on the body, but no beetles were appeared during the fresh and bloat stages except for *Sa. chalcites*, which appeared on the blat stage of the A3 rabbits. Again, *Sa. chalcites*

**Table 2**  
Kruskal-Wallis test results for insects that were collected from rabbit carcasses during the decomposition stages at different alcohol concentrations in the two seasons.

Comparison	Kruskal-Wallis Test Statistic	d.f.	P-Value	Notes
No. of insects by Alcohol concentration	2.79	3	0.915	NS
No. of insects by season	16.44	1	0.001	*
No. of insects by decomposition stage	73.48	3	0.002	*

\*Significant, NS; Not significant ( $\alpha = 0.05$ ).

**Table 3**  
Absolute (n) and relative (%) frequency of insect families collected from rabbit carcasses during winter and summer of 2018.

Order	Family	Number of insects			
		Winter	%	Summer	%
Diptera	Muscidae	2541	57.55	310	30.01
	Sarcophagidae	75	1.7	26	2.52
	Calliphoridae	1100	24.92	430	41.63
	Anthomyiidae	64	1.45	3	0.29
	Heleomyzidae	5	0.11		
	Ulidiidae	73	1.65	164	15.87
	Chloropidae	11	0.25		
	Sepsidae	3	0.07	11	1.06
	Total number of Diptera	3872	87.7	944	91.38
Coleoptera	Melyridae	9	0.2		
	Cleridae	20	0.45	8	0.77
	Dermestidae	86	1.95	10	0.97
	Tenebrionidae	7	0.16		
	Carabidae	4	0.09		
	Histeridae	39	0.88	9	0.87
	Coccinellidae	8	0.18		
	Nitidulidae	1	0.02	6	0.59
	Staphylinidae	2	0.04		
	Total number of Coleoptera	176	3.99	33	3.2
Hymenoptera	Formicidae	367	8.31	56	5.42
Total number of insects	4415	100	1033	100	

**Table 4**  
Total richness (total number of scavenger species) of recorded insects at different alcohol concentrations in the two seasons.

Season	Insects	Species richness			
		C	A1	A2	A3
Winter	Flies	12	11	8	11
	Beetles	8	7	5	7
	Ants	6	6	5	5
	Total	26	24	18	23
Summer		30			
	Flies	8	5	7	6
	Beetles	3	2	3	2
	Ants	2	1	4	3
	Total	13	8	14	11
		21			

and *D. frischii* were the only beetles appeared on the decay stage of all carcasses.

Six species of ants were recorded on all types of carcass with only slight differences in numbers, except of *Messor wasmanni* which was appeared only on the A1 and control carcasses (Table 5). *Cataglyphis ibericus* was the least common species of ant appeared on all carcasses. A few numbers of ants appeared on the fresh stage such as *Camponotus fellah*, *Monomorium* sp. and *Me. wasmanni*. During the bloat stage, no ant species were recorded, while during the decay stage, four ant species were appeared with a small number on different rabbit types. Like beetles, most of ants arrived at the dry stage.

In the summer study, ten fly species were recorded in total. Nine of these were found on the treated carcasses and eight on untreated carcasses (Table 6). In terms of differences in species distributions between the different carcass types, five fly species were recorded on the A1 carcasses, with *Ch. megacephala* only appearing on this group of carcasses. Seven species of flies were recorded on the A2 carcasses, with *Physiphora alcaea* appearing only on this group. Six fly species were recorded on the A3 carcasses and eight on the control carcasses, with *Sarcophaga dux* appearing only on the latter. *Coenosia attenuata* appeared on A3 and control carcasses but not on the A1 or A2 carcasses, while *Delia platura* appeared on A2 and control carcasses but not on A1 or A3 carcasses. *Chrysomya albiceps* was the only fly that appeared during the decomposition stages on all rabbit types followed by *Physiphora clausa* which appeared also during the

decomposition stages on all rabbit types except of the dry stage in the A1 and control rabbits. During the fresh stage, *Ch. albiceps* was the first flies to arrive, followed by *Phy. clausa* in all rabbits except of the control rabbit, where the Sarcophagid fly *Wohlfahrtia nuba* was accompanied. *Musca domestica* and other fly species arrived at the bloat stages on all rabbits, and the most fly abundance was during the decay stage. The numbers of fly individuals and taxa decreased during the dry stage.

The abundance of the beetle was very low in the summer study (Table 6). Only six species of beetles with a small number reported. Furthermore, none of these species were recorded on all carcass types. Around 2 to 3 beetle species appeared on different carcass types, where control carcasses attracted three beetle species, *Sa. chalcites*, *D. frischii* and *N. rufipes*. *Dermestes frischii* and *N. rufipes* were found only on the A1 carcasses, *Sa. splendens*, *D. maculatus* and *N. rufipes* on the A2 carcasses, and *D. frischii* and *Urophorus humeralis* on the A3 carcasses. Beetles arrived at the dry stage in all carcass types, except *Sa. chalcites*, which appeared at the fresh stage on the control carcasses and *N. rufipes*, which arrived at the bloat stage of the A2 carcasses.

Five species of ants were recorded on all types of carcass with only slight differences in numbers. *Cataglyphis holgerseni* was the only species recorded on the A1. *Camponotus fellah* and *Anochetus grandidieri* were reported only on the A2 and A3, respectively (Table 6). Some ants appeared during the fresh and decay stages, but the abundance of ants was concentrated with a few numbers during the dry stage.

**Table 5**  
Species of insects attracted to alcoholic beverages-treated and untreated rabbit carcasses during the winter, with reference to the decomposition stages of the rabbit carcasses and the degree of insect attraction.

Insects	Family	Species	Conc./ decomposition stage/ Insect no.															
			A1				A2				A3				C			
			F	B	E	D	F	B	E	D	F	B	E	D	F	B	E	D
Flies	Muscidae	<i>Musca domestica</i>	++	++++	++	+	++	++++	+	+	+++	++++	++	++	++++	++		
		<i>Coenosia attenuate</i>		+++	+			+++	+		++	+++	+		+++	+		
		<i>Atherigona orientalis</i>		+								+			++	+		
	Calliphoridae	<i>Chrysomya albiceps</i>	+	++	++++	++	++	++	++++		+++	+++	++++		+	++	+++	++
		<i>Chrysomya megacephala</i>		+					++			+	++			+	++	
		<i>Paralucilia paraensis</i>										++					++	+
	Sarcophagidae	<i>Sarcophaga dux</i>			+				++		+	+	++			+	++	
		<i>Wohlfahrtia muba</i>			+				+			+					+	
	Ulidiidae	<i>Physiphora clausa</i>		+	+	+	+	+	++			+	++				++	
	Anthomyiidae	<i>Delia platura</i>	+		++		+	+	++				+			+	++	
	Heleomyzidae	<i>Trixoscelis fumipennis</i>			+												+	
	Chloropidae	<i>Chloropidae</i> sp.	+		+													+
	Sepsidae	<i>Sepsis dissimilis</i>																+
Stage total			++	+++	++++	++	++	+++	++++	+	+++	+++	++++	++	+	++	++++	++
Beetles	Tenebrionidae	<i>Pimelia boyeri</i>															+	
		<i>Microdera kraatzii alashanica</i>				+						+						
	Staphylinidae	<i>Philonthus longicornis</i>																+
	Histeridae	<i>Saprinus chalcites</i>				+						+	+	+			+	++
		<i>Saprinus splendens</i>															+	
	Dermestidae	<i>Dermestes frischii</i>				+			+	++			+	++				++
		<i>Dermestes maculatus</i>				+								+				+
	Melyridae	<i>Aplocnemus virens</i>				+								+				+
	Carabidae	<i>Harpalus reversus</i>				+												
	Cleridae	<i>Necrobia rufipes</i>				+							+					+
Coccinellidae	<i>Nephus reunion</i>																+	
Stage total						++		+	++	+	+	+++		+	++	+	++	
Ants	Formicidae	<i>Camponotus fellah</i>				++				++	+		+	++		+	++	
		<i>Cataglyphis holgerseni</i>			+	++				++			+	++				++
		<i>Monomorium</i> sp.			+	++	+			++			+	++			+	++
		<i>Messor wasmanni</i>				+										+		+
		<i>Camponotus sericeus</i>			+	++				+				+				+
		<i>Cataglyphis ibericus</i>				+				+				+				+
Stage total					++	+++	+		+	+++	+		+	+++	+	+	+++	

Fresh, B: Bloat, E: Decay, D: Dry; +: 1–10, ++: 11–50, +++: 51–100, ++++: 101–200, +++++: 201–500, ++++++: ≥501; Boxes that are highlighted in gray indicate taxa trapped on decomposition stage.

**4. Discussion**

Decomposition is the consequence of identifiable physical, biological and chemical changes, which process through a series of stages over time (Henssge et al., 1995). The results showed four stages of decomposition in all the carcasses, but there were two variations in the decay process between the rabbits fed with an alcoholic beverage antemortem and those that were not. Namely, the treated rabbits took two days longer than the untreated ones to reach the dry stage of decay in winter, and one day longer in summer. These findings agreed with those of Monthei (2009), who reported differences in the rate of carcass decomposition between ethanol-treated and untreated pigs. In addition, results indicated that, decomposition was faster in the summer than in the winter, which may be attributed to the high temperatures in the summer. This was discussed with Goddard and Lago (1985) that temperature is the most important factor, when temperatures are high, the process of decomposition becomes faster. Turner (1991) also stated that seasonal variation could be one of the factors affecting the rate of carrion decomposition. In addition, Li et al. (2016) found that pig carcasses were skeletonized in a short period of time in summer relative to winter.

Many insects use the corpse as a food or oviposition site, while others are attracted by a large aggregation of other insects that they use as a food resource (Byrd and Castner, 2010). Insect succession was identified by Schoenly and Reid (1987) as directional and predictable changes in species composition occurs in carrion insect communities during decomposition of carrion. The current study, suggested that, insect visitation and colonisation occurred at the

same rate for alcohol treated and untreated rabbits. Antemortem ingestion of alcoholic beverages by rabbits does not appear to alter insect succession patterns on decomposing carcasses in the winter since the same species were recorded on both treated and untreated rabbits. Relatively few studies have been done to explore the effects of antemortem ingestion of ethanol on insect succession (Tabor et al., 2005; Monthei, 2009).

Avila and Goff (1998) said that ecological succession and distribution of insects is affected by the physical state of the remains. In addition, Anderson (2000) reported that many factors can affect insect, such as temperature, season, time of day, and accessibility and physical position of a carcasses. We found that the number of insects differ due to season and stage of decomposition. The number of insects was higher in winter than in the summer, and the decay stage had the highest number of insects. The low number of occurrences of insect species in summer was not sufficient to create statistical differences in occurrence matrices, as indicated by the results. The exception to this is *Ch. albiceps*, which was represented on all the rabbit carcasses (treated and untreated). The small number of insects in summer compared with winter may be due to the seasonal difference, where the temperature in Riyadh is very high in summer, as recorded during the study. Reed (1958) stated that the insect taxonomic groups have been shown to vary seasonally, as well as in different field types. In fact, Gruner et al. (2007) observed that the relative abundance of the collected species varied significantly by decay day, by season and day, season and species, and day and species. Furthermore, in an experiment in Baghdad city, Abdul-Rassoul et al. (2009) recorded a low number of forensic insects during summer compared to other seasons.

**Table 6**

Species of insects attracted to alcoholic beverages-treated and untreated rabbit carcasses in the summer, with reference to the types of the decomposition stages of the rabbit carcasses and the degree of insect attraction.

Insects	Family	Species	Conc./ Insect no.																
			A1				A2				A3				C				
			F	B	E	D	F	B	E	D	F	B	E	D	F	B	E	D	
Flies	Muscidae	<i>Musca domestica</i>	+	++	+		+	++	+		++	++	+	+	+++	+			
		<i>Coenosia attenuate</i>									+	+++	++						
	Calliphoridae	<i>Chrysomya albiceps</i>	+	+	+++	+	+	+	+++	+	+	++	+++	+	+	+	+++	+	
		<i>Chrysomya megacephala</i>		+	++	+													
	Sarcophagidae	<i>Sarcophaga dux</i>																+	
		<i>Wohlfahrtia nuba</i>		+	++				+				+		+			+	
	Ulidiidae	<i>Physiphora clausa</i>	+	+	++		+	+	++	+	+	+	++	+	+	+		++	
		<i>Physiphora alceae</i>							+	+									
	Anthomyiidae	<i>Delia platura</i>							+									+	
	Sepsidae	<i>Sepsis dissimilis</i>							+				+		+	+	+	+	
Stage total			+	++	++++	++	+	++	++++	++	++	++++	++	+	++	++++	++		
Beetles	Histeridae	<i>Saprinus chalcites</i>													+	+	+		
		<i>Saprinus splendens</i>								+									
	Dermestidae	<i>Dermestes frischii</i>				+									+			+	
		<i>Dermestes maculatus</i>									+								
	Cleridae	<i>Necrobia rufipes</i>				+			+	+								+	
	Nitidulidae	<i>Urophorus humeralis</i>													+				
Stage total						+		+	+				+	+		+	+		
Ants	Formicidae	<i>Camponotus fellah</i>																+	
		<i>Cataglyphis holgerseni</i>				+	+		+	+								+	+
		<i>Monomorium</i> sp.									+		+	+					
		<i>Cataglyphis ibericus</i>									+			+					+
		<i>Anochetus grandidieri</i>													+				
Stage total						+	+		+	++			+	++			+	+	

Fresh, B: Bloat, E: Decay, D: Dry; +: 1–10, ++: 11–50, +++: 51–100, ++++: 101–200, +++++: 201–500, ++++++: ≥501; Boxes that are highlighted in gray indicate taxa trapped on decomposition stage.

During decomposition, each of the stages is attractive to a particular group of insect arthropods. As indicated in the results, the most diverse of insects was found in the decay stage, and Prado e Castro et al. (2013) described the same results. Also, Archer and Elgar (2003) found that most flies were collected during the decay stage. Calliphorid flies arrived first, during the fresh stage of decomposition of most carcass types as previously suggested by Goff (1993). Insect activity, particularly flies, increased through the bloat stage due to odors decomposition (Paczkowski et al., 2012; Brodie et al., 2014). We also observed the largest numbers of house flies and blow flies, along with previous work of Goff (1993). Beetles and ants began to appear during the decay stage and reach the peak level in the dry stage, where the beetles prey on the available fly larvae (Goff, 1993) and ants feeds on the decomposing tissues and insect larvae (Mashaly et al., 2018). The insect activity was decreased during the dry stage as indicated by Cruise et al. (2018).

The species collected in this study were identical to those collected in other studies conducted in Riyadh (Mashaly et al., 2018, 2020). Three types of insects were recorded during the studies in both seasons, flies were the dominant insects, representing 87.7% of the total in winter and 91.38% in summer. The current study reported Muscidae as the dominant family, followed by Calliphoridae in winter, whereas the opposite was the case in summer. Blow flies lay eggs on the corpses where immatures feed directly on animal tissues during their growth (Goff, 1993). Furthermore, immature house flies could also be found colonizing corpses and

carcasses (Barbosa et al., 2009). Mashaly et al. (2017) noted house flies and blow flies as dominant forensic flies in Saudi Arabia.

Beetles were the least common insects collected during the study, 3.99% of the total in winter and 3.2% in summer. This may be due to the short period of decomposition, habitat or the number of insects attracted to the carcasses. Beetles are known to be attracted to carcasses both to feed on the carcass itself or on fly larvae (Catts and Goff, 1992). Perveen and Khan (2013), meanwhile, concluded that flies and ants arrived at *Canis domesticus* carcasses before beetles. The Dermestid was the most represented beetles in our study and its well-known worldwide distributed family (Byrd and Castner, 2010; Omer, 2014) and its species are considered primarily to be scavengers. These beetles feed on dry tissue, that is found on the carrion at this stage decomposition (Villet, 2011). Mashaly (2017) reported Dermestidae in three different habitats in Riyadh. In addition, Omer (2014) also reported six species belonging to four Coleoptera families, including Dermestidae, from different animals in Sudan.

Ants were the second dominant insects, representing 8.31% of the total in winter and 5.42% in summer and appearing on all types of carcasses in both seasons. Ants also tended to be abundant at the dry stage of decomposition, and to be dispersed with a small number on other stages. Arnaldos et al. (2005) said that ants use the corpse as a place to obtain moisture and food. Furthermore, Campobasso et al. (2009) reported that ants appear at all stages of cadaver decomposition, and Mashaly et al. (2018) reported that ants were attracted to carcasses irrespective of decomposition or

carcass status. In contrast, Park and Moon (2020) indicated that the species richness and species composition of ants were significantly different at the bloated and decay stages compared to the other stages of decomposition, regardless of season. Also, Chen et al. (2014) said that ant species composition differed according to the study site.

Overall, this study has detailed the effect of the antemortem ingestion of alcoholic beverages on insect succession patterns and the decomposition of rabbit carcasses in Riyadh, Saudi Arabia, during the winter and summer of 2018. Results suggest that while the consumption of alcoholic beverages did not have a significant impact on the succession patterns, there was a slight effect on the decomposition period. Muscidae was the dominant insect family in the winter, while Calliphoridae was the dominant in the summer. In winter, *M. domestica* was the dominant species, followed by *Ch. albiceps* and *C. attenuata*. Whereas *Ch. albiceps* was the dominant species in the summer, followed by *M. domestica*. Various developmental instars were recorded in flies and beetles, while ants were described as adults only. This study provides entomotoxicological information to enhance the database of forensic literature in respect to Riyadh, Saudi Arabia.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

This work was supported by Researchers Supporting Project number (RSP-2019-104) King Saud University, Riyadh, Saudi Arabia.

### References

Abdul-Rassoul, M.S., Augul, R.S., Al-Saffar, H.H., 2009. Seasonal abundance of adult fly species on the exposed carcasses in Baghdad city, Ibn Al-Haitham. *J. Appl. Sci.* 22 (4), 16–25.

Akbarzadeh, K., Wallman, J.F., Sulakova, H., et al., 2015. Species identification of Middle Eastern blowflies (Diptera: Calliphoridae) of forensic importance. *Parasitol. Res.* 114, 1463–1472.

Anderson, G.S., 2000. Minimum and maximum developmental rates of some forensically significant Calliphoridae (Diptera). *J. Forensic Sci.* 45 (4), 824–832.

Anderson, G.S., 2001. Forensic entomology in British Columbia: a brief history. *J. Entomol. Soc. B.C.* 98, 127–135.

Archer, M., Elgar, M., 2003. Effects of decomposition on carcass attendance in a guild of carrion-breeding flies. *Med. Vet. Entomol.* 17 (3), 263–271.

Arnaldos, M.I., García, M.D., Romera, E., Presa, J.J., Luna, A., 2005. Estimation of postmortem interval in real cases based on experimentally obtained entomological evidence. *Forensic Sci. Int.* 149, 57–65.

Avila, F.W., Goff, M.L., 1998. Arthropod succession patterns onto burnt carrion on two contrasting habitats in the Hawaiian Islands. *J. Forensic Sci.* 43, 581–586.

Barbosa, R.R., Mello-Patiu, C.A., Mello, R.P., Queiroz, M.M., 2009. New records of calyptrate dipterans (Fanniidae, Muscidae and Sarcophagidae) associated with the decomposition of domestic pigs in Brazil. *Mem. Inst. Oswaldo Cruz.* 104, 923–926.

Baselt, R.C., Cravey, R.H., 1980. Forensic toxicology. In: *Toxicology, the Basic Science of Poisons*. second ed. MacMillan, New York, p. 663.

Beyer, J.C., Enos, W.F., Stajic, M., 1980. Drug identification through analyses of maggots. *J. Forensic Sci.* 25, 411–412.

Bolton, B., 1994. *Identification Guide to the Ant Genera of the World*. Harvard University Press, Cambridge.

Borror, D.J., Triplehorn, C.A., Johnson, N.F., 1989. *An Introduction to the Study of Insects*. Saunders College Publishing, Philadelphia.

Brodie, B., Gries, R., Martins, A., VanLaerhoven, S., Gries, G., 2014. Bimodal cue complex signifies suitable oviposition sites to gravid females of the common green bottle fly. *Entomol. Exp. Appl.* 153 (2), 114–127.

Brown, K., Thorne, A., Harvey, M., 2012. Preservation of *Calliphora vicina* (Diptera: Calliphoridae) pupae for use in post-mortem interval estimation. *Forensic Sci. Int.* 223, 176–183.

Büttiker, W., Attiah, M.D., Pont, A.C., 1979. Insect of Saudi Arabia, Diptera: synanthropic flies. *Fauna Saudi Arabia* 1, 352–367.

Byrd, G.H., Castner, G.L., 2010. *Forensic Entomology: The utility of arthropods in legal investigations*. CRC Press, Boca Raton, Florida.

Campobasso, C.P., Merchetti, D., Introna, F., 2009. Postmortem artifacts made by ants and the effect of ant activity on decomposition rates. *Am. J. Forensic Med. Pathol.* 30, 84–87.

Caplan, Y.H., Ottinger, W.E., Park, J., et al., 1985. Drug and chemical related deaths: incidences in the state of Maryland, 1975–1980. *J. Forensic Sci.* 30, 1012–1021.

Catts, E.P., Goff, M.L., 1992. Forensic entomology in criminal investigations. *Ann. Rev. Entomol.* 37, 253–272.

Catts, E.P., Haskell, N.H., 1990. *Entomology and Death*. Joyce's Print Shop, Clemson, SC.

Chen, C.D., Nazni, W.A., Lee, H.L., et al., 2014. A preliminary report on ants (Hymenoptera: Formicidae) recovered from forensic entomological studies conducted in different ecological habitats in Malaysia. *Trop. Biomed.* 31, 381–386.

Chick, A.I.R., Cassella, J.P., Terrell-Nield, C., 2008. A Study of the effects of common insecticides on the colonisation and decomposition of carrion by invertebrates. *Global Forensic Sci. Today* 6, 18–26.

Collingwood, C.A., 1985. Hymenoptera: Fam. Formicidae of Saudi Arabia. *Fauna of Saudi Arabia* 7, 230–301.

Collingwood, C.A., Agosti, D., 1996. Formicidae (Insecta: Hymenoptera) of Saudi Arabia (part 2). *Fauna of Saudi Arabia* 15, 300–385.

Cruise, A., Watson, D.W., Schal, C., 2018. Ecological succession of adult necrophilous insects on neonate *Sus scrofa domestica* in central North Carolina. *PLoS ONE* 13 (4), e0195785.

Goddard, J., Lago, P.K., 1985. Notes on blow fly (Diptera: Calliphoridae) succession on carrion in Northern Mississippi. *J. Entomol. Sci.* 20, 312–317.

Goff, M.L., 1993. Estimation of postmortem interval using arthropod development and successional patterns. *Forensic Sci. Rev.* 5 (2), 81–94.

Goff, M.L., 2009. Early post-mortem changes and stages of decomposition in exposed cadavers. *Exp. Appl. Acarol.* 49, 21–36.

Goff, M.L., Lord, W.D., 2001. Entomotoxicology. In: Byrd, J.H., Castner, J.L. (Eds.), *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. CRC Press, Boca Raton.

Gruner, S.V., Slone, D.H., Capinera, J.L., 2007. Forensically important Calliphoridae (Diptera) associated with pig carrion in rural north-central Florida. *J. Med. Entomol.* 44, 509–515.

Henssge, C., Madea, B., Knight, B., Nokes, L., Krompecher, T., 1995. *The Estimation of the Time Since Death in the Early Postmortem Period*. Arnold, London.

Introna, F., Campobasso, C.P., Goff, M.L., 2001. Entomotoxicology. *Forensic Sci. Int.* 120, 42–47.

Keh, B., 1985. Scope and applications of forensic entomology. *Ann. Rev. Entomol.* 30, 137–154.

Li, L., Wang, J., Wang, Y., 2016. A comparative study of the decomposition of pig carcasses in a methyl methacrylate box and open air conditions. *J. Forensic Leg. Med.* 42, 92–95.

Mashaly, A., 2017. Carrion beetles succession in three different habitats in Riyadh, Saudi. *J. Biol. Sci.* 24, 430–435.

Mashaly, A., Alajimi, R., Mustafa, A., Rady, A., Alkhedi, H., 2017. Species abundance and identification of forensically important flies of Saudi Arabia by DNA barcoding. *J. Med. Entomol.* 54 (4), 837–843.

Mashaly, A., Mahmoud, A., Ebaid, H., 2020. Relative insect frequency and species richness on sun-exposed and shaded rabbit carrions. *J. Med. Entomol.* 57 (4), 1006–1011.

Mashaly, A., Sharaf, M., Al-Mekhlafi, F.A., Al-Subaie, M., Aldawood, A.S., Anderson, G., 2018. Ants (Hymenoptera: Formicidae) attracted to rabbit carcasses at three different habitats. *Sociobiol.* 65 (3), 433–440.

Monthei, D.R., 2009. *Entomotoxicological and Thermal Factors Affecting the Development of Forensically Important Flies*. Doctor of Philosophy. Blacksburg, Virginia Tech, USA.

Omer, S.S.A., 2014. *The succession of forensic beetles on exposed and wrapped carcasses during winter and summer in Khartoum State*. Master thesis.

Paczkowski, S., Maibaum, F., Paczkowska, M., Schütz, S., 2012. Decaying mouse volatiles perceived by *Calliphora vicina* Rob.-Desv. *J. Forensic Sci.* 57 (6), 1497–1506.

Park, S.-H., Moon, T.-Y., 2020. Positive and negative impact to decomposition of pig cadavers by ants (hymenoptera: Formicidae) at the Islet Yeongdo in Busan, South Korea. *Entomol. Res.* <https://doi.org/10.1111/1748-5967.12419>.

Payne, J.A., 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecol.* 46, 592–602.

Perveen, F., Khan, A., 2013. Ecological succession pattern of the insect species associated with the carcass of the free-ranging urban dog, *canis domesticus* (L): a tool for forensic entomology. *Mintage J. Pharm. Med. Sci.*, 31–36.

Pont, A.C., 1991. A review of Fanniidae and Muscidae of Arabian Peninsula. *Fauna Saudi Arabia* 12, 312–365.

Prado e Castro, C., García, M.D., Martins da Silva, P., Faria e Silva, I., Serrano, A., 2013. Coleoptera of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal. *Forensic Sci. Int.* 232, 73–83.

Ramchandani, V.A., Bosron, W.F., Li, T.K., 2001. Research advances in ethanol metabolism. *Pathologie et Biologie* 49, 676–682.

Reed, H.B., 1958. A study of dog carcass communities in Tennessee, with special reference to the insects. *Am. Midland Nat.* 59, 213–245.

Schoenly, K., Reid, W., 1987. Dynamics of heterotrophic succession in carrion arthropod assemblages: discrete series or a continuum of change?. *Oecologia* 73, 192–202.

- Tabor, K.L., Brewster, C.C., Fell, R.D., 2004. Analysis of the successional patterns of insects on carrion in southwest Virginia. *J. Med. Entomol.* 41, 785–795.
- Tabor, K.L., Fell, R.D., Brewster, C.C., Pelzer, K., Behonick, G.S., 2005. Effects of antemortem ingestion of ethanol on insect successional patterns and development of *Phormia regina* (Diptera: Calliphoridae). *J. Med. Entomol.* 42, 487–489.
- Turner, B.D., 1991. Forensic entomology. *Forensic Science Progress* 5, Division of Biosphere, Kensington, London, pp. 131–150.
- Villet, M.H., 2011. African carrion ecosystems and their insect communities in relation to forensic entomology. *Pest Technol.* 5 (1), 1–15.
- King, S.J., Vanin, S., 2016. Effects of Ethanol on the Development of *Megaselia scalaris* (Diptera: Phoridae). 13th Meeting of the European Association of Forensic Entomology. Budapest, Hungary.