



RESEARCH ARTICLE - ANTS

Ants (Hymenoptera: Formicidae) Attracted to Rabbit Carcasses in Three Different Habitats

ASHRAF MASHALY^{1,3}, MOSTAFA SHARAF², MEDGHOM AL-SUBEAI¹, FAHD AL-MEKHLAFI, ABDULRAHMAN ALDAWOOD², GAIL ANDERSON⁴

1 - Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

2 - Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

3 - Department of Zoology, Faculty of Science, Minia University, El-Minia, Egypt

4 - School of Criminology, Simon Fraser University, University Drive, Burnaby, BC, Canada

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Corresponding author

Ashraf Mashaly

Department of Zoology, College of Science

P. O. Box 2455, King Saud University

Riyadh 11451, Saudi Arabia.

E-Mail: mmashely@ksu.edu.sa

Abstract

This study reports the ant species that were attracted to rabbit carcasses in three different habitats (agricultural, desert, urban) in the city of Riyadh, Saudi Arabia from May to July 2014. Rabbit carcasses were used as a model for decomposition. Carcasses were categorized as exposed, clothed, shaded and burnt. A total of 726 ants belonging to three subfamilies and 14 species were collected during the decomposition process. *Trichomyrmex mayri* (Forel) was the only ant species attracted to the carcasses placed in the desert site. At the agricultural site, there was one ponerine species, five formicine species, and three myrmicine species were attracted, while at the urban site, five formicine species and one myrmicine species were recorded. The agricultural site attracted the highest number of ants. In contrast, the desert site attracted the lowest number of ants. *Tr. mayri* was the most prevalent species occurring in both the agricultural and desert sites. The bloated stage of decomposition attracted the highest number of ants followed by the decay, fresh and dry stages, respectively. Clothed carcasses attracted the highest number of ants followed by the exposed and burnt carcasses, respectively. The shaded carcasses attracted the fewest number of ants. This study found that ants are attracted to carcasses at all stages of decomposition and are common components of the necrofauna of central Saudi Arabia.

Introduction

The combined weight of all living ants has been estimated to constitute half the mass of all extant insects (Sleigh, 2003) and individual ants outnumber most other terrestrial animals (Taylor, 2007). Ant species constitute the largest family in the order Hymenoptera, which, in itself, is the third most numerous order of insects after Diptera (true flies) and Coleoptera (beetles) (Taylor, 2007). Ants are one of the most successful groups of insects, exploiting a variety of habitats around the world (Sleigh, 2003) and are absent from only a few areas, even able to exist in extreme arid and hot desert environments. For example, species of the genus *Cataglyphis* have developed very long legs so as to lift their

bodies up off the substrate, together with hairs which reflect light, allowing them to cope with air temperatures as high as 55 °C (Keller & Gordon, 2009; Shi et al., 2015).

Ants can have an impact on overall carrion ecology. Catts and Haskell (1990) reported that ants may feed directly on carcasses or prey on other arthropods associated with carcasses. Ants can also accelerate the decomposition process by producing abrasions or injuries facilitating the attraction of other species of necrophagous insects (Gunn, 2006). Sometimes, these ants cause lesions that can be misinterpreted as premortem mutilations, which can lead to errors in forensic analyses (Moretti & Ribeiro, 2006; Campobasso et al., 2009; Lindgren et al., 2011; Moretti et al., 2011). For example, the Red Imported Fire Ant, *Solenopsis invicta* (Buren) can



cause damage to tissue that could be mistaken for pre-mortem burns (Byrd & Castner, 2001). In some instances, initial colonization of dipterans or other organisms on carcasses may be delayed by two to three days due to the high predation rate of fly eggs by ants (Byrd & Castner, 2001); these instances appear more common if the carcass is located close to an ant nest. For example, acrobat ants of the genus *Crematogaster* feed on immature stages of many flies.

The literature shows that there are a number of factors that may affect insect colonization of carcasses, including habitat, the presence or absence of clothing, sun exposure and whether the carcass has been burned. Clothing may affect insect diversity, abundance, and feeding activities and consequently may increase the decomposition rate, by offering protection from ambient conditions and predators (Mann et al., 1990; Dillon, 1997; Campobasso et al., 2001; Card et al., 2015). It has been reported that carcasses exposed to the sun decompose faster than ones in the shade, as well as exhibiting different patterns of insect succession (Joy et al., 2006; Sharanowski et al., 2008; Castro et al., 2011). Heo et al. (2009) studied ant succession in Malaysia and noted differences in species attracted to burned and unburned pig carcasses.

In Saudi Arabia only one published study recorded ants attracted to the carcasses, where Shaalan et al. (2017) recorded *Pheidole megacephala* (F.) in Al-Ahsaa Oasis, in the Eastern Region of the Kingdom. Hence, the importance of the present article is that it is the first to provide a qualitative assessment of the major ants taxa attracted to decomposing carcasses placed in an agricultural area, a typical desert site, and an urban site, in the city of Riyadh using four carcass treatments, namely, exposed, clothed, shaded and burnt.

Material and Methods

Study sites

The study was conducted in three different habitats: an urban area (24°43'40.89" N, 46°36'54.92" E) located within 200 m of human dwellings; an agricultural area (24°44'36.54" N, 46°33'45.12" E), with many palm trees (*Phoenix canariensis* Chabaud) and typical grasses (*Setaria verticillata* L.), where the nearest human dwelling was approximately 1 km from the study site, and a desert site (24°44'36.54" N, 46°33'45.12" E) with a substrate made up primarily of hard packed dry rock, where the nearest human dwelling was approximate 4.5 km away. The experiment was repeated three times during May to July 2014. Temperatures (Fig 1) were recorded using a Lascar EL-USB-2 data logger. Each data logger was attached to the top of a 1 m vertical post at the centre of each of the experimental sites.

Carcasses

Most carrion research utilizes pig (*Sus scrofa* L.) carcasses, but pigs cannot be used in KSA. Therefore, thirty-three (n = 33) live mature rabbits, *Oryctolagus cuniculus* (L.),

were obtained. Each animal was euthanized using chloroform inhaled in a chamber. The treatment of the rabbits followed the regulations of the Animal Research and Ethics Committee (FU-CARE). Several studies in KSA and other countries have used rabbit carcasses to study the process of decomposition and insect succession; for example, Tantawi et al. (1996); Abou Zied (2014); Silahuddin et al. (2015) and Shaalan et al. (2016). Each rabbit weighed 1.57 ± 0.44 kg. At each of the agricultural and urban sites, twelve rabbits were divided into three groups of four. At the desert site, nine rabbits were divided into three groups of three, since the experiment in shaded conditions was excluded from the desert habitat. At each study site, rabbit carcasses were arranged in a regular, symmetrical pattern of three parallel rows, with four carcasses in each row, with a 2 m space between rabbits. Three rabbit carcasses (one from each row) were partially burned at the site using wood without accelerant to a level 2 on the Crow-Glassman Scale (Glassman & Crow, 1996). Three carcasses were clothed in a white cotton short-sleeved clean T-shirt to simulate a clothed body. Three rabbit carcasses were placed in a shaded area under a tree which was 2 °C cooler than in direct sun and three rabbits were exposed, with no additional treatment. Each carcass was placed inside a steel cage (55cm x 40cm x 24cm) to exclude scavengers. The cages did not have a bottom, allowing the carcasses to be in direct contact with the ground. All carcasses were monitored from initial exposure to almost complete skeletonization. Four stages of decomposition were recognized following Payne (1965), Gennard (2007) and AbouZied (2014), namely fresh, bloated, active decay, and dry remains.

Sampling and identification

Each carcass was examined for 10 minutes hourly from 9 AM to 4 PM, for the first three days, then daily, at 9 am from the fourth day after death, until the carcasses were almost completely skeletonized. Ants were collected with forceps (for the running ants around the carcasses) from all parts of the rabbit carcasses including underneath

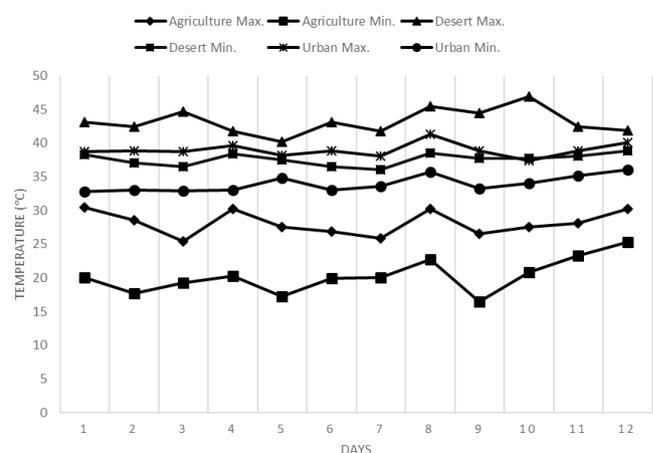


Fig 1. Summary of the temperature data at the study sites over the duration of the experiment (May to July 2014).

the carcasses. Pitfall traps were also used to reduce carcass disturbance and to continue to monitor beyond the active collection times. Collection was carried out without depleting the ants on the carcass so as to avoid any impact on the next collection. All ant specimens were preserved in 70% ethanol. The specimens were identified by the second author (M. R. Sharaf) using Collingwood (1985), Hölldobler and Wilson (1990), Bolton (1994) and Collingwood and Agosti (1996). Voucher specimens of each species are deposited at the King Saud University Museum of Arthropods, King Saud University, Riyadh, Saudi Arabia.

Statistical analysis

Significant differences in diversity (as quantified by the number of ant species) among habitats, decomposition stages and carcass status was evaluated using the Kruskal-Wallis test (Minitab 2017).

Results

In our study, the rabbit carcasses attracted different species of ants during the decomposition stages. The carcasses in the desert and urban sites progressed from the fresh to the dry remains stage in six days and in approximately 12 days at the agricultural site (Fig 2). Ants were observed feeding on immature insects on the rabbit carcasses.

Ants were attracted significantly to the carcasses at the agricultural site (454 ants) than the urban and desert sites, with 165 ants and 106 ants, respectively. The abundance of ants varied between treatments and sites. By far, the greatest number of ants were collected from the carcasses at the agricultural site and ants were present in all decompositional stages on the clothed and exposed carcasses and in the fresh, bloat and decay stages on the shaded and burnt carcasses, but were not present during the dry remains stage of these treatments. At the desert site, ants were much rarer in general. The urban site was the only one of the three in which ants were collected in all decay stages and on all treatments, although far fewer were collected than in the agricultural habitat.

Table 1 presents a summary of ant species collected at the three different sites from the rabbit carcasses. Only one species, *Trichomyrmex mayri* (Forel) (Myrmicinae) was collected from carcasses placed at the desert site. Nine species of ants belonging to three subfamilies were found on carcasses placed at the agricultural site, *Brachyponera sennaarensis* (Mayr), *Camponotus aegyptiacus* (Emery), *C. maculatus* (Fabricius), *C. sericeus* (Fabricius), *C. xerxes* (Forel), *Cataglyphis semitonsa* (Santschi), *Pheidole* sp., *Tr. mayri*, and *Tetramorium lanuginosum* (Mayr). At the urban site, six species were attracted, *C. aegyptiacus*, *C. sericeus*, *Ca. holgerseni* (Collingwood and Agosti), *Ca. livida* (André), *Ca. savignyi* (Dufour) and *Monomorium venustum* (Smith).

Habitat	Post-mortem interval (Days)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Agricultural														
Urban														
Desert														
<p style="text-align: center;">Key: Fresh Bloating Decay Dry</p> <div style="display: flex; justify-content: space-around; width: 100%;"> <div style="width: 20%; background-color: #cccccc; height: 10px;"></div> </div>														

Fig 2. The duration of the decomposition stages in the three different habitats.

Ant species were very habitat specific, with only *Tr. mayri* observed in two different sites (agricultural and desert). At the agricultural and urban sites, ants were attracted to all decomposition stages, but at the desert site, ants were active only during the bloated and decay stages. Also, at the agricultural and urban sites, ants were attracted to all types of carcasses although, at the urban site, *Ca. livida* was the only ant species attracted to all types of carcasses and during all stages of decomposition.

At the agricultural site, *Tr. mayri* was attracted in high numbers followed by *T. lanuginosum*, *C. sericeus* and *C. xerxes*. *Cataglyphis holgerseni*, *Ca. livida*, *Ca. savignyi* and *M. venustum* were not attracted to the carcasses at this site (Fig 3). At the desert site, *Tr. mayri* was the only ant species attracted in large numbers in comparison with other species at the other sites (Fig 3). At the urban site, *Ca. savignyi* was

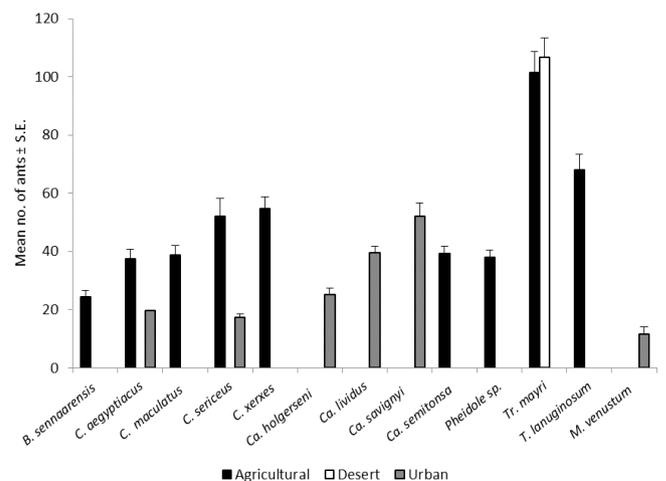


Fig 3. Abundance of ants according to the habitat in Riyadh, Saudi Arabia from May to July (2014).

Table 1. Ant attracted to the rabbit carcasses in three different habitats.

Subfamily	Species	Occurrence of ants / Agricultural habitat																			
		Fresh				Bloated				Decay				Dry							
		E	C	S	B	E	C	S	B	E	C	S	B	E	C	S	B				
Ponerinae	<i>Brachyponera sennaarensis</i>	√							√								√				
	<i>Camponotus aegyptiacus</i>	√			√								√				√				
	<i>Camponotus maculatus</i>			√					√								√				
Formicinae	<i>Camponotus sericeus</i>	√							√									√		√	
	<i>Camponotus xerxes</i>								√								√				
	<i>Cataglyphis semitonsa</i>	√											√							√	
Myrmicinae	<i>Pheidole sp.</i>	√	√		√	√	√										√				√
	<i>Trichomyrmex mayri</i>	√	√				√						√	√						√	√
	<i>Tetramorium lanuginosum</i>	√	√				√							√							
Urban habitat																					
Formicinae	<i>Cataglyphis livida</i>	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	<i>Cataglyphis holgerseni</i>	√	√	√	√	√	√	√	√												
	<i>Cataglyphis savignyi</i>						√	√					√	√				√	√		
	<i>Camponotus sericeus</i>			√					√				√	√						√	√
	<i>Camponotus aegyptiacus</i>				√	√	√		√	√				√	√			√	√		
Myrmicinae	<i>Monomorium venustum</i>						√						√							√	
Desert habitat																					
Myrmicinae	<i>Trichomyrmex mayri</i>												√							√	

E: Exposed, C: Clothed, S: Shaded, B: Burnt
 √: Presence of ant

attracted in significantly higher numbers than the other ants, followed by *Ca. livida*, *Ca. holgerseni* and *M. venustum*. *Camponotus aegyptiacus* was attracted significantly lower numbers than the other species (Fig 3). The species richness of ant species showed significant difference among the three habitats (Kruskal-Wallis test, table 2).

In the fresh stage, all ant taxa were represented except *C. xerxes*, *Ca. savignyi* and *M. venustum*. *Tetramorium lanuginosum* was the most abundant species attracted to the fresh stage. In the bloated stage, only *Ca. semitonsa* was not attracted. In the bloat stage, *Trichomyrmex mayri* was the most abundant species collected (Fig 4). In the active decay stage, all of the ant taxa reported here were present, with the exception of *Ca. holgerseni*. *Camponotus xerxes* and *C. aegyptiacus* were attracted in a higher numbers in this stage.

Table 2. Kruskal-Wallis test results.

Comparison	Kruskal-Wallis Test Statistic	d.f.	P-Value	Notes
No. of ant species by Habitat	9.57	2	0.008	*
No. of ant species by decomposition stage	6.74	3	0.081	NS
No. of ant species by carcass status	3.74	3	0.291	NS

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

In the dry stage, only six ant species were still present, *C. sericeus*, *Ca. livida*, *Ca. savignyi*, *Ca. semitonsa*, *Pheidole sp.* and *Tr. mayri*, the latter being the most abundant (Fig 4). The Kruskal-Wallis test indicated that there were no significant differences in the number of ant species between the different stages of decomposition (Table 2).

The exposed and clothed rabbit carcasses attracted the greatest number of ants (237 and 289, respectively). In contrast, the burnt and shaded rabbit carcasses attracted relatively few ants (98 and 101, respectively). All species

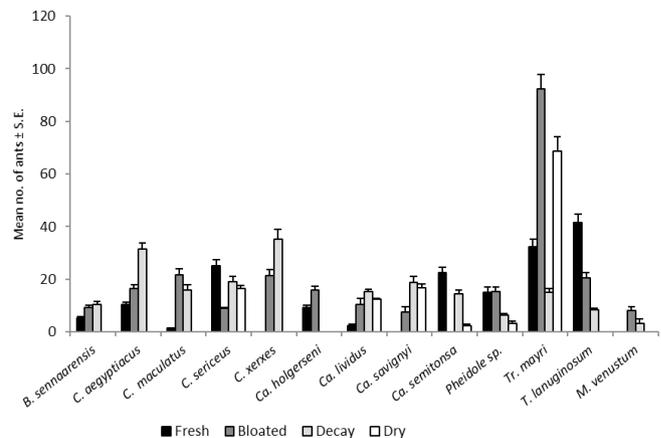


Fig 4. Abundance of ants according to the decomposition stage of rabbit carcasses placed at the three habitats in Riyadh, Saudi Arabia from May to July (2014).

of ants were attracted to the exposed carcasses (control and clothed carcasses), except *C. maculatus*, *C. xerxes*, *Ca. savignyi* and *M. venustum*. *Brachyponera sennaarensis*, *C. maculatus*, *C. sericeus* and *Ca. semitonsa* were not apparently attracted to the clothed carcasses (Fig 5). Only *C. maculatus*, *C. sericeus*, *Ca. livida*, *Ca. savignyi* and *M. venustum* were attracted to the shaded carcasses. The burnt carcasses attracted *C. aegyptiacus*, *C. sericeus*, *C. xerxes*, *Ca. livida*, *Ca. savignyi*, *Pheidole* sp. and *Tr. mayri*. *Brachyponera sennaarensis* and *Ca. semitonsa* were present only on exposed carcasses and *C. maculatus* was only attracted to shaded carcasses (Fig 5). Also, no significant difference in the number of ant species was indicated among the different carcass status using the Kruskal-Wallis test, table 2).

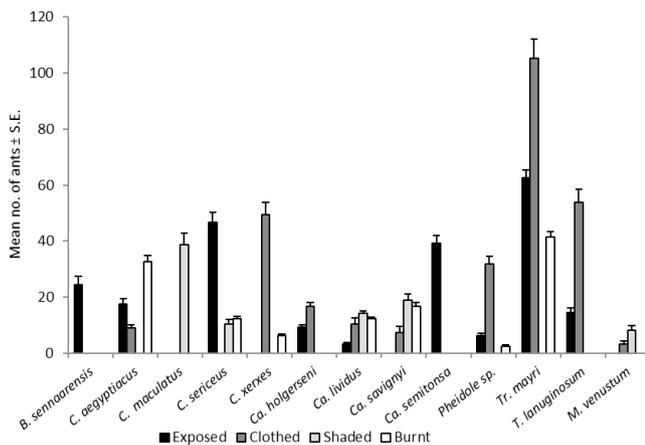


Fig 5. Abundance of ants according to the condition if the carcasses placed at the three habitats in Riyadh, Saudi Arabia from May to July (2014).

Discussion

In forensic entomological studies, ants attracted to corpses are categorized as omnivorous species (Tabor et al., 2005). Ants can feed on both the corpse and associated fauna. Luederwaldt (1926) reported collecting several species of ants from vertebrate carrion. In addition, Smith (1986) reported that, in the context of forensic entomological analyses, ants are considered to be predators. Throughout our study period, ants were observed actively preying upon the eggs, larvae and newly emerged flies of Calliphoridae, Muscidae and Sarcophagidae. Campobasso et al. (2009) and Chen et al. (2014) reported similar observations. Mashaly et al. (2013) stated that *B. sennaarensis* preferred proteinaceous food over other types of food. Carpenter ants of the genus *Camponotus* are generally considered to be predators (Sanders & Pang 1992), and others feed on live and dead arthropods (Dejean, 1988). Although Sharaf et al. (2016) recorded workers of *Tr. mayri* in areas rich in decaying organic matter. In our study, we recorded ants on the carcasses from shortly after placement during the early post-mortem period and in all the subsequent stages of decomposition, apart from the dry stage.

We did not observe ants feeding directly on the carcasses, but rather, feeding only on the immature stages of flies (*Lucilia sericata* Meigen, *Musca domestica* L. and *Sarcophaga hirtipes* Wiedemann) during the succession process, as has also been reported in the studies of Campobasso et al. (2004), Heo et al. (2009), Nazni et al. (2011) and Chen et al. (2014).

Smith (1986) stated that, habitat and ambient temperatures are important factors that determine the composition and structure of carrion fauna. In our study, high air temperatures affected the rates of decomposition at all study sites. Almost complete decomposition of the rabbit carcasses took six days in the desert and urban sites and about 12 days at the agricultural site. Similar results were reported by Mashaly (2016). Anderson (2009) indicated that the length of time it takes for decomposition to complete is very variable, depending on a number of parameters, including geographical region.

Abou Zied (2014) conducted a decomposition study in the southwestern mountains of KSA and found that the rate of decomposition of rabbit carcasses was affected by high temperature (about 30 °C). In Kuwait, Al-Mesbah et al. (2012) illustrated that carcasses at an urban site were found to decompose significantly faster than in the agricultural, coastal or desert sites (as measured by percentage weight loss).

Throughout the course of the study, a total of 726 ants were identified as being attracted to the rabbit carcasses. The agricultural site attracted a significantly higher number of ants compared to the desert and urban sites. Al-Mesbah et al. (2012) did not collect ants from rabbit carcasses at an urban site in Kuwait. We recorded only one species of ant from the carcasses placed at the desert site, but nine and six species of ants from the agricultural and urban sites, respectively. This may be due to the high temperature and relatively barren substrate of the desert site. *Trichomyrmex mayri* is the most prevalent species compared with other species in all habitats and this is consistent with Sharaf et al. (2016), who said that *T. mayri* is the most widely-distributed species in the Arabian peninsula. The differences in diversity and numbers of ants attracted to the carcasses in the three different habitats are probably due to the differences in biogeoclimatic zones or ecozones as each habitat was very different (Anderson, 2009). Conditions in the desert habitat are very extreme and only species highly adapted to the high temperatures and arid conditions are able to survive there. The ant species richness is sensitive to plant cover and diversity (Morrison, 1998), soil type (Peck et al., 1998).

In our study, no differences were found in the rate of decomposition between the four types of exposure (exposed, shaded, clothed, and burned) during the study time. Heo et al. (2008), Gruenthal et al. (2012). Mashaly (2016) reported that burning not to have an effect on the rate of decomposition but to have an effect on insect succession. Beckerdite et al. (2014) found that burning chicken carcasses accelerated the decomposition process and affected the succession of insects on the bodies. Clothes can increase the rate of decomposition

and insect activity (Mann et al., 1990; Campobasso et al., 2001), although Anderson (2009) said that clothes slowed the decomposition and the insect succession, whereas Kelly et al. (2009) found no difference in the succession of insects between unclothed and clothed carcasses. Castro et al. (2011) found a significant effect of the interaction between the decomposition stage and insolation regime. Carcasses exposed to the sun decompose faster than the ones in the shade (Shean et al., 1993; Joy et al., 2006) and show different patterns of insect succession (Sharanowski et al., 2008). Anderson (2009) reported that clothing cadavers affects relative humidity and provides protection for some insects, which may increase insect abundance and diversity. In contrast, Matuszewski et al. (2015) reported that clothing has little importance when considering insect abundance and diversity.

Also, ant species differ according to the condition of the carcass. Some ant species were restricted by the treatment types. For example, *B. sennaarensis* and *Ca. semitonsa* were only attracted to the exposed carcasses and *C. maculatus* only to the shaded carcasses. Heo et al. (2009) reported six species of ants associated with pig carcasses placed on the ground. *Oecophylla smaragdina* (Fabricius) was collected only from the burned carcass and was found throughout decomposition, *Tetramorium* sp. was only collected from the burned carcass but only in the dry remains stage, whereas *Odontoponera* sp., *Diacamma* sp. and *Anoplolepis gracilipes* F. Smith were specific to the unburned carcass; *A. gracilipes* was found only in the fresh and bloated stages and *Odontoponera* sp. and *Diacamma* sp. only in the fresh and dry remains stage. *Pheidologeton* sp. was the only species found on both burned and unburned carcasses, primarily in the dry remains stage (Heo et al., 2009). As in our study, Heo et al. (2009) reported that the ants predated on fly larvae. Kolver (2009) stated that ants preyed on the fly eggs, fly larvae and adult beetles, but also fed on the burnt skin and soft tissue of the burnt carcasses, causing feeding damage in the form of a characteristic pitted appearance formed by the removal of minute portions of tissue.

In conclusion, this is the first study to report the species of ants that are attracted to rabbit carcasses exposed in four different conditions (exposed, clothed, shaded and burnt) and in three different sites (agricultural, desert and urban) in Riyadh, KSA. The study recorded a total of 726 ants belonging to three subfamilies and 14 species. The agricultural site attracted the highest number of ants. *Trichomyrmex mayri* was the most abundant species in both the agricultural and desert sites. Decomposition stage or the carcass status did not effect on the number of ants attracted.

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