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Beetles (Insecta, Coleoptera) associated with rabbit carcasses in three habitats in Jeddah, Kingdom of Saudi Arabia

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ABSTRACT
A preliminary study on insects from Order Coleoptera associated with rabbit carcasses was conducted in Jeddah city, Kingdom of Saudi Arabia. Three different habitats were chosen to carry out this study; agriculture, desert and costal habitats. Five species belong to three families of Coleoptera were collected. These species were *Dermestes frischii, Dermestes macultus* from family Dermestidae, *Necrobia rufipes* belong to family Cleridae and *Saprinus splendens, Saprinus chalcites* represented family Hestridae. All these insects were collected from dray stage of carcass decomposition. The highest mean numbers of beetles significantly were collected from agriculture and desert habitats, whereas the lowest mean number was in coastal habitat. In general, *Dermestes frischii* represented the highest mean number of individuals significantly followed by *Dermestes macultus* and *Necrobia rufipes*, then each of *Saprinus splendens* and *Saprinus chalcites.* These findings may provide data for further use in legal investigations in Kingdom of Saudi Arabia.

INTRODUCTION

Carrion beetles perform vital ecosystem functions (Wolf & Gibbs, 2004) by support the breakdown and recycling of organic matter into terrestrial ecosystems (Kalinova *et al*., 2009). During legal investigation of crime scene, it is very important to determine time since death. Blowflies lay their eggs on the corpses directly after death (Smith, 1986), therefore, they used to determine time since death in the early post-mortem interval (PMI) (Goff, 1993; Byrd & Castner, 2009). Beetles are the most useful insects for the estimation PMI in the later stages of decomposition particularly when skeletonised corpses are found (Kulshrestha & Satpathy, 2001; Midgley *et al*., 2010). Due to their succession pattern, Coleoptera can also be used to support the PMI estimate from Diptera data (Goff & Flynn, 1991). Many published reports are focused on Diptera pattern colonization and few shed light on Coleoptera succession (Kocarek, 2003; Matuszewski *et al*., 2008). Several families of Coleoptera typically are associate139d with carrions; some of them feed directly on carcasses, whereas others are predators of maggots or beetles on carrions (Byrd & Castner, 2001). Dermestidae, Cleridae and Histeridae were among the important beetle families which associated with carcasses in different countries (Haskell *et al*., 1997; Wyss & Cherix, 2006). So far, carrion beetles are poorly known in the kingdom of Saudi Arabia. The objective of this research was to obtain data concerning Coleopteran entomofauna at different habitats in Jeddah city, kingdom of Saudi Arabia. This knowledge will be an invaluable tool for the forensic entomologist to use them in criminal investigations to determine the time of death and place of death.

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MATERIALS AND METHODS

Sites and study period:

This study was conducted during autumn 2015 to winter 2016 in Jeddah city (latitude 29.21 north & longitude 39.7 east) at the west of the Kingdom of Saudi Arabia. Three different habitats were chosen to carry out this study: (1) an agricultural habitat which was a farm located at 50 km northwest of Jeddah city, the plants in this area composed of palm trees and grasses, with dark and moist soil, the duration of study in this location lasted from 28 November to 12 December 2015, (2) desert area presented at one km away from Dahban Highway main road, the soil was sandy with pale yellow color, and the duration from 13 to 27 December 2015, (3) coastal habitat found at South Obhor district in a place at 300 m away from the Red Sea shore, There were no plants, no soil in this area, and the study period lasted from 28 December 2015 to11 January 2016.

Animals and experimental protocol:

Domestic rabbits (*Lepus cuniculus*) weightings between 1.4 and 2.5 kg were used as models and were killed by cutting trachea without decapitation. After death was confirmed and to protect from scavenger vertebrates, each rabbit carcass was placed inside a metal cage measured 65x55x45 cm^3 with 2 cm^2 mesh. In each site, 10 rabbit carcasses were placed in tow lines and distance between them was 10 m. Each line included 5 cages which located2 m from each other.

Insect collection and identification:

Daily, adult insects were captured off the carrion with hand picking forceps and directly transferred to a 'killing jar' containing 70% ethyl alcohol for preservation. All collected samples were examined using dissecting stereomicroscope from Leica Company (Leica M205C stereomicroscope). Beetles specimens were identified using different keys given by Bousquet (1990) Leavengood (2008) and Peacock (2013)

Environmental parameters:

Ambient temperature (maximum, minimum temperatures) and relative humidity were recorded every day using digital thermohygrometer. The rainfall rate was obtained from faculty of meteorology environment and arid land agriculture of King Abdulaziz University.

Statical analysis:

Factorial experiments analysis method was used in this study which achieved in randomized complete block design with three replicates for two factors. These factors were beetle species which represented by five levels and study habitats included three levels (agriculture, desert and coastal). The statistical analysis included using "F test", and its results summarized in "ANOVA (analysis of variance) table", then "Dancun's test" was used to compare means of significant factors, according to Snedicor (1958).

Results:

In the recent study, insects from order Coleoptera were found associated with rabbit carcasses in three different habitats (agriculture, desert and coastal) in Jeddah city. Five species of beetles belong to three families were collected. These species were; *Dermestes frischii* Kugelann,1792 and *Dermestes maculatus* DeGeer, 1774 from family Dermestidae, *Necrobia rufipes* De Geer,1775 represented family Cleridae, *Saprinus chalcites* Illiger, 1807 and *Saprinus splendens* Paykull,1811 from family Histeride. All these species were found only in dray stage and were absent from other decomposition stages. The climatic conditions during study period were represented table (1). Results of statistical analysis in ANOVA table (table 2) showed that there were highly significant differences in the treatments of the experiment. Each factors; beetle species, study habitats affected significantly on mean numbers of beetles, but interaction between them was not significant. When Dancun's test was used to compare treatments' means (by L.S.D Bayesian test) (table 3), and with respect to the tow studied factors beetle species and habitats we found that in agriculture habitat *Der. macultus* represented the highest mean number of beetles significantly (2.44), it was not differ significantly than each of *Der. frischii* (2.20), *Nec. rufipes* (2.23) and *Sap. chalcites* (1.99), which in turn was not different significantly than *Sap. splendens* (1.72). In desert habitat, *Der. frischii* (4.0) had the highest mean number significantly, followed by *Nec. rufipes* (2.23) and *Der. macultus* (1.72), but each of *Sap. splendens* and *Sap. chalcites* had the lowest mean number (1.0). In coastal habitat, also *Der. frischii* (2.23) was higher than the rest of species *Nec. rufipes* (1.38), *Der. macultus, Sap. splendens* and *Sap. chalcites* (1.0). By comparing mean numbers of beetle species in the three habitats it was clear that, *Der. frischii* in desert recognized by higher mean number significantly (4) than agriculture and coastal habitat (2.20 and 2.23, respectively), but *Nec. rufipes* in agriculture and desert habitat (2.23) was more significant than in coastal habitat (1.38). Whereas, both *Sap. splendens* and *Sap. chalcites* in agriculture habitat (1.72, 1.99) included higher number significantly than each of desert and coastal (1.0). *Der.*

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macultus had mean numbers differed significantly in three habitats, they were in descending order; agriculture (2.44), desert (1.72), coastal (1.0) (fig.1).

Overall, table (3) also illustrated that, *Der. frischii* represented the highest mean number of beetles significantly (2.81), then each of *Der. macultus* (1.72) and *Nec. rufipes* (1.95),but each *of Sap. splendens* and *Sap. chalcites* represented the lowest mean number of beetles significantly (1.24 and 1.33, respectively) (fig. 2).

In general, agriculture and desert habits both had the highest mean number of beetles significantly (2.12 and 1.99) with mean of maximum, minimum temperatures and relative humidity were 29.40^{0}C, 22.2^{0}C, 39.75% and 26.88^{0}C, 19.92^{0}C, and 49.40%, respectively. Whereas, coastal habitat included the lowest mean number significantly (1.32) with climatic condition 20.55^{0}C, 28.8^{0}C, 53.5% (fig.3).

Table 1: Climatic conditions in the different habitats during the period of study

Habitat	Date	Temperature (^{0}C)			R.H (%)
		Mini.	Maxi.	Mean	
Agriculture	2-4/12/2015	*22.2±2.01*	*29.40±1.19*	*25.625±0.71*	*39.75±19.05*
Desert	21 25/12/2015	*19.92±2.08*	*26.88±0.88*	*23.66±0.74*	*49.40±5.77*
Coastal	4-7/1/2016	*20.55±0.53*	*28.80±0.91*	*24.50±0.88*	*53.5±6.61*

Table 2: ANOVA for adult beetles which collected from rabbit carcasses during dray stage in different habitats

Source of Variance	Degree of Freedom	Sum of squares	Mean sum of squares	F-cal	F-table	Notes
Replicates	2	0.014377	0.007189	0.009136036	3.34, 5.45	NS
Treatment	14	28.16075	2.011482	2.556399031	2.04, 2.75	**
Beetle Species	4	14.2669	3.566725	4.532962026	2.71,4.07	**
Study Habitats	2	5.456092	2.728046	3.467082506	3.34, 5.45	**
Spices*Habitats	8	8.437758	1.05472	1.340446665	2.29,3.23	NS
Error	28	1.563759	0.055849	0.009136036	3.34, 5.45	
Total	44	29.73889				
S^2	0.0558485				0.01	0.05
$S^2_x$for 3	0.0037232	$S_x$for 3	0.0610183	Bayesian L.S.D. for 3	0.2752329	0.154441
$S^2_x$for 5	0.0062054	$S_x$for 5	0.0787743	Bayesian L.S.D. for 5	0.3553242	0.2528482
$S^2_x$for 15	0.0186162	$S_x$for 15	0.1364411	Bayesian L.S.D. for 15	0.681035	0.4147947

NS; Not significant. **; Highly Significant (0.01)

Table 3: Comparison of treatments' means (by L.S.D Bayesian test) with respect to the tow studied factors beetle species and habitats, and the interaction between them.

Habitats	Beetle species					
	Der. frischii	*Der. macultus*	*Nec. rufipes*	*Sap. splendens*	*Sap. chalcites*	Habitats' means
Agriculture	2.20±0.46 Bab	2.44±0.21 Aa	2.23±0.22 Aab	1.72±0.29 Ab	1.99±0.25 Aab	2.12±0.36 A
Desert	4.00±0.13 Aa	1.72±0.29 Bb	2.23±0.22 Ab	1.00±0.00 Bc	1.00±0.00 Bc	1.99±1.16 A
Coast	2.23±00.22 Ba	1.00±0.00 Cb	1.38±0.37 Bb	1.00±0.00 Bb	1.00±0.00 Bb	1.32±0.52 B
Species' means	2.81±0.93 a	1.72±0.65 b	1.95±0.49 b	1.24±0.39 c	1.33±0.51 c	1.81

Small letters for the horizontal comparisons. Capital letters for the vertical comparisons.
(L.S.D. Bayesian) for 3 Means =0.2752329 (L.S.D. Bayesian) for 5 Means=0.3553242
(L.S.D. Bayesian) for 15 Means=0.68103

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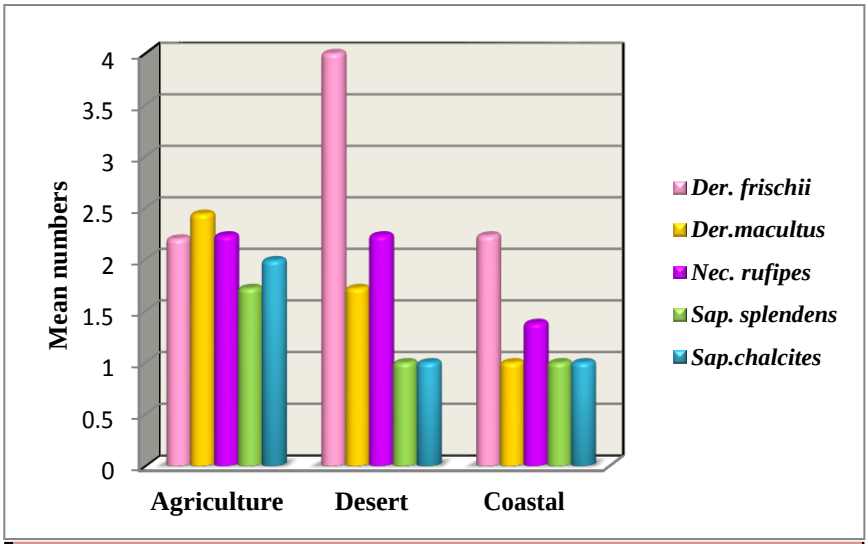

Fig. 1: Mean numbers of beetle species in different habitats

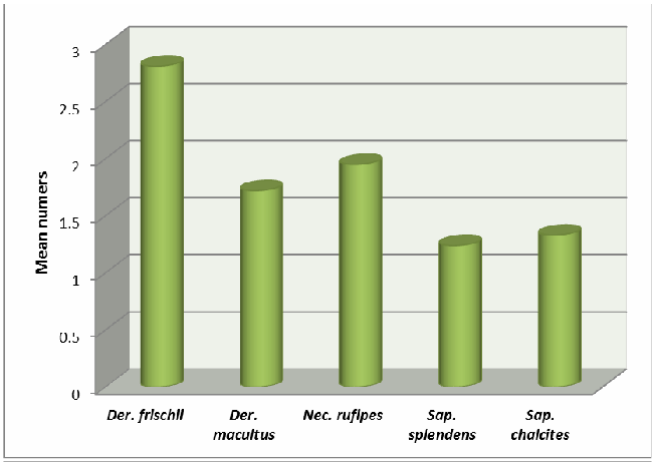

Fig. 2: Mean numbers of different species

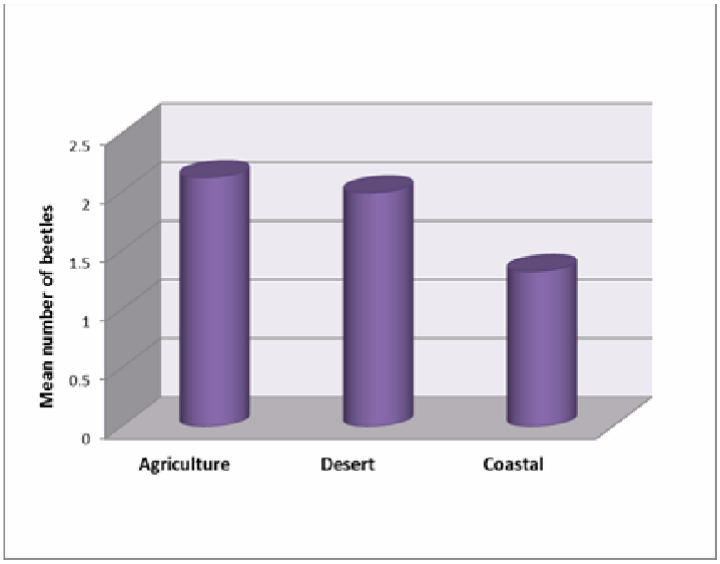

Fig. 3: Mean numbers of all beetle in different habitats

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Discussion:

In the recent study three families of Coleoptera were collected from rabbit carcasses; Dermestidae, Cleridae and Histeride. These families also recorded as most important beetles often found on carrions in different countries around the world; in Brazil, Carvalho *et al*. (2000) reported Histeridae in São Paulo, Mise *et al.*(2007) found Histeridae and Cleridae in State of Paraná. While, Dermestidae, Histeridae and Cleridae were reported in State of Minas Gerais in Brazil by Rosa *et al.*(2011) and in Turkey by Bana &, Beyarslan (2012).

We found two species of Dermestidae on the rabbit carcass, *Der. freschii* and *Der. maculate.* Dermestids are known as necrophagous insects feed on carcasses in later stages of decomposition and accelerate the skeletonisation process (Voigt, 1965; Schroeder, *et al*., 2002), even so, adults and larvae can be found associated with the remains years after death (Byrd & Castner, 2010). Several authors recorded the presence of larvae and adults of *Der. freschii* on animal decomposition matter*;* in Assiut, Egypt on human leftover parts by Galal *et al.* (2009), and in Turkey on pig carcass by Bana & Beyarsalan (2012). *Der. maculatus* was reported in different area of the world; in Thailand on animal cadavers (Vitta *et al*., 2007), in Australia on pig carcass (Voss *et al*., 2008), in Brazil (Rosa *et al*., 2011) and Neotropical region (Valdes-Perezgasga *et al*., 2010; Horenstein & Linhares, 2011; Aballay *et al*., 2012). *Nec. rufipes* in this study represented family Cleridae. Most of Clerids are Omnivores, feeding on carcass in dry stage of decomposition and act as predators of flies and other beetles (Payne & King, 1970; Arnaldos *et al*., 2004). Adults and larvae of *Nec. rufipes* were collected from carcasses and corpses in Brazil (Carvalho *et al*., 2004; Mise *et al*., 2007; Silva & Santos, 2012) and elsewhere in the Neotropical region (Valdes-Perezgasga *et al*., 2010 ; Horenstein & Linhares, 2011; Aballay *et al.* 2012). This species was known as predator of Dermestid (Simmons & Ellington, 1925), and live larvae of *Der. frischii* were recorded to be eaten by *Nec. rufipes* by Peacock (2013). Whereas, Histeride in the recent experiment included two species *Sap. splendens* and *Sap. chalcites.* Histeridae individuals are necrophiles preyed on necrophagous insects on the carcasses (Payne, 1965; Carvalho & Linhares, 2001). Arnaldos *et al.* (2005) stated that Cleride species recorded to be eaten by Hestrid species and they present together in the same area.

Many previous experiments collected most of our specious together from carcasses; Centeno *et al*. (2002) collected *Der. maculatus; Nec. rufipes* and Hestir species from pig carcasses in Argentina. In Ohio, John (2005) recorded *Der. maculatus, Nec. rufipes* and Hister beetles from genus *Saprinus* on pig carcasses. In northern Thailand Vitta *et al*. (2009) found *Der. maculatus, Nec. rufipes* and *Hister* sp. on pig carcasses. Martin-Vegai & Baz (2012) recorded *Der. freschii* in central Spain throughout a year using carrion-baited traps.

This study proved that, the species of family Dermestidae presented in higher mean number significantly more than species of other families in most habitats (*Der. macultus* in agriculture habitat, *Der. frischii* in desert and coastal habitat). Türkiye'de *et al.*(2014) found that Dermesid bettles *Der. frischii, Der. undulatus* and *Der. maculatus* were the most numerous than species of other families on carcasses.

All Coleopteran species in this study were found in dry stage of carcasses decomposition. Several authors recorded these insects in the same decomposition stage or in other stages. Koc˘árek (2003) found insects from genus *Dermestes* and genus *Necrobia* in dry stage of decomposition and genus *Saprinus* in bloated to decay stage, on rat carcasses in Czech Republic. Özdemir & Sert (2009) observed Dermestidae and Cleridae species in advanced decay and dry stages. Bana & Beyarslan (2012) found Histeridae species in active and advanced decay stages, but Cleridae and Dermestidae species in advanced decay and dry stages of decomposition. The *Der. frischii* adults were reported on human leftover parts in fresh, bloat and dry stages in Egypt by Galal *et al.*(2009), and in the advanced decay and dry stages of decomposition on pig carcasses by Bana & Beyarsalan (2012) in Turkey. Adults of *Der. maculates* were recorded in the advanced decay stage on a pig carrion in Thailand (Vitta *et al*., 2007), and in bloated and decay stages on animal cadavers in Australia (Voss *et al*., 2008). Türkiye'de *et al.* (2014) collected Cleridae, Dermestidae and Histeridae, among families of Coleoptera from active decay stage to the end phases of the dry stage.

In this study, highest mean numbers of individuals were found in agriculture and desert habitats then coastal habitat. It is known that each geographical region is characterized by its temperature, humidity, vegetation, soil type and environmental conditions (Dillon, 1997; Anderson, 2000). These parameters affect the attraction and colonization of insects to carrions. This seems especially clear at coastal habitat where soil and vegetation were absent and characterized with lowest mean numbers of beetles. In general, our results agree with John (2005) who proved that coast habitat had the lowest species richness, and the lowest diversity.

Deference in tow Dermestid species preference was appeared; while *Der. freschii* preferred desert habitat where the soil was sandy, no plants in the site and the maximum temperature was low 26.88^0C, *Der. macultus* preferred agriculture habitat where the vegetation composed of palm trees and grasses with dark moist soil and temperature rose to 29.4^0C, this result is corresponding with John (2005) in Ohio who recorded *Der. maculatus* in agricultural area with clay soil and vegetation consists of grasses and miscellaneous herbaceous. The species

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Nec. rufipes during our study occurred in each agriculture and desert habitats higher significantly than in coast habitat, rising relative humidity in coastal habitat (53.5%) compared to agriculture (39.75%) and desert habitat (49.4%) may be led to low numbers of *Nec. rufipes.* This result agreement with John (2005) who recorded *Nec. rufipes* in agriculture area without the site along lake. Whereas, *Sap. splendens* and *Sap. chalcites* were both found in agriculture habitat with mean number higher than desert and coasta habitats significantly. In contrast, John (2005) collected Hister beetles from genus *Saprinus* in a site along lack and sandy soil in high numbers.

Coclusion:

It is necessary to establish Coleoptera fauna for illumination of forensic investigation in Kingdom of Saudi Arabia. For this aim, this study provides information about Coleoptera species in three habitats using rabbit carcasses. Five species were recorded, *Dermestes frischii*, *Dermestes macultus, Necrobia rufipes,Saprinus splendens* and *Saprinus chalcites*. This species were more present in agriculture and desert habitat. *Dermestes frischii* was the most species abundance.

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