Arthropods associated with human remains and determination of postmortem interval in Jeddah, kingdom of Saudi Arabia

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Abstract: In this study arthropods fauna which attracted to an exposed human corpse was detected in summer season at Jeddah city, west region of the kingdom of Saudi Arabia. The fauna were; third instar larvae in post-feeding phase and pupae of the blowfly *Chrysomya albcipes* (Diptera; Calliphoridae), adults of each beetles *Dermestes frischii* Kugelann, 1792 (Coleoptera; Dermestidae) and *Necrobia rufipes* De Geer, 1775 (Coleoptera; Cleridae), and adult stage of spiders. The post-mortem interval was estimated based on the age of largest unadult stage of the blowfly *Chrysomya albcipes;* pupa; which was 9.5 days. This study Confirmed presence of *Dermestes frischii* and *Necrobia rufipes* together on human corpsein later stages of decomposition. Also, presence of spiders as adventives visitor to the corpse.

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1. Introduction

Forensic or medico-legal entomology is the study of insects and arthropods associated with a dead body. Seventy-two hours after death, insects become the most accurate and sometimes the only tool for determining the time of death (Anderson & VanLaerhoven, 1996). Likewise, insects frequently used for clarifying murders, suicides, identifying both the criminal and the victim, provide information about the place of death (Anderson, 2001; Gordh & Headrick, 2001). The reasons for using insects in criminal investigations, based on the fact that insects are the first life organisms to find a corpse and they present in all stages of decomposition and, furthermore, some species are specific for certain region and season (Matuszewski *et al.*, 2011).Another important reason to be considered is that oviposition occur directly after death (Smith, 1986).

Flies and beetles are the most important groups of insects which predictably attract to corpses and provide the majority of information in forensic investigation (Benecke & Seifert 1998; LaMontte & Wells, 2000). Due to the fact that the flies (Diptera) particularly the blowflies (family Calliphoridae), have been recognized as the first wave of the faunal succession on human cadavers (Nuorteva,1977; Goff, 1993), they have been the group that used for estimating Post Mortem Interval (PMI) within short periods after death. Whereas, Beetles (Coleoptera) can provide useful forensic entomological evidence particularly with regard to dry human skeletal remains in the later stages of decomposition (Berenbaum, 1999; Kulshrestha &Satpathy, 2001).

The blowfly *Chrysomya albiceps* (Wiedemann 1819) is normally carrion breeder. It is very important fly for forensic entomologists because it's larvae are always present on a dead body and it is facultative predators and therefore can alter the composition of species present at the carcass (Mendonca *et al*., 2010). The life cycle of *Chrysomya albiceps* consists of three larval instars and pupal stage. At the end of the third larval instar larvae stopped feeding and moving before pupate and this phase called post-feeding (Dhang *et al.*, 2008). The first stage larvae feed on the decomposing flesh, but the second and third-stage larvae are predaceous, feeding on other blowfly larvae (Del BiancoFaria *et al.*, 1999), this behavior may possibly lead to a decline in the population numbers of other species.

Order Coleopteran is distinct from others by the presence of a pair of hardened and thickened wings or "elytra". Beetles are holometabolous insects and their lifecycle consists of an egg stage, three to five larval stages depending on species and pupal stage (Gennard, 2007). The most important families of Coleoptera mentioned in the forensic entomology studies are Dermestidae and Cleridae. Family Dermestidae are small to medium sized beetles (3.5-12 mm long), round to oval body shape and covered with setae or scales which are sometimes conspicuously colored and form distinctive patterns (Bousquet, 1990). This family contains 1400 species and subspecies belong to about 50 genera worldwide (Ress, 2004; Hava, 2011). *Dermestes frischii* return to Genus Dermestes, and it is necrophagous beetle feed on everything dry such as hairs, bones, skin and dry tissues for human corpse or animal cadavers (Haskell *et al*., 1997; Kulshrestha & Satpathy, 2001). Family Cleridae is small to medium sized (5-9 mm long) and brightly colored beetles, recognized by wide head,

usually wider than the pronotum, the pronotum often rather cylindrical and narrower than the elytra (Leavengood, 2008). The Clerid species *Necrobia rufipes*is predaceous beetle and often association with *Dermestes* spp. on corpses where it feeds on other insects like blowfly and Dermestid larvae (Simmons & Ellington, 1925; Kočárek, 2003). This study aimed to identify arthropods fauna associated with human corpse in summer season at Jeddah city, which locates at west region of the kingdom of Saudi Arabia, and to detect the time elapsed since death (PMI) using the insect evidence which was collected from the corpse.

2. Materials and Methods:

At summer 2015, a family complained about absence of their mother which was an old woman (81-year-old) lives in a village.On21 July 2015, the corpse of this woman was found in a goat yard far about 100 m from their house which located in a village on the outskirts of Jeddah city (latitude 29.21 north and longitude 39.7 east) west of the Kingdom of Saudi Arabia. The corpse was clothed and in the decomposition stage between advanced decay and skeletal, there were goat feces on the corpse and around it. The temperature and relative humidity data were obtained from Faculty of Meteorology, Environment and Arid Land Agriculture. The mean of temperature and relative humidity assessed for a period of two weeks ago before discovering the corpse were33.43^{0}C to39.13^{0}C and 30% to60% RH. The corpse was transferred to the Forensic Medicine Center and Mortuary Affairs for autopsy, where it was examined on the same day. The arthropods which collected from the corpse were; fly larvae and pupae, adult beetles and spiders. Life fly larvae were put in near-boiling water (85-90°C) for 2-3 minutes, for killing them and prevent larval shrinkage, then were preserved in 75% ethyle alcohol. Whereas, adult beetles were killed with ethyle acetate in glass jars and were mounted in the laboratory, each of fly pupae and adult spiders were directly reserved in 75% ethyle alcohol. Then specimens brought back to laboratory of Entomological Research Unit at Science Collage for girls, king Abdulaziz University in Jeddah. The specimens were examined using dissecting stereomicroscope from Leica Company (Leica M205 C stereomicroscope). Digital photographs of the specimens were taken with Leica IC80 HD camera adapted to a Leica M205 C stereomicroscope. Measurement given in millimeters. Determination of fly species was conducted using taxonomical keys for third larval instar according to Spradbery (2002), Szpila (2010) and Thyssen (2014), based on features of the body structure for patterns of spinulation and shape of body spines, anterior and posterior spiracles, prominent papillae. Identification species for pupae based on morphological characters according to Mendonca *et al*. (2010). The species of adult beetles was determinated using taxonomical keys based on Bousquet (1990), Leavengood (2008) and Peacock (2013).

3. Results and Discussion:

Following death, dead body goes through a series of physical and biochemical changes (Gennard, 2007).Four stages of decomposition (fresh stage, bloated stage, decay stage and dray stage)defined by Rodriguez & Bass (1983), Braack (1986). Other studies described five stages of decomposition; fresh stage, bloated stage, decay stage, advanced decay stage and skeletal or remains (Centeno *et al*., 2002). In the recent study, the corpse was found out door in summer season and the decomposition stage was advanced decay at some parts of the body and skeletal stage for others. Saukko & Knight (2004) noted that, the decomposition differs from body to body, and even from one part of the same corpse to another. Marks &Tersigni (2005) stated that length of stages of decomposition depends on climatic condition such as temperature and humidity, and decomposition rate was fast and carcasses was dried up in a short period in summer, whereas these periods in winter was longer, it also varies by the size of carcasses (Kumara *et al*., 2009).

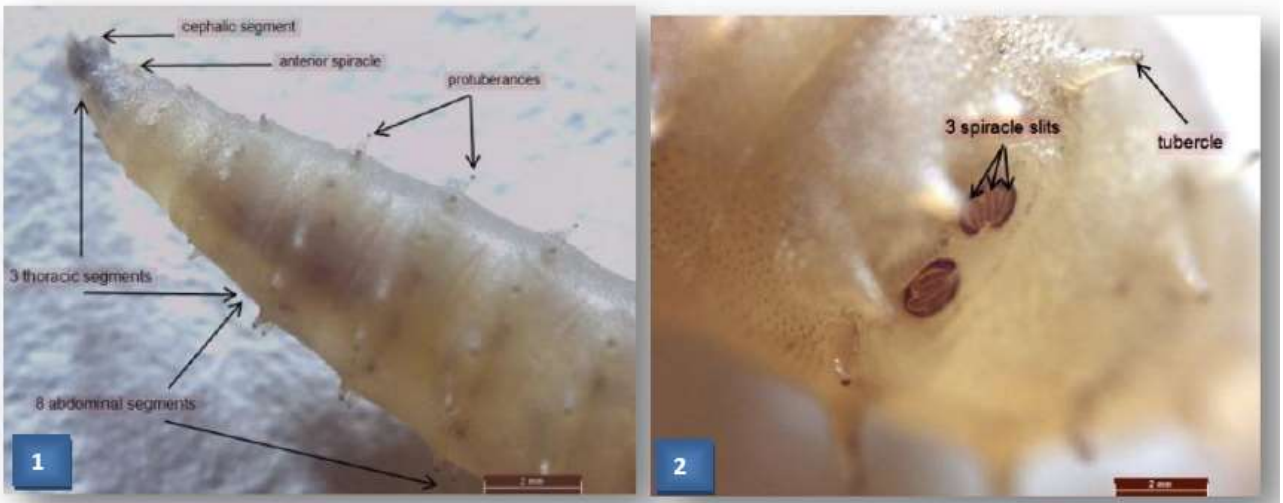
By examination the specimens it was clear that arthropods which collected from the corpse were third instar larvae in post-feeding phase and pupae of the blowfly *Chrysomya albcipes* (Diptera; Calliphoridae), adults of each the beetles *Dermestes frischii* Kugelann, 1792 (Coleoptera; Dermestidae) and *Necrobia rufipes* De Geer, 1775 (Coleoptera; Cleridae), and adult stage of spiders (table1).

It is known that arthropods visiting corpse or carrion in succession form at a certain time (Centeno *et al*., 2002; Villet, 2011). Flies particularly the blowflies (family, Calliphoridae), have been recognized as the first wave of insects attract to human cadavers (Nuorteva,1977; Goff, 1993).In this study *Chrysomya albcipes* unadult stages were collected as insect evidence.The larvae of *Chrysomya albicep*sa re morphologically very distinct from all other Calliphoridae larvae by possessing prominent fleshy protruberances along their body (“hairy maggots”) (Baumgartner & Greenberg, 1984) (fig. 1,2).In addition, pupae have the same protruberances (Mendonca *et al*., 2010) (fig. 3). Female of *Chrysomya albcipes* lays the eggs on the corpse through minutes after death (Higley & Haskell, 2010). Therefore, it is an important tool to estimate the minimum postmortem interval (PMI_{min}) by detecting the age of the larger immature stage found on the corpse (Carvalho *et al*., 2004; Reibe *et al*., 2009; Niederegger & Spiess, 2012). In the recent study the largest immature stage collected from the corpse was pupa. The corpse was found exposed in summer season and the temperature was not constant, but ranging from33.43^{0}C to 39.13^{0}C, the mean of egg stage and each larval instar periods for the specimens found on the corpse were estimated based on that recorded in previous studies (table2). The age of the 3rd instar larvae in post-feeding was calculated as 133.96 hours (5.582 days) (table2). The duration of pupal stage was not the same in previous studies at the same temperature, so we

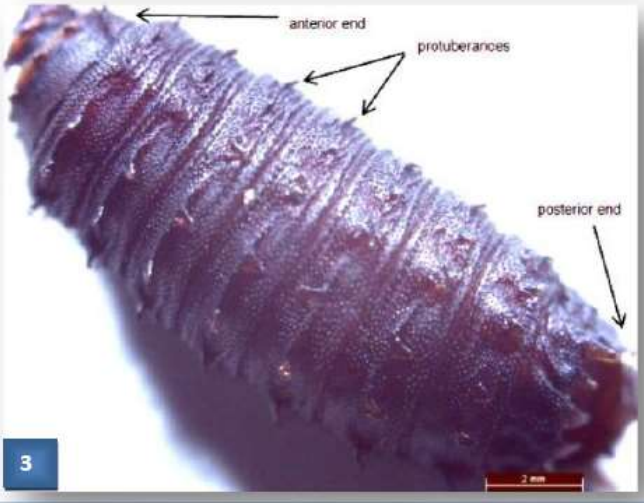
calculated the mean of pupal stage period at each temperatures from deferent studies, which were4.967, 3.75and 3.05 day at 30°C, 32°C and 35°C, respectively (table3).The mean of pupal stage period at the time we found the corpse was 3.922 day. The time passed from laying eggs to getting largest stage, pupae, was 9.5 days. Being the blowfly *Chrysomya albcipes* laid their eggs minutes after death we can estimate PMI as 9.5 days. Whereas, the time of death was estimated by forensic medicine as 14days.

Table (1): Arthropods fauna which collected from an exposed corpse in summer season, at Jeddah city.

Class	Order	Family	Species	stage
Insecta	Diptera	Calliphoridae	*Chrysomya albiceps* (Wiedemann 1819)	Third larval instar in Post-feeding phase
Insecta	Diptera	Calliphoridae	*Chrysomya albiceps* (Wiedemann 1819)	Pupa
Insecta	Coleoptera	Dermestidae	*Dermestes frischii* Kugelann, 1792	Adult
Insecta	Coleoptera	Cleridae	*Necrobia rufipes* De Geer, 1775	Adult
Arachnida	Araneae	Unidentified	Unidentified (Spider)	Adult



Figs. 1-2: The blowfly *Chrysomya albiceps*. 1. Anterior end of the third larval instar (post-feeding phase). 2. Posterior end of the third larval instar (post-feeding phase).



Figs. 3: Dorsal lateral view for the pupa of the blowfly *Chrysomya albiceps* .

Table (2): Time of development obtained from literatures for *Chrysomya albcipes.*

Source	Stage	Temperatures(°C)			The mean
		30	32	35	
Augul&Jassim (2009)	Egg stage	20 h (0.833 day)	18 h (0.75 day)	—	19 h (0.792 day)
Al-Shareef & Al-Qurashi (2016)	1st larval instar (L1)	24 h (1 day)	__	24 h (1 day)	24 h(1 day)
Al-Shareef & Al-Qurashi (2016)	2nd larval instar (L2)	24 h (1 day)	__	24 h (1 day)	24 h(1 day)
Al-Shareef & Al-Qurashi (2016)	Young L3 (feeding phase)	24 h (1 day)	__	24 h (1 day)	24 h (1 day)
Al-Shareef & Al-Qurashi (2016)	Mature L3 (feeding phase)	24 h (1 day)	__	24 h (1 day)	24 h (1 day)
Al-Shareef & Al-Qurashi (2016)	Post-feeding phase	19.92 h (0.830 day)	—	18 h (0.75 day)	18.96 h (0.790 day)
The total					133.96 h (5.582 day)

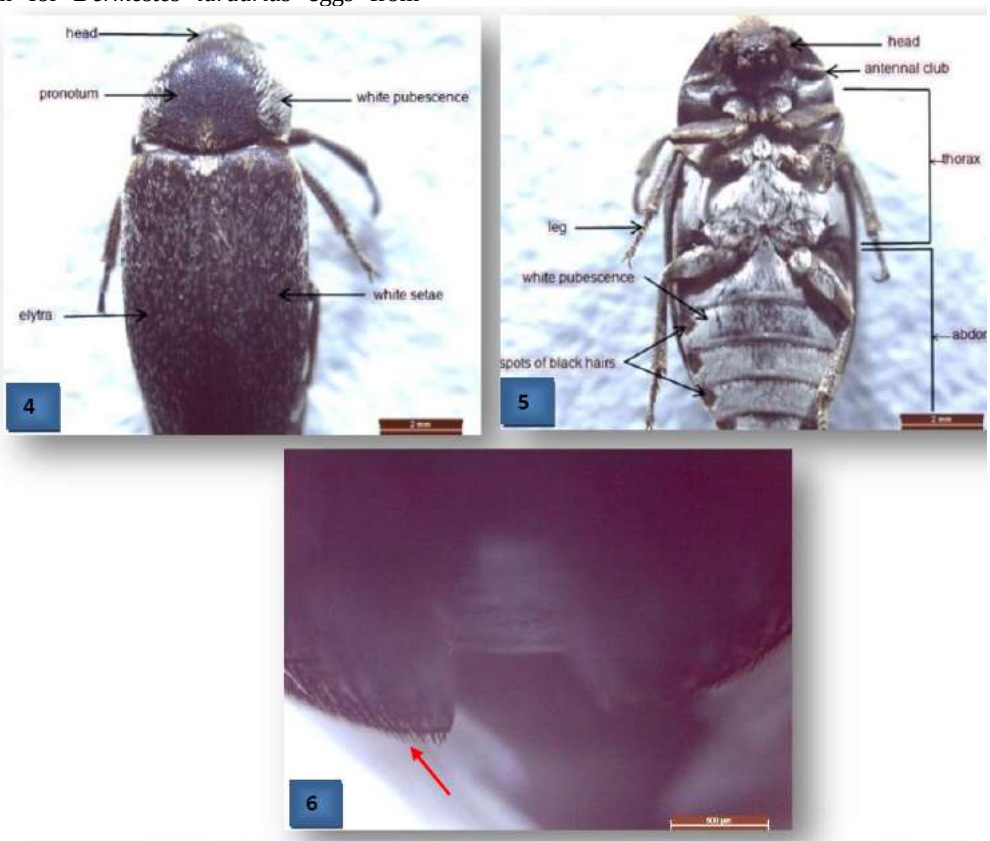
Table (3): Time of development for *Chrysomyaalbcipes*pupal stage obtained from literatures.

Source	Stage	Temperatures(°C)		
		30	32	35
Queiroz (1996)	Pupal stage	__	3.0day	__
Grassberger *et al*. (2003)		5.9Day	__	4.6Day
Augul & Jassin (2009)		5.0±0.5day	4.5±0.5 day	__
Al-Shareef & Al-Qurashi (2016)		4 day	__	1.5 day
The mean		4.967 day	3.75 day	3.05 day

In the recent study *Dermestes frischii* beetle which collected from the corpse can easily recognizable by its aspect; the elongate oval body shape, dark brown elytra covered with white sparse setae (fig. 4), the underside of abdomen covered with white pubescence and dark spots of black hairs at margin of each segment and at tip of final segment (fig. 5), the apical of the elytra without spine (fig. 6), the head is deflexed and can usually be retracted into the prothorax, the antennae are usually clubbedand no median ocellus on its forehead (fig. 5).The corpse examined was between advanced decay and skeletal stage of decomposition, *Dermestes frischii* was found in this stage of decomposition because of their feeding habitat, where each of adult and larvae of *Dermestes frischii* were known to feed on dry tissues such as skin, hairs and bones which containing animal proteins necessary for their full development (Kulshrestha & Satpathy, 2001). Various workers mentioned different time periods in the infestation of the Dermestid on human corpse and animal cadavers, but most workers detected Dermestid beetles species at the advanced stage of decomposition (Anderson &VanLaerhoven, 1996; Wolff *et al.*, 2001), or the dry stage (Rodriguez & Bss1983; De souza & Linhares, 1997). Whereas, few authors recorded adult of Dermestes earlier in the stages of bloated and decay (Voss *et al*., 2008). In this study, PMI was estimated using the pupae of *Chrysomya albiceps* as 9.5 days, and the presence of *Dermestes frischii* proved that the corpse reached to the later stages of decomposition, due to the presence of the corpse exposure under the sun light and higher temperature in summer season, this conditions accelerate decomposition processes. This result was agreement with Kökdener & Polat (2014) who collected *Dermestes frischii* from dog carcasses in the active decay stage, advanced decay and dry stages in summer season. Zhantiev (2009) stated that hot and dry summer and relative humidity in winter are the preferable climatic conditions for this species. *Dermestes frischii* had been associated with remains years after death (Byrd &Castner, 2001), it was detected in Egypt in tomb of Egyptian mummies by several authors (Lesne, 1930; Hinton, 1945; Alfieri, 1976; Strong, 1981; Panagiotakopulu, 2001).

Although, *Dermestes freschii* was collected from this corpse neither larvae nor pupae were found. It seems that the collected adults represented the first beetles which arrived and may be they could not laid eggs or laid little eggs which did not hatch due to the higher temperature which was from 33.43^{0}C to 39.13^{0}C. Temperature and humidity are with heavily influence on insect activity and rates of oviposition and development (Smith, 1986; Gillot, 1995; Anderson & Cervenka, 2002). The adult of Dermestid was reported to tolerate temperatures between 29^{0}C to 42^{0}C with the relative humidity 40% to 70% (Gennard, 2007). Whereas, Coombs (1981) reported that, last larvae and adults of *Dermeses ater* were found to avoid a temperature range of 30^{0}C to 45^{0}C. Eggs were heavily influenced by temperatures, no eggs of *Dermestes lardarius* were laid

at 30^{0}C (Coombos, 1978; Jacob & Fleming, 1984a), and the eggs of *Dermestes haemorrhoidalis* failed to hatch at temperature above 32.5^{0}C (Jacob & Fleming, 1984b), and increasing temperature from 15^{0}C to 32.5^{0}C decrease the percentage hatch for *Dermestes lardarius* eggs from 50.8% to 12.5%. (Jacob & Fleming, 1980b). Rising temperature to 35^{0}C decreasing number of *Dermestes maculatus* eggs to one third from that at 21^{0}C (83 eggs instead of 214 eggs) (Azab *et al*., 1973c).



Figs. 4-6: Adult of *Dermestes frischii* beetle. 4. Dorsal view. 5. Ventral view. 6. Margin of the elytra (red row points to the absence of spine).

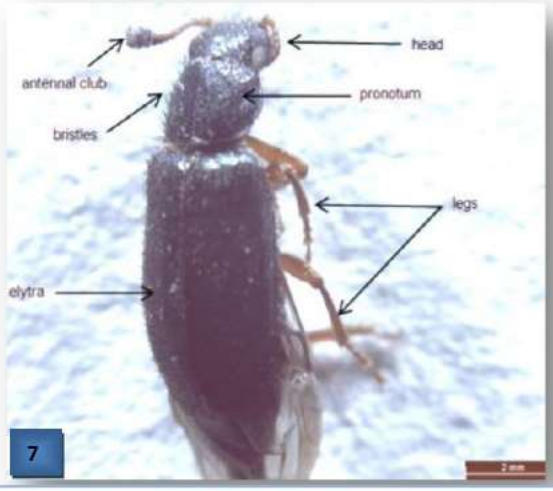
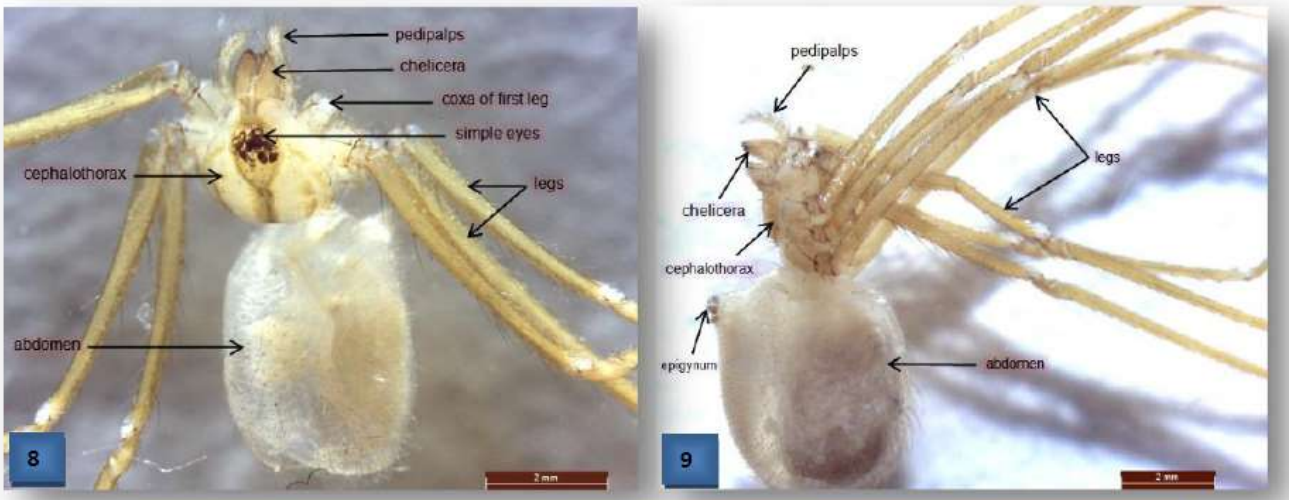


Fig. 7: Dorsal view of adult *Necrobia rufipes* beetle

In this study adult *Necrobia rufipes* was collected with *Dermestes frischii* from the corpse. The Clerid species *Necrobia rufipes* was distinguished by their appearance; flattened paralled-sided, about 5 mm long, the antennae have a very distinct three-segmented club, the sides of the thorax have bristle-like hairs pointing outwards, the antennae and legs are reddish-orange in colour, but rest of body (upper surface of the head, thorax and elytra) metallic blue (fig. 7). Several authors recorded *Necrobia rufipes* associated with *Dermestes frischii* on the corpse, such as Özdemir & Sert (2009) who found them on pig carcasses in spring and summer season in Ankara, Turkey. Bana & Beyarslan (2012) detected *Dermestes frischii* and beetle from Genus *Necrobia* in the advanced and dry stages of decomposition on pig carcasses in Turkey. Shalaby *et al.* (2000) in Hawaii recorded *Dermestes frischii* in the bloated stage, decay stage, post decay stage and skeletal stage, and *Necrobia rufipes* in just decay and post decay stages of decomposition on pig carcasses. *Necrobia rufipes* is recorded as a predator for the blowfly and dermestid larvae; therefore, it arrives before the fly larvae migrate far from the body (Peck & Thomas, 1998; Kočărek, 2003).

For estimation of long post-mortem interval using adult beetles in Jeddah city in the kingdom of Saudi Arabia it is important to study the rates of development for these species because the rates of development for *Dermestes frischii* and other species of the Dermestidae family is different strains and different foodstuffs (Amos, 1968; Woodroffe & Coombs, 1979).

Another pointer for a long PMI in this study, was the presence of spiders, which were commonly found in exposed cadavers in desiccated remains.Spiders can easily recognize by their aspect (fig. 8,9). The spider's body consisted of two sections; the head and thorax, and both were fused to form the cephalothorax region which joined by a waist (pedicel) to the second body region, the abdomen. The cephalothorax bearing mouthparts, simple eyes and four pairs of legs. Spider had two short appendages, one on each side of the face, that are called pedipalps. Spiders were known to visit corpses accidentally to find place for rest or get humidity (Price, 1975).Several authors listed spiders as arthropods visit cadavers; Vitta *et al.* (2007) reported spiders on pig carcass at bloated stage, active stage, advanced stage and dry stage, in Thiland. Dupont *et al.* (2011) reported spiders on rat carcasses at decay stag, dray stage and skeletal stage in Cameroon. Spiders were recorded in a checklist of arthropods associated with African carrion (Prins, 1983; Ekanem& Dike, 2010). However, because this specimen was not essential arthropod on carrion, the identification to the family level was not being done and its presence provided little additional help.



Figs. 8-9: Adult of a spider. 8. Dorsal view.9. Lateral view.

This study proved that the insect fauna which could be found on an exposed corpse in Jeddah city in Saudi Arabia at summer season were larvae and pupae of the blowfly *Chrysomya albcipes*, adults of each beetles *Dermestes frischii* and *Necrobia rufipes* and adult stage of spiders. This result agreement with several authors; Arnaldos *et al.* (2004) found insect evidences; empty puparia of *Chrysomya albiceps*, live adult beetles of *Dermestes maculatus* and *Necrobia rufipes* on skeletal human corpses in February at Southeastern Iberian Peninsula in Spain, and he determined minimum of PMI as 6 months since *Chrysomya albiceps* was the most abundant species of Diptera in this region at the end of summer and beginning of autumn. Grisales *et al*. (2010) recorded *Chrysomya albiceps* on exposed decomposing human corpse as adult and larval stage in bloated stage,

active stage, advanced stage, and adult of Demestid on the active stage, advanced stage, and remains on exposed pig carcasses in Colombia. Kumara *et al.* (2009) reported adult of *Dermested maculatus* and its larvae, pupae of *Chrysomya megacephala* (Diptera: Calliphoridae), larvae of *Piophilacasei* (Diptera: Piophilidae), *Megaselia sclaris* (larvae and pupa) (Diptera: Phoridae) in advanced stage of decomposition (with the maximum PMI was 13 days).

Conclusion:

Arthropods which attracted to human corpse in later stages of decomposition were detected in summer season at Jeddah city, west region of the kingdom of Saudi Arabia. They were; third larval instar and pupae of the blowfly *Chrysomya albcipes*, adults of each beetles *Dermestes frischii* and *Necrobia rufipes*, and adults of spiders.The post-mortem interval was estimated based on the age of pupa of the blowfly *Chrysomya albcipes*; which was 9.5 days. In the recent study, PMI estimated by insect evidence is more accurate than those using forensic medicine which was two weeks. This study proved the importance of using forensic entomology in legal investigation for human corpses, and it can help police in criminal cases.

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