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Comparative insect fauna succession on indoor and outdoor monkey carrions in a semi-forested area in Malaysia

Nazni Wasi Ahmad[1*], Lee Han Lim[1], Chen Chee Dhang[1,2], Heo Chong Chin[3], Abdullah AG[3], Wan-Norjuliana Wan Mustaffa[3], Chew Wai Kian[1], John Jeffery[4], Rosli Hashim[2], Sofian Mohd Azirun[2]

[1]*Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia*
[2]*Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[3]*Universiti Teknologi Mara, 40450 Shah Alam, Selangor, Malaysia*
[4]*Parasitology Department, Medical Faculty, University of Malaya, 50603 Kuala Lumpur, Malaysia*

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ABSTRACT

Objective: To study the occurrence of insects fauna obtained in a semi forested area in Malaysia using the carcass of the monkey *Macaca fascicularis*, Raffles, and to compared the wave of insect succession collected from the carcass placed outdoor and indoor. **Methods:** The outdoor and indoor studies were conducted at a location of 3°17′57.86″N, 101°47′00.78″E. The euthanized monkeys were placed indoor and outdor. The insect seccession visited the carcass, the envirenmental temperature and relative humidity were recorded. **Results:** The main insects attracted to specific stages of decomposition were mainly members of Diptera and Coleoptera. There was a delay of fly arrival by two days in indoor carcass. Nocturnal oviposition behaviour was not observed in this study. The flies left the carcass during the twilight zone as noted during a 3 days observation period in outdoor study. The dipteran species found outdoor and indoor were similar but more diverse coleopteran species was found indoor. **Conclusions:**Information and evidence from this study can be used to improve the estimation of the post mortem interval in forensic cases.

1. Introduction

Forensic entomology refers to the insects and related arthropods and their association with legal matter. Medicolegal forensic entomology is a discipline of forensic entomology interpreting information concerning death using insects as the first witness in order to estimate the minimum time elapsed since death or the post mortem interval (PMI).

Insects are the primary fauna associated with carrion[1,2]. It is known that there is an assemblage of insect species that are attracted to decomposing animal remains and play an active role in the decay process[3,4]. Certain species in the orders of Diptera and Coleoptera represent the majority of the total necrophagous faunae found on carrion[5].

Insect colonisation of carrion is dependent on many factors. Each geographical region is characterized by its season, temperature, humidity, habitat, vegetation, soil type and environmental conditions[6-8]. These regional characteristics influence insect species composition, development times of insects and thus affect the rate of carrion decomposition. Many years of research on succession pattern indicate that the difference is really dependent on the geographical location under question.

Insect species do not occur on the carrion at the same time but in succession patterns and some variations are observed in different habitats[9,10]. Forensic entomology has been practiced at the Institute for Medical Research (IMR), Malaysia since 1950[11]. Reid from IMR was the first to mention the use of forensic entomology to determine the PMI in a female corpse in Penang in 1950[11]. Although forensic entomology has since been practiced in Malaysia for the past 60 years, the discipline, however, still needs more local information such as fauna succession data. There were several succession studies conducted in outdoor situations in Malaysia to establish baseline data to be used in estimation of PMI[12-14]. However, such studies had never been conducted indoor. Hence, the present study aims to determine and compare the arthropod succession associated

*Corresponding author: Nazni Wasi Ahmad, Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
E-mail: nazni@imr.gov.my
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with monkey remains in indoor and outdoor ecological habitats.

2. Materials and methods

2.1. Study site

The present study was conducted at the Wildlife Research Centre, University of Malaya, 16th Mile off Jalan Gombak, Gombak District, in the state of Selangor. This site is located at 3°17′57.86″N, 101°47′00.78″E which is 400 m above sea level. The average temperature of the site ranged from 23.0 ℃ – 31.9 ℃ with relative humidity of 67% – 90%. The outdoor and indoor study was conducted in two different sites located at the same region having similar vegetation. Both sites were separated by a distance of 200 m. The outdoor site was partially shaded while the indoor site was inside a brick – wooden hut of size 10 feet (length) x 10 feet (wide) x 8 ft (height), simulating a medium room size in Malaysia.

2.2. Study animal and field procedures

The monkey (*Macaca fascicularis*, Raffles) was used exclusively as a model for human decomposition since they are known to be phylogenetically related to human. Monkeys weighing 5.4–5.6 kg were used in this study.

The study protocol was approved by the Animal Care and Use Committee, Ministry of Health Malaysia [Permit No. ACUC/KKM/02(2/2008)]. Prior to commencement of project, approval was obtained from the Department of Wildlife and National Parks (PERHILITAN) of Malaysia to euthanise the monkeys. All euthanization was administered by the experts from the Department of Wildlife and National Parks (PERHILITAN) of Peninsular Malaysia.

The monkeys were euthanized by a single shot to the forehead from a handgun individually. The euthanisation was conducted at 11:30, but two monkeys were euthanized after sunset at about 23:00 to observe possible nocturnal fly oviposition. After death was confirmed, the monkeys were immediately clothed to simulate human cadavers. Monkey carcass was placed indoor and outdoor, respectively. The monkey carcass used for the outdoor study was placed inside a metallic cage (1 × 0.75 × 0.5 m) with mesh (3 ×3 cm) on all four sides which allowed the free access of insects and also ensured the close contact of the monkey carcass with the ground and protection from scavengers.

For the indoor study, after death was confirmed, the monkey was immediately placed inside a wooden – brick hut. All the windows in the hut were partially opened to allow the entrance of the flies. The indoor carcass was also clothed to simulate human cadavers. Both the outdoor and indoor studies were conducted simultaneously.

In all the experiments, environmental parameters of temperature and relative humidity were recorded daily at 11:00 in the morning by using probe thermometer (Eutech Eco Scan Temp 5) and digital humidity reader (Oregon Scientific Weather Station BAR 629HG). The temperatures were recorded. These factors are important for oviposition and growth of the immature stages of insects. The temperature and humidity for monkeys euthanized during the nocturnal period was (24.9 ± 0.1) ℃ and (81.5 ± 0.5) %, respectively.

The monkeys which were euthanized during diurnal period were monitored hourly for the first 3 days, and daily from Day 4 onwards until larvae were no longer observed, leaving only bones. Monkeys that were euthanized during the nocturnal period were monitored hourly throughout the night until sunrise on the next day and continued to be monitored hourly for the next two days and then daily.

Adult insects which visited the monkey carcasses were collected by using an insect net. A representative larval sample of the total insects from different larval masses was collected so that the natural populations were not disturbed. Larvae were collected by using art-brush and forceps and immediately placed into glass vials containing 20 mL of 70 % ethanol. Pupae were also collected and brought to the laboratory for adult emergence. All specimens were mounted according to the in-house entomological mounting techniques. Specimens were identified according to Greenberg and Kurahashi[15,16].

3. Results

During the respective study period of 15 and 34 days for 2 replicates outdoor, the minimum and maximum temperature were 24.9 ℃ and 29.7 ℃ with relative humidity in the range of 71% – 84% (replicate 1) and 23.0 ℃ and 31.9 ℃ with relative humidity of 67% – 90% (replicate 2) (Figure 1).

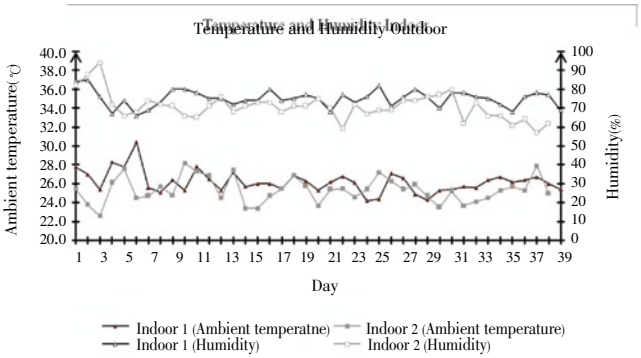
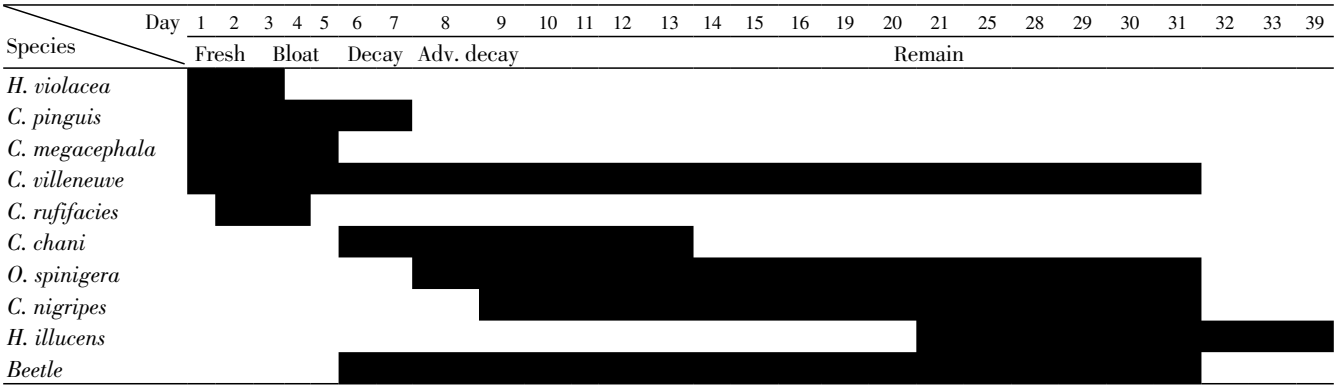


Figure 1. Ambient temperature and relative humidity of 2 replicates of outdoor forested sites in Ulu Gombak, Selangor.

Decomposition of monkeys either euthanised during diurnal or nocturnal period was determined by 5 stages: fresh, bloating, decay, advanced decay and remain as indicated by the morphological changes of the carcasses. In the outdoor fresh decomposition stage (Day 1–2), within 5 minutes of death, ants of the species *Pheidologeton* sp. and *Odontoponera* sp. were attracted to the bloodstain. Within

Table 1
Decomposition stage, number of days and insect succession on monkey carcass in outdoor forested site in Ulu Gombak, Selangor.



the following 30 minutes the first blowflies of the family Calliphoridae, *Hypopygiopsis violacea (H. violacea)* arrived and oviposition took place after 1.5 h in the oral cavity. These were big robust greenish metallic-coloured flies about 1.50 – 1.75 cm in length. Within hours later many calliphorines arrived and began ovipositing in the ear and the neck region. Within 6 hours after death, the eggs oviposited in the oral cavity (1st oviposition site) hatched. We observed many ants (*Pheidologeton* sp. and *Odontoponera* sp.) predating actively on the eggs. Ants were observed on carcass on the fresh blood. However, the presence of ovipositing flies were far more in numbers compared to the ants. The limited number of ants may not have great impact on the predation of the fly maggots.

We also observed that adult flies began to move away from monkey carcasses during sunset or during the twilight zone period when it was getting darker. Not a single adult fly was found on carcasses. The flies left the monkey carcasses during the dusk period and this phenomenon was observed in the following 3 consecutive days.

In the bloated stage lasting 3 days (Day 3 – 5), *Chrysomya megacephala* (*C. megacephala*), *Chrysomya villeneuve* (*C. villeneuve*) and *Chrysomya pinguis* (*C. pinguis*) were collected. During this period the abdomen of the monkey was swollen. The decay stage lasted for 2 days (Day 6 – 7), during which many maggot masses were seen in the mouth, anus and armpit region. The stomach contents were exposed after the skin and flesh at the stomach region were consumed by maggots. Dissociation of body hairs was also observed. At this stage, the body was emitting extremely strong odours and adult beetles of family Scarabaeidae were also found in the carcass. The advanced decay stage (Day 8–9) had lesser odour with many maggots pupated under the carcasses. On Day 8 the first bone was exposed, the majority of maggots were in the late 3rd instar and many were undergoing pupation. Beetles of the family Silphidae were observed. From Day 10 onwards, the remain stage appeared but this varied from 10 – 16 days (replicate 1) and 10–40 days (replicate 2) where transformation to skeletons took place and many maggots and pupae as well as beetles were observed.

Table 2
Summary of insect succession recovered from monkey carcasses placed outdoor in forested area of Ulu Gombak, Selangor.

Insect fauna	Order	Family	Species
Flies	Diptera	Calliphoridae	*C. megacephala*
			C. rufifacies
			C. villeneuve
			C. pinguis
			C. nigripes
			C. chani
			H. violacea
		Muscidae	*O. spinigera*
		Stratiomyidae	*H. illucens*
		Sarcophagidae	Unknown
Beetles	Coleoptera	Scarabaeidae	Unknown (Species 1)
			Unknown (Species 2)
			Unknown (Species 3)
		Silphilidae	Unknown (Species 1)
Ant	Hymenoptera	Formicidae	*Pheidologeton* sp.
			Odontoponera sp.

Note: A total of 4 species of beetles belonging to 2 families could not be identified.

Table 1–3 showed the fly maggots and Coleopterans genus recovered from monkey carcasses. Many adult flies were observed on Day 13 after death. These were the flies which had newly emerged. On Day 16 there was a complete absence of maggots in the replicate 1, whereas in the replicate 2, we found maggots of the family Stratiomyidae, *Hermetia illucens* (*H. illucens*) on Day 21 to Day 39. On Day 40 there was a complete absence of maggots.

Table 3
Beetles recovered from different decomposition stages of monkey carcasses placed outdoor in forested area of Ulu Gombak, Selangor.

Decomposition stages	Period (Day)	Replicate 1	Replicate 2
Fresh	1 – 2	Nil	Nil
Bloating	3 –5	Nil	Nil
Decay	6– 7	Scarabaeidae	Scarabaeidae Silphilidae
Advanced decay	8 – 9	Scarabaeidae	Scarabaeidae Silphilidae
Remains	10 – 31	Scarabaeidae	Scarabaeidae

The diversity of insects collected from outdoor condition

Table 4
Decomposition stage, number of days and insect succession on monkey carcass in indoor forested site in Ulu Gombak, Selangor.

Species \ Day	1 2 3 4 5 6 7 8 9 10 11 12 15 16 19 20 21 22 29 30 31 32 33 39 41 61 77 86
	Fresh Bloat Decay Adv. Decay Remain
C. megacephala	
C. pinguis	
C. villeneuve	
C. chani	
H. ligurriens	
C. rufifacies	
O. spinigera	
H. violacea	
Sarcophagidae	
H. illucens	
M. domestica	
Beetle	

is shown in Table 1. It is obvious that the Diptera peaked during the initial stages of decomposition whereas the Coleopteran as well as the Stratiomyidae appeared during the final decay and remain stages. Table 2 and Table 3 indicated the diversity of orders, family and species of insects collected from the outdoor condition. These insects belonged to 3 orders, namely Diptera, Coleoptera and Hymenoptera consisting of 7 families. The decmposition process of carcasses in 2 replicates of outdoor study were shown in Figure 2.

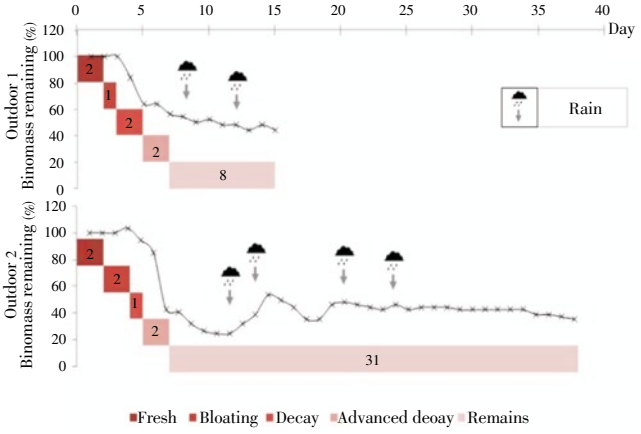


Figure 2. Decomposition process, duration of each stage and percentage of biomass remaining of carcasses in 2 replicates of outdoor forested sites in Ulu Gombak, Selangor.

It is noteworthy to mention that maggots of *H. violacea* were only found during fresh and bloated stage. The species *O. spinigera* oviposited during advanced decay stage when there was lesser odour and the carcass had minimum tissues left.

When euthanization was conducted during the nocturnal period, there was a complete absence of oviposition by flies, as evidenced by hourly observation throughout the night. The flies started to oviposit the next morning by sunrise at 8:00 am and the temperature and humidity recorded at that time was 23.80 ℃ and 88.0%. The minimum and maximum temperature and relative humidity data for the decomposition period of 42 and 86 days for 2 replicates for the indoor studies ranged from 24.2 ℃ and 30.4 ℃ with relative humidity of 61 − 85 % (replicate 1) and 22.6 ℃ and 28.2 ℃ with relative humidity of 57 − 94 % (replicate 2) as shown in Figure 3. In the indoor studies, the decomposition period was prolonged especially in the second replicate where the decomposition was even longer due to the lower temperature and high humidity (Figures 3, 4). There was also a delay of fly arrival for at least 3 days for both the replicates as exhibited in Table 4. Our studies indicated that the various decomposition stages in indoor condition were prolonged (Figure 4). Decomposition rate of carcass placed outdoor was faster than indoor by 3 folds. Mummification of carcass was observed during the advanced decay stage in indoor condition. The present study in which the two dipteran species found during the mummification and remain stage between Day 15 − Day 50 and Day 29 − Day 86 were *O. spinigera* and *H. illucens*, respectively.

Species of maggots recovered at different stages of decomposition in indoor condition and the summary of insect succession recovered from monkey carcasses placed indoor in forested area are tabulated in Table 4 and Table 5. It was noted that most of the calliporines found in outdoor carcass also visited the indoor carcass except for *C. nigripes*. In the indoor studies *H. violacea* was found during the bloating and decay stage in indoor condition (Table 4).

Four orders of insects found indoors were: Diptera, Coleptera, Hymenoptera and Dermaptera. The order Dermaptera was found at the remain stage, while the order Diptera consisted of the family Calliphoridae, Muscidae, Sarcophagidae and Stratiomyidae. The order Coleoptera was more diverse with 6 families recovered in indoor condition: Lampyridae, Lycidae, Scarabaeidae, Sliphidae, Staphylinidae and Tenebrionidae (Table 6). The order Hymenoptera comprised only 1 family with 3 different species of ants. The order Dermaptera could not be identified.

Table 5
Summary of insect succession recovered from monkey carcasses placed indoor in forested area of Ulu Gombak, Selangor.

Insect Fauna	Order	Family	Species
Flies	Diptera	Calliphoridae	*C. megacephala*
			C. rufifacies
			C. villeneuve
			C. pinguis
			C. chani
			H. violacea
			H. ligurriens
		Muscidae	*O. spinigera*
			M. domestica
		Stratiomyidae	*H. illucens*
		Sarcophagidae	Unknown
Beetles	Coleoptera	Lampyridae	Unknown (Species 1)
		Lycidae	Unknown (Species 1)
		Scarabaeidae	Unknown (Species 1)
			Unknown (Species 2)
			Unknown (Species 3)
		Sliphidae	Unknown (Species 1)
		Staphylinidae	Unknown (Species 1)
			Unknown (Species 2)
		Tenebrionidae	Unknown (Species 1)
Ant	Hymenoptera	Formicidae	*Odontoponera* sp.
			Pheidologeton sp.
			Tetramorium sp.

Note: A total of 9 species of beetles belonging to 6 families could not be identified.

Table 6
Beetles recovered from different decomposition stages of monkey carcasses placed indoor in forested area of Ulu Gombak, Selangor.

Decomposition stage	Period (Day)	Replicate 1	Replicate 2
Fresh	1 – 3	Nil	Nil
Bloating	4 – 7	Staphylinidae Silphidae	Silphidae
Decay	8 – 11	Staphylinidae Silphidae Scarabaeidae	Silphidae
Advanced Decay	12 – 21	Staphylinidae Scarabaeidae Lampyridae	Silphidae Lycidae Lampyridae
Remains	22 – 59	Scarabaeidae	Tenebrionidae

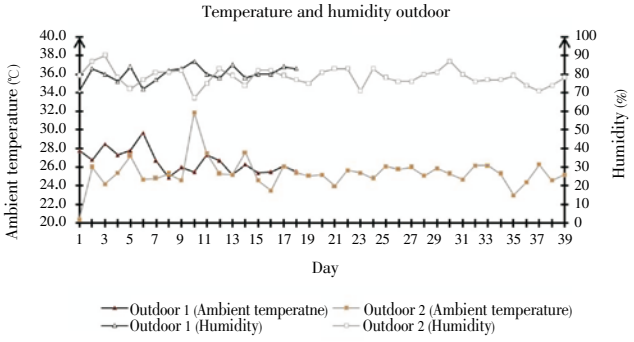


Figure 3. Ambient temperature and relative humidity of 2 replicates of indoor forested site in Ulu Gombak, Selangor.

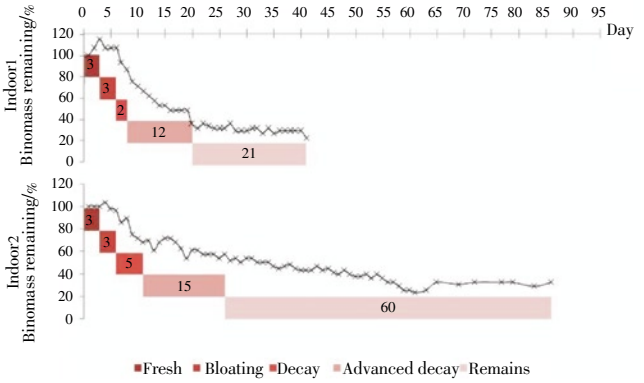


Figure 4. Decomposition process, duration of each stage and percentage biomass remaining of carcasses in 2 replicates of indoor forested sites in Ulu Gombak, Selangor.

4. Discussion

In the indoor and outdoor decomposition studies, 5 decomposition stages were discerned. There are many outdoor carcass decomposition studies[17-20]. But indoor studies are rare. In the outdoor studies, flies visited the monkey carcass within 30 minutes after death. Similar findings were reported by Smith who stated that oviposition could occur within a few minutes after death[21]. On the other hand, Wolff observed that flies arrived within 30 minutes after death but they oviposited during the bloated stage[22]. The majority of insects faunae found on monkey carcasses were those belonging to Diptera at the early stages of decomposition and Coleoptera at the remain stage. *H. illucens* was the only dipteran found in the later stage of remains when only bones were available. From this study, *H. illucens*larvae were found on Day 21 in the outdoor study. This was in line with other findings that *H. illucens* was only attracted to remains of cadaver 20 to 30 days after death[20,23]. This information is pivotal in the determination of PMI in the Malaysian forensic cases because often samples of *H. illucens* were received for PMI determination. Lord also stated that the presence of *H. illucens* in a cadaver can be of paramount importance in determining PMI in the advanced decay decomposition stage[23].

In our study it was observed that there were marked differences in the decomposition of the 2 replicates in the outdoor studies. The reason could be due to the increased frequency of raining days and the cooler temperature in the second replicate (Figure 2) that had extended the decomposition stages and hence attracting more flies to oviposit. Matoba and Terazawa mentioned that among other factors, temperature and weather were known to be the major factors[24]. Similarly, Mahat showed that rain had prolonged the pupation period of flies by 1–34 days[25].

In the succession patterns of insects arriving in the outdoor studies, it exhibit that Diptera peaked during the initial stages of decomposition whereas the Coleopteran

and Stratiomyidae appeared during the final decay and remain stages. This study showed similar pattern in insect succession as reported by Wolff in which the flies were the first necrophagous wave to oviposit and the first immature stages collected[22].

The 7 species of Calliphoridae found in the outdoor study were all of forensic importance and had been repeatedly recovered from human cadavers in Malaysia[26-29]. It is noteworthy to mention that maggots of *H. violacea* were only found during fresh and bloated stage. These maggots would eventually leave the breeding medium early and have a long post feeding as well as pupation stage as observed in our previous laboratory life cycle study[30]. Only recently, *H. violacea* was reported for the first time on human cadaver in Malaysia[31]. This rare observation was due to the behaviour of its maggot *i.e.* leaving the carrion early during the fresh stage.

Another issue to answer was that if blowflies oviposit at night. If flies oviposited at night, the estimation of PMI needs to take nocturnal oviposition into account since the calculation of PMI is based on the oldest larvae which could come from nocturnal oviposition resulting in as much as a 12 h difference in the estimation of PMI[32]. On the other hand, evidence of nocturnal oviposition had been reported in species of blowflies[32,33]. Blowflies were reportedly inactive and did not oviposit at night which is agreeable with our findings which indicated that blowflies did not oviposit at night[34]. Pritem stated that limited colonisation due to nocturnal oviposition occurred in a decomposition bait only after the fourth or fifth day[35]. He suggested that such delayed and limited nocturnal oviposition was not a forensically significant phenomenon when estimating PMI. Even though this may be true, however, as long as nocturnal oviposition occurred on a limited scale, such occurrence would still affect estimation of PMI. Although our study supported findings of Tessmer JW, *et al*[36] that blowflies failed to lay eggs during nocturnal period, the possibility of this activity occurring should be taken into consideration as has been reported by Amendt[37].

For the indoor studies, our study showed that there was also a delay of fly arrival for at least 3 days for both the replicates as exhibited in Table 4. This is similar to the findings by Amendt and Goff for indoor cadavers[37,38].

Mummification of carcass was observed during the advanced decay stage in indoor condition. Although, it has been reported that Diptera did not oviposit in dehydrated or mummified tissues[39], this was in contrast to the present study in which the two dipteran species found during the mummification and remain stage between Day 15 – Day 50 and Day 29 – Day 86 were *O. spinigera* and *H. illucens*, respectively.

The diversity of insects collected in indoor condition was slightly more than outdoor habitat. In the outdoor habitat, 4 families of Diptera with 10 species of flies were recovered compared to 4 families of Diptera with 11 species recovered in indoor. Hence, there was little variation in the outdoor and indoor dipteran species. However, Goff observed more dipteran species in indoor compared to outdoor[38]. This could be due to the fact that blowflies are strong fliers that can follow an odour plume over long distance and easily enter building[40]. In Malaysia the outdoor and indoor ambient temperature and humidity usually do not differ much and hence both outdoor and indoor condition are conducive for fly breeding.

In contrast, although only 2 Coleopteran families with 3 unidentified species were found outdoor, 6 families with nine species of beetles were found in indoor condition (Table 5). However, Goff showed more Coleopteran species in outdoor compared to indoor in contrast to what we have observed in our study[38].

C. villeneuve maggot was the dominant species obtained from both indoor and outdoor carcasses, in bloating, decay and advance decay stages. The family Tenebrionidae which is normally associated with stored product pest was also found in the carcasses. The presence of *Musca domestica* (*M. domestica*) larvae during the remain stages was noteworthy, since housefly could be considered as an incidental visitor as has been reported and the presence of *M. domestica* was of minor significance in estimation of PMI[38]. In Malaysian forensic cases (1972 – 2002) reported and reviewed, not a single case involved the presence of *M. domestica* larvae[28].

This is the first evidence-based study in Malaysia indicating that there is a delay in fly arrival in indoor condition. Data obtained from this study provided practical knowledge of the insect faunae associated with carrion and the possible application in the interpretation and estimation of PMI for human cadavers found in outdoor and in indoor conditions in the Malaysian context.

Conflict of interest statement

We declare that we have no conflict of interest.

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