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Forensic investigation of carcass decomposition and dipteran fly composition ver the summer and winter: a comparative analysis of indoor versus outdoor at a multi-story building

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This study aimed to explore the rate of decomposition of rabbit carcasses and the succession pattern of the associated dipteran flies outdoor, indoor, and on the roof of a 4-story building during the summer and winter. A total of 6,069 flies were recorded, with 30.91% reported as 2 waves outdoor and on the roof in the summer and 69.09% as 4 waves outdoor in the winter. The roof showed the most flies in the summer but the least in the winter, whereas the outdoor showed the most in the winter but the least in the summer. The ground and fi st floo s showed the most indoor flies, while the second and third floo s showed the least in both seasons. Indoor carcasses decomposed slower than those outdoor, and those on the second and third floo s decomposed slower than those on the ground and fi st floo s. Ten fly species from 8 families were identified in the winter, compared to 6 from 5 families in the summer. The most abundant species was *Musca domestica* Linnaeus (Muscidae) on the roof in the summer, while it was *Chrysomya albiceps* (Wiedemannn) (Calliphoridae) outdoor in the winter. The rare species (singletons) were *Musca sp.* (Muscidae) and *Megaselia scalaris* (Loew) (Phoridae) on the fi st floor in both seasons, *Scaptomyza pallida* (Zetterstedt) (Drosophilidae) on the ground floor in the summer, and *Atherigona orientalis* Schiner (Muscidae) outdoor in the winter. These data highlight the variance in carcass decomposition and fly composition across outdoor, indoor, and the roof of human dwellings, which could be of forensic importance.

Key words: forensic, Diptera, outdoor, indoor, roof

Introduction

Cases of criminal deaths are reported at various crime scenes, including both indoor and outdoor human dwellings (Pohjoismäki et al. 2010, Pechal et al. 2015, Byrd and Castner 2019, Kadej et al. 2020). Necrophagous insects seek a corpse to colonize and form distinct communities depending on the location and environmental conditions (Anderson 2019, Byrd and Castner 2019, Wiltshire 2019). These insects can find a corpse within minutes after confirmed death (Amendt et al. 2011, Byrd and Castner 2019), and thus are the first arrivals at the corpse (Tantawi et al. 1996). Therefore, understanding their succession pattern, behavior, ecology, taxonomy, and physiology, as well as the process of carcass decomposition, can be helpful for developing forensically important evidence (Farrell et al. 2015, Charabidze et al. 2017, Joshi and Kumar 2020, Singh et al. 2022). Entomologists utilize these insect species to unravel suspicious crimes due to their successional behavior to the corpse. This could be helpful for providing physical evidence of the time, location, and the way death occurred at the crime scene (Byrd and Castner 2019, Ali et al. 2020, Aly and Aldeyarbi 2020, Hu et al. 2020). This forensic approach has rapidly evolved with several studies recently, for example (Abdullah et al. 2017, Bugelli and Campobasso 2017, Bugelli et al. 2018, Al-Qahtni et al. 2019, Al-Khalifa et al. 2020b, Mashaly et al. 2020c, Al-Qurashi et al. 2023, Khalil et al. 2023).

Carcass-seeking insects are categorized into 4 groups based on their feeding habits (Smith 1986, Byrd and Castner 2019). The first group is the sarcosaprophages, which feed on decomposing cadavers and their fluids, such as dipteran flies from Muscidae, Sarcophagidae, and Calliphoridae, and beetles from the Dermestidae. The second group is coprophages, which are attracted to the rumen material of herbivores, such as flies from Muscidae and beetles from Scarabaeidae. The third group is the dermatophages, which feed on dried skin, ligaments, bones, and hair, such as beetles from Dermestidae and moth larvae from Tineidae. The fourth group is the predaceous, which feed solely on carcass colonizers, particularly dipteran larvae such as beetles from Histeridae and Staphylinidae and ants from Formicidae.

Dipteran flies of the families Sarcophagidae, Piophilidae, Muscidae, and Calliphoridae are the first arrivals at carcasses and, hence, are considered the most common forensically important carcass-colonizers (Ren et al. 2018, Byrd and Castner 2019, Byrd and Brundage 2020). Their larvae feed on carcass remains, thus accounting for the vast bulk of carcass-colonizing insects. Thus, they can provide forensically valuable proof for the location of the crime and whether the cadaver was moved from its original crime location (Cruz 2016, Byrd and Castner 2019), estimation of the time since death (the postmortem interval "PMI") (Goff 1993, Matuszewski 2017, Ramos-Pastrana and Wolff 2017, Bajerlein et al. 2018), and determination of the link between the suspect and the crime (Amendt et al. 2011). Moreover, cadaver-colonizing insects constitute a determining factor in the rate of decomposition process (Soon et al. 2017).

The decomposition process of corpses is an important step in redistributing organic nutrients and minerals across the food chain in the nutrition cycle (Parmenter and MacMahon 2009, Weathers et al. 2012). This process passes through 4 main stages: fresh, bloated, decay, and dry (skeletonized) stages (Byrd and Castner 2019), which involve many interacting organisms, including insects (Hau et al. 2014, Byrd and Castner 2019). The rate of the decomposition process varies depending on several factors: (i) the environmental conditions such as temperature and humidity (Mahat et al. 2009, Iancu et al. 2018b, Hwang et al. 2022), (ii) the type of habitat (Mashaly and Al-Mekhlafi 2016, Mashaly 2017, Haddadi et al. 2019), (iii) type of soil (Carter and Tibbett 2008, Diaz-Aranda et al. 2018), (iv) the chemical constituents of the body (Coe 1974, Torres et al. 2023), (v) the cause of death (Al-Khalifa et al. 2020a, Al-Qahtni et al. 2020a, El-Aziz et al. 2022, Khalil et al. 2023), (vi) the bacterial activity within the corpse (Carter and Tibbett 2006, Carter et al. 2008, Cobaugh 2013, Lauber et al. 2014, Iancu et al. 2018a), and (vii) barriers such as coverage or clothes (Card et al. 2015, Mashaly et al. 2019). These factors can be classified as environmental or non-environmental, with insect activity and temperature being the most influential on the decomposition of a deceased body (Campobasso et al. 2001, Soon et al. 2017).

In addition to the aforementioned factors, the conditions of the enclosed indoor environment can also affect the decomposition process and limit, or delay, the access of cadaver-seeking insects (Campobasso et al. 2001, Anderson 2011). This, in particular, could be a significant factor influencing the decomposition process and insect colonization, and, thus, it can bias the insect-dependent PMI estimation (Mann et al. 1990). In Saudi Arabia, some studies have recently investigated insects colonizing human corpses at both outdoor and indoor sites (Alajmi et al. 2016, Al-Qahtni et al. 2019, Al-Khalifa et al. 2020b, Mashaly et al. 2020c). Indeed, those studies constitute an important initiative step toward understanding the local fauna associated with casework at various places, particularly indoors and outdoors. There is evidence of variation in outdoor and indoor temperature and humidity, which may induce bias in the investigation of weather-related biological impacts (Nguyen and Dockery 2016, Chiesa et al. 2019, Habeebullah et al. 2021) such as the decomposition of corpses and, consequently, the PMI estimation (Reibe and Madea 2010). This, in turn, necessitates extensive research into carcass decomposition and the colonizing insect taxa at indoor versus outdoor sites in human dwellings. Therefore, the current study was conducted to comparatively investigate the decomposition process of rabbit carcasses and the succession pattern of carcass-attracted flies at a large multi-story building over the summer and winter seasons. The findings from this study may help us understand not only the impact of this site variation on carcass decomposition and associated insect succession but also determine which fly species dominate each site. It may also contribute to the update and expansion of the database on the indoor carcass decomposition rate and the associated flies' taxa in Saudi Arabia.

Material and Methods

Meteorological Parameters

This study ran for 11 days in the summer (from the 1st to the 12th of August 2021) and 23 days in the winter (from the 6th to the 28th of January 2022). The climate in Riyadh city is often hot and dry in the summer and cold in the winter, with rare rains. The summer and winter seasons, as well as the atmospheric parameters during the study periods, were determined via the Saudi National Center for Meteorology (National Center for Meteorology 2021). Because the temperatures and relative humidity (RH) vary between outdoor and indoor sites (Nguyen and Dockery 2016, Chiesa et al. 2019, Habeebullah et al. 2021) which may induce bias in studies of weather-related biological impacts, they were additionally measured manually on-site in a daily manner (at midday) following Al-Qurashi et al. (2023).

Experimental Building

This study was carried out in Building 35 at the students' campus of King Saud University (24°43'23.2"N 46°37'16.2"E), Riyadh, Saudi Arabia (Fig. 1A), with the approval of the Deanship of Student Affairs. This building had been vacant (out of service) for 2 years prior to carrying out this study. It was targeted because of the difficulty of carrying out such a study in a human-occupied building. It is a large building with an area of 1,911.2 m^2 and composed of 4 identical multi-story sections of 477.8 m^2 each (Vice Presidency for Projects, King Saud University, https://projects.ksu.edu.sa/en) (personal communication). Each section of the building contains 4 stories: the ground floor (Gr), first floor (F1), second floor (F2), and third floor (F3) (Fig. 1B). The western front section (Part 1) (Fig. 1A) was selected for carrying out this study.

Experimental Sites

Three experiments were conducted in parallel at 3 experimental sites: the outdoor (Ot), the indoor (4 stories), and the roof (Ro) (≈ 12 m high). The experiment at each site was carried out in triplicate ($n = 3$); each replicate was done on 1 of the 3 assigned front (southern), right (eastern), and left (western) sides of the building.

On the Ot site, the 3 experimental replicates were carried out 10 m outside the building in each of the surrounding areas of the 3 assigned sides. Each area was sandy with no grasses or plants and was designated for 1 carcass (Fig. 1C).

On the indoor site, the 3 experimental replicates were carried out in 3 individual living rooms on each of the 4 floors (Gr, F1, F2, and F3). One room was chosen on each of the assigned sides (1 room per side). All rooms were dusty, similar in dimensions,

Fig. 1. The experimental sites: A) a Google Map represents the location of building 35 and its 4 multi-story parts (1, 2, 3, and 4) (inside the square). B) The internal view of the right (eastern) side of part 1, as an example of the 3 experimental sides, shows the experimental rooms arranged in reciprocal successive order (arrows). C) The left (western) side, as an example of the 3 Ot sites, shows a metal cage containing a carcass (inside the circle) located 10 m away from the building (the double-headed arrow). D) An example of the experimental rooms showing the furniture components: the door (1), a wardrobe (2), a study table (3) a chair (4), a single bed (5), a small sofa (6), 1 window (7) with its fabric curtain (8). A metal cage containing 1 rabbit carcass and 2 pitfall traps (9) was placed in the free space in the room center. E) An example of the 3 Ro sides (the western side) showing a metal cage containing a carcass (inside the circle) placed equidistance from both ends. Gr: ground floor; F1: first floor; F2: second floor; F3: third floor; and Ro: the roof.

had the same furniture components, and had no carpets on the ground. As shown in Fig. 1D, each room has dimensions of 350 cm long × 220 cm wide × 300 cm high, a door of 220 cm high × 80 cm wide, and 1 window located on the opposite side facing the door with dimensions of 115 cm high × 50 cm wide and a brown fabric curtain. All of the furniture was brownish and included a large wardrobe (270 cm long × 50 cm wide × 300 cm high) on the left side, a single bed (200 cm long × 90 cm wide × 50 cm high) on the right side, a study table (100 cm long × 75 cm wide × 70 cm high) located on the front side underneath the window along with a chair, and a small setting sofa on the right side of the door next to the bed. The doors and windows of the experimental rooms were left wide open during the experiments to provide easy access for the carcass-seeking insects. One carcass was assigned to each room (1 carcass per room) (Fig. 1D).

On the Ro site, the 3 experimental replicates were carried out in the space areas on top of the 3 assigned building sides. Each area was exposed to the sun and open air, with dimensions of 10 m long × 3 m wide. Each direction was designated for 1 rabbit carcass (Fig. 1E).

Experimental Animals

The experimental animal model used in this study was the rabbit, *Oryctolagus cuniculus* (Linnaeus, 1758), according to Mapara et al. (2012) and following previous studies (Silahuddin et al. 2015, Dautartas et al. 2018, Al-Qurashi et al. 2023, Khalil et al. 2023). Male rabbits were purchased from a local farmer's market prior to the experimental use in each season. Animals were euthanized with CO_2 at the Animal House of Zoology Department, College of Science, King Saud University, according to Conlee et al. (2005) and following Al-Qurashi et al. (2023). Carcasses were immediately transported to the experimental building to carry out the experiments. At the experimental sites, each carcass was placed in an iron cage (50 × 40 × 25 cm each) (Fig. 1C–E) for protection against predators and scavengers, as detailed in (Al-Qurashi et al. 2023, Khalil et al. 2023). Killing animals and carrying out the experiments took place in accordance with the Research Ethical Committee at King Saud University (approval code: KSU-SE-22-82).

Experimental Design

For carrying out the 3 experiments in each season, a total of 18 adult rabbits (2.8–3.0 kg each) were divided into 3 groups. The first group (3 carcasses) was used for the Ot experiment. One carcass was placed 10 m away from the building on each of the 3 assigned experimental sides (Fig. 1C). The second group (12 carcasses) was used for the indoor experiment on the 4 successive floors, Gr, F1, F2, and F3. On each floor, 3 different rooms (1 room on each of the 3 sides) were assigned to 3 different carcasses (1 carcass per room) (Fig. 1D). The 12 experimental rooms were chosen in a reciprocal successive order (Fig. 1B) to increase the distance between them in order to provide isolated resources for carcass-seeking insects and to minimize the effect of odor interference, which could affect insect succession (Lewis and Benbow 2011, Al-Qurashi et al. 2023, Khalil et al. 2023). During the experiment, the light and the conditioning system were turned off, and no one entered the room except to inspect the carcasses at the experimental time intervals. The third group (3 carcasses) was used for the Ro experiment, as 1 carcass was placed on top of each of the 3 assigned sides (Fig. 1E). Carcasses at all experimental sites were observed and investigated on a daily basis until they dried completely (skeletonized).

Carcass Decomposition Process

Each carcass was examined for 10 min, from the first day of exposure (the day of rabbits' euthanasia) until completely skeletonized (Parmenter and MacMahon 2009, Al-Qurashi et al. 2023, Khalil et al. 2023). The 4 categorized decomposition stages—fresh, bloated, decay, and dry—were observed and investigated. The duration (in days) of each decomposition stage was reported. In addition, carcasses' temperature (°C) was measured daily (for 1 min) via the anus using a Lascar EL-USB-2 digital thermometer (Omron, China) according to the instruction manual and following Al-Qurashi et al. (2023).

Flies Collection and Identificatio

Carcass-attracted flies were collected from the experimental sites over 10 minutes period on hourly basis from 8 am to 5 pm during the first 3 days postmortem. Afterward, insects were collected once a day at 9 am until the end of the experiment in each season, according to (Mashaly and Al-Mekhlafi 2016, Khalil et al. 2023). Flies were collected using both sweeping nets (5 repeated nets per visit) and pitfall traps. Pitfall traps were used to extend monitoring beyond the collection time and to minimize the disturbance of carcass-attracted insects, as recommended by Majer (1997). Sampling was performed without significant consumption of flies to avoid adverse impacts on the subsequent collection and decomposition processes, as recommended by Michaud and Moreau (2013). Collected flies were immediately preserved in 70% ethanol, labeled, transported to the laboratory, and stored at 4 °C for subsequent investigation and identification as detailed in Khalil et al. (2023). At the end of the study period of each season, collected flies were counted, labeled, and morphologically identified at the King Saud University Museum of Arthropods (https://cfas.ksu.edu.sa/en/node/3075) by the third and fourth authors, H. Al-Dhafer and M. Abdel-Dayem.

Statistical Analysis

Data were statistically analyzed using MINITAB software (MINITAB, State College, PA, version 18.1, 2018, UK). The Anderson–Darling Normality Test was used for testing data normality, according to Morrison (2002), prior to any further analysis. All results are presented as means (of 3 replicates) ± standard errors (SE), as determined by basic statistical analyses. Data pertaining to the atmospheric wind speeds and the on-site recorded RH were not normally distributed, and, thus, were analyzed using the non-parametric Mann–Whitney *U*-test. Data pertaining to the atmospheric temperature and RH were normally distributed and thus, a 2-sample *t*-test was used to compare differences between the summer and winter. Data on fly counts were normally distributed, and thus, comparisons between the experimental sites were made using one-way ANOVA, and differences between means were analyzed using the multiple Tukey's pairwise comparison test, according to Morrison (2002). Finally, when only 1 or 2 individuals of particular fly species were reported, they were categorized as singleton or doubleton, respectively, according to Novotný and Basset (2000) and following Mashaly et al. (2020a), Al-Qurashi et al. (2023), and Khalil et al. (2023). All experiments were carried out in triplicate using 3 individual rabbit carcasses ($n = 3$).

Results

Meteorological Parameters

Atmospheric parameters.

The atmospheric parameters during the studied periods of the summer and winter in Riyadh city are represented graphically in Fig. 2. The maximum and minimum temperatures were constant over the course of the 10-day summer experiment (Fig. 2A), with averages of 44.3 °C and 29.2 °C, respectively (Fig. 2B). They were, however, comparably variable and significantly lower over the course of the 23-day winter experiment (Fig. 2C), with averages of 21.1 °C and 9.5 °C, respectively (Fig. 2D) (*t*-test, $P < 0.05$).

The RH varied significantly between the summer and winter (Fig. 2A and C), with an average of 13.1% in the summer (Fig. 2B) and 48.5% in the winter (Fig. 2D) (Mann–Whitney *U*, $P < 0.05$, $n = 10$). Wind speed varied significantly in winter (Fig. 2C) compared to summer (Fig. 2A), with a lower average of 10.4 km/h in winter (Fig. 2D) compared to 14.6 km/h in summer (Fig. 2B) (*t*-test, $P < 0.05$, $n = 10$).

On-site recorded weather parameters.

In the summer, the one-way ANOVA revealed significant differences in the mean temperatures between the Ot and both the Gr and the Ro, the F1 and both F3 and Ro, the F2 and Ro, the F3 and Gr, and the Gr and Ro ($F_{5,60} = 14.29$; $P < 0.05$, $n = 11$) (Figs. 3A and 5A), with an overall average range from 38.1 °C to 43.0 °C (Fig. 3B). Whereas, winter showed no variation ($F_{5,132} = 1.69$; $P > 0.05$, $n = 23$) (Figs. 3C and 5B), with an overall average range from 18.0 °C to 19.6 °C (Fig. 2D). The winter temperatures fluctuated, with 2 peaks on days 7 and 20 and a low record on day 13 (Fig. 3C).

The RH was constant in the summer at all sites, recording 10% throughout the study period (Fig. 3E). However, in the winter, it

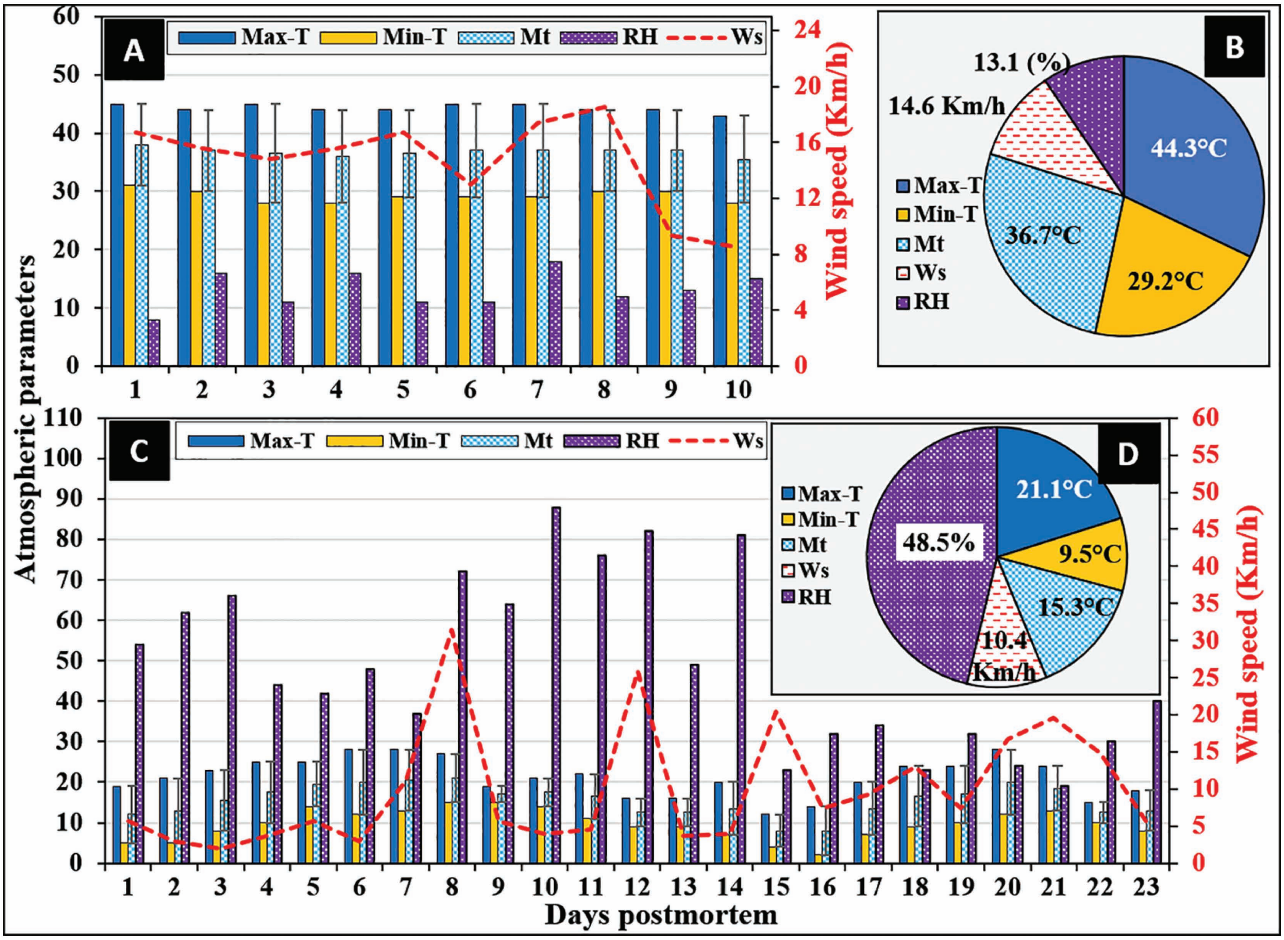

Fig. 2. The atmospheric parameters and their overall averages during the study periods in the summer A) and B) and winter C) and D). Max-T: maximum temperature, Min-T: minimum temperature, Mt: the mean of maximum and minimum temperatures ($n = 10$), RH: relative humidity, and Ws: wind speed.

recorded 10% on the first day, increased until it reached 75% on days 9 and 12, which might be attributed to the rain occurring on these 2 days, and then returned to 10% again from day 13 until the end of the experiment (Fig. 3F). All of the experimental sites had similar recorded RH (Mann–Whitney *U* test, $P > 0.05$, $n = 23$), with an overall average range from 18% to 19% (Fig. 3G).

Profile of carcass decomposition process.

Carcasses temperature.

The temperatures of the indoor and outdoor rabbit carcasses throughout the experimental periods in both seasons are presented in Fig. 4. The one-way ANOVA revealed an overall significant difference in the mean carcass temperatures between the 3 experimental sites in the summer ($F_{5,54} = 23.9$, $P < 0.05$, $n = 10$) (Figs. 4A and 5C) and winter ($F_{5,132} = 3.04$, $P < 0.05$, $n = 23$) (Figs. 4C and 5D). In the summer, Tukey's simultaneous 95% confidence levels revealed significant variation in the carcass' temperatures between both the Ot and Ro, as well as between each of the Ot and Ro compared to the other sites (Fig. 5C), with an overall average ranging from 36.66 °C to 42.36 °C (Fig. 3B). While in the winter, it revealed a significant variation in the mean carcasses' temperatures only between the Gr and Ro (Fig. 5D), with an overall average ranging from 14.7 °C to 17.6 °C (Fig. 4D). Moreover, carcasses' temperatures fluctuated across all sites during the study period, with 2 peaks on days 8 and 20, and lowest record on day 13 (Fig. 4C). This fluctuation corresponds to the on-site measured ambient temperatures (Fig. 3C).

Decomposition stages.

The data in Supplementary Table 1 represent the 4 reported decomposition stages: fresh, bloating, decay, and dry, in both the summer and winter. The fresh stage showed features similar to those before euthanasia (all body parts were still fresh and soft) and lasted only for a few hours (≈ 4 h) in the summer compared to 24 h in the winter at all sites. The bloating stage showed abdominal inflation and fluid leakage from the natural orifices, associated with a weak offensive putrefaction odor. This stage lasted for 1.5 days at all sites in the summer. While in the winter, it lasted 4 days (at Ro), 5 days (at the Ot), and 8 days (at the Gr, F1, F2, and F3). The decay stage showed moistened and rotten carcasses' bottoms a distinct offensive bad odor. This stage lasted 5 days at both Ot and Gr in the summer and increased at the subsequent floors toward the Ro, reaching 8 days at the F3 and Ro. While in the winter, it lasted 5 days (at the Ro), 6 days (at the Ot and Gr), and 7 days (at the F1, F2, and F3). The dry stage showed completely dried (skeletonized) and mummified carcasses with no bad smell, rottenness, or dampness. In the summer, this stage began on day 8 at the Ot, Gr, and F1, compared to days 9 and 11 at the other subsequent floors. While in the winter, dryness was recorded at the RO on day 11, but it began later on days 13, 16, and 17 at the Ot, Gr, and both the F1 and F2, respectively.

These findings suggest that the decomposition process was faster in the summer than in the winter, as the dry stage was reached in an average of 8.1 days compared to 14.1 days, respectively. Moreover, the decomposition process was faster at the Ot, Gr, and F1 than at

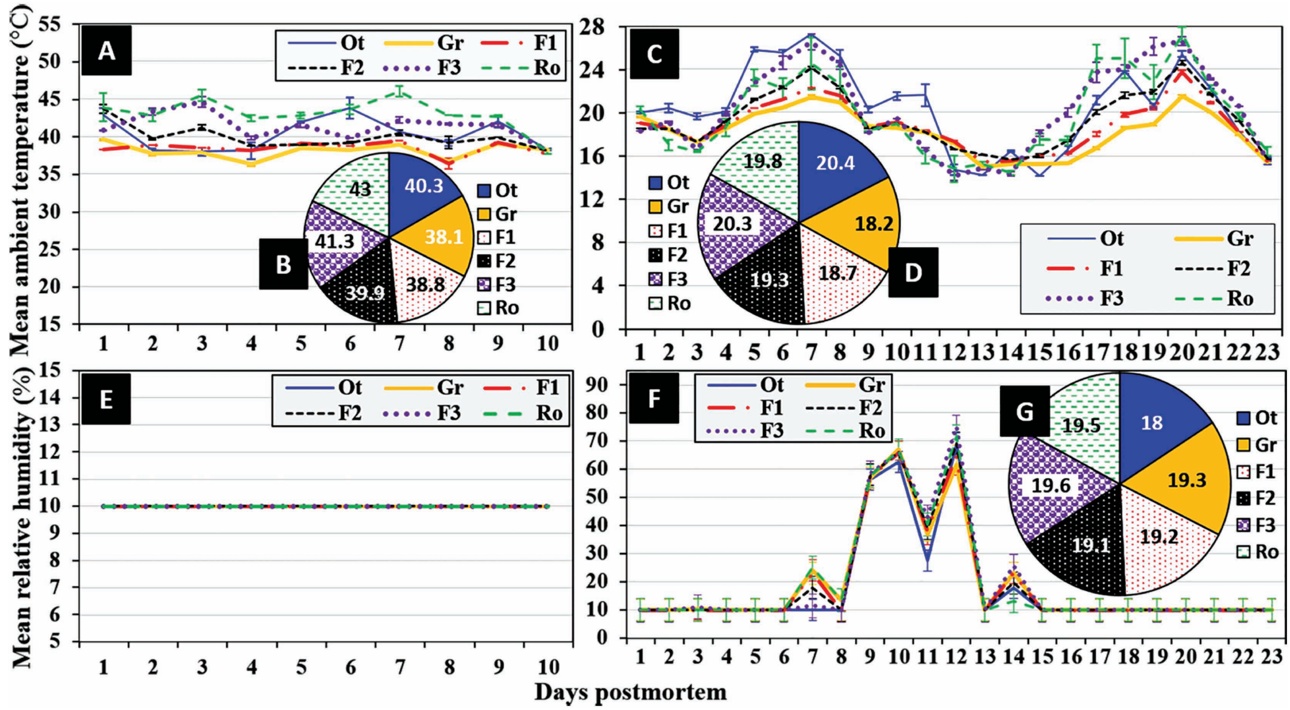

Fig. 3. The on-site manually recorded daily ambient weather factors. The temperatures and their overall averages at the experimental sites in the summer A) and B) and winter C) and D), respectively, and the RH and its overall average in the summer E) and winter F) and G), respectively. Ot: outdoor; Gr: ground floor; F1: fi st floor; F2: second floor; F3: third floor; an o: the roof.

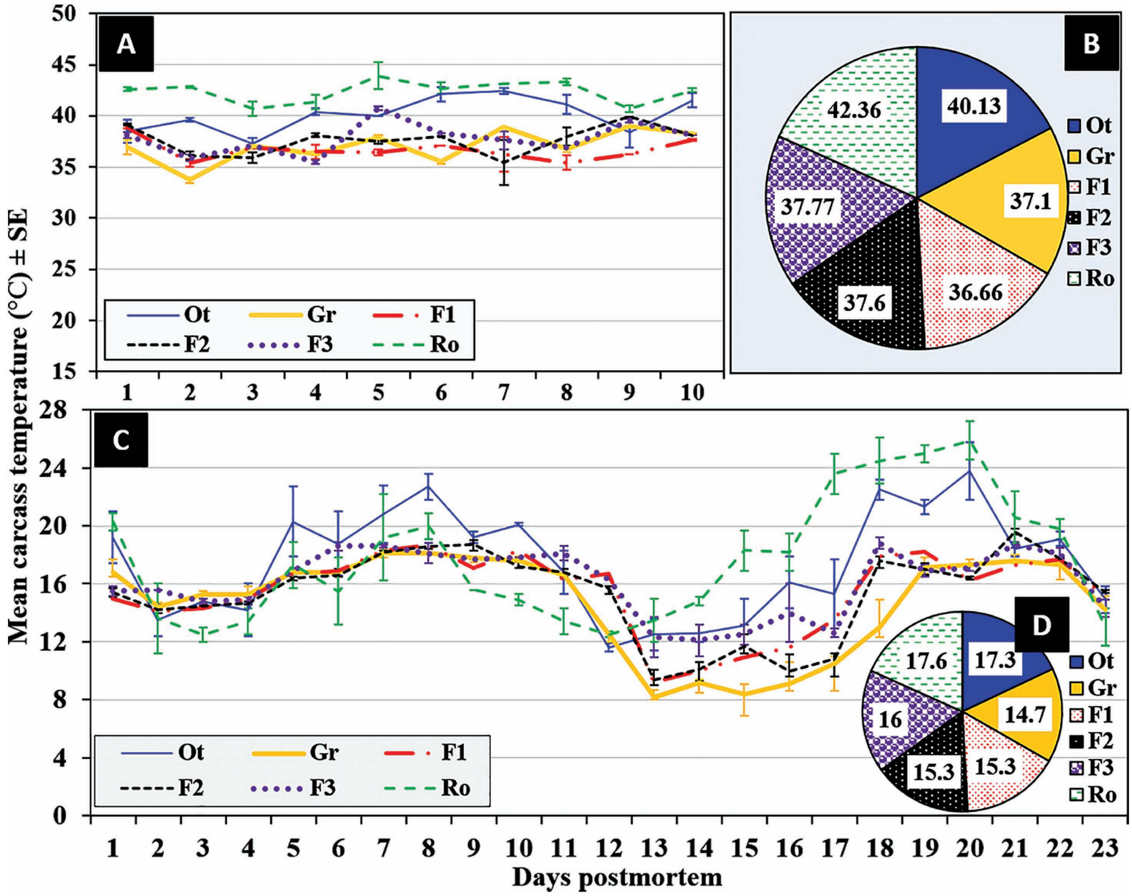

Fig. 4. The mean carcasses' temperatures and their overall averages over the experimental period at the experimental sites in summer A) and B) and winter C) and D). Ot: outdoor; Gr: ground floor; F1: first floor; F2: second floor; F3: third floor; an o: the roof.

the other sites in the summer, while in the winter, it was faster at the Ot and Ro than at the other sites. Finally, the fastest decomposition process lasted 7 days at the Ot in the summer, compared to 10 days at the Ro in the winter. These findings suggest that indoor carcass decomposition process was slower than that of the outdoor, and that the upper floors (F2 and F3) showed slower decomposition than the lower ones (Gr and F1).

Abundance of Carcasses Attracted Flies

A total of 6,069 carcass-attracted flies were collected in this study during the experimental periods of both the summer and winter and were distributed across the 3 experimental sites, as shown in Table 1. Data revealed that flies collected in the winter are 2.22 times more than those in the summer. The numbers of collected flies from Ot and indoor were similar in the summer. At the same time, the Ro showed a greater number than both indoor and Ot collectively (53.48% versus 46.5%), indicating that flies were more abundant on the Ro than on the other sites. In the winter, the Ot showed the largest number (47.01%), followed by the indoor (35.73%), and the Ro had the lowest (17.27%). These data could imply that the Ro had the highest percentage of flies collected in the summer but the lowest in the winter, and that the Ot had the highest percentage in the winter but the lowest in the summer.

The indoor collected flies in the summer (437 flies) were distributed across the 4 floors Gr, F1, F2, and F3 as 69.6 (3.71%), 179.53 (9.57%), 48.4 (2.58%), and 139.2 (7.42%), respectively, while those in the winter (1,498 flies) were distributed as 584.7 (13.95%), 498.4 (11.89%), 172.3 (4.11%), and 241.9 (5.77%) respectively. These data may indicate that F1 and Gr had the largest number of indoor collected flies in summer and winter, respectively, while F2 had the lowest in both seasons (Fig. 6A–C).

The one-way ANOVA and the Interval Plot of the pooled standard deviations both revealed that flies were attracted to carcasses in 2 significant peaks (waves) in the summer ($F_{31,64} = 17.94$, $P < 0.05$, $n = 3$) (Fig. 6D) and 4 in the winter ($F_{61,124} = 8.28$, $P < 0.05$, $n = 3$) (Fig. 6E). In the summer, the first wave was recorded at both the Ot and Ro on day 4, while the second was the largest and reported at the Ro on day 7 (Fig. 6A and D). Moreover, only a singleton (1 fly) was

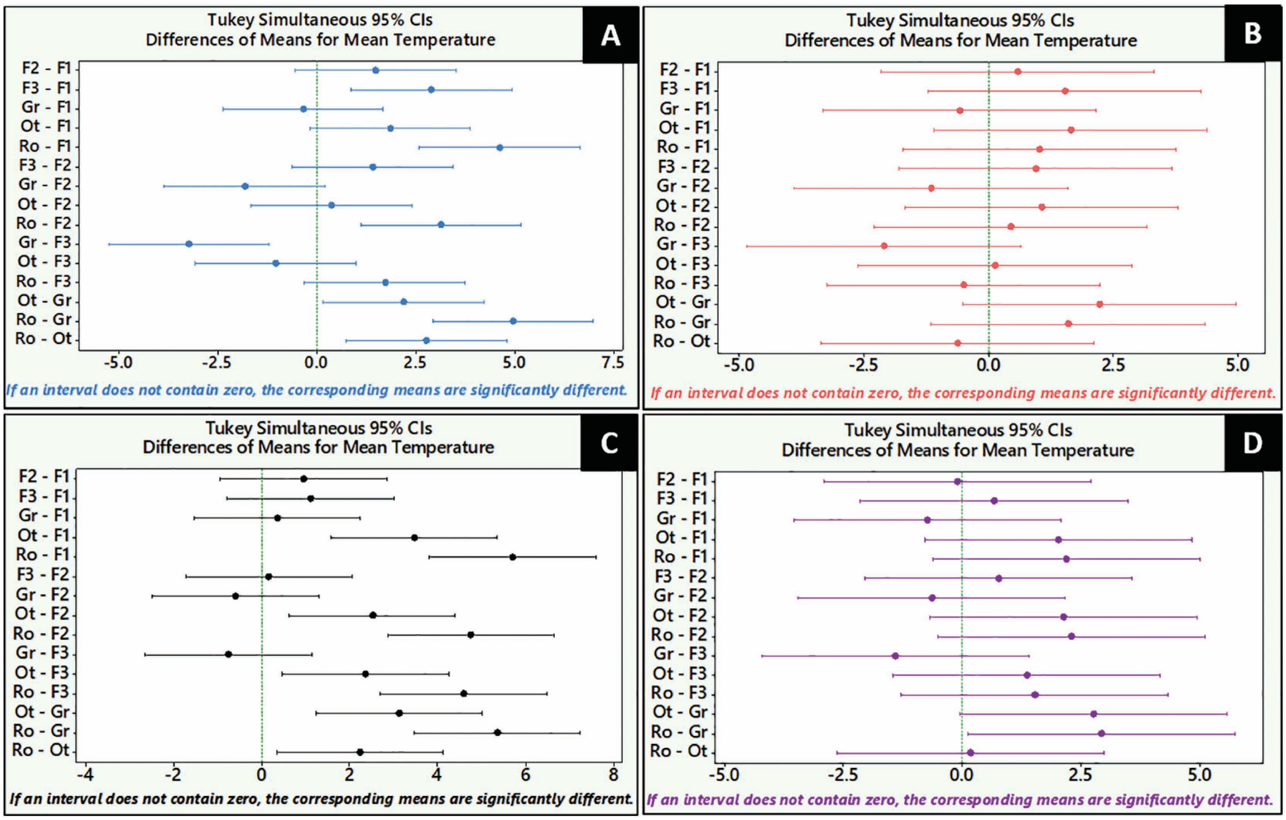

Fig. 5. One-way ANOVA showing Tukey's simultaneous 95% confidence levels for the means of the on-site recorded atmospheric temperatures during the experimental period in the summer A) and winter B) and the carcasses' temperatures in the summer C) and winter D), each versus the experimental sites. Intervals that do not contain zeros correspond to means that are significantly different. While those that contain zeros correspond to means that are not significantly di ferent. Ot: outdoor, Gr: ground floo , F1: fi st floo , F2: second floo , F3: third floo , and Ro: the roof.

Table 1. Numbers of collected flies from the experimental sites ver the summer and winter seasons

Sites	Outdoor		Indoor (4 floors)		Roof		Number/season	
Seasons	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Number	436	1,971	437	1,498	1,004	724	1,876	4,192
Percentage	23.22%	47.01%	23.28%	35.73%	53.48%	17.27%	30.91%	69.07%
Total number of collected flies							6,069	

recorded at the F1 on day 6 and at the F2 and F3 on day 7. While only 1 doubleton (2 flies) was recorded at the F3 and Ro on day 9 (Fig. 6A). In the winter, the first, second, third, and fourth waves were recorded only at the Ot on days 7, 8, 9, and 10 (Fig. 6B, C, and E). This may indicate that the carcasses at the Ot attracted significantly more flies than the other floors. Singletons were recorded at the Ot

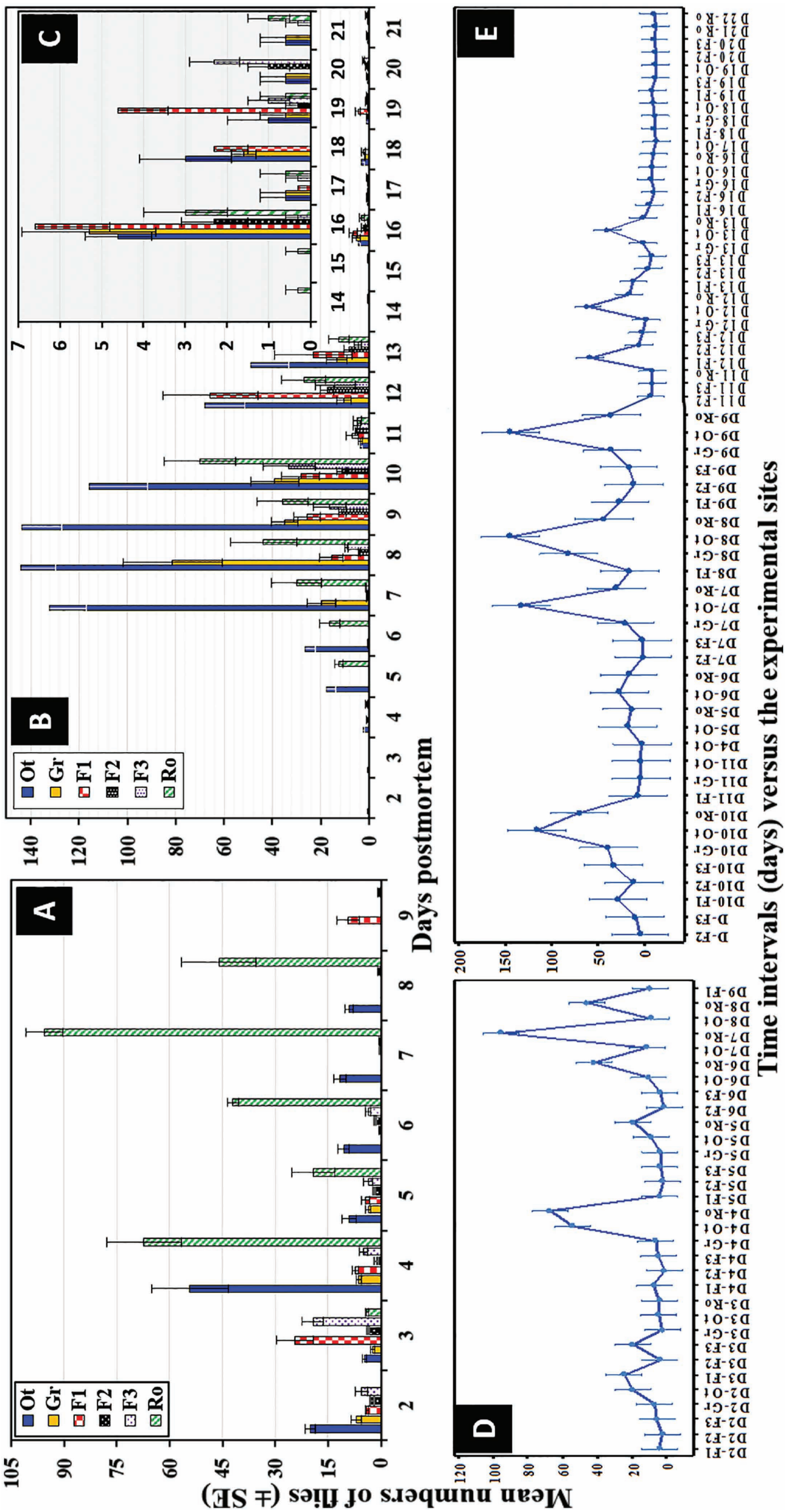

Fig. 6. The mean numbers of attracted flies to rabbit carcasses in summer A) and winter B) and C) at the experimental sites over the experimental periods (days postmortem). The pooled standard deviation was used to calculate and create the interval plot of mean numbers of flies at the experimental site versus days postmortem in the summer D) and winter E). Ot: outdoor; Gr: ground floor; F1: first floor; F2: second floor; F3: third floor; an o: the roof.

in days 2 and 3, Gr in day 6, F1 in day 17, F2 in day 19, F3 in days 16, 17, and 21, and Ro on days 14 and 15. While doubletons were only recorded at the Ot on days 17, 20, and 21, Gr on days 17, 19, 20, and 21, F1 on days 4 and 7, and Ro on days 17 and 19.

As an overall comparison, the overall number of collected flies in the winter was 2.2 times more than those in the summer (4,192 versus 1,876, respectively). The highest percentages of flies were collected from the Ro in the summer but from the Ot in the winter (53.48% and 47.01%, respectively) compared to the other sites of each season. On the indoor floors, the highest percentages of collected flies were collected from the F1 in summer and the Gr in winter (9.57% and 13.95%, respectively). In comparison, the lowest percentages were collected from the F2 in both summer and winter (2.58% and 4.11%, respectively). Finally, the carcasses at the Ot attracted more flies in the winter than in the summer (47.01% versus 23.20%, respectively), while those in the Ro attracted more flies in the summer than in the winter (53.51% versus 17.27%).

Differential Abundance of Flies

The differential abundance of carcass-attracted flies during each of the decomposition stages at the experimental sites in both seasons is presented in Fig. 7. No flies were recorded during the first 24 h postmortem (fresh stage) at all sites (Fig. 7 and Supplementary Table 1). Flies were observed and recorded starting from the bloating stage, reached maximum abundance during the decay stage, and declined to the minimum (no record in most sites) during the dry stage in both seasons. The one-way ANOVA revealed that the highest peak of flies' abundance was recorded during the decay stages at the Ro in the summer, as it accounted for 43.6% ($df_{14,30} = 82.6$, $P < 0.05$, $n = 3$ carcasses/site) (Fig. 7A), while it was at the Ot in the winter, as it accounted for 43.4% ($df_{17,36} = 27.09$, $P < 0.05$, $n = 3$ carcasses/site) (Fig. 7B). The second peaks were reported at the Ot during both the decay and dry stages, accounting for 16.2% and 14.4%, respectively, in the summer, and at the Gr and F1, accounting for 9.7% and 8.8% during the bloating and decay stages, respectively, in the winter. While the least abundance of flies was recorded during the bloating stages at the Gr (1.1%), F3 (0.8%), F1 (0.6%), and F2 (0.4%) in the summer, and at the Ro (0.9%), F2 (1.1%), and F3 (1.6%) in the winter. Finally, the dry stage showed an overall 15.9% of recorded flies at the Ot and F1 in the summer, compared to 9.1% at all sites in the winter. These findings could imply that no flies were observed at the fresh stage in either season, while they reached their peak abundance at the Ro in the summer and the Ot in the winter, flowed by the Ot in the summer and the Gr in the winter.

Differential Succession of Flies

As shown in Fig. 8 and Supplementary Table 2, 10 fly species belonging to 8 families were identified: Calliphoridae, represented by *Chrysomya albiceps* (Wiedemann, 1819); Drosophilidae, represented by *Scaptomyza pallida* (Zetterstedt, 1847); Ephydridae, represented by *Atissa pygmaea* (Haliday, 1833); Milichiidae, represented by *Desmametopa sp*.; Muscidae, represented by *Atherigona orientalis* Schiner, 1868, *Musca domestica* Linnaeus, 1758, and *Musca sp*.; Phoridae, represented by *Megaselia scalaris* (Loew, 1866); Sarcophagidae, represented by *Wohlfahrtia nuba* (Wiedemann 1830); and Ulidiidae, represented by *Physiphora alceae* (Preyssler, 1791) (Fig. 8 and Supplementary Table 2).

In the winter, all those species were recorded except *Masca sp.* (Muscidae). Moreover, *M. scalaris* (Phoridae) was recorded as a singleton at F3 during the bloating stage, and *A. pygmaea* (Ephydridae) was recorded as a doubleton at the Ot during the bloating and decay stages (Fig. 8B and Supplementary Table 2). In the summer, only 6 species belonging to 5 families were recorded, since Ephydridae, Milichiidae, and Phoridae were absent, and Muscidae was represented only by *M. domestica* and *Musca sp.* Moreover, *S. pallida* (Drosophilidae) was represented as a singleton only during the decay stage at the Gr. (Fig. 8A and Supplementary Table 2).

It was noticeable that *M. domestica* (Muscidae) and *C. albiceps* (Calliphoridae) dominated all the experimental sites in both seasons. The former was the most abundant in the summer, accounting for 46.9% of the reported flies at the Ro (Fig. 8A), while the latter was the most abundant in the winter, accounting for 40.3% at the Ot (Fig. 8B). The *S. pallida* (Drosophilidae) and *Musca sp.* (Muscidae) that were recorded in the summer, and the *A. orientalis* (Muscidae) and *M. scalaris* (Phoridae) that were recorded in the winter are not shown in Fig. 8A and B, respectively, since each is recorded as a singleton only (Supplementary Table 2).

Discussion

The present study constitutes an important step toward developing an experimental scenario for the indoor–outdoor-related crime scene in human dwellings. The rate of the decomposition process of rabbit carcasses and the quantitative and qualitative assessments of the associated fly species were investigated indoors versus outdoors and on the roof over the summer and winter seasons. In this regard, we would explain 4 points: (i) the experimental building had been vacant for 2 years prior to conducting this study; (ii) the indoor experiments were carried out inside the students' living rooms (3 solitary rooms in each of the 4 floors) with doors and windows wide-opened for providing access to the carcass-seeking insects; (iii) lights and air conditioning system were turned off during the experimental periods; and (iv) the university campus was uninhabited during the summer months due to the summer break, while it was actively inhabited (except the experimental building) during the winter experiments.

Deceased human bodies are reported in various environments, including indoor (Charabidze et al. 2014, Byrd and Castner 2019, Lutz et al. 2021). Consequently, indoor corpse-colonizing insects could be successfully utilized for estimating the PMI (Ren et al. 2017, Kadej et al. 2020), which might be variable according to the building's type, size, and construction. There are many studies reporting human corpses in various indoor sites and their associated insects in various countries (Campobasso et al. 2009, Pohjoismäki et al. 2010, Baz et al. 2015, Bugelli et al. 2015, Keshavarzi et al. 2016, Abdullah et al. 2017, Kadej et al. 2022a, b). In addition, there is an increasing interest in such studies in Riyadh city during autopsy (Mashaly et al. 2020c), outdoor (Al-Qahtni et al. 2019) as well as randomly reported indoor and outdoor cases (Alajmi et al. 2016, Al-Khalifa et al. 2020b, Al-Qahtni et al. 2020b). More importantly, the PMI was successfully estimated using the corpses-associated dipteran larvae in some of these case studies, which ranged from 5 to 12 days (Al-Qahtni et al. 2019), 4 days (Al-Qahtni et al. 2020b), and 1–15 days (Al-Khalifa et al. 2020b).

Although those aforementioned studies are forensically significant, other studies conducted by Campobasso et al. (2001) and Anderson (2011) proved that the indoor environment limits insects' access to the cadaver, which affects the decomposition rate and, in turn, could bias the PMI estimation. From the point of view of Forensic Ecology (Wiltshire 2019), this, in fact, emphasizes the need for more in-depth studies using animal models to determine the impact of various enclosed indoor environments on carcass decomposition and colonization by insects. Yet, the very few studies conducted using various animal models were limited to the ground floors in

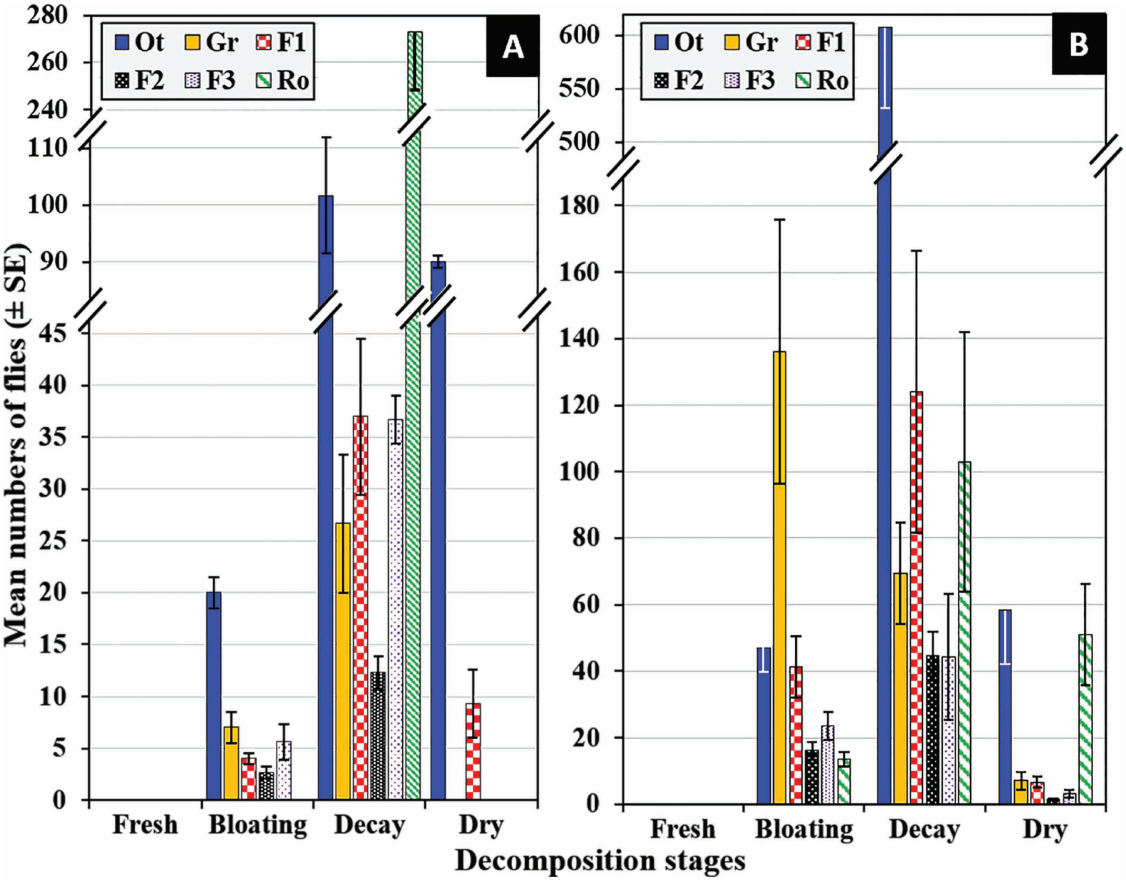

Fig. 7. The differential abundance of carcass-attracted flies during the decomposition stages at the experimental sites over the study periods in both the summer A) and winter B). Ot: outdoor; Gr: ground floor; F1: first floor; F2: second floor; F3: third floor; an o: the roof.

small houses (Ahmad et al. 2011, Yin et al. 2014, Keshavarzi et al. 2019) or on the ground floor versus the roof in a college building (Mashaly et al. 2020b). Therefore, more intensive studies on various shapes, levels, and constructions of indoor versus outdoor sites within human dwellings would help us to better understand not only the effect of indoor–outdoor site variation on insect succession and carcass decomposition but also to determine which fly species dominate each site over different seasons. This, in fact, necessitates carrying out the present study, which revealed that most flies were collected during the decay stages in both seasons and that those collected in the winter were 2.2 times more than those collected in the summer. In winter, the majority of flies were collected from the outdoor, while those in the summer were collected from the roof. In addition, most of the indoor flies were collected from the lower floors (first floor in the summer and ground floor in the winter), while the lowest number was collected from the upper floors (second and third floors) in both seasons. These data may indicate significant seasonal variations between outdoor and indoor, as well as between the successive indoor floors.

Four decomposition stages were observed in this study, as reported in previous studies in different habitats (Al-Mesbah et al. 2012, Chen et al. 2014, Richards et al. 2015, Al-Qurashi et al. 2023, Khalil et al. 2023). The recorded duration of each stage in the present study was different and site-dependent. Similar results were reported in different habitats (Dillon 1997, Al-Mesbah et al. 2012, Abouzied 2014, Anderson 2019). The overall duration of the carcass decomposition process was shorter in the summer (8.1 days on average) than in the winter (14.1 days on average). Various studies also reported this seasonal variation (Jarmusz and Bajerlein 2015, Rodriguez and Liria 2017, Oliveira and Vasconcelos 2020, Al-Qurashi et al. 2023). In addition, the decomposition duration in the outdoor carcasses was faster than that in the indoor, and on the indoor lower floors, it was faster than that on the higher floors in both seasons. This variation in the duration of the decomposition process could be attributed to the variation in temperature between indoor and outdoor sites (Nguyen and Dockery 2016, Chiesa et al. 2019, Habeebullah et al. 2021). This is supported by the on-site manually reported variation in the ambient and carcasses temperatures during the experimental period. Moreover, temperature variation has been proven to impact not only the decomposition process but also larval growth and development (Martin-Vega et al. 2017, Yang et al. 2017, Cervantes et al. 2018, Iancu et al. 2018b, Charabidze and Hedouin 2019, Chen et al. 2019, Wang et al. 2020, Alajmi et al. 2021). Taking all these findings, they may indicate that the duration of the decomposition process and flies' succession could be considered as site and seasonal markers, which should be addressed during forensic investigations. In addition, the lower temperatures of the experimental carcass compared to the atmospheric and on-site recorded ambient ones could be attributed to the postmortem biochemical alteration and degradation in the carcass tissues (Micozzi 1986, Goff and Win 1997).

As recorded in previous studies (AbouZied 2016, Shaalan et al. 2017, Al-Qurashi et al. 2023), the present study recorded significant seasonal variation in the atmospheric parameters that affected flies' succession and carcasses' decomposition. It has been proven that dipteran flies have a particular carcass decomposition-stag-dependent

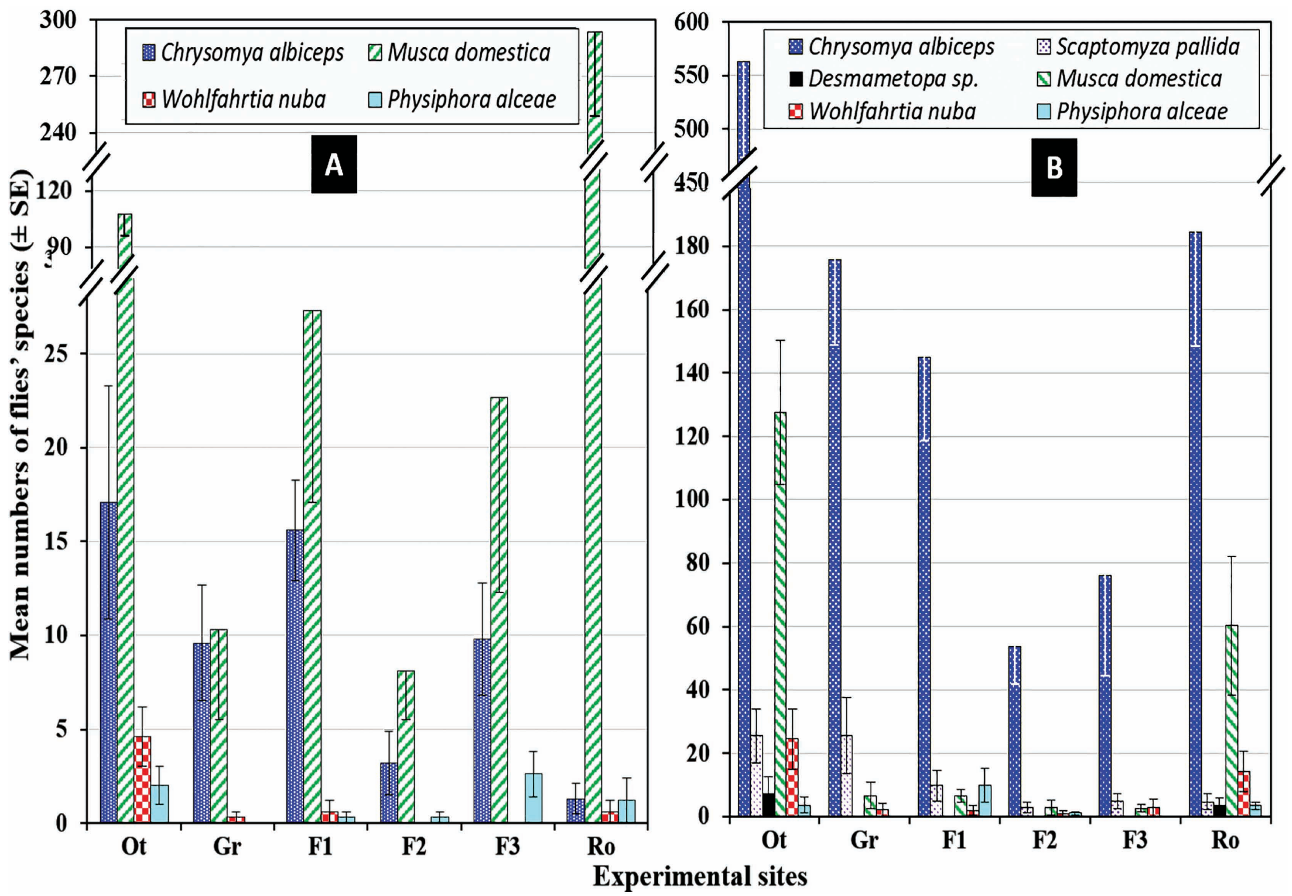

Fig. 8. The differential abundance of carcass-attracted fly species at the experimental sites over the study period in both the summer A) and winter B). Ot: outdoor; Gr: ground floor; F1: first floor; F2: second floor; F3: third floor; an o: the roof.

succession and, thus, could be used as decomposition-stage markers (Greenberg 1991, Mashaly and Al-Mekhlafi 2016, Khalil et al. 2023). In this context, the current study revealed that *Musca domestica* (Muscidae) was the most abundant species on the roof in the summer, whereas it was *Chrysomya albiceps* (Calliphoridae) outdoor in the winter, both during the decay stage. Furthermore, it has been reported that Hippoboscidae, Sphaeroceridae, Drosophilidae, Fanniidae, Phoridae, Piophilidae, and Ulidiidae are the dipteran families that contain the majority of rarely reported species, which arrive at carcasses in late decomposition stages (Novotný and Basset 2000). In the current study, the reported rare species (as singletons) were *Scaptomyza pallida* (Drosophilidae) on the ground floor in the summer during the decay stage on the ground floor, and *Atherigona orientalis* (Muscidae) outdoor in the winter during the decay stage. These data may suggest those species as decomposition stage-indoor–outdoor indicators.

In conclusion, a total of 6,068 flies were collected in this study; of them, 30.91% and 69.09% were collected during the summer and winter, respectively. Most of the flies were collected from the roof in the summer and from the outdoor in the winter, compared to the indoor site. Most indoor flies were collected from the lower floors (ground and first floors), while the minority were collected from the upper floors (the second and third floors) in both seasons. Ten fly species belonging to 8 families were identified in the winter, while only 6 species belonging to 5 of those families were recorded in the summer. *M. domestica* (Muscidae) was the most abundant in the summer reported on the roof, while *C. albiceps* (Calliphoridae) was the most abundant in the winter outdoor. Whereas, the least abundant species were *S. pallida* (Drosophilidae) and *Musca sp*. (Muscidae), in the summer, and *A. orientalis* (Muscidae) and *M. scalaris* (Phoridae), in the winter, since each is recorded as a singleton on various floors.

The decomposition process of rabbit carcasses was faster in the summer (8.1 days) than in the winter (14.1 days). The shortest decomposition duration was 7 days on the outdoor, ground, and first floors in the summer, while it was 10 days on the outdoor and roof in the winter. This study provided evidence of differences in carcass decomposition and the associated flies' community between outdoor, indoor floors, and the roof of a multi-story building over the summer and winter. It also constitutes an important step forward toward more research at human dwellings of different types, constructions, and sizes to fill in this gap in forensic Entomology and to provide additional knowledge to the context of indoor forensic insect fauna in Saudi Arabia, which, in turn, may contribute to providing helpful forensic evidence to justice in the field of criminology.

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Masha'el Y. Almutawa (Data curation [equal], Investigation [lead], Methodology [lead], Resources [equal], Visualization [equal], Writing—review & editing [equal]), Mohammed Al-Khalifa (Supervision [equal], Validation [equal], Writing—review & editing [equal]), Hathal Al Dhafer (Investigation [equal], Methodology [equal], Validation [equal], Writing—review & editing [equal]), Mahmoud Abdel-Dayem (Investigation [equal], Methodology [equal], Validation [equal], Writing—review & editing [equal]), Hossam Ebaid (Data curation [equal], Software [equal], Visualization [equal], Writing—review & editing [equal]), and Ashraf Ahmed (Conceptualization [equal], Data curation [equal], Formal analysis [lead], Funding acquisition [lead], Project administration [lead], Resources [equal], Software [equal], Supervision [equal], Validation [equal], Visualization [equal], Writing—original draft [lead], Writing—review & editing [equal])

Supplementary Material

Supplementary material is available at *Journal of Medical Entomology* online.

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