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Cover: Nilgiri Large Burrowing Spider *Haploclastus nilgirinus*. Acrylic on canvas. © Aakanksha Komanduri.

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ARTICLE

Seasonal study on succession of forensically significant entomofauna under indoor environment in Punjab, India

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Abstract: This study was focused on the prevalence of carrion insects under indoor environment, during the five seasons, i.e., winter, summer, monsson, post-monsoon, and spring. The pig carcasses were placed inside a room and daily (morning and evening) observations were made to collect the insect in addition to room temperature and humidity. A total of 1,187 insect specimens belonging to three insect orders: Diptera (692), Coleoptera (467), and Hymenoptera (28), 10 families, and 32 species were collected during the five seasons. The abundance and richness of each species varied in each season. However, it has been observed that the decomposition of pig carcasses was prompt in the monsoon season followed by summer, spring, post-monsoon, and winter seasons. *Sarcophaga* (*Liosarcophaga*) *aegyptica, Boettcherisca bengalensis*, *Calliphora vicina*, *Megaselia scalaris* (Coffin fly) were the exclusive species collected during experimentation. The data collected from these seasonal experiments can serve as the baseline data for indoor homicides, suicides, and related crime investigations as the insects collected from these experiments will help in determining the post-mortem interval of corpses belonging to that geographic location.

Keywords: Carcasse, Carrion, Coleoptera, decomposition, Diptera, Hymenoptera, indoor cases, insects, observation, post-mortem interval.

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Competing interests: The authors declare no competing interests.

Author details: Madhu Bala, assistant professor, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, working in the field of forensic entomology form the last 15 years. Succession studies were carried out to collect and study the biology of locally available blow flies, flesh flies and beetles of forensic importance that can be used in crime investigation. Morphological as well as molecular identifications of these arthropods have been done. Pawandeep Kaur, assistant professor, Sri Guru Gobind Singh College, Chandigarh, working with systematics of forensically relevant blowflies, flesh flies and beetles.

Author contributions: PK—data collection, manuscript preparation. MB—data authentication, manuscript editing.

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ज्ञान-विज्ञान विमुक्तये

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INTRODUCTION

The field of forensic entomology is concerned with using insects in legal investigations. Earlier studies have shown that insects are one of the major components of legal investigations (Ahmad & Ahmad 2002; Ahmad et al. 2011). Medico-legal forensic entomology is used in the estimation of post-mortem interval (PMI) along with the physiological and chemical changes that occur in the body before and after death, which are called antemortem and post-mortem changes respectively (Varatharajan & Sen 2000; Campobasso & Introna 2001). There are integral pathological changes such as rigor mortis, algor mortis, lividity, tissue autolysis, putrefaction, and bacterial activity that are accountable for PMI estimation, but they are reliable for just 72 hours (Henssge et al. 1995). However, the entomological methods of estimation of PMI are more rivaled in this field of science and help in finding the occurrence, distribution, abundance, richness, and species diversity of insect fauna visiting the carcasses during different decomposition stages. These entomological parameters are more reliable and undisputed compared to pathological methods, specifically during later stages of decay (Bala et al. 2016). Insects are ectothermic and specifically susceptible to climatic changes. Diversity and succession waves are affected by weather, temperature, relative humidity, body decomposition stage, size, and location of the carrion (Mann et al. 1990; Turchetto & Vanin 2004). Insect succession pattern in an indoor environment is quite different from outdoor environment. Under the indoor condition, the accessibility of carrion to entomofauna is restricted. This limitation can be attributed to factors such as physical barriers, lack of suitable entry points, or the absence of the necessary cues that attract decomposer insects. As a result, the decomposition process may be delayed or altered, leading to differences in the stages and patterns of decay compared to outdoor scenarios (Reibe & Madea 2010; Anderson 2011). It has been observed that many carrion insects in indoor environments are synanthropic (Anderson 2011). Various animals (pig, rabbit, cat, and rat) have been used as a research model to study successional and decomposition patterns (Early & Goff 1986; Tomberlin & Adler 1998; Anderson 2011; Bala & Kaur 2014; Zeariya et al. 2015; Kaur et al. 2020a). So, the aim of this study is to determine the insect succession pattern using pig carrion as a research model under indoor environmental conditions.

MATERIALS AND METHODS

Biological material

Two to six months old piglet carcasses (*Sus scrofa* L.) were used as a research model to collect the adult and immature insects from indoor. Piglet carcasses were procured from local slaughterhouse, and it was placed on the floor inside a room individually. There is no ethical issue, since dead piglets were utilized in this study. The experiments were conducted during winter, summer, monsoon, post-monsoon (2015), and spring season (2017). Five piglet carcasses, weighing 20 kg, were utilized during the study. The pig carcasses were placed on the floor inside the room (10’16’’ × 8’-16’’). The door of the room was kept closed. The window remains open to allow insect access and to allow some airflow. The observations were made two times a day, i.e., morning and evening. The experimental site was an agricultural land (30.46574º N, 75.53275º E) at Ghawaddi village of Ludhiana, Punjab (India). The land was mainly used to grow paddy crop in summer and wheat crop in winter. Eucalyptus trees were the main flora of the area.

Sampling of carrion insects

The flies were captured by sweeping hand net over the pig carcasses and then transferred to the killing jar. The insect collection was done within 15 minutes to avoid disturbance to carcass. Beetles and ants were collected with the help of forceps and fine brushes enriched with alcohol. After killing, the flies were preserved in 70% ethanol. The vials were placed into wooden collection boxes. For the identification, specimens were first relaxed in the relaxing boxes and then stretched with the help of entomological pins. After stretching, the specimens were placed in the fumigated wooden collection boxes, with the labeling of date, time, and temperature. Naphthalene balls were also placed inside the wooden collection box to avoid fungal growth. The collected insects were identified with a Stereo zoom microscope (Rescholar, Model No.RI-90-01) and by running the keys based on their morphological characters (Nandi 2002).

Sampling and rearing of immature stages

Larvae were collected from the carcasses with the help of forceps and brought into the rearing lab. The larvae were then placed on the moist sawdust in the rearing jar provided with a piece (15 g) of chicken meat for feeding. The developmental stages were observed until the emergence of adult flies from the pupae. After the emergence, the flies were identified with the help of standard keys based on their morphological characters provided by the following sources and experts.

Identification of insects

Flies were identified based on morphological characters like anterior spiracle, gena, calypter etc. Dipteran flies were identified by following the fauna of British India. For the identification of adult flesh fly, the abdomen of the male was cut off and soaked in a 10% KOH solution for 24 hours for softening. The terminalia was dissected and structures like, fifth sternite, inner and outer forceps, phallus were separated. These structures were kept in clove oil for better visibility. Species were identified by utilizing the keys given by Senior-White et al. (1940) and Nandi (2002). Hymenopterans were identified by Prof. Himender Bharti, Department of Zoology and Environmental Sciences, Punjabi University, Patiala. Members of the Histeridae were identified by Dr. Tomas Lackner, Zoologische Staatssammlung Munich, Germany. For Demestidae, keys of Royal Entomological Society, London (Peacock 1993) were used. Scarabaeidae was identified with the help of Dr. Devanshu Gupta, Zoological Survey of India, Kolkata. Staphylinidae was identified by comparing it with the collection at the Department of Entomology, IARI, Delhi. Cleridae was identified with the keys from a thesis entitled "The checkered beetles (Coleoptera: Cleridae) of Florida" (Leavengood Jr. 2008).

RESULTS

During the period of five seasons such as winter, summer, monsoon, post-monsoon, and spring, nearly 692 dipterans, 467 beetles (Coleoptera), and 28 hymenopterans were collected from the indoor pig carcasses (Table 2). The observation has clearly indicated the remarkable differences in terms of occurrence, abundance, and richness of insect species in indoor pig carcasses during different decomposition stages and seasons. As mentioned by Goff (2009), four stages of decomposition were recognized, i.e., fresh, bloated, advanced decay, and dry decay stages.

1. **Fresh stage**: It begins a few moments after the placement of the carcasses in the room. A few calliphorid flies appeared on the carcasses during the first day of carcasses placement; later, fly eggs, and a few calliphorid larvae were collected from the body's natural orifices, i.e., eyes, mouth, ear, nose, and genital areas. The body colour changes to pale (Pallor mortis), and a strong odour of decomposition were noticed at the end of this stage. This stage persists for four days in winter three days each in post-monsoon and spring, two days in monsoon, and one day in summer (Table 1)

2. **Bloated stage**: The carcasses turn bluish and greenish (Rigor mortis), and the limbs get stiffened. Putrefaction begins with a robust putrefying odour, and fluid seepage starts from the mouth and genital organs. To prevent the spread of foul odour, door was always closed, and experimental site was away from residential area. The body turned balloon-like due to gas accumulation (methane, hydrogen sulphide, carbon dioxide, and hydrogen). The larvae of calliphorid species were also collected and found in abundance. During this stage maggot mass appeared on the mouth and abdomen, especially in the trunk region. This stage remained for six days in winter, three days in both summer and post-monsoon seasons, for a single day in monsoon, and four days in the spring season (Table 1).

3. **Active decay stage**: This stage begins with the origination of "maggot mass". Hundreds of fly larvae form maggot mass over the neck and abdominal region of the carcasses. Maggot mass elevates the body temperature of carcass, thus, enhancing decomposition. The abundance of beetles and fewer flies were also an indication of the beginning of this stage. The flesh was reduced, and the bony carcass became visible. Several histerid beetles were seen in this stage, while many post-feeding larvae and fewer pupae of dipteran flies were also noticed. The ants were also found feeding on the dried skin and fluid seepage. The stage stayed on the carcasses for six days in the winter season, four days in summer, for three days during monsoon, five days in post-monsoon, and four days in the spring season respectively (Table 1).

4. **Dry decay stage**: It begins when the bones of the carcasses are visible. The dry decay stage had no significant end and continued for several months. This stage had not shown any abundance of flies; only a few newly emerged flies and some specimens of coleopteran were collected from this stage during this season. The decay stage on indoor carcasses were observed for six days in the winter season, four days each during summer and monsoon seasons, five days in the post-monsoon season, and three days in the spring season respectively (Table 1).

Comparative account of insect succession and rate of decomposition

Winter season: It took 22 days for the carcass to decompose completely (Table 1). The rate of decomposition was slow because of low temperature.

Table 1. Duration of decomposition of pig carcasses at different seasons (2015 & 2017) in Punjab, India.

Stages of Decomposition	Winter (Feb, 2015) (Days)	Summer (June, 2015) (Days)	Monsoon (July, 2015) (Days)	Post-Monsoon (Nov, 2015) (Days)	Spring (March, 2017) (Days)
Fresh stage	0–4	0–1	0–2	0–3	0–3
Bloated stage	4–10	1–3	2–3	3–6	3–7
Active decay	10–16	3–7	3–6	7–11	7–11
Dry decay stage	16–22	7–11	6–10	11–16	11–14
Total	22	11	10	16	14

Table 2. Number of insects collected from indoor pig carcasses during diverse seasons.

Insect order	Season					Total fauna
	Winter	Summer	Monsoon	Post-monsoon	Spring	
Diptera	176 (51.7%)	127 (54.9%)	141 (69.1%)	110 (55.2%)	138 (64.7%)	692
Coleoptera	153 (45%)	97 (41.9%)	61 (29.9%)	89 (44.7%)	67 (31.4%)	467
Hymenoptera	11 (3.2%)	07 (3%)	2 (0.98%)	0	08 (8.2%)	28
Total fauna	340	231	204	199	213	1187

The average temperature and relative humidity during the experimental period are given in Table 4. A total of 340 specimens belonging to 24 species and 10 families of three insect orders (Diptera, Coleoptera, and Hymenoptera), were collected during winter season from indoor pig carcass, of which, a total of 176 specimens belonging to 15 species from five families under Diptera (Calliphoridae, Sarcophagidae, Muscidae, Anthomyiidae, and Phoridae) were collected from the fresh, bloated, advanced decay and dry decay stages of decomposition. While from order Coleoptera, 153 specimens of beetles belonging to nine species were collected during different decomposition stages. However, only 11 specimens of Hymenoptera belonging to *Anochaetus graeffei* were reported (Table 3).

Summer season: Pig carcass decomposed in 11 days, and the rate of decomposition was significantly faster than that of the winter season (Table 1). This could be attributed to the ambient temperature reached above 35^{0}C and ranged 31–35 ^{0}C. Increased temperature and optimum relative humidity which ranged 65–81 % (Table 4) accelerated the decomposition, thus the pig carcasses decomposed faster in the summer season. The record indicated the collection of 231 specimens belonging to 21 species of dipterans, beetles and Hymenoptera during the summer season's indoor pig carcass. Of which, 127 specimens belonging to order Diptera, 97 to order Coleoptera, and seven specimens from the order Hymenoptera were collected (Tables 2 & 3).

Monsoon season: The carcass decomposed in 10 days, which was faster than the summer as well as winter (Table 1). The reason for the faster decomposition could be due to high relative humidity ranged from 60 to 80% and temperature ranged 29–32 ^{0}C (Table 4). Heavy rainfall prevails in the monsoon season that tend to rise the humidity which accelerate the decomposition process as these are favourable conditions for bacterial activity as well as for insect development. A total of 204 insect specimens (Diptera – 141; Coleoptera – 61; Hymenoptera – 02) belonging to 14 species were collected from the indoor pig carcass during monsoon season (Tables 2 & 3).

Post-monsoon season: The carcass took 16 days to decompose completely during post-monsoon period (Table 1). The decomposition process was slow because of low temperature. The ambient mean temperature ranged 15–26 ^{0}C, change in relative humidity ranged 70–88 % (Table 4) and change in duration of day-night length, slowed the process of decomposition. A total of 199 specimens (Diptera – 110; Coleoptera – 89) belonging to 18 species were collected from this season. No hymenopteran species was observed over the carcass during this season (Tables 2 & 3).

Spring season: The carcass took 14 days to decompose completely (Table 1). The maximum temperature recorded was 25^{0}C and 90% relative humidity (Table 4). Rate of decomposition was appreciably increased as compared to winter season because of rise in room temperature. This happened because of sunny days during the earlier stages of decomposition (Figure 5).

Table 3. Diversity of insect fauna collected from indoor pig carcasses at different seasons in Punjab, India.

Order	Family	Winter	Summer	Monsoon	Post-monsoon	Spring
Diptera	Calliphoridae	*Chrysomya megacephala* *Chrysomya rufifacies* *Chrysomya albiceps* *Lucilia cuprina* *Lucilia sericata*	*L. cuprina* *L. sericata*	*C. megacephala* *C. rufifacies* *Calliphora vomitoria* *L. cuprina* *L. sericata*	*C. megacephala* *C. rufifacies* *L. cuprina* *L. sericata*	*C. megacephala* *C. rufifacies* *C. albiceps* *C. vomitoria* *Calliphora vicina* *L. cuprina* *L. sericata*
	Sarcophagidae	*Sarcophaga misera* *Sarcophaga dux* *Sarcophaga aegyptica* *Sarcophaga albiceps* *Boettcherisca nathani*	*Sarcophaga hirtipes* *S. misera* *S. dux* *S. albiceps* *B. nathani*	*S. albiceps*	*Sarcophaga sericea* *S. dux* *S. albiceps*	*S. misera* *S. dux* *S. sericea* *S. albiceps*
	Muscidae	*Musca domestica* *Musca sorbens* *Synthesiomyia nudiseta*	*M. domestica* *M. sorbens* *S. nudiseta*	*M. domestica* *M. sorbens*	*M. domestica*	*M. domestica* *M. sorbens*
	Anthomyiidae	*Anthomyia* sp.	*Anthomyia* sp.	*Anthomyia* sp.	*Anthomyia* sp.	*Anthomyia* sp.
	Phoridae	*Megaselia scalaris*	*M. scalaris*	*M. scalaris*	*M. scalaris*	*M. scalaris*
Coleoptera	Histeridae	*Saprinus pensylvanicus* *Saprinus quadriguttatus* *Saprinus splendens* *Atholus maindroni*	*S. pensylvanicus* *S. quadriguttatus* *S. splendens* *Saprinus interruptus* *Merohister jekeli*	*S. pensylvanicus* *S. quadriguttatus*	*S. pensylvanicus* *S. quadriguttatus*	*S. pensylvanicus* *S. quadriguttatus* *S. splendens*
	Scarabidae	*Caccobius vulcanus* *Onthophagus cervus*	*C. vulcanus*		*C. vulcanus* *O. cervus*	
	Dermestidae	*Dermestes maculatus*	*D. maculatus*	*D. maculatus*	*D. maculatus*	*D. maculatus*
	Cleridae	*Necrobia rufipes*	*N. rufipes*	*N. rufipes*	*N. rufipes*	*N. rufipes*
	Staphylinidae		*Creophilus maxillosus*		*Creophilous flavipenis* *C. maxillosus*	*C. flavipenis*
Hymenoptera	Formicidae	*Anochaetus graeffei*	*A. graeffei*	*A. graeffei*		*A. graeffei*
						Camponotus compressus

A total of 213 specimens (Diptera – 138; Coleoptera – 67; Hymenoptera – 08) belonging to 29 species were collected during this season (Tables 2 & 3).

DISCUSSION

The present study revealed that the rate of decomposition was slow under the indoor environment, during winter as compared to all other seasons. Decrease in temperature and relative humidity are factors responsible for gradual decomposition with a range of 16–24 ^{0}C and relative humidity range of 30–80 % (Table 4). Similar results were reported by Ahmad & Ahmad (2002) and Ahmad et al. (2011) by using monkey and pig carcasses, where it had been concluded that the indoor carcasses took longer to decompose than the outdoor carcasses. The observations made by Kumara et al. (2012) on human cadaver were quite similar to the indoor pig carcasses. Observations of that study also reported similar dipteran fauna, i.e., *Chrysomya megacephala* (Fabricius 1794) (46%), followed by *C. rufifacies* (Macquart, 1842) (22%), *Sarcophaga* (*Liopygia*) *ruficornis* (Fabricius 1974) (5%), *Sarcophaga* sp. (4%), *Synthesiomyia nudiseta* Wulp, 1883 (6%), *Megaselia* sp. (3%) and *Megaselia scalaris* (Loew, 1866), (2%). Al-Khalifa et al. (2020) and Al-Qahtni et al. (2020) had also made similar observations from the indoor human carcasses and found *Chrysomya albiceps*, *Musca domestica*, and *Dermestes maculatus* as the most abundant species and determined the PMI from the larvae of *Musca domestica* and *Dermestes maculatus*. Succession patterns spanning three seasons for the insect fauna on pig cadavers were studied in Changwon, South Korea in 2018 and 2019.

Table 4. Data on average temperature and humidity in case of indoor pig carcasses during various seasons in Punjab, India.

Days	Winter		Summer		Monsoon		Post-monsoon		Spring	
	Average temperature ^{0}C	h (%)	Average temperature ^{0}C	h. (%)	Average temperature ^{0}C	h. (%)	Average temperature ^{0}C.	h. (%)	Average temperature ^{0}C	h. (%)
1	16.95	80	31.7	75	31.45	66	25.15	70	19.25	76
2	19.85	44	32.55	78	31.4	80	24.7	76	18.35	68
3	18.25	49	28.25	70	30.2	76	24.65	79	17.6	63
4	25.15	30	31.65	65	30.25	60	25.3	77	18.7	54
5	18.15	33	33.0	75	32.6	63	25.3	75	18.3	60
6	18.6	35	34.65	81	31.85	70	24.0	80	18.4	70
7	16.8	76	34.1	80	30.5	68	25.3	79	18.35	64
8	23.4	28	34.05	81	31.1	67	26.35	76	11.45	94
9	24.55	41	33.35	80	31.75	64	23.4	83	14.7	80
10	19.8	42	33.1	80	30.15	72	25.3	77	13.0	94
11	16.65	58	33.0	79	29.15	60	25.4	72	14.8	80
12	16.5	56					24.55	78	14.6	94
13	16.1	66					23.25	81	15.65	86
14	17.3	62					15.35	88	16.3	78
15	19.3	66					23.25	83		
16	18.25	66					23.15	88		
17	18.8	56								
18	17.2	52								
19	22.0	65								
20	22.05	36								
21	23.25	39								
22	22.9	70								

Park et al. (2022) collected and identified 107 species belonging to 41 families in six orders. The sequence of insect succession followed a general pattern in which Diptera peaked initially and followed by Coleoptera which is akin to the present study. *Chrysomya pinguis* was identified as the most frequently visiting species, and act as forensic indicator in Changwon. *Calliphora nigribarbis* occurred exclusively in spring and autumn, and *Pheropsophus javanus* occurred exclusively in one locality only. Therefore, these species may be important for characterizing the different seasons or locations during PMI estimations. Almutava et al. (2024) explored the rate of decomposition of rabbit carcasses and the succession pattern of the associated dipteran flies in outdoor, indoor, and on the roof of a 4-story building during the summer and winter. A total of 6,069 flies were recorded. From roof maximum flies were collected in summer but the least in the winter, whereas the outdoor showed the most in the winter and least in the summer. In present study also maximum flies' diversity is in winter as compared to summers. Ten fly species belonging to eight families were identified in the winter, whereas six species from five families were collected in the summer. The most abundant species was *Musca domestica* Linnaeus (Muscidae) on the roof in the summer, while it was *Chrysomya albiceps* (Wiedemann) (Calliphoridae) outdoor in the winter.

CONCLUSION

It has been observed that *Sarcophaga* (*Liosarcophaga*) *aegyptica* was reported for the first time from India during this study. It was collected during the bloated and active decay stage of the winter and spring seasons. Adding to it, *Boettcherisca bengalensis* has been reported for the first time from northwestern India. *Boettcherisca bengalensis* was observed from the fresh and bloated stages of pig carcasses during the monsoon season. Furthermore, *Synthesiomyia nudiseta* was reported for the first time in India from pig carcasses and collected from active decay stage during winter, summer, and spring seasons only. *Calliphora vicina* and *Megaselia scalaris* were restricted to the seasons where *C. vicina* was only found in winter, spring, and summer seasons while *M. scalaris* (Coffin fly) was observed in all the seasons. *Chrysomya albiceps*, *Musca domestica*, and *Dermestes maculatus* were found to be the most abundant species throughout the study during all the seasons. It has been observed that the decomposition was appreciably quick during the monsoon followed by summer season, however it was slow or delayed in the winter followed by spring and post-monsoon season. The maximum species richness was observed during summer and spring seasons, followed by the winter, post-monsoon, and monsoon seasons. Maximum species abundance has been observed in the winter followed by summer, spring, post-monsoon, and monsoon season. From the elucidated uses of carrion insects in forensic entomology, it is evident that forensic entomology is an interesting aspect of entomology with useful application to investigation and in aiding justice. So, the data collected from this study will definitely form a reference or baseline data to solve crime cases. It will help in PMI estimation, especially in murders and suicides in indoor environments. Further, the chances of error in calculating the PMI will be less by using these kinds of studies as references.

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