Decomposition of the Free-Ranging Urban Dog, *Canis Domesticus* (L.) Carcass with Reference to Insect Fauna Identification: A Tool for Legal Investigations

Farzana Perveen[a*], Pir Asmat[a]

[a] *Department of Zoology, Shaheed Benazir Bhutto University (SBBU), Main Campus, Sheringal, Khyber Pakhtunkhwa, Pakistan*

ABSTRACT: In the present study, insect fauna were identified in the carcass of the free-ranging urban dog, *Canis domesticus* (L.) in tropical region, *i.e.*, Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011. Decomposition of the carcass has been divided into 5 stages: fresh (1-12 h), bloat (13-36 h), active decay (37-84 h), advanced decay (85-166 h) and dry stage (167-286 h) that can be used as an important tool in legal investigations. The collected insects were identified into 11 species of 3 orders. First arrived species were the blow flies, *Chrysomya rufifacies* (Macquart) and jummper ant, *Myrmecia pilosula* (Smith) to the carcass. The species dominating in the early (fresh and bloat) stages of decomposition was *C. rufifacies* with their adults, pupae and larvae. The species dominating in the later (active and advanced decays) stages of decomposition was the clown beetle, *Hister sp.* (Gullenhal) with their adults, pupae and larvae. The average temperature (min.: 28.3±1.8; max.: 40.4±1.7), humidity (38.8%), and rainfalls (3.8 mm) were found affecting insect larvae, pupae and adults, and rate of decomposition of *C. domesticus*. This research showed fast rate of decomposition of *C. domesticus* carcass in Mardan region and the ecological succession pattern of insects were indicator for post mortem interval time estimation of death.

Keywords: Carcass, decomposition, ecological succession pattern, forensic entomology, tropical region

Introduction

The district of Mardan, Khyber Pakhtunkhwa, Pakistan lies between 34°-05' and 34°-32' north latitudes, 71°-48' and 72°-25' east longitudes and altitude of 283 m (928 ft) in the south-west. It is humid and heat is oppressive, due to intensive cultivation and artificial irrigation. A rapid fall of temperature has been recorded from October onwards. The coldest months are December and January. The minimum temperature recorded for January is 2.09 °C. Most of the rainfall occurs July-January. August is the rainiest month with 125.85 mm. There are occasional thunder and hail storms at the end of cold weather. The relative humidity is quite high throughout the year while maximum was recorded in December, *i.e.*, 73.33% [1].

The time and arrival of insect colonies can be estimated in carcasses [2]. These studies are commonly attempted to suspected criminal, for the purpose of knowing hidden facts for the investigation [3-5]. Insect life cycles begin within minutes of death which act as precise clocks [6]. Two dependent processes for the calculation of death time period are involved. The first is the growth of insect larvae that feed upon the victim. Most of the carrion insects rarely deposit offspring on a live person, therefore, the age of a larva provides a minimum time since death. The second is the succession of carrion arthropod species found in the body, which has the potential of providing both a minimum and maximum estimated post mortem interval (PMI) [7].

Post mortem interval estimations are based on the body decomposition, faunal evidence analysis and environmental influences [6]. Forensic entomological methods have been successfully applied in many cases, with the calculations of the PMI by entomological techniques fitting well with the time intervals established by other means [3-4]. However, these insects are considered to be the vultures of the insect world, with the ability to recognize decomposing bodies over vast distances and in any landscape type. They can travel for 20 km or more in a day [2]. In fact, almost every aspect of their biology suggests intense competition for rapid location and consumption of decomposing bodies [8-9].

Most commonly known insect's fauna for ecological succession pattern included the flies, beetles, wasps and ants. These species can simply be using the carcasses as for life cycles, food, shelter and site for oviposition etc. Adults of the flies use carrion for feeding. Larvae of a beetle *Hister sp.* are dominated and responsible for the maximum consumption of terrestrial carrion [9-13]. Hymenoptera species like European hornet wasp, *Vespa crabro* (Linnaeus) [14] and Jummper ant, *Myrmecia pilosula* (Smith) are predators, parasitoids, parasites, opportunistic feeders and scavengers [10, 11, 15].

Some taxa were restricted to bodies discovered inside, while others were found only associated with bodies in outdoor situations [16]. The pig, *Sus domesticus* L. carcasses that are free of insect's successions, decomposition occurred very slowly, retaining their form for many months. While the carcasses that are open to insect colonisation had 90% of their tissues remove in six days [17]. A carcass exposes to full sunlight decomposed faster than a carcass that is in shaded woodland. The shaded carcass has slightly less species (11 species) than the exposed one (16 species) [18]. Another factor may be the condition of bodies as Grisbaum *et al.* [19] reported that refrigeration of *S. domesticus* carcass does not alter the insect succession. Avila and Goff [20] reported that there was no marked differences being noted in insect fauna present or the duration of the stages of decomposition between burnt and control carcasses. However, the major oviposition by blow flies, *Chrysomya spp.* occurred one day earlier on burnt carcasses. All of these factors are continually being researched [21].

A death investigator in China, Sung Tz'u wrote a book entitled "The Washing Away of Wrongs" that reports wounds inflicted by a sickle. The death investigator assembled all the farmers from the neighborhood and had them lay their sickles on the ground. It was mid-summer, the weather was hot and *Chrysomya sp.* landed on only one sickle, whose owner confessed to the murder [22]. Francesco Redi rejected the idea of spontaneous generation based on the appearance of maggots on rotten meat [23]. In a study on ecological succession pattern of insect fauna on carcass, Orfila [24] reported 30 insects and other arthropods that visited to corpse for feeding and oviposition.

The objective of present research is to determine decomposition of the free-ranging urban dog, *Canis domesticus* (L.) carcass with reference to associated insect fauna identification as a tool for legal investigations.

Materials and methods

Experimental animal

The free-ranging urban dog, *Canis domesticus* (L.) was used as experimental animal.

Study design

The present research was based on observation of the ecological succession pattern of the insect fauna appeared and associated with the carcass of *C. domesticus* in tropical region, *i.e.*, Takht, Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan that can be used as an important tool in legal investigations.

Place and duration of study

The present research was done in Government Degree College, Mardan Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011 (ca. 11 d).

Methodology

Canis domesticus from the street of Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan, was put dazed using chloroform and the carcass was kept under the wire gaze cage (length: 54"; width: 36"; height: 32") in the ground of Government Degree College, Takht Bhai, Mardan under natural environmental conditions. A 5 kg stone was put on the cage to ensure that it was not disturbed by other living scavengers.

Temperature and humidity variations were noted daily for 3 times and 3 readings at interval of 30 min during each collection period were taken. Rainfall was observed to know the effects on the carcass decomposition. Temperature and humidity were confirmed from Government Metrological Centre, Mardan, Khyber Pakhtunkhwa, Pakistan and the rain-fall data was confirmed from Takkar Tehsil Branch of Government Irrigation Department, Mardan, Khyber Pakhtunkhwa, Pakistan on daily bases.

Data collection

Insects, *i.e.*, larvae, pupa and adults of different species were collected which associated with the carcass of *C. domesticus* 3 times in a day, *i.e.*, morning (ca. 0700-0800 h), noon (ca. 1200-1300 h) and evening (ca. 1800-1900 h). They were kept in labeled transparent glass bottles/jars containing 70% formalin each day separately for preservation.

The wire gaze cage was removed aside at every sampling time that the carcass was not disturbed during the study. It was exercised to the period of total fleshy tissues of dog until the dead body was exhausted. Flies were active in later morning and larval growth was more rapid in noon while beetles movement was more in evening. Adults of insects were collected through insects net while larvae and pupae and beetles crawling were collected through forceps. It was ensured that all species of insects with their all developmental stages were sampled. Gloves and mask were used to secure the self-body.

The pictures of each stage of decomposition of the carcass, adult insects and their developmental stages due to the expanding insect's masses were taken through digital camera (5 mega pixel, Sony, Tokyo, Japan). Collected insects were identified by Dr Ather Rafi, Senior Scientist, National Insect Museum, National Agriculture Research Council, Islamabad, Pakistan and forensic methods were used [25-29].

Results

Decomposition of the free-ranging urban dog, *Canis domesticus* (L.) carcass was determined by 5 stages, *i.e.*, fresh, bloated, active, advanced and dry [6]:

Fresh stage

During the first day after the death up to the evening, it was declared as fresh stage (12 h). The local insect fauna was restricted on the dead body. This stage showed maximum adult insect fauna than other stages of decomposition. In this stage, body structure was remained in original form except of dead. The outside appearance of the bodies was similar to those of live *C. domesticus*, however, a number of eggs and larvae of insects were observed (Figure 1a).

Bloated stage

This stage started from 1st day evening of death up to 3rd day morning (13-48 h), *i.e.*, 36 h. On 2nd day the body of *C. domesticus* emitted very strong smell that was higher than 1st day and it was characterised by swelling, over-large and cumbersome of the intestinal tract, therefore, it was named as bloat stage of decomposition. *Canis domesticus* abdomen became scratched and blackened. The abdominal parts were removed out of the body, and its internal parts were completely disappeared during night activity of the larvae. Upper parts of mouth, head, fore-legs and upper-abdomen region was completely decomposed except the some parts of lower-legs and ground located side of abdomen portion were left. In this stage, larvae were dominant which was occupied the body. Decomposition during bloat stage was very fast, while it was lasted for 3 or 4 days. Maximum numbers of larvae were observed at this stage. Their numbers were higher since morning and reached to the highest at noon (Figure 1b).

Active decay stage

This stage was completed in 3-4 days after death (49-96 h). The skin of *C. domesticus* blackened, emitted less smell and 80-90% of the body was decomposed. There were traces of flesh present on ground side of the body. Fast rain for 4 minutes at evening and random slow rain was recorded at day time for 10 min. At the morning, numbers of larvae were greatly reduced due to the affect of rain. During raining day, no adult species of insects was observed, as the larvae reduced its activities and showed migration (Figure 1c).

Advanced decay stage

Duration of this stage was 5-6 days after death (97-144 h) and was characterised by minor deep odor and removal of the soft internal tissues. The body was decomposed up to 90-95% (Figure 1d).

Dry stage

Duration of dry stage was 5 (7th-11th) days (145-265 h) of decomposition. The smell was very minor or absent. There were minor traces of skin and hairs in first 2 days of this stage, but after that the bones were totally exposed. At this stage flies were absent. For first 2 days few larvae of hide beetle, *Dermestes maculatus* De Geer; skin beetle *Trox sp*. F. and clown beetle, *Hister sp*. L. were rarely noted. In later days (9-11), no insect was collected due to their absence (Figure 1e and f).

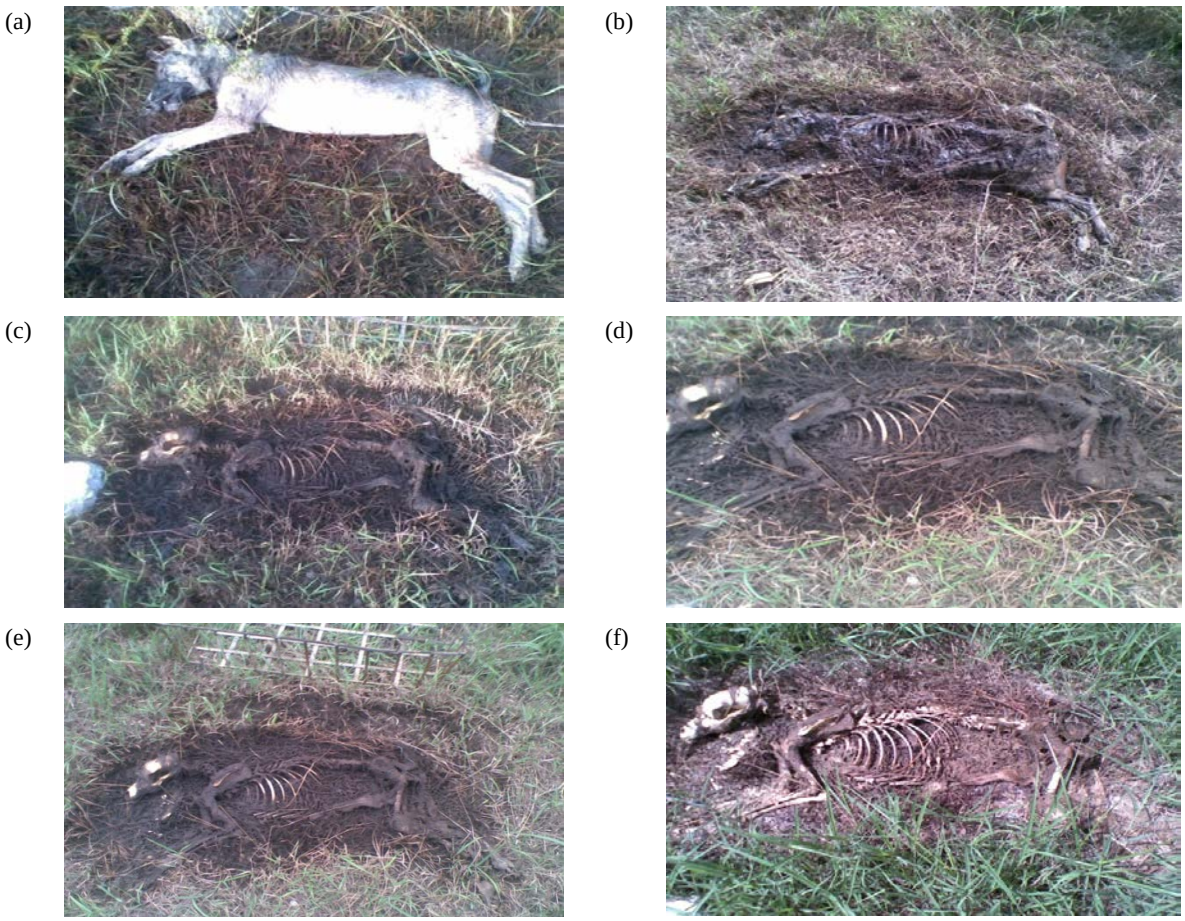

Figure 1 Stages of decomposition of the carcass of the free-ranging urban dog, *Canis domesticus* (L.) in tropical region, *i.e.*, Takht Bhai, Mardan Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011 has been divided into 5 stages; a: fresh (1-12 h); b: bloat (13-36 h); c: active decay (37-84 h); d: advanced decay (85-166 h); e and f: dry stage (167-286 h)

Species Collected

The collected insects were identified into 11 species of 3 orders. The blow flies, *Chrysomya rufifacies* (Macquart) and *C. megacephala* (Fabricius); flesh fly, *Parasarcophaga ruficornis* (Meigen); house fly, *Musca domestica* (Linnaeus), cheese fly, *Piophila casei* (Linnaeus) were belonging to order Diptera. The hide beetle, *Dermestes maculates* (Geer), clown beetle, *Hister sp.* (Gullenhal), ham beetle, *Necrobia rufipes* (Fabricus), skin beetle, *Trox sp.* (Harold) were belonging to order Coleoptera. The hornet wasp, *Vespa orientalis* (Linnaeus), jamper ant, *Myrmecia pilosula* (Smith) were belonging to order Hymenoptera.

In brief, *Chrysomya rufifacies* and *M. pilosula* that first arrived to the carcass could be used as a forensic indicator in tropical region. *Chrysomya rufifacies*, adults, larvae and pupa dominated the early stages (fresh and bloat) of decomposition. *Parasarcophaga ruficornis* and *M. domestica* were found during fresh, bloated and active stages. *Piophila casei* was only found in bloated stage and *Dermestes maculatus* was only found in active stages. *Necrobia rufipes* and *Trox sp.* were found in active, advanced and dry stages. *Histers* sp. adults, larvae and pupa dominated the later stages (active, advanced and dry) of decomposition. *Vespa orientalis* and *Myrmecia pilosula* were rare and only found in fresh stage (Table 1).

Table 1: The insect fauna associated with the free-ranging urban dog, *Canis domesticus* (L.) carcass observed during 5 different decomposition stages (fresh: 12 h; bloated: 13-48 h; active: 49-96 h; advanced: 97-144 h; dry: 145-265 h) in tropical region, *i.e.*, Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011

No	Associated insect fauna		Decomposition stages of *Canis domesticus*				
	Common names	*Scientific names*	fresh (12 h)	bloated (36 h)	active (3-4 d)	advanced (5-6 d)	dry (7-11 d)
1	Blow fly	*Chrysomya rufifacies*	P*	P*	P*	A*	A*
2	Blow fly	*Chrysomya megacephala*	P	P	P	A	A
3	Flesh fly	*Parasarcophaga ruficornis*	P	P	P	A	A
4	House fly	*Musca domestica*	P	P	P	A	A
5	Chese skipper	*Piophila casei*	A	P	A	A	A
6	Hide beetles	*Dermestes maculatus*	A	A	P	A	A
7	Clown beetles	*Hister sp.*	A	A	P	P	P
8	Ham beetle	*Necrobia rufipes*	A	A	P	P	P
9	Skin beetle	*Trox sp.*	A	A	P	P	P
10	Hornet wasp	*Vespa orientalis*	P	P	A	A	A
11	Jummper ants	*Myrmecia pilosula*	P	A	A	A	A

*(number in parentheses): duration of stage in h: hour; d: day; P or A: presence or absence of associated insect fauna in the stage mentioned in row.

Temperature

The study was conducted in summer to investigate the maximum insect fauna in *C. domesticus* carcass. Temperature variations were observed, it was lower during morning due to evaporation and higher during day time due to sun. Temperature changes were noted at morning, noon and evening. This study showed that the number of different insects species growth, rate of eggs lying, larvae development and abundance of insect were directly proportional to temperature. During experimental period (15-25 May 2011), minimum and maximum temperatures in the morning were 24.6±1.5 and 31±2 °C on 19 May 2011 and 24, 25 May 2011, respectively, minimum and maximum temperatures at noon were 36.6±1.5 and 41.6±1.5 °C on 19 May 2011 and 23, 24 May 2011, respectively, while, in the evening, minimum and maximum temperatures were 28.3±3.2 and 41.3±2.1 °C on 18 May 2011 and 25 May 2011, respectively. Averaged minimum and maximum temperatures of the day were 30.9±1.9 and 37.8±1.7 °C on 18 May 2011 and 25 May 2011, respectively. The averaged temperature of 11 days was 35.34±1.54 (Table 2).

Table 2: Temperatures variations associated with the insect fauna appeared on the free-ranging urban dog, *Canis domesticus* (L.) carcass were observed during 5 different decomposition stages in tropical region, *i.e.*, Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011

No	Date	n*	Temperatures (M±SD)*			
			Morning	Noon	Evening	Total
1	15/05/11	3	27.3±1.5	40±2	36.3±2.5	34.5±2.1
2	16/05/11	3	27.6±2.5	39.3±2.1	36.6±1.5	34.5±0.4
3	17/05/11	3	27.3±1.5	40.6±1.5	38.6±1.5	35.5±1.5
4	18/05/11	3	27.6±1.5	37±1	28.3±3.2	30.9±1.9
5	19/05/11	3	24.6±1.5	36.6±1.5	33.3±1.5	31.5±1.5
6	20/05/11	3	30.3±1.5	41±2	40.3±1.5	37.2±1.6
7	21/05/11	3	27.3±1.5	40.3±1.5	39.6±1.5	35.8±1.5
8	22/05/11	3	27.6±1.5	41±2	40.3±1.5	36.6±1.6
9	23/05/11	3	29.6±1.5	41.6±1.5	40±1	37.1±1.3
10	24/05/11	3	31±2	41.6±1.5	40.6±1.5	37.7±1.6
11	25/0511	3	31±1	41.3±2.1	41.3±2.1	37.8±1.7

*n: number of visits; temperature was noted 3 times in morning (8:00, 8:30, 9:00 am) afternoon (12:30, 1:00, 1:30 pm) and evening (6:00, 6:30, 7:00 pm); Mean±SD: mean ± standard deviation.

Humidity

Humidity had effect on insect fauna succession. It was observed that high humidity in the morning stopped earlier arrival of insect species due to rise of moisture of carcass. It was observed that the numbers of insect larvae were also reduced due to rise of humidity in morning and evening. The averaged minimum and maximum humidity of the day were 17.7±15.9 and 63±24.3 °C on 5 May 2011 and 7 May 2011, respectively. The average dhumidity was 38.8% (Table 3).

Table 3: Humidity variations associated with the insect fauna appeared on the free-ranging urban dog, *Canis domesticus* (L.) carcass were observed during 5 different decomposition stages in tropical region, *i.e.*, Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011

No	Date	n*	Humidity (M%±SD)*
1	15/05/11	3	32.6±24.1
2	16/05/11	3	44±27.2
3	17/05/11	3	39.3±22.1
4	18/05/11	3	57.3±7.4
5	19/05/11	3	63±24.3
6	20/05/11	3	49.3±19.7
7	21/05/11	3	17.7±15.9
8	22/05/11	3	41.5±25.6
9	23/05/11	3	30.3±18.4
10	24/05/11	3	34±22.9
11	25/05/11	3	28±20.2

*n: number of visits; humidity was noted 3 times in a day, morning (8:00 am), afternoon (1:30 pm) and evening (7:15 pm); M±SD: mean ± standard deviation.

Rainfall

The rainfalls ceased and slowed down adults insects and larval activities, respectively. The accumulative rainfall during the present research was 3.8 mm (Table 4).

Table 4: Rainfall variations associated with the insect fauna appeared on the free-ranging urban dog, *Canis domesticus* (L.) carcass were observed during 5 different decomposition stages in tropical region, *i.e.*, Takht Bhai, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011

No	Date	n*	Morning	Noon	Evening	Total
1	15/05/11	3	A*	A*	A*	A*
2	16/05/11	3	A	A	A	A
3	17/05/11	3	A	A	A	A
4	18/05/11	3	A	A	2.6 mm	2.6 mm
5	19/05/11	3	1.2 mm	A	A	1.2 mm
6	20/05/11	3	A	A	A	A
7	21/05/11	3	A	A	A	A
8	22/05/11	3	A	A	A	A
9	23/05/11	3	A	A	A	A
10	24/05/11	3	A	A	A	A
11	25/05/11	3	A	A	A	A

*n: number of visits; rainfalls was noted 3 times in a day, morning (8:00 am), afternoon (1:30 pm) and evening (7:15 pm); A: absence of rainfalls.

Discussion

In the present study, insect fauna on carcass of the free-ranging urban dog, *Canis domesticus* (L.) placed in tropical region, *i.e.*, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011 were identified. 3 orders, 10 families and 11 species were found. In comparison, Nidya *et al.* [30] reported that 12 kg pig, *Sus domesticus* L. carcass was placed in Colombia and collected 11 species belonging to 2 orders and 7 families. Shi *et al.* [31] observed the insect fauna succession on the rabbit, *Oryctolagus cuniculus* (L.) carcass in China, 4 stages of decomposition were divided into fresh, boat, decay and dry. In which 49 species were recorded of 3 orders and 15 families. In our study, the fast rate of decomposition of *C. domesticus* carcass was found because flies' numbers were abundance. However, *C. domesticus* carcass was the source of food and matting for insect species; if the flesh tissues were not quickly removed, may be more diversity of insects would be observed. Moreover, due to fast decomposition rate of carcass, the most of insect species were not come.

Shean *et al.* [18] examined differential rate of decomposition of *S. domesticus* carcass. In which they concluded that low and high temperatures retarded and fasted insect successions, respectively. Low temperature slowed decomposition and insect fauna succession and high temperature increased them. Introna *et al.* [32] reported sarcosaprophagous fly, *Sarcophaga carnaria* (L.) activity on carrion. They reported that temperature had highly influenced on flies larval development on carrion and its size. Tantawi *et al.* [33] examined arthropod succession on *O. cuniculus* cadaver. They showed a direct correlation between the rate of decomposition and temperature. It influenced the rate of decay and also affected the types of insects that arrived at a carcass in a given season. In the present study, it was observed that the averaged temperature was 35.35±1.54 °C, which could have increased insect succession rates. This accelerated larvae

development into adults and the carcass decomposition rate. Under high temperature, development of adults from eggs of *C. rufifacies* appeared in 1.5 d, *M. domesticus* attained 3.2 d, *C. megacephala* required 2.3 d, *P. ruficornis* achieved 2.9 d, *M. pilosula* required 3.5 d, *V. carabro* reached in 4.5 d, *P. casei* obtained 3 d, *Histers sp.* completed in 4 d, *D. maculates* required 5.4 d, *N. rufipes* attained 2.9 d and *Trox sp.* reached in 2.5 d, as compared to other regions.

Payne [10] concluded that high humidity produced cool conditions that reduced insect fauna carrion. However, low humidity showed rapid depletion of cadaver. A slowed decomposition rate decreased insect activity and altered the timing of insects arrival to carcass was due to high humidity. High humidity delayed flies and ceased larvae development. In the present study, average humidity was 38.58%; it was observed that high and low humidity produced low and high temperatures, respectively, which had effect on adults and larvae of insects. In all these studies, it was observed that low humidity developed warmer condition and high temperature which increased adults flies arrivals, larval growth and development of eggs into larvae.

Tantawi *et al.* [33] studied arthropods succession and concluded that rainfall fasted the rate of carrion decay. Shean *et al.* [18] examined differential rate of decomposition of *S. domesticus* cadaver and reported that differences in decomposition rates were seen due to rainfall. This decreased the insect fauna activities of the under wet conditions. It also decreased body weight, bloat size and number of insects. In the present study, rainfall was not occurred in fresh and bloat stages of decomposition and was noted a great diversity of flies species. The rainfall was occurred in active decay and advanced decay stages, therefore, accelerated decay stages. Further, in the present research, the fluctuation in temperature, low and high humidity and rainfall that associated the insects to the carcass and only a limited diversity of insects were observed. This may explain why only 10 families and their 11 species were collected belonging to 3 orders. If such research may be conducted in different seasons, more information and insect species will be obtained for forensic used.

Conclusion

The decomposition of *C. domesticus* carcass can be differentiated into 5 stages due to clear cut changes occurred from one stage to another stage in the tropical region, *i.e.*, Mardan, Khyber Pakhtunkhwa, Pakistan. The high temperature increased decomposition rate, as well as the number and growth of insects' larvae. However, the low humidity increased adults and larval activities of insects. Moreover, the rainfalls inhibited insects' larvae and adults to come on carcass, while it softened the tissues for fast decomposition.

Recommendation

It is recommended to formulate a chart for insect developmental stages under different temperatures, humidity and rainfall in different parts of the country. It is also recommended to study insects fauna on carcasses of different animal phyla and classes to get the knowledge about diversity of the ecological succession pattern of insect species.

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Additional information and reprint request:
Farzana Perveen
Email: farzana_san@hotmail.com
Chairperson and Associate Professor
Department of Zoology
Shaheed Benazir Bhutto University
Main Campus, Sheringal,
Khyber Pakhtunkhwa, Pakistan;
Office Phone: (092)-944-885529
Mobile: (092)-300-2253872
Fax: (092)-944-885805