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RESEARCH ARTICLE

ECOLOGICAL SUCCESSION PATTERN OF THE INSECT SPECIES ASSOCIATED WITH THE CARCASS OF THE FREE-RANGING URBAN DOG, *Canis domesticus* (L.): A TOOL FOR FORENSIC ENTOMOLOGY

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ABSTRACT

The free-ranging urban dog, *Canis domesticus* (L.) carcass decomposition in tropical region, i.e., Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011 was characterized by 5 stages, i.e., fresh (12 h), bloated (13-48 h), active (49-96 h), advanced (97-144 h) and dry (145-265 h) and the ecological succession pattern of insect species collected were identified into 11 species of 3 orders. The insect species associated with the carcass of *C. domesticus* found that the blow flies, *Chrysomya rufifacies* (Macquart) and house fly, *Musca domestica* (Linnaeus, 1758) were found in the fresh, bloat and active decay stages. However, the blow fly, *C. megacephala* (Fabricius); flesh fly, *Parasarcophaga ruficornis* (Meigen) and the hornet wasp, *Vespa orientalis* (Linnaeus) were existed in the fresh and bloat stages. Moreover, cheese fly, *Piophila casei* (Linnaeus) was found bloat and active decay stages. Further, the hide beetle, *Dermestes maculates* (Geer), clown beetle, *Hister sp.* (Gullenhal); ham beetle, *Necrobia rufipes* (Fabricus) and skin beetle, *Trox sp.* (Harold) completed their life cycle in active decay, advance decay and dry stages. Furthermore, the jummper ant, *Myrmecia pilosula* (Smith) was collected from only fresh stage. The specimens of insect species were collected minimum 0.8% of *V. orientalis* and maximum 21.3% of 2 species, i.e., *C. rufifacies* and *M. domestica*. The temperature, humidity and rainfalls were found affected insect larvae, pupae and adults, and rate of decomposition of *C. domesticus*. The average temperature, humidity and rainfalls were 35.34±1.54, 38.8% and 3.8 mm, respectively, of 11 experimental days. This research will be helpful for forensic entomologist for a case study of death to investigate of crimes in tropical region of Pakistan.

Keywords: *Canis domesticus*, carcass, decomposition, ecological succession pattern, forensic entomology, insect fauna, tropical region.

INTRODUCTION

They ecological succession pattern represents an important tools in legal investigations [1, 2]. Forensic entomology is divided into three main branches: Urban entomology is legal investigations involving insects and related animals that disturb manmade involving insects infesting stored commodities such as cereals and grains; Medico legal death; entomology also known forensic medical entomology because of its focus on crime, relates primarily to determine the time of death, place or region of death, way of causes involving of sudden death, accidents of traffic time (day or night) [3, 4].

The existence of the arthropods in particular insects is facilitated by the protein rich carcass resources [5]. The decomposers of carcasses can be divided into two groups. Those that consume the soft tissues of fresh carcasses i.e. Diptera and those that utilize the skin and hair material of carcasses are Coleoptera [6, 7]. They complete every stage of their life cycle on the carcasses but mostly in initial stages of decomposition and not in decay [8-10]. Coleoptera, is the second decomposers, become the most important forensic indicators during later stages of decomposition, as they feed upon the skin and hairs [11-12].

Three significant differences showed in gross appearances of 3 cases of human remains found in different environments. The postmortem intervals (PMI) were similar in all cases. In some cases forensically important arthropods can provide clues about the place of death (rural or urban; indoors or outdoors) or if the body has been moved [13]. The number of taxa on a body indoor decreased rapidly. However, it showed that there may be a greater variety of Diptera associated with them [10, 14-15]. This contrasts with the situation outdoors, where is an increase in the number of taxa through the first 3-4 weeks of decomposition [10, 14]. Coleoptera species have greater varieties that present worldwide [16, 17].

The season may influence on the existence or competition between the carcass insect communities [18, 19]. Flies are usually very inactive on cloudy days due to high humidity [20]. Seasonal changes of the species of blow flies, *Chrysomya spp.* and flesh flies, *Parasarcophaga spp.* have been recorded that these arthropods are poikilothermic; their body temperature corresponds to the ambient temperature. This means that temperature exerts a strong influence on the reproduction and development of the insects [21-23].

The history of forensic entomology is sporadic but a short overview of the known milestones in it is as, in 1600 B.C., a collection of blow flies, *Chrysomya spp.* of clay was published in the oldest known book on zoology. First mention of “green” and “blue” fly [24]. In 907 B.C., court officer heard woman’s endless weeping and inquires of her troubles. Woman says her husband was killed by fire but officer sees flies clustered around the head of the corpse. An autopsy revealed a snag, which the wife later confessed to placing in the head of her husband [25].

William Brittle accused of the murder of Peter Thomas, whose decaying body was found in a wood near Bracknell, Berkshire. Brittle’s alibi was destroyed when Dr. Keith Simpson testified that maggots of the common blue bottle flies, *Calliphora vomitoria* (L.) found on the remains had not pupated which, given the life cycle of the insect, established time of death [26]. Nabity et al. [27] reported précised developmental rate of the blow fly, *Phormia regina* Meigen for accurate estimation in PMI, but PMI results conflicts with time. For this purpose, constant temperature trails were conducted for *P. regina* at minimum and maximum temperaturs 8-32 °C. This measured by rearing external and internal temperatures of the body through thermocouples and rate of larval growth to degree day model. There were no differences of larval growth to temperature affect. The former and current estimates ignored degree day assumptions. The objective of present research is to determine the ecological succession pattern of the insect species associated with the carcass of the free-ranging urban dog, *Canis domesticus* (L.) as a tool for forensic entomology.

The district Mardan, Khyber Pakhtunkhwa, Pakistan is bounded on the east by Swabi, west by Charsadda, north by Buneer districts and Malakand protected area, south by Nowshera district. The total area of the district is 1632 km. It 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. The district is consisted of 2 Tehsil

Mardan and Takht-i-Bahi. The summer season is extremely hot. A steep rise of temperature is observed during May-June. Even during July-September recorded quite high temperatures. During May-June dust storms are frequent at night. The temperature reaches its maximum in June 45.50 °C (Fig. 1) [28].

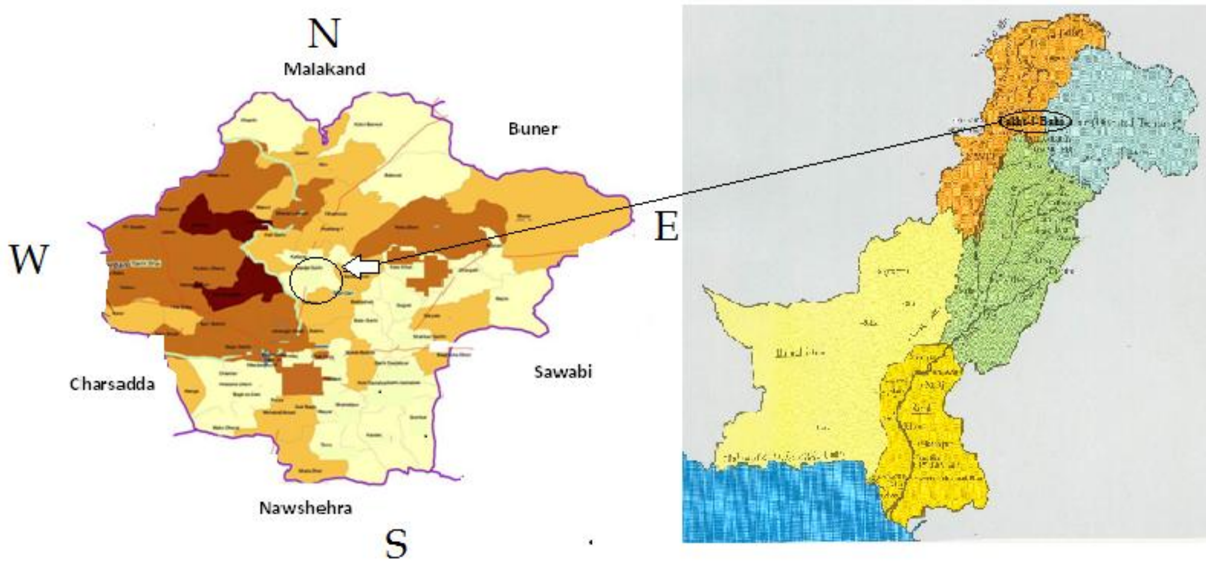

Fig 1: Map of Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan where the free-ranging urban dog, *Canis domesticus* (L.) carcass was placed for observation of ecological succession of insect faunal pattern during 15-25 May 2011 [28]

MATERIALS AND METHODS

Study design

The present study was based on observation of the ecological succession pattern of the insect fauna appeared with the dead body of an experimental animal, free-ranging urban dog, *Canis domesticus* (L.) in tropical region, i.e., Mardan, Khyber Pakhtunkhwa, Pakistan that can be used as an important tool in legal investigations.

Methodology

The present study was conducted in Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011. *Canis domesticus* was hunted alive from the street of Takht-i-Bahi. It was killed by chloroform and the carcass was kept under the wire gaze cage (height: 32"; length: 54"; width: 36") in the ground of Government Degree College, Takht-i-Bahi, 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.

Physical environmental conditions, 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.

Sampling

Insect fauna, i.e., larvae, pupa and adults of different species were collected which appeared on *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.

At every sampling time, the wire gaze cage was removed aside that dog was not disturbed during the study. It was exercised to the period of total fleshy tissues of dog dead body 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. To secure the self-body, gloves and mask were used.

Collected insects have been identified by Dr Ather Rafi, Senior Scientist, National Insect Museum, National Agriculture Research Council, Islamabad, Pakistan and forensic methods were used [29-33]. 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).

RESULTS

The free-ranging urban dog, *Canis domesticus* (L.) carcass decomposition in tropical region, i.e., Takht-i-Bahi, Mardan Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011 was characterized by 5 stages [34-35] (Catts and Haskell, 1990; Kashyap and Pillai, 1989), i.e., fresh (12 h), bloated (13-48 h), active (49-96 h), advanced (97-144 h) and dry (145-265 h) and the ecological succession pattern of insect species collected were identified into 11 species of 3 orders in descending order: Diptera: 5 species > Coleoptera: 4 species > Hymenoptera: 2 species.

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 (Table 1).

Table 1 : The insect fauna associated with the free-ranging urban dog, *Canis domesticus* (L.) carcass 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-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011

Order	Family	Common names	Genus/Species	Authority	Year
Diptera	Calliphoridae	Blow fly	*Chrysomya rufifacies*	(Meigen)	1826
		Blow fly	*Chrysomya megacephala*	(Fabricius)	1794
	Sarcophagidae	Flesh fly	*Parasarcophaga ruficornis*	(Meigen)	1826
	Muscidae	House fly	*Musca domestica*	(Linnaeus) [36]	1758
	Piophilidae	Cheese skipper	*Piophila casei*	(Linnaeus) [36]	1758
Coleoptera	Dermestidae	Hide beetles	*Dermestes maculatus*	(Geer)	1774
	Histeridae	Clown beetles	*Hister sp.*	(Gullenhal)	1808
	Cleridae	Ham beetle	*Necrobia rufipes*	(Fabricus)	1781
	Trogidae	Skin beetle	*Trox sp.*	(Harold)	1872
Hymenoptera	Vespidae	Hornet wasp	*Vespa orientalis*	(Linnaeus)	1758
	Formicidae	Jummper ant	*Myrmecia pilosula*	(Smith)	1858

The number of specimen of insect species were collected from *C. domesticus* carcass from were in descending order: *C. rufifacies*: 21.3% = *M. domestica*: 21.3% > *C. megacephala*: 19.9% > *P. ruficornis*: 12.4% > *Hister sp.*: 7.1% > *P. casei*: 5.3% > *N. rufipes*: 4.3% > *D. maculates*: 3.5% > *Trox sp.*: 2.5% > *M. pilosula*: 1.77% > *V. orientalis*: 0.8% (Fig. 2).

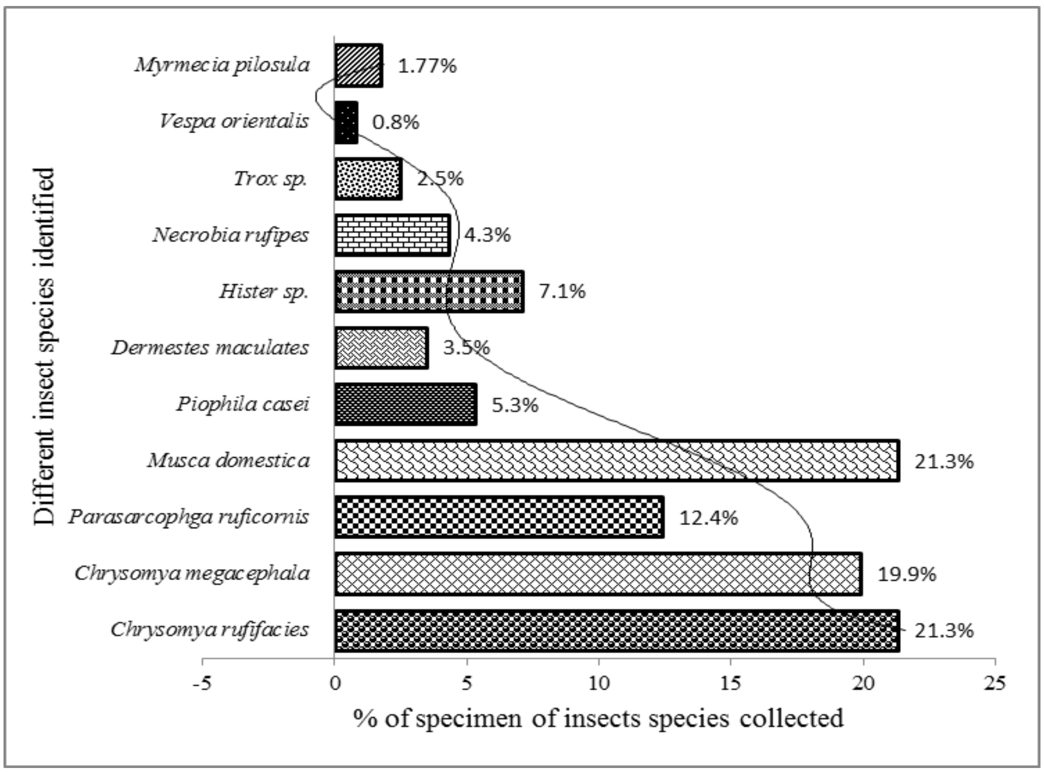

Fig. 2 Number of specimen of insect species were collected from the free-ranging urban dog, *Canis domesticus* (L.) carcass during 5 different decomposition stages (fresh: 12 h; bloated: 13-48 h; active: 49-96 h; advanced: 97-144 h; dry: 145-265 h) in tropical region, i.e., Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011; a: blow fly, *Chrysomya rufifacies* (Macquart); b: blow fly, *Chrysomya megacephala* (Fabricus); c: flesh fly, *Parasarcophga ruficornis* (Meigen); d: house fly, *Musca domestica* (Linnaeus); e: cheese skipper fly, *Piophila casei* (Linnaeus); f: hide beetle, *Dermestes maculates* (Geer); g: clown beetle, *Hister sp.* (Gullenhal); h: ham beetle, *Necrobia rufipes* (Fabricus); i: skin beetle, *Trox sp.* (Harold); j: hornet wasp, *Vespa orientalis* (Linnaeus); k: jumper ant, *Myrmecia pilosula* (Smith);

The ecological succession pattern of insect species associated with the carcass of *C. domesticus* observed during 5 different decomposition stages in tropical region, i.e., Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011, it was found that *C. rufifacies* and *M. domestica* were existed in the fresh, bloat and active decay stages. However, *C. megacephala*, *P. ruficornis* and *V. orientalis* were found in the fresh and bloat stages. Moreover, *P. casei* was bloat and active decay stages. Further, *D. maculates*, *Hister sp.*, *N. rufipes* and *Trox sp.* were found in active decay, advance decay and dry stages. Furthermore, *M. pilosula* was existed in only fresh stage (Fig. 3).

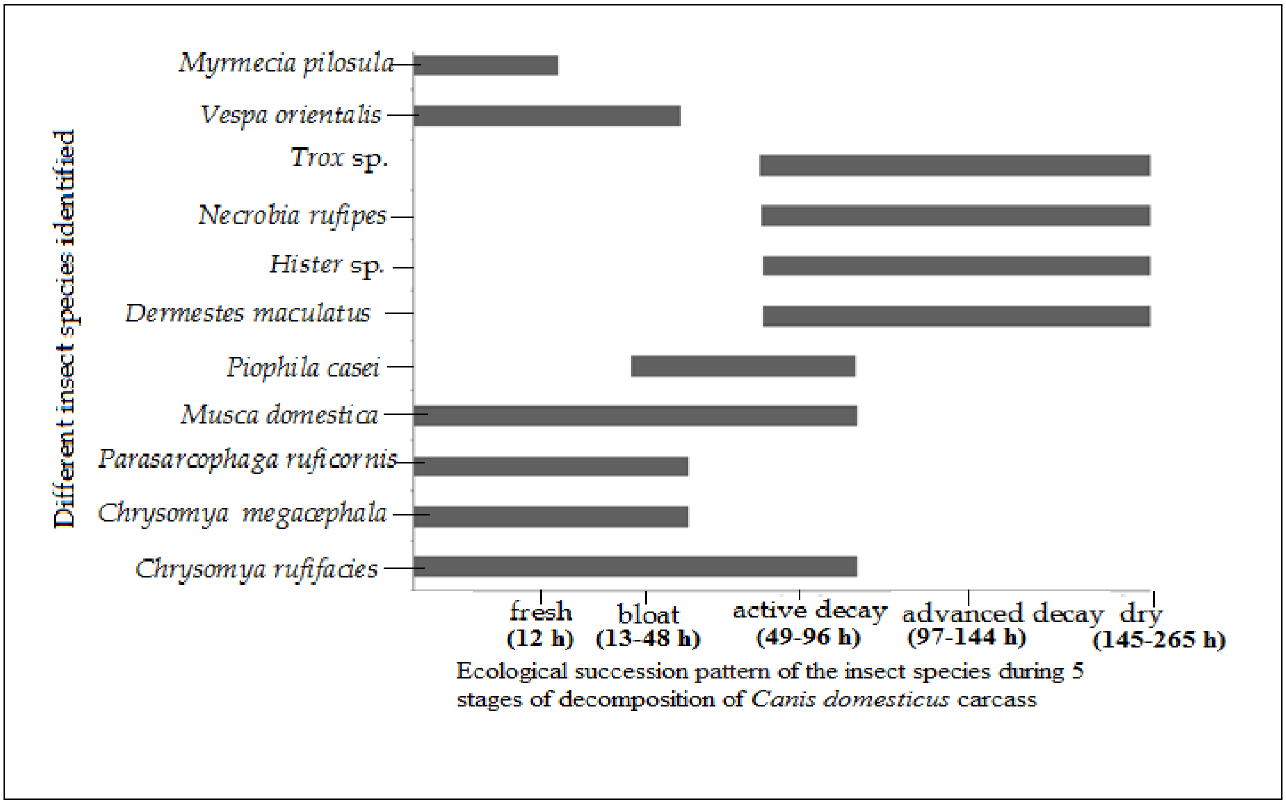

Fig. 3 The ecological succession pattern of the insect species associated with the carcass of the free-ranging urban dog, *Canis domesticus* (L.) observed during 5 different decomposition stages (fresh: 12 h; bloated: 13-48 h; active: 49-96 h; advanced: 97-144 h; dry: 145-265 h) in tropical region, i.e., Takht-i-Bahi, Mardan, Khyber Pakhtunkhwa, Pakistan during 15-25 May 2011; a: blow fly, *Chrysomya rufifacies* (Macquart); b: blow fly, *Chrysomya megacephala* (Fabricus); c: flesh fly, *Parasarcophga ruficornis* (Meigen); d: house fly, *Musca domestica* (Linnaeus); e: cheese skipper fly, *Piophila casei* (Linnaeus); f: hide beetle, *Dermestes maculates* (Geer); g: clown beetle, *Hister sp.* (Gullenhal); h: ham beetle, *Necrobia rufipes* (Fabricus); i: skin beetle, *Trox sp.* (Harold); j: hornet wasp, *Vespa orientalis* (Linnaeus); k: jumper ant, *Myrmecia pilosula* (Smith).

DISCUSSION

In the present study of insect fauna identification for forensic use, the free-ranging urban dog, *Canis domesticus* (L.) carcass has been placed in tropical region, i.e., Mardan, Khyber Pakhtunkhwa, Pakistan. *Canis domesticus* carcass was divided into 5 stages of decomposition and 3 orders, 10 families and 11 species were found. Grassberger and frank [37] examined insect fauna in Austria in the pig, *Sus domesticus* L. carcass. In which 42 species were found belonging to 3 orders and 20 families. Carlvalho et al. [38] observed insect fauna in *S. domesticus* carrion in Brazil. They examined 31 species of 3 orders and 6 families. Szymon et al. [39] reported that insect fauna in *S. domesticus* in Poland. They included above mentioned 5 stages of decomposition. They collected 12 species of 7 families and 2 orders from cadaver. At the present, in *C. domesticus* carcass, flies' numbers were abundance, therefore, the fastened rate of decomposition. However, due to fast decomposition rate of carcass, the most of insect species were not reached. *Canis domesticus* carcass was the source of food and matting for insect species.

In the present research, *C. rufifacies* adults and larvae were dominated the whole carcass in fresh and bloat stages of decomposition, due to their nature of earlier arrival, the highest in numbers, fast rates of eggs lying and emergence of adults. *Hister sp.* dominated active decay and advanced decay stages of decomposition. Carvalho et al. [40] reported in the study of insects' succession on *S. domesticus* carcass that abundant *Calliphoridae* species was *C. vomitoria* adults and larvae, while *D. maculates* was dominated in later stages of decomposition. In both studies, *C. rufifacies* arrived first of all insects and was the highest in numbers in the carcass in the regions. It showed diversity of family Calliphoridae and first arrival of *C. rufifacies*. Its adults and larvae were dominated the whole carcass, due to its fast rates of eggs hatching and emergence of adults (1.5 d) in fresh and bloated stages of decomposition. It was noted that *C. rufifaacies* adults and larvae would be helpful for an earlier time estimation of dead body in this region. *Histers sp.* adults and larvae were dominated in active, advanced decay and dry stages of decomposition. This research also showed deviation from above that *Histers sp.* would be helpful for later time duration estimation for death in tropical region like Pakistan.

Kom et al. [41] examined the emergence of larvae at 31.4 °C required 84 h, while at 27.4 °C, it emerged at 96 h. Damien et al. [42] concluded that at high temperature, increased the rate of development of larvae, its length and rate of oviposition. Slone and Gruner [43] reported from 2.5 year of study of *S. domesticus* cadaver. They studied that the volume of a larval mass had a strong influence on its temperature. It showed that if cadaver mass was large, it regulated high internal temperature which fasted the larvae development. Barbara et al. [44] examined succession of insects in *S. domesticus* carcass. This study was divided in spring, summer and fall seasons and observed ambient and internal temperatures of cadaver. The ambient temperature was the chief factor for determining quick decay rate. Maximum carcass' internal temperature was due to external temperature that fasted development of larvae. In the present study, it was observed that the average temperature was 35.35±1.54 °C and it played important role in development of adults, decomposition rate and insect succession.

This fasted larvae development into adults and carcass decomposition rate. Development of adults from eggs under high temperature for *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. It was fastest as compared to other regions.

In the present study, average humidity was 38.58%, which was effected on adults and larvae growth. Shean et al. [45] reported differential rate of decomposition of *S. dometicus* carrion that maximum and minimum humidity disturbed insect activities. Maximum humidity stopped insect fauna but fasted rate of decomposition than minimum humidity. In both studies, it was observed that low humidity developed warmer condition and high temperature which increased adults flies arrivals and larval growth. The development of eggs into larvae was very fast due to high humidity. Further, in the present research, the fluctuation in temperature, low and high humidity and rainfall that associated insects to carcass and only limited diversity of insects were observed. Therefore, at the present 10 families and their 11 species were collected belonging to 3 orders.

In the present study rainfall was not occurred in fresh and bloated but occurred in active decay and advanced decay stages. It was noted that fast rainfall increased decay stage, stopped insect succession and larvae mass was disintegrated. Flies stopped their arrival to carcass and larvae migration was also observed. In the present research, it was also observed that moistening of tissues due to rain increased eggs hatching and fasted decomposition. As rainfall produced low temperature, insects are cold blooded, its adults stopped movements and larvae came close to each other to regulate heat. It was also observed that moistening of tissues produced fluidly condition for insect eggs and larvae; therefore, fast hatching and emergence of adults with maximum flesh decomposition occurred. Archer [46] reported that rainfall reduced the duration of decomposition by increasing biomass loss. Early and Goff [10] reported that rainfall effected decomposition and insect succession. Rain stopped the insect colonization and increased rate of decomposition of carcass. Greenberg [47] observed of oviposition of flies and concluded that in the rain, flies did not fly, therefore, no oviposition occurred. Therefore, effect of temperature, humidity and rainfall on insects succession can be used for legal investigation.

CONCLUSION

It was concluded that different species of flies and ants first arrived to carcass as compared to beetles. However, the first arrived species within 10 min of death was *C. rufifacies* adults. Moreover, the last arrival species after 81 h of death was *Trox sp.* Further, the adults of *M. pilosula* were abundant in early (fresh and bloat) stages of decomposition. Furthermore, the beetles species and their larvae dominated later (active and advanced decay) stages of decomposition.

Recommendation

Detail study on insects' diversity and their comparison are recommended for forensic studies in different ecological and geographical regions of Pakistan. It is recommended to study the ecological succession pattern of insect fauna in different seasons of Pakistan.

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