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Necrophagous Insects Succession on Carrions' of Two Tropical Animals

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ABSTRACT

Many species of insects are known to be associated with carrion and impacts greatly on the decay rate as well as provide useful data on the mode and time taken for death of that organism. In this study, the diversity of insect associated with the different stages of decomposition, the effect of insects' presence on the decay rates due to difference in mode of death and some climatic factors that influence the rate of decay of toad (Amietophrynus regularis) and guinea pig (Cavia porcellus) were assessed at the University of Cape Coast (UCC) Science Botanical gardens. Six guinea pigs and six toads were killed by drowning in sea water in a plastic container and the others by chloroform soaked in cotton wool. Results obtained when carrions were exposed to insects while others were placed in an insect free cages indicated approximately 14 species and 461 adult insects associated with the carrions. Oecophylla sp was the most abundant representing 256 (55.51%) with Piophila sp being the least (0.65%). It was also found that it is the presence of insects alone, via the consumption or the feeding behavior on the carcass by maggots that accelerate decomposition. Tukey statistics at 95% CI showed a significant difference between the number of insects and average ambient temperature (˚C), while no significant difference was observed between number of insects and average relative humidity (%) in ANOVA. Temperature and relative humidity influenced the stages of carcass decay and insect activity and abundance. These results show that necrophagous insect are important in carcass decay and therefore can contribute to forensic entomological studies especially within the tropics.

Keywords: *insects, diversity, decomposition, effect, carrion, temperature, humidity*

INTRODUCTION

Necrophagous insects are arthropods that forage directly on decomposing organisms, or the fluids released from these organisms during the decomposition process [1]. Often, necrophagous blowfly species are the first to reach and colonize a decomposing organism for oviposition as such are considered the most important for post-mortem interval estimations [2].

Although no two organisms decompose the same way, they all undergo the various successive stages involved in decomposition. Organisms undergo five different stages of decomposition. The fresh stage begins immediately the organisms' heart stops pumping blood [3]. The process called algor mortis sets in when the body begins to loose heat to the surrounding environment [4]. Further, this is also known as autolysis, thus, the breaking down of an organism's cell by internal chemicals and enzymes produced by the organisms itself. Certain observable features caused by decomposition are restricted during the fresh stage, although the process of autolysis may cause blisters at the surface of the skin [5]. Insects such as blowflies and flesh flies are the first to arrive at carrion, and seek appropriate oviposition site.

The second is the bloat stage where anaerobic metabolism takes place, resulting in the amassing of gases, such as carbon dioxide, hydrogen sulphide, methane, and nitrogen. Gases produced by the metabolic activities of anaerobic bacteria results in an inflammation of the abdomen and the 3 carcass forming a balloon-like appearance [6]. The gases produced also cause natural liquids and liquefying tissues to become frothy [7]. Many insect in varied developmental stages are known to be actively involved in this stage. Openings in the skin allow oxygen to re-enter the body and make available more surface area for the development of fly larvae and the activity of aerobic microorganisms. The purging of gases and fluids results in the strong distinctive odours associated with decay [8].

Active decay stage follows the bloated stage and is characterized as the period of greatest mass loss. This loss happens as a result of the release of decomposition liquids into the surrounding environment and feeding habits of maggots

[6]. Decomposition liquids amass around the body and generate a cadaver decomposition island [3]. The conversion of tissues into liquid becomes obvious during this time and strong odors continue [8]. The end of active decay stage is seen by the moving away of maggots from the decomposing body to pupate [3].

In the advance decay stage, decomposition process is reduced due to the loss of readily available cadaveric material [6]. The activity of insects is reduced when the decaying organisms is found on soil. The area surrounding it will then show signs of vegetation death [6]. The cadaver decomposition island (CDI) surrounding the carcass will show an increase in soil carbon and nutrients such as potassium, calcium, phosphorus, and magnesium, variations in pH; and an important increase in soil nitrogen [9].

Dry/remains stage is the last stage in which reappearance of plant growth around the CDI may provide a sign that nutrients available in the surrounding soil have not yet reverted to their regular levels [6]. The residue of the cadaver at this stage is dry skin, bones and cartilage which will become dry and faded if exposed to environmental elements [8].

Temperature, humidity, amount of light, accessibility, physical position of a carcass, mass and type of carcass, vertebrate scavengers, insect abundance, biology and geographical distribution of the necrophagous insects and season can influence the time of arrival and duration insects stay on the carrion [10-14]. These factors play an important role by making it easier to study insect succession on carrion in different regions and diverse conditions [15]. It is only within the last twenty years that the study of insects has been extensively used in homicide cases in North America. The better attention in forensic entomology has resulted in many studies that have been beneficial and informative with respect to the development of insect species lists in certain parts of the world [12,16-19]. Studies of fauna connected with corpses and other decomposition organisms are the most important in the application of entomology to legal medicine, whereby stored data can be used as forensic indicators [20].

Currently, studies dealing with successional patterns in decomposition of animals have been conducted in temperate regions [8,21-23] with comparatively few relevant data generated from the tropics. Undertaking more research is essential to examine the effects of the the factors that influence species composition, insect succession and carrion decomposition [24]. This study therefore objectively assessed necrophagous insects succession associated with the different stages of decomposition on carrions of toad (*Amietophrynus regularis*) and guinea pig (*Cavia porcellus*) and the effect of insects' presence on the decay rates as well as some climatic factors that influence the rate of decay of the two studied animals.

MATERIAL AND METHODS

Study area

The study was conducted in the University of Cape Coast (UCC) Botanical Garden. The site lies within the co-ordinates 5º06'59" N and 1°17'42" W. UCC is located in the Cape Coast in the Central Region of Ghana. Cape Coast is located between latitude 05º 0'00" to 05º10'0" North and longitude 01º07'00" to 10º15'0" West (Figure 1). The vegetation is tropical rainforest type. There are two large forest reserve namely Subri river forest which occupies 375 km^2 and Pra-Suhyen Forest Reserve with 204 km^2 area. The hottest months are February and March while the coldest months are between June and August. The major rainy season occurs between May to July and minor rainy season falls within September to October [25]. Cape Coast has a double maximal rainfall, with annual rainfall between 750 mm and 1000 mm. The natural vegetation of Cape Coast consists of shrubs of about 1.5 m high, grasses and a few scattered trees. The original vegetation of dense shrubs has been replaced by secondary vegetation as a result of clearing for farming, charcoal burning, bushfires and other human activities.

The University of Cape Coast Botanical Gardens is one of the several botanical gardens for education and research with diverse plant and animal species. These include; vines, herbs, shrubs, trees and also different species of birds, reptiles with unique characteristics that may be of importance to the university and the local community in general. UCC Botanical garden also has research and aesthetic values, and incorporates conservation of a wide range of plant species which are important in traditional medicine as well as other aspects of life.

Study design

Six toads (*Amietophrynus regularis*) and six guinea pigs (*Cavia porcellus*) were used as decomposition organisms in this study. Cylinder shaped metal cages of area 0.0534 m^2 were used for the exclusion of necrophagous invertebrates. Each cage was placed at a depth of 10 cm, for carcass contact with the soil. At each collection site, a small area was cleared to improve observation of specimens and fixing of the cage (Figure 2A). Insect exclusion cages were used as control for preventing insect contact with the experimental animals (Figure 2B).

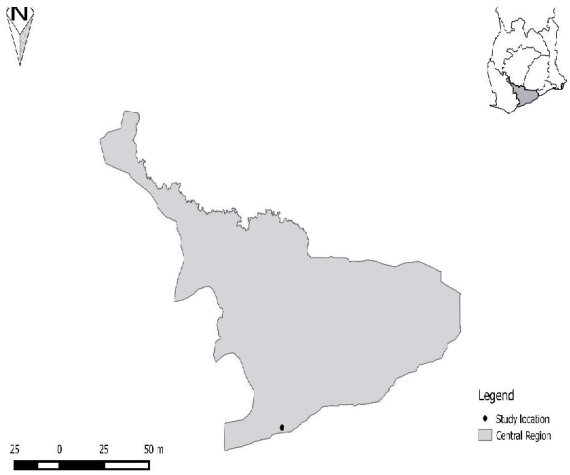

Figure 1: Map showing location of study site.

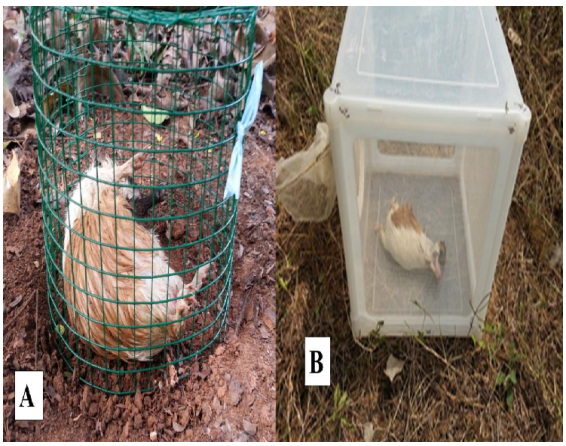

Figure 2: (A) Experimental animal in exposed to insect visit. (B) Guinea pig in an insect free cage (control experiment).

Killing procedure

A total of 12 experimental animals were used. Three guinea pigs were killed using chloroform and other three by drowning in sea water and deposited at different sites in the gardens. Similar procedure was conducted on six toads. Killing method by chloroform was achieved by placing the studied animals in a plastic container and then 45 ml of chloroform soaked in cotton wool was introduced into the plastic container with a lid. Time of complete knock down was observed as recorded for each of the studied animals. Killing by drowning method was done by drowning the studied animals in sea water in two separate plastic containers and recording time for knockdown for each animal.

After death, all the studied animals were weighed and their masses recorded before placement. One set up consisted of three (3) specimen. Thus, two (2) guinea pigs which were covered with cylinder-shaped metal cages and the last one placed in an insect-free cage to prevent vultures and other scavengers from picking up the decomposing organisms [8]. Specimens were placed 5 m apart in a triangular form. Same procedure was used for the toads. Guinea pigs and toads of the same killing method were placed 80 m to 100 m apart and the distance between the different killing methods was 40 m-50 m apart.

Insect collection

In assessing the diversity of insect associated with the different stages of decomposition of toad (*Amietophrynus regularis*) and guinea pig (*Cavia porcellus*), insect visitors on the dead experimental animals were sampled at the various stages of decomposition. Collection was performed in respect to a defined sampling protocol [26]. The carcasses were observed daily, with a minimum observation time of 30 minutes per set up. The resulting data on carcasses' condition were collected as: presence of larvae, decomposition stage and insects. The collections were conducted with low harmful impact on the fauna, particularly on the immature specimens. The actively flying insects

were collected with an aerial net. Insects not captured with the aerial net and immature specimens in larvae stage were handpicked on the carcass in the cages. All adult insects were killed with soapy water and sent to the laboratory.

The effect of insects' presence on the decay rates of the two studied animals were also assessed by excluding carcasses of two specimens of each animal from insect visitation as control. This was achieved by placing a specimen from each experimental treatment in an insect free cage and recording the time it takes for each carcass to enter into the various phases of decomposition. Data was compared with the other set up that received insect visitation and impacts of insect presence on carcasses of the studied animals were ascertained.

Site scale climatic factors such as temperature and relative humidity were recorded using a digital thermo-hygrometer.

LABORATORY PREPARATIONS

Rearing of larvae

The larvae collected were placed in deli cups filled with pieces of smoked tuna and moist soil. This was done to enhance the development of the reared individuals. The deli cups were given the appropriate labeling (including the date) and covered with a perforated cardboard. All emerged adult insects were sorted out and killed by placing them in soapy water.

Pinning and mounting

Pinning was achieved by pushing entomological pins gently through the right side of the mesothorax until the pin emerges on the underside of the insects. Using a pinning block made of hard wood with vertical holes drilled to different heights, the insects were adjusted on the pin at the height which leaves the top 8-10 mm of the pin projecting above the insects to facilitate handling. The pin with the insect was pushed into the mounting board until the underside rested on the board. The legs and the antennae were arranged close to the body (to reduce possibilities of damage) and were secured in that position with bracing pins. Data label was secured next to the insects. The bracing pins were removed when the insects were dried. The dried insects were placed in a curating box containing naphthalene ball for identification. Insects not mounted were preserved in alcohol (70%).

Statistical analysis

Statistical software Minitab (version 17) and Microsoft excel (2013) was used to analyze the data. The main factors were the total number of insects per treatment, maximum average temperature and average humidity. The species diversity index on the insects was determined using the Simpson's diversity index, D

N=total number of samples.

n=total number of individual species

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

RESULTS

Assessment of necrophagous insects' succession associated with the different stages of decomposition on carrions of toad (*Amietophrynus regularis*) and guinea pig (*Cavia porcellus*) indicated that, a total of 14 insect species representing 9 families, 14 genera and 461 adult insects visited the two carrions (Table 1).

In both carrions, there seems to be similar insect species encountered at each stage of decomposition. Stage one was associated with *Dinoponera* sp, *Dysdercus* sp, *Oecophylla* sp and *Lucilia* sp. Stage two *Oecophylla* sp, *Lucilia* sp, *Hermetia* sp, *Necrobia* sp and *Dysdercus* sp were observed visiting carrions. *Hermetia* sp was observed only in this stage. Stage three recorded highness richness of insects, comprising *Oecophylla* sp, *Lucilia* sp, *Necrobia* sp, *Dysdercus* sp, *Musca* sp, *Dinoponera* sp, *Solenopsis* sp, *Phaenicia* sp, *Cynomyopsi* sp, *Calliphora* sp and *Piophila* sp. *Solenopsis, Phaenicia, Musca* and *Cynomyopsi* species were observed visiting carrions only in decomposition stage three. *Dinoponera* sp, *Oecophylla* sp, *Lucilia* sp, *Calliphora* sp, *Piophila* sp, *Sarcophaga* sp, *Necrobia* sp and *Philonthus* sp wererecorded in stage four of decomposition of the carrions with *Sarcophaga* sp and *Philonthus* sp observed only at this stage of decomposition. At stage five of decomposition, only *Calliphora* sp were recorded. (Table 2).

Table 1: Diversity of insects collected on all the guinea pigs killed by drowning in sea water and by chloroform

Genus	Genus	n(n-1)	N
1	*Dinoponera*	37	1332
2	*Oecophylla*	256	65280
3	*Solenopsis*	37	1332
4	*Phaenicia*	9	72
5	*Cynomyopsi*	12	132
6	*Lucilia*	37	1332
7	*Calliphora*	9	72
8	*Sarcophaga*	8	56
9	*Hermetia*	4	12
10	*Musca*	5	20
11	*Necrobia*	27	702
12	*Philonthus*	4	12
13	*Dysdercus*	13	156
14	*Piophila*	3	6
	Total	461	76516
Simpson's diversity index the diversity of the insects in the botanical gardens was estimated to be 0.36.			

Table 2: Families of insects collected for both guinea pigs and toads treatment. [1=fresh stage; 2=bloat stage; 3=active decay stage; 4=advance decay stage; 5=dry/remains stage].

Order	Family	Stage present	Genus
Hymenoptera	Formicidae	1,3,4	*Dinoponera* (Kempf, 1971)
		1,2,3,4	*Oecophylla* (Fabricius, 1775)
		3	*Solenopsis* (Fabricius, 1775)
Diptera	Calliphoridae	3	*Phaenicia* (Wiedemann, 1814)
		3	*Cynomyopsi* (Robineau-*Desvoidy*, 1830)
		1,2,3,4	*Lucilia* (Robineau-*Desvoidy,* 1830)
		3,4,5	*Calliphora* (Robineau-Desvoidy, 1830)
Coleoptera	Piophilidae	3,4	*Piophila*
	Sarcophagidae	4	*Sarcophaga* (Meigen, 1826)
	Stratiomyidae	2	*Hermetia* (Linnaeus)
	Muscidae	3	*Musca* (Linnaeus)
	Cleridae	2,3,4	*Necrobia* (Fabricius,1781)
Hemiptera	Staphylinidae	4	*Philonthus* (Stephens, 1892)
	Pyrrchocoridae	1,2,3	*Dysdercus* (Fallen,1814)

In estimating insect diversity on carrions of the guinea pigs and toads, Simpson's diversity indices of 0.29 and 0.37 were respectively recorded for each studied animal (Tables 3 and 4). Among the carrions, guinea pig carrions had the most diverse insect visitors both in evenness and richness.

Table 3: The diversity of the insects collected on the guinea pigs killed by drowning in sea water and chloroform was found to be 0.29 using Simpson's index. See appendix.'

	Genus	N	n(n-1)
1	*Dinoponera*	25	600
2	*Oecophylla*	145	20880
3	*Solenopsis*	21	420
4	*Phaenicia*	7	42
5	*Cynomyopsi*	7	42
6	*Lucilia*	24	552
7	*Calliphora*	6	30
8	*Sarcophaga*	5	20
9	*Hermetia*	4	12
10	*Musca*	5	20

11	*Necrobia*	15	210
12	*Philonthus*	4	12
13	*Dysdercus*	10	90
14	*Piophila*	3	6
	Total	**281**	**22934**

Table 4: Diversity of insects collected on all the Toads killed by chloroform and sea water.

	Genus	**N**	**n(n-1)**
1	*Dinoponera*	12	132
2	*Oecophylla*	107	11342
3	*Solenopsis*	16	240
4	*Cynomyopsi*	5	20
5	*Lucilia*	13	156
6	*Calliphora*	3	6
7	*Sarcophaga*	3	6
8	*Hermetia*	4	12
9	*Necrobia*	12	132
10	*Dysdercus*	3	6
11	*Phaenicia*	2	2
	Total	**180**	**12054**

The data gathered shows that the diversity of the insects collected on the toads killed by drowning in sea water and chloroform was found to be 0.37 using Simpson's index. See appendix.

Generally, the most abundant of insect visitors on both carrions were observed to be Hymenopterans belonging to the genus *Oecophylla.* We recorded a total of 256 individuals. The least recorded group was members of the Dipteran of the genus *Piophila* (Figure 2A and 2B).

In determining the effects of insect presence on the decomposition rate of the two studied animals, an increased decomposition rates were observed in all stages apart from decomposition stages 1 and 5 that recorded approximately 24 hours in all the set ups and control. Control set up that excluded any insect visitation on the carcass however recorded increasing time taken for each of the carcass to enter into the next stage of decomposition for stages 2, 3 and 4 (Figures 3 and 4).

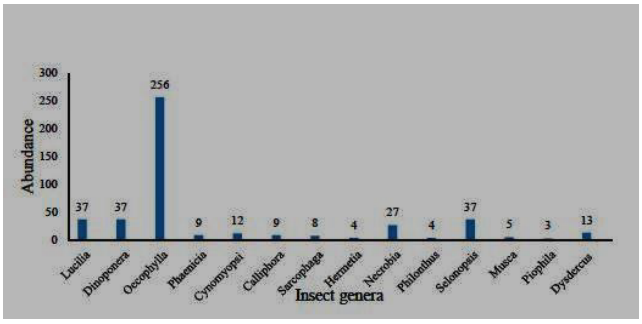

Figure 3: Total number of individual genus collected during the different stages of decomposition.

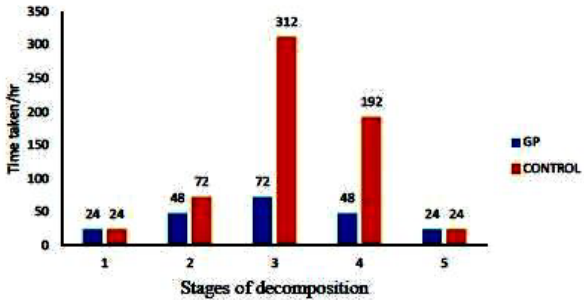

Figure 4: Total hours spent by each specimen during the various stages of decomposition for guinea pigs killed by chloroform.

The two methods used in killing the animals presented similar trends of increasing time of decomposition for stages 2, 3 and 4, while stages 1 and 5 recorded approximately similar duration of decomposition (Figures 5 and 6). In assessing some climatic factors that may influence the rate of decay of the two studied animals, analysis of Variance (ANOVA) was performed, with the main factors being the total number of insects per treatment, maximum average temperature and average humidity using the statistical software Minitab (version 17) which provided the same results with P-value of 0.052, F-value of 4.20 and DF of 2. Grouping using the Tukey method and 95% confidence showed a significant difference between the number of insects, average temperature (˚C) and relative humidity meaning the higher the temperature, the less number of insects and also the higher the relative humidity (%) the higher the insect number (Tables 5A, 5B and 6).

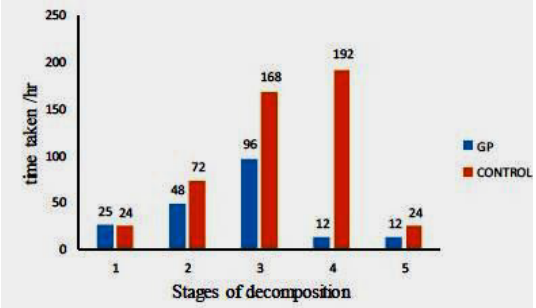

Figure 5: Total hours spent by each specimen during the various stages of decomposition for guinea pigs killed by drowning in sea water.

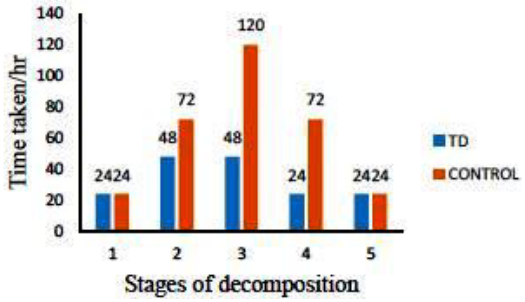

Figure 6: Total hours spent by each specimen during the various stages of decomposition for toads killed by chloroform

DISCUSSION

Many arthropods including insects are known to influence decomposition processes, essential for recycling the finite matter that occupies physical space in the biome [27]. The various successive stages of decomposition are also known to be highly reliant on climate and seasonal variations [28]. In this study that determined the succession pattern of necrophagous insects on toad (*Amietophrynus regularis*) and guinea pig (*Cavia porcellus*), highly diverse groups of insects were associated with the successive stages of decomposition and influenced the rate of these stages.

All the insect families recorded in this work have been known to be associated in carcass decomposition. For instance, six out of the nine insects families (Formicidae, Calliphoridae, Sarcophagidae, Stratiomyidae Muscidae and Staphylinidae) recorded in this work were also reported to have been associated with carcass of pigs in Brazil [29]. In [30] families Calliphoridae, Muscidae, Formicidae, Cleridae, Staphylinidae and Pyrrchocoridae were also observed visiting carcasses of pigs [30]. In a work in Brazil, six of the insect families Formicidae, Calliphoridae, Sarcophagidae, Muscidae, Cleridae and Staphylinidae were also observed visiting carrions of rabbits while insect family Piophilidae have also been observed visiting carcasses of pigs [31].

Comparison of both studied animals reveals that diversity appears to be high among carrions of the guinea pigs than the carcasses of toads. Various insects are attracted to different carcasses. This could be due to various factors mentioned in subsequent paragraphs. While insects such as *Solenopsis* sp, *Phaenicia* sp, *Cynomyopsi* sp *Hermetia* sp, *Musca* sp, *Philonthus* sp and *Sarcophaga* sp seems to visit carcass in only one of the stages of decomposition, several other species of insects *Dinoponera* sp, *Oecophylla* sp, *Lucilia* sp, *Calliphora* sp, *Piophila* sp, *Hermetia* sp, *Musca* sp, *Necrobia* sp and *Dysdercus* sp rather visit carcass in more than one of the stages of decomposition. Similar

results have been reported on carcasses of rabbit [28], where many insects were known to be associated with the various stages of decomposition. The degree of putrefaction, developmental stage of insect, feeding requirements and environmental factors are known to contribute to differential presences of these insects on carrions [23,32-34].

Insect presence appears to be an important factor that hasten rate of decomposition. Carrions that received insect visitation spent fewer days in each of the stages of decomposition than those carcasses that were excluded from insect visitation. Several research findings support this outcome. In the presence of insects alone, aided the consumption of the carcass by maggots, which accelerated decomposition. Rate of decomposition on the carcasses is also influenced by the biology of the necrophagous insects, appetite and reproductive state of the insects whether they are ready to use carrion as a food source or medium for oviposition [35]. Rates of oviposition and development of insects is influenced by temperature and humidity [14, 32]. This influences the overall rate of decomposition since insect activity is either accelerated or inhibited depending on temperature and humidity. In assessing temperature and humidity and its effect on decay rate, it was found that high temperatures resulted in fewer numbers of insect's visitors. In contrast [8] found that the reduction of carrion was slower on cool, cloudy days. The opposite was recorded on warmer days since high temperatures intensified insect activity, resulting in a rapid depletion of the carrions. Therefore, slower decomposition rates and reduced insect activity alter the timing of insect arrival to the carcass, which in turn affects the rate of decay. Temperature and humidity influence insects' behavior towards an animal immediately after death and detection of the remains [32]. Temperature was found to be an important climatic factor that influences insect abundance on the studied carrions.

Environmental factors thus significantly influence the succession and colonization of insects on carrions and are unique to different regions and can even vary from one animal to another [8,36].

CONCLUSION

This study provides evidence that the presence of insects hasten decomposition rate of carrions. Climatic factors especially temperature significantly influences decomposition rates and the diversity of necrophagous insects on carrions. Type of carrion and method of death have influence on the diversity of necrophagous insects on carrions. Data obtained contributes to our knowledge on succession and colonization of necrophagous insects on carrion decay and are, therefore, also of forensic importance.

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REFERENCES

[1] Goff, ML., and Odom, CB., *The American journal of forensic medicine and pathology*, **1987**. 8: 45-50.
[2] Watson, E., and Carlton, C., *Journal of Medical Entomology*, **2003**. 40: 338-347.
[3] Carter, DO., Yellowlees, D., and Tibbett, M., *Naturwissenschaften*, **2007**. 94: 12-24.
[4] Samuel, EJ., TS Manoharan, and Pearl Mary, S., *Putrefaction of Innumerable Aborted Fetuses*, **2015**.
[5] Pinheiro, J., *Forensic anthropology and medicine*, **2006**.
[6] Tibbett, M., and Carter, DO., *CRC Press*, **2008**.
[7] Payne, JA., *Ecology*, **1965**.
[8] Vass, AA., et al., *Journal of Forensic Sciences*, **1992**. 37: 1236- 1253.
[9] Williams, H., and Richardson, A., *Australian Journal of Ecology*, **1984**. 9: 141-152.
[10] Tullis, K., and Goff, ML., *Journal of Medical Entomology*, **1987**. 24: 332-339.
[11] Hall, RD., and Doisy, KE, *Annals of the Entomological Society of America*, **1993**. 86: 589-593.
[12] Tessmer, J., and Meek, C., *Journal of Medical Entomology*, **1996**. 33: 665-669.
[13] Anderson, G., *Entomological Society of British Columbia*, **2001**. 98:127-136.
[14] Dent, BB., Forbes, S., and Stuart, B., *Environmental Geology*, **2004**. 45: 576-585.
[15] Greenberg, B., *Journal of Medical Entomology*, **1991**. 28: 565-577.
[16] Aspoas, B., *Medical and veterinary entomology*, **1994**. 8: 292-294.
[17] Wells, JD., and Kurahashi, H., *Japanese Journal of Sanitary Zoology*, **1994**. 45: 303-303.

[18] De Souza, AM., Linhares, AX., *Medical and veterinary entomology*, **1997**. 11: 8-12.
[19] Catts, E., and Goff, ML., *Annual review of entomology*, **1992**. 37: 253-272.
[20] Bornemissza, G., *Australian Journal of Zoology*, **1957**. 5: 1-12.
[21] Reed Jr, H., *American Midland Naturalist*, **1958**. 213-245.
[22] Nuorteva, P., et al., *Annales Entomologici Fennici***, 1974**.
[23] Anderson, GS., and VJ Cervenka., *Advances in forensic taphonomy*, 2002: 173-200.
[24] Faanu, A., Ephraim, JH., and Darko, EO., *Environmental monitoring and assessment*, 2011. 180: 15-29.
[25] Amendt, J., et al., *International journal of legal medicine*, **2007**. 121: 90-104.
[26] Weigelt, J., *Chicago Press*, **2009.**
[27] Souza, AS., Kirst, FD., and Krüger, RF., *Revista Brasileira de Entomologia*, **2008**. 52: 641-646.
[28] De Carvalho, LL., and Linhares, AX., *Journal of Forensic Sciences*, **2001**. 46: 604-608.
[29] Kyerematen, RA., Boateng, BA., Haruna, M., Eziah, VY., *Sus scrofa L*, **2006**.
[30] Vasconcelos, SD., et al., *Journal of Insect Science*, **2013**. 13: 145.
[31] Smith, K., *A manual of forensic entomology*, **1986.**
[32] Bourel, B., et al., *Journal of Medical Entomology*, **1999**. 36: 420-425.
[33] Anderson, GS., JH Byrd, and Castner, JL., *Forensic entomology*, **2001**. : 143-175.
[34] Dethie,V., and Bodenstein, D., *Zeitschrift für Tierpsychologie*, **1958**.15: 129-140.
[35] Turner, B., and Howard, T., *Medical and veterinary entomology*, **1992**. 6: 179-181.
[36] Tantawi, TI., et al. *Journal of Medical Entomology*, **1996.** 33: 566-580.