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Effect of Killing Methods on the Insect Fauna and Succession on African Giant Rat *Cricetomys gambianus*

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Abstract

The rise in insecurity and criminal activities remains one of the major challenges facing Nigeria. This has resulted in loss of lives in violent crimes, in addition to the deaths occurring naturally and from suicide deaths using chemicals. Collecting evidences at a crime scene requires that the evidences are accurately interpreted for proper investigation and use in court of law. The knowledge gap on the insect fauna of cadaver and accurate interpretation of insect evidence at crime scene hampers criminal investigations and the use of insect evidence in the court of law. This research provided data on insect fauna and succession on cadavers to unravel cause of death. The research was conducted from March to May 2017 in the University of Nigeria Nsukka. Three different killing methods - slaughtering with knife, oral injection of 5ml of 2, 2-dichlorovinyl dimethyl phosphate and oxygen deprivation were used to simulate violent, suicide, and natural death respectively on African giant rat. A total of 5,036 arthropods comprising 10 Orders, 23 families and 50 species were collected. Among the necrophagous families collected Calliphoridae is the most important as they are the first to colonize and breed on the carrion. Arthropod successions on the carcass were in the order Formicidae, Muscidae, Calliphoridae, Sarcophagidae, Histeridae, Dermestidae and others. Natural death recorded the highest number of species (36 species), followed by suicide death (35 species) and violent death (27 species). Insect activity clearly differentiated the three killing methods and offer useful information in criminal investigation.

Keywords: Crime; Carrion; Fauna; Death; Insect; Succession

Introduction

Dead animals attract insects especially flies which feed and eventually lay eggs on the body. These behaviours of feeding and laying eggs on dead bodies make insects the first witness to a crime and, the eggs deposited, and the subsequent larval development offer useful information on time since death or the postmortem intervals (PMI) [1-3]. Insect arrival and life cycle on a cadaver serve as a clock those records when death occurred, manner and cause of death, and this is what forensic entomologists are exploiting in criminal investigation using insects [4-6]. Insects arrive at a crime scene few minutes after death and remain with the carrion until the end of decomposition [7]. They feed, live, and breed on carrion from fresh stage to skeletonization [8].

Insect fauna and succession on carcass are influenced by the killing method and several other factors such as temperature, humidity, mummification, burial, and bio-geoclimatic zones [9]. The number and composition of insect species on a cadaver vary according to the killing methods or the cause of death. Violent death which left open wound on cadaver will result in massive colonization by Diptera and formation of maggot masses. Suicide death due to drinking of common home pesticides, cocaine, heroin etc. can prevent insects from colonizing the cadaver until after many days hence few species can breed on the cadaver [10]. The insect species found on a cadaver are specific to geographical regions and seasons [11-19]. Thus, insects of forensic importance for each geographical region need to be characterized for wider application of forensic entomology in criminal investigations [16,20].

The spate of violent crimes such as ritual killings, ethnic clashes, suicide bombing, religious killings, farmers—herders clash etc. have resulted in several deaths across Nigeria [21,22] yet, the use of arthropods which are silent witness to death scene has been ignored in criminal investigation in Nigeria [3]. This is largely due to lack or paucity of knowledge on the arthropod fauna of decomposing remains in many geographical zones of Nigeria. The species composition and succession of arthropods on a cadaver are useful in criminal investigation [19,23]. In some developed countries, arthropod species composition and succession on cadavers are well documented [24,25]. However, in Nigeria, earlier researchers [6,19,26-29], have established some data on insect succession on carcass in some localities but there is still the paucity of knowledge or lack of information on the insect fauna of cadavers on many other geographical zones of Nigeria and the information provided by these insects when different killing methods are involved.

Thus, the objectives of this study are to provide data on the effects of different killing methods on the insect fauna and succession of decomposing African giant rant, *Cricetomys gambianus*, and to use insect evidence to establish the mode of killing in the study area.

Materials and Methods

Study Area

The study was carried out in Nsukka, Enugu State Nigeria. Nsukka Local Government Area (LGA) is located between latitude 6.5148 and 6.5256 °N and longitude 7.2388 and 7.2568° E (Figure 1) with land mass of 1,810km² and a population of 309,633 according to 2006 national census [30]. Nsukka is characterized by green grassy vegetation consisting of grasslands, farmland, wooden shrub and mature natural forests [31]. Nsukka LGA shares boundary with other LGAs in Nsukka Senatorial Zone which include; Udenu, Igboeze South, Isi Uzo, Igboetiti and Uzo Uwani. The two prominent climatic seasons in the area are the wet season, lasting from April to October and the dry season lasting from November to March [32]. The area is in the humid tropical region with average monthly temperature fluctuating between 24 °C and 29 °C and mean annual rainfall ranging from 786 mm to 2098.2 mm [31].

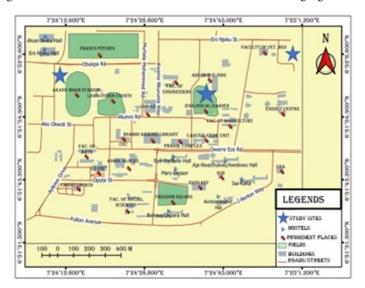


Figure 1: Map of University of Nigeria Nsukka, Enugu State showing the study sites

Killing and setting out the experimental sites

Nine (9) African giant rats (*Cricetomys gambianus*) were used as a model for human decomposition. The animals were killed using 3 different methods thus:

- A. Slaughtering the rat with knife which was used to simulate violent death with open wound,
- B. Narcotizing the rat with 5 ml of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) (a common home pesticide) used to simulate suicide death, and
- C. Depriving the rat of oxygen (suffocation), used to simulate natural death [6,33].

Three sites within the University of Nigeria Nsukka (UNN) were used for the study which lasted from March to May 2017. The sites were: grassy area behind UNN Stadium, UNN Zoological Garden and UNN Veterinary Medicine Farm (Figure 1). The distance between two sites was at least 1 Km apart. Three giant rats each with a different killing method were deposited 15 m apart within each study site. Carcass was kept in a cage of 10 cm long and 4 cm high made of wire gauze [6,34]. This was to prevent disturbances by the vertebrate scavengers. These cages were placed on the ground for easy access by the crawling insects [6,29]. The time of death and deposition at the sites were recorded and this day was designated as day 0 [35].

Collection of Insects at the Mock Crime Scene

After setting the sites, the first species of insect that arrived at the scene was recorded. The first species that arrived, the time of arrival of the first species, the killing method visited, and the part of the body that was first colonized were recorded. Insects were collected from the rat carrions twice daily during the first eleven days of killing and at 2 days interval during the rest periods of decomposition [6,34]. Insects were collected at the scene from 7 – 10 am and 3 – 6 pm when most of the insects were active [36]. This was to ensure that all ranges of insects that visited the scene were sampled as some might be active at different times of the day. Insect net was used to collect flying insects while eggs, larvae and pupae were collected from the remains using brush and forceps [29]. Eggs and larvae collected were reared on beef liver in the laboratory until they grew to adults for ease of identification. Two pitfall traps were set on each site to catch nocturnal crawling insects as well as wandering larvae [37]. The samples collected from each remain were preserved in 70% ethanol and labeled appropriately. Insects were collected from the natural openings on the body as well as other parts of the body.

Identification of the Arthropods Collected

The arthropods collected were identified in the Entomology Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka with the aid of microscope/hand lens and using morphological keys [38-48]. The samples were, thereafter, sent to Entomology Museum, Department of Biological Sciences, Ahmadu Bello University Zaria where they were compared with voucher specimens and the identities confirmed by a taxonomist in the Museum. The number of each species collected was recorded while voucher specimens of all species collected were deposited in the Entomology Laboratory, Department of Zoology and Environmental Biology, University of Nigeria Nsukka.

Statistical Analysis

Data collected on insect fauna and successions were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Analysis of variance (ANOVA) was used to compare the arthropod emergence from the reared larvae on the different killing methods. The analysis was done at 95% confidence interval.

Results

Arthropod Fauna of the Decomposing Rat Carcasses

A total of 5,064 arthropods were collected during the period of study. They comprised the necrophagous arthropods, predators, parasites, adventives and accidental arthropods. Some of the necrophagous species include; *Chrysomyia chloropyga, C. albiceps, Lucillia sericata, L. cuprina* (Calliphoridae), *Oestrus ovis* (Oestridae), *Sarcophaga inzi, S. exuberans* (Sarcophagidae), *Musca domestica, Morellia nilotica* (Muscidae), *Dermestes maculatus, D. frichii, D. atar* (Dermestidae) and *Necrobia rufipes* (Cleriidae). Adults of Dermestidae, Histeridae, Scarabaeidae and Formicidae were predatory on necrophagous species. The adventives and accidental species were *Sandalus niger, Harpalus honestus, Prosoestus* sp., *Lema dentipes, Lethocerus* sp., *Laemophaeus fasciatus, Gryllus* sp., *Scolopendra gigantean* (centipede), *Anadenobolus* sp. (millipede), *Araneus* sp. (spider), *Periplaneta* sp. and *Lymantria dispar* (moth). They were termed adventives or accidentals because they did not breed on the carcass. All the arthropod species collected on the decomposing giant rat remains are shown in Table 1. The most preponderance insect species from the three killing methods was *Chrysomyia albiceps. Chrysomyia albiceps* was significantly higher across the killing methods (P < 0.05) with violent death being highest (581) and the least found in natural death (74).

Order	Family	Species	Total number of species
	Calliphoridae Chrysomyia chloropyga (Wied.) Chrysomyia albiceps (Wied.) Stomorhina rugosa (Bigot.) Bengalia peuhi (Villen.) Lucillia sericata (Meig.) Lucillia cuprina (Wied.) Pollenia sp. (Fab.) Megaselia scalaris (Loew.) Sarcophagidae Sarcophaga exuberans (Pand.) Sarcophag aginzi (Curron) Muscidae Musca domestica (Linn.) Morellia nilotica (Walk.) Oestridae Oesterus ovis (Linn.) Dermestidae Dermestes frischii (Klug.) Dermestes ater (DeG.) Trogoderma granaries (Everts.) Cleriidae Necropis rufipes (DeG.) Curculimidae Prosoestus sp. (Faust.) Chrysomelidae Gymnopleurus fulgidus (Oliv.) Gymnopleurus laevicollis (Cast.) Phanaeus igneus (Mac.)	Chrysomyia chloropyga (Wied.)	460
		1189	
		Stomorhina rugosa (Bigot.)	25
Dinton		Bengalia peuhi (Villen.)	21
Diptera		Chrysomyia chloropyga (Wied.) Chrysomyia albiceps (Wied.) Stomorhina rugosa (Bigot.) Bengalia peuhi (Villen.) Lucillia sericata (Meig.) Lucillia cuprina (Wied.) Pollenia sp. (Fab.) Megaselia scalaris (Loew.) Sarcophaga exuberans (Pand.) Sarcopha gainzi (Curron) Musca domestica (Linn.) Morellia nilotica (Walk.) Oesterus ovis (Linn.) Dermestes frischii (Klug.) Dermestes maculatus (DeG.) Trogoderma granaries (Everts.) Necropis rufipes (DeG.) Prosoestus sp. (Faust.) Lema dentipes (Jac.) Gymnopleurus fulgidus (Oliv.) Gymnopleurus laevicollis (Cast.)	122
		Lucillia cuprina (Wied.)	5
		Pollenia sp. (Fab.)	7
	Phoridae	Megaselia scalaris (Loew.)	28
	Sarcophagidae	Sarcophaga exuberans (Pand.)	97
		Sarcopha gainzi (Curron)	448
Diptera	Muscidae	Musca domestica (Linn.)	325
		Morellia nilotica (Walk.)	161
	Oestridae	Oesterus ovis (Linn.)	3
	Dermestidae	Dermestes frischii (Klug.)	195
		Dermestes maculatus (DeG.)	99
		Dermestes ater (DeG.)	18
		Trogoderma granaries (Everts.)	16
	Cleriidae	Necropis rufipes (DeG.)	43
	Curculimidae	Prosoestus sp. (Faust.)	1
0.1	Chrysomelidae	Lema dentipes (Jac.)	1
Coleoptera	Scarabaeidae	Gymnopleurus fulgidus (Oliv.)	5
		Gymnopleurus capensis (Ferr.)	11
		Gymnopleurus laevicollis (Cast.)	39
		Phanaeus igneus (Mac.)	2
		Ateuchetus laticollis (Fab.)	3
		Euphoria kernii (Hald.)	5
	Trogoidae	Omorgus bachorum (Eric.)	8

	Histeridae	Platysoma leconti (Mars.)	16
		Saprinus sp. (Erichson)	9
		Euspilotus assimilies (Pay.)	8
		Euspilotus scrupularis (Fisher)	3
		Margarinotus brunneus (Fab.)	2
		Harpalus honestus (Dufisch.)	1
	Carabidae	Galeritiola africana (Dejean)	2
	Cucujidae	Laemophoeus fasciatus (Melsh.)	1
	Rhipiceridae	Sandalus niger (Knoch)	1
Hymenoptera	Formicidae	Oecophylla longinoda (Fab.)	378
		Camponotus maculatus (Erich.)	155
		Camponotus sericus (Fab.)	866
		Monomorium minimum (Buckley)	205
	Vespidae	Vespa sp. (Linn.)	2
Orthoptera	Gryllidae (Cricket)	Gryllus bimaculatus (Linn.)	31
Lepidoptera	Erebidae (Moth)	Lymantria dispar (Linn.)	3
Hemiptera	Belostomatidae	Lethocerus sp. (Mayr)	4
Dictyoptera	Blattidae	Periplaneta sp. (Fab.)	11
Arachnidae	Araneae (spider)	Araneus sp. (Clerck)	23
	Scolopendridae (Centipede)	Scolopendra gigantea (Linn.)	3
Scolopendromorpha	Rhinocricidae (Milipede)	Anadenobolus sp. (Von Porat)	3
Total			5064

Table 1: Arthropod Fauna of the Giant Rat Carcass during the Study Period

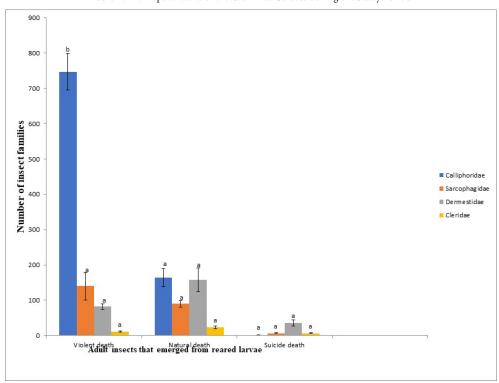


Figure 2: Insect families that emerged from the reared insect larvae

The insect families that bred on the carcasses are shown in Figure 2. The families Calliphoridae and Sarcophagidae were higher in violent killing method compared with other killing methods but only Calliphoridae was significantly different (P < 0.05). Natural death had the highest number of Dermestidae and Cleriidae while the least was observed in suicide death but there was no significant difference in the number of the beetles among the killing methods (P > 0.05). The family Calliphoridae formed maggot masses in violent death but no maggot mass was observed in natural death. The suicide death had the least number of Calliphoridae and other insect families that breed on the carcass. The calliphorids and other arthropods were recorded in suicide death during the early stages of decomposition, but they all died on the spot. Within 48 hours of killing the rats, the suicide death recorded dead Calliphoridae (773), Muscidae (156), Sarcophagidae (74), Coleoptera (5), Formicidae (*Camponotus sericeus*) (402) and Formicidae (*Oecophylla* sp.) (1).

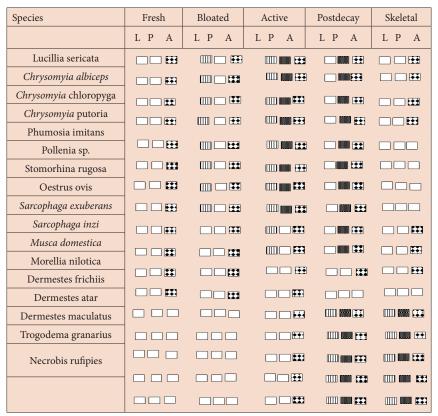
Insect Succession on the Decomposing Giant Rat Carcass

In violent death *Monomorium minimum* (Hymenoptera: Formicidae) was the first species to visit the rat carrion after 5 minutes. They were seen licking the blood on the carcass. The blowfly (family Calliphoridae) arrived at the scene after 29 minutes and perched on the open wound. In natural death, *M. minimum* visited the carcass after 7 minutes. It was crawling and licking the sweat on the carcass. *Camponotus perrisi* also visited the carcass at the early stage. *Musca domestica* visited the carcass after 20 minutes. It only perched on the carcass and left after few seconds. In suicide death, *Camponotus sericeus* visited the carcass after 2 hours 45 minutes but died instantly on the scene. The flesh fly (Sarcophagidae) was seen after 24 hours though it still died on the spot. The blowflies that colonized the carcass laid eggs on the natural orifice of the body within 24 hours of death. In violent and natural deaths, dipteral eggs were found on the mouth, head, open wound (only on the violent death), anus (only on the natural death) and on other body parts. Neither eggs nor larvae were found on the suicide death during the fresh and bloating stages of decomposition. The Sarcophagidae also visited the carcass during this early stage of decomposition but did not lay eggs immediately.

In suicide death, eggs and larvae were collected during the late active decay stage but, they were never found on the mouth, anus or on the head rather, eggs and larvae were found on other parts of the body apart from the natural orifices. The head was not colonized by any insect species until the post decay stages. In suicide death, the insect visitors stopped dying on the 4th day, but no egg was collected until the 6th day. All the larvae collected on the 6th day were dead, the viable eggs and larvae were collected on the 7th day of decomposition. In natural and violent deaths, eggs and larvae of Sarcophagidae were collected on the evening of the 3rd day but in suicide death, it was collected on the 11th day of decomposition. The muscid larvae were never collected over the nine carcasses though the adults were present from the fresh stage to the end of carcass decomposition. Other arthropod families that visited the carcass during this stage were Histeridae, Dermestidae, Cleridae, Scarabaeidae, Belostomatidae, Trogidae and Rhipiceridae. The family Dermestidae, Cleridae and Histeridae were found in all the killing methods. The family Trogidae and Scarabaeidae were found in the natural and suicide death while Belostomatidae and Rhipiceridae were only found in the natural death. Dermestidae visited the carcass of violent and natural death on the 5th day but on suicide death, it was found on the 10th day. They were present till the skeletonization stage in all the killing methods though their eggs and larvae were only found during post decay stage. In suicide death, Dermestidae visited the carcass on the 10th day but their larvae were found from the 20th day of decomposition till the end of decomposition.

Post decay stage was marked by the gradual decrease in dipteral larvae or pupae and presence of coleopteran adults and larvae. The coleopteran adults fed on the bones, fur and other dry flesh that was left by the dipteral larvae.

During the skeletal stage of decomposition, the greatest number of coleopteran larvae was found on the natural death and the least larvae were observed on the suicide death though it took the longest period of decomposition. The successional pattern of the necrophagous species during different stages of decomposition is shown in Table 2.



Legend: Neither larvae (L), Pupa (P) nor Adult (A) were present; Larva present; Pupa present; Adult present. Table 2: Stages of insect (Larvae, Pupae and Adult) species present during decomposition in their successional pattern

Discussions

The results of this study provided a clear interpretation of insect evidences at a crime scene for the three major means of death in the society. Dipteran eggs and larvae in the natural orifices (mouth, anus, ears, eyes) but no maggot mass in the body is indicative of natural death. Dipteran eggs and larvae on the mouth and maggot masses on the body is suggestive of violent death with open wound. The maggot masses indicate sites of open wound in the body. On the other hand, absence of eggs and larvae, and presence of many dead adult insects around a body is evidence of suicide death involving chemicals. Dead dipteran eggs, larvae and absence of eggs and larvae in the natural orifices is also suggestive of suicide death using chemicals.

The delay in the colonization of the rat carcasses in suicide death will affect determination of PMI using maggot ages. However, this study showed that viable eggs and maggots were collected on the rat carcasses on the 7th day of death. Hence, absence of viable eggs and maggots on a decomposing remain plus other evidences such as many dead insects on the body, absence of eggs and larvae on the natural orifices indicates that the body has been dead for less than 7 days and is a suicide death involving chemicals. However, presence of viable eggs and larvae plus other evidences such as many dead insects and absence of eggs and larvae on the natural orifices indicates a suicide death from chemical and that the body has been dead for at least 7 days.

The most abundant insect Orders were: Hymenoptera, Diptera and Coleoptera. This result is in line with [6,27,49] who observed the same insect Orders as the most abundant in their studies. The family Formicidae was found in all the killing methods and was represented by *Monomorium minimum*, *Camponotus sericeus*, *Oecophylla* sp., and *Camponotus consobrinus*. This result corroborates the findings of [6] who also recorded Formicidae in all the killing methods and stages of decomposition. The family Formicidae are the earliest visitors recorded in the different killing methods however, they are less important in determining PMI since they do not breed in the carcass. They are adventive species and do not offer useful information on the cause of death except in the suicide death where they will die and suggest the presence of chemical.

The family Calliphoridae remains the most important of other families of Diptera in forensic entomology. The genera of the family Calliphoridae that first colonized the carcass are *Lucilia* and *Chrysomyia*. They occurred in all the killing methods in the study sites. This finding agrees with [6] who also recorded the genus *Chrysomyia* in all killing methods and throughout the decomposition stages in Ugbor Village, Benin City Edo State. *Chrysomyia albiceps* was the most abundant and most diverse species in the whole killing methods. This observation agrees with the work of [29,50,51] who also recorded *C. albiceps* as the most abundant and distributed species in carcasses. This species has been widely recorded in carrion across Nigeria [19,26,29].

Two species of the family Sarcophagidae - Sarcophaga inzi and Sarcophaga exuberans were recorded in the study. They visited the carcasses during the fresh stage and laid eggs on the caresses on the 3rd day after depositing the carcasses. These species of sarcophagids were also recorded by [19,29] in Kaduna State and Okija in Anambra State respectively. Similarly, [6] observed species of sarcophagids between 2-4 days in Ugbor Village, Edo State. Sarcophagids are less important in determining PMI since they arrive after some days of death.

The family Dermestidae dominated the beetles collected in the study and comprised *Dermestes frischii*, *D. maculatus*, *D. atar* and *Trogoderma granarium*. They occurred on the 5th day and the post decay and skeletonization periods. Of all the beetles, only *N. rufipes* bred on the carcasses. These observations agreed with [6,52] who observed dermestid beetles between 3-11 days, and [29,33,54] who recorded *D. rufipes* breeding on the carrion. The observations indicate that the beetles can be used to determine a window of period when death occurred since they visited later during decomposition stage.

The family Histeridae was observed during the bloating stage and the six families recorded were also reported by [9,26,37,51,54,55]. The species visited the carcasses but did not breed on the carcasses hence, they are of less importance in PMI determination. The larvae of the family Scarabaeidae were not collected over the carrion; therefore, they only used the carcasses for food and shelter which agrees with the observations of [28,53]. Other species encountered in this study were *Gryllus bimaculatus* (cricket), *Lymantria dispar* (moth), *Lethocerus* sp., *Periplaneta* sp. (cockroach), *Araneus* sp. (spider), *Scolopendra gigantea* (centipede) and *Anadenobolus* sp. (millipede). These species only feed on the carcasses and used the carcasses as an extension of their habitats but do not breed on the carcasses. They are regarded as predators, parasites and accidental or opportunistic arthropods in a crime scene. The earlier researchers also reported similar opportunistic arthropods in carrion; [26] collected Orthoptera (Acrididae, Gryllidae), Dictoptera (Perisphaeridae), Arachnida (Aranaea) and Diplopoda (Juliforma) while [27] observed Araneidae (spider), Lepidoptera (*Danaus plexippus*) and Orthoptera (Gryllidae, *Monopsis argillacea*). Similarly [29], observed Orthoptera (Gryllidae), Dictyoptera (Prygomorphidae, Mantidae) and Hemiptera (Plastaspidae, Coreidae, Tiphiidae) in the studied carrions.

Conclusion

This study established the arthropod fauna, arthropod succession and interpretation of insect evidence in a crime scene in Nsukka Nigeria. The result of the study offers salient and robust means of investigating causes of deaths in the society. This study when reproduced in many other regions across Nigeria where data on insect fauna and succession on decomposing remains are lacking will improve criminal investigations and aid criminal justice in law courts in Nigeria.

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