

Entomotoxicological Analysis Of Pig Carcass With 2, 3-Dichloro Vinyl, Dimethyl Phosphate

Research Article

Cosmas, Augustus Uhuo^{1*}, Ewuim Sylvanus², Elebe Florence Amarachineke¹, Imakwu, Cyril³, Oliver Odikamnor¹, Onwe, Chikadiri¹, Elom, Michael¹

¹ Department of Biological Sciences, Ebonyi State University Abakaliki, Ebonyi State, Nigeria.

² Department of Zoology Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

³ Department of Parasitology and Entomology Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

⁴ Department of Animal and Environmental Biology, University of Portharcourt, River State, Nigeria.

Abstract

Background: Entomotoxicology an aspect of Forensic Entomology is a critical approach of forensic toxicology that addresses the presence of toxic chemicals in insects that have contact with a decomposing cadaver. Toxic substances in or on a dead body may accumulate in the tissues of insects while feeding and either kill or alter their developmental cycle and affect successional pattern. This study was aimed to determine the effects of poisonous chemicals on the insect succession of pig carrion decomposition in Abakaliki Ebonyi State at latitude 6.333oN 8.100oE using pig (*Sus scrofa* Linn.) that weighed 22.3 ± on the average.

Results: The analysis revealed that the AAS detected and quantified phosphate, ethylether and chloride ion in the maggot samples from the poisoned cadavers. High humidity (93.2 6.5) buffered the effect of the poisonous chemical into killing the insect witnesses because it slowed the decomposition rate. The inconsistency in insect attendance in carrion decomposition time interval and insect assembly was due to poisonous effects of the chemical on the carrion, this is because insect colonization brings about decomposition. The effects of the chemical killed the insects and destroyed and destroyed and decomposition rate of the carrion. The arthropod assemblies were identified and classified into 4 phyla, 9 families and 26 genera, where dipterans were recorded the highest in attendance pattern.

Conclusion: It is interesting to note that metabolisms of insecticides are usually rapid, hence, the phosphide rapidly metabolized in the cadaver body, thus, liberating the phosphorus into phosphine gas which caused acute kidney and heart failures. The chemical poisons was assumingly affected the cadaver decomposition pattern as well distorted insect colonization mainly dipteran flies after death. This understanding would enhance the global data to be acceptably applied during coronary investigation of cases associated with poisonous deaths as entomological data.

Keywords: Entomo-Toxicological; Pig Carcass; 2, 3-DVDP; Insects; Abakaliki; Nigeria.

Background

Insects are the most abundant animals on earth and are present in all ecological systems, especially terrestrial environments [5]. Because of their ubiquity, they are usually present as silent witnesses at crime scenes [11]. Some sarcophagous flies are very agile and can reach a carcass and oviposit within a few hours of death. Forensic entomology, the scientific discipline which uses the presence of insects at sites of crime to establish the causes and the time of such death while entomotoxicology is the analysis of carrion feeding insects to detect toxic substances and to investigate their effect on the insect development. Forensic entomology can

assist the pathologists in narcotic or drug intoxication of the adverse as the insect specimens may serve as alternate reliable specimens for the examination of toxicologically relevant substances in the absence of appropriate sources such as tissue, blood or urine in highly decomposed or badly skeletonized bodies (Joy et al.; 2004). The insects encountered or their larvae may reveal through toxicological assessment the cause of death, as they feed on the corpses, sequester drugs and other toxicants that may have been ingested by the deceased person.

The use of insects in death investigation has been extended in crime scenes on land and water (Keiper and Casamatta 2001)

*Corresponding Author:

Cosmas, Augustus Uhuo,
Department of Biological Sciences, Ebonyi State University Abakaliki, Ebonyi State Nigeria.
E-mail: coscusanas@gmail.com

Received: March 14, 2023

Accepted: April 12, 2023

Published: April 28, 2023

Citation: Cosmas, Augustus Uhuo, Ewuim Sylvanus, Elebe Florence Amarachineke, Imakwu, Cyril, Oliver Odikamnor, Onwe, Chikadiri, Elom, Michael. Entomotoxicological Analysis Of Pig Carcass With 2, 3-Dichloro Vinyl, Dimethyl Phosphate. *Int J Forensic Sci Pathol.* 2023;10(1):503-509.

Copyright: Cosmas, Augustus Uhuo[©]2023. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

[13]. It is also evolving into entomotoxicology, which is a critical approach in forensic toxicology that alternatively assesses presence of toxins in insects that have eaten a decomposing cadaver. Apparently, the use of insects in crime scene investigation has been well documented [8, 21], and the history is revalidated [3, 9]. Toxic substances in or on a dead body may accumulate in the tissues of fly maggots while feeding on the body and thus may provide evidence of the toxin that killed the dead body. Dichlorvos (2,3-dichlorovinyl dimethyl phosphate, commonly abbreviated as an DDVP) is an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored products from insects. The compound has been commercially available since 1961 and has become controversial because of its prevalence in urban waterways and the fact that its toxicity extends well. 2,3-Dichloro-1-propanol belongs to the group of chloropropanols. Inhibitory effects of 2,3-dichloro-1-propanol on T cell both in vivo and in vitro is reported. Improved enantioselective resolution of (R,S)-2,3-dichloro-1-propanol to (S)-2,3-dichloro-1-propanol by whole cells of a recombinant *Escherichia coli* in n-heptane-aqueous biphasic system is reported.

Since it is an acetylcholinesterase inhibitor, symptoms of dichlorvos exposure include weakness, headache, tightness in chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhea, abdominal cramps, eye and skin irritation, miosis (pupil constriction), eye pain, runny nose, wheezing, laryngospasm, cyanosis, anorexia, muscle fasciculation, paralysis, dizziness, ataxia, convulsions, hypotension (low blood pressure), and cardiac arrhythmias.

The insect specimens incorporate and bioaccumulated chemical metabolites of drugs into their tissues such as barbiturates, cocaine, amphetamines, and even poisons. The insect tissues or even remnants of pupal/larval specimens can be macerated and processed to detect these substances even after several years of death. But the ingested drug or toxicant can influence or modify the development of the necrophagous flies, thus causing a risk of calculating incorrect PMI. So before using the insect specimens for PMI determination, the forensic entomologist should be aware of the extent of effects of drugs and toxins on the developmental stages of the insects or their delayed invasion of the tissues [10].

Secondly, chemical analysis of maggots found on, in or around the cadavers can reveal the presence of specific drugs/chemicals/poisons, particularly in cases where no human tissues are available for investigation.

Apparently, determining the time and cause of a questionable death of a corpse is always difficult if the insect evident died in contact with the body fluid emitting from the decomposing animal, attendance was missed and emerging larval stages were dead. But still, it is an important component of coronal death investigation. In this study therefore, the decomposition duration in days of poisoned pig cadavers as models to human corpse were studied behind College Mortuary at Ebonyi State University Abakaliki Ebonyi State, Nigeria. This study was aimed to determine the effects of poisonous chemicals on the insect succession of pig carrion decomposition using AAS.

Methods

Study area: This research was conducted at the topographical

area in Presco Campus behind College mortuary, Ebonyi State University, Abakaliki, within a geographical area with longitude and latitude of 6o201N8o061/6.333oN8.100oE owned by Ebonyi State University Abakaliki Nigeria. The surrounding area behaves semi-aquatic plant planted along the ridge of the channel and shrubs, tall trees, and palm trees which provided shade against sunlight to the pig carrion. This area mapped was 34m² with predominantly sandy clay soil with densely plants and shrubs. The site was selected to ensure limited public access and minimize potential human interference to represent dead bodies under trees at the back of the building. This represents a trial which started at the late rainy season in 2019.

Cage: The cage is locally fabricated wire gauze stalked to the ground to prevent scavengers feeding on the carrion and other animals from feeding on the insects.

Pitfall trap: There are four proximal pitfall traps that surrounded the sample carcass and were positioned one meter from the cage. These pitfall traps were used to record the time at which the first maggot entered the wandering stage of development and how long the maggot stage lasted. These traps were also used to record the directional movement of calliphorid larvae and to collect an astronomical number of maggots associated with the pig carrion. They were placed into the soil that the lid of the pitfall containers was flushed at ground level. The insects in the bottles were collected every day and strained into a sample bottle for preservation. The collection of maggots ceased after the peak period of maggot dispersal was completed from the pitfall traps.

Chemical used for poisoning the pig: The chemical used was 2ml of 2, 3-dichloro vinyl, dimethyl phosphate (sniper) purchased from the local market in Abakaliki. The chemical was injected into the pig using syringe at the heart region at 0700 hours in the morning. After forty minutes of the injection, the pig started tumbling, hiking up and gasping air and later started producing fumes at 45mins of this exercise, dozed off and died.

The pigs were sacrificed with 2, 3-dichloro vinyl, dimethyl phosphate (poison). 2, 3-dichloro vinyl, dimethyl phosphate (sniper) is an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored products from insects. This is sold in open market at Abakaliki, Nigeria, as insecticide with a trade name as "sniper". It is a systemic poison that is used to different insects. Occasionally, the poison has been alleged to be used to commit suicide because of its availability, accessibility, and affordability. Each of the pigs was injected with 2ml of the poison to mimic drug poison. The purpose is to mimic a dead body through poisonous injection or chemical poisoning. Some samples of maggots collected between day 4 and day 20 were killed with hot water and preserved with 70 % ethanol. One gram of each of the preserved maggots for each day which were morphologically dissimilar and representing three dipteran maggot families was washed with distilled water. The washed maggots were digested with 10 ml of 70 % chloric acid (HOCl₄) and 10 ml of concentrated nitric acid (HNO₃) by indirectly heating with a water bath at 60°C for 1 h in a fume cupboard. The filtered solution of the digested maggots was analyzed with an atomic absorption spectrophotometer (AAS-model: BUCK Scientific 210GP) to assess and quantify chlorine, ethyle ether and phosphate contained in the maggots collected during the study.

Waves/Stages of decomposition

The decomposition stages are commonly described as fresh, bloat, active, advanced and dry decay stages [13, 20].

Fresh stage: The fresh stage of decomposition is generally described as the period between the moment of death and when the first signs of bloats are apparent. There are no outward signs of physical change, though internal bacteria have begun to digest organ tissues. No odour is associated with the carcass.

Bloat Stage: The first visible sign of the bloat stage is slight inflation of the abdomen and some blood bubbles at the nose. Activities of anaerobic bacteria in the abdomen create gases which accumulate and results in abdominal bloating.

Active Decay Stage: The beginning of active decay stage is marked by the deflation of 'the carcass as feeding dipteran larvae pierce the skin and internal gases are released. During this stage, the carcass has a characteristic wet appearance due to the liquefaction of tissues.

Advanced Decay Stage: Most of the flesh is removed from the carcass during the advanced decay stage, though some flesh may remain in the abdominal cavity. Strong odors of decomposition begin to fade. This stage marks the first mass migration of third instar calliphorid larvae from the carcass.

Dry Decay: The final stage of decomposition is dry remains. Very little remains of the carcass is mainly bones, cartilage and small bits of dried skin. There is little to no odor associated with remains. Any odor present may range from that of dried skin to wet fur.

Insect Sampling

The carcass was collected and recorded during the time of arrival and lasting duration at different waves of decomposition of the pig carrion. During this trial, insects were collected by using sweep net and handpicking using forceps inside and around the cage. Also, all insects collected were put into vials of 4% formalin for preservation and returned taken to Applied Biology laboratory for proper identification and preservation.

Measurement of environmental variables

The research site has a data logger (KT908) which was used to re-

cord ambient temperature (AMT) and Ambient humidity (AMH), at 10mins in each visit throughout the experiment. Glass in mercury thermometer probe used was inserted between the carrion and the ground, in and around the carcass to record the ground temperature. The physical features of the carcass were observed at each point during the time of specimen investigation. The carcass was visited between 7:00 am and 5 pm local time (GMT) every day till the end of the experiment. Adult insects on the carcass were collected by sweep netting. Eggs, Larvae, Pupae and ground crawling arthropods were collected by hand using forceps from, on, in, under and around the carcass (head, abdomen, and anus).

Larvae were found on and near the carcass especially dipteran larvae in large masses. The temperature of larval mass was recorded using mercury in glass thermometer. The sampled larvae were killed by immersion for 10-15mins in boiled water and then transferred to 4% formalin.

Ethical Clearance

This research titled: The Entomotoxicological analysis of pig carcass with 2, 3-dichloro vinyl, dimethyl phosphate followed ethical approval from Animal Welfare Committee, Ebonyi State Directorate of Veterinary Services, Ministry of Agriculture, Abakaliki, Ebonyi State. The use of pig for this research was approved to be sacrificed for the purpose of this study with strict adherence to the ethical best practices.

Data Analysis Data were generated from the insect samples collected from the decomposing pig carcass. Also, data were collected from the Thermo hygrometer logger and other instruments used for the microenvironmental variables and combined to form values and figures for the analysis. Data generated from insect colonization were analyzed among waves of decomposition using ANOVA while the microenvironmental variables of weather parameters determined during this study were subjected to analysis using Chi-square test to separate the samples mean of the values with respect to microenvironment when significant statistically ($p < 0.05$).

Results

The table above showed the effect of ambient temperature and ambient humidity on the pig carrion decomposition stages. It was observed that decomposition was delayed at the bloat stage and active stage of decomposition. The ANOVA result showed that

Table 1. Effects of temperature and humidity on the time interval of decomposition stages.

Stages	Time Interval in Sec	HMax (%)	TMax(0C)
Fresh	14400	91	31.2
	40	7	9.4
Bloat	18000	93.2	27.6
	50	6.5	5.8
Active	21600	90	28.9
	49	11.5	3.3
Advance	21600	85.3	28.2
	28	3	4.3
Dry	28600	70.1	32.2
	56	19.5	5.4
Total	20880	85.92	29.62
	46.6	9.5	5.64

there is a significant difference between ambient humidity at [P 0.05].

Two stages i.e. fresh and bloat stages recorded a high mean number of AMH than other stages at the decomposition period (91%) and (93%) respectively. The ANOVA result revealed that increased ambient humidity decreased ambient temperature and prolonged decomposition of the carrion while decreased AMH increased AMT shortened decomposition interval.

The analysis of the pig carrion decomposition with respect to geographical weather conditions showed that decomposition processes were prolonged due to the effect of weather conditions. It was observed that the fresh stage took only one day before it transited to bloat where the maximum temperature recorded highest at 32.2°C and lowest at 27.60°C respectively. This study showed that high humidity and low temperature as shown in fig-

ure 2 where highest recorded at 93.2 % and lowest at 70.1 %. It was also observed that active and advance stages of decomposition witnessed prolonged decay process due to high humidity and low temperature recorded. Dry decay did not last longer time interval due to rise in temperature, although it did not attain skeletonization stage easily because of a variable in the weather condition.

Discussion

The AAS detected and quantified phosphide ions, ethyle ether and chloride ion in the maggot samples from the poisoned cadavers. Dipteran maggots are allogenic based on their behavioral adaptability. Hence, they usually take residence on vertebrate cadavers especially when the cadaver is accessible by adult flies. They are known to consume cadaver tissues of all sources, but consuming poisoned cadavers was expected to affect their devel-

The time interval in days of decomposition with respect to temperature and humidity

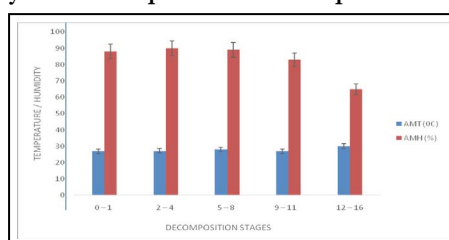


Table 2. The pattern of insect checklists on pig carrion in Abakaliki.

Order	Family	genus/sp
Diptera	Calliphoridae	<i>Chrysomyia albiceps</i> Wied
		<i>Chrysomyia chloropyga</i> Wied
		<i>Chrysomyia regalis</i> Rod-Desv
	Muscidae	<i>Musca domestica</i> Linn
		<i>Musca sorbens</i> Wied
	Syrphidae	<i>Microdus</i> sp.
	Sarcophagidae	<i>Sarcophaga inzi</i>
		<i>Sarcophaga exuberans</i> Pandelle
		Calliphoridae
	Coleoptera	Dermestidae
<i>Vanemdenia africana</i> Peris		
Dermestidae		<i>Dermestes frischii</i> Klug
		<i>Dermestes ater</i> Deg
Chrysomelidae		<i>Haltica</i> sp
		<i>Lema affinis</i> Cik
Staphylinidae		<i>Philonthus</i> sp.
Dermestidae		<i>Dermestes maculates</i> Deg
Chrysomelidae		<i>Buphonella</i> sp.
		<i>Buphonella nigraviolacea</i> All
	Hymenoptera	<i>Camponotus acvapimensis</i> Mayt
Hymenoptera	Formicidae	<i>Dorylus affinis</i> . Emy
		<i>Messor galla</i> Emy
		<i>Myrmicaria striata</i> Stilz
		<i>Pheidole</i> sp.
Orthoptera	Tetrigidae	<i>Paratetix</i> sp.

Table 3. Arthropod Succession on Pig Carrion among decomposition stages.

Decomposition Stage	Arthropod succession
Fresh stage	Musca domestica, Musca sorbens, Chrysomyia albiceps, Chrysomyia regalis, Chrysomyia chloropyga, Sarcophaga inzi, Myrmecaria striata, Lema affinis.
Bloat stage	Chrysomyia albiceps, Chrysomyia regalis, Chrysomyia chloropyga, Sarcophaga inzi Sarcophaga exuberance Buphonella sp, Messor galla,
Active stage	Chrysomyia regalis, Microden sp, Rhyncomya cassotis, Haltica sp, Musca domestica, Musca sorbens, Vanemdenia africana, Buphonella sp, Camponotus acvapimensis, Messor galla
Advance stage	Chrysomyia albiceps, Lema affinis, Philonthus sp. Musca domestica, Musca sorbens, Dermestes frischii, Dermestes ater , Buphonella nigraviolacea, Messor galla
Dry stage	Dermestes frischii, Dermestes ater Chrysomyia regalis, Musca domestica, Musca sorbens , Sarcophaga sp. Philonthus sp. Buphonella sp, Paratetix sp, Lema affinis

Plate 1. Death of larval mass.



Plate 2. Death of different species of flies.



opment. In this study, the chemical poisons was assumingly expected to affect the cadaver decomposition but was not observed as decomposition commenced immediately after death and as well attracted distorted insect colonisation mainly dipteran flies after death. The differences observed in the decomposition duration of the cadavers was as a result of environmental changes that influenced the temperature and the relative humidity. This agrees with the report (Gunatilake and Goff 1989) that malathion in the tissues of a decomposing body delayed insects’ colonization and oviposition for several days. This was contained in a study of a suicide case where malathion pesticide had been consumed. The developmental stages of blow flies found on the dead body indicated a minimum postmortem interval of 5 days while the victim was last seen alive 8 days prior to the discovery of the body.

Pig cadaver was chosen for the study as alternative for human model because of its analogue to human cadaver and has been reported to attract similar arthropod fauna recorded for human cadavers in studies relating to forensic entomology [22]. The pigs were sacrificed with 2, 3-dichloro vinyl, dimethyl phosphate (poison). This chemical 2, 3-dichloro vinyl, dimethyl phosphate (sniper) is an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored products from insects. This is sold in open market at Abakaliki, Nigeria, as insecticide with a trade name as “sniper”. It is a systemic poison that is used to different insects.

In forensic entomology, insects are used as a potential source of evidence in cases of murder or suspicious death. This is because many insects are associated with the human body after death and their pattern of colonization occurs at a predictable sequence [20]. This study has become immensely important because they are the most diversified animals ever known to use than men. Murder cases associated with chemical poisons can destroy evidence testifying to the coronary investigation about the sequence of events and time elapsed during death with the true cause of death and event in our diverse ecological regions.

The temperature curve in relation to the decomposition stages of the carcass (Fig 1) showed that the ambient temperature was high at the active decay and declined again at the dry decay/remains. In this case, the temperature determines the rate of decomposition from the fresh to dry remains and showed whether the decomposition is faster or slower.

The decomposition stages of carcasses Table 1 showed that the decomposition rate was faster in the active decay (5-7) days and advanced decay. This is because the stage is characterized by a heat period at which rainfall had ceased with low ambient humidity and high temperature which may have assisted in the carcass degradation by the succeeding insect fauna on the carrion. However, irrespective of the carcass environment condition, the dry decay

stage of decomposition generally recorded the longest decomposition period (>11 days). This is similar to the work of Ekraekene and lloba (2011) [6] which states that dry decay/remains recorded averagely higher decomposition process when compared to other stages but recorded low insect succession. They also recorded that the longest period of decomposition was in dry decay while the least was in fresh decay irrespective of the season.

The insect checklists picked from the pig carrion in Table 2 showed that four orders, 9 families and twenty-six genera were recorded during the study. Life and dead Calliphoridae were observed dominating in attendance within the stages of decomposition. Coleopteran order recorded second to the highest insect with 4 families and 8 genera. The hymenopteran recorded only 1 family, 5 genera while orthoptera recorded only 1 family and 1 genus. The study reveals that dipterans recorded the highest genera in the diversity of arthropod than any other insects observed in the study. This is because dipteran insects especially the Calliphoridae and Sarcophagidae were the first to witness the presence of decomposing carcass and were the first to oviposit and develop a chemical substance which can dispel other insects giving them a lead to other insects. It was observed that most eggs did not hatch into adults due to effect of the poisonous chemical and pupation did not complete its cycle due to poisonous interference of the chemicals in the soil. Most predatory Coleopteran and Hymenoptera that appeared during the decay process fed on the eggs, maggots and adult insects were as well seen dead and their presence could not compete with other insects. It is interesting to note that metabolisms of pesticides are usually rapid, hence, they rapidly metabolized in the cadaver body, thus, liberating the chemicals which caused acute kidney, lung, and heart failures. This biochemistry might be the reason the chemical component was heavy in the invertebrate's body. Therefore, this metabolic pathway may be the reason why the chemical component of the poison was assimilated in the maggots irrespective of the climatic difference between the two seasons. However, directly or indirectly consuming it in large amount will cause acute or chronic toxicity or poisoning of vital organs in the body. Consumption of chemical agents such as dimethyl phosphate becomes toxic when they are not metabolized by the body and accumulate in the soft tissues of the body [7].

The components of the poison especially phosphides were assumed to accumulate in the cadaver tissues and were stored in the cuticles of the maggots that fed on the poisoned pig cadavers, thus, supporting the reports (Amendt et al. 2004) that larvae feeding on a corpse may accumulate drugs and toxicants which had been ingested by the dead person. It is therefore interesting to note that if a toxic substance is ingested by a deceased person, the probability is that dipteran maggots found on the body will accumulate the substance. This may be necessary when maggot samples collected on the body is glaring that the victim was suspected to die of suspected toxic substance. The 2, 3-dichloro vinyl, dimethyl phosphate (sniper) is an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored products from insects. This "sniper" killed the pigs and was assessed in the fly maggots, is thus an evidence base report that the substance is toxic and capable of killing human being.

The detection of poisonous metals such as mercury in the larvae of various species of blowflies reared on tissues containing

known concentrations of mercury has been reported [19]. Toxicological data from fly larvae was reported to be reliable as well as those from cadaver tissues [15]. A study of Nolte et al., [18] reported that cocaine and its breakdown products have been found in small quantity in the puparium of a calliphorid fly, thus, validating the present report which assessed phosphide in the maggots that fed on 2, 3- dichloro vinyl, dimethyl phosphate-poisoned pig cadavers. It is interesting to note that metabolisms of insecticides are usually rapid, hence, the phosphide rapidly metabolized in the cadaver body, thus, liberating the phosphorus into phosphine gas which caused acute kidney and heart failures. However, directly or indirectly consuming it in large amount will cause acute or chronic toxicity or poisoning of vital organs in the body. Consumption of heavy metals such as phosphide becomes toxic when they are not metabolized by the body and accumulate in the soft tissues of the body [7]. The acute toxicity of the phosphide caused the death of the pigs, but the non-metabolism of other components in the pig tissues may have led to its recovery on the fly maggots that fed on the poisoned cadavers.

The study in plate 1 and 2 revealed that after the fresh stage which recorded low fauna of arthropod assembly followed liquification of chemical substances in the bloat stage during decomposition process. The chemical is assumed to have mixed with leachate from the carcass degradation in killing the various stages of the insects starting from the egg to the adult insects. This continued till the advance decay stage and progressed to post skeletonization stage. This stage recorded low or few arthropod faunae distorting the decomposition sequence and prolonging the decay process of the carrion. Dipteran insects observed at the cause of the succession were recorded with high mortality and these were majorly the insects which would have aided in a little way the slow decomposition of the carrion tissue.

They were no assembly of carrion beetle between fresh stage to advance until they started visiting the dry decay and post skeletonization stage. This may be because the eggs which they came to feed on were seen dead around the carcass and this could lead to distorted assembly. Therefore, poisonous chemicals can kill insect witnesses as evidence in murder cases as observed in this study.

The arthropod succession on pig carrion among decomposition stages Table 3 showed that arthropod observed in this study was inconsistent among the stages of decomposition. The fresh stage was dominated by Calliphorids. The bloat stage was also succeeded by other insects apart from Calliphorids and inclusive of the coleopterans and hymenopterans. There was a similar succession pattern of insects among all the decomposition stages which continued till dry decay stage and lastly at the dry decay/remains were recorded with few coleopteran insects which endured the wet-dry condition of the carcass.

The flies in attendance to the carrions of each season were *Chrysomya albiceps* at 13 minutes after carrion deposition followed by *Musca domestica* after 15 minutes and *Sarcophaga inzi* at 18 minutes. The dipteran insects under the family Calliphoridae attracted were as follows: *Stomorphina* sp, *Vanemdenia africana*, *Chrysomya chloropyga*, *Chrysomya regalis*, and *Chrysomyia albiceps*. The Sarcophagidae in attendance were *Sarcophaga exuberans* and *Sarcophaga inzi*. Muscidae in attendance includes *Musca domestica* and *Musca sorbens* which were regularly present even at the

dry decay stage. Therefore, the unavoidable infestation of the pig cadavers by these flies with their maggots via their laid eggs in an indoor scene and on poisoned cadavers, present them as useful samples for toxicological analysis in forensic toxicology of a badly decomposed corpse suspected to die of a toxic substance.

The most outstanding challenges from this research were the fact that none of the implicated insect species were present at the last stage of decomposition (dry decay). They came feed on the carcass and died, not living up to another stage in the decay process. Others who attended independently and encountered the same, were noticed in another decomposition stage. Observation reveals that none of the insect species identified from the scene of the study had more than one generation of life cycle throughout the waves of decomposition.

Conclusion

Insect's role in the decomposition process of carrion can provide useful information to determine the time after deaths associated with poisonous cadaver. On account of this study, species of insect taxonomically identified showed that most of the insects are considered with forensic values due to their behavior and role in the decay process. Their life cycle of generation was not completed to attest the reason and time of the suspicious death due to the volatility of the chemical agents. The post mortem interval was not complete due to sudden death occasioned by the chemical used in poisoning the animals.

This observation, therefore, has prompted the conclusion that 'the Entomotoxicological analysis of pig carcass with 2, 3-dichloro vinyl, dimethyl phosphate are predictable circumstances that can undermine the true evidence in murder scenes and thus can enhance entomological evidence if properly harnessed. Therefore, poisonous chemicals can kill and destroy the life cycle stages of the insects who gives witness to the cause of death in the case of a coronary investigation. This understanding would enhance the global database to be acceptably applied as entomological data. Further research focusing on the bioaccumulation and metabolism of drugs in necrophagous insects and their effects on their rate of development would be reason for case proof.

References

[1]. Abajue MC, Ewuim SC, Akunne CE. Preliminary checklist of flies associ-

- ated with pig carrions decomposition in Okija, Anambra State, Nigeria. *An Res Int.* 2014 Apr 17;11(1):1899-904.
- [2]. Abajue MC, Ewuim SC. Evaluation of activities of dipteran maggots on a poisoned pig cadaver at Nnamdi Azikiwe University Awka, Nigeria. *Egypt J Forensic Sci.* 2020 Dec;10(1):1-8.
- [3]. Benecke M. A brief history of forensic entomology. *Forensic Sci Int.* 2001 Aug 15;120(1-2):2-14. PubMed PMID: 11457602.
- [4]. Campobasso CP, Gherardi M, Caligara M, Sironi L, Introna F. Drug analysis in blowfly larvae and in human tissues: a comparative study. *Int J Legal Med.* 2004 Aug;118(4):210-4. PubMed PMID: 15106008.
- [5]. Catts EP, Goff ML. Forensic entomology in criminal investigations. *Annu Rev Entomol.* 1992;37:253-72. PubMed PMID: 1539937.
- [6]. Ekrakene T, Iloba BN. 2011;8(1):27-39.
- [7]. Ezeonu FC. Poisons in the Nigeria environment: within our reach, beyond our control. 25th Inaugural Lecture, Nnamdi Azikiwe University, Nigeria. Rex Charles and Patrick limited, Nimo, Anambra State; 2015. p. 92.
- [8]. Gennard DE. Forensic entomology: An introduction. Wiley, New York Goff ML; 2007.
- [9]. Greenberg B, Kunich JC. Entomology and the law: flies as forensic indicators. Cambridge University Press; 2002 Aug 29.
- [10]. Greenberg B. Nocturnal oviposition behavior of blow flies (Diptera: Calliphoridae). *J Med Entomol.* 1990 Sep 1;27(5):807-10.
- [11]. Greenberg B. Flies as forensic indicators. *J Med Entomol.* 1991 Sep;28(5):565-77. PubMed PMID: 1941921.
- [12]. Greenberg B, Singh D. Species identification of calliphorid (Diptera) eggs. *J Med Entomol.* 1995 Jan;32(1):21-6. PubMed PMID: 7869338.
- [13]. Hobischak NR, Anderson GS. Time of submergence using aquatic invertebrate succession and compositional changes. *J Forensic Sci.* 2002 Jan;47(1):142-51. PubMed PMID: 12064642.
- [14]. Introna F, Campobasso CP, Goff ML. Entomotoxicology. *Forensic Sci Int.* 2001 Aug 15;120(1-2):42-7. PubMed PMID: 11457608.
- [15]. Kintz P, Tracqui A, Mangin P. Toxicology and fly larvae on a putrefied cadaver. *J Forensic Sci Soc.* 1990 Jul-Aug;30(4):243-6. PubMed PMID: 2262784.
- [16]. Goff ML, Lord WD. Entomotoxicology. A new area for forensic investigation. *Am J Forensic Med Pathol.* 1994 Mar;15(1):51-7. PubMed PMID: 8166117.
- [17]. Matuszewski S, Bajerlein D, Konwerski S, Szpila K. An initial study of insect succession and carrion decomposition in various forest habitats of Central Europe. *Forensic Sci Int.* 2008 Sep 18;180(2-3):61-9. PubMed PMID: 18715728.
- [18]. Nolte KB, Pinder RD, Lord WD. Insect larvae used to detect cocaine poisoning in a decomposed body. *J Forensic Sci.* 1992 Jul;37(4):1179-85. PubMed PMID: 1506834.
- [19]. Nuorteva P, Nuorteva SL. The fate of mercury in sarcosaprophagous flies and in insects eating them. *Ambio.* 1982 Jan 1:34-7.
- [20]. Payne JA. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology.* 1965 Sep;46(5):592-602.
- [21]. Smith KG. A manual of forensic entomology. Trustees of the British Museum (Natural History) and Cornell University Press, London; 1986.
- [22]. Tomberlin JK, Byrd JH, Wallace JR, Benbow ME. Assessment of decomposition studies indicates need for standardized and repeatable research methods in forensic entomology. *J Forensic Res.* 2012;3(5):147.