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FORENSIC ENTOMOTOXICOLOGICAL EVALUATION OF CARRION INSECTS FOUND ON PIGS EUTHANIZED WITH SNIPER INSECTICIDE DICHLORVOS IN IBADAN

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ABSTRACT

Dichlorvos is a commonly used suicide agent in Nigeria that needs to be forensically evaluated. A forensic entomotoxicological evaluation of carrion insects found on pigs euthanized with sniper insecticide was carried out at the far backend of the University of Ibadan. Two domestic pigs, Sus scrofa weighing 5.2kg and 10kg were used as surrogate human models for the study. One of the pigs was euthanized with 0.157ml/kg of sniper insecticide while the other was sacrificed without poison and used as the control experiment. Arthropods were collected from the decomposing carrions using a sweep net and were preserved in 70% ethanol for identification. Larvae and pupae were also collected and preserved in 70% ethanol. The families of arthropods namely; Calliphoridae, Muscidae and Dermestidae were found, while five species namely; Chrysomya albiceps, Chrysomya megacephala, Lucilia sericata, Dermestes maculatus and Musca domestica were found. The succession pattern and abundance of the carrion insects, effects of poison and environmental variables such as temperature and humidity on them were studied. The family Calliphoridae were the most abundant and the first to colonize the decomposing carrion. The Calliphoridae had 103 individuals followed by the Muscidae with 17 individuals and the Dermestidae with 4 individuals. It was observed from this study that carrion insects are very important in the decomposition of pig carcass. It was also observed that these insects are of forensic importance and can be used in forensic investigations since pigs are biologically similar to humans, this can be linked to solving human crime cases

Keywords: Entomotoxicology, Sniper insecticide, Pigs, Decomposition, Carrion insects

INTRODUCTION

Forensic entomology is a branch of science, which uses the knowledge of arthropods in civil proceedings and criminal trials (Byrd, 2010). It is the study of the arthropods with the developmental stages of different species found on decomposed corpse during legal investigations (Verma and Paul, 2016). This can be used to determine how much time has passed since death (Post-Mortem Index or Interval, PMI) and the cause of death. Despite being primarily associated with death investigations, it can be used to determine the location of an incident and to know if the corpse was moved to another place, and to detect drugs and poisons (Byrd *et al*., 2001).

However, medico-legal forensic entomology is the subfield of interest here. This subfield deals with the aspect of using arthropods gathered at crime scenes for investigation (Catts and Goff, 1992). In murder cases, it deals with the appearance, location and order of appearance of insect stages. It can be used to determine the post-mortem index and location of death (Benecke, 2001).

Carrion insects are insects found on decomposing bodies. Decomposition process begins within a few minutes of death (Vass *et al,* 2002). Carrion insects are commonly described based on their ecological role (Goff, 1993).

Necrophagous species are the arthropods/insects that feed on the remains or the fluids released from remains during the decomposition process (Goff, 1993). These species are very important in forensic investigations. They include many species of the Dipterans (true flies) from family Callliphoridae (blowflies) and Sarcophagidae (flesh flies) and some species of the Coleopterans (beetles). Some examples of common blowflies include *Calliphora vicina*, *Phormia regina, Protophormia terraenovae and Lucilia sericata.* However, specific arthropod species present on carrions will vary from one geographic location to another (Ekrakene and Iloba, 2011).

The Calliphoridae are often the first species to arrive and colonize decomposing remains (Goff, 1993). They develop from eggs laid directly on the carrion and complete their life cycle on or near the remains, hence, they are considered the most important for estimating post-mortem intervals (Watson and Carlton, 2003, 2005; Tabor *et al* 2005). The first colonizers of great importance are those of the families Calliphoridae, Sarcophagidae and Muscidae, as these are the first insects to lay eggs at remains (Tabor *et al*, 2005).

The fresh stage of decomposition is characterized by the arrival of necrophagous blowflies and flesh flies. The blowflies are strongly attracted at the bloat stage of decomposition (Goff, 1993). Each group of insect will be attracted to decomposing remains at different stages of decomposition that follows namely fresh stage, bloat stage, active stage, advanced stage and dry decay stages (Ekanem and Dike, 2010).

The succession pattern of the different carrion insect groups can be used to estimate the post-mortem interval in forensic investigation (Anderson and VanLaerhoven, 1996); this is most useful in later stages of decomposition (Tabor *et al*, 2004). Another method of determining post-mortem interval, in early decomposition stages is using the developmental rates of the arthropods (Goff, 1993).

Sniper insecticide (Dichlorvos) is widely used to control household pests, in public health, and protecting stored products from insects. It 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 beyond insects and is widely used as a suicide agent (Das, 2013). Dichlorvos, like other organophosphate insecticides, acts on acetylcholinesterase associated with the nervous systems of insects. Evidence for other modes of action, applicable to higher animals, have been presented (Pancetti *et al*, 2007). It is claimed to damage DNA of insects (*Espeland et al, 2010).There is need for this study because* *sniper insecticide dichlorvos is widely used as a suicide agent in Nigeria hence the need to evaluate its forensic entomotoxicological profile in pigs for extrapolation to human cases.*

MATERIALS AND METHODS

The Study Site

The far backend of the University of Ibadan stadium was used for this study. This is because the place is not frequently visited hence the foul smell from the decomposing pigs would minimally affect humans and the environment. The area has a latitude of 7.434022 and a longitude of 3.890303, on the digital compass.

According to Audu *et al.* (2015), Ibadan experiences a tropical climate characterized by distinct wet and dry seasons and relatively stable temperatures throughout the year. The city's wet season extends from March to October, though there is a noticeable decrease in rainfall during August, creating a brief pause that effectively splits the wet season into two separate periods of high precipitation. The dry season spans from November to February, during which Ibadan is affected by the West African harmattan winds. On average, the city receives 1,420.06 mm of rainfall over about 109 days annually. Rainfall peaks in June and September. The average maximum temperature in Ibadan is 26.46°C, the minimum is 21.42°C, and the relative humidity averages 74.55%.

Ethical Approval

Ethical approval to conduct the research using pigs was sought and obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC) with assigned number as UI-ACUREC/19/132.

Sample Collection and Processing

Two domestic pigs (*Sus scrofa*), tagged PG1 and PG2 weighing 5.2kg and 10kg respectively were used as human models and baits for carrion insects. A spacious vehicle was used to transport the pigs from the farm to the study site in order to prevent stress on them. One of these pigs was sacrificed by administering 0.157ml/kg of the sniper insecticide orally based on its body weight and lethal dose calculations and left to die, while the second pig, sacrificed by stunning through hitting its head with a heavy object was left as control experiment.

After the pigs were confirmed dead, each pig was immediately mounted to allow for arthropod colonization. The pig carcasses were mounted on a board layered with sawdust, the boards were then placed on wooden stools to prevent feeding by other soil arthropods. A container with spent engine oil was also placed on each leg of the stool to trap other arthropods that are of no forensic importance.

The experiment was carried out at two different locations at a distance of 50 meters apart, to represent two different replicates of the same study and to also prevent crossing over of the flies during assemblage on the carcasses.

Sampling for Insects

The adult insects were sampled using a sweep net and an insecticide, the sweep net was swept clockwise and anticlockwise over the decomposing carcass after which the open-end of the net was quickly folded to prevent the insects from escaping. The insecticide was then sprayed over the net to immobilize the insects and they were then transferred into appropriately labelled clean and clear sample bottles and fixed in 70% ethanol in case of immature insects. The collection of samples was done twice a day: morning between 08.00 to 10.00 hours and evening between 17.00 to 19.00 hours) until the carcasses reached the skeletonized stage, which marked the end of decomposition.

Sampling for Maggot

The larvae stage of insects is referred to as maggot. Larvae samples were collected by using a sampling spoon to collect a substantial amount of maggots from the decomposing carcass. These maggots were collected from key areas such as the eye, mouth, stomach and anus for each pig. The active ones were then transferred into a small bowl and hot water was used to demobilize, kill and render the maggots inactive. The water was then sieved away from the maggots using a sieve and the maggots were transferred into well labelled sample bottles containing 70% ethanol for preservation, using a spatula.

Sampling for Pupa

Presence of pupa was checked for by searching underneath the sawdust, the ones present were collected and stored in well labelled sample bottles containing 70% ethanol

Measurement of Environmental Variables

An infrared thermometer (Model: IRT 1050P,Meco, India) was used for taking the ambient and carcass temperature readings. The humidity of the environment was also taken and recorded in order to study the effects of temperature and humidity on the abundance and growth of carrion insects during decomposition.

Sample Analyses

Identification of the adult insects was done with the use of identification keys. The abundance of adult insects found on the poisoned pig and control pig was tested for and compared. The insects were directly counted to determine their abundance on each carcass. The length and weight of the larvae were also compared. Length measurements of the larvae were taken with the use of a divider and a transparent ruler. The divider was extended to the length of each larva and was then placed on the ruler to check for the actual length. A digital weighing balance (Model: WT-S, Want, China) was used to determine the weight of *Chrysomya albiceps* larva sample.

RESULTS AND DISCUSSION

Abundance and Species Composition of Carrion Insects on Pig Carcass

Table 1 shows the abundance of arthropods of forensic importance found on the pig carrion that was treated with sniper insecticide. Two families of the Dipteran order (families Calliphoridae and Muscidae) and 1 family of the Coleopteran order (family Dermestidae) were collected from the pig carrion. Arthropods of the family Calliphoridae were the first to colonize the decomposing carrion, while those of the families Dermestidae and Muscidae appeared at the later stages of decomposition. The family Calliphoridae was found to be the most dominant family in all stages of decomposition, while the Dermestidae had the lowest number of individuals.

At the fresh stage of decomposition, the Calliphoridae had total of 4 individuals, while the Dermestidae and Muscidae were absent.

At the bloat stage, the Calliphoridae had a total of 32 individuals present, while the Muscidae and Dermestidae were absent at this stage.

At the active decay stage, the Calliphoridae had a total of 17 individuals, the Muscidae had a total of 7 individuals present, while the Dermestidae were absent.

At the advanced decay stage, the Calliphoridae had a total of 7 individuals, the Muscidae had 2 individuals and the Dermestidae also had a total of 2 individuals present.

At the dry stage, the Calliphoridae had a total of 12 individuals, while the Muscidae and Dermestidae had 1 individual each present.

Table 1: Abundance of forensically important insects collected from pig carrion treated with sniper insecticide

Family	Genus/species	Fresh	Bloat	Active	Advance	Dry
Calliphoridae	*Chrysomya albiceps*	0	15	6	0	1
	Chrysomya megacephala	0	0	3	2	6
	Lucilia sericata	4	17	8	5	6
Dermestidae	*Dermestes maculatus*	0	0	0	2	1
Muscidae	*Musca domestica*	0	0	7	2	1
	Total	4	32	24	11	15

Table 2 shows the abundance of arthropods of forensic importance found on the pig carrion slaughtered without sniper insecticide. Two families of the Dipteran order (families Calliphoridae and Muscidae) and 1 family of the Coleopteran order (family Dermestidae) were collected from the pig carrion. Arthropods of the family Calliphoridae were the first to colonize the decomposing carrion, arthropods of the family Dermestidae appeared at the advanced decay stage of decomposition, while those of the family Muscidae appeared at the bloat stage of decomposition. The family Calliphoridae was found to be the most dominant family in almost all stages of decomposition, while the Dermestidae had the lowest number of individuals.

At the fresh stage of decomposition, the Calliphoridae had total of 5 individuals, while the Dermestidae and Muscidae were absent.

At the bloat stage, the Calliphoridae had a total of 9 individuals present, the Muscidae had 1 individual while Dermestidae were absent at this stage.

At the active decay stage, the Calliphoridae had a total of 6 individuals, the Muscidae had a total of 3 individuals present, while the Dermestidae were still absent.

At the advanced decay stage, the Calliphoridae had a total of 11 individuals, the Muscidae had 2 individuals and the Dermestidae also had 1individual present.

At the dry stage, the Calliphoridae and Dermestidae had no individual present, while the Muscidae had 1 individual present.

Table 2: Abundance of Forensically Important Insects Collected From Pig Carrion Slaughtered Without Sniper Insecticide (Control)

Family	Genus/species	Fresh	Bloat	Active	Advance	Dry
Calliphoridae	*Chrysomya albiceps*	0	1	2	2	0
	Chrysomya megacephala	0	2	2	3	0
	Lucilia sericata	5	6	2	6	0
Dermestidae	*Dermestes maculatus*	0	0	0	1	0
Muscidae	*Musca domestica*	0	1	3	2	1
	Total	5	10	9	14	1

From the Table 3, it was observed that the family Calliphoridae were the first to arrive on the pig carrion and were present till the dry stage of decomposition. The Dermestidae arrived later at the advanced stage of decomposition and were also present till the dry stage. The family Muscidae arrived at the active stage of decomposition, they were also present till the dry stage. The larva of Calliphoridae were observed at the bloat stage, while the pupae were observed at the advanced stage. The larvae of the Muscidae were observed at the active and advanced decay stage.

Table 3: Occurrence Matrix of Carrion Insect Stages in the Pig Treated with Sniper Insecticide

	Days postmortem	**0 1 2**	**3 4 5 6 7**	**8 9 10 11**	**12 13 14**	**15 16 17**
Family	Genus/specie	Fresh	Bloat	Active	Advance	Dry
Calliphoridae	*Chrysomya albiceps*	0 0 0	a a a l al	al al l l	p p p	p ap p
	Chrysomya megacephala	0 0 0	0 0 0 a 0	l l al l	al p p	ap p ap
	Lucilia sericata	0 a a	a a a al al	al al al al	l ap p	ap ap ap
Dermestidae	*Dermestes maculatus*	0 0 0	0 0 0 0 0	0 0 0 0	a 0 a	0 0 a
Muscidae	*Musca domestica*	0 0 0	0 0 0 0 0	a a a al	l a a	a 0 0

Key: a= adults, p= pupae, l= larvae, 0= absent.

From the Table 4, it was observed that the family Calliphoridae were the first to arrive on the pig carrion during the fresh stage and were present till the dry stage of decomposition. Their larvae were found at the bloat stage, while the pupae were found at the active decay stage.

The Dermestidae arrived later at the advanced stage of decomposition but were absent at the dry stage.

The family Muscidae arrived at the bloat stage of decomposition and were also present till the dry stage. The larvae of the Muscidae were observed at the active decay stage while the pupae were later observed at the advanced decay stage.

Table 4: Occurrence matrix of carrion insect stages in the control pig

	Days postmortem	0 1 2	3 4 5 6 7	8 9 10 11	12 13 14	15 16 17
1Family	Genus/specie	Fresh	Bloat	Active	Advance	Dry
Calliphoridae	*Chrysomya albiceps*	0 0 0	0 a 0 1 1	1 1 1 al	alp p p	p p p
	Chrysomya megacephala	0 0 0	0 a 0 1 1	1 1 lp alp	al p p	p p p
	Lucilia sericata	0 a a	a a 1 1 1	al alp lp lp	al p ap	p p p
Dermestidae	*Dermestes maculatus*	0 0 0	0 0 0 0 0	0 0 0 0	0 0 a	0 0 0
Muscidae	*Musca domestica*	0 0 0	0 0 a 0 0	al 1 al 1	1 al alp	lp lp lp

Key: a= adults, p= pupae, l= larvae, 0= absent.

Effect of Sniper Insecticide on the Length and Weight of Larva

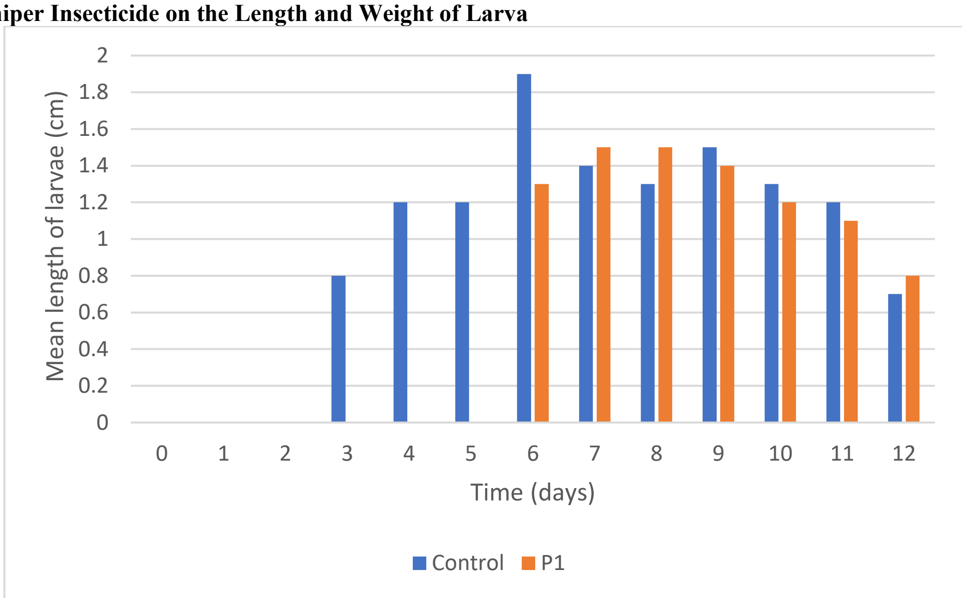

Figure 1: Mean body length of the larvae of *Chrysomya albiceps* collected from pig carrion

The effect of sniper insecticide on the mean body length of selected larvae collected from pig carrion is shown in fig. 1. For the control pig, values were not recorded for the first two days because larvae were not present till the third day. There was an increase in the larvae length from 0.8 to 1.9cm until the sixth day, followed by a decrease on the seventh day and eighth day. There was an increase on the ninth day, which was then followed by another decrease from the tenth day until the twelfth day.

For the pig that was treated with sniper insecticide (P1), no larva was gotten until the sixth day. The larvae length increased from 1.3 to 1.5cm for the first three days of collection. This was followed by a decrease from the ninth day to 0.8cm on the twelfth day.

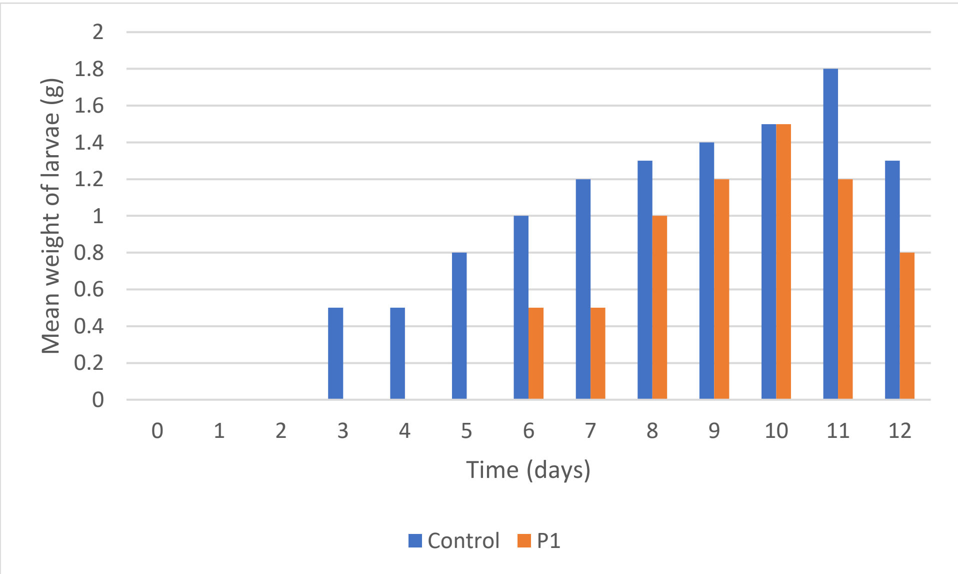

Figure 2: Mean body weight of larvae of *Chrysomya albiceps* found on pig carrion

The effect of sniper insecticide on the mean body weight of the larvae gotten from pig carrion is shown in fig 2.
As observed for the control pig in the mean body length, values were not recorded until the third day because no larva was present for the first two days. The weight of the larvae increased gradually until the later stages of decomposition where a decrease in weight was observed.

For the pig treated with sniper insecticide, no values were recorded until the sixth day because of the absence of larvae for the first five days. There was also an increase in the body weight as decomposition progressed, but there was a decrease in the body weight at later stages of decomposition.

Variations in environmental variables at the field site

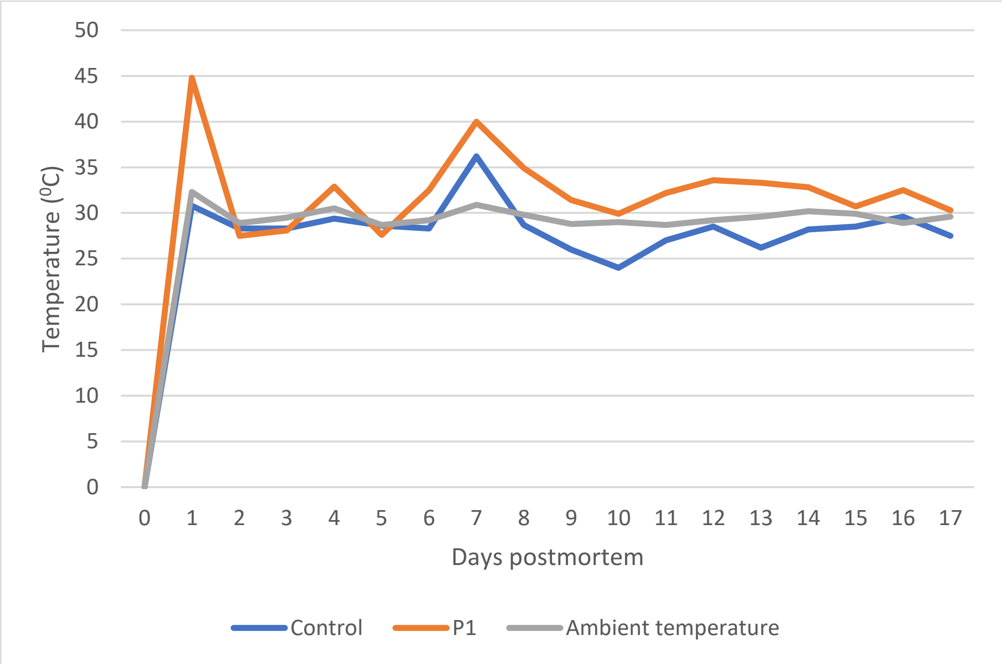

Figure 3: Mean carcass temperature recorded at the field experiment

It was observed that the carcass temperature for the control pig ranged from 24^{0}C to 36.2^{0}C while that of the pig treated with sniper insecticide ranged from 27.5^{0}C to 44.8^{0}C. A gradual increase and decrease in the ambient temperature was observed during the decomposition process. This had an effect on the decomposition as an increase in ambient temperature causes a high rate of decomposition, while a decrease in ambient temperature leads to a decreased rate of decomposition.

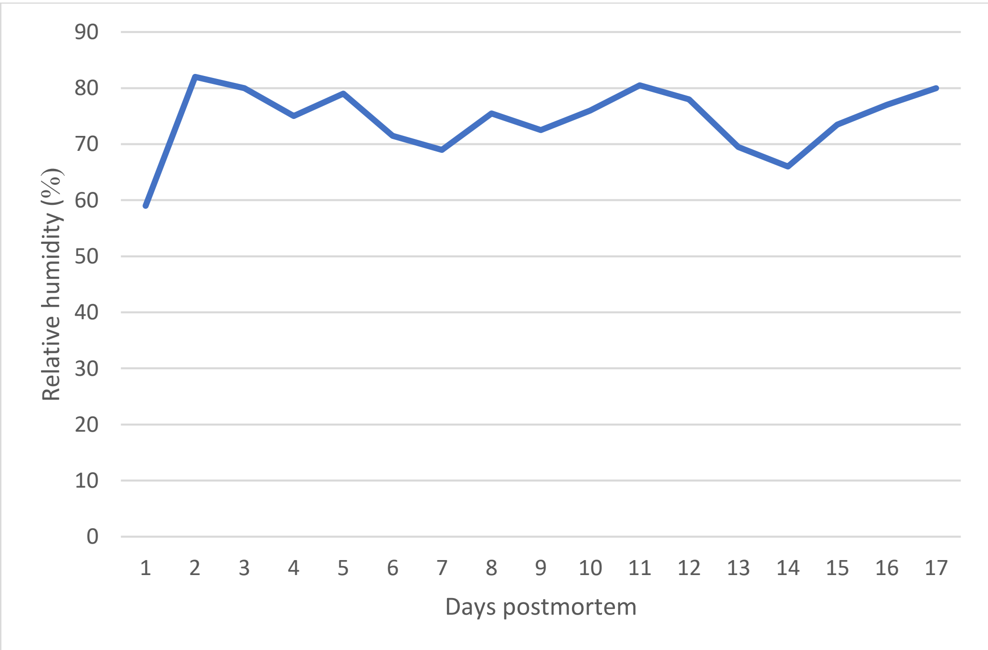

Figure 4: Mean relative humidity of carcass environment

Fig 4 shows the mean relative humidity of the carcass environment. A gradual increase and decrease in the relative humidity of the carcass environment was observed during the decomposition process which ranged from 59% to 80.5%.

Discussion

Abundance and Species Composition of Carrion Insects on Pig Carcass

Five stages of decomposition were observed during the study. Adults of two families of the order Diptera, which include the families Calliphoridae and Muscidae, and the family Dermestidae of the order Coleoptera were gotten from the pig carrions corroborating findings by Onyishi *et al*, (2020),. The family Calliphoridae and family Muscidae were the first to arrive on the pig carrions; this aligns with earlier reports by Abajue *et al* (2013) in another ecological zone in Nigeria. The family Dermestidae arrived at the later stages of decomposition.

The Calliphorids were the most abundant of all the insects found on the pig carrions. Three species of the family Calliphoridae were gotten from the carrions, with *Lucilia sericata* being the most abundant, the *Chrysomya* species were also found. Only one species each from the families Muscidae and Dermestidae was found on the decomposing carrion. Abundance of the family Calliphoridae is due to their ability to perceive dead animal matter from a distance (Janaway *et al*, 2009, Onyishi *et al*, 2020)).

Carrion Insect Succession on Pig Carcass

The Calliphoridae adults were the first to arrive on the pig carcass during the fresh stage, this observation is in line with what has been reported by Abajue *et al* (2013) where a study was carried out on insect larvae found on decomposing pig carrions in Okija, Anambra State, Nigeria. *Musca domestica* was the only species from the family Muscidae that was found on the pig carrion and they started arriving during the bloat stage and were present till the dry stage. This arrival time has been previously reported by Ekanem and Dike (2010) that muscids are usually observed from the bloat stage when the odour from the decomposing carrion becomes perceivable. The family Dermestidae was found during the advanced decay and the dry stage and the only species that was found was the *Dermestes maculatus.* Previous researchers such as Benecke (1998), Ekrakene and Iloba (2011), Okiwelu *et al* (2013), Abajue *et al* (2013) and Iloba and Odo (2021) also observed this order of insect succession.

Effect of Sniper Insecticide on the Length and Weight of Larva

It was discovered that the length and weight of the larva of *Lucilia sericata* was influenced by the concentration and presence of toxin (sniper insecticide) that was used. Larger sizes and weights were observed from the larvae gotten from the control group compared to those gotten from the pig treated with sniper insecticide. The larvae gotten from the pig treated with sniper insecticide were smaller in size and lesser in weight, and this indicates that presence of toxins has an effect on the development process of larvae gotten from the pig carrions as earlier reported by Tyokumbur and Ogunlade (2023). The presence of sniper insecticide in an arthropod damages the DNA of that arthropod and this can lead to the alteration of the development process of larvae gotten from the arthropod (*Espeland et al, 2010).*

Variations in Environmental Variables at the Field Site

It was observed, according to the reports from the previous studies by Ekanem and Dike (2010), that a higher temperature and relative humidity leads to a faster and increased rate of decomposition, and also, increased rate of insect abundance. However, the presence and concentration of toxin in the pig treated with sniper insecticide delayed the rate of decomposition. The higher the temperature and relative humidity, the higher the decomposition rate and the lower the relative humidity and temperature, the lower the decomposition rate.

CONCLUSION

This study confirms that flies are very essential in the decomposition process of carrions following predictable succession patterns. The species of arthropod gotten from this study have been previously reported to be forensically important. These arthropods can be used to estimate the postmortem interval of the carrion, the cause and also the place of death. The developmental process of the larvae gotten from these carrions can also be used for forensic investigations related to suicide especially the recurring use of sniper insecticide as a suicide agent in Nigeria.

It can be concluded from this study that the arthropods associated with carrion have an effect on their decomposition and can be used to solve crime cases, and for forensic investigations. Relative humidity and temperature also play important roles in the decomposition process.

It was discovered that there was a greater abundance of insects on the pig that was poisoned with sniper insecticide than that of the control group.

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