FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 7 No. 2, April, 2023, pp 72 - 78
DOI: https://doi.org/10.33003/fjs-2023-0702-1712

FORENSIC APPRAISAL OF CARRION INSECTS FOUND ON DOMESTIC FOWLS POISONED WITH SNIPER INSECTICIDE

***Tyokumbur Emmanuel, Ogunlade Fredrick**

Department of Zoology, University of Ibadan, Ibadan, Nigeria.

*Corresponding authors' email: emmanuel_tyokumbur@yahoo.com

ABSTRACT

A study was carried out on the forensic entomotoxicological appraisal of carrion insects of domestic fowl poisoned with dichlorvos (sniper insecticide) between October and December 2019 on the campus of University of Ibadan. Sniper as a common suicide agent in Nigeria needs to be studied using animal models. Two domestic fowls were euthanized with 3 ml and 5 ml of dichlorvos respectively. Adult carrion insects were collected from the carrions using a sweep net and stored in silica gel. The larvae were collected and preserved in 70% ethanol while pupae were collected using forceps. Ambient and carcass temperature were measured using infrared thermometer while relative humidity was recorded from digital hygrometer. Calliphoridae and Muscidae were the initial colonizers of the decomposing carcass and were dominant followed by Sarcophagidae. The defeathered domestic fowl had more abundance and species composition of carrion insects than the feathered. The length and weight of the larvae as indices of growth were comparatively higher in the defeathered domestic fowl. The highest mean carcass temperature was 30.5^0 C while the lowest was 22.6^0C. The highest carcass temperature was recorded on the seventh day during the active decomposition stage. The highest mean relative humidity value was 85% while the least recorded was 60%. Overall, fast decomposition rate was recorded due to high ambient and carcass temperatures. It can be deduced that carrion insects are essential in providing the essential ecosystem service of decomposition and can be used in solving suicide puzzles through the extrapolation of post mortem intervals.

Keywords: Entomotoxicology, Dichlorvos, Domestic fowl, Carrion insects, Decomposition

INTRODUCTION

Entomotoxicology is the application of toxicological testing of carrion-feeding insects to classify drugs and toxins in toxic tissues (Catts and Goff, 1992). Many situations where entomological evidence has been used for criminal activities takes place on land and are found within a limited period of time (Chophi *et al*, 2019). Whenever conventional matrices such as blood, urine or internal organs are no longer available, use of insects as an alternative matrix for drug detection is well documented and recommended (Nuorteva, 1977). Entomotoxicology's main interest is determining substance abuse just before death, particularly in skeletonized remains where there is no tissue or fluid left (Gennard, 2012). Toxicological analysis can also be used in highly decomposed remains using insects as less intrusion is found in the analytical process due to the decomposition of the matrix (Steinhaus, 2012). From a purely practical viewpoint, insects are of interest because they are found in high quantities and their life stage remains are preserved for a long time, even if toxicological specimens are no longer available (Bourel 2011; Ekrakene and Odo, 2017).

Flies are used in legal investigations to estimate the postmortem interval (PMI) by collecting immature larvae, pupa and insects from the body to identify the insect along with its size and stage of development (Gosselin *et al*, 2004).. The postmortem interval is also determined from the succession of insects on the corpse which depends on factors such as climatic conditions, season, geographical area, exposure to solar radiation, synanthropy, type of substratum, latitude, elevation, location and position of the body, size of the body, cause of death, presence of clothing, intra and inter-specific competition and larval migration (Anderson and VanLaerhoven, 1996; Anderson *et al*, 2001; Campobasso *et al*, 2001; Carvalho *et al*, 2004; Klong-Klaew *et al*, 2018).

Carrion insects are the insects associated with the decomposition of the remains which begins within a few minutes after death (Watson and Carlton, 2005). Decomposing remains provide a temporary and evolving site of concentrated resources that are used by a wide range of species, frequently the first to arrive are arthropods and the predominant exploitative community (Introna *et al*, 2001; Tabor *et al*, 2004). Notably, not all arthropods found on or nearby decomposing remains will play an active role in the process of decay (Goff, 1993).

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate), an organophosphate compound known as sniper, is commonly used as an agricultural insecticide among the vast majority of pesticides which is currently approved for use with worldwide concerns for a number of reasons including use as a suicide agent (Mennear, 1998**;** Espeland *et al*, 2010; Das, 2013). Dichlorvos is used in this study because lately many Nigerians have committed suicide using dichlorvos. The animal used for this study is the domestic fowl. In this study, the domestic fowl is covered by feathers thus by removing them in one of the fowls will mimic removal of clothes (nakedness) in humans, while the others will be left with feathers (mimicking clothes) in suicide events.

MATERIALS AND METHODS

Study Site

The site to be used for the study is located at the back of Awolowo Stadium, University of Ibadan. The site is suitable because it is at the south end of the university and just a few people visit the place. So the smell emanating from the decomposition of the carcass will pose no threat to humans. The latitude and longitude of the area as read on a digital compass are 7.434022, 3.890303 respectively. The location of the area when imputed into internet maps shows Oyo, Akinyele, Ibadan, The Polytechnic Main Road, Nigeria and this is reasonable as the Polytechnic Ibadan shares boundary with University of Ibadan particularly along Awo stadium road.

Ethical approval
Ethical approval for animal use was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC) with Assigned Number UI-ACUREC/19/131.

Sample Collection and Processing
The domestic fowl used for the experiment were acquired from poultry farmers in Ibadan, Oyo State. The experiment was not sex-specific as the poison affects both male and female domestic fowl in the same way. The domestic fowl acquired were inspected for injuries or disease and the domestic fowl with obvious body injury were returned and replaced with a healthy one. The domestic fowl which were to serve as bait for the carrion insects were sacrificed by administering the lethal poison which is dichlorvos (sniper).

Preparation of Domestic Fowl for Set-up
The fowls were transported from the farm to the field site. The domestic fowl will be transported in cages. In the process of transporting the fowls, a spacious vehicle was used so that the fowls can move freely and the stress on each fowl is reduced. Also the windows of the vehicle were opened in such a way that cross ventilation is enhanced. At the site, the fowls were weighted and the weight of each fowl was recorded. The fowls were then tied with ropes to prevent them from escaping. 5 ml and 3 ml of dichlorvos is administered to the fowls orally in such a way that it causes little or no pain during the administration.

Mounting the Fowls
The fowls after death were placed on separate mounts. The mounts consisted of a board lined with polythene sack, covered with sawdust and placed on a stool. The sawdust is important because it mimics sand and it provides shelter for the developing larvae and pupae of the insects. The polythene sacks are perforated so that rainwater can easily drain through them. The stool is important as it provides a platform in which other animals that are not of entomotoxicological importance are not able to visit the carcass. Also the container with the spent engine oil placed on each leg of the stool is able to trap other arthropods that are not of entomotoxicological importance that might also visit the carcass. 'Research in progress', 'Keep off' signs were also placed on each carcass mount to better inform people who might encounter the field set-up.

Sampling of carrion insect stages
Decomposition sampling is divided into three phases namely: sampling for insects, maggot/larvae and pupa.

Sampling for insects
Sampling for adult insect was done by using a sweep net and insecticide. The sweep net was swept clockwise and anticlockwise at an angle of almost 180^0 arc over the decomposing carcass after which the tip was quickly folded by using the second hand so as to prevent the escape of the trapped insects. The insecticide was then sprayed over the sweep net to immobilize the insects and then they were transferred into appropriately labelled clean and clear sample bottles. The sampling procedure was carried out twice a day (in the morning and in the evening) until the skeletonization of the carcass. The adult insects were then preserved and stored in 70% alcohol.

Sampling for maggot
Sampling for larvae involves using a sampling spoon to collect adequate amount of the maggots from the decomposing carcass. The maggots were collected from crevices and other areas such as the eye, mouth, stomach and anus from each fowl. The active maggots were then transferred into small bowl. Hot water kept in a flask was then poured into the bowl containing the maggots. The hot water kills and renders the maggots inactive. The water was then sieved from the maggot by using a sieve and the maggots were put into appropriately labelled sample bottles containing 70% formalin solution using a spatula.

Sampling for pupa
When the maggots on the decomposing carcass start reducing, the sawdust was checked for the presence or absence of pupa. If pupa were present, they were retrieved from the sawdust and were also kept in appropriately labelled sample bottles.

Measurement of larval body length and weight
Measurement of larvae body length and weight was carried out at regular intervals of 12 hours, amounting to twice in a day. Five larvae were randomly sampled from each carrion group and demobilized in the boiled water according to the method of Adams and Hall (2003). The lengths and weights were measured and mean values recorded for each carrion group at different stages of decomposition. An electrical weighting balance with sensitivity (of readability) of 0.001 g-1 g was used to measure the weight of the larvae. Length of larvae from second instar stage were obtained by a pair of divider and read on a transparent meter rule.

Measurement of carcass temperature and humidity
The temperature of the carcass were read and recorded daily using infrared thermometer that can accurately measure between -50^0C to 330^0C. The thermometer was used by pointing the infrared beam to the decomposing fowl carcass. Readings were then generated and shown on the thermometer screen. The readings were then recorded into the field experiment book. Measurement of relative humidity was done using a digital hygrometer. The hygrometer is placed within the vicinity of the carcass, readings are generated on the screen of the hygrometer and the readings were then recorded into the field experiment book.

RESULTS
Abundance and species composition of carrion insects on domestic fowl carcass
The result of the abundance of forensically important insects collected from domestic fowl carcass treated with 5ml of dichlorvos is shown in Table 1. From the table, Platystomatidae family was dominant in the fresh stage with 6 representatives. During the bloat stage of decomposition, Calliphoridae dominated this stage with about 12 individuals. In the active stage of the decomposition, Calliphoridae was also dominant with 20 individuals; Platystomatidae and Sarcophagidae were absent. In the advanced stage of decomposition, there was a drastic reduction in the occurrence and abundance of carrion insect that visited the decomposition set-up, Muscidae was the most abundant followed by Calliphoridae. At the dry stage of decomposition, Sarcophagidae was the most abundant with only 6 individuals.

Table 1: Abundance of forensically important insects collected from feathered domestic fowl treated with 5ml of dichlorvos

Family	Genus/species	Fresh	Bloat	Active	Advance	Dry
Platystomatidae	*Lule sp*	6	0	0	0	0
Muscidae	*Musca domestica*	0	0	4	8	0
Calliphoridae	*Chrysomya albiceps*	2	12	20	2	2
Calliphoridae	*Chrysomya megacephala*	0	0	2	0	0
Sarcophagidae	*Sarcophaga sp*	0	0	0	0	6

The result of the abundance of forensically important insects collected from domestic fowl carcass treated with 3ml of dichlorvos is shown in Table 2. From the table, Calliphoridae family was dominant during the fresh stage with 2 representatives. During the bloat stage of decomposition, Calliphoridae also dominated this stage with about 12 individuals. In the active stage of decomposition, Calliphoridae also dominated with 20 representatives; Sarcophagidae was the least dominant with 0 individuals. In the advanced stage of decomposition, there was a drastic reduction in the occurrence and abundance of carrion insect that visited the decomposition set-up, Calliphoridae was the most abundant with 6 individuals. At the dry stage of decomposition, Calliphoridae, Silphidae and Sarcophagidae were briefly seen to visit the domestic fowl carcass, while Muscidae was not seen at all.

Table 2: Abundance of forensically important insects collected from defeathered domestic fowl carrion treated with 3ml of dichlorvos

Family	Genus/species	Fresh	Bloat	Active	Advance	Dry
Calliphoridae	*Chrysomya albiceps*	2	12	20	6	2
Silphidae	*Necrodes littoralis*	0	0	4	2	2
Muscidae	*Musca domestica*	0	0	2	2	0
Sarcophagidae	*Sarcophaga sp*	0	0	0	2	4

Carrion insect succession on domestic fowl carcass

From Table 3 which shows the occurrence matrix of carrion insect stage in 5ml of dichlorvos treated domestic fowl, we can observe that the adult and egg stage were present in the fresh stage of Calliphoridae and Platystomatidae and these life forms were also present in large quantities hence the deep shading. At the bloat stage, eggs and larva of most of the forensically important carrion insect were observed. During the stage of active decomposition among Calliphoridae, adult and egg were only seen. During the advanced decomposition stage, adult and egg forms of Muscidae were found. Towards the end of the advanced stage, all Calliphoridae life forms disappeared from the decomposition set-up. Some adult and egg sarcophaga was found at the dry stage. The *Sarcophaga* was only observed in the dry stage while the Muscidae were only observed in the active and advanced stage of decomposition.

Table 3: Occurrence matrix of carrion insect stage in 5ml of dichlorvos treated domestic fowl

	Days of Postmortem	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Family	**Genus/species**	**Fresh**			**Bloat**			**Active**							**Advance**			**Dry**	
Platystomatidae	*Lule sp*	AE	AE	AE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscidae	*Musca domestica*	0	0	0	0	0	0	0	0	0	0	AE	AL	0	AE	AE	AE	0	0
Calliphoridae	*Chrysomya albiceps*	AE	0	0	AE	AE	AE	AE	AE	AE	AE	AE	AE	AE	0	AE	0	0	AE
Calliphoridae	*Chrysomya megacephala*	0	0	0	0	0	0	0	0	0	0	0	0	A	0	0	0	0	0
Sarcophagidae	*Sarcophaga sp*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	AE	AE

Key; A= Adult, E= Eggs, L= Larva, 0= Absent

Places with dark shading represent high abundance, while those with light shading represent low abundance.

From Table 4, we can see the occurrence matrix of carrion insect in the 3 ml dichlorvos poisoned domestic fowl. Sarcophaga was not found in the fresh stage, it was found as adult in advanced and dry stage. Calliphoridae was not found in the early fresh stage, but was later found in the late fresh stage as adult and egg forms. Calliphoridae was found as adult and egg in bloat stage. In the active and advanced decay stage Calliphoridae was found as adult, egg and larva. Calliphoridae was found as adult, egg, larva and pupa in the dry stage. Muscidae was not found in the fresh and bloat decay stage. Muscidae was found as adult, egg and larva in the active stage while it was found as adult, egg, larva and pupa in the advanced stage. Silphidae was not found in the fresh and bloat stage. They were found as adult and larva in the active stage while they are found as adult, egg, larva and pupa in the advanced and dry stage.

Table 4: Occurrence matrix of carrion insect stage in 3ml of dichlorvos treated domestic fowl

	Days of Postmortem	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Family	**Genus/species**	**Fresh**			**Bloat**			**Active**							**Advance**			**Dry**	
Calliphoridae	*Chrysomya albiceps*	0	0	AE	AE	AE	AE	AE	AE	AEL	AEL	AE	AEL	0	0	AEL	AE	AELP	0
Silphidae	*Necrodes littoralis*	0	0	0	0	0	0	AL	0	0	0	0	0	0	AELP	0	0	AELP	0
Muscidae	*Musca domestica*	0	0	0	0	0	0	AL	0	0	0	AEL	AE	0	0	AELP	0	0	0
Sarcophagidae	*Sarcophaga sp*	0	0	0	0	0	0	0	0	0	0	0	0	0	A	0	0	0	A

Key; A= Adult, E= Eggs, L= Larva, P= Pupa, 0= Absent

Places with dark shading represent high abundance, while those with light shading represent low abundance.

From Fig.1, we can observe that the larva passed through some certain developmental stages and we got our first larva on the ninth day. On the ninth day, the mean length of the larva in the carcass poisoned with 3 ml of dichlorvos is 1.2 cm while that of the 5 ml fowl was 1.0 cm. On the tenth day, the mean length of the larva in the carcass poisoned with 3ml of dichlorvos is 1.3 cm while that of the 5 ml fowl was 1.1 cm. On the eleventh day, the mean length of the larva in the carcass poisoned with 3ml of dichlorvos is 1.4cm while that of the 5ml fowl was 1.2 cm.On the twelfth day, the mean length of the larva in the carcass poisoned with 3 ml of dichlorvos is 1.5 cm while that of the 5 ml fowl was 1.3 cm. On the thirteenth day, the mean length of the larva in the carcass poisoned with 3ml of dichlorvos is 1.65 cm while that of the 5 ml fowl was 1.45 cm.On the fourteenth day, the mean length of the larva in the carcass poisoned with 3ml of dichlorvos is 1.4 cm while that of the 5 ml fowl was 1.2 cm. On the fifteenth day, the mean length of the larva in the carcass poisoned with 3 ml of dichlorvos is 1.4 cm while that of the 5 ml fowl was 1.2 cm.On the sixteenth day, the mean length of the larva in the carcass poisoned with 3 ml of dichlorvos is 1.4 cm while that of the 5 ml fowl was 1.2 cm. On the seventeenth day, the mean length of the larva in the carcass poisoned with 3 ml of dichlorvos is 1 cm while that of the 5 ml fowl was 0.18 cm.

Effect of dichlorvos on the length and weight of *Chrysomya albiceps* larva

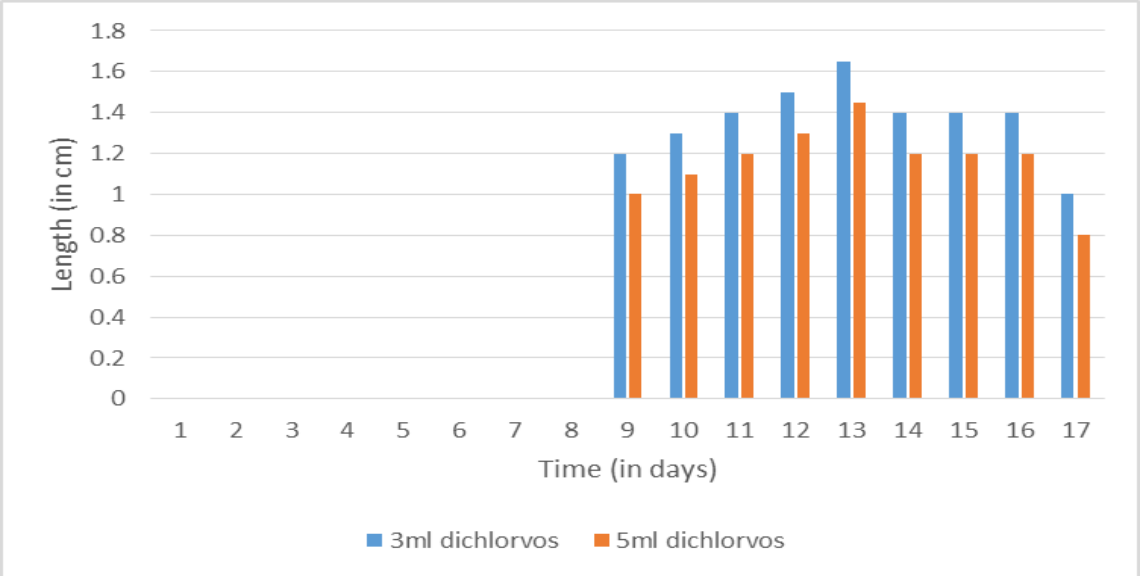

Figure 1: Histogram showing the mean body length of *Chrysomya albiceps* larva collected from domestic fowl carcass

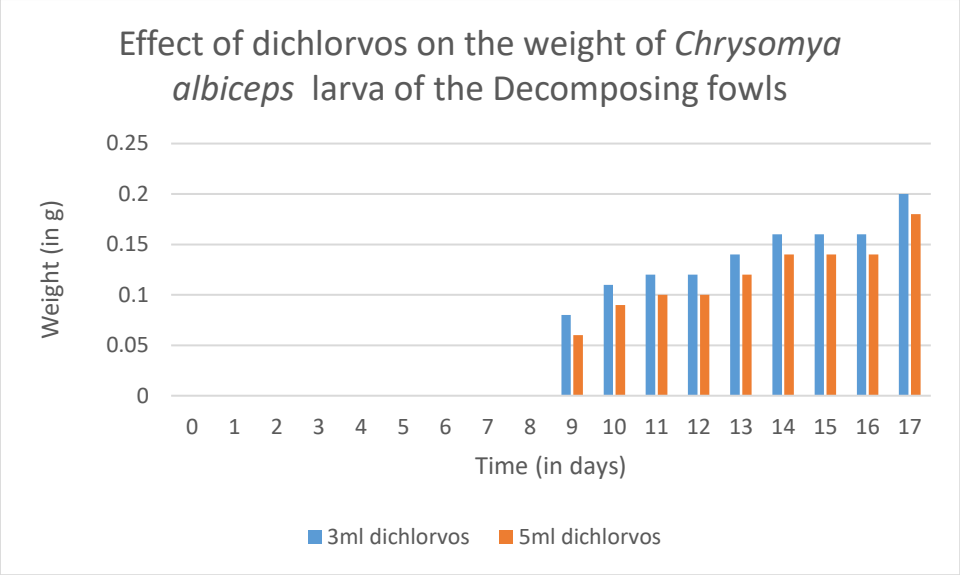

Figure 2: Histogram showing the mean body weight of Chrysomya *albiceps* larva collected from fowl carcass

The histogram in Fig.2 which shows the effect of dichlorvos on the weight of larva of the decomposing domestic fowl is similar to that of fig.1 which shows the effect of dichlorvos on the length of the larva of the decomposing domestic fowl. There were no values for the first eight days as larva were still developing. The larva weight in the 3ml group is found to be higher than that of the 5ml group in most days.

From the graph in Fig.3 we notice that the mean temperature of the 3ml group ranges from 33ºC which was the highest value recorded to 26ºC which was the lowest value recorded. The highest mean temperature value for the decomposing domestic fowl treated with 5ml dichlorvos was 31ºC while its lowest mean value was 23ºC. It should be noted that in both the 3ml and 5ml dichlorvos treated fowl, the temperature at the seventh day was higher than the temperature on other days. This might be because the seventh day and some days before and after it are part of the days of active decomposition in which insect activity particularly maggot is high and the high insect activity have been observed to generate heat.

Variations in environmental variables at the field site

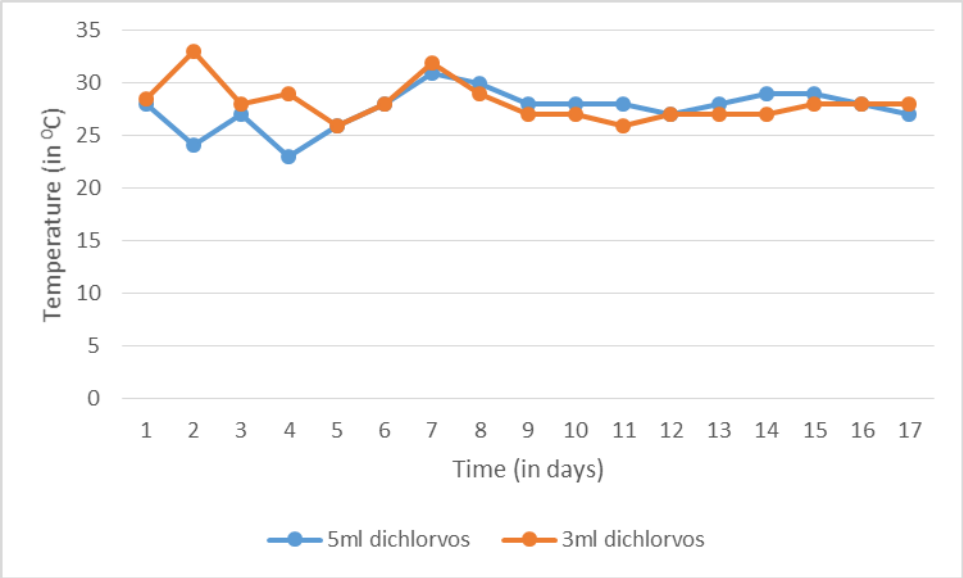

Figure 3: Comparison of 3ml and 5ml dichlorvos poisoned domestic fowl carcass temperature recorded in the field experiment

DISCUSSION

Abundance and specie composition of carrion insects on domestic fowl carcass

Five stages of decomposition were found at the end of the experiment, and this corroborates earlier observations and studies such as the one performed by Carvalho *et al*. (2004), where they discovered that the fresh stage, the bloated stage, the decay stage, the post-decay stage and the skeletal stage are five essential stages of decomposition. Also reported in the Carvalho *et al*. (2004) experiment were the four major carrion insect families in the study, although Carvalho *et al* (Campobasso, 2001) recorded some families we did not observe such as Formicidae, Histeridae and Staphylinidae particularly in the post-decay stage. Abajue *et al*., (2013) reported that Calliphoridae, Sarcophagidae and Muscidae are the arthropods that arrived on a carcass from the beginning of the decomposition. This statement is corroborated by our experiment as these families were very dominant among the

carrion groups particularly the Calliphoridae. In the 5ml and 3ml feathered and defeathered domestic fowl carcass, respectively, the Calliphoridae was very dominant.

Carrion insect succession on domestic fowl carcass

During the experiment, it was observed that the Calliphoridae and Platystomatidae were the initial pioneers of the decomposing carcass and were seen during the fresh stage, while Sarcophagidae arrived after the fresh stage of decomposition. Silphidae was seen later in the advanced stage of decomposition and it was also observed at the dry stage. The trend observed here was similar to the trends observed by Abajue *et al.,* (2014) and Ekrakene and Iloba (2011). They observed that the sarcophagids and calliphorids were the first to arrive on the carrions and deposited their first instar larvae and eggs respectively, during the fresh stage. Followed by the clerids and demestids during the bloated stage. The ulidiids and stratiomyiids arrived on the carrions during the active decay stage.

Effect of dichlorvos and feather on the length and weight of larva

In this study, dichlorvos was found to retard the growth of *Chrysomya albiceps.* The feathered domestic fowl had lesser colonization by carrion insects compared to the defeathered domestic fowl. According to an experiment by Chick (2014), where the abundance and occurrence of carrion insects on pigs poisoned with nicotine was studied, it was discovered that the low dose group had lesser occurrence and abundance of carrion insects when compared with the control group. According to an experiment by Kelly *et al.,* (2008), where they studied the influence of clothing and wrapping on carcass decomposition and arthropod succession, they discovered that wrapping of a carcass delayed the oviposition of the adult Diptera by four days. They also discovered that wrapping results in a significant delay in the drying out of a carcass. In this study, it was discovered that the feather of domestic fowls delayed oviposition and succession of carrion insects.

Variations in environmental variables at the field site

The experiment was conducted in Ibadan, Nigeria with a tropical climate, temperature and relative humidity values, hence the decomposition rates of the carrion domestic fowl was fast. The result of the fast decomposition agrees with Ekanem and Dike (2010) where it was established that higher air temperature leads to faster decomposition rates and increase in the abundance of insects colonizing the carcass. Similar findings have been reported by Abril *et al*, (2010) on the effect of temperature on the development and survival of the Argentine ant, *Linepithema humile*. Findings from this study on insect carrion abundance corroborates findings by Oguche *et al*, (2022) that showed correlation between environmental variables and mosquito abundance in some selected locations within Kaduna metropolis.

CONCLUSION

In this study, the effect of feather covering on the decomposition of the domestic fowl carcass was shown to delay insect colonization. At the end of the experiment it was established that indeed cloth or body covering will affect the abundance of carrion insects found on decomposing domestic fowl as models for human cadaver. Also, as the dosage of the dichlorvos poisoning increases, the composition, abundance and diversity of carrion insects on the decomposing domestic fowl will also reduce. In addition, this study has shown that domestic fowl can also attract a significant assemblage of carrion insect and therefore recommended for future forensic studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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