SUCCESSION AND DEVELOPMENT STUDIES OF CARRION INSECTS ON *CLARIAS GARIEPINUS* POISONED WITH SNIPER (DICHLORVOS)

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[1]Tyokumbur Emmanuel Teryila, [1]Okediran Rasaq Dolapo
[1]Department of Zoology, University of Ibadan, Ibadan, Nigeria.
Corresponding author: emmanuel_tyokumbur@yahoo.com

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Abstract

A study was carried out on the forensic entomotoxicological evaluation of carrion insects found on fish poisoned with Sniper (Dichlorvos) on the main campus of the University of Ibadan. Fish (*Clarias gariepinus*) for the study were obtained from the fish farm on the University Campus. The fish were then euthanized with 5ml of Dichlorvos while the control fish were sacrificed by percussive stunning but without the poison. Adult carrion insects were collected from the fish carrion using a sweep net. The larvae were collected into a bowl by using a scoop, immobilized with hot water and later placed in sample bottles containing 70% ethanol. Pupae were collected using forceps. Carcass temperature was measured using infrared thermometer while relative humidity was recorded with a digital hygrometer. Calliphoridae and Muscidae were the initial pioneers of the decomposing carcass and were seen during the fresh stage, sarcophagidae was seen shortly after the fresh stage of decomposition. The highest mean temperature value for the fish treated with 5ml Dichlorvos was 31.3^0C while its lowest mean value was 22.6^0C. It was observed that the temperature on the ninth day was higher than the other days. This is attributed to the heat generated by the active maggots at that stage of decomposition. Dichlorvos was found to retard the growth of carrion larva as shown in *Musca domestica* larvae when compared with the control. Fast decomposition rate was recorded due to high ambient and carcass temperature. It can be concluded from this study that carrion insects can be used in solving crime puzzles through the extrication of post mortem intervals in conjunction with environmental variables. Since fish carrion in this study attracted a sizable number of carrion insects, it is recommended that fish be used in future forensic case and experimental studies.

Keywords: Entomotoxicology, Dichlorvos, *Clarias gariepinus*, Carrion insect, Decomposition

Introduction

Entomotoxicology is derived from "entomo" which literally means insects and "toxicology" which means the study of toxins. Therefore entomotoxicology can simply be defined as the study of toxins or poisons in insects (Nuorteva, 1997; Amendt *et al*, 2004). Forensic entomology is the scientific study of the invasion and succession pattern of arthropods with their developmental stages of different species found on the decomposed cadaver during legal investigations. It is the application and study of insect and other arthropods, including insects, arachnids, and centipedes to criminal or legal cases. It is primarily associated with investigations (Benecke, 2001; Amendt *et al*, 2004)

Forensic entomotoxicology is most commonly used to estimate Post Mortem Interval (PMI) in cases involving homicide (Wallman, 2003: Dadour and Morris, 2009). The initial signs of soft tissue decay occurs within the first 72-98 hours after death. After the initial stages of decomposition are complete, the accurate determination of PMI is not possible but insects found on the deceased can enable entomologist to provide an estimation of time since death ranging from one day up to more than 2 months (Schoenly *et al*, 1992). Carrion insects are insects that are associated with decomposing or deteriorating remains (Nuorteva, 1977). Carrion insects are very important in the ecosystem as they help in the breakdown of dead and decomposing organisms and they also return nutrients to the soil (Campobasso *et al*, 2001). During this decomposition, the carcass goes through dramatic, physical, biological, and chemical changes (Henssge *et al*., 1955; Vaqn den Oever 1976; Coe and Curran 1980). Each of these stages of decomposition is attractive to a different group of Sarcosaprohagous arthropods, primarily insects. Some are attracted directly by the corpse, which is used as food or an oviposition medium, whereas other species are attracted by the large aggregation of other insects they use as food resource (Von Zuben *et al*, 1996; Anderson *et al*., 2002).

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) commonly abbreviated as DDVP is an organophosphate widely used as an insecticide to control household pests, in public health, and protecting stored foods although it could have genetic effects on human health (Wells *et al* 2009; Howard, 2017) Dichlorvos is widely used as a suicide agent in developing countries like Nigeria hence the need for this study to understand its forensics. The oral LD_{50} of dichlorvos in its various forms in rats ranges from 25 to 80mg/kg, 61 to 175mg/kg in mice, 100 to 109mg/kg in dogs, 15mg/kg in chickens, 157mg/kg in pigs, and 11 to 12.5mg/kg in rabbits. The dermal LD_{50} for dichlorvos in rats is 70.4 to 250mg/kg, 206mg/kg in mice, and 107mg/kg in rabbits. The 96-hour LC_{50} for dichlorvos in fathead minnow is 11.6mg/L, 0.9mg/L in blue gill, 5.3mg/L in mosquito fish. The 24-hour LC_{50} for dichlorvos in blue gill sunfish is 1.0mg/L (Howard, P. 2017)

Materials and Methods

Study site

The site to be used for the study is located on the departmental grounds of the Department of Zoology near the Animal House. The site is suitable as there is limited movements and activities around the site. So the smell emanating from the decomposition of the fish carcass will pose little or no threats to humans. The location reading on the digital compass is $7^0$26'37"N $3^0$53'46"E.

Ethical approval

The 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/19/0137.

Preparation of fish for setup

The fish were transported from the farm to the field site using a bucket containing water. The bucket was covered with mesh net to prevent the fishes from jumping out. Measuring cylinder was used to measure out 5ml of Dichlorvos and administered to the two fishes through the mouth and left to die. The control fishes (2) were sacrificed by hitting the back of their heads on a hard surface without dichlorvos intake. This was achieved by percussive stunning to induce immediate insensibility by administering a severe blow to the skull of the fish. The fish then remains unconscious until death.

Mounting the fishes

The fish after death were placed on separate mounts (Two fish per mount). The mounts consisted of a tray covered with sawdust and placed on a stool. The essence of the sawdust is to provide q hiding medium for the mobile larvae and pupae of the insects. The stool is important as it provides a platform that excludes other animals that are not of entomological importance that might also visit the carcass. Also a container with spent engine oil was placed on each leg of the stool to trap crawling arthropods that are not of entomotoxicological importance that might also visit the carcass. "Keep Off" signs were also placed on each carcass mount to better inform people who might encounter the setup.

Carrion insect collection and processing

The carrions will be collected in the following order: First, those flying over and/or landing on the carcass; then those found in natural cavities and finally those that are under the carcass and in the sawdust upon which the carcass is placed. Sampling for adult insects will be done by using a sweep net and insecticide. The net will be swept clockwise and anticlockwise at an angle of almost 180^0 arc over the decomposition carcass after which the open end will be quickly folded by using the second hand so as to prevent the escape of the trapped insects. The insecticide will then be sprayed over the sweep net to immobilize the insects and they will be transferred into appropriately labelled clean and clear sample bottles. The collected samples will be fixed in 70% alcohol.

Sampling for larvae

Maggot is the larvae stage of carrion insects. This involves using a sampling spoon to collect adequate amount of the maggots from the decomposing carcass. The active maggots will then be transferred into a small bowl. Hot water kept in a flask will then be poured into the bowl containing the maggots. The hot water kills and renders the maggots inactive. The maggots will then be isolated by using a sieve and then placed into appropriately labelled sample bottles with 70% ethanol (Adams and Halls, 2003).

Sampling for pupae

When the maggots on the decomposing carcass start reducing, the sawdust was checked for the presence or absence of pupae. If pupa were present, they were collected and also kept in appropriately labelled sample bottles with 70% ethanol (Adams and Halls, 2003).

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Measurement of larval body length

The lengths and weights were measured and mean values recorded for each carrion group at different stages of decomposition. Mean values were used for statistical analysis. Time of pupation and adult emergence were recorded for each carrion group. This was followed by group mean developmental period. Length of larvae from the 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 an infrared thermometer that can accurately measure between -50^0C to 330^0C. The thermometer was used by pointing the infrared beam towards the pig 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 as close as possible to the carcass, readings are generated on the LCD screen of the hygrometer and the readings are then recorded into the field experiment book.

Results

Abundance and species composition of carrion insects on fish carcass.

The result of the abundance of forensically important insects collected from *Clarias* treated with 5ml of Dichlorvos is shown in Table 1. From the table, calliphoridae and sarcophagidae lead the fresh stage with 2 representatives each, muscidae had one representative. In the bloat stage, calliphoridae proved to be the most abundant with 8 representatives, sarcophagidae had 4 representatives and muscidae lagged with 2 representatives. At the dry stag of decomposition, muscidae was the most abundant with 6 representatives and a new family; dermastidae came up with 2 representatives.

Table 1. Abundance of forensically important insects collected from fish carcass treated with 5ml of Dichlorvos

Family	Genus/ species	Fresh	Bloat	Active	Advanced	Dry
Calliphoridae	*Lucilia sericata*	2	4	8	2	0
Sarcophagidae	*Sarcophaga spp*	2	4	4	6	0
Muscidae	*Musca domestica*	1	2	2	2	6
Dermastidae	*Dermastes maculates*	0	0	0	1	2

Table 2 shows the abundance of forensically important insect collected from fish carrion in the control group. Calliphoridae is the abundant family with 4 representatives, the muscidae follows with 2 representatives. In the bloat

stage, family calliphoridae was still found to be dominant. The calliphoridae was found dominating all stages except advanced and dry stages. The family dermastidae was found dominating all stages except advanced and dry stages. The family dermastidae was found in the active, advanced and dry stages with representatives in the format 1, 1, 2 respectively.

Table 2. Abundance of forensically important insects collected from control fish carcass

Family	Genus/ species	Fresh	Bloat	Active	Advanced	Dry
Calliphoridae	*Lucilia sericata*	4	8	8	0	0
Sarcophagidae	*Sarcophaga spp*	1	1	4	1	0
Muscidae	*Musca domestica*	2	4	1	0	0
Dermastidae	*Dermastes maculates*	0	0	1	1	2

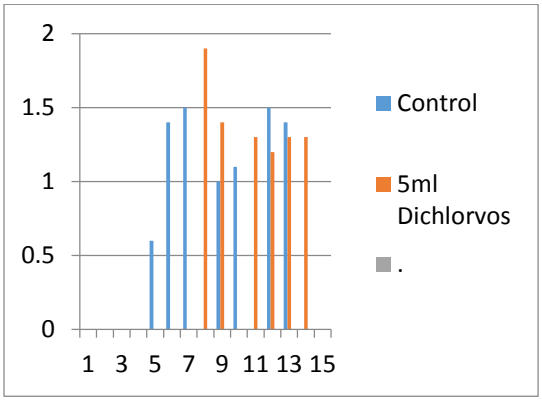

Fig. 1: Histogram showing the mean body length of the larvae of *Musca domestica* collected from fish carcass

Figure 1 shows the effect of Dichlorvos on the mean body length of the larvae stage of *Musca domestica* collected from the fish carcass. We can observe that the first four days after death of fish was required for transformation of *Musca domestica* into the second instar stage. The second instar larva were collected from the decomposing fishes on the fifth day.

On the sixth day, the mean length of larvae from the control group was 1.4cm increasing by 0.8cm from the mean larva length of the fifth day.

On the seventh day, the mean length of the larvae from the control was increased by 0.1cm on the eighth day, the mean length of the larvae in the poisoned carcass shot up to 1.9cm and no mean larva length was recorded for the control group.

On the ninth day, the mean larvae length for the control group was 1.0cm and the mean larvae length for the poisoned fish was 1.4cm

There was no record for the poisoned group on the tenth day but the mean larvae length for the control group was placed at 1.1cm

On the eleventh day, there is a record for the mean larvae length for the poisoned fish and was found to be 1.3cm. The length reduced in comparison to the mean larvae length for the poisoned fish on the eight day.

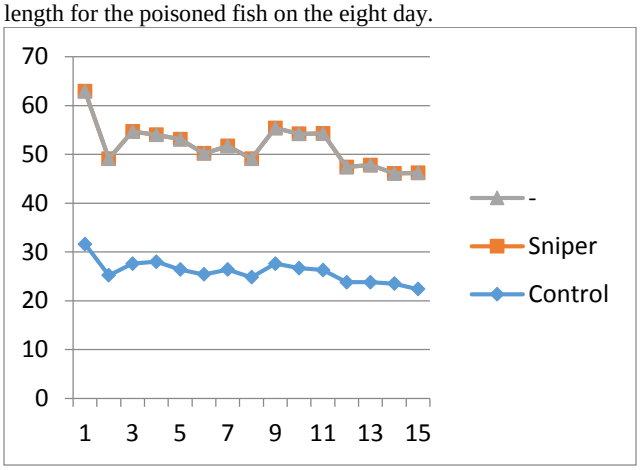

Fig 2: Comparison of Dichlorvos poisoned and control fish

carcass temperature recorded in the field experiment

From the graph in Fig 2. We notice that the mean temperature of the control group ranges from 31.6^{0}C which was the highest value recorded to 22.4^{0}C which was the lowest value recorded. The highest mean temperature value for the fish treated with 5ml Dichlorvos was 31.3^{0}C while its lowest mean value was 22.6^{0}C. It was observed that the temperature from day 9-11 was higher than the other days. This is attributed to the heat generated by the active maggots at that stage of decomposition.

Discussion

Abundance and species composition of carrion insects on fish carcass

At the end of the field work, five stages of decomposition were observed and this corroborates earlier findings and experiments such as the one done by Carvalho *et al*., (2004) where they found out that the five important stages of decomposition are the fresh stage, bloated stage, active stage, active stage, advanced stage and the dry stage. The four major families of carrion insects in the study were also reported in Carvalho *et al*., (2004) experiment, although Carvalho *et al* recorded some families that were not observed in this study such as Formicidae, Histeridae, Staphylinidae, etc. especially in the post decay stage. Abajue *et al*., in 2013 also recorded that the arthropods that arrived on a carcass from the beginning of the decomposition are the Calliphoridae, Sarcophagidae and Muscidae.

Carrion insect succession on fish carcass

During the experiment, Calliphoridae and Muscidae were the initial pioneers of the decomposing carcass and were seen at the fresh stage, while Sarcophagidae arrived shortly after the fresh stage of decomposition. Dermastidae was later seen in the advanced stage of decomposition and it was observed till dry decay stage. The trend we observed here was similar to the trends observed by Bharti and Singh

(2002), Abajue *et al*., (2013) and Ekrakene and Iloba (2011)

Effects of Dichlorvos on the length and weight of larva

In this study, dichlorvos was found to inhibit the growth of *Musca domestica* larva and when compared with the control this corroborates earlier findings by Ekrakene and Odo (2017) which they assessed effects of varying volumes of cypermethrin pesticide on the larval body length, weight, and development time of blowfly *Chrysomya albiceps* (Diptera: Calliphoridae) reared on rabbit carrions.

According to an experiment by Andrew (2014), where he studied the abundance and occurrence of carrion insect on pigs poisoned with nicotine, he discovered that the low dose group had lesser occurrence and abundance of carrion insects when compared with the control group, he also discovered that the high dose group nicotine poisoned pigs had more occurrence and abundance of carrion insects than both the low dose group and control group. However, in this study, dichlorvos was found to inhibit larva growth.

Variation in environmental variables at the field site

The high tropical temperature and relative humidity of Ibadan where the experiment was conducted aided in the faster decomposition of the fishes. The result of the fast decomposition corroborates findings by Ekanem and Dike (2010) where it was established that higher air temperature leads to a faster decomposition rates and increase in the abundance of insects.

Conclusion

At the end of the study, we have established the fact that indeed suicidal poisons will affect the diversity, abundance and decomposition of carrion insects found on decomposing fishes. As the dosage of Dichlorvos poisoning increases, the composition, abundance and diversity of carrion insects on the decomposing fish will also reduce. These findings can be used to extrapolate cause of death and PMI of fish and other aquatic animals suddenly found dead floating on the surface of water bodies for the purpose of biodiversity conservation. Insect larva found from crime scenes can also be cultured in fish carcass to get further forensic information for judicial purposes.

Conflict of Interest

The authors declare that there is no conflict of interest.

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