IOSR Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 3, Issue 1 (Jan. - Feb. 2013), PP 64-71
www.Iosrjournals.Org

Toxicological Effect of Diazinon on African Catfish (*Clarias anguillaris*)

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Abstract: *Fish are sensitive to a wide range of herbicides and toxic conditions as a result of spillage or deliberate discharge of chemicals to water ways. In addition, water pollution may arise from agricultural practices with attendant negative effect on aquatic organisms. The toxicological effect of diazinon (pesticide) on Clarias anguillaris was carried out, using the 96hours bioassay test to determine its acute toxicity. Varying concentrations of Diazinon, 0.0 ml (control), 0.2, 0.4 and 0.6ml/70L of water, at ten fish per test concentration in two replicates were subjected to 96 hours exposure. The median lethal concentration (LC_{50}) value of Diazinon on C. anguillaris was 0.5ml. Changes in water quality and mortality in all treatments including the control were monitored. Except the control, there were significant differences ($p>0.05$) in values of pH (7.30±0.03-5.60±0.02) and dissolve oxygen (5.40±0.03 - 2.03±0.02)mg/L before and after the experiment in all treatments. Mortality was highest(100%) in 0.6ml/L concentration at 48hrs exposure. As concentration and time of exposure increased, fish showed restlessness, rapid opercular movement, erratic swimming before death. Major histopathological effects were hypertrophy, necrosis of hepatocyte and secondary lamella of the liver, gills and intestine. It is obvious from this study that diazinon negatively affects respiration and produce nervous signs in the test fish.*

Key words*: Diazinon, Clarias anguillaris, lethal concentration, histopathological effecs, water pollution, mortality*

I. Introduction

Aquatic environment is polluted when there are both quantitative and qualitative changes in the physical, chemical and biological properties of water. Herbicides or pesticides can enter water courses intentionally or unintentionally through spray drift from terrestrial application on treated agricultural fields, thus, posing serious problem to fauna and human health (Rahman, 2002).Contamination of water with chemicals results in bio- accumulation in fish and other biota, sometimes to biologically active levels, which may be evaluated in a short term test and death represents unequivocal end point.

According to Adedeji *et al*., (2009), diazinon is an organophosphate insecticide that is used to control insects on agricultural farm lands, households and urban settings. It is mobile and moderately persistent in the environment and does not bio-accumulate. Due to its chemical properties and widespread use, diazinon is frequently found in point and non-point sources in urban and agricultural areas. Although the aquatic environment is not the main target and the aquatic invertebrates are not the target organisms, but the insecticide, after application easily enters to current and underground water resources. Studies have shown that no matter the route or duration of exposure, diazinon is a potential danger to aquatic fauna or flora and a threat to biodiversity conservation (Fadina *et al*,1991).

Damaging effect of diazinon on aquatic organisms has been reported by De Vlaming *et al*., (2000). Some of the visible effects of this chemical are reduction in fish survival, growth and reproduction. The toxicity effects of diazinon are due to the inhibition of an enzyme needed for proper nervous system function. The range of doses that result in toxic effects varies widely with formulation and with the individual species being exposed (Fernandes and Mazon, 2003).Toxicity to different species of fish is affected by age, sex, body size, climatic conditions, chemical formulation, and chemistry of the environment among others (Adedeji *et al*., 2009). Macroscopically, signals of toxicity are almost always preceded by changes of the organs, tissues, cellular and molecular levels (Fadina *et al*,1991). Important organs like the kidney, liver, gills, stomach, brain muscles and genital organs are mostly damaged. *Clarias anguillaris* was used as test fish in this study because of its endemic nature in Nigeria waters and economic relevance. The study determines the (LC_{50}) values and the behavioral responses of *C. auguillaris* to acute effects of diazinon, an organophosphate pesticide.

II. Materials and Methods

Range Finding Test

Range finding test was performed on the fish before the introduction of different concentrations of the chemical. Lethal Concentration (LC_{50}) is the concentration of toxic material (Diazinon) which kills 50% of the

test organisms in 96hrs (four days). LC_{50} is somewhat similar to LD (Lethal dosage). It is the concentration, usually in the air or water surrounding test organism that cause 50% mortality. It is intended to provide data with predicted environmental effects. Range finding Test for Diazinon was conducted with lethal concentrations of between 0.5ml and 0.6ml. The test concentrations were selected based on Observable Effect of Concentration (NEC) and the rate of mortality, using Organization for Economic Cooperation and Development (OECD) standard guidelines for testing of chemicals (OECD, 1993).

Experimental procedure

The experiment was carried out in the Research Farm of Faculty of Agricultural Sciences, Ekiti State University, Ado Ekiti in April, 2012. Fourty (40) *Clarias anguillaris* of average standard length and weight of 16cm and 52.5g respectively were collected from fish pond in the Faculty Research Farm. The fish were acclimatized at room temperature in aquaria tanks before the commencement of the experiment. Fish were divided into four groups based on the experimental treatments. Eight glass aquaria tanks of size 75cm x 45cm x 40cm each, filled with 70L of well water were used in the experiment. The fish were weighed with a metler top loading balance and measured with mounted ruler. Within 30 minutes of addition of Diazinon, fish were introduced randomly to three concentrations of the test substance at 0.0 ml (control), 0.2, 0.4 and 0.6ml. Two replicates with an equal number of fish (10) per test concentration were used to provide strong statistical baseline. No feed was administered within the 96hrs of testing.

Water parameters and fish mortality

Temperature, Dissolve oxygen and pH of the water in the experimental tanks were monitored, while mortalities were recorded at 24hrs, 48hrs, 72hrs and 96hrs. Abnormal behaviours of fish were also monitored. The physiochemical parameters of water were analyzed at different stages; before the introduction of toxicant, just after the introduction of toxicant and at the end of the experiment. pH was determined by dipping the electrode of the pH meter into each tank while dissolve oxygen was measured using standardized YSI DO Meter. The numbers of dead and living test fish in each tank were counted every 24 hours and general observations were made.

Tissue Samples Preparation

Dead fish samples were dissected to remove some organs; liver, gill and intestine. The tissues were preserved in 10% buffered formalin for 3 days to prevent pigmentation which could occur as a result of blockage in blood circulation. Tissues were then dehydrated by passing through different grades of alcohol (70%, 95% and 100%) to ensure complete dehydration. This involved clearing the tissues in three changes of molten paraffin wax for one hour each and finally embedded in paraffin wax at temperature of 50^0C for solidification. Trimming was done to expose the surface area of tissue inside the block and supported on a piece of blocking wood. The blocks were then sectioned at 4 microns using rotary microtone and the sections floated out into a slide pre coated with glycerin egg lumen in water bath. The re- coating enables sections to adhere firmly as to withstand the staining procedure. They were them oven fixed for one hour and allowed to cool staining. The sections were dewaxed in 3 changes of xylene and hydrated in alcohol for 3 minutes. Sections were further place in water to stop action of alcohol and xylene and stained with haematoxylene for 15 minutes, rinsed in water for 3 minute and diffentiated in 10% acid alcohol to separate cytoplasm and nuclei. The sections were allowed to blue in scott tap water for 5 minute and counterstained using 10% alcohol eosin for a few seconds. This was followed by quick dehydration in 70%, 90% and 100% of alcohol and clearing in several changes of xylene to remove alcohol.

III. Result

Different concentrations and exposure time of diazinon on *Clarias anguillaris* fingerlings in this study showed negative effects on physico-chemical parameters and fish organs.At higher doses, abnormal responses such as restlessness, rolling movements and finally fish settled at the bottom leading to death was observed. However, normal values and reactions were observed in the control, an evident that physical and chemical properties of aquaria water were within desirable range of fish culture (Boyd 1981). In Table 1, the water quality parameters such as temperature, dissolved oxygen and pH varied a little but significant at different concentrations of Diazinon.The decline in oxygen and pH levels from the lower to higher concentrations of Diazinon in the experimental tanks affected the gas exchange of fish, leading to death (Table 2)

Table 1: Comparison of water quality parameters of experimental tanks before, during and after the introduction of Diazinon

Treatments	pH			Temp (°C)			DO (mg/ L)		
	before	during	after	before	During	After	before	during	after
(0.0 ml) Control	7.20 ± 0.01^{a}	7.20 ± 0.03^{a}	7.20 ± 0.01^{a}	25.20 ± 0.12^{e}	25.20 ± 0.10^{e}	25.91 ± 0.12^{e}	5.40 ± 0.03^{j}	5.20 ± 0.01^{i}	5.07 ± 0.02^{h}
0.2ml	7.30 ± 0.03^{a}	6.75 ± 0.01^{b}	5.42 ± 0.03^{c}	25.22 ± 0.15^{e}	24.75 ± 0.10^{g}	24.07 ± 0.14^{g}	5.40 ± 0.01^{j}	4.28 ± 0.02^{j}	3.56 ± 0.03^{j}
0.4ml	7.20 ± 0.01^{a}	6.63 ± 0.04^{b}	5.50 ± 0.01^{c}	25.30 ± 0.11^{e}	24.33 ± 0.13^{g}	24.93 ± 0.11^{g}	5.42 ± 0.02^{j}	3.50 ± 0.04^{j}	3.50 ± 0.01^{j}
0.6ml	7.30 ± 0.04^{a}	6.42 ± 0.02^{b}	5.60 ± 0.02^{c}	25.20 ± 0.13^{e}	24.11 ± 0.12^{g}	23.27 ± 0.10^{g}	5.37 ± 0.01^{j}	2.99 ± 0.02^{j}	2.03 ± 0.02^{k}

Mean values for each parameter with similar superscript are not significantly different (P>0.05)

At varying hours (24hrs, 48hrs, 72hrs and 96hrs), the effect of different concentrations and exposure time of Diazinon on mortality and survivability of *Clarias anguillaris* is presented in table 2.

Table 2: Mortality and survival data of experimental fish after the experiment.

Concentration of Diazinon (ml)	24hrs		48hrs		72hrs		96hrs	
	M	S	M	S	M	S	M	S
0.0 ml(Control)	00	10	00	10	00	10	00	10
0.2ml	00	10	01	09	03	06	03	03
0.4ml	00	10	02	08	04	04	03	01
0.6ml	00	10	10	00	10	00	10	00

NB: M = Mortality
S = Survival

It was observed that at concentration of 0.0ml (control), the rate of mortality was 0% within the 96hrs duration of experiment. At concentration of 0.2ml, 70% mortality was recorded at 96hrs. Mortality began at 48hrs, at concentration of 0.4ml, while 100% mortality was recoded within 48hrs at a concentration of 0.6ml. Abnormal behavior which includes erratic swimming started minutes after the introduction of toxicant, settling at the bottom and increase in peculiar beat, movement at the side of the container, swelling of stomach, sudden and quick movement, rolling movements, swimming on the back (at higher doses) swimming at the water surface were observed. The affected fish became very weak, settled at the bottom and died in increasing numbers at the higher doses. Normal colour and behavior were observed in the control tanks. However, the colour became pale progressively with higher doses at the end of 96hrs of exposure time.

Histopathological Examination

The results of the histopathological examination are shown in Plate 1 to 12.

Liver: Hepatocytes and other cell of the liver in control treatment (0.0ml) were normal and systematically arranged (Plate1). Hypertrophy of hepatocytes, mild necrosis and minor vacuolation were found at the dose of 0.2ml (Plate 2). At the dose of 0.4ml and 0.6ml, severe necrotic hepatocytes, pyknosis and vacuolation were observed in the fish(Plate 3 and 4)

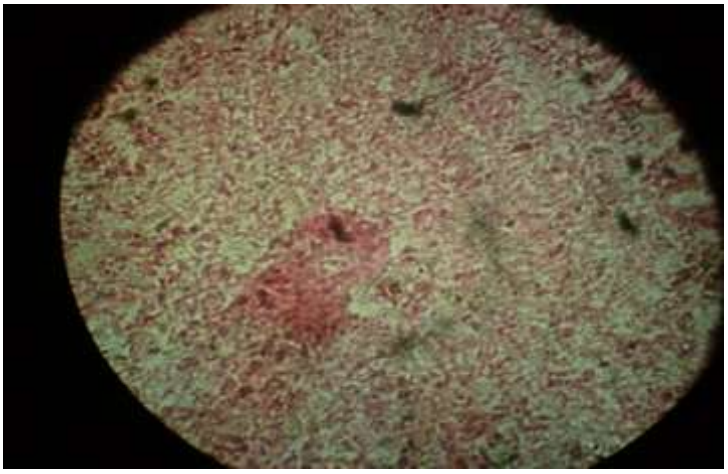

Plate1: Liver of *Clarias anguillaris* exposed to 0.0ml of diazinon showing normal hapatocytes and other cells

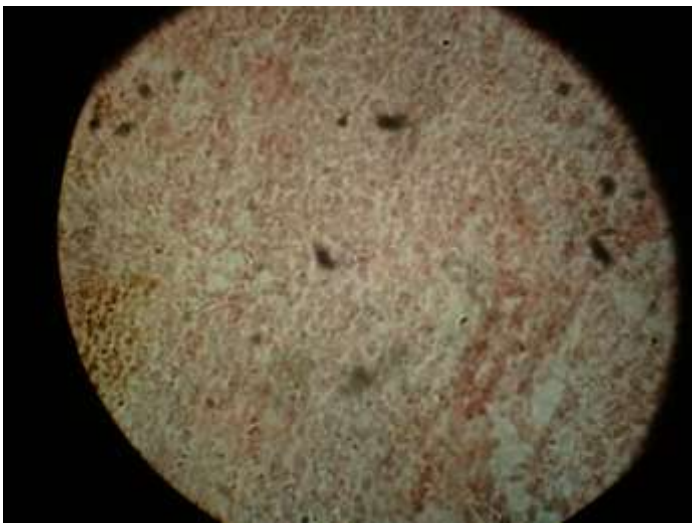

Plate 2: Liver of *Clarias anguillaris* exposed to 0.2ml of diazinon showing mild necrosis and minor vacuolation

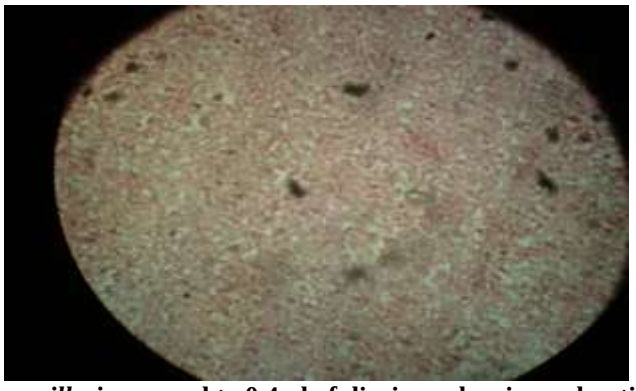

Plate3: Liver of *Clarias anguillaris* exposed to 0.4ml of diazinon showing pyknotic cells and vacuolation.

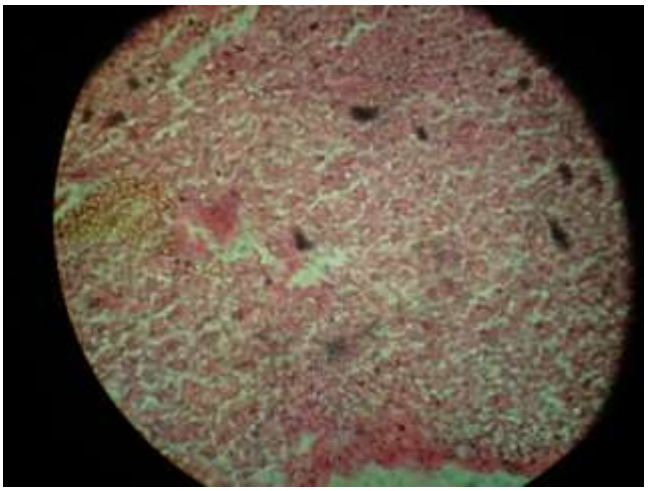

Plate 4: Liver of *Clarias anguillaris* exposed to 0.6ml of diazinon showing severe necrotic hapatocytes, pyknotic cells and vacuolation.

Gills: Changes were not observed in the gills of fish in the control treatment (Plate 5). Each gill consists of a primary filament and secondary lamellae. There were infiltration of the secondary lamellae distortion, and numerous mucus cells at the dose of 0.2ml (Plate 6). At the dose of 0.4ml and 0.6ml, total blockage of secondary lamellae, distortion, numerous mucus cells and separation of layers were observed in the test fish (Plate 7 and 8)

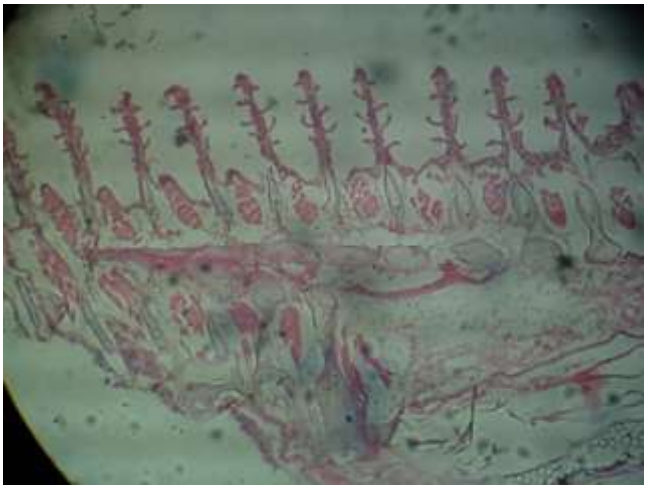

Plate 5: Control experiment (0.0ml diazinon) showing the normal gill of *Clarias anguillaris.*

Plate 6: The gill of *Clarias anguillaris* exposed to 0.2ml of diazinon showing distortion of numerous mucus cells

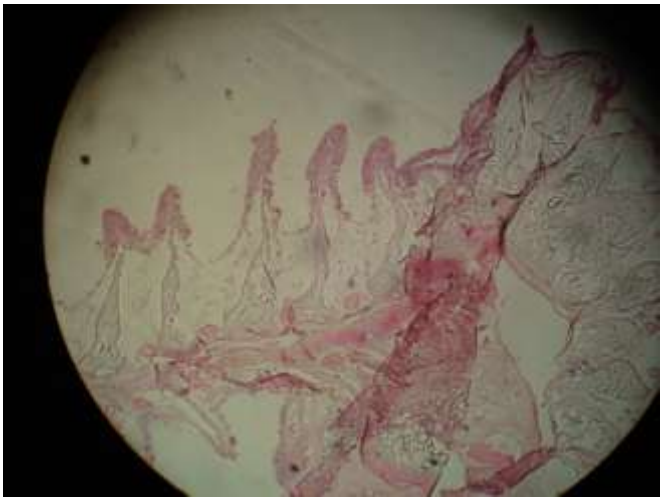

Plate 7: The gill of *Clarias anguillaris* exposed to 0.4ml of diazinon showing blockage of secondary lamellae, distortion, and separation of layers

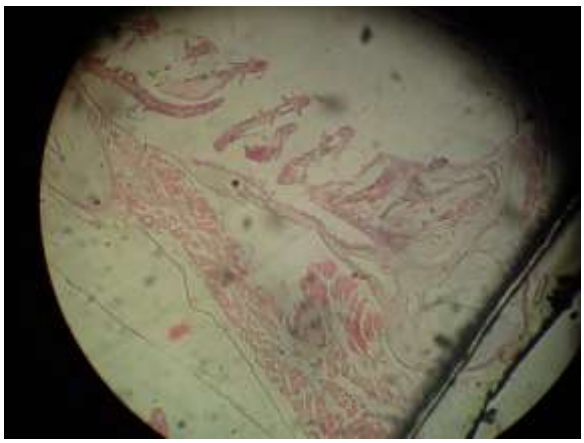

Plate 8: The gill of *C. anguillaris* exposed to 0.6ml of diazinon showing total blockage of secondary lamellae, separation of layers.

Intestine: Changes were not observed in the intestine of control fish which show no yellow bodies (Plate 9). At the doses of 0.2ml, 0.4ml and 0.6ml, yellow bodies were shown at the lamina propria of the mucosal fold (plate 10,11 and 12) respectively

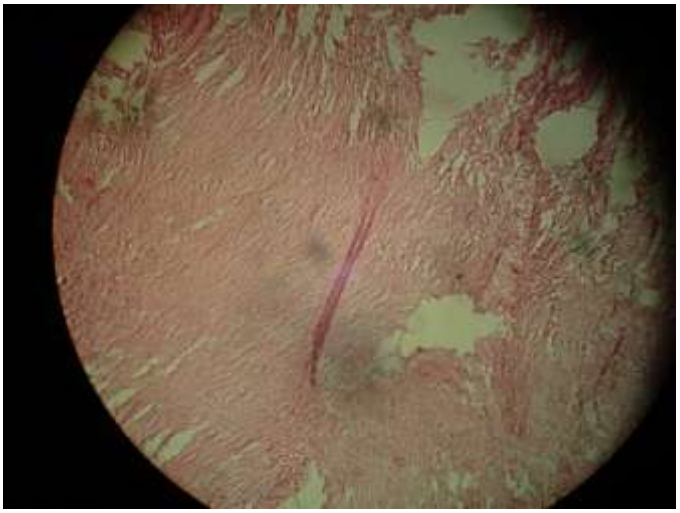

Plate9: The intestine of *Clarias anguillaris* exposed to 0.0ml of diazinon showing no yellow bodies.

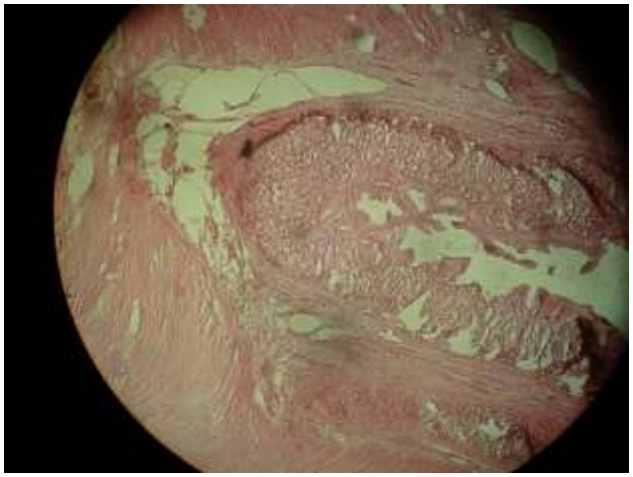

Plate10: The intestine of *Clarias anguillaris* exposed to 0.2ml of diazinon showing yellow bodies.

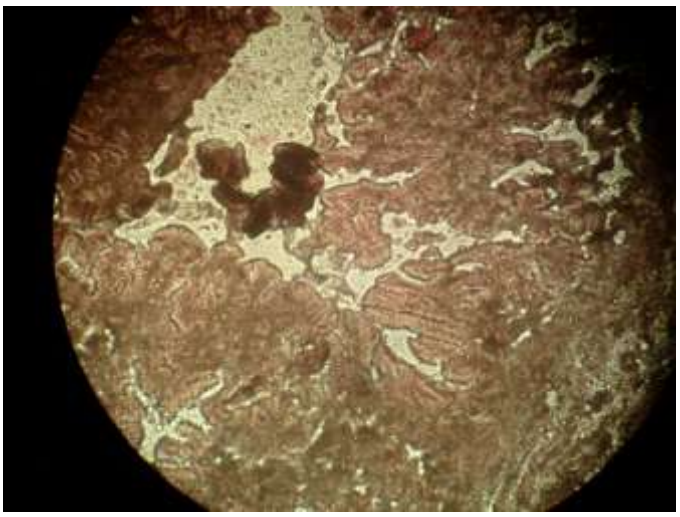

Plate11: Intestine of *Clarias anguillaris* exposed to 0.4ml of diazinon showing yellow bodies at lamina propria of the mucosal fold.

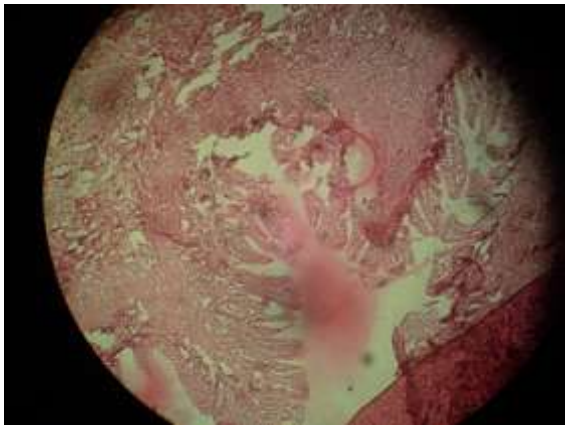

Plate12: Intestine of *Clarias anguillaris* exposed to 0.6ml of diazinon showing yellow bodies at lamina propria of the mucosal fold.

IV. Discussion

The observable effects of different concentrations of this toxicant (diazinon) on water and test fish (*Clarias anguillaris)* were compared with earlier work done on water pollution by pesticides/ insecticides. It was observed in this study, that, the higher the concentration of the toxicant, the higher the mortality rate. This was in line with the observation of Jezierska and Witeska (2004) that all toxicant has a threshold reach, below which the animal is in a tolerance zone and above the tolerance zone is the zone of resistance where there is no drastic survival of animal. Several abnormal behaviours such as restlessness, loss of equilibrium, increased opercular activities, surface to bottom movement and resting at the bottom as recorded in this study were similar to the observations of Watanabe *et al* (2005). However swelling in the abnormal region and gas-filled stomach were not observed which is contrary to the finding of Mido and Satake (2003). It is an indication that the effect of pesticide is species – specific. Histopathological results indicated that gill was the primary target effect by diazinon. Damage to the gills indicated that the sub lethal concentrations of the toxicant caused impairment in gaseous exchange efficiency of the gills as reported by (Rahman, 2002) and Omitoyin (2006).

Liver is the main organ for detoxication that suffers serious morphological alteration in fish exposed to pesticide (Dutta et al., 1997). In this study, observed necrosis of some portion of the liver tissue resulted from excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification as reported by Adedeji *et al*., 2009). At the doze of 0.4ml, mild pyknotic and necrotic hepatocytes, hypertophy and in a few cases vacuolation were recorded for the fish. At the highest dose of 0.6ml generative changes of hepatocytes and severe degenerative changes like necrosis, pyknosis, vacuolation, rupture of blood vessel casing haemorrhage were recorded in the fish. Adedeji *et al.*, 2009) also reported cytoplasmic degeneration, pyknotic nuclei in liver tissues, vacuolation in hepatic cells and rupture of blood vessels; degenerative hepatic cells and necrotic nuclei when *Heteroneutus fossils* was exposed for 25 days to 5, 10 and 20ppm Diazinon, respectively The intestinal tract is not easily divided into small and large intestine as it is in mammal. In this study, the

lamina propria at the tip of mucosal fold of the intestine show yellow bodies in diazinon- treated fish. Lovely (1998) reported similar effect on *Barbodes gonionotus* treated with diazinon.

Diazinon toxicity varies widely within and among species. In a study by Svoboda *et al* (2001), the diazinon LC_{50} for common carp *Cyprinus carpio* was $1mgL^{-1}$ and $4mgL^{-1}$ for golden carp *Carassius auratus* respectively, while in this study, LC_{50} was at 0.5ml to 0.6ml/70L of water. In rainbow trout, the diazinon LC_{50} is 2.6 to 3.2 mg/L Sensitivity of fish to diazinon may be due to the difference in diazinon uptake by the different fish species, size and weight of the fish among other factors. Warm water fish such as fathead minnows and goldfish are even more resistant with diazinon LC_{50} value ranging up to 15mg/L.

This study had shown that diazinon is toxic to African Catfish and that the toxicity varies with the duration of exposure. Investigation over time has shown that pesticide (herbicide) at higher levels in the aquatic environment can accumulate in the tissue of fish and aquatic organisms disrupting physiological processes. It is however incorrect to suggest that all pesticides are dangerous to fish as many are unstable in water (Holden, 1973) or have low toxicity relation to other chemical. Concentration toxic to fish can never arise with the normal agricultural usage of many such chemicals although spillages of concentrations can, as with the chemicals cause fish kills. Understanding the mechanisms of body defense system against diazinon and reactive agent should be understood, hence the need for more studies is suggested. Establishment of farms very close to water bodies should be discouraged to control run off from treated farms..

V. Conclusion

The results obtained in this study showed that varying levels of diazinon application had harmful effect on fish and aquatic environment. Since the use of agrochemicals can not be totally faced out in agricultural fields, it is hereby suggested that efforts should be intensified on the use of biological control of insects and weeds especially in ponds situation. It is a non- toxicant means of pest and weed management. Introduction of herbivorus fish species such as Grass Carp controls aquatic weeds effectively, why aquatic insects are eradicated through constant desilting of pond bottom.

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