Nwanna, L. C.; Ajani , E. K.; Omitoyin, B. O.; Magawata, I. Ayoola, S. O.; Orisasona, O.; Kareem, O. K and Abubakar, M. Y. (Eds.) (2004) ***Proceedings of the Fifth Conference of Association of Nigerian Fisheries Scientists.*** **"SUSTAINABLE FISHERIES AND AQUACULTURE PRODUCTION FOR ENHANCING LIVELIHOOD IN NIGERIAN ECONOMIC DEVELOPMENT"** 9TH – 11TH JULY, 2024 In the University of Lagos, Lagos, Nigeria

Genotoxic Effects and Haematological Profile of Catfish (*Clarias gariepinus)* Exposed to Varying Concentrations of Lambda-Cyhalothrin

***Moses, D.U.[1], Ayoola, S. O[2] and Truscott, A. O.[2]**

[1]College central research laboratory, Yaba college of Technology,Lagos Nigeria

[2]Department of Marine Sciences, Faculty of Science, University of Lagos, Nigeria

*Corresponding Author: ugomoses77@yahoo.com, ugochukwu.moses@yabatech.edu.ng, 234(90)90079951

ABSTRACT

Employing the use of pesticides in the cultivation of commercially valued crops seems to cause a deleterious effect on the inhabitants of neighboring aquatic environments through the release of metabolically active compounds into such water bodies via genotoxic and physiologically altering mechanisms. Juveniles of *Clarias gariepinus*, which serves as a very good test organism, were subjected to acute and sub-lethal exposures to Lambda-cyhalothrin, which belongs to a highly active group of pesticides, known as pyrethroids. From the acute toxicity test, the LC_{50} of the pesticide used was derived as 1.0µg/L. A 28-day sub-lethal assay was carried out, to analyze Haematological and Cytogenetic effects, of five sub-lethal concentrations (0.00, 0.20, 0.40, 0.60 and 0.80µg/L) of the pesticide. All the haematology parameters analyzed showed a positive correlation with the concentration of exposure, except for Total Platelet count which showed no significant correlation ($p \geq 0.05$). The haematology profile reveals that the highest parameters were recorded in fish exposed to 0.80µg/L including Haemoglobin (12.1±0.36g/dl), PCV (37.1±4.07%), RBC (2.86±1.2million per mm^3), WBC (13,670±500 per mm^3) and Total Platelet Count (215,300±197,900 per mm^3). The cytogenetic analysis showed that micronucleus aberrations were relatively higher than binucleated and fragmented nucleus cells. The Binucleated and fragmented nucleus cell frequency, unlike micronucleus frequency, were not found to significantly correlate with the dose of exposure. These prove the genotoxic ability of Lambda-cyhalothrin, and its influence on the haematology profile of *Clarias gariepinus*.

Keywords:

Bioindicator, lambda-cyhalothrin, Clarias gariepinus, toxicological effects

Introduction

Anthropogenic activities in agriculture, manufacturing, power generation, development, and transport sectors of a nation result in the release of various wastes products in solid, liquid or gaseous forms into the aquatic environment (Ayoola and Alajabo, 2012). In a country like Nigeria, where sectors are being developed in an attempt to improve the quality of basic amenities, the agricultural sector is not left behind in this advancement. Pesticides are widely used throughout the developing world (Like Nigeria) and pesticide demand is increasing due to the current system of crop production, which prioritises high agricultural yields. Made up of chemicals that can control pests or regulate plant growth (Sarkar *et al*.,

2021). The use of Pesticides is to eliminate pests to increase production and improve the quality of agricultural products (Yekeen and Adeboye, 2013). Public health organisation use pepticide to reduce ill health as well as enhance productivity and yield of crops. They could be used as insecticides, rodenticides, fungicides, herbicides, and fumigants. Rafeal *et al.,* (2021) reported that due to the simultaneous fight against different pest species, agricultural workers are exposed to pesticide mixtures with different mechanisms of action and, although with the lower pesticide dose currently used than in the past, the effect of long-term exposure on various aspects of human health is not clearly defined. Various biomarkers are used in the biomonitoring of human populations exposed to environmental pollutants, among them the comet (primary DNA damage detection), The indiscriminate use of pesticides, careless handling, accidental spillage, or discharges of untreated effluents into natural waterways has harmful effects on the fish population and other forms of aquatic life, as well as long term effects on the environment. The continuous discharge of agricultural effluent into water bodies often leads to a variety of pollutant accumulation, which becomes apparent when considering toxic pollution (Ayoola, 2008).

The comet assay is a versatile method for measuring DNA strand breaks in individual cells. It can also be applied to cells isolated from treated animals. Modified versions of the standard protocol detect oxidized DNA bases and may be used to reveal sites of DNA base loss (Eugenia *et al.,* 2021). The mutagen tests used for bio-monitoring contaminated environments, including the comet assay, nuclear aberrations, alterations in erythrocytes and chromosomal aberrations; the micronuclei assay (MN) is relatively simple, reliable and sensitive, and has been used to evaluate the effects of genotoxic compounds in various environments (Galindo and Moreira, 2009). For genotoxicity in fish and its use for environmental screening and biomonitoring. As a result of an increase in marine contamination, aquatic fauna and flora have been significantly exposed to adverse effects of harmful contaminants. In environmental surveillance evaluation, MN assaying has become an easy, low cost and fast technique for evaluating genotoxic effects as evidently, the activity of any genotoxic agent might trigger an increase in MN frequency (Santana *et al*., 2020). Fishes are used as a biological indicator to determine the physiological changes which serve as biomarkers of environmental pollution, the health of the aquatic ecosystem and ecotoxicological studies. *Clarias gariepinus* is among the most widely used fishes in evaluating the health of the aquatic ecosystem and physiological changes that arise from environmental pollution. This is because it is hardy and can tolerate both well and poorly oxygenated waters (Ogundiran *et al.*, 2010). In Lagos for example, the use of pesticides in the cultivation of *Corchorus olitorius* – commonly known as 'ewedu. Nevertheless, how their interaction and cumulative effects along the ever-evolving field production to postharvest continuum contribute towards food insecurity remain scant in the literature. To address this within the context of Africa, we focus on the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (*Lepidoptera: Noctuidae*), and the larger grain borer, Prostephanus truncatus (Horn) (*Coleoptera: Bostrichidae*). Both insect pests have shown high invasion success, managing to establish themselves in >50% of the African continent within a decade post-introduction (Shaw *et al*., 2024). Most farmers now employ the use of various pesticides in the cultivation of this commercially valued crop. Freshwater fishes are faced with increasing threats due to intensification of agriculture with the use of pesticides like, abamectin, carbofuran, chlorpyrifos, cypermethrin, deltamethrin, dichlorvos, dimethoate, fipronil, lambda-cyhalothrin and paraquat (Nnamdi *et al.,* 2020). This study evaluated the haematological and genotoxic effects of exposed (African Catfish) *Clarias gariepinus* to sublethal concentrations of commonly used pesticides in agricultural settings.

Materials and Methods

Experimental design

300 healthy juveniles of African catfish (*C. gariepinus*) with mean weight 7.5±0.2 g and a standard length range of 8±0.2 cm were purchased from a Alagemo fish farm at Igbe Ikorodu, Lagos State and were placed in well-aerated plastic containers, in the basement of the marine science of the Department of Marine Sciences, University of Lagos. The fish were then kept in these plastic containers for two weeks before the assessment, to allow acclimatization to the environment and they were fed twice daily with commercially prepared feed, until a day before the bioassay test. The test compound was a pesticide used in the control of pests that attack the productivity of Ewedu (*Corchorus clitoris*), purchased from a nearby market. The pesticide used was 'RE- LAMBDA' which contained the compound *Lambda-cyhalothrin*, at an identifiable percentage (2.5%). A stock solution containing 0.1% of the pesticide was prepared by diluting 1ml of the pesticide in 999ml of water that is serial dilution. This meant that 1ml of the stock solution contained 10^{-3}ml (1µg) of the pesticide. This made the measure of the amount of pesticide used in testings up to a specific level of 0.0001ml of the pesticide.

Acute Toxicity Testing

Range Finding test was first carried out to determine the range of concentration that will be used for the acute toxicity assay by the method described by solbe (1996). An assessment and record of mortality were carried out every 24h over the 96h of the preliminary range-finding test. Fish were assumed to be dead when there was no operculum or body movement even when prodded with a glass rod. The fish were not fed throughout the experimental period and 24 hours before the experiment. The ranges of concentrations used for range finding include; 0, 0.0012, 0.0014, 0.0015, 0.0016 and 0.0018 ml^{-1}; which were carefully introduced into 2Litres of water, by measuring 0, 2.4, 2.8, 3.0, 3.2 and 3.6 ml respectively, of the stock solution, using a syringe. The results gotten from the range findings test were used in deriving the concentrations used for this test. These include 0, 0.0008, 0.0010, 0.0012, 0.0014 and 0.0015 ml^{-1}, which were carefully gotten by measuring 0, 10, 12, 14.4, 16.8 and 18 ml respectively, into 12Litres of water. Ten fishes were introduced into the test medium containing the various concentrations and were set in triplicates, giving a total of 30 fish per test medium, including control media. The result of mortality gotten from this test was then used to calculate the 96h lethal concentration(LC_{50}) that is the concentration of the pesticide (Lambda-cyhalothrin), which will cause the death of 50% of the fish population, in 96hours.

Sub-Lethal Assay

This was a 4 week (28 days) long assay, where the fish were exposed at sub-lethal concentrations that are concentrations less than the derived LC_{50}. The experiment was set up in triplicates, with each tank containing 12 Litres of water and 10 fish. It is aimed at exposing the fish to various sub-lethal concentrations and assessing certain endpoints of the fish response to the pesticide other than mortality. These include changes in haematological composition, and micronucleus aberrations, in response to various concentrations of the pesticide. The concentrations used for the sub-lethal poisoning include 0, 0.2, 0.4, 0.6 and 0.8 μgL^{-1} of the stock solution. The exposure to the sub-lethal concentration was done every 24 hours, and the fish were fed throughout the experimental period.

Haematology Analysis

This analysis is aimed at identifying the haematology profile of fish exposed to Lambda-Chyalothrin at sub-lethal concentrations. Components of the blood tissue are quantified and recorded including Haemoglobin Concentration (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White Blood Cells (WBC) count and Total Platelet count. These parameters are common for being altered by exposure of fish to toxic substances. At the end of the 28-day sub-lethal assay, blood samples were collected from fish in each tank and immediately stored in Lithium heparin bottles – which contains an anti-coagulant. These samples were then transferred to the Blood and Haematology Laboratory, Lagos University Teaching Hospital (LUTH), Idi Araba, Lagos state, where they were analyzed for the afore-mentioned components.

Cytogenetic (Micronucleus) Analysis

It is aimed at investigating the rate at which *Clarias gariepinus* accumulates the pesticide, and aberrations in the nucleus content of the cells of the fish blood after the exposure period (28days). These aberrations can be caused by the genotoxic effects of the pesticide on the division of the cell nucleus during mitosis, resulting in more than two nuclei before splitting. At the end of the 28-day exposure, blood samples were collected from fish in each tank. These were smeared on microscopic slides, fixed and then stained with 10% Giemsa stain for 20minutes. After these, the slides were rinsed off with absolute ethanol and left to air-dry for 24 hours, before viewing with a microscope in oil immersion (x1500). In total, 15 blood samples were collected, 3 from each concentration.

Statistical Analysis

Statistical packages were used for computation, representation and analyzing the results of the assays. Microsoft Office's Excel Worksheet and IBM's SPSS Statistics 20 were the packages used to carry out processes like sums, errors, deviations, probit analysis, correlations, and One-way ANOVA analysis.

Results

Results of Acute Toxicity Testing

Mortality of fish was recorded when exposed to lethal concentrations of Lambda-cyhalothrin in an attempt to evaluate its acute toxicity and derive the LC_{50} of the pesticide. The mortality table is as follows:

Table 1: Mortality recorded during acute toxicity testing after 96 hours

Stock Solution	Concentration	Mortality											
(ml)	(µg/l)	24h			48h			72h			96h		
0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0.8	0	0	0	0	0	0	0	0	1	1	1	1
12	1.0	2	2	2	3	3	3	4	5	5	6	5	6
14.4	1.2	2	3	3	4	5	5	5	6	6	7	7	7
16.8	1.4	3	4	4	5	6	6	6	7	7	8	8	9
18	1.5	5	4	5	7	6	6	9	9	8	10	10	10
		Note: 12 Litres of water and 10 fish were used per tank											

The average mortality of the concentrations is given in Table 2;

Table 2: Average Mortality Table

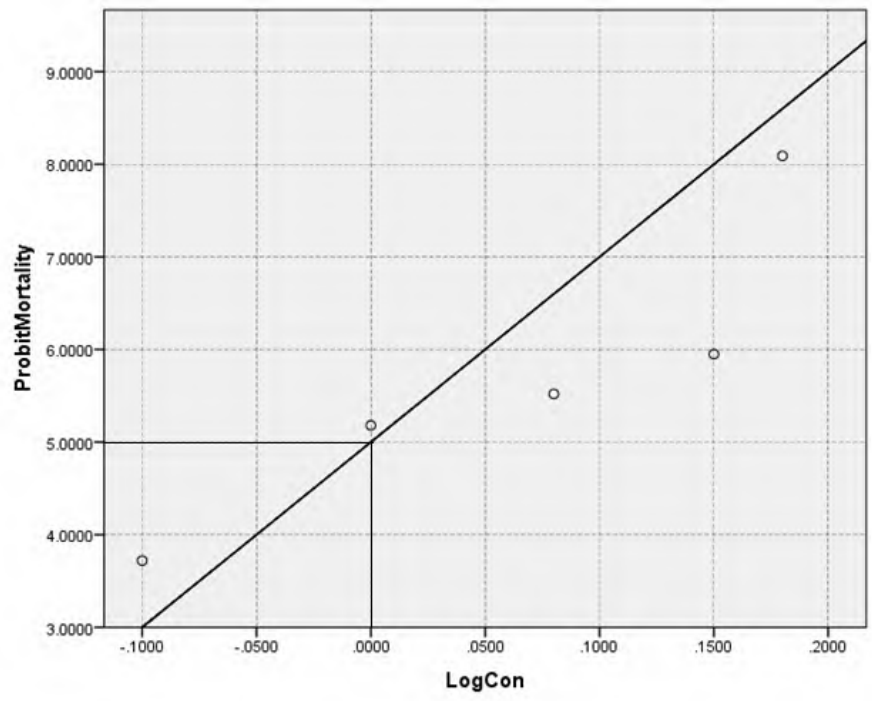
Stock Solution	Concentration	Mortality			
(ml)	(µg/l)	24h	48h	72h	96h
0.0	0.00	0.00	0.00	0.00	0.00
10.0	0.80	0.00	0.00	0.33	1.00
12.0	1.00	2.00	3.00	4.67	5.67
14.4	1.20	2.67	4.67	6.67	7.00
16.8	1.40	3.67	5.67	6.67	8.33
18.0	1.50	4.67	6.33	8.67	10.00

In Table 3, Percentage mortality is transformed to a probit value. This is then plotted against Logarithm of Concentration as illustrated in Figure 1

Table 3: Probit Transformation of Average Mortality recorded from Acute Toxicity Test

Stock Solution (ml)	Concentration (µg/L)	Mortality (96h)	Percentage Mortality	Log Concentration	Probit Mortality
0.0	0.00	0.00	0.00	-	-
10.0	0.80	1.00	10.00	-0.10	3.72
12.0	1.00	5.67	56.70	0.00	5.18
14.4	1.20	7.00	70.00	0.08	5.52
16.8	1.40	8.33	83.30	0.15	5.95
18.0	1.50	10.00	100.00	0.18	8.09

Figure 1: A plot of Probit Mortality against Logarithm of Concentration to derive LC_{50}.



From the graph above, the LC_{50} is derived as 1.0 µg/L. This is gotten by finding the Inverse Logarithm of the intercept of 'Probit = 5.0' on the x-axis.

$LC_{50} \rightarrow Log^{-1}$ (0.00) = 1.0 µg/L

Five concentrations, less than the LC_{50} were selected for exposure during the sub-lethal assay. These include 0.80, 0.60, 0.40, 0.20 and 0.00µg/L.

Results of Haematology Analysis

Haemoglobin

The haemoglobin level was measured in g/dl and was increasing as the concentration of exposure increased, showing a significant positive correlation with concentration ($p \geq 0.05$). The highest haemoglobin was recorded in fish fed with 0.8µg/L of the pesticide, with a value of 12.1 ± 0.36 µg/dl.

Packed Cell Volume

The Packed Cell Volume was measured in percentage (%) and was increasing as the concentration of exposure increased, showing a significant positive correlation with concentration ($p \geq 0.05$). The highest Packed Cell Volume was recorded in fish fed with 0.8µg/L of the pesticide, with a value of 37.1±4.07%.

Red Blood Cells Count

The Red Blood Cell count was measured per mm^3 and was increasing as the concentration of exposure increased, showing a significant positive correlation with concentration ($p \geq 0.05$). The highest Red Blood Cells count was recorded in fish fed with 0.8µg/L of the pesticide, with a value of 2.86 ± 0.12 million per mm^3.

White Blood Cells Count

The White Blood Cell count was measured per mm^3 and was increasing as the concentration of exposure increased, showing a significant positive correlation with concentration ($p \geq 0.05$). The highest White Blood Cells count was recorded in fish fed with 0.8µg/L of the pesticide, with a value of 13,670±500 per mm^3.

Total Platelet Count

The Total Platelet count was measured per mm^3 and showed no significant correlation with concentration ($p \geq 0.05$). The highest Total Platelet count was recorded in fish fed with 0.8µg/L of the pesticide, with a value of 215,300±197,900 per mm^3.

The results of the analysis carried out to determine the components of the blood tissue of fish exposed to sub-lethal concentrations of Lambda-Cyhalothrin are represented in Table 4.5 below;

Table 4: A Summary of the Haematology profile of fish exposed to sub-lethal concentrations of Lambda-Cyhalothrin.

Concentration	**Hb**	**PCV**	**RBC**	**WBC**	**PLATELET**
(µg/L)	(g/dl)	(%)	(*10^5/mm^3)	(*10^3/mm^3)	(*10^4/mm^3)
0.00	**7.77±1.39**	**23.23±7.32**	**15.97±3.54**	**7.33±2.70**	**21.5±11.08**
0.20	**10.1±0.26**	**27.43±2.99**	**23.8±4.23**	**11.09±1.72**	**10.27±2.52**

0.40	**10.7±1.01**	**31.2±3.67**	**25.37±4.29**	**12.69±1.82**	**9.77±3.05**
0.60	**10.9±0.35**	**32.7±2.52**	**26.27±1.47**	**12.40±0.17**	**14.8±12.96**
0.80	**12.1±0.36**	**37.1±4.07**	**28.6±1.2**	**13.67±0.5**	**21.53±19.79**

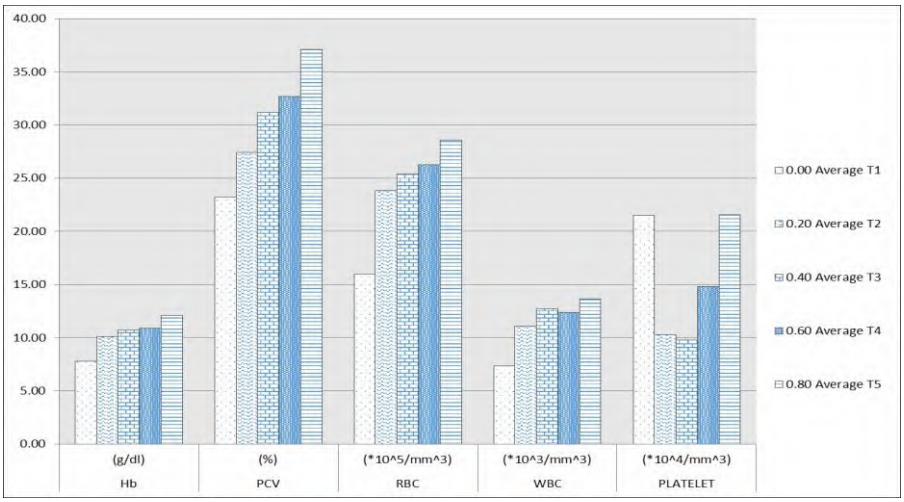


Figure 2: A Comparison of the analyzed haematology parameters for the various sub-lethal concentrations of exposure.

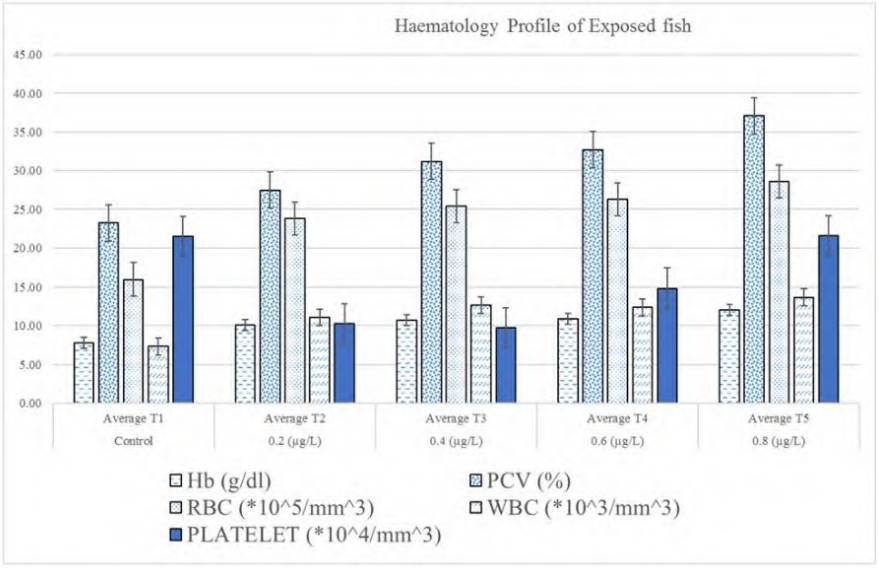


Figure 3: A graphical illustration of the haematology profile of fish exposed to sub-lethal concentrations of lambda-cyhalothrin.

Results of Cytogenetic Analysis

After viewing slides with the blood of exposed fish smeared on them the following result was obtained for the kinds of micronucleus aberrations observed in them, as shown in Table 4.6.

Table 5: **Kinds of aberrations observed in Microscopic slides of smeared blood of exposed fish (per 500 cells).**

Slide	Concentration (µg/l)	Micro-Nucleated	Nuclear Bud	Bi-Nucleated	Fragmented Nucleus
T1 (Control)	0.00	0.00	0.00	0.00	0.00
T2	0.20	1.67±0.58	0.00	0.33±0.58	0.00
T3	0.40	4.33±1.53	0.00	1.33±0.58	0.00
T4	0.60	5.33±1.53	0.00	0.67±0.58	0.67±0.58
T5	0.80	6.33±1.53	0.00	2.00±1.00	2.67±1.15

The following plates (Plates 1-4) show examples of the three kinds of cytogenetic aberrations resulting from the sub-lethal assay.

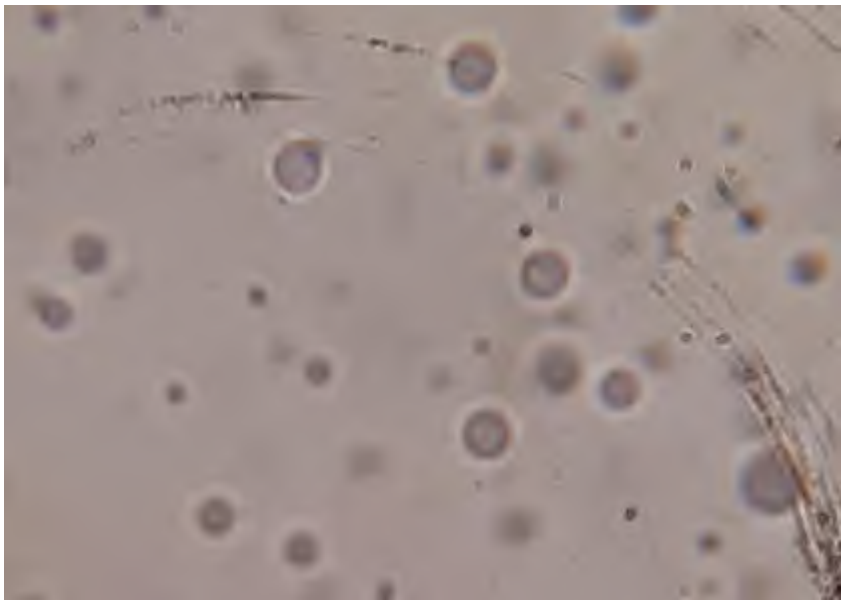


Plate 1: A micrograph showing Normal Differentiation of cells observed from the result of the cytogenetic analysis.

Plate 2: A micrograph showing Binucleated aberration in a cell observed from the result of the cytogenetic analysis.

Plate 3: Micrographs showing Micronucleus aberrations of cells observed from the result of the cytogenetic analysis.

Plate 4: A micrograph showing a Fragmented Nucleus in a cell observed from the result of the cytogenetic analysis.

Discussion

The acute toxicity test indicates that mortality varies based on the concentration of Lambda-cyhalothrin exposed to the fish, over the 96 hours. The result confirms the lethality of the pesticide to the fish population. Ayoola (2012), described toxicity testing as a measure of how poisonous and how much of a dose of a substance would kill or cause damage in an organism. This agrees with the premise of the acute toxicity test result obtained from this research, seeing that the doses of pesticide the fish were exposed to result in damage and ultimately death of the fish. The mortality recorded has a strong positive relationship with the dose, expressing its lethality. The LC_{50} was derived as a value of 1.0µg/L of the pesticide used, containing 2.5% of Lambda-cyhalothrin. The result of the haematology analysis reveals that the highest parameters were recorded in fish exposed to 0.80µg/L including Haemoglobin (12.1±0.36g/dl), PCV (37.1±4.07%), RBC (2.86±1.2million per mm^3), WBC (13,670±500 per mm^3) and Total Platelet Count (215,300±197,900 per mm^3). However, not all the parameters were in correlation with the concentration of exposure. This showed that the most abundant blood component is the Erythrocyte, ranging from 1.20 to 2.98 million per mm^3 of the blood sample. This is similar to the work of Małgorzata *et al.*, (2022), who worked on the characterization of blood cells and

haematological parameters of some fish species. Małgorzata *et al.,* also described the White blood cells as the second most abundant blood component, which is in agreement with the result of this research. The trend of the haematology parameters indicates the fish's response to stress arising from the various concentrations of the 28-day exposure. Ogundiran *et al*. (2009), describes *Clarias gariepinus* as a hardy fish with the ability to tolerate conditions of low aeration. This is indicated by the increase in the production of blood components that aid the oxygenation of the organism, in the condition created by the exposure. However, haematology parameters of poisoned fish were discovered to be lower than control by Olushola *et al.,* (2021), when analyzed after a sub-lethal, short-term (96 hours) exposure to ammonia. This is probably because the fish's response to the effect of toxin in its blood system is not yet effectual at such a short term, but is realized after 28-days of consistent exposure to a toxin in this research, as indicated by increasing heamatology parameters compared to control. The cytogenetic analysis, however, was similar to the result of Grisolia *et al* (2009) who worked on the profile of micronucleus frequencies and DNA damage in different species of fish in an eutrophic tropical lake. This indicates that the formation of micronucleus and related aberrations is a phenomenon that is not limited to freshwater fishes only. At much more sub-lethal levels, the damage caused by the pesticide was limited to aberrations in the fish's cells and nucleus, arising from chromosomal aberrations. The frequency of micronuclei formation increased with an increasing dose of pesticide. This aligns with the work of Campana *et al.* (2003), in evaluating the micronucleus frequency of an amphibian bullfrog (*Rana catesbeiana*), exposed to Lambda-cyhalothrin. However, this finding is not in concordance with the result of the work of Ayoola (2012), in evaluating the genotoxicity of spent engine oil. This should be because the test compound used for the cytogenetic assay – spent engine oil, does not belong to the same class of pyrethroids as Lambda-cyhalothrin, but is a mixture of branched alkanes, cycloalkanes, Polyaromatic hydrocarbons (PAHs), linear alkanes and decomposition products, all at unidentified proportions. This pattern of positive correlation with concentration was not observed in the frequency of binucleated cells, which is in agreement with Ayoola (2012). There was no significant correlation between the frequency of binucleated cells and the dose of exposure, while the fragmented nucleus was present only in concentrations relatively close to the LC_{50} (concentrations 0.60 and 0.80 µg/L).

Conclusion

The result of this project work could be a good indication of the genotoxic potential of Lambda-cyhalothrin in living organisms. Therefore, environmental assessments and investigations studies should be on the lookout for pyrethroid chemicals, including Lambda-cyhalothrin, which has proved itself potential enough to cause physiological stress as well as alterations to the nucleic portions of living organisms.

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