HISTOPATHOLOGICAL EFFECTS OF A PYRETHROID MIXTURE ON THE FINGERLINGS OF *Clarias gariepinus* (BURCHELL, 1822)

LELEI, K.E.*, W. V. EBIKEBUNA, N. L. EZAMGBA AND O. S. NANAKUMO

Department of Biological Sciences, Faculty of Science, Niger Delta University, Amassoma, Bayelsa State, Nigeria
**Correspondence Author: kariyelelei@gmail.com; +2348055409784; 0009-0002-2321-440X*

ABSTRACT

The toxic effects of a commonly sold pyrethroid insecticide on Clarias gariepinus fingerling were assessed. One hundred and sixty fingerlings of mean weight and length, 2.620 ± 0.370g and 6.480 ± 0.598cm respectively were procured, acclimatized for seven days and randomly allocated to five exposure concentrations of 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L in triplicates, and a control (0ml/L) for 96hours in the laboratory using Completely Randomized Design. The gills, muscles, intestines and brain were excised for histopathological examination. Some physicochemical parameters of the test media were determined. Mortalities per exposure concentration were recorded 24hourly, and the 96hr-LC_{50} of the pyrethroid mixture was calculated using Probit analysis. The results showed that, the mean values of the physicochemical parameters varied significantly ($P< 0.05$) and were concentration dependent. The histopathology of the tissues reflected concentration- dependent degenerations and alterations. Fish mortality varied with time, mortalities were 6.67, 10.0, 56.67, 30.0 and 63.33% in the 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L concentrations respectively, indicating concentration-dependent effects. The lethal concentration (96hr-LC_{50}) of the toxicant was 0.0128ml/L which implied that, the insecticide was highly toxic, and will pose risk to fish and aquatic life by extension if introduced into aquatic ecosystems. These findings underscore the importance of understanding the toxicological effects of such insecticides on non-target species, so as to develop effective environmental management strategies to mitigate their ecological risks.

Keywords: *Clarias gariepinus,* pyrethroid, lethal concentration, tissues, histopathology.

INTRODUCTION

Pyrethroid pesticides are synthetic insecticides derived from pyrethrins, an insecticidal chemical present in natural pyrethrum, in *Chrysanthemum cinerariaefolium* (Evan and Evans, 2009). They are amongst the important classes of pesticides mostly used in the control of pest populations and domestic insects (Ayaz and Kumar, 2023). These pesticides are the only class of pesticides recommended for application on insecticide-treated nets (ITNs) and are the cheapest pesticides for controlling malaria vectors (Van den Berg *et al*., 2021). These pesticides are present in all WHO-pre-qualified types of ITNs (WHO, 2020; Lissenden *et al*., 2021), used in the production of long-lasting insecticidal nets (LLINs), hence, its wide usage in areas where such vectors are prevalent.

Pyrethroids are neurotoxic, they target the receptor site of the voltage-gated sodium channel, specifically in insects (Valmorbida *et al.,* 2022), and cause changes in the membrane potential resulting in abnormal state of hyper-excitability in the nerve cell. These alterations in insects have sub-lethal, incapacitating 'knockdown' effects, and kill exposed insects by binding to sodium channels resulting in excitatory paralysis (Davies *et al*., 2007). There are over twenty pyrethroids widely used in insecticide formulations; some are combined to reduce insect resistance and improve the effectiveness of the formulations. There are two types of pyrethroid pesticides- Type I and Type II. The Type I pyrethroid pesticides cause Type I Poisoning syndrome, or "T syndrome." The symptoms include tremors, poor coordination, prostration, seizures and death. The Type II pyrethroid pesticides cause Type II 'Choreoathetosis syndrome' or "CS syndrome." The symptoms include hyperactivity, hunched back, salivation, tremors, and progress to sinuous writhing movements (Gupta and Crissman 2013). The wide use of pyrethroids pesticides especially for insects control, threatens the health of aquatic organisms, as they end up in aquatic ecosystems through spray drifts, run-offs and discharges (Bashir *et al*., 2020; Galadima *et al*., 2021), with their resultant toxic effects on the environment and non-target organisms amongst which are fish.

There is an influx of assorted 'labeled and unlabeled', 'powder and liquid' insecticide formulations into markets in Bayelsa State, Nigeria. This gave rise to the need to assess the toxic effects of one of these 'labeled', 'liquid,' locally produced insecticide formulations (a Type II Pyrethroid, classed as moderately hazardous), which was a mixture of Deltamethrin, Cypermethrin and Cyhalothrin (pyrethroid mixture) using *Clarias gariepinus* fingerlings.

MATERIALS AND METHOD

Test Organisms

Two hundred and forty fingerlings of *C. gariepinus* of mean weight and length, 2.620 ± 0.370g and 6.480 ± 0.598cm respectively were procured from a fish farm in Akenfa, Yenegoa, Bayelsa State, Nigeria. These were kept in holding in plastic tanks of 50L capacity to acclimatize for seven days in the laboratory of the Department of Biological Sciences, Niger Delta University, Bayelsa State using borehole water (Reish and Oshida, 1987). They were fed twice daily with Coppen® feed (0.8-1.2mm) at 5% body weight during the holding period, with change of media to prevent stress and fouling of the water. The fingerlings were monitored for mortality and behavioural changes to allow for stabilization before exposure. No mortality was observed during the holding

period.

Toxicant
In this study, the toxicant used is a commonly sold and used pyrethroid mixture with the assigned name '**B**' (to protect the 'trademark'). The insecticide is locally produced in the State and was procured from a market in Yenegoa metropolis in Bayelsa State, Nigeria. This pyrethroid mixture-'**B**' (thus, named) was a mixture of three pyrethroids: Deltamethrin (0.5%), Cypermethrin (0.2%) and Cyhalothrin (0.4%).

Range Finding Test
This was carried to determine the concentrations at which responses will be elicited from the exposed fish to the pyrethroid mixture- '**B**'. The concentrations of 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L respectively were determined as the acute toxicity exposure concentrations after 0.025, 0.05, 0.10 and 0.20ml/L respectively modified after Yidi *et al*. (2021) and Mohammad *et al*. (2022), resulted in one hundred percent (100%) mortality within three hours of exposure.

Experimental Design
A static toxicity test with five exposure concentrations of 0.0045, 0.0085, 0.0110, 0.0125 and 0.015ml/L, and a control (0ml/L) made up to 15L with borehole water of acceptable quality in the 50L plastic tanks was carried out for 96hours (during which the fish were not fed to reduce fouling of media). The experiment consisted of one hundred and sixty (160) fingerlings of *C. gariepinus*, randomly allocated to the five exposure concentrations in triplicates, each having ten fish; and a control using Completely Randomized Design.

Physicochemical Parameters of Test Media (Water)
Some physicochemical parameters- dissolved oxygen (DO), temperature, total dissolved solids (TDS), pH, electrical conductivity (EC) and salinity were evaluated to ascertain the suitability of the water for fish survival (Boyd, 2015) before and after exposure. These were measured *in-situ* using Hanna HI 9828 pH/ORP/EC/DO water analyzing device.

Toxicity Testing
The toxicity testing was based on the determination of the mean values of the evaluated physicochemical parameters of the exposure media and the control compared with standards, assessment of the mortalities of the fish in the exposure concentrations and control with time, determination of the lethal concentration (96hr $LC_{50;}$ the concentration at which 50% of the test population will die), and histopathological examinations of the excised gills, muscles, intestines and brain of the exposed fish compared with the control.

Histological Analysis
The muscles, gills, intestines and brain were excised from the *C. gariepinus* fingerlings harvested from the exposure concentrations. Sections of the excised muscles, gills, intestines and brain of *C. gariepinus* were prepared by dehydration, clearing, impregnating, embedding, microtomed and stained with Hematoxylin and Eosin. The prepared slides were then viewed under the microscope and microphotography was done using Olympus CX31 binocular microscope at x100 for general examination and x400 magnification for detailed view.

Mortalities
During the 96-hr exposure period, observations were made and records taken of the mortalities in the exposure concentrations every 24hrs, and the percentage mortalities deduced with respect to time. Probit Plot of mortality was used to determine the median/lethal concentration (96hr LC_{50}) of the toxicant.

Statistical Analysis
Data obtained were analyzed for means ± standard deviation. One-way Analysis of Variance (ANOVA) was used to compare the differences in the means at $P < 0.05$ and Duncan's Multiple Range Test was used for the post hoc test using SPSS® version 21.

RESULTS

The results of the mean values determined for the physicochemical parameters evaluated in the test media (Table 1) showed that, there were significant differences ($P<0.05$) with respect to concentrations with obvious differences in the mean values of the TDS (which ranged from 92.00±1.0 to 124.50±2.50mg/L in the exposure concentration while the control was 119.30±3.33mg/L), EC (from 183.50±1.50 to 249.00±5.00µS/cm with 253.00±5.23µS/cm in the control) and salinity values (from 133.76±3.20 to 159.36±3.20mg/L, and 151.50±11.37mg/L in the control).

Table 1: Result Showing the Mean ± Standard Deviation of the Physicochemical Parameters of the Test Media of the Different Exposure Concentrations of the Pyrethroid Mixture –'B'

Exposure Concentrations (ml/L)	Temperature (?)	pH	DO (mg/L)	TDS (mg/L)	EC (µS/cm)	Salinity (mg/L)
0	26.90±0.10^{a}	6.95±0.04^{cd}	4.73±0.09^{ab}	119.30±3.33^{d}	253.00±5.23^{d}	151.50±11.37^{d}
0.0045	27.85±0.05^{b}	6.83±0.10^{ab}	5.37±0.25^{ab}	110.50±8.50^{bc}	221.50±17.50^{bc}	141.76±11.20^{b}
0.0085	27.95±0.05^{bc}	6.59±0.08^{a}	5.31±0.70^{ab}	104.00±3.00^{b}	209.00±5.00^{b}	133.76±3.20^{ab}
0.0110	27.95±0.05^{bc}	6.92±0.03^{c}	4.94±0.04^{a}	92.00±1.00^{a}	183.50±1.50^{a}	117.44±0.96^{a}
0.0125	28.00±0.00^{c}	6.79±0.12^{bc}	5.62±0.30^{b}	124.50±2.50^{d}	249.00±5.00^{c}	159.36±3.20^{cd}
0.0150	28.10±0.00^{d}	7.09±0.01^{d}	5.68±0.17^{b}	115.50±11.50^{cd}	231.00±22.00b^{c}	147.84±14.80^{cd}
WHO (2008)	<40	6.5–8.5	> 4	500	70	<600
USEPA (2011)	-	6.5–8.5	-	500	-	-

Key: Means with the same superscripts down the columns were not statistically different at P<0.05; DO =Dissolved Oxygen; pH = Potential Hydrogen; EC = Electrical Conductivity; TDS = Total Dissolved Solids; WHO = World Health Organization; USEPA = United States Environmental Protection Agency

For the mortality, there were 32, 9, 6 and 4 dead fish at the 24th, 48th, 72nd, and 96th hour. The exposure concentrations of 0.0045, 0.0085, 0.011, 0.0125 and 0.015ml/L had 6.67, 10.0, 56.67, 30.0 and 63.33% mortality respectively while the control had none (Fig. 1).

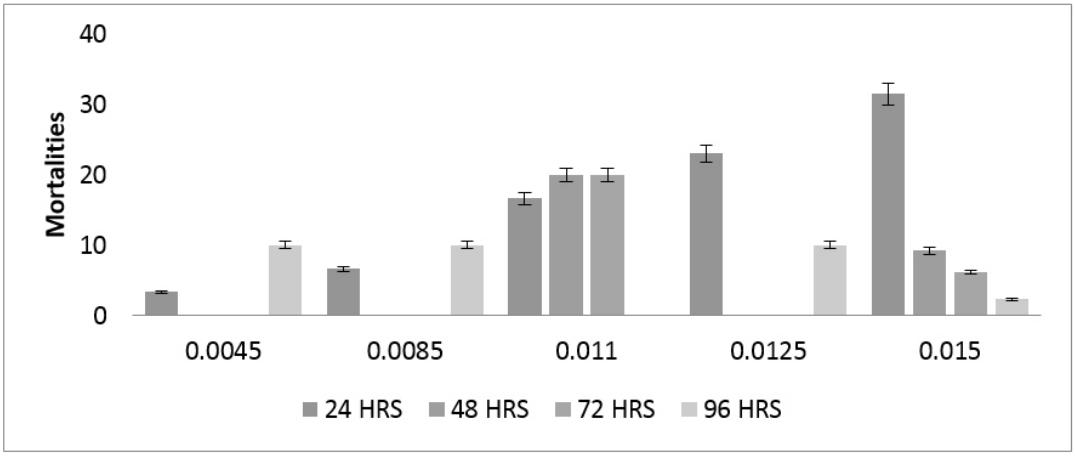
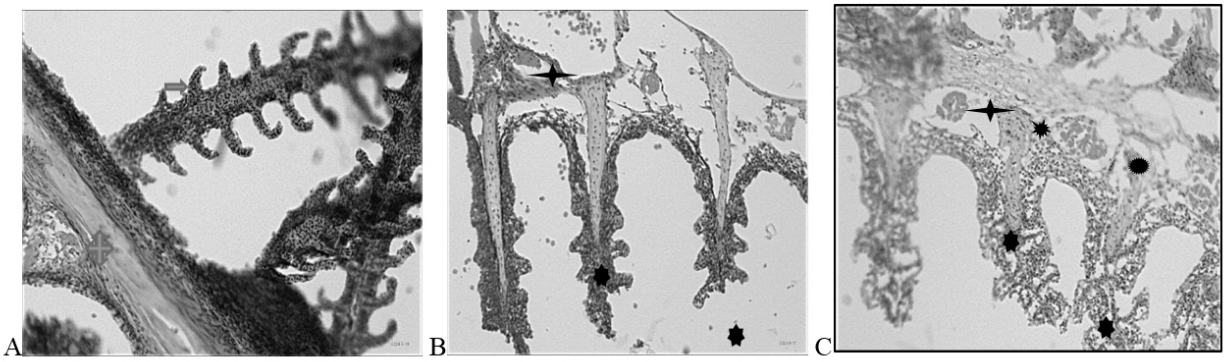


Fig. 1: Mortalities of *Clarias gariepinus* fingerlings in the Different Exposure Concentrations of the Pyrethroid Mixture – '**B**' with time.

Histology of the Gills, Muscles, Intestines and Brain

Microscopic examinations of the prepared slides showed histopathological changes in the muscles, gills, intestines' and brain of *C. gariepinus* from the exposure concentrations when compared with the control (Plates A - L).

The gills in the fish from control had the gill filaments arranged in parallel rows, the gill archs and gill filaments/rakers were intact with the secondary branchial lamellae evenly spaced (Plate A). The gills from the exposed fish showed acute necrosis and lesions of the epithelial cells, damaged gill filaments and rakers (shortening of and eroded secondary lamella), congestion of secondary gill lamella, and diffusion of mucous cell. The changes increased with increase in the concentration of the toxicant that is, the alterations were concentration-dependent (Plates B and C).

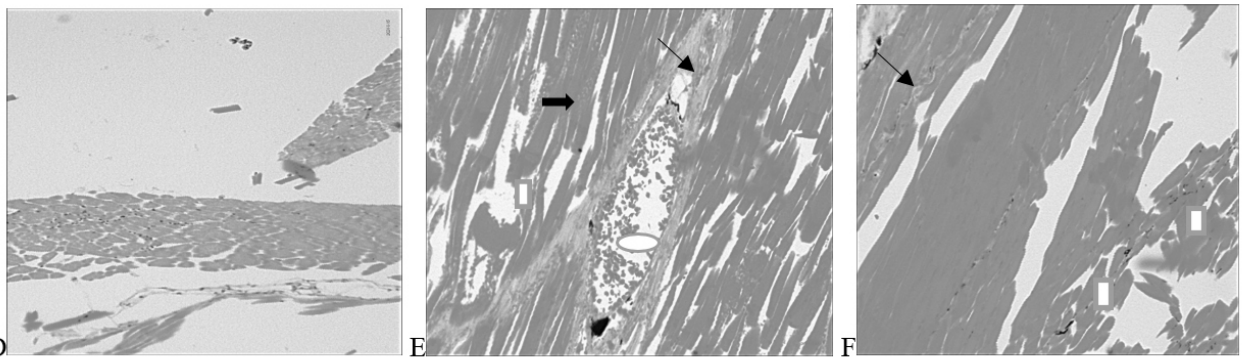


A = Control (0ml/L); B = 0.0045ml/L; C = 0.0150ml/L

: Intact gill archs, ➡ : Intact Gill filament/rakers

= Necrosis and Lesions of epithelial cells; : Damaged gill filaments/rakers (shortening of and eroded secondary lamellar); : Congestion of secondary gill lamella; : Diffusion of mucous cell.

The muscles of fish are striated musculature arranged in myotomes which exhibit sloping patterns. These were the patterns observed in the fish tissues from the control (Plate D). The muscle tissues in the fish from the exposure concentrations showed slight thickening or atrophy of the tissues with increased sloping, necrosis, vacuoles and splitting of muscle fibers. The vacuoles and degenerated muscles increased with increase in concentration of the toxicant (Plates E and F).



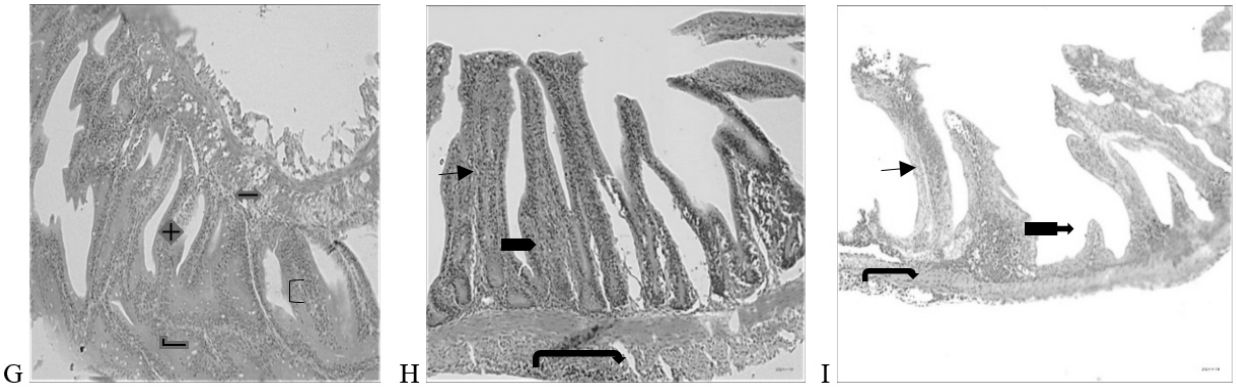
D E F

D = Control (0ml/L); E = 0.0045ml/L; F = 0.0150ml/L

= Necrosis; = Sloping/Degeneration of muscle fiber; = Splitting of muscle; = Vacuole

The small intestine has large circular folds called plicae circular ( ) that has the mucosa ( ), and the villi ( ) which are finger-like projections of simple columnar epithelium and goblet cells ( ). These were intact in the control (Plate G).

The villi showed alterations (degeneration/damages) in the intestines from the exposed fish (Plates H and I) which increased with increase in concentration when compared with the control. Tissue alterations included erosion of the villi, clumps/atrophy in the villi, shrinkage and deadening of the goblet cells (darker colour), lesions in the mucosa which may result in intestinal dysfunction.



G H I

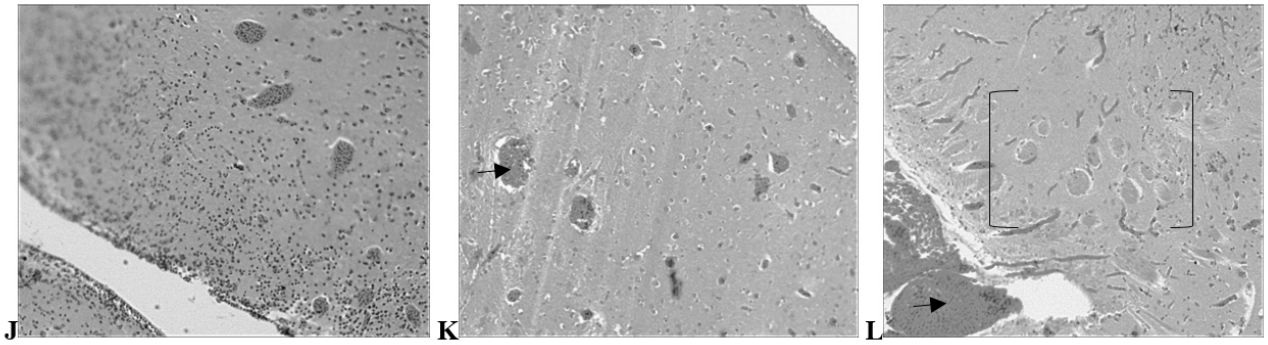
G = Control (0ml/L); H = 0.0045ml/L; I = 0.0150ml/L

Control had normal plicae circular showing mucosa and villi with the goblets.

= Lesions in the mucosa; = Shrinkage and deadening of the goblet cells;

= Erosion of villi; = Clumped/atrophied in the villi.

The sectioned brain showed atrophy of tissues in the grey matter (lighter shade) and white matter (darker shade), with vacuolation and necrosis which were obvious in the tissues of the fish from the exposure concentrations (Plates K and L) and were different from the control (Plate J).



J K L

J = Control (0ml/L); K = 0.0045ml/L; L = 0.0150ml/L
→ = Vacuolation and necrosis; [ ] = Atrophy of tissues

DISCUSSION

The values of the physicochemical parameters determined were juxtaposed and compared with the World Health Organization (2008) and United States Environmental Protection Agency (2011) Guidelines. The values showed that, the parameters were affected by the toxicant and the variations were concentration-dependent. The obvious differences in the values of the total dissolved solids (TDS), electrical conductivity (EC) and salinity among the exposure concentrations were likely due to the interaction between the toxicant and test media, and resultant physiological responses of the fish which increased with increased concentrations of the toxicant. The DO and EC levels exceeded the recommended WHO (2008) and USEPA (2011) water quality guidelines, which could have implications for fish physiology and stress responses of the fish in the test media. These values also indicated increased presence of solutes in the test media which could indicate stress on and in the fish. These findings had similar trends with the reports of Yidi *et al*. (2021), who studied the effects of Deltamethrin on *Chanas argus* and Hossain *et al*. (2022), who reported on the effects of Chlorpyrifos on *Oreochromis niloticus.*

The mortalities observed in the exposure concentrations increased with the exposure concentrations, and was highest in 0.015ml/L by the 24th hour. The observations indicated that, fish mortality varied with time. This was attributed to the degradation (effects of photolysis and oxidation) of the insecticide; mortalities increased with increase in concentration, thus, was concentration-dependent.

The lethal concentration (96hr LC_{50}) of the insecticide was estimated to be 0.0128ml/L which implied that, this pyrethroid insecticide was highly toxic. Since, the lower the LC_{50} value, the more toxic the substance (Shefali *et al*., 2021), in this case, the pyrethroid mixture. Prudencio *et al*. (2023), investigated the acute toxicity of increasing concentrations of the insecticides- Pyrinex Quick 212 EC (Deltamethrin 12g $L^{-1}$and Chlorpyrifos 200g L^{-1}) and Pyro FTE 472 EC (Cypermethrin 72 $gL^{-1}$and Chlorpyrifos 400g L^{-1}) on *C. gariepinus*. The reported 96hr LC_{50} values were 0.004 and 0.012µL L^{-1} for Pyrinex and Pyro respectively, indicating very high toxicity to *C. gariepinus* juveniles. These values were similar to the findings of this study for the Cypermethrin, Cyhalothrin and Deltamethrin mixture, implicated in the toxicity of this insecticide as reflected in the mortalities observed. The 96hr LC_{50} value obtained in this study was lower than the 1.94 µg/L 96-hour LC_{50} reported by Yidi *et al*. (2021) for Deltamethrin alone, suggesting that the combined formulation of Deltamethrin, Cypermethrin, and Cyhalothrin may have contributed to the higher toxicity of the insecticide used in this study.

The low propensity of pyrethroid pesticides to accumulate in organisms (Ayaz and Kumar, 2023), their quick photo-biodegradation (Agnieszka *et al*., 2018), and efficiency (Li *et al*., 2017) have resulted in their abuse with predictable resultant effects. In insects, Type II pyrethroid insecticides cause 'choreoathetosis syndrome' (Gupta and Crissman, 2013). Characterized by symptoms that include hyperactivity, hunched back, salivation, tremors, and progressing to sinuous writhing movements. Paul and Simonin (2006), reported that, pyrethroids were 10–1000 times more toxic to aquatic animals than to mammals and birds due to their lower ability to degrade pyrethroid pesticides. Fish have reduced esterase-mediated hydrolysis of pyrethroids compared to mammals, making them more susceptible to pyrethroid toxicity (Yang *et al*., 2016). Several studies have shown that, pyrethroid pesticides are hazardous to fish in reproductive and early development phases (Farag *et al*., 2021).

The histological changes in the gills of the exposed fish observed in this study as acute necrosis and lesions of the epithelial cells, damaged gill filaments and rakers (shortening of and eroded secondary lamellar), congested secondary gill lamella, and diffusion of mucous cell were similar to the reports of Hossain *et al.* (2022). These histological changes in the gills could imply respiratory dysfunction, alterations in osmoregulation and excretion; and may lead to death. Li *et al*. (2023), observed swellings in the muscle layer of the intestinal tissue of juvenile *Trachinotus ovatus* in response to stress. The folds showed slight swelling to shedding and deformation. The intestinal mucosa was necrotic, had vacuoles which increased with the stress, erosion of the villi was also observed. Prudencio *et al*. (2023), investigated the acute toxicity of increasing concentrations of the insecticides- Pyrinex Quick 212 EC (Deltamethrin 12g $L^{-1}$and Chlorpyrifos 200g L^{-1}) and Pyro FTE 472 EC (Cypermethrin 72 $gL^{-1}$and Chlorpyrifos 400g L^{-1}) on *C. gariepinus* with emphasis on liver histopathological effects for 96hr. And reported on the degenerative effects of the insecticides on the liver tissues. Uptake of pyrethroids in fish may be due to the lipophilic and hydrophilic properties (Clasen *et al*., 2018) and the ease of entry into exposed fish through the gills, skin, during feeding, and their transport to tissues through blood circulation thereby, resulting in the toxic effects. Deltamethrin, cypermethrin, and lambda-cyhalothrin have been shown to cause histopathological abnormalities in tissues such as, fish gills, liver and muscles (Ogueji *et al*., 2019; Yang *et al*., 2020) which were comparable to the effects of Chlorpyrifos in *Oreochromis niloticus* (Hossain *et al*., 2022), and as observed in the alterations in the excised gills, muscles, intestines and brain in this study. The observations in the intestines of the exposed fish in this study were in line with the study of Wu *et al.* (2022), who studied Deltamethrin disruption of the intestinal health of Crucian Carp. The intestines are important in the digestion, uptake and absorption of nutrients after ingestion (Sæle *et al*., 2018). They are the first barrier in

the body's defense system, important in maintaining the body's normal nutrition, metabolism, and immune defense (Lall, 2020), functioning as a barrier against foreign antigens and pathogens from entering the mucosal tissues. The intestinal mucosa in fish is an effective barrier to maintain intestinal health due to its integrity of morphology, structure, and function (Lin *et al.,* 2020). It is also associated with mediating and stimulating optimal gastrointestinal growth (Hassenrück *et al*., 2020). The microvilli, mucosal fold height, villus width, and muscularis thickness of the intestines are important indicators for evaluating intestinal function (Hassenrück *et al*., 2020; Cheng *et al*., 2022). The villi increases the internal surface area of the intestine, facilitating the efficient absorption of nutrients. The integrity of the goblet cells and their secretions-mucins play vital role as intestinal barrier in maintaining intestinal homeostasis. Stress leads to a significant decrease in the number of goblet cells and an increase in the number of vacuoles in the intestinal tissue. With the prolonged stress time, the intestinal villi in fish become arranged disorderly and eroded (Cheng *et al*., 2022). As observed in the histological examination of the intestines in this study, the villi and goblet cells were altered, attributed to the effects of the toxicant. The villi were eroded which may reduce the internal surface area of the intestine, thereby, affecting the efficient absorption of nutrients. The damaged goblet cells in the intestines of the affected fish can result in goblet cell dysfunction which may impair mucin secretion, compromising the intestinal mucosal barrier, thereby, increasing susceptibility to opportunistic bacterial colonization and inflammatory responses in fish (Johansson and Hansson, 2014; Birchenough *et al*., 2015; Yang and Yu, 2021). These can pose threat to fish health and well-being.

The observed histological changes in the excised gills, intestines, muscles and brain of the exposed fish as observed in the sectioned tissues can impair the behaviour, respiratory function, nutrition and development, swimming performance and balance, and wellness of the fish. These can have significant implications on the survival, health, well-being and even reproductive successes of fish and other non-target organisms when exposed to such insecticides in aquatic ecosystems. Therefore, further studies on the responses of aquatic organisms to insecticide mixtures are suggested to understand their toxicological implications on non-target organisms and aquatic environment.

CONCLUSION

Following the environmentally relevant concentrations of the pyrethroid mixture-'**B**' in this study, the findings have clearly shown histopathological alterations that are detrimental to the health and well-being of *C. gariepinus.* The insecticide mixture is highly toxic to fish and can be toxic to other aquatic organisms by extension, as evident in the fish mortalities. The presence of the toxicant had negative impacts on the evaluated physicochemical parameters of the test media (water), and can affect aquatic productivity by implication. Having understanding of the toxicological effects of such insecticides on non-target organisms is important, so as to develop effective environmental management strategies to mitigate their ecological risks.

ACKNOWLEDGEMENT

This research had no external funding hence, no conflict of interest.

CONTRIBUTION OF AUTHORS

LKE conceptualized and designed the experiment, and prepared the manuscript. WVE was involved in samples and data collection, NLE carried out samples and data collection and OSN was also involved in sample and data collection.

REFERENCES

Agnieszka C., I. Hoły ´nska-Iwan, I. Dziembowska, J. Bogusiewicz, M. Wróblewski, A. Cwynar and D. Olszewska-Słonina (2018). Current Research on the Safety of Pyrethroids used as Insecticide. *Medicina*, 54: 61. https://doi:10.3390/medicina54040061

Ayaz, A. and J. Kumar (2023). Pyrethroid pesticides: An overview on classification, toxicological assessment and monitoring. *Journal of Hazardous Materials Advances*, 10: 100284. https://doi.org/10.1016/j.hazadv.2023.100284

Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A. and Dar, S.A. (2020). Concerns and Threats of Contamination on Aquatic Ecosystems. *In:* Hakeem, K., Bhat, R. and Qadri, H. (Eds.). Bioremediation and Biotechnology. Springer, Cham. https://doi.org/10.1007/978-3-030-35691-0_1

Birchenough, G.M., Johansson, M.E., Gustafsson, J.K., Bergstrom, J.H. and Hansson, G.C. (2015). New developments in goblet cell mucus secretion and function. *Mucosal Immunol*ogy, 8(4):712–719. https://doi:10.1038/ mi.2015.32 5.

Boyd, C. E. (2015). Water Quality: An Introduction. 2nd Edition. Springer International Publishing. New York, 374p. https://doi 10.1007/978-3-319-17446-4

Cheng, J-H., Li, Y., Shen, M-H., Yang, Z-R., Zhang, W-J., Zhang, K., Wang, T., Zhang, G-S. and Yin, S-W. (2022). Effects of hypoxia stress and recovery on intestinal tissue of hybrid Yellow Catfish, "Huangyou-1" (*Pelteobagrus vachelli* ♂ × *Pelteobagrus fulvidraco* ♀) [J]. *Acta Hydrobiologica Sinica*, 46(11):1598–1608. https://doi: 10.7541/2023.2021.0342

Clasen, B., Loro, V.L., Murussi, C.R., Tiecher, T.L., Moraes, B. and Zanella, R. (2018). Bioaccumulation and oxidative stress caused by pesticides in *Cyprinus carpio* reared in a rice-fish system. *Science of The Total Environ*ment, 626: 737–743. https://doi.org/10.1016/j.scitotenv.2018.01.154

Davies, T.G.E., Field, L.M., Usherwood, P.N.R. and Williamson, M.S. (2007). DDT, Pyrethrins, Pyrethroid pesticides and insect sodium channels. *IUBMB Life,* 59 (3):151–162. https://doi:

10.1080/15216540701352042

Evans, W.C. and Evans, D. (2009). Pesticides of natural origin. *Trease and Evans' Pharmacogn*osy, 533–536. https://doi:10.1016/b978-0-7020-2933.

Farag, M.R., Alagawany, M., Bilal, R.M., Gewida, A.G., Dhama, K., Abdel-Latif, H.M. and Naiel, M.A. (2021). An overview on the potential hazards of pyrethroid insecticides in fish with special emphasis on Cypermethrin toxicity. *Animals,* 11 (7): 1880. https://doi: 10.3390/ani11071880

Galadima, M., Singh, S., Pawar, A., Khasnabis, S., Dhanjal, D.S., Anil, A.G. and Singh, J. (2021). Toxicity, microbial degradation and analytical detection of Pyrethroid pesticides: A Review. *Environmental Adv*ances, 5: 100105. https:// https://doi.org/10.1016/j.envadv.2021.100105

Gupta, R.C. and Crissman, J.W. (2013). Chapter 42-Agricultural chemicals. *In*: Haschek and Rousseaux's Handbook of Toxicologic Pathology. 3rd Edition. Volume II. Academic Press. Pp. 1349–1372. https://doi.org/10.1016/B978-0-12-415759-0.00042-X

Hassenrück, C., Reinwald, H., Kunzmann, A., Tiedemann, I. and Gärdes, A. (2020). Effects of thermal stress on the gut microbiome of juvenile Milkfish (*Chanos chanos*). *Microorganisms,* 9 (1): 5. https://doi: 10.3390/microorganisms9010005

Hossain, M. A., L. Sutradhar, T. R. Sarker, S. Saha and M. M. Iqba (2022). Toxic effects of Chlorpyrifos on the growth, hematology, and different organs histopathology of Nile tilapia, *Oreochromis niloticus. Saudi Journal of Biological Sciences,* 29: 103316. *https://*doi: 10.1016/j.sjbs.2022.103316

Lall, S. J. P. (2020). Intestinal alkaline phosphatase in the gastrointestinal tract offish: Biology, ontogeny, and environmental and nutritional modulation. *Rev. Aquacult.,* 12 (2): 555–581. https://doi: 10.1111/raq.12340

Li, R-X., Amenyogbe, E., Lu, Y., Jin, J-T., Xie, R-T. and Huang. J-S. (2023). Effects of low temperature stress on intestinal structure, enzyme activities and metabolomic analysis of juvenile Golden Pompano (*Trachinotus ovatus*). *Frontiers in Marine Science*, 10:1114120. https://doi: 10.3389/fmars.2023.1114120

Li, Z. H., Velisek, J., Grabic, R. and Machova, J. (2017). Effects of Pyrethroid and Oxadiazine Pesticides on Common Carp (*Cyprinus carpio* L.), Hematology and Plasma Biochemistry. *Environmental Toxicology and Pharmacology*, 52:159-165 [Cross Ref].

Lin, S.-M., Zhou, X.-M., Zhou, Y.-L., Kuang, W.-M., Chen, Y.-J., Luo, L., et al. (2020). Intestinal morphology, immunity and microbiota response to dietary fibers in Largemouth bass, *Micropterus salmoide*. *Fish Shellfish Immunol*., 103: 135–142. https://doi: 10.1016/j.fsi.2020.04.070

Lissenden, N., Armistead, J.S., Gleave, K., Irish, S.R., Martin, J.L., Messenger, L.A., Lees, R.S. (2022). Developing consensus standard operating procedures (SOPs) to evaluate new types of insecticide-treated nets. *Insects*, 13 (1): 7. https://doi.org/10.3390/insects13010007

Mohammad, A. H., Lipi, S., Tumpa, R. S., Shuva, S. and Mohammed M. I. (2022). Toxic effects of Chlorpyrifos on the growth, hematology, and different organs histopathology of Nile tilapia, *Oreochromis niloticus Saudi Journal of Biological Sciences*, 29: 103316. https://doi.org/10.1016/j.sjbs.2022.103316

Ogueji, E. O., Jumaine, E. O. and Auta, J. (2019). Histopathological changes in the gill and muscle of *Clarias gariepinus* (Burchell, 1822) exposed to sub-lethal concentrations of Cypermethrin. *Bulletin of the National Research Centre*, 43(1): 1-8 [Cross Ref].

Paul, E. and Simonin, H. (2006). Toxicity of Three Mosquito Insecticides to Crayfish. *Bulletin of Environmental Contaminaation and Toxicology*, 76: 614–621. https://doi.org/10.1007/s00128-006-0964-4

Prudencio, A., L. Olowo, B. Degila, G. Houedjissi, I. I. Toko, S. N. M. Mandiki and P. Kestemont (2023). Comparative assessment of acute toxicity and histological changes in liver of African Catfish, *Clarias gariepinus* exposed to Cotton insecticides. Journal of Environmental Science and Health, Part B, 58 (1): 31-44. https://doi: 10.1080/03601234.2023.216844

Reish, D.J. and P.S. Oshida (1987): Manual of Methods in Aquatic Environment Research. Part 10. Short-term Static Bioassay. Food and Agriculture Organization Fisheries Technical Paper (247):62.

Sæle, Ø., Rød, K. E. L., Vanessa, H. Q., Shengrong, L. and Farber, S. (2018). A novel system to quantify intestinal lipid digestion and transport. *Biochim. Biophys. Acta (BBA)-Molecular Cell Biol. Lipids,* 1863 (9): 948–957. https://doi: 10.1016/j.bbalip.2018.05.006

Shefali, G., R.Kumar, M. S. Sankhla, R. Kumar and Swaroop S. S. (2021). Impact of Pesticide Toxicity in Aquatic Environment. Biointerface Research in Applied Chemistry, 11 (3): 10131–10140 https://doi.org/10.33263/BRIAC113.1013110140

USEPA (2011). USEPA Regional Screening Level (RSL) Summary Table. http://www.epa.gov/regshwmd/risk/human/index ht m.

Valmorbida, I., Hohenstein, J.D., Coates, B.S., Bevilaqua, J.G., Menger, J., Hodgson, E.W. and O'Neal, M.E. (2022). Association of voltage-gated sodium channel mutations with field- evolved pyrethroid resistant phenotypes in Soybean Aphid and genetic markers for their detection. *Science Reports*, 12 (1): 1–14. https://doi: 10.1038/s41598-022-16366-1

Van den Berg, H., da Silva Bezerra, H.S., Al-Eryani, S., Chanda, E., Nagpal, B.N., Knox, T. B. and Yadav, R.S. (2021). Recent trends in global insecticide use for disease vector control and potential implications for resistance management. *Science Reports*, 11 (1): 1–12.

https://www.nature.com/articles/s41598-021-03367-9

WHO (2020). Prequalified vector control products. WHO—Prequalification of medical products (IVDs, medicines, vaccines and immunization devices, vector control). https://extranet.who.int/pqweb/vector-control-products/prequalified product-list.

World Health Organization (2008). Guideline for drinking water quality. Health Criteria and other supporting information. 3rd Edition, Geneva.

Wu, H., J. Gao, M. Xie, J. Wu , R. Song, X. Yuan, Y. Wu and Dongsheng Ou (2022). Chronic exposure to Deltamethrin disrupts intestinal health and intestinal microbiota in juvenile Crucian carp. *Ecotoxicology and Environmental Safety*, 241: 113732. https://doi.org/10.1016/j.ecoenv.2022.113732

Yang, C., Lim, W. and Song, G. (2020). Mediation of oxidative stress toxicity induced by Pyrethroid pesticides in fish. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*., 234: 108758. https://doi: 10.1016/j.cbpc.2020.108758

Yang, S. and Min Yu (2021). Role of Goblet Cells in Intestinal Barrier and Mucosal Immunity. *Journal Inflammation Research*, 14: 3171–3183. https://doi: 10.2147/JIR.S318327

Yang, Y., Ye, X., He, B. and Liu, J. (2016). Cadmium potentiates toxicity of Cypermethrin in Zebrafish. *Environmental Toxicology and Chem.,* 35 (2): 435–445. https://doi: 10.1002/etc.3200

Yidi K., M. Li, X. Shan, G. Wang and G. Han (2021). Effects of Deltamethrin Sub-acute exposure in Snakehead fish, *Channa argus*: Biochemicals, antioxidants and immune responses. *Ecotoxicology and Environmental Safety*, 209: 111821. https://doi.org/10.1016/j.ecoenv.2020.11182