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Genotoxicity evaluation of Chlorfenapyr in exposed freshwater African catfish *Clarias gariepinus* using micronucleus test

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ABSTRACT

Chlorfenapyr is a new formulated insecticide targeted against insecticide resistant species. Its application in targeting harmful aquatic insects may potentially contaminate aquatic environment. This study is to evaluate its genotoxic effects on non-targeted aquatic biota. The study assessed the genotoxic potentials of chlorfenapyr in African catfish (Clarias gariepinus) using the micronucleus (MN) test. Juvenile stage of C. gariepinus were exposed to different concentrations; 5.00 – 15.00 mgL^{-1} of chlorfenapyr for 96 hours, peripheral blood was collected through the caudal vein and examined for micronucleus induction in the erythrocytes. The tested concentrations of chlorfenapyr did not elicit significant [$F(5, 18) = 0.167$, $p < 0.971$] increase in MN formation and other nuclear abnormalities in the peripheral erythrocytes of C. gariepinus. The outcome of this study gives credence to the view that chlorfenapyr is not clastogenic and or aneugenic to non-targeted aquatic vertebrate with reference to fish (C. gariepinus).

Introduction

Over the years, the effects and control of environmental pollution has been a global issue of concern. Advancement in science and technology is beneficial to the society and also comes with some adverse effects which include contamination of the environment by chemicals and radiations, hence making it a double edge sword. For instance, Ahmed (2007) reported that both animals and humans are victims of the adverse effects of pollution. These adverse effects include several diseases (asthma, cancer), threat to habitat loss, death and extinction of endangered or rare species. Heavy metals, pharmaceuticals and pesticides are common pollutants that contaminates aquatic environments and hence affecting aquatic life-forms. These contaminants enter the aquatic environment through industrial effluent discharge, agricultural runoff and direct discharge of chemicals into aquatic environment by humans (Muralikrishna and Manickam, 2017).

Insecticides are common pesticides widely used both as household and industrial chemicals for controlling insect pests and or insect vectors. During applications, the effectiveness of various insecticides in killing pests and their vectors had long been used to evaluate the potency of these chemicals. However, their consequences on non-targeted species are rarely considered (Fumio, 1975). The potencies of many insecticides including organochlorides, organophosphates, carbamates and pyrethroids have been greatly resisted by many species of insects (Ansari *et al.*, 2011). This warrants that new insecticides with better efficiencies against the resistant species be synthesized. Chlorfenapyr, a member of the class of insecticides known as pyrroles, is a new insecticide synthesized against insects and insect vectors (Black *et al.*, 1994; Raghavendra *et al.*, 2011). It was considered a suitable

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alternative to synthetic pyrethroids due to a lower toxicity to mammalian and aquatic life (Ingham *et al.*, 2012). Generally, pyrroles including Chlorfenapyr are pro-insecticides which require activation in *in vivo* by mixed function oxidative elimination of its N-ethoxymethyl group to produce the active compound (Black *et al.*, 1994). Unlike other classes of insecticide that act by disrupting insect nervous system, pyrroles act at the cellular level and disrupt respiratory pathways and proton gradients via uncoupling the mitochondrial oxidative phosphorylations, interrupting the conversion of ADP to ATP, which might generate reactive oxygen radicals (Black *et al.*, 1994).

The deliberate and or accidental discharge of insecticides into aquatic environment causes molecules of the insecticides to bind onto suspended particles and sediments, and accumulate in the aquatic environment. This increases exposure of non-targeted species to the chemicals threatening their survival via interacting with physiological processes and nuclear material that may lead to pathophysiological problem and carcinogenesis (Corredor-Santamaría *et al.*, 2016). Piscine micronucleus test, a sensitive and suitable cytogenetic bioassay, has long been utilised to monitor chemical contaminants in aquatic environment. Micronuclei are extra-chromosomal bodies that contain acentric chromatid fragments or chromosomes that were not incorporated ino either of the daughter nuclei after cell division (Ali *et al.*, 2014). Micronucleus (MN) assay is the most widely used technique for the detection of mutagenic effects of individual and mixture of chemical compounds in erythrocytes of *Clarias gariepinus* exposed both in *in vivo* and *in situ* (Alimba *et al.*, 2015, 2017, 2019; Fagbenro *et al.*, 2019).

Contradicting the report by Al-Sarar *et al.* (2015), chlorfenapyr (and its metabolite, tralopyril) are non-genotoxic (WHO, 2017; Horibe *et al.*, 2018). The genotoxicity of chlorfenapyr viewpoints remain unresolved since studies on its genotoxicity in different model organisms are very limited. Hence, this study was conducted to assess the genotoxic potential of chlorfenapyr in freshwater African catfish, *C. gariepinus* using the MN test.

Materials and Methods

Fish collection and acclimatization

Juvenile stages of the freshwater African catfish *C. gariepinus* (Burchell, 1822) (Family: Clariidae, Order: Siluriformes) were procured from Tilapia Resorts at Abak Local Government Area of Akwa Ibom State. They were transported in aerated containers, in the morning, from the Resort to the Department of Animal and Environmental Biology Laboratory, University of Uyo. The fish were acclimatized for 48 h and fed twice daily with commercial feed (Coppens commercial feed, Coppens International Helmond, Netherlands) containing 35% crude protein. To maintain hygienic condition and prevent pollution during the period of acclimatization, the water in the tanks was renewed every other day with well aerated and dechlorinated tap water.

Experimental design and preparation of Chlorfenapyr stock solution

Chlorfenapyr powder, obtained from the University of Uyo Insectary Laboratory, was used to prepare the stock solutions. Chlorfenapyr of 500mg was weighed using an electronic weighing balance and dissolved in 10 L of distilled water to give a stock solution of 50 mgL^{-1}. Dilution of the stock solution using dechlorinated tap water into five different concentrations; 5, 7, 9, 11 and 15 mgL^{-1} were used for the sublethal exposure. Values used in this present study were obtained from the work of Rand (2004) on chlorfenapyr and catfish; and these values were used for definitive test and the acute genotoxicity observations. Bioassay and LC_{50} value of the toxicant were determined following previously established methods (Yekeen and Fawole, 2011; Mahdi, 2018). Ten (10) fish per group, randomly selected from the acclimatized stock, were used for the exposure which lasted for 96 h. Ten fish per group were exposed to dechlorinated tap water and cyclophosphamide (4 mgL^{-1}) solution as negative (-ve) and positive (+ve) controls, respectively. Prior to exposure by immersion, the length and weight of the exposed and control fish, polarity of hydrogen ion (pH) and dissolved oxygen (DO) level of the water were measured in all the treatment and control (except in cyclophosphamide group). The physico-chemical parameters of the water were measured intermittently throughout the period of exposure.

Analysis of the micronucleus (MN) assay

Four fish per group were randomly selected for the MN analysis. Peripheral blood was collected with heparinized syringe at the caudal vein region. The blood was used to prepare a thin and uniform smear on ethanol pre-cleaned microscopic slides and left to air-dry at room temperature for about 3 hours. The slides were then fixed in absolute methanol for 10 minutes and left to air-dry at room temperature for 1 hour. Finally, the slides were stained with 6% Giemsa in phosphate buffer for 30 minutes. Excess stain was thoroughly rinsed using double distilled water, air-

dried, and mounted with DPX-mounting. Slides were prepared in replicate per fish. The slides were observed under Olympus light microscope and a total of 1200 erythrocytes per fish were scored for MN and other nuclear abnormalities such as blebbed nucleus (BL), lobed nucleus (LB), notched nucleus (NT) and binuclear (BN) cell (Carrasco *et al.*, 1990) were examined.

Statistical analysis

The data obtained were analyzed using the statistical package SPSS 21.0 computer program (SPSS Inc., Chicago, IL). Difference in the biomarkers between treated and control were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. The $p<0.05$ was considered statistically significant. Results were expressed as means ± standard deviation.

Results

Physiochemical properties of test water

The mean value of DO for all the groups was 0.667 mgL^{-1}, with the negative control group recording the least DO of 0.4 mgL^{-1} and the 9 mgL^{-1} experimental group recording the highest DO of 1.0 mgL^{-1} (Table 1). There was no continuous trend of increase in DO from the lowest experimental group (5 mgL^{-1}) to the highest experimental group (15 mgL^{-1}). Increase or decrease in DO did not show a corresponding increase or decrease in pH i.e. DO of 0.4 mgL^{-1} had a corresponding pH of 8.16, DO of 0.6 mgL^{-1} had a corresponding pH of 7.73 (showing a reduction) but DO of 1.0 mgL^{-1} had a corresponding pH of 7.84 (showing further increase). The mean value for the pH was 7.905, with the experimental group of 15.0 mgL^{-1} recording the least pH of 7.73.

Physiochemical parameters of water in the container. DO reduces with depth i.e. the closer the meniscus is to the bottom of pool, the lower the DO. The lowest level of DO in the control group showed the rate of oxygen consumption by the experimental organisms due to their activity. pH was normal to slightly alkaline.

Induction of micronucleus (MN) and other nuclear abnormalities

The mean value of DO for all the groups was 0.667 mgL^{-1}, with the negative control group recording the least DO of 0.4 mgL^{-1} and the 9 mgL^{-1} experimental group recording the highest DO of 1.0 mgL^{-1} (Table 1). There was no continuous trend of increase in DO from the lowest experimental group (5 mgL^{-1}) to the highest experimental group (15 mgL^{-1}). Increase or decrease in DO did not show a corresponding increase or decrease in pH i.e. DO of 0.4 mgL^{-1} had a corresponding pH of 8.16, DO of 0.6 mgL^{-1} had a corresponding pH of 7.73 (showing a reduction) but DO of 1.0 mgL^{-1} had a corresponding pH of 7.84 (showing further increase). The mean value for the pH was 7.905, with the experimental group of 15.0 mgL^{-1} recording the least pH of 7.73.

Table 1. The results of the proximate analysis of *Artemia* sp.

Parameters	Concentrations (mgL^{-1})						Mean ± SE
	Control	5	7	9	11	15	
DO (mgL^{-1})	0.4	0.5	0.8	1.0	0.7	0.6	0.667 ± 0.088
pH	8.16	8.03	7.9	7.84	7.77	7.73	7.905 ± 0.067

Table 2. Frequencies of Micronuclei in the erythrocytes of *C. gariepinus*

Treatments	Total No of Micronucleus	Micronucleus (Mean ± SD)	Binucleated	Tailed Nucleus	Bud Nucleus
5mg/L	10	2.50 ± 1.00	0.00	0.00	0.00
7mg/L	9	2.25 ± 0.95	0.00	0.00	0.00
9mg/L	8	2.00 ± 0.82	0.00	0.00	0.00
11mg/L	9	2.25 ± 0.50	0.00	0.00	0.00
15mg/L	9	2.25 ± 0.96	0.00	0.00	0.00
-ve control	8	2.00 ± 1.15	0.00	0.00	0.00
+ve control	25	6.25 ± 0.96	0.00	0.00	0.00

Values are expressed as mean ± standard deviation
Number (N) of *C. gariepinus* per treatment group analyzed after mortality = 4
Value of $p>0.05$ when comparing treatment groups to the negative (-ve) control at 0.05 level of significance

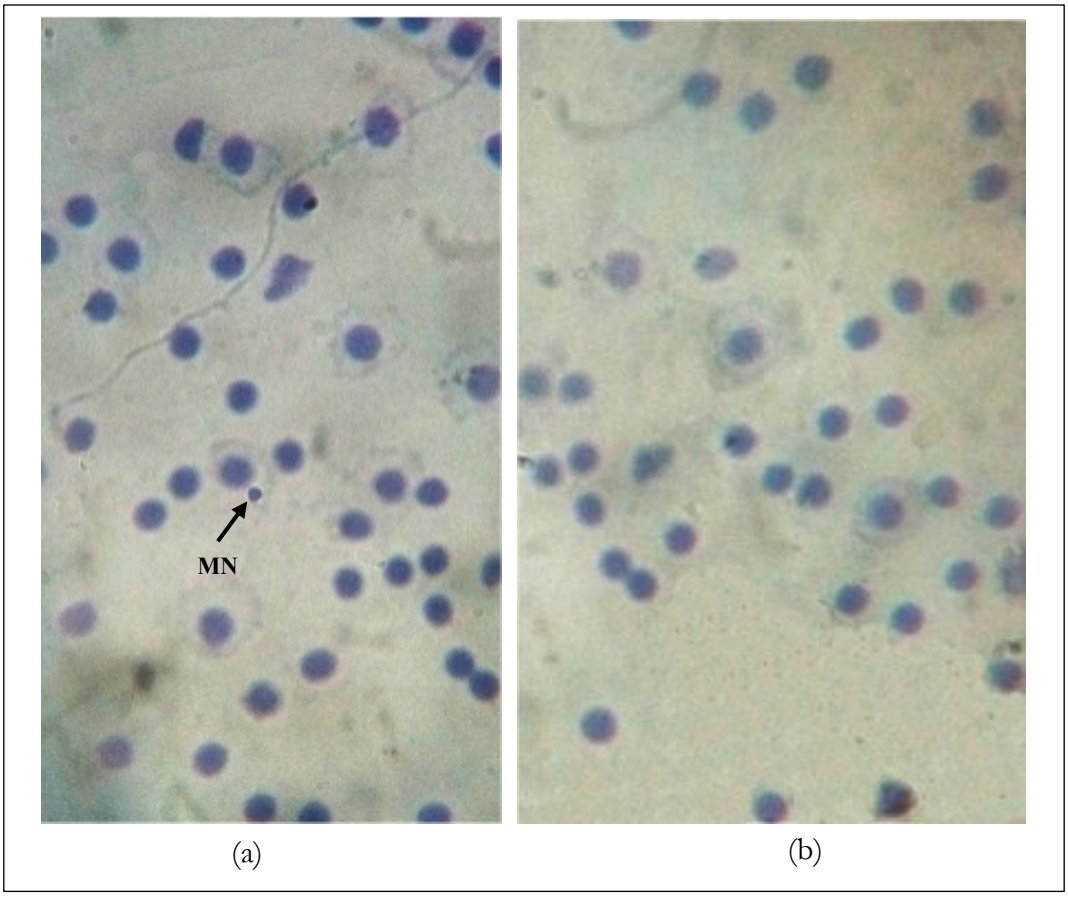

Figure 1. Peripheral blood film of catfish, *C. gariepinus*, showing (a) the manifestation of micronucleus (MN) and (b) normal erythrocyte.

Discussion

Genotoxic assays have been considered useful tools to evaluate the effects of pollutants in fish (Mazzeo and Marin-Morales, 2015) and other aquatic organisms (Ansari *et al.*, 2011). Micronucleus is formed by the elimination of amplified genetic materials from the cell (Fenech, 2000) and chromosomal breaks or losses that are not incorporated to the main nucleus during cell division cycle (Renu and Saxena, 2015). The present study indicated that exposure to sublethal concentrations of chlorfenapyr was not sufficient to induce severe nuclear abnormality in the haematological parameters of the catfish, *C. gariepinus*; hence, chlorfenapyr was non-genotoxic. This study supports the already-held view that chlorfenapyr (and its metabolite, tralopyril) are not genotoxic, owing to non-evidence of mutagenic, clastogenic or aneugenic activity in assays (WHO, 2017; Horibe *et al.*, 2018), but opposes that held that it caused significant inductions of chromosomal aberrations and MN (Al-Sarar *et al.*, 2015).

A study by Sonoda and Tsumuki (2007) showed the induction of stress protein genes in cultured cells of cabbage armyworm, *Mamestra brassicae,* treated with chlorfenapyr, providing evidence of defence of cells from the reactive oxygen radicals. Earlier studies conducted by Li and Chen (2004) on mice reported inducement of DNA damage of spleen, liver, and kidney cells on gavage administration of chlorfenapyr. Inducement of genotoxicity in mice administered intraperitoneally (IP) has also been reported by Gao *et al.* (2006). As observed, there are indications of chlorfenapyr being both genotoxic *in vitro* and *in vivo*. However, according to Nohmi (2018), some chemicals yield negative results in *in vitro* bacterial mutation assays, but positive results in the *in vivo* transgenic rodent gene mutation assay. We believe same can be said *vice versa*. This assertion provides reasonable viewpoints for the positive genotoxic effect of chlorfenapyr observed in one study using one model system and the non-genotoxic effect in another study using a different model system.

Genotoxic chemicals possess the chemical properties that allow them interact with DNA inducing DNA damage and mutation irrespective of exposure threshold or dose (Nohmi, 2018). Different

studies have shown concentration- and duration-dependent increase of MN in the peripheral blood cells of *C. gariepinus* and other organisms exposed to various pollutants (Mekkawy *et al.*, 2011; Ayoola *et al.*, 2012; Mahboob *et al.*, 2013; Nwani *et al.*, 2014, 2017; de Morais *et al.*, 2019). High production of reactive oxygen species (ROS) has been observed to be the major culprit that produces oxidative stress resulting in the formation of MN. In our study, the insignificant formation of MN indicated a negligible formation of ROS. ROS such as superoxide (O_2), hydrogen peroxide (H_2O_2), and hydroxyl (OH^-) radicals are free radicals that contain oxygen atoms that are highly reactive due to the presence of unpaired electrons (Sharma *et al.*, 2014). The inability of the body to temporally eliminate ROS by the antioxidant system would lead to oxidative stress (Dar *et al.*, 2015). Our study shows that chlorfenapyr was not sufficient to breach the antioxidant system that could have resulted in the formation of MN.

Irrespective of the non-formation of MN by chlorfenapyr, as observed in this study, there were observable behavioural changes on introducing the experimental organisms into the different constituted treatment groups. The *C. gariepinus* in the negative control group were active and responded rapidly to stimuli at all times, while those in the experimental groups gradually became docile, responded slowly to external stimuli and exhibited vertical movements towards the water surface to gulp in air. This vertical movement indicated a low level of dissolved oxygen (DO). The physiochemical parameter, DO, fell within the minimum standard required for the survival of aquatic organisms. According to Matthews and Berg (1997), DO levels at the bottom of pools can fall below 1.0 mgL^{-1}. In our study, DO was below 1.0 mgL^{-1}, since the depth (distance between the meniscus and bottom of container) was below 20 cm. Temperature (DO reduces with increase in temperature) and atmospheric pressure also had its contributory role. Since the control group had the lowest DO, this signified the consumption rate of the experimental organism due to their active nature. DO is a key determinant of oxidation–reduction (redox) state of the water in which compounds are dissolved; if absent, then redox is low and electrons are plentiful (because the DO does not react with them) (Hauer and Lamberti, 2017). DO concentrations are constantly affected by the forward and backward reaction of photosynthesis and respiration, diffusion and decomposition. Its levels fluctuate with salinity, temperature and pressure changes (Jantzen, 1978), ranging from less than 1 mgL^{-1} to more than 20 mgL^{-1} depending on how these factors interact (Hong *et al.*, 2009). In freshwater systems such as lakes, rivers and streams, DO concentrations will vary by season, location and water depth.

According to Hauer and Lamberti (2017), oxygen levels that remain below 1 to 2 mgL^{-1} for a few hours can result in large fish kills – on the contrary, *C. gariepinus* is one of the exceptions to this rule. This catfish is uniquely endowed with fascinating ability to endure extremely harsh conditions, exhibiting tolerance to very low oxygen concentrations and can even survive for considerable periods out of water (*Van der* Waal, 1998). Catfish can tolerate hypoxia for a long period without any changes in biochemical parameters, indicating the use of an anaerobic pathway by the fish (Braun *et al.*, 2006). Generally, catfishes do this using a specialized suprabranchial organ (Hecht *et al.*, 1988), which is a large, paired chamber with branches above the gill arches specifically adapted for air breathing (Maina and Maloiy, 2011) enabling them to move over land even when not forced to do so by drought. Although, exposure to 2.5 mgL^{-1} DO levels has been shown to promote loss of ions and lower ammonia excretion in silver catfish juveniles, but these losses were rapidly stabilized for Na^+ and Cl^- (Rosso *et al.*, 2006). Catfishes are also able to endure a wide range of pH balances (Hecht *et al.* 1988; *Van der* Waal, 1998). However, fishes raised at pH of 7 gained significant weight higher than that of 6 and 8 (Ivoke *et al.*, 2007). In this study, *C. gariepinus* endured and survived within the different groups with the pH range of 7.73 – 8.16. This observation supports the finding that catfishes can tolerate a wide range of pH balances.

Conclusions

The present study showed that chlorfenapyr did not induce significant MN in the blood erythrocytes of *C. gariepinus*, hence it is non-genotoxic. Despite varying views on the genotoxicity of chlorfenapyr in different model systems, it has been claimed that some pollutants tend to exhibit positive genotoxic potentials *in vivo* and negative genotoxic potentials *in vitro* and *vice versa*. Further studies on the toxicokinetics and dynamics of chlorfenapyr are necessary for a greater perception on the mechanisms of action that results in induction of MN formation. Meanwhile, since our study was conducted on somatic cells, there is a need to investigate the genotoxic effect of chlorfenapyr on germline (sex) cells.

Declarations of interest

The authors declare that there are no conflicts of interest.

References

Ahmed, D. E. 2007. Environmental pollution and its impact on human and animal health: a review. *The Sudan Journal of Veterinary Research,* 22: 37 – 46.

Ali, D., P. G. Kumar, S. Kumar, M. Ahmed. 2014. Evaluation of genotoxic and oxidative stress response to dimethoate in freshwater fish *Channa punctatus* (Bloch). Chemical Speciation and Bioavailability, 26(2): 111–118.

Alimba, C. G., K. O. Adekoya, O. O. Soyinka. 2019. Exposure to effluent from pharmaceutical industry induced cytogenotoxicity, hematological and histopathological alterations in *Clarias gariepinus* (Burchell, 1822). Experimental and Clinical Sciences (EXCLI) Journal, 18: 63–78.

Alimba, C. G., R. D. Ajiboye, O. S. Fagbenro. 2017. Dietary ascorbic acid reduced micronucleus and nuclear abnormalities in *Clarias gariepinus* (Burchell 1822) exposed to hospital effluent. Fish Physiology and Biochemistry, 43(5): 1325–1335.

Alimba, C. G., J. K. Saliu, O. A. Ubani-Rex. 2015. Cytogenotoxicity and histopathological assessment of Lekki lagoon and Ogun River in *Synodontis clarias* (Linnaeus, 1758). Toxicological and Environmental Chemistry, 97:221–234.

Al-Sarar, A. S., Y. Abobakr, A. E Bayoumi, H. I. Hussein. 2015. Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid on CHO_{K1} cells. Environmental Science and Pollution Research, *22*(21): 17041–17052.

Ansari, R. A., A. Rahman, M. Kaur, S. Anjum, S. Raisuddin. 2011. *In vivo* cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish, *Channa punctata* (Bloch). Ecotoxicology and Environmental Safety, *74*(1): 150–156.

Ayoola, S. O., B. O. Bassey, C. G. Alimba, E. K. Ajani. 2012. Textile effluent induced genotoxic effects and oxidative stress in *Clarias gariepinus*. Pakistan Journal of Biological Sciences, *15*(17): 804–812.

Black, B. C., R. M. Hollingworth, K. I. Ahmmadsahib, C. D. Kukel, S. Donovan. 1994. Insecticidal action and mitochondrial uncoupling activity of AC-303,630 and related halogenated pyrroles. Pesticide Biochemistry and Physiology, 50(2): 115–128.

Braun, N., R. L. De Lima, B. Moraes, V. L. Loro, B. Baldisserotto. 2006. Survival, growth and biochemical parameters of silver catfish, *Rhamdia quelen* (Quoy and Gaimard, 1824), juveniles exposed to different dissolved oxygen levels. Aquaculture Research, 37(15): 1524-1531.

Carrasco, K. R., K. L. Tilbury, M. S. Myers. 1990. Assessment of the piscine micronucleus test as air *in situ* biological indicator of chemical contaminant effects. Canadian Journal of Fisheries and Aquatic Sciences, *47*: 2123–2136.

Corredor-Santamaría, W., M. Serrano Gómez, Y. M. Velasco-Santamaría. 2016. Using genotoxic and haematological biomarkers as an evidence of environmental contamination in the Ocoa River native fish, Villavicencio-Meta, Colombia. SpringerPlus, *5*: 351. doi:10.1186/s40064-016-1753-0

Dar, S.A., A. R. Yousuf, M. Balkhi, F. A. Ganai, F. A. Bhat. 2015. Assessment of endosulfan induced genotoxicity and mutagenicity manifested by oxidative stress pathways in freshwater cyprinid fish crucian carp (*Carassius carassius* L.). Chemosphere, *120*: 273–283.

de Morais, C. R., B. B. Pereira, P. C. A. Sousa, V. S. V. Santos, C. F. Campos, S. M. Carvalho, M. A. Spanó, A. A. A. de Rezende, A. M. Bonetti. 2019. Evaluation of the genotoxicity of neurotoxic insecticides using the micronucleus test in *Tradescantia pallida*. Chemosphere, *227*: 371–380.

Fagbenro, O. S., C. G. Alimba, A. A. Bakare. 2019. Experimental modeling of the acute toxicity and cytogenotoxic fate of composite mixtures of chromate, copper and arsenate oxides associated with CCA preservative using *Clarias gariepinus* (Burchell 1822). Environmental Analysis Health and Toxicology, 34(3): e2019010.

Fenech, M. 2000. The *in vitro* micronucleus technique. Mutation Research, *455*(1-2): 81–95.

Fumio, M. 1975. *Toxicology of Insecticides*. Springer Science and Business Media, UK.

Gao, S., Y. Lu, P. Zhou. 2006. Study on genotoxicity of three chlorfenapyr formulation. Journal of Environmental and Occupational Medicine, 23: 11–14.

Hauer, R., G. Lamberti. 2017. *Methods in Stream Ecology* (3rd ed.). Academic Press

Hecht, T., W. Uys, P. J. Britz. (Editors). 1988. Culture of sharptooth catfish, *Clarias gariepinus*, in southern Africa. National Scientific Programmes Unit: CSIR, *SANSP Report 153*:146.

Hong, C. X., J. D. Lea-Cox, D. S. Ross, G. W. Moorman, P. A. Richardson, S. R. Ghimire, P. Kong. 2009. Containment basin water quality fluctuation and implications for crop health management. Irrigation Science, 27(6): 485–496.

Horibe, A., S. Odashima, N. Hamasuna, T. Morita, M. Hayashi. 2018. Weight of contribution of *in vitro* chromosomal aberration assay for evaluation of pesticides: Experience of risk assessment at the Food Safety Commission of Japan. Regulatory Toxicology and Pharmacology, 95: 133–141.

Ingham, L., U. Audoer, T. Victor. 2012. The Chlorfenapyr. Biology of Insecticides, *20: 131-136*.

Ivoke, N., B. O. Mgbenka, O. Okeke. 2007. Effect of pH on the growth performance of *Heterobranchus bidorsalis* (♂) x *Clarias gariepinus* (♀) hybrid juveniles. Animal Research International, 4(1): 639-642.

Jantzen P. G. 1978. Investigating factors that affect dissolved oxygen concentration in water. The American Biology Teacher, 40(6): 346-352. doi: 10.2307/4446266

Li, X., X. Chen. 2004. Effects of chlorfenapyr on the DNA damages of three kinds of cells in mice. Journal of Xianning College (Medical Sciences), 18: 164–167.

Mahboob, S., H. F. A. Al-Balwai, F. Al-Misned, Z. Ahmad. 2013. Investigation on the genotoxicity of mercuric chloride to freshwater *Clarias gariepinus*. Pakistan Veterinary Journal, 34: 100–103.

Mahdi, B. 2012. Adverse effect of insecticides on various aspects of fish's biology and physiology, Insecticides - Basic and Other Applications, Dr. Sonia Soloneski (Ed.), ISBN: 978-953-51-0007-2, InTech, Available from:.

Maina, J., G. Maloiy. 1986. The morphology of the respiratory organs of the African air-breathing catfish (*Clarias mossambicus*): A light, electron and scanning microscopic study, with morphometric observations. Journal of Zoology, 209: 421–445. 10.1111/j.1469-7998.1986.tb03602.x.

Matthews, K. R., N. H. Berg. 1997. Rainbow trout responses to water temperature and dissolved oxygen stress in two southern California stream pools. Journal of Fish Biology, *50*: 50–67.

Mazzeo, D. E. C., M. A. Marin-Morales. 2015. Genotoxicity evaluation of environmental pollutants using analysis of nuclear alterations. Environmental Science and Pollution Research, 22(13): 9796–9806.

Mekkawy, I. A., U. M. Mahmoud, A. E. H. Sayed. 2011. Effects of 4-nonylphenol on blood cells of the African catfish *Clarias gariepinus* (Burchell, 1822). Tissue Cell, 43: 223–229.

Muralikrishna, I. V., V. Manickam. 2017. *Environmental Management: Science and Engineering for Industry*. Butterworth-Heinemann, Elsevier Inc.

Nohmi, T. 2018. Thresholds of genotoxic and non-genotoxic carcinogens. Toxicology Research, 34(4): 281–290. doi:10.5487/TR.2018.34.4.281

Nwani, C. D., P. O. Somdare, E. O. Ogueji, J. C. Nwani, J. A. Ukonze, A. O. Nwadinigwe. 2017. Genotoxicity assessment and oxidative stress responses in freshwater African catfish *Clarias gariepinus* exposed to fenthion formulations. Drug and Chemical Toxicology, 40(3): 273–280.

Nwani, C. D., M. C. Nnaji, S. N. Oluah, P. C. Echi, H. O. Nwamba, O. E. Ikwuagwu, M. N. Ajima. 2014. Mutagenic and physiological responses in the juveniles of African catfish, *Clarias gariepinus* (Burchell 1822) following short-term exposure to praziquantel. Tissue and Cell, 46(4): 264–273.

Raghavendra, K., T. K. Barik, P. Sharma, R. M. Bhatt, H. C. Srivastava, U. Sreehari, A. Dash. 2011. Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. Malaria Journal, 10: 16.

Rand, G.M. 2004. Fate and effects of the insecticide–miticide chlorfenapyr in outdoor aquatic microcosms. Ecotoxicology and Environmental Safety, 58: 50–60.

Renu, C., K. Saxena. 2015. Genotoxic evaluation of fenvalerate in *Channa punctatus* by micronucleus test. Indian Journal of Scientific Research and Technology, *3*: 30–33.

Rosso, F., K. C. S. Bolner, B. Baldisserotto. 2006. Ion fluxes in silver catfish (*Rhamdia quelen*) juveniles exposed to different dissolved oxygen levels. Neotropical Ichthyology, 4(4): 435-440.

Sharma, S., A. Sharma, N. D. Tasuja, S. C. Joshi. 2014. Organophosphorous compounds and oxidative stress: a review. Toxicological and Environmental Chemistry, 96: 681–698.

Sonoda, S., H. Tsumuki. 2007. Induction of heat shock protein genes by chlorfenapyr in cultured cells of the cabbage armyworm, *Mamestra brassicae*. Pesticide Biochemistry and Physiology, 89: 185–189.

Van der Waal, B. 1998. Survival strategies of sharptooth catfish *Clarias gariepinus* in desiccating pans in the northern Kruger National Park. Koedoe, 41(2): 131-138.

WHO. 2017. Specifications and Evaluations for Public Health Pesticides. Chlorfenapyr.*https://www.who.int/whopes/quality/Chlorfenapyr_specs_eval_WHO_December_2017.pdf?ua=1*

Yekeen, T. A., O. O. Fawole. 2011. Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus*. African Journal of Biotechnology, 10(64): 14090-14096.