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Genotoxic Potential of The Cypermethrin-Based Pesticide Best® On African Catfish *Clarias gariepinus*

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ABSTRACT

Due to industrialization and agricultural developments, various chemical substances have been released into the environment causing pollution.The accumulation and persistence of these pollutants in the aquaticenvironment constitute a serious threat to biological life and to humanbeings indirectly through the food chain.Two hundred and fifty specimens of *Clarias gariepinus* having a mean weight of 0.2 kg and length of 2.50 cm were exposed to synthetic pyrethroid pesticide, cypermethrin in concrete tanks.Acute toxicity testing was carried out to determine the 96 hours lethal concentration. Genotoxicity was evaluated using the micronucleus assay method.Behavioral abnormalities such as hyperactivity, faster opercula activity, and erratic swimming movements were observed following the exposure of the test fish to pesticide during the acute toxicity test.The median lethal concentration value (LC_{50}) of cypermethrin was determined to be 7.23µg/l.There was a significant decrease ($P < 0.05$) in the frequency of induction of micronuclei as the concentration of the pesticide and period of exposure increased. Formation of micronuclei in the erythrocyte of *Clariasgariepinus* in this study is an indication of genetic damage.

Keywords: Toxicity, Cypermethrin, Genetic damage, *Clarias gariepinus.*

INTRODUCTION

The use of pesticides in agriculture to control weeds and pests is highly toxic to non-target organisms such as fish. This toxicity normally results through the impairment of the fish metabolism which in most cases leads to the death of the fish [1]

Application of synthetic pesticides is one of the methods used to increase agriculture production. Pesticides in agricultural runoff affect all the aquatic organisms [1].Synthetic pyrethroids are considered to be safe over other insecticides, however, animal data indicate their use may pose a great risk to environmental biota [2].All manmade chemicals eventually find their way into the aquatic environment. These pollutants are domestic wastes, untreated or semi treated from industries and different chemicals such as pesticides used in agricultural sector [3]*;* [4];[5]; [6]; [7]. The accumulation and persistence of these pollutants in the aquaticenvironment constitute a serious threat to biological life and to humanbeings indirectly through the food chain [7].

Cypermethrin is a type II synthetic pyrethroid insecticide widely used in commercial agricultural applications as well as food storage. Its structure is based on pyrethrum, a natural insecticide which is contained in chrysanthemum flowers. The pyrethroids containing alpha- cyano group are grouped as type II with toxicity solely being attributed to their alpha-cyano constituent [8]. Pesticides containing highly toxic chemicals like cypermethrin through effluents from industries and agricultural runoffs lead to pollution of aquatic environments such as rivers, ponds, lakes, streams, e.t.c.

This work is aimed at evaluating the toxicity of the cypermethrin- based agricultural pesticide BEST® on the African catfish, *clarias gariepinus*.

MATERIALS AND METHODS

A total number of 250 post fingerlings of *C. gariepinus* of average length 10± 2.5cm and average weight were purchased from renowned fish farm. They were then transported live in a black plastic can (25 liters), to the fish farm in Federal University of Technology, Owerri to be acclimatized. Fishes were acclimatized in a concrete tank under normal farm conditions, for a period of 3 weeks. During acclimatization, water was changed every two days and the fishes were fed with commercial fish pellets twice daily, using Scretin (2mm) as starter feed and completing the acclimatization with Coppens (4mm).

The physic- chemical properties of the test water were analyzed using standard methods.Prior to the acute toxicity testing, a range finding test was carried out. Next, Prior to the acute toxicity testing, a range finding test was done. The experiment was conducted in a plastic bowl containing 15 liters of borehole water. 2 plastic bowls were filled with water and the test chemical introduced, one containing a higher concentration and the other a lower concentration. Varying volumes of the test chemical were used to determine the range that can cause 100% death of the test species. 4 fishes were exposed in each of the bowls for 24hours. After determination of the range of the test chemical, the acute toxicity was then carried out in which 10 experimental fishes were randomly assigned to each of the 18 treatment bowls. 15 of these bowls contained the different pesticide concentrations ranging from 2µg/l-10µg/l, while 3 bowls served as control with no test chemical introduced. Fishes were allotted to treatment as follows:

Table 1: Experimental Fish simultaneously assigned to treatment units.

	T_0	T_1	T_2	T_3	T_4	T_5	
REPLICATE	CONTROL	2µg/l	4µg/l	6µg/l	8µg/l	10µg/l	TOTAL
R_1	10	10	10	10	10	10	60
R_2	10	10	10	10	10	10	60
R_3	10	10	10	10	10	10	60
No. of Fish	30	30	30	30	30	30	180

The experiment lasted for 4 days (96hrs).The solution for each concentration was renewed every alternate day with changing of water.

Fishes were not fed during the experiment as recommended by [2]. Exposure time was 96hrs after which the mean mortality from each dose and its replicate was calculated. Fishes were considered dead when it sinks to the bottom of the bowl; do not move at agitation of water and changes to a whitish colouration. Dead fishes were removed immediately from the water. The median lethal concentration (LC_{50}) of the test chemical was obtained from the acute toxicity bioassay, following the probit analysis method as described by [9]. The safe level estimate was based on [10]. For the sub-lethal *in vivo* exposure, two sub-lethal concentrations of cypermethrin was calculated and used.

Sub- Lethal Exposure

After the calculation of the LC_{50}, the test fishes were exposed to the two sub-lethal test concentrations of cypermethrin, one-tenth LC_{50} = 0.72 µg/l and one- fifth LC_{50} =1.45µg/l. Each concentration was exposed in triplicates with the test water changed every alternate day to maintain the concentration of the pesticide. The control set was maintained in normal water without introducing the test chemical. The exposure lasted for 15 days and blood samples were collected at intervals of 0, 5, 10 and 15days.On each sampling day, blood was collected from the caudal vein of the cypermethrin treated fishes and control using the puncture technique with the aid of a 2ml syringe and transferred immediately into a heparin bottle. The whole blood samples, collected, were used to prepare slides for the identification of the formation of micronucleus under the microscope.

Estimation of Micronucleus

Thin smear of the heparinized blood was made in a clean-grease free glass slides and air dried for 10 minutes. 4 drops of Leishmann stain was placed on the smear and allowed to stand for 2 minutes followed by 5 drops of distilled water and allowed to stand for 7 minutes after which the slides were washed off with distilled water and then ordinary water. The slides were allowed to air dry for one hour and then viewed under oil immersion under x1000 and result was expressed as percentage. Also, cell lesions of different shapes were also checked and recorded.

Percentage frequency of induction of micronuclei = $\frac{Number\ of\ micronucleated\ cells}{Total\ number\ of\ cells\ counted} \times 100$

RESULTS

Result of Pysico-chemical Parameters of Water

TABLE 2: Physiochemical characteristics of test water

Parameters	00µg/l	02 µg/l	04 µg/l	06 µg/l	08 µg/l	10.0µg/l
Temperature (°C)	26	26.2	26.0	26.4	26.3	26.7
Dissolved oxygen (ppm)	8.1 ± 0.10	4.69± 0.03	4.19 ± 0.02	3.91 ± 0.03	3.84 ± 0.03	3.43 ± 0.03
Ph	6.7	6.7	6,8	6.8	6.8	6.9

TABLE 3: Cumulative mortality of *Clarias gariepinus* exposed to various concentrations of Cypermethrin after the acute toxicity test.

Concentration (µg/l)	No. exposed	24hr	48hr	72hr	96hr	Survival (%)	Mortality (%)
10	30	4	8	7	25	16.67	83.33
8	30	8	0	10	22	26.67	73.33
6	30	4	2	5	14	53.33	46.67
4	30	2	0	6	13	56.67	43.33
2	30	2	2	3	1	73.33	26.67
00	0	0	0	0	0	100	0

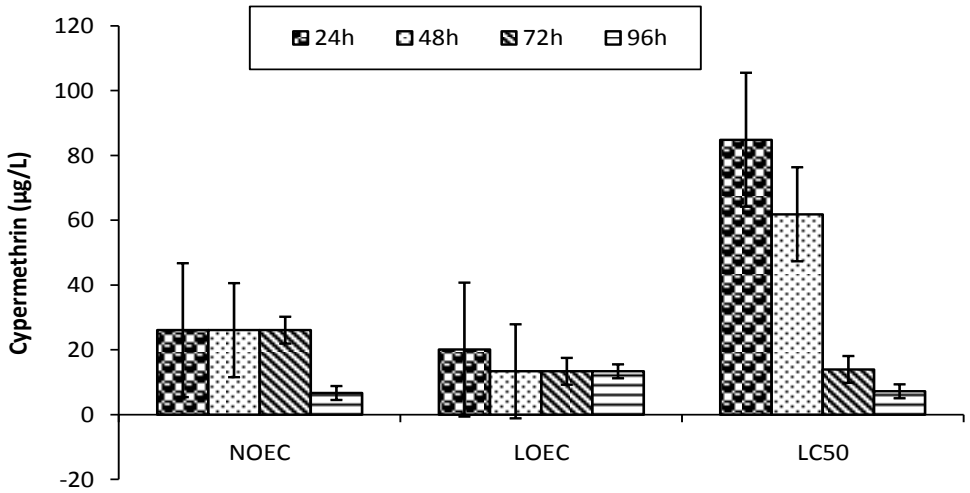

Fig 1: Statistical end points of acute toxicity testing in *Clariasgariepinus* exposed to cypermethrin at different durations (24h, 48h, 72h and 96h)

- **Legend**

NOEC: No Observed Effect Concentration

LOEC: Lowest Observed Effect Concentration

LC_{50}: Median Lethal Concentration

Table 4: Induction of Micronuclei in the Erythrocyte of Fish Exposed to sub-lethal concentration of cypermethrin for 15 Days

Pesticide Concentration (µg/L)	**Micronuclei Induced (Percentage)**			
	Day 0	Day 5	Day 10	Day 15
A	0.00	0.00	0.00	0.00
B	0.00	6.20 ± 0.50	5.70 ± 0.50	5.20 ± 0.50
C	0.00	6.80 ± 0.56	6.40 ± 0.56	5.73 ± 0.60

Values are expressed as mean ± standard deviation.

- **Legend**

A : Control exposure

B: 1/5 LC_{50} (2µl)

C: 1/10 LC_{50} (4µl)

DISCUSSION

The 96h LC_{50} value obtained for the commercial formulations of cypermethrin BEST® was 7.23µg/l. This LC_{50} value obtained was higher than the 2.6µg/l value reported by [3], when *Cyprinuscarpio* was exposed to a formulation of cypermethrin. This was also observed to be lower than the 21.37µg/l recorded by [2] when *Poecilia reticulate* was exposed to a commercial formulation of cypermethrin. This may be attributed to many reasons such as the fish species, the size of the fish, or the cypermethrin formula [7]. The abnormal behavioral responses observed during cypermethrin acute and sub-acute exposure of *C. gariepinus*correspond to the findings of Tendulka and Kulkarni (2012). Abnormal behavioral responses observed in this study included hyperactivity, increased erratic swimming, and rapid opercula movement. These behavioral alterations may occur as a result of disruption of transmission along the nerve impulse and various effector sites. This can also arise due to ATP and GABA inhibition caused by the synthetic pyrethroid [11]. Toxicity of chemicals to aquatic organisms has also been reported to be affected by dissolved oxygen, size, age, water quality and formulations of chemicals [12]. The safe level of cypermethrin formulation (BEST®) in the present study varied from 3.49µg/l to 3.62 x 10^{-1}µg/l.

The action of any genotoxic agent may give rise to an increase in micronuclei frequency. In the present study, the micronucleus formation in the erythrocytes of *C.gariepinus* to the sub-lethal concentrations of the cypermethrin based pesticide increased significantly ($p< 0.05$) with increase in concentration. In comparison to earlier investigations, [13], recorded a time and concentration dependent response in *L.rohita* chronically treated with sub- lethal concentration of cypermethrin and noticed a marked decrease in (Hb) content and oxygen carrying capacity of blood. [14], showed that two sub-lethal concentrations of cypermethrin induced significantly ($p<0.001$) higher number of micronuclei than the control and its frequency increased with increase in concentration. The same induction was also reported when *Gambusiaaffinis* and*Cheirodoninterruptusinterruptus* was exposed to pyrethroidlamda-cyhalothrin [12]; [15], respectively. The result of this study emphasizes the importance of the micronucleus assay and suggests that this assay be applied on a broader scale as abiological marker of exposure of fish, to aquatic pollutants.

CONCLUSION

The results of this study provide evidence that cypermethrin pollution may have adverse impacts and were highly toxic to fish. The BEST® pesticide has the potential to impair physiological activities of the organism leading to observed

changes in behavioural pattern, and consequent dose dependent mortality. This chemical also exhibited the potential to induce the formation of micronucleus which can lead to mutation in aquatic organisms. Hence, the use of BEST® pesticide should be strongly monitored regularly to avoid cypermethrin related hazards.

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