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Changes in glucose and phospholipids level of *Oreochromis niloticus* fingerlings exposed to varying concentrations of Propoxur.

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Abstract: *The effects of Propoxur (a carbamate pesticide) on the fingerlings of Oreochromis niloticus are reported. Mortality was found to increase with concentration and exposure duration, and the LC_{50} value obtained was 20.906mg/l and the safe concentration was 2.091mg/l after the acute toxicity test. The sub lethal toxicity test recorded a mean increase in glucose and phospholipids level. However, the increase in glucose level was statistically significant at $P_{0.05}$ while the increase in phospholipid level was not statistically significant. The result obtained indicated that the pesticide, Propoxur had slight effect on the glucose and phospholipids levels of the fish*

***Keywords**: Propoxur, survival, glucose, phospholipid, Oreochromis niloticus*

I. Introduction

Pesticides are compounds or mixtures of substances intended of preventing, destroying, repelling or mitigating any pests, including not only insects but also other animals, unwanted plants, fungi or micro organism (Ezemonye *et al.*, 2009). At the traditional level, agricultural activities in river basins are known to have little effect on water quality and fisheries and in certain cases, may be regarded as a non-competing joint-use of the resource. However, with increasing human population and the need for more crop production, Agricultural methods have become more advanced, which results in desiccation of downstream farmlands to the detriment of traditional agricultural and loss of Fishery resources as a result of habitat loss (Welcome, 1983). In order to feed these ever increasing human populations in Nigeria for example, it becomes necessary to use pesticide to minimize loss due to pests both on the farm and after harvest. Hence, pesticides play an important role in agriculture and in pest control worldwide (Castillo *et al.*, 2000). Propoxur-Phenol, 2-(1-Methylethoxy) -methyl carbamate, is a carbamate and molluscide characterized by a fast knock down and long residual effect. It is a non-systematic insecticide which was introduced in 1959.

It is compatible with most insecticides and fungicides except alkalines and may be found in combination with azinphosmethyl, chlorpyrifos, cyfluthrin, dichlorvos, disulfoton or methiocarb.

It is used on a variety of insect pests such as chewing and sucking insects, ants, cockroaches, crickets, flies and mosquitoes, and may be used for control of these in agriculture or (as Baygon) in non-agricultural (e.g. private or public facilities and grounds) applications. Propoxur has toxicological and Ecological effect. Acute toxicity, chronic toxicity, reproductive effects, teratogenic effects and organ toxicity have been reported for Propoxur. It inhibits the production and action of cholinesterase, an essential nervous system enzyme. These materials quickly paralyze the nervous system of the insects, gaining the reputation of having a rapid "knockdown" effect. Non target organisms are exposed to Propoxur when it is used to control insects, both indoors and outdoors.

Modern agricultural practices result in indiscriminate use of various agrochemicals, which usually enter into the aquatic environment. The use of agrochemicals in the field has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystem. These agrochemicals adversely affect the non-target organisms, especially plankton and fish. The present study reports the acute and sublethal toxicity of Propoxur on survival, glucose and phospholipids levels of *Oreochromis niloticus*, a freshwater fish.

II. Materials and Method

Fingerlings collection

Healthy samples of *O. niloticus* fingerling with mean length of 5.76±0.7cm and a mean weight of 4.83±1.5g were collected at sydclifen and jr's agro projects, a fish farm at Aduwawa in Benin City, Edo State. The fish were transported in a well aerated large container to the laboratory, and then kept in well aerated plastic tanks prior to the acclimatization period.

Acclimatization

The collected fingerlings (*O. niloticus*) were placed on three (3) plastic tanks each containing 20 litres of dechlorinated tap water. They were allowed to acclimatize for 7 days in the holding tank prior to the bioassay (ASTM 1985). They were fed daily with pelleted fish meal. A photoperiod of 10:14 hours of light:dark cycle was maintained to mimic natural condition and room temperature was also maintained at 30 ± 2^0C throughout the experimental period. The water in the tank was changed every 3 days

Experimental design

A total of 15 glass tanks were used for the experiment, each holding a total of 10 fingerlings and were fully aerated.

Test solution

Stock solution of the required concentration was prepared by adding 950ml of dechlorinated tap water to 50ml of liquid Propoxur to make a stock solution of 50mg/l. The stock was then diluted into environmental relevant treatment concentrations of 0, 5, 10, 15 and 20µg/l and 0, 0.5, 1.0, 1.5 and 2.0µg/l for acute and sub lethal tests respectively.

Acute toxicity tests

Acute toxicity test were conducted according to standard procedures (ASTM, 1996).

Mortality rate

Mortality was recorded at an interval of 24 hours over a period of 4 days (96hours).

Sublethal toxicity test

The chronic toxicity test was carried out in similar manner as the acute test. However, for chronic toxicity tests, very low sublethal concentrations were used. 1/10 of the concentrations used in the acute toxicity test were used for the sublethal test. The *O. niloticus* fingerlings were exposed to concentrations of 0, 0.5, 1.0, 1.5 and 2.0µg/l of Propoxur. Exposure lasted for 28 days. Changes in glucose and phospholipids levels were used as assessment end point.

Glucose level bioassay

The glucose levels were estimated using digestion based on glucose oxidase method of (Trindax 1969).

Phospholipid level bioassay

The phosphlipid levels were estimated using the method of (Wagner *et al.* 1968). The phospholipid is digested with a sulphoric- acid – perchloric acid reagent. The inorganic phosphate is determined colorimetrically.

Statistical Analysis

The susceptibility of the *O. niloticus* fingerling to Propoxur pesticide was determined using the probit (probit software) method of analysis (Finney, 1971). This was used to determine the LC_{50}. Safe concentrations at 96 hours were obtained by multiplying the lethal concentration by factor of 0.1 (EIFAC, 1998). Experimental results were subjected to one-way analysis of variance by Snedecor and Lochran (1982), at $P<0.05$ level of significance.

III. Results

The results of the Acute and Sublethal toxicity of *Oreochromis niloticus* fingerlings exposed to varying concentrations of Propoxur pesticide are represented in Tables and figures below.

Control

No mortality or morphological changes were observed in the control for the 96 – hour acute toxicity test. Fingerlings in the control experiment for both acute and sublethal toxicity tests appeared active and healthy throughout the test period.

Acute toxicity

The fingerlings of *Oreochromis niloticus* exposed to varying Propoxur concentrations recorded mortality in all the concentrations. The mean percentage mortality increased with increase in concentration and duration of exposure (figure 1,). This indicated that mortality was concentration dependent. Sixty percent (60%) mortality was observed at 96-hours in the highest concentration (20µg/L).

Propoxur was found to be slightly toxic. LC_{50} value was found to be 20.906mg/l after a 96hours exposure time. This indicates an increase in toxicity with increase in concentration and exposure duration.
Estimated safe concentrations at 96-hour was 2.091mg/L.
Mean percentage (%) mortality of *Oreochromis niliticus* fingerling was significantly affected by concentration at $P < 0.05$.

Sublethal toxicity

Glucose levels: Glucose levels were used as assessment endpoint in the sublethal toxicity test. The result showed that glucose levels of *O. niloticus* fingerling varied with concentrations of the test chemical. The value obtained for the pesticide increased with increase in concentration of the pesticide (Table 1). Glucose values were observed to be lower in the control than the treated groups. The mean glucose level increase with concentration was significant at $P < 0.05$. There was a positive correlation between the glucose level and the concentration with $r = 0.8431$ at ($P < 0.05$).

Phospholipid levels

The results obtained showed that phospholipid levels of the *O. niloticus* fingerling varied with concentration of the test chemical. The values obtained increased with increase in concentration (Table 2). Phospholipid levels were found to be lower in the control than in the treated groups.
Mean phospholipid level showed no significant increase with exposure to varying concentration of $P > 0.05$, but there was a positive correlation between the phospholipid level and the concentration with $r = 0.9192$ at ($P < 0.05$).

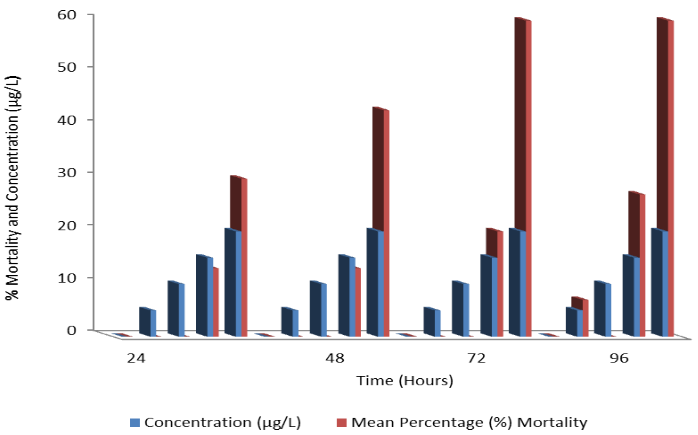

Figure1: Mean percentage mortality of *Oreochromis niloticus* fingerling exposed to different concentrations of Propoxur

Concentration (µg/L)	Mean Glucose Levels (mg/dl)
Control	3.9±0.24
0.5	4.7 ±0.41
1.0	6.4± 0.74
1.5	10.8 ± 1.98
2.0	8.3 ± 1.37

Table 1: Mean glucose levels (mg/dl) and S.E of *O. niloticus* exposed to different sublethal concentrations of Propoxur after 28 days

Concentration (µg/L)	Mean Phospholipid Levels (mg/dl)
Control	8.7±0.12
0.5	9.1 ± 0.37
1.0	9.0 ± 0.47
1.5	9.3 ± 0.97
2.0	9.9 ± 0.49

Table 2: Mean phospholipid levels (mg/dl) and S.E of *O. niloticus* fingerling exposed to different sublethal levels of Propoxur after 28 days.

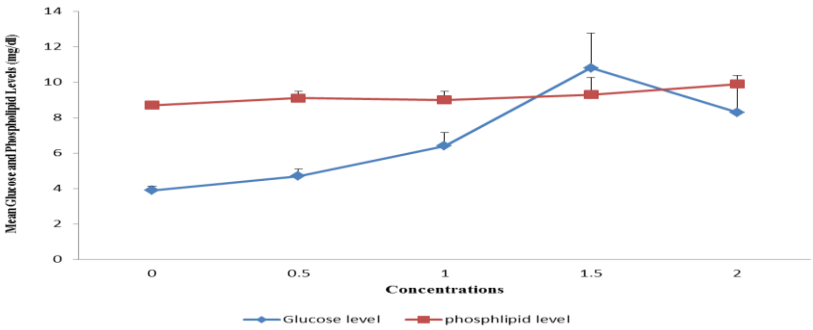

Figure 2: Mean Glucose and Phospholipid Levels of *Oreochromis niloticus* exposed to different sublethal concentrations of Propoxur after 28 days.

IV. Discussion

Variation in percentage mortality of o. niloticus fingerling with concentrations

No observable mortality was reported in all the control tests in this study, while varying degrees of mortality were reported in the tests concentrations. This is a clear indication that the effects of the pesticide could be regarded as possible cause of death of the test organism.

However, mortality increased as concentration and time increased. Thirteen (13) percent mortality was recorded in 15µg/L concentration tank at 24hrs, this value remained constant after 48hrs exposure, but it increased to 20% in 72hrs and 27% in 96hrs respectively. Seven percent mortality was recorded in 5µg/L concentration at only at the 96hrs exposure. At 20µg/L concentration, 30% mortality was observed in 24hrs exposure. This increased to 43% and 60% for 48hrs and 72hrs respectively. No mortalities recorded in the 10µg/L concentration. The LC_{50} value at the end of the 96hrs acute toxicity test was 20.906µg/L and was found to increase with concentration as the time increased. This can be compared to the LC_{50} value of 30.40mg/L of Propoxur when *Oreochromis niloticus* was exposed (Hanson *et al.,* 2007). The influence of Propoxur concentration on mortality was significant with ($P < 0.05$, $F = 3.998$) after 72hrs exposure. There was no significant mortality after 96hrs exposure.

This observation agrees with the finding of Chindah *et al.,* (2000) who reported on the toxicity of organophosphate pesticide (chloropyrifos) on a common Niger Delta wetland fish *Tilapia guineensis*, that ten (10) percent mortality was recorded in 0.0125mg/L concentration tank at 24hrs, this value increased to 32% in 36hrs, 38% in 48hrs, 52% in 60hrs, 62% in 72hrs, 78% in 84hrs and 85% in 96hrs respectively, thus indicating increased mortality over time. At 0.025mg/L concentration, 22% mortality was observed in 24hrs followed by 45%, 50%, 67%, 77%, 82%, and 92% for 36hrs, 48hrs, 60hrs, 72hrs, 84hrs, and 96hrs respectively. The influence of chlorphyfos concentration on mortality of *Tilapia guineensis* was significant with F cal (23.85) > P =3.26 0.05.

The results of this study indicate that the pesticides, Propoxur varied greatly with its effect on survival of *O. niloticus* with concentrations. The highest mortality was found at the highest concentrations, suggesting dose-dependent survival and concentration graded lethality.

Different acute toxicity levels observed in the pesticide at different concentrations and exposure duration was a reflection of the effect of toxicity modifying factors. Test organisms showed better tolerance at lower concentrations, which did not necessarily mean complete compensation for the pesticide. Resistance may have at least added metabolic cost and negatively influenced energy budget (Langston and Spence, 1995).

Grillitsch *et al.,* (1999) reported that organisms exhibit behavioural responses to chemical stress both at acute and sublethal toxicity. This elicits the potency and sensitivity of the fish to the test chemical. The ecological importance of this is that the damage to non-target species in the environment and such attribute of the organism could be effectively used as toxicity biosensor of chemical stress.

Sublethal and chronic effects associated with exposure may be more significant in mediating ecological effects (Sparling *et al.,* 2000). Sublethal toxicity test exposes test organisms to a series of dilutions site's medium and measures sublethal effects and in some cases lethal effects as well. Sublethal effects may include growth, reproduction impairments, and nerve function impairments, lack of motility, behavioural changes and structural abnormalities (Weber *et al.,* 1989).

Glucose ($C_6H_{12}O_6$) is a ubiquitous fuel molecule in biology. It is oxidized through a series of enzyme-catalyzed reactions to form carbon dioxide and water, yielding the universal energy molecule ATP. Due to its importance in metabolism, glucose level is a key diagnostic parameter for many metabolic disorders. Increased glucose levels have been associated with diabetes mellitus, hyperactivity of thyroid, pituitary and adrenal glands. Decreased levels are found in insulin secreting tumors, myxedema, hypopituitarism and hypoadrenalism. Glucose level, an ecological end point of oxidative stress was assessed in this study. Glucose is stored in organism basically as glycogen.

The glucose levels of O. *niloticus* fingerling exposed to varying concentrations of Propoxur pesticide were observed to increase with concentrations. Glucose level was lowest in the control experiment. This increase in blood glucose can be viewed as part of stress response triggered by the presence of Propoxur in water. The mechanism may be attributed to the stimulation produce by Propoxur to secrete epinephrine. This is because epinephrine has been reported to induce hyperglycemia due to its dual action on carbohydrate metabolism; it causes increased liver glycogenolysis and reduction in peripheral utilization of glucose (Feldman and Lebovitz, 1970). Similar results were reported by Srivatava and Singh were the treated fish elicited hyperglycemia and glycogenolysis in their blood when exposed to 5.20 and 2.608ppm of formothion and Propoxur for 8 days respectively. In another work by Srivatava and Singh (1982) on the acute toxicity of Propoxur on carbohydrate metabolism of India catfish, *Heteropneustis fossilis*, showed a significant increase in blood pyruvate levels at 12 and 48hrs.

Conversely, the present results also agree with those of (Winkaler *et al.,* 2007) who reported higher plasma glucose levels in the fish *Prochilodus lineatus* exposed to neem extract. This increase in blood glucose can be viewed as part of a stress response triggered by the presence of neem extract in water (Winkaler *et al.*, 2007). Hyperglycemia has also been found in *P. lineatus* acutely exposed to lead for 6, 12 and 24 h (Martinez *et al.*, 2004). When glucose tolerance tests were performed in tilapia, the mean glucose disappearance rates were very low (James *et al.,* 2010)

Phospholipid levels, as ecological endpoint of oxidative stress was assessed in this study. Phospholipids acts as building blocks of the biological cell membrane in virtually all organisms, they participate in the transduction of biological signals across the membrane, acts as efficient store of energy as with triglycerides, plays an important role as a source of acetylcholine which is the most commonly occurring neurotransmitter substance occurring in organisms. All cell membranes are built up of phospholipids each of which contains two fatty acids. Fatty acids are an important source of energy for many organisms. Excess glucose can be stored efficiently as fat. Fatty acids are also used for protein modification. The metabolism of fatty acids therefore consist of catabolic process which generate energy and primary metabolite from fatty acids and anabolic process which create biologically important molecules from fatty acids and other dietary carbon sources.

The phospholipid levels of O. *niloticus* fingerling exposed to varying concentrations of Propoxur in this study were observed to vary with concentrations. Phospholipid level was lowest in the control experiment. There was a corresponding increase in the phospholipid level as the concentration increased. This is probably due to toxicological effect as observed in oxidative stress.

This result agrees with that of (Saxena *et al.,* 1986) whose study on the effect of safe application rate (SAR) concentrations of some biocides (fenitrothion an organophosphate and carbofuran, a carbamate) on the gonads of the fresh water murrel, *Channa punctatus* have revealed that the levels of total proteins, RNA, total lipids, and ascorbic acid decreased, while those of the cholesterol and the phospholipids increased following exposure to the two biocides. However, the result was not consistent with the findings of (Rao and Rao 1984) who reported a decrease in phospholipd and total lipids levels of fish (*Oreochromis mossambicus*) exposed to methyl parathion pesticide.

V. Conclusion

Fingerling of *O. niloticus* exposed to sublethal concentrations of Propoxur pesticides showed increased level in glucose and phospholipid. The results obtained indicated that the pesticide Propoxur is toxic and could bioconcentrate along the food chain, therefore, it is imperative that their use should be closely monitored.

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