IJABR Vol. 6(2):71 – 79 (2014)

Original Article

ACUTE TOXICITY OF PROPANIL TO THE JUVENILES OF NILE TILAPIA (*Oreochromis niloticus*)

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Submitted: August, 2014; Accepted: October 06, 2014; Published: December 30, 2014.

ABSTRACT

The widespread increase of pesticides application in crops frequently leads to the contamination of the fresh water ecosystem. One of the most commonly used pesticides in Nigeria is propanil. Acute toxicity of propanil (3, 4-dichloropionanilide) to juveniles of *Oreochromis niloticus* was determined in a 96-hour static bioassay. During the assay opercular ventilation rate of the fish was determined. The 48-hour and 96-hour LC_{50} of propanil to the juveniles of *O. niloticus* were observed to be $11.22mgL^{-1}$ and $5.79mgL^{-1}$ respectively. The physico-chemical properties of the borehole water used were also observed for the duration of the experiment. The highest and lowest of all observed parameters were as follows: Temperature recorded 27.73 ± 0.30^0C in 12.50 mg/l and 26.05 ± 0.44^0C in the control, pH recorded 7.27 ± 0.02 and 6.54 ± 0.09 for 15.00 mg/l and control respectively, DO record was high in the control with 7.45 ± 0.19 and low in 15.00 mg/l with 5.58 ± 0.10, while conductivity was also 3.37 ± 0.02 for15.00 mg/l and 3.17 ± 0.01 for control, 7.5 mg/l had 33.46 ± 0.02 and control had 31.13 ± 0.08 for alkalinity, and finally hardness recorded 24.41 ± 0.04 and 20.30 ± 0.10 for 15.00 mg/l and the control experiment respectively. Other behavioural response of the exposed fish include loss of equilibrium, agitated swimming, air gulping; visible blood lines on the gills, pectoral and pelvic fins, swollen and ruptured swim bladder. These results show that the contamination of water by pesticides either directly or indirectly be lethal to fish and may reduce fish productivity. Elevated **concentrations** of undesirable chemicals in edible fish tissue can affect the health of humans eating these fishes.

Keywords: Fish toxicity, *Oreochromis niloticus*, propanil, physico-chemical parameters.

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INTRODUCTION

Pesticides are chemical substances used by humans to control pest. The term pesticide is an all-embracing word for herbicides (which kills all plants), insecticides (which kills insects), and fungicides (which act on fungi). Most Pesticides are poisons and aim to kill the target species (Taylor *et al*., 1998).
In Nigeria pesticides used are mainly associated with agriculture and horticulture, though pesticides are also widely used in food storage and to protect wood, wool and other natural products. In many countries, pesticides are used in forestry and they are also used extensively to control human and animal disease vectors (Taylor *et al*., 1998).

Agricultural activities had little effect on water quality and fisheries in the past. However, with increasing human population and the need for increased crop production, agricultural methods have become more advanced. The application of pesticides is known to lead to aquatic pollution which have caused fish kills, and has aroused concern about possible long-term and sub-lethal effects on fish, including accumulation of chemicals in edible fish tissue with resultant adverse effects on human (Oloruntuyi *et al*., 1992). Herbicides are widely used in agriculture and in landscape turf management; these account for about 70% of all agricultural pesticides used (Kellogg *et al*., 2000). Propanil is commonly used in rice farming which is usually in a water logged area and can be washed downstream during intense flooding and runoff.

Tilapia has become the third most important fish in aquaculture worldwide after Carps and *Salmonids* with production reaching 1,505,804 metric ton in 2002 (Fessehaye, 2006) and *Oreochromis niloticus* is a common food fish in Nigeria, hence its choice in investigating the effect of acute toxicity of propanil on fish and its environment. This research work aims fill the information gap and have a sound knowledge of the effects of propanil on the juveniles of *Oreochromis niloticus.* Daniel *et al*. (2005) reported that propanil is moderately more toxic to rainbow trout (*Salmo gairdneri*) with 48 and 96-hr LC_{50} of 8.6 mgL^{-1} and 3.4 mgL^{-1} respectively. Villarroel *et al*. (2003) also reported the acute toxicity LC_{50} of propanil to *Daphnia magna* to be 43.74 mgL^{-1} and 5.01 mgL^{-1} for 24 and 48 hours respectively. Opercula hyperventilation has been reported to be an index of stress when fish is in an unfavorable environment (Sprague, 1971). This indicates damage being caused by propanil to the gills. The fish thus increases its ventilation rates in an attempt to make up for the loss in the efficiency of the gills for oxygen uptake as observed by Omoregie and Ufodike, (2011) when they exposed *Clarias gariepinus* to 2,4-Dichlorphenoxyacetic acid (2,4 D) in a 96hr acute test and the

LC_{50} obtained was 86 mgL^{-1}. The loss of buoyancy could be attributed to the ruptured swim bladder. Experiments on the behaviour of *O. niloticus* to several other toxicants and differences in reaction times have been observed as due to the effect of chemicals, their concentrations, species size and environmental conditions (Babatunde, 2008; Bobmanuel *et al*., 2006; Henry and Kishinba, 2005; Babatunde *et al*., 2001).

MATERIALS AND METHODS

The static methods of acute toxicity test of pollutants to fish described by Sprague (1971) and APHA (2005) were employed and all tanks were aerated during the period of study.

Test Animals

Oreochromis niloticus used for the study were primarily selected for its availability and ability to thrive in captivity. The 4-week old test specimens used for the experiment which were of weight range of 6.02-6.21g and mean weight of 6.11$\pm$ 0.26g were obtained from the School of Aquaculture and Fisheries Technology Fish farm, Federal University of Technology Minna. The fish were fed on pellet diet of imported feed called "Coppens" during the 14 days acclimatization period.

Test Chemical

The test chemical used for the assay was a commercial propanil (3, 4-dichloropropionanilide) of solution with effective concentration (EC) of 360g/liter. The chemical was obtained from a licensed agro-chemical shop in Minna.

Water: Bore-hole water obtained from the premises of Federal University of Technology Minna, was used for all bio-assays. Physico-chemical properties of the well-water; such as Temperature, pH, hardness, alkalinity, conductivity, dissolved oxygen were analyzed following the methods of APHA (2005).

Preliminary Studies (Pilot assay): Following the techniques of APHA, (2005), a preliminary study was carried out to determine the range of concentrations of the test chemical and density of test animal to be used for the experiment proper. Five juvenile fish each were exposed to propanil in 24 liters of water contained in eight 65-litre glass aquaria. The fish were exposed to seven concentrations of the toxicant 0.50, 1.00, 2.00, 2.50, 4.00, 5.00, 10.00 mg/l and control with 2 replicates for the 24-hour static bioassay. Feeding stopped 24h prior to the commencement of the assay. The concentrations listed above were obtained using the formula:

$$V_2 = \frac{C_1 \times V_1}{C_2}$$

Where

V_2 = Volume of test chemical to be added to required volume of water in the aquaria.

C_1 = the desired concentration of the test chemical.

V_1 = Required volume of water in the aquaria.

C_2 = 1ml of the test chemical.

The assay

Test solutions were prepared shortly before the commencement of assays. The five concentrations of propanil (7.50, 10.00, 12.50, 15.00 mg L^{-1} and control) to which the fish (mean weight 6.11$\pm$ 0.26g) were exposed were replicated. One hundred juveniles were randomly distributed into the toxicant concentration to give ten in each aquarium including the control tanks of 30 liters each that contained bore-hole water. Physico-chemical parameters of test solutions were determined daily as described by APHA (2005).

Mortality was recorded at experimental periods of 12, 24, 48, 72 and 96 hours. The LC_{50} was determined graphically using probit analysis (Dick and Dixon 1985). The opercula ventilation rate of 4 fishes, sampled from each concentration, was determined once daily during the assay. Other behavioral changes such stress, irregular movement of the fish prior death were also noted and recorded.

Disposal of test materials: All test solutions were disposed of in a sink that goes to a septic tank, and from the septic tank it goes to the waste water treatment plant. Dead specimens were removed from the solution using nets, wrapped in cellophane bag before being disposed of to avoid further contamination of the solution and the environment.

Data analysis: The statistical package for social sciences (SPSS) version 20.0 of which descriptive statistics was used to compare means and standard error of means, ANOVA was used to test the association in hours between groups and DMRT was used to separate means.

RESULTS AND DISCUSSION

The physico-chemical properties of the solution during the assay are presented in Table 1 and from the findings in this study indicated that slight variations did occur during the study and within concentrations of toxicant affected water quality which could in turn further quicken fish mortality. Although dissolved oxygen concentration (DO) was above approved minimum value of 5.0 mg/l, variations did occur among the different concentrations but with the control having the highest of 7.45 ± 0.19 mg/l than other treatment tanks. Conductivity, pH and hardness increased with increase in toxicant concentrations in all treatments. Fig. 1: shows dose response between propanil and probit mortality of *Oreochromis niloticus* for 48 hours where the LC_{50} was calculated to be 11.22mgL^{-1} with 95% confidence limit and Fig. 2 also shows the dose response between propanil and the probit mortality of *Oreochromis niloticus* exposed for 96 hours where the LC_{50} was also calculated to be 5.79 mgL^{-1} with 95% confidence limit. The computed regression equation for probit kill at 48 hrs was found to be $y = 4.832x - 0.093$ with $R^2 = 0.989$ and at 96 hrs was also found to be $y = 6.7777x - 0.171$ with $R^2 = 0.970$, where x = log of conc., y = probit kill. The R^2 values obtained in the regression equation showed that there was a strong correlation between probit kill and toxicant concentration implying that the higher the concentration of propanil, the higher the mortality. This observation agrees with the findings of Mallum *et al.* (2015) that stated that juveniles of *O. niloticus* were sensitive to propanil as concentrations increase. Sancho *et al.* (2009) determined the 96-h lethal toxicity of propanil in European eel (*Anguilla anguilla*) to be 31.55ppm and this value for propanil was higher to that obtained for *O. niloticus* in this study. The difference could be for various reasons such as the geographical location of the species, type of species and size of the species.

After the 24 hours pilot assay, organisms that showed signs of toxic effect were placed in a propanil free environment, returned to normal within 4 hours. During the 96 hours assay all organisms were knocked off with visible opercula beats except for those in the control aquaria and after recovery, the organisms' started to exhibit abnormal swimming habits such as loss of equilibrium, agitated swimming and air gulping. These behavioral response to the toxicant were similar to findings reported by Annune *et al*. (1994); Oti (2000); Okayi *et al*, (2013) on acute toxicity of toxicants exposed to fishes. Some of the organisms had swollen abdomen and protruding swim bladders and visible bloodlines around the eyes, pectoral and pelvic fins and along the gills which resulted to loss of balance and poor swimming. This observation also conforms to that of Sancho *et al*., (2009) who observed lethargy, swimming reduction, torpor, and presences of small dots all over the scales of *Anguilla anguilla* exposed to propanil after two hours, which further increased with increase in exposure time.

Opercula ventilation rate as shown in Fig.3, were lower in fish exposed to higher concentrations of propanil after the first 24 and 48 hours while the control maintained relatively high constant rates throughout the period of the experiment, which further implies that the decrease in opercula ventilation observed during the first 24 and 48 hours of exposure to propanil in this study indicates odious effect of the toxicant and acclimation to the unfamiliar environment followed by hyperventilation and eventually death. The reduction of respiratory rate implies that the fishes had become fatigued as a result of attempts to escape from the medium to facilitate more oxygen intake (Mallum *et al*., 2015) and also the pattern of behaviour are indicative of respiratory impairment, due to the effect of the toxicant on the gills and general metabolism (Chindah *et al*., 2004). At 72 and 96 hours, increase in opercula ventilation rate above the 48-hour values was observed in fish exposed to higher concentration of propanil prior death and these could be attributed to the need for more oxygen consumption from the environment which off course is much more reduced or absent due to the toxicant and its lack, resulted in energy loss and subsequently death. Matsumura, (1975) and Mallum *et al*., (2015) explained that hyperactivity is a primary sign of failure of the nervous system due to the toxicant affecting physiological and biochemical activities.

CONCLUSION

The study has revealed the effect of propanil on juveniles of *O. niloticus*, which was found to be toxic with increase in concentrations. To avoid bioaccumulation and biomagnifications there is the need for proper and regular monitoring of toxic levels and concentrations in water and fish. A system for screening pesticides for safety and for giving advice on safe use, including information on toxicity to fish needs to be put in place.

Table 1: Water quality parameters of different test tanks by concentrations during toxicity assay with *Oreochromis niloticus* exposed to propanil in 96 hours.

Conc. (Mg/l-1)	Temp. (^{0}C)	pH	DO (Mg/l^{-1})	Conductivity (µmhos x10^2)	Alkalinity (Mg/l^{-1})	Hardness (Mg/l-1)
control	26.05 ± 0.44^a	6.54 ± 0.09^a	7.45 ± 0.19^c	3.17 ± 0.01^a	31.13 ± 0.08^a	20.30 ± 0.10^a
7.50	26.45 ± 0.19^a	6.56 ± 0.17^a	6.23 ± 0.09^b	3.22 ± 0.01^a	33.46 ± 0.02^c	20.52 ± 0.17^a
10.00	27.40 ± 0.13^b	6.56 ± 0.11^a	6.28 ± 0.14^b	3.32 ± 0.01^b	32.61 ± 0.26^b	23.35 ± 0.08^b
12.50	27.73 ± 0.30^b	7.09 ± 0.07^b	6.00 ± 0.32^{ab}	3.35 ± 0.02^b	32.56 ± 0.24^b	23.46 ± 0.04^b
15.00	26.84 ± 0.32^{ab}	7.27 ± 0.02^b	5.58 ± 0.10^a	3.37 ± 0.02^b	33.18 ± 0.01^c	24.41 ± 0.04^c

Values in the same column with the same letter superscript are not significantly different ($p > 0.05$)
DO = Dissolved oxygen concentration

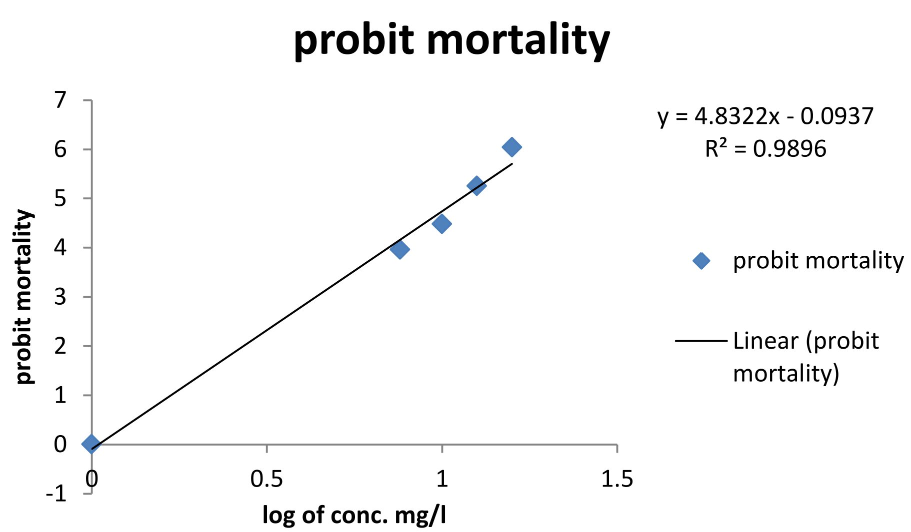

Fig 1: Linear relationship between probit mortality and log of concentration in *O.niloticus* exposed to propanil for 48 hours.

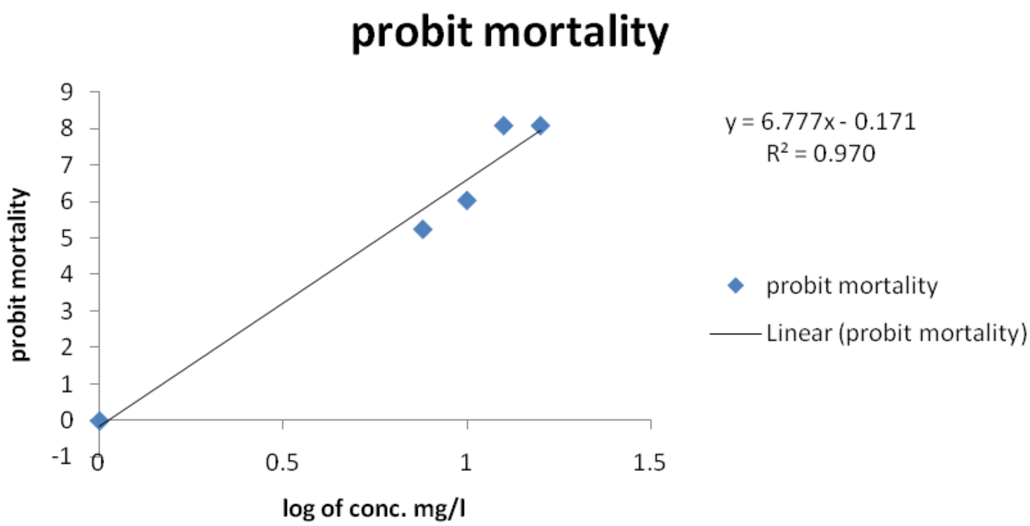

Fig 2: Linear relationship between probit mortality and log of concentration in *O.niloticus* exposed to propanil for 96 hr

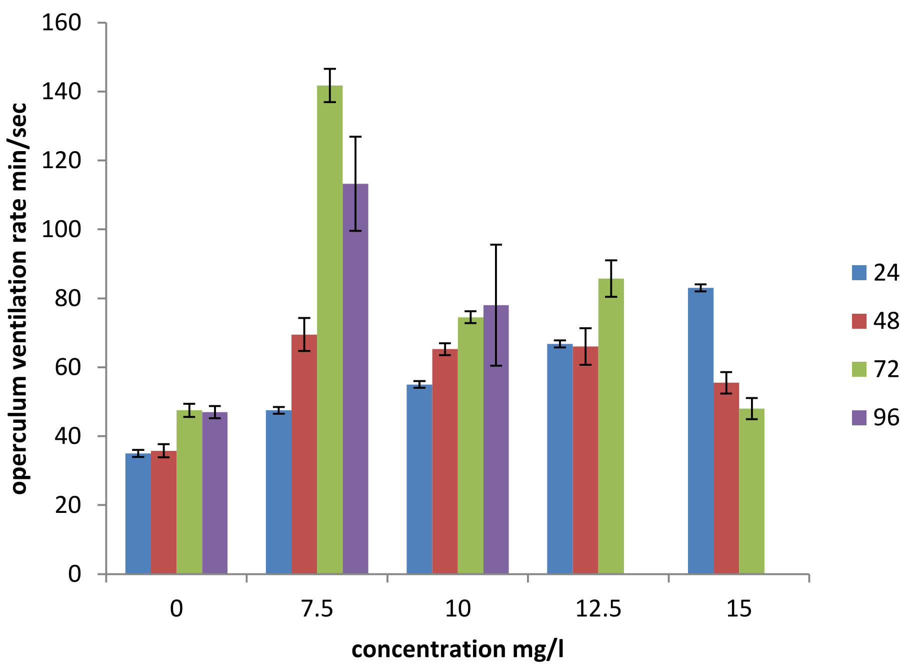

Fig 3: Opercular vertilation rate of *O. niloticus* exposed to various concentrations of propanil

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