American-Eurasian J. Agric. & Environ. Sci., 10 (5): 884-892, 2011
ISSN 1818-6769

Acute Toxic Effects of Endosulfan (Organochlorine Pesticides) to Fingerlings of African Catfish (*Clarias gariepinus*, Burchell, 1822)

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Abstract: The acute toxicity of endosulfan to African catfish (*Clarias gariepinus*) was assessed in a static bioassay for 96 hours. The fish specimens were acclimatized in the laboratory for 21 days. There were initial range-finding test to determine the concentrations of endosulfan to be administered on the test organisms in the definitive tests. Five concentrations of the toxicant were prepared as 1.0, 2.2, 4.8, 11.0, 23.0 µg/l and a control experiment (0 µg/l). The median lethal concentration (LC_{50}) at 24-hr, 48-hr, 72-hr and 96-hr was 16.22, 4.68, 2.45 and 2.09 µg/l, respectively. The median lethal time (LT_{50}) of endosulfan at concentrations of 4.8 and 11.0 µg/l were approximately 37 hours and 48 hours respectively while LT_{50} at concentrations of 1.0 and 2.2 µg/l was zero. The minimum concentration of endosulfan that can cause the death of *Clarias gariepinus* was 4.79 µg/l and the minimum time required for this concentration to cause the death of *Clarias gariepinus* was approximately 48 hours. Mortality increased with increase in the concentration of endosulfan and the difference was significant ($p<0.05$). The use of endosulfan as pesticide should be discouraged.

Key words: Endosulfan · *Clarias gariepinus* · Median lethal concentration (LC_{50}) · Median lethal time (LT_{50}) · Minimum concentration · Minimum time

INTRODUCTION

Pesticide is defined by United Nations Environment Programme [1] as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Lee [2] also defined pesticide as any substance used to control pests. It is used directly or indirectly for controlling or preventing, destroying, mitigating or repelling any pest or of altering their growth, development and characteristics. Pesticides are widely used to control pest species and increase crop yields for economic gain. However, they are also commonly found in aquatic habitats, including streams, rivers and ponds, at varying concentrations because of direct overspray, drift, atmospheric transport, agricultural and residential runoff, individual misuse and improper disposal [3].

The cyclodienes like endosulfan are persistent insecticides which are stable in soil and relatively stable to the ultraviolet action of sunlight. This organochlorine insecticide, endosulfan is an organic compound with chlorine atoms attached to the ring structures. The Chlorine atoms prevent the organic compounds from being rapidly degraded in the environment, thus this pesticide is "persistent" and is active for long periods of time after application. They are neurotoxicants that have effects similar to those of DDT and HCH. Endosulfan is particularly neurotoxic to both insects and mammals, including humans. It was classified by the United States Environmental Protection Agency (US EPA) as Category Ib: "highly toxic", based on an LD_{50} of 30 mg/kg for rats [4], while the World Health organization (WHO) puts it in Class II "moderately hazardous", based on an LD_{50} of 80 mg/kg for rats [5]. Many *in vitro* studies have demonstrated the oestrogenic activity of endosulfan and have concluded that endosulfan is an endocrine disruptor [6, 4].

Fish is usually affected by toxicants in aquatic environment. The moment effects on the exposed fish is well pronounced, abnormal behaviors such as incessant gasping for air, backward swimming and secretion of mucus on the skin of fish would set in [7]. Fafioye [8] reported erratic swimming behavior at different concentrations of toxicants in which fresh water fish species is exposed to polluted water.

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Mortality is obviously not the only end point to consider and there is growing interest in the development of behavioral markers to assess the lethal effects of toxicants on fish. Behavior is considered a promising tool in ecotoxicology [9]. A wide range of chemical pollutants is more toxic to fishes at low levels of dissolved oxygen than at higher levels [9]. Low level of dissolved oxygen may increase toxicity by increasing ventilation rates which increases the flux of toxicant to the gill epithelium, a major site for respiration. Invariably, toxicants affect the oxygen consumption of fish and their initial response to effect may be an increase or decrease in rate of opercula movement. This is dependent on nature of the toxicants since some could be depressants and some stimulant [8]. Akinbulumo [10] reported slow opercula movement and settling at the bottom motionless in *Oreochromis niloticus* exposed to acute toxicity.

Bioassays are used to determine the toxicity of chemical substances and to indicate which organisms are the most sensitive to such chemicals [11]. These data are used to rank chemicals, determine their water quality criteria and set standards for effluent discharges [12].

The objective of this study is to examine the acute toxicity of endosulfan to *Clarias gariepinus* so as to ascertain their level of tolerance and their suitability as bio-indicator in freshwater ecosystems.

MATERIALS AND METHODS

A 96 hour static bioassay was conducted in the laboratory of the Department of Wildlife and Fisheries Management, University of Ibadan, Ibadan, Nigeria between August, 2010 and February, 2011 to determine the acute toxicity of endosulfan to fingerlings of *Clarias gariepinus*. The pesticide was obtained from the stocks available at Agro chemicals sales and distribution centre, Ogunpa market, Ibadan, Nigeria. The fish used for the study were obtained from Folabex Fish Farm, Agodi, Ibadan, Nigeria and were transported in 50 litres jerrican in the early hours of the morning to the study site. The experimental fish were randomly distributed into 18 aquaria of 30 liters capacity each. The fish were acclimated for 21 days in rectangular plastic aquaria prior to tests. During the acclimation, 3 quarters of the test water were changed daily by siphoning out the spent water. The tanks were monitored daily for fish mortality. The experimental fish were fed twice daily with 2mm commercial pelleted feed of 45% crude protein purchased from Adoms Ltd Ibadan, Nigeria. Unconsumed feed and faecal wastes were siphoned daily with a rubber hose and the water replenished regularly as recommended by Oyelese and Faturoti [13]. Feeding was stopped 24 hours before the commencement of the experiments.

The water used for the definitive test was made free of chlorine by exposing it to air for 24 hours. The experimental set-up consists of 18 circular plastic tanks with various concentrations of pesticides in dechlorinated tap water.

Preliminary screening test otherwise called the range finding or limit test was carried out to allow for comparison of chemicals and provide data with which a definitive test can be based.

The range finding test was carried out according to the method described by Odiete [14] and percentage mortality in each concentration was recorded every 3 hours for 24 hours. Concentrations of pesticides which caused fish death within 30 minutes were omitted from the test. 10 litres of water was measured into each glass aquarium representing each replicate. A factor of 2.2 was used as recommended by Reish and Oshida [15] to obtain the final concentration used for the definitive test which lasted for 96 hours. The concentrations used for the definitive test are: 0.0 (control), 1.0, 2.2, 4.8, 11.0, 23.0 μg/l.

In each treatment, 10 fingerlings of *C. gariepinus* were distributed randomly into each tank with known concentration of pesticides. The fish were subjected to photoperiod of 12 hours light and 12 hours darkness. Observations for behavioral responses of the fish to the toxicant such as loss of equilibrium, changes in swimming behavior, respiratory malfunction, pigmentation and death were done in each replicate. The fish were considered dead when respiratory movement of gills (opercula movement) stopped. Mortalities were confirmed when fish do not react to touch stimuli. The dead fish were removed during the experiment and the mortality recorded at 3 hour intervals until 96 hours.

Physico-Chemical Parameters: Comprehensive analyses of six important physico-chemical parameters (pH, temperature, dissolved oxygen, nitrite, nitrate and ammonia) were carried out before, during and after the definitive test. Temperature and pH values were determined in the laboratory using a mercury-in-glass thermometer and a Griffin pH meter (Model 400) respectively, while dissolved oxygen, nitrite, nitrate and ammonia were determined according to Boyd [16].

Statistical Analyses: The experimental design was completely randomized design (CRD) with six treatments representing the five different concentrations (1.0, 2.2, 4.8, 11.0, 23.0 μg/l) of toxicant, endosulfan and the control. The set-up was replicated thrice with each experimental

tank having 10 fingerlings of *C. gariepinus,* which were randomly assigned into each glass aquarium. Each test concentration was converted into a logarithm and the corresponding percentage mortality was transformed into probit [17]. The median lethal toxicity (LC_{50}), median lethal time (LT_{50}), minimum lethal concentration and minimum lethal time were determined according to the method described by Finney [12]. Analysis of variance (ANOVA) was used to test for significant differences in the number of survivors in different concentrations of the toxicants (endosulfan).

RESULTS

The results of the physico-chemical qualities of experimental units before, during and after the experiment (that is 96 hours) is presented in figures 1-6. pH and dissolved oxygen decreased with increase in the concentration of the toxicant while temperature, nitrite, nitrate and unionized ammonia increased with increase in the concentration of toxicant. During the experiment, pH and dissolved oxygen varied from 5.03-7.00 and 4.12-5.20 mg/l respectively. The lowest values of temperature (24.90°C), nitrite (0.33 mg/l), nitrate (0.45 mg/l) and unionized ammonia (0.34 mg/l) occurred in the control while the highest values (temperature, 26.52°C; nitrite, 4.10 mg/l; nitrate, 14.20 mg/l and unionized ammonia, 33.3 mg/l) was recorded in treatment with the highest concentration (0.023 mg/l) of endosulfan.

The 24, 48, 72 and 96 h median lethal concentration (LC_{50}) of endosulfan to *Clarias gariepinus* are 16.22, 4.68, 2.45 and 2.09 µg/l respectively (Table 1). The coefficient of determination (r^2) between log concentration of the toxicant (endosulfan) and probit mortality (Fig. 7) showed that there were strong and positive correlations between concentration of endosulfan and mortality values for 48, 72 and 96 h. The coefficient of determination (r^2) for 48, 72 and 96 h are ($r^2 = 0.90$; N = 5; $\alpha = 0.05$); ($r^2 = 0.93$; N = 5; $\alpha = 0.05$); and ($r^2 = 0.95$; N = 5; $\alpha = 0.05$) respectively. The coefficient of determination (r^2) between the concentration of endosulfan and mortality of test organism (*Clarias gariepinus*) at 24 hour was weak ($r^2 = 0.33$; N = 5; $\alpha = 0.05$) although, it was also positive. Table 2 shows the number of survivors of *C. gariepinus* exposed to different concentrations of endosulfan. The number of survivors in each concentration differ significantly ($p<0.05$) from others except for concentrations of 11.0µg/l and 23.0µg/l.

Table 1: Median lethal concentrations (LC_{50}) of endosulfan to African catfish (*Clarias gariepinus*)

Time (HR)	LC_{50} (µg/l)
24	16.22
48	4.68
72	2.45
96	2.09

Table 2: Survivors of African catfish (*Clarias gariepinus*) exposed to different concentrations of endosulfan.

Concentration of Endosulfan (µg/l)	No of survivors (Mean±SD)
0.0 (Control)	10.00 ± 0.00^{a}
1.0	8.33 ± 0.33^{b}
2.2	5.67 ± 0.33^{c}
4.8	2.00 ± 0.58^{d}
11.0	0.00 ± 0.00^{e}
23.0	0.00 ± 0.00^{e}

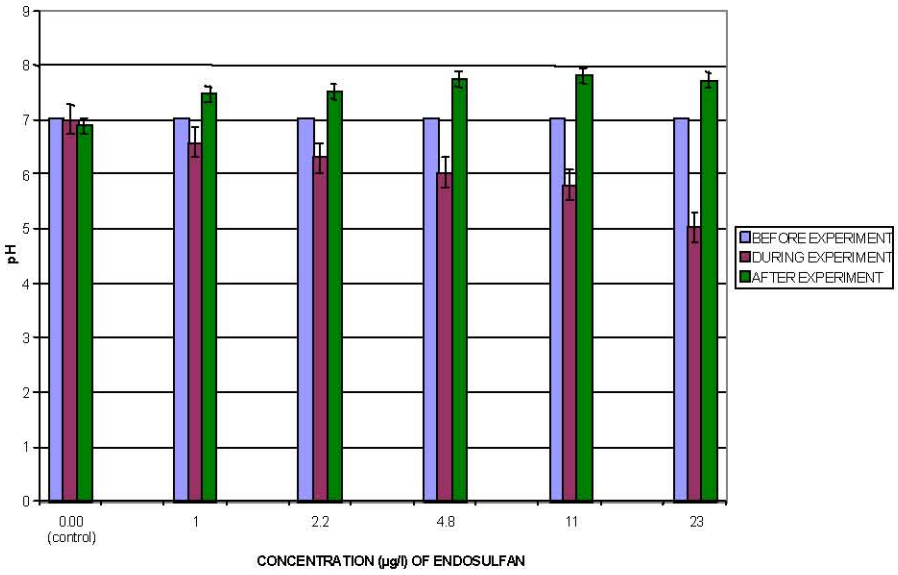

Fig. 1: pH values before, during and after exposure of *C. gariepinus* to various concentrations of endosulfan.

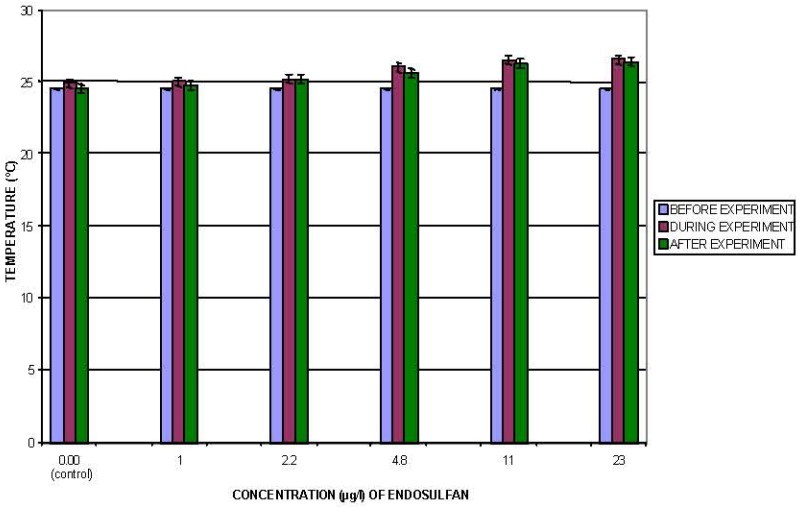

Fig. 2: Temperature values before, during and after exposure of *C. gariepinus* to various concentrations of endosulfan.

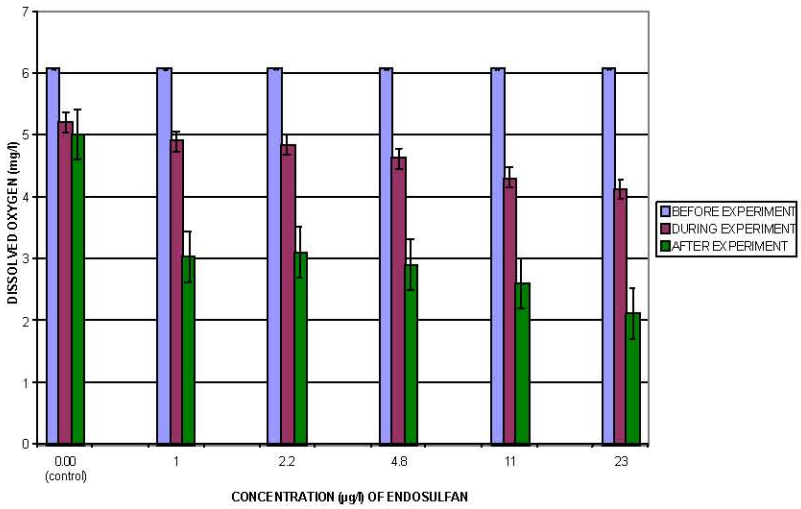

Fig. 3: Dissolved oxygen values before, during and after exposure of *C. gariepinus* to various concentrations of endosulfan.

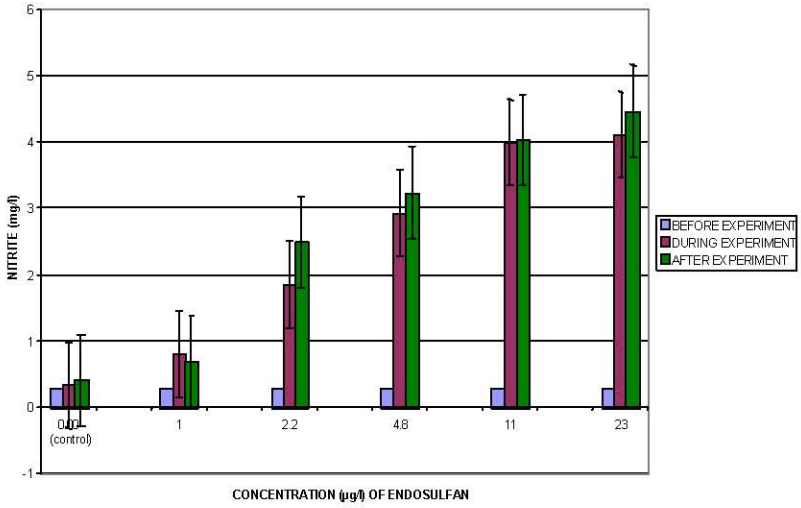

Fig. 4:Nitrite values before, during and after exposure of *C. gariepinus* to various concentrations of endosulfan.

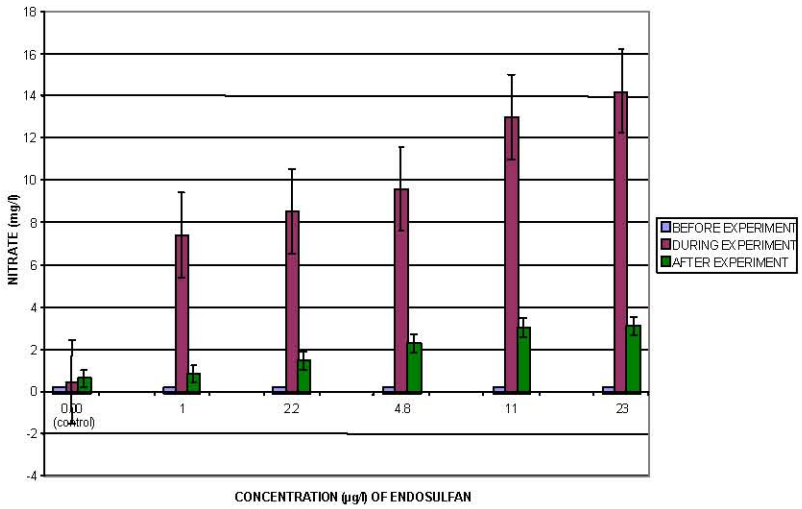

Fig. 5: Nitrate values before, during and after exposure of *C. gariepinus* to various concentrations of endosulfan.

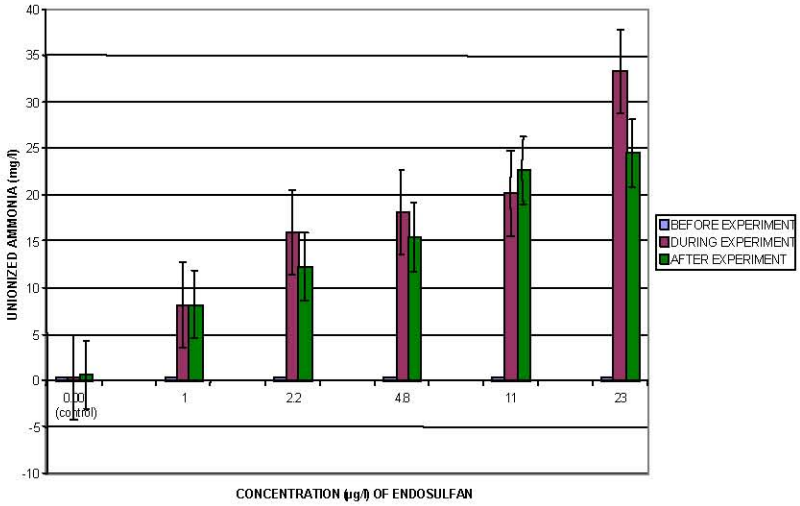

Fig. 6: Unionized ammonia values before, during and after exposure of *C. gariepinus* to various concentrations of endosulfan.

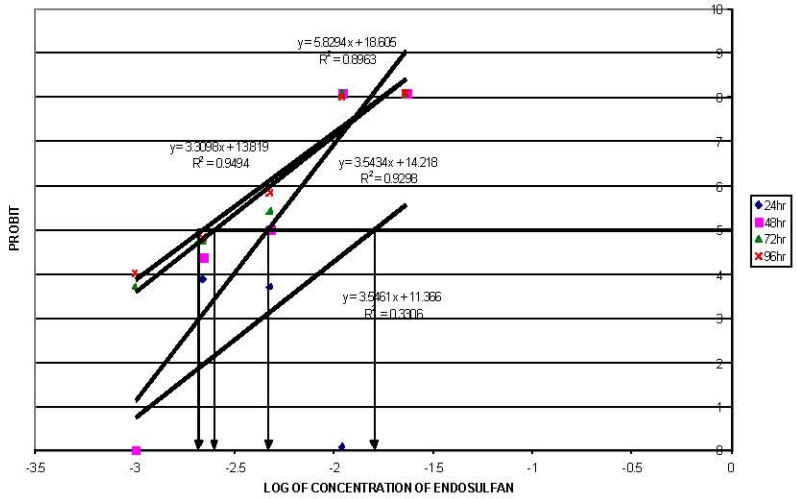

Fig. 7: Median Lethal Concentration (LC_{50}) of endosulfan to *Clarias gariepinus*.

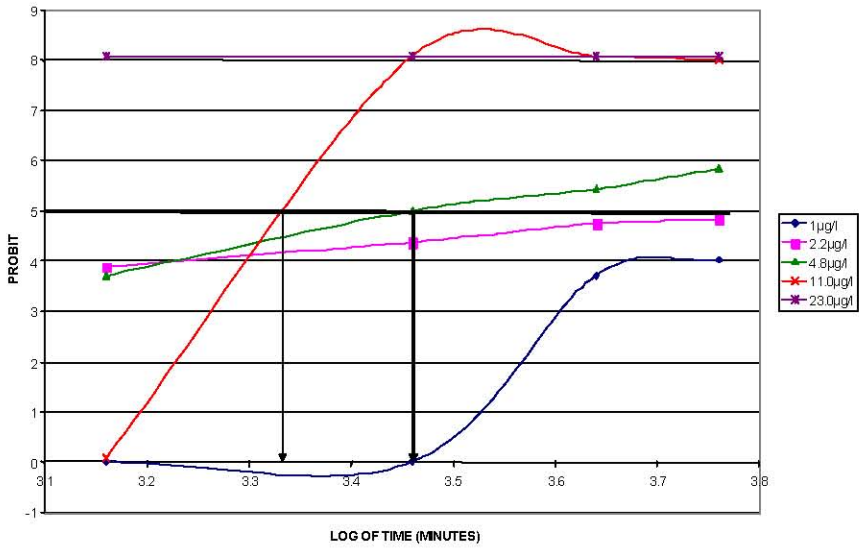

Fig. 8: Median Lethal Time (LT_{50}) of endosulfan to *Clarias gariepinus*

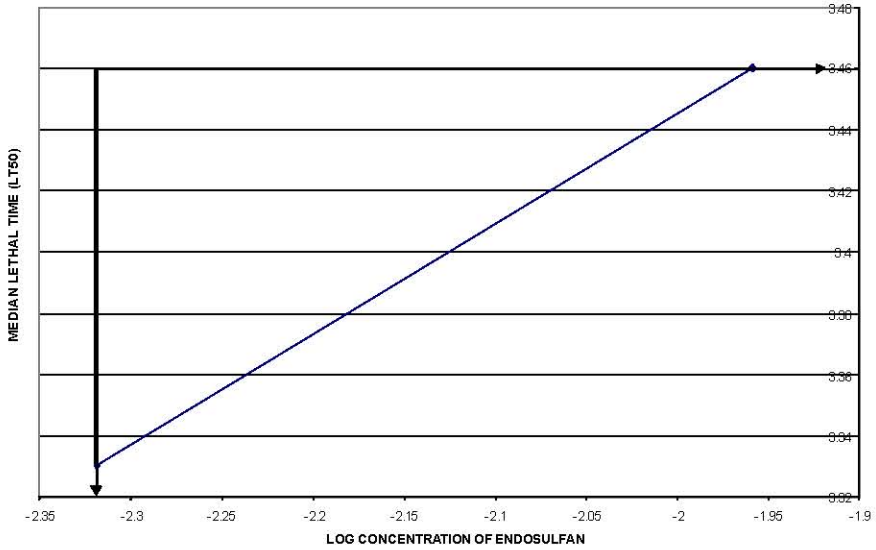

Fig. 9: Minimum lethal concentration and minimum lethal time of endosulfan to *Clarias gariepinus*

The median lethal time (LT_{50}) of 1.0 and 2.2µg/l of endosulfan is zero (Fig. 8) indicating that at these concentrations of endosulfan, the mortalities of the test organism (*Clarias gariepinus*) were less than 50%. However, for endosulfan concentration of 2.3µg/l, the curve of probit against log of time was above the 50% mark, indicating that at this concentration, the percentage of test organism that would be killed even within 24 hours is more than 50%. The mean lethal time of 4.8 and 11.0 µg/l are 2138 min (~37 hrs) and 2884 min (~48 hrs) respectively (Fig. 8). The minimum lethal concentration was 4.79 µg/l while the minimum lethal time was 2884 min (~48 hrs) (Fig. 9).

DISCUSSION

The observed behavioural changes are directly proportional to the concentration of the toxicant as the fishes became inactive at higher concentrations with increased time of exposure to the toxicant. Erratic swimming and excessive jumping observed in the fish on immediate exposure to the toxicant especially in tanks with 11.0 and 23.0 µg/l of endosulfan could be due to skin irritation, respiratory rate impairment or a response to altered locomotor activity which is an indication of the effect of toxicant on the nervous system as was reported by Ayoola [18]. Erratic swimming and settling at

the bottom of the tank and subsequent immobilization before death observed in the study agree with the reports of Omitoyin *et al.* [7], Fafioye [8] and Adesina [9] who reported erratic swimming behavior at different concentrations of toxicant when freshwater fish were exposed to the toxicants. The stressful behavior exhibited by the fish may be attributed to the effect of the toxicant on the gill which agrees with the report of Jenyo-Oni *et al.* [19]. Several authors have reported similar patterns of abnormal behavioral responses in fish exposed to toxicant [20-23].

The physico-chemical parameters of the experimental water studied revealed a significant response to the effect of the toxicant. A decrease in dissolved oxygen after the addition of the toxicant and further decrease at the end of the experiment compared to the control (Fig. 1-6) was identified. This is in consonance with earlier report by Warren [24] that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration, which will impair respiration leading to asphyxiation. The decrease in the dissolved oxygen during the experiment could be as a result of the chemical reaction between endosulfan and the experimental water as the toxicant is very soluble in water or the increase in respiration as a result of stress induced by the toxicant. This is similar to the report of Adesina [9] and Omitoyin *et al.* [7] that increased ventilation leads to the flux of toxicants to the gill epithelium which is a major site for absorption. Moreover, Adesina [9] also reported that a wide range of chemical pollutants are more toxic to fishes at low dissolved oxygen. In the same vein, Rahmann *et al.* [20] stated that various abnormal behavioral patterns displayed by fish during toxicity bioassay indicated that the cause of death might be due to insufficient supply of oxygen.

The increase in temperature observed during and after the experiment as the concentration of the toxicant increased could be as a result of increased metabolic activity and osmoregulatory mechanisms exhibited by the fish in an attempt to counter the effect of the toxicant [25, 26]. The nitrate, nitrite and ammonia increased significantly during the experiment. These are as a result of the reduction of the dissolved oxygen which plays a major role in fish survival, growth and development [16].

No mortality was reported in all the control tests in this study, while varying degrees of mortality were reported in the test concentrations. This is a clear indication that the effects of the pesticide (endosulfan) could be regarded as possible cause of death of the test organism. The results clearly indicate that endosulfan varied greatly in their effects on survival of *Clarias gariepinus*. The highest mortality was found at the highest concentrations, suggesting dose-dependent survival and concentration graded lethality. The 96hr-LC_{50} of endosulfan obtained in this study using the probit method was 2.09 μg/l. Comparing this value with the standard set by United States Environmental Protection Agency (US EPA), puts endosulfan as very highly toxic (LC_{50}<100μg/l) [4] to *Clarias gariepinus*. These values are similar to the findings of Richard [27] who reported LC_{50} of 1.42 μg/l for *Oreochromis niloticus* exposed to different concentrations of endosulfan. Nowak and Sunderam [28] also reported LC_{50} values of 2.0 μg/l at 30°C and 4.6 μg/L at 35°C when mosquito fish was exposed to technical grade endosulfan. Smith [29] reported LC_{50} for rainbow trout to be 1.4 μg/l. However, some studies have also reported values lower or higher than the values obtained in the present study. Richard [27] reported an LC_{50} value of 0.32μg/l for *Tilapia zilli* while Werimo and Seinen [30] reported 10.20μg/l for *Oreochromis niloticus*.

Other aquatic organisms have also been tested with endosulfan with similar levels of reported toxicity. Endosulfan 96 hr-LC_{50} studies have been done on crustaceans, including amphipods and crayfish. Static 96 hr-LC_{50} values for the amphipods (*Gammarus palustris*) [31] and *Hyalella azteca* [32] are 0.43 and 5.7 μg/l, respectively, whereas the static LC_{50} value for the red swamp crayfish (*Procambarus clarkii*) is 120 μg/l [31]. The cause of the variation in toxicity of endosulfan may be due to differences in testing protocols [3] and/or differences in susceptibility and tolerance related to its accumulation, biotransformation and excretion [7]. For instance, Harris *et al* [33] used static renewals in which endosulfan concentrations were renewed every 24 hr, whereas Gopal *et al* [34] used static tests in which replicates were dosed with endosulfan at the start of the experiment but the concentration was never renewed. Differences in metabolic pathways among species may result in different patterns of biotransformation, leading to more or less toxic metabolites [35]. The magnitude of toxic effects of pesticide also depends on length and weight, corporal surface/body weight ratio and breathing rate [36]. Metabolic differences between different animal classes may also be responsible for differential toxicity of chemicals.

CONCLUSION

The purpose of acute toxicity test with fish species under laboratory conditions is to help in the assessment of possible risks to similar species in natural environments. It also aids the determination of possible water quality criteria for regulatory purposes and for use in correlation with acute testing of other species for comparative purposes. It was established through this study that short-term exposure to endosulfan resulted in negative alterations in behavior and mortality. These indicate that endosulfan is highly toxic to fingerlings of *Clarias gariepinus*. Therefore, the use of endosulfan insecticide on or near fish farms or in area close to aquatic environment should be discouraged.

ACKNOWLEDGEMENT

The authors are very grateful to the Head, Department of Wildlife and Fisheries Management, Faculty of Agriculture and Forestry, University of Ibadan for providing logistics for the successful conduct of the research.

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