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Study of the Physiochemical changes analysis of endosulfan on Indian cat fish *Heteropneustes fossilis*

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Abstract
The effect of various concentrations of endosulfan, an organochlorine pesticide on the haematological and biochemical changes of *Hetrobranchus bidorsalis* was investigated in static bioassay under laboratory conditions. The concentrations the product used were 0.05, 0.10, 0.20, 0.30 and 0.0 (control) ml/L. The result of water quality parameters showed there were no significant difference (P>0.05) between the test media and the control. Abnormal behaviour such as imbalance position, secretion of mucus, erratic swimming, respiratory distress flashing and lethargy were observed before death during the exposure period. The exposure leads to a decrease in the levels of erythrocytes, leucocytes, haemoglobin, total protein and an increase in alkaline phosphatase, total bilirubin and plasma glucose when compared with the control. The implications of the findings in relation to the exposed fish and the aquatic environment are discussed.

Keywords: endosulfan, haematology, biochemical, toxicity, *Clarias gariepinus*

Introduction
With rapid global economic development and need for more food production, pollution from agricultural practices through pesticides remain a major threat to aquatic ecosystem. Highly effective pesticides are used indiscriminately, which result in aquatic pollution on entering the aquatic environment and brings multiple changes in organism by altering growth rate, nutritive value, and behavoural pattern of the fish (Ramesh and Saravanan, 2008, Gaafer *et al.*, 2010) [11, 7]. Endosulfan (6,7,8,9,10-hexachloro-15,5,9,9a-hexahydro-6,9-methano- 2,4,3-benzodioxathiepine-3-oxide) is an organochlorine compound introduced in the 1950's with a blood spectrum effects against insects and ants in agriculture and allied sectors (Robert and Mat Calf, 2008) [14].
Kay (2006) reported that acute toxicity exposure to endosulfan produces symptoms like hyperactivity, convulsions, staggering, difficulty in breathing, nausea and vomiting, diarrhea and unconsciousness.
Widespread application of various pesticides has aggravated the problem of pollution to aquatic environment. Silva and Gammon (2009) noted that runoff of pesticides like endosulfan from agricultural field killed fish in various parts of the world. Repeated exposure to sub lethal dose of some pesticides can cause physiological and behavioural changes in fish and reduces their population such as its abandonment of nests and broads decreased immunity to disease and increased failure to avoid predators. Erickson *et al.* (2008) [6] observed that the breakdown particles of these pesticides can be 10 – 100 times more toxic to fishes than the original pesticides. Tripathi and Verma (2004) documented that the exposure of sub lethal concentration of endosulfan decreased the activities of citrate sythase (CS) and glucose 6-phosphate dehydrogenase (G6-PDH) in the brain, liver and skeletal muscle of the freshwater fish catfish, *C. batrachus.*
Toxicity of endosulfan to non- target animals have reported thoroughly by Naqvi and Vaishanavi (1993) [9] and fishes are extremely sensitive to endosulfan and mortality has been reported a number of time as a result of the pesticide leakage into river. WHO(1986) [17] reported that endosulfan is a central nervous system (CNS) poison and had gain significance as a potential environmental pollutant due to its wide spread use in agricultural pests. In this study the effects of endosulfan on an economically freshwater fish *Heterobranchus bidorsalis* was investigated on static condition.

Materials and Methods

Juveniles of *Hetrobranchus bidorsalis* of the same brood stock were collected from Louis Fish Farms Warri, Delta State, Nigeria. They were transported to Applied Biology and Biotechnology Research Laboratory, Enugu State University of Science and Technology with the aid of oxygenated bag. The fish were held in plastic tanks and acclimated for a period of two weeks and were fed with laboratory formulated feed.

The concentrations of the endosulfan used were 0.05, 0.10, 0.20, and 0.30mlL^{-1} while de-chlorinated tap water served as control (0.00). For the experimental set up, fifteen plastic tanks of 90 litres capacity were used comprising five treatments replicated thrice in order to minimize experimental error. Each tank was stocked with ten fish. The tanks were examined on a daily basis and dead fish were removed and recorded immediately from the solution to avoid polluting the test media. Physicochemical analysis of the test water such as temperature, dissolved oxygen, alkalinity, pH and free carbon dioxide were determine using analytical methods in APHA (1995) [4].

For blood analysis, the caudal peduncle was cut; blood was collected in heparinized tubes. Total red blood cell count was determined according to the classical method using the Neubauer haemocytometer. Haemoglobin was estimated colourmeterically according to the method of Maile (1972). The plasma glucose fish was determined using the anthrone method as modified for use in fish tissues by Wedemeyer and Yasutake (1977). Alkaline phospatase, total protein total bilirubin was done according to method mention by the kits manufacturer.

The significance of the sample means between the control and the endosulfan treated fish was tested by using student's t test.

Results

The result of the values of water quality parameter monitored in represented in Table 1. The result indicates that dissolved oxygen ranged between 4.98 to 6.25mg/l, temperature and pH ranged between 25 to 26^{0}C and 6.65 to 7.88 respectively. There was no significant difference (P>0.05) between the means of the values recorded. Fish exposed to various concentrations of the endosulfan exhibited some abnormal behaviour changes like respiratory distress, erratic swimming, loss of equilibrium and secretion of mucus in the body among others. The result of haematological and biochemical examination of the fish is presented in Table 2. The result shows there was a reduction in the levels of erythrocytes, leucocytes, haemoglobin, and total protein while alkaline phosphatase, total bilirubin and plasma glucose increased when compared with the control.

Discussion

The result of water quality parameter recorded in this investigation is within the range for the survival of fresh water fish (Relyen 2009).The changes in the haemalotogical and biochemical parameters as observed may be attributed to the toxic effects of the endosulfan on the fish. More so, several abnormalities in behaviour of test fish such as imbalance position, restlessness in movement, erratic swimming, tremor, flashing and lethargy etc. are similar to the observations of (Hoque and Omotoyin 1998; Omoregie *et al.*, 2009; Akpa *et al.*, 2010) [8, 10, 1]. Respiratory irregularities noticed in the exposed fish could have been caused by mucus precipitation on the gill epithelia response to the toxicant which resulted in abnormal behaviour as earlier reported by Banerjee (2007) [5].

Table 1: Mean values of water quality parameters recorded during the exposure of endosulfan to *Heterobranchus bidorsalis* for 96 hours.

Parameters	Minimum values	Maximum values
Dissolved oxygen(mg/l)	4.98±0.04	6.25±0.05
pH	6.65±0.08	7.88±0.07
Free carbon dioxide(mg/l)	1.22±0.05	1.42±0.04
Temperature(^{0}C)	25.00±0.01	26.00±0.02
Alkalinity(mg/l)	21.18±0.02	22.20±0.02

In this present investigation, the haematological changes showed a significant decrease in the haemoglobin, erythrocyte and leucocytes. The reduction in haematological values indicated anaemia in the pesticides exposure due to an increase in the rate of erythrocyte destruction in haematopoietic organs (Seth and Saxena 2003) [16]. This revealed the prominent anaemic effects of the blood indices which in turn revealed the haemolysing power of endosulfan. The haemolytic effects and destructive effects of pesticides on blood cells were supported by (Robert, 2001; Al-Atar, 2005) [13]. Low-Jinde and Niimi (1986) stated that the anaemia was probably not due to increased destruction of erythrocytes since the spleen size was unchanged but might be due to a decrease in the synthesis or release of erythrocytes into the circulatory system. The decrease in the haemoglobin content in this study result from rapid oxidation of haemoglobin to methaemoglobin or release of oxygen radical brought about by the stress of toxicant (Ramsesh and Saravanan, 2008) [11]. The haematological indices (red blood cell count) concentration of haemoglobin has been reported to indicate secondary responses of an organism to irritant. Rogers *et al.* (2003) [15] noted that mechanism of toxicant occurs by ion regulatory disruption. The decrease in the total protein values as observed may be due to the severity of the endosulfan which causes osmotic imbalance in the fish. Alkahem *et al.* (1998) [2] attributed the reduction in the protein to its conversion to the fulfilling an increase energy demanded by the fish to cope with detrimental condition imposed by the toxicant. The significant increase the level of alkaline phosphatase agrees with findings of Daadees *et al.* (1992) who supported the hypothesis that increase in serum transaminase may reflect hepatic toxicity which leads to extensive liberation of the enzymes into the blood circulation. The increase in plasma glucose reported in this investigation indicated that *Heterobranchus bidorsalis* exposed to endosulfan pesticide has become hyperglycaemic. Wedemeyer (1973) observed that fish show a marked hyperglycaemic response to stressful environmental conditions as a result of incomplete metabolism of the blood sugar.

Table 2: Changes in haemaological and biochemical parameters in *Heterobranchus bidorsalis* exposed to endosulfan for 96 hours.

Parameters	Control	Experiment
Erythrocytes (10^6/ml^{-1}	2.34±0.45	1.53±0.33
Leucocytes (10^3/ml^{-1}	30.60±0.05	19.32±0.06
Haemoglobin (g/dl)	12.41±0.55	8.09±0.52
Plasma glucose(mg/100ml	0.85±0.07	1.88±0.08
Alkaline phosphatase (i.u/l)	10.32±0.06	18.48±0.03
Total bilirubin (mg/100l)	0.92±0.01	0.94±0.02
Total protein(g/dl)	4.45±0.03	2.850.05

Q``5The reports from investigation revealed that endosulfan causes drastic reduction in the haematolgical and biochemical indices of *Heterobranchus bidorsalis* juveniles. Indiscriminate uses of

endosulfan in agriculture should be avoided in order to reduce the adverse effect it may cause in aquatic biota.

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