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Size Related Changes in Enzyme Activities in Heterobranchus longifilis Exposed to Dimethoate in the Laboratory

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Abstract

Variations in enzyme activities in two sizes of Heterobranchus longifilis exposed to dimethoate in the laboratory were assessed. Two sizes of H. longifilis comprising of 60 each of juveniles (mean total length 14.77 cm ± 3.09 SD; mean weight 250.99g ± 11.07 SD) and adult (mean total length 24.07 cm ± 11.06 SD; mean weight 780.03g ± 21.99 SD) were treated with different concentrations of dimethoate (0.00-control; 0.25; 0.50;0.75;1.00) for a period of 15 days. The blood plasma collected from the fish was analyzed for five different enzymes namely: aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH). The results indicated a concentration dependent increase in all the enzymes above the control value, with a peak at 1.00mg/l of the chemical. The study suggests that sublethal level of dimethoate concentrations could cause enzyme imbalance in the tissue of the exposed fish. The enzymes from the blood plasma of the exposed fish have shown clearly that the chemical dimethoate is capable of inducing adverse effects and impacting on the health of fish. Therefore, the presence of this chemical in aquatic environment could be hazardous to fish and invariably inimical to human health.

Key Words: Aquatic Environment; Toxicants; Plasma Enzymes; Dimethoate; Fish; Aquatic Pollution

Introduction:

Various concentrations of contaminants in the aquatic environment are believed to have some levels of noxious consequence on fish enzymes, and general physiological status of exposed fish [1]. In recent years, enzyme activities variables are being used in fish when clinical diagnosis is carried out to determine the effects of external stress and toxic substances as a result of the close association between the circulatory system and the aquatic environment [2] Moreover, Gabriel et al. [3] also suggested that biochemical changes such as enzymes profiles can be used in determining the toxicity of pollutants in fish. This is because biochemical evaluation of environmental pollution using enzymatic profiles in fish are responsive as timely notification of short- or long-term harmful consequences of toxicants in aquatic environment [4].

Pesticides are commonly used in agricultural field operations to improve quality and quantity of food products [5]. The unhampered and unhindered use of synthetic chemical pesticides by farmers in different parts of the country, have resulted in various damaging effects such as bad odour of water and deadly consequence on different non-target organisms in aquatic environment [6]. In recent times, divers eco-friendly methodologies for pest control which include biopesticides, integrated pest management system, application of plant based biopesticides and other natural biodegradable methods are being utilized for pest management system in different parts of the world [7], Conversely, most farmers in both rural and urban areas in developing countries still depends largely on the usage of chemical pesticides in their farming operations [8], because it is cheap, easy to applied and readily available [9].

Most of the aquatic organisms normally bio accumulate different degrees of toxicants in various forms from the surrounding water [10]. Serious pollution of aquatic environment by synthetic herbicides in most cases results in chronic and acute destruction of fish and other organisms [11]. In the system of the fish, the applied chemicals can impair the skeletal system, damage some important organs and cause biochemical modifications of

the exposed fish [12]. However, the minimal solubility of these chemicals enhances their high level of their deposit in different species of fish and finally bioaccumulation in human body on consumption of contaminated fish. In spite of their low concentrations in aquatic environment, pesticides display high toxicity to animals and humans as their occurrence in food raises different safety concerns among the populace [13].

It is a known fact that any category of stress; not resulting in total modification and death of an organism, creates some definite alterations in the fish blood parameters [14]. Conversely, different techniques of scientific diagnosis have been used in fish ecology to evaluate the resultant effects of contaminants [15]. Furthermore, Nte et al. [16] observed that plasma enzyme activities have been utilized expansively to give uncomplicated precise values of organ dysfunction in aquatic animals especially fish. Furthermore, enzymes have been described as catalysts that lower the activation energy of a reaction, that is, the required amount of energy needed for a reaction to occur. They do this by binding to a substrate and holding it in a way that allows the reaction to happen more efficiently [17]. They also serve as indicators of altered physiological or stress condition in fish. Moreover, enzyme activities have been used to demonstrate tissue damage in fish. Hence, in the present study, the degree of enzyme responses of an economically important fish, H.longifilis to dimethoatein the laboratory toxicity was investigated.

Materials and Methods:

Experimental Location and Fish:

The study was carried out in Aqua Green Integrated Aquaculture Center, Eliozu, Port Harcourt, Rivers State, Nigeria. A total of 120 H.longifilis comprised of 60 each of juvenile and adult size were sourced from a fish farm in the State. The fishes were transported in six open 50l containers to the laboratory and acclimated for a period of seven days.

Preparation of Test Solutions and Exposure of Fish:

Dimethoate, used in this experiment was purchased from a commercial outlet in Port Harcourt, Nigeria. H.longifilis were exposed to the chemical at the concentrations of 0.00 control, 0.25, 0.50, 0.75 and 1.00 mg/L in triplicates. Four fish were randomly distributed into each test tank. The experiment lasted for a period of 15 days. The water in the tanks was exchanged on daily basis. The fish were fed two times in a day at 3% body weight with Vital fish feed.

Evaluation of Physico-Chemical Parameters of Water in Experimental Tanks:

During the exposure period which lasted for 15 days, some water quality parameters namely pH, dissolved oxygen, temperature and ammonia were taken daily using the methods described by APHA [18].

Enzyme Analysis:

At the end of experimental period, 2ml of fresh blood sample was collected by making a caudal puncture with the help of needle and poured in heparinized sample bottles. Blood samples were centrifuged immediately for 15 minutes at 5000 rpm. Plasma specimens were separated, pipetted into eppendorf tubes and stored in a refrigerator at -20°C until assayed [19]. The results were read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405). Five enzymes namely, Aspartate amino transaminase (AST), Alanine amino transaminase (ALT), Alkaline phosphatase (ALP), Acid phosphatase (ACP) and Lactate dehydrogenase (LDH) were analyzed in the blood of the exposed H.longifilis. The Reitman and Frankel [20] method was used to analyze AST, because it can be performed as a manual colorimetric end-point technique. While, ALP, ACP and LDH was done by method described by Huang [21].

Statistical Analysis:

All the data were expressed as mean and standard deviation of mean. The statistical package, SPSS Version 22 was used for the data analysis. The means were separated using two ways ANOVA and the two means were considered significant at 5 %($P<0.05$).

Results:

The water quality parameters (Table 1) were within the same range except in DO, where a lesser value was obtained at higher concentration of the chemical. The effects of dimethoate on the enzymes in the plasma of H.longifilis juveniles are presented in Table 2. It was observed that the values of aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) significantly ($P<0.05$) increased with increasing concentrations of the chemical. The same trends were observed in the enzyme profiles of adult fish exposed to the chemical dimethoate (Table 3).

Concentration	DO (mg/l)	Temperature (°C)	pH	NH3 (mg/l)
0.00	5.86±0.65 ^{c}	29.89±2.66 ^{a}	6.67±1.19 ^{a}	0.02±0.00 ^{a}
0.25	5.41±0.48 ^{c}	29.90±3.52 ^{a}	6.00±1.88 ^{a}	0.02±0.00 ^{a}
0.50	4.39±0.82 ^{b}	29.81±2.04 ^{a}	6.64±1.81 ^{a}	0.02±0.00 ^{a}
0.75	4.10±0.99 ^{b}	29.99±3.99 ^{a}	6.65±0.88 ^{a}	0.02±0.00 ^{a}
1.00	3.45±0.78 ^{a}	29.98±5.71 ^{a}	6.66±0.71 ^{a}	0.03±0.00 ^{a}

Means within the same column with different super scripts are significantly different ($P<0.05$).

Table1: Physico-Chemical Parameters of Water in Experimental Tanks of H.longifilis Exposed to Dimethoate.

CONC	AST	ALT	ACP	ALP	LDH
0.00	71.48±1 1.99^{a}	51.02± 12.51 ^{a}	25.77± 9.51 ^{a}	65.02± 8.64 ^{a}	310.00± 21.00 ^{a}
0.25	74.22±1 1.02 ^{a}	58.66± 11.01 ^{a}	27.90± 8.29 ^{a}	67.07± 9.73 ^{a}	334.66± 28.08 ^{a}
0.50	80.42±1 2.51 ^{b}	69.33± 13.52 ^{b}	32.33± 7.98 ^{b}	78.02± 9.88 ^{b}	361.66± 34.45 ^{b}
0.75	85.02±1	79.56±	43.95±	79.66±	364.33±

	2.67 [b]	11.09 [b]	7.00 [c]	9.55 [b]	40.79 [b]
1.00	99.22±1 0.16 [c]	82.99± 14.77 [c]	48.00± 8.09 [c]	87.07± 8.04 [c]	390.99± 41.59 [b]

Means within the same column with different super scripts are significantly different (P<0.05).

Table2: Enzymes Activities in Juveniles of H.longifilis Exposed to Dimethoate (Mean±SD).

CONC	AST	ALT	ACP	ALP	LDH
0.00	81.24±2 1.09 [a]	65.00± 19.00 [a]	30.92± 6.71 [b]	73.33± 13.09 [a]	350.82± 29.16 [a]
0.25	91.65±2 6.02 [b]	78.66± 21.52 [b]	37.90± 7.29 [a]	78.66± 15.69 [a]	374.51± 28.08 [a]
0.50	95.89±2 2.11 [b]	83.33± 24.52 [b]	42.61± 8.01 [b]	87.00± 13.67 [b]	381.71± 24.72 [b]
0.75	106.73± 19.04 [b]	87.00± 23.39 [b]	47.99± 7.00 [b]	95.66± 10.57 [c]	394.61± 24.02 [b]
1.00	118.98± 32.55 [b]	91.00± 22.77 [c]	54.24± 9.99 [c]	97.00± 11.02 [c]	426.99± 25.03 [b]

Means within the same column with different super scripts are significantly different (P<0.05).

Table 3: Enzymes Activities in Adults of H.longifilis Exposed to Dimethoate (Mean±SD)

Discussion:

Evaluations of various enzymes such as aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) are parts of standard laboratory tests to detect abnormalities in animals [22]. Also, studies of enzyme activity in fish can serve as an important biomarker of toxicants exposure and effect in the aquatic environment [23]. Pollutants in the aquatic settings can inhibit and elevate enzymes activities at very specific sites or effects can be stir up by less specific interactions with various cells [24]. In this study, increase in the values of ALT and AST in the exposed fish suggests an increase in energy demand, metabolic pathway and amino acids. The increase in the activities of ALT and AST in H.longifilis exposed to dimethoate agrees with the findings of Nte et al. [16] who reported similar results in Sarotherodon melanotheron exposed to industrial effluents but contradicts that of Luscova et al. [25] in common carp treated with sub-lethal levels of diazinone. An increase in the transaminases suggests that there was tissue damage, the parenchymatous tissue and skeletal muscles were altered.

Variations in ACP and ALP enzymes in fish exposed to dimethoate in the present work resulted in alterations in their activities which agree with the findings of Udeme-Naa and Erondu [26], who reported that when hepatocytes are damaged, enzymes located in cytosol are released into the extra cellular space and enter the circulation due to membrane defects causing increased permeability. Chemical induced alterations in ACP and ALP activities in fish have been reported and this elevation was directly attributed to toxic action of chemical on gill [27]. LDH is a tetrameric glycolytic enzyme and recognized as a potential marker of tissue damage [28]. In the present study the increase in LDH activity in the exposed fish indicate a higher glycolysis rate under pesticide stress. Conversely, chemical application may inhibit the aerobic and anaerobic metabolism of fish resulting in elevated LDH activity. The increase in LDH activity of fish as observed in this work may be due to binding of the pesticides or its metabolites with the enzyme molecule [29]. Moreover, Dimou et al. [30] observed that prevalence of anoxia during stress conditions may lead to an increase in LDH activity in tissue. Hence, the observed elevation of LDH activity in the plasma of the exposed fish may be due to damage of these tissues.

Conclusion:

The exposure of juveniles and adult sizes of H.longifilis to sub-lethal concentrations of dimethoate can induce various toxicological effects in the form of enzymatic degradation. It can be concluded that the presence of chemical in aquatic environment can stimulate enzymatic alterations, which might make fish in polluted environment vulnerable to stress, and eventually lead to death. Therefore, enzyme activities can be suitably used to determine the effect of chemical on the physiology of fish under sub-lethal condition prior to sudden death of the fish exposed to toxicants.

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