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ORGANOSOMATIC INDEX AND BEHAVIORAL RESPONSE OF *HETEROBRANCHUS BIDORSALIS* EXPOSED TO RHONASATE 360SL CONTAINING GLYPHOSATE (ISOPROPYLAMINE SALT GLYCINE)

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ABSTRACT

This study evaluated the organosomatic and behavioral response of Heterobranchus bidorsalis exposed to rhonasate 360SL containing glyphosate (isopropylamine salt glycine). The fish samples were purchased from private fish farm in Okaka, Yenagoa metropolis, Bayelsa state. The fish samples have mean weight of 97.00g and length of 14.02cm. The fish samples were exposed to various concentrations i.e. 0.00, 0.01, 0.02 and 0.03ppm of rhonasate 360SL for 14 days. Behavioral response was checked after the introduction of the toxicant for 2 hours. At the end of the experimental period the fish were dissected and spleen, heat, kidney and liver were obtained and weighed using electrical weighing balance. The study found that the behaviors (i.e. swimming pattern, body pigmentation, surfacing and air gulping, operulcar movement) were altered as the toxicant was introduced. The initial and final condition factor of the fish showed significant variation (P<0.05). Also, there was no significant difference (P>0.05) among the various concentration of the toxicant in each of the organosomatic indices i.e. Renatosomatic, Spleenosomatic and Hepathosomatic studied apart from Cardiosomatic index. This suggested that toxicants i.e. rhonasate 360SL did not significantly increase the organs of fishes. However, caution should be exercise in the use of herbicides such as rhonasate 360SL close to aquatic ecosystem.

Keywords: Fisheries, Herbicides, Pesticides, Toxicity, Water pollution.

1. INTRODUCTION

Globally, the use of pesticides for eradicating, mitigating, repelling and controlling pest in homes and agricultural field has increased. According to Nwani et al. [1], pesticides usage has been acknowledged as part of integral agricultural practices world-wide. The type of pesticides used depends on the pest. Pesticides are also grouped on their target organisms including insects, miticides, larvicides, pediculicides (for controlling insects), fungicides (for controlling fungi and molds), herbicides (for controlling weeds), rodenticides (for controlling rodents) and molluscides (for controlling snails, other mollusks causing disease or inhabiting parasites that cause disease conditions). The group of commonly used herbicides includes chlorophenoxy and bipyridyl compounds, chloroacetanilides, triazines and phosphonomethyl amino acids. The phosphonomethyl amino acids oriented herbicides are majorly grouped into glyphosate and glufosinate

Glyphosate is a major chemical herbicides used to control annual, biannual and perennial weeds, sedges, broad leaved weeds and woody shrubs [2, 3]. According to Inyang et al. [4], Annett et al. [5], glysophate is a post emergent, systemic and non-selective herbicide used in both agricultural and non-agricultural areas. It's widely used in developing countries such as Nigeria. Glyphosate is majorly absorbed into the plant through the leaves and then transported throughout the plant where it acts on plant enzyme system [4].

Glyphosate is an aminophosphoric analogue of the natural amino acid glycine and is commonly formulated in its form of isopropylamine salt [3]. Glyphosate (N-(Phosphoromethyl) glycine (IUPAC) is an acid that belongs to chemical group of N-phosphonomethyl glycine [2, 4, 6].

Typically, glyphosate has low toxicity to life forms in its environment. But it could be highly toxic when it combines with other surfactants based salts in lethal and acute doses [3, 6]. Glyphosate have been implicated in the distortion of physiological functions of the nervous system [3]. However, careless handling, accidental spillage, unsustainable discharge of empty cans of pesticides on the aquatic ecosystem may have adverse effect on the fish composition, abundance, metabolism and physiology

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depending on the dosage/concentration. Also when the herbicides are applied on land, it could find its way to aquatic ecosystem via runoff after precipitation [7, 8].

Several studies have been conducted with regard to toxicity of pesticides on aquatic ecosystem. Some common parameters widely studied in fisheries include biochemical, haematological, electrolytes, enzymes, histological, physiological and behavioral changes. Also, effects of toxicants i.e. herbicides have been widely studied in the Clariidae family. On *Clarias gariepinus*, 2, 4-Dichlorophenoxyacetic acid [7], Fluaxifop-p-butyl [9- 11] has been documented. Similarly, several studies have been conducted on the effects of herbicides i.e. rhonasate 360sl containing glyphosate (isopropylamine salt, glycine) on the enzymes, urea and creatinine of *Heterobranchus bidorsalis* [3], haematological parameters [6], enzymes and electrolytes [4]. On a broad spectrum, the effect of glyphosate on histopathological activity on neotropical fish sensitivity (*Piaractus mesopotamicus, Phallocerus caudimaculatus, Hyphessobrycon eques* and *Brachydanio rerio*) [12], morphology of the gills and liver of the Neotropical fish, *Piaractus mesopotamicus* [13]. Hence, this study focus on the organosomatic and behavioral response of *Heterobranchus bidorsalis* exposed to rhonasate 360sl containing glyphosate (isopropylamine salt glycine).

2. MATERIALS AND METHODS

2.1 Source of fish and experimental location

Thirty health African cat fish (*Heterobranchus bidorsalis*) purchased from private fish farm at Okaka, Yenagoa metropolis, Bayelsa State, Nigeria. The fish samples were transported to the Department of Biological Sciences, Niger Delta University, where the experiment was conducted. The fish samples have mean weight of 97.00g and length of 14.02cm. The fish were acclimatized individually in a circular aquaria covered with fishing net for 7 days during which they were fed once a day (10.00-11.00hrs) with normal fish diet. During the acclimatization, the water was renewed daily.

2.2 Experimental design

The Completely Randomized Design experimental design was used for this study. The *Heterobranchus bidorsalis* used for this study were randomly divided into four experimental groups of three fishes each, representing one control group and three treatments i.e. 0.00ppm (control group), 0.01, 0.02 and 0.03ppm of the toxicant.

2.3 Preparation of toxicant solution

The toxicant i.e. rhonasate 360SL was prepared with reference to the formula;

$N1\ V2 = N2V2$ [7,8, 14 – 16]

N1 = Manufacturer concentrated (480g/l)

N2 = Concentration of test solution desired.

V1 = Volume of the original solution added.

V2 = Volume of the test solution (30L).

3.4 General bioassay technique

Based on the concentration of the toxicant prepared, sublethal concentrations of toxicant for the assay (0.01, 0.02, 0.03ppm) were determined based on the range finding test [17]. The concentrations of the toxicant were made up with 30L of borehole water in the test aquaria. Replications of each treatment group were set up by introducing fishes individually into each aquarium. The exposure period lasted for 2 weeks during which the exposure media were renewed daily. The water quality parameters used for the bioassay were carried out using standard methods of American Public Health Association (APHA) [18]. The values of the resultant parameters ranged from 24.00 – 24.17 ºC (Temperature), 6.17 - 6.34 (pH) 4.36-7.19mg/l (dissolved oxygen), 98.49 – 132.08 µS/cm (conductivity), 0.15 – 0.48 NTU (Turbidity) and 10.30 – 16.07mg/l (alkalinity).

At the end of the experimental period (i.e. 14 days), the fish samples were dissected and the organs i.e. heart, kidney, liver and spleen were obtained and weight for organosomatic indices using weighing electrical balance. The organ indices were computed based on the method of Jekins [19] foulton's condition factors values.

F (foulton condition factors) = weight of fish x $100L^2$.

The behavioral changes checked for by observing the reaction of the fishes as the toxicant at different concentration were introduced into the aquarium for about 2 hours daily.

3.5 Statistical analysis

Statistical Package for Social Sciences (SPSS) software version 20 was used to carry out the statistical analysis. The data were expressed as Mean ± standard error. One-way analysis of variance was carried out at $\alpha = 0.05$, and Duncan statistics was used for multiple comparison. The charts was plotted using Paleontological statistics software package by Hammer *et al*. [20], the standard error bar was determined at 95% interval level

3. RESULTS AND DISCUSSION

Table 1 present the behavioral characteristics of *Heterobranchus bidorsalis* exposed to Rhonasate 360SL for 14 days. The exposed fish showed normal swimming in the control which declined as the concentration of the toxicant increases. The fishes exposed to the toxicant showed changes in body pigmentation, erratic swarming and jerky movement which increases as the concentration of the toxicant increases. Similarly opercular movement decreased as the toxicant concentration decreases. Also surfacing and air gulping increased as the concentration of the toxicant increases. The observed variation from the control suggested the effect of the herbicides Rhonasate 360SL on the behavior of the fish. This finding of this study was compared to the work of previous authors. Nwani et al. [2] studied *Tilapia zilli* exposed to Glyphosate, N-(phosphoromethyl) glycine and reported that fish exposed to higher concentration of the herbicide showed uncoordinated behavioral changes including erratic and jerky swimming, increased surfacing and air gulping, decline in opercula movement and secretion of mucus on the body and gills. Ladipo [21] studied African Catfish (*Clarias gariepinus*) juvenile exposed to Paraquat Dichloride and reported that fishes erratic swimming behavior, sudden quick movements, restlessness and weakness at high concentration of the toxicants. Ayoola

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[22] reported that *Oreochromis niloticus* exposed to glysophate suffer tissues changes at 4 days exposure, respiratory stress, erratic swimming and instant death depending on the concentration of the toxicants. This trend has been reported in *Channa punctatus* exposed to carbosulfan, glyphosate and atrazine [1], *Clarias gariepinus* exposed to diazinon [23]. As such behavioral changes have been seen as the most sensitive indicator in toxicity studies in fisheries [1, 2, 21 - 23].

Table 1: Behavioral characteristics of *Heterobranchus bidorsalis* exposed to Rhonasate 360SL for 14 days

Characteristics	Concentration of Rhonasate 360SL, ppm			
	0.00	0.01	0.02	0.03
Swimming	++	++	+	+
Colour (body pigmentation)	-	+	++	++
Intermittent swarming	-	+	+	+
Jerky movement	-	+	+	++
Opercular movement	++	+	+	+
Surfacing and air gulping	+	+	++	++

- Absent; + Low; ++ Moderate; +++ High characteristics

The condition factor of *Heterobranchus bidorsalis* exposed to Rhonasate 360SL for 14 days are presented in Figure 1 and Figure 2 for initial and final condition respectively. The initial condition factor were 1.73 at 0.00ppm and 0.03 at 0.03ppm, being not significantly different (P>0.05) apart from the control (Figure 1). At the final condition factor, the highest value of 1.75 was observed at 0.00ppm and least (0.06) at 0.03ppm. Apart from the control there was no significance variation (P>0.05) among the various experimental groups (Figure 2). The condition factor showed apparent elevation between the initial and final condition factor. However, there was significant decline as the toxicant was introduced. The observations were comparable to the findings of Inyang et al. [23] on *Clarias gariepinus* exposed to diazinon. This suggested an effect of toxicant on the condition factor of the experimental fish [23].

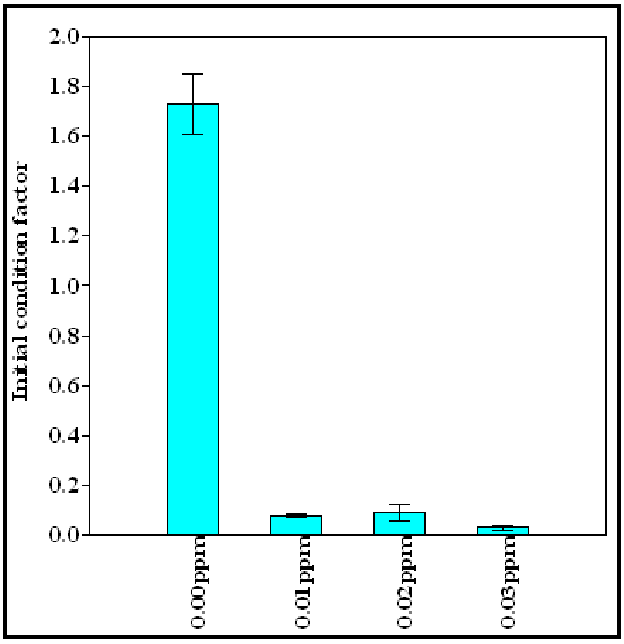

Figure 1: Initial condition factor of *Heterobranchus bidorsalis* exposed to Rhonasate 360SL for 14 days

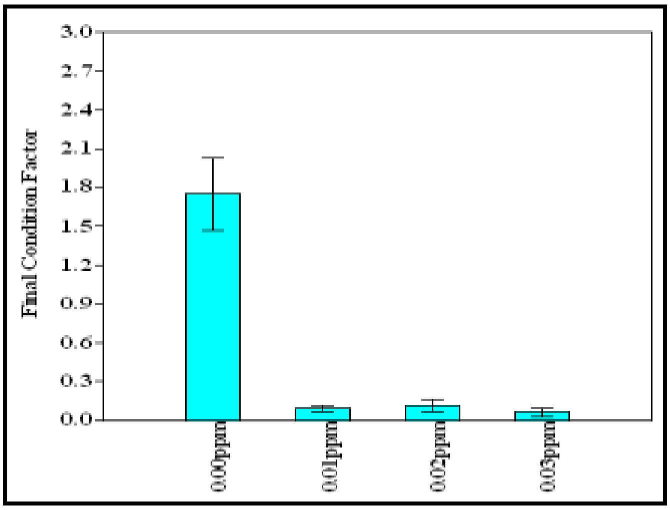

Figure 2: Final Condition factor of *Heterobranchus bidorsalis* exposed to Rhonasate 360SL for 14 days

Table 2 presents the Organosomatic indices of *Heterobranchus bidorsalis* exposed to Rhonasate 360SL for 14 days. There was no significant difference (P>0.05) among the various concentration of the toxicant in each of the organosomatic indices i.e. Renatosomatic, Spleenosomatic and Hepathosomatic studied apart from Cardiosomatic index. The significant variation (P<0.05) suggested that at increased concentration of Rhonasate 360SL over a long period of time it could cause heart related problem fisheries. Within the various concentration of the toxicant, there was fluctuation in each of the parameters. Fluctuation in biochemical, enzymes, haematological, electrolytes in fisheries exposed to pesticides have been variously reported by authors [7,8, 15, 17, 23-29]. Rhonasate 360SL did not cause a significant change in the weight of the organs in *Heterobranchus bidorsalis* exposed up to 0.03ppm of the toxicant. However, significant variations have been widely reported in different tissues, organs and system of fishes exposed to sublethal concentration of pesticides.

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Table 2: Organosomatic indices of *Heterobranchus bidorsalis* exposed to Rhonasate 360SL for 14 days

Concentration of Rhonasate 360SL, ppm	Renatosomatic (Kidney), g	Cardiosomatic (heart), g	Hepathosomatic (liver), g	Spleenosomatic (spleen), g
0.00	0.25±0.03a	1.53±0.27a	0.43±0.11ab	0.97±0.21ab
0.01	0.26±0.02a	1.70±0.35a	0.28±0.11a	1.11±0.06b
0.02	0.18±0.03a	3.65±0.23c	0.51±0.11ab	0.97±0.04ab
0.03	0.28±0.04a	2.73±0.06b	0.70±0.10a	0.67±0.04a

Data are expressed as mean± standard error (n=3); Different letters along the column indicate significance difference ($P<0.05$) according to Duncan multiple Range statistics

4. CONCLUSIONS

In the recent times the use of herbicides to control weeds has increased. Herbicides have been widely used for the control of weed in developing countries like Nigeria. This study assessed organosomatic and behavioral response of *Heterobranchus bidorsalis* exposed to rhonasate 360SL containing glyphosate (isopropylamine salt glycine). The study found that even at low concentration, it could cause alteration in behavior and condition factors of fishes. Also, beside Cardiosomatic index, there was no significance variation ($P>0.05$) in the organosomatic indices (i.e. Renatosomatic, Spleenosomatic and Hepathosomatic). This suggested that at 0.03ppm, there was no weight alteration in the organs apart from the heart. Notwithstanding, caution should be exercised in the use of the rhonasate 360SL containing glyphosate (isopropylamine salt glycine) close to fish habitats i.e. water bodies.

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