Effects of Glyphosate Herbicide on Serum Growth Hormone (GH) Levels and Muscle Protein Content in Nile Tilapia (*Oreochromis niloticus* L.)

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Abstract: Glyphosate herbicide is one the most toxic pollutants for aquatic ecosystem. Changes in serum concentrations of growth hormone (GH) and muscle protein content in *O. niloticus* treated with different concentrations of glyphosate herbicide under laboratory conditions were evaluated. Nile tilapia *Oreochromis niloticus* (mean initial weight of 57.61 ± 4.8g and initial length 14.87±1.7cm) were exposed to different concentrations of Roundup, a glyphosate (acid equivalent) herbicide: 0 (control), 1, 3, and 5 mg/L for 96 h (short-term) of exposure. Unexposed fish (control) exhibited significantly higher serum GH levels (p<0.05) compared to treated fish. The GH levels and muscle protein content decreased significantly with increasing glyphosate concentrations. GH levels and muscle protein percentage may be the good early indicators of herbicide contamination in tilapia species.

Key Words: Glyphosate, herbicide, *Oreochromis niloticus*, GH, muscle protein.

INTRODUCTION

Pollution in the world has received considerable attention. It is becoming a greatest problem with increasing the human activities. The determination of toxic compounds present in the aquatic environment and its effect on fish is a basic issue in aquatic toxicology [7]. Toxic substances may be determined with chemical analysis but its effects on fish and ecological risks for aquatic ecosystem could not be determined by chemical analysis. That is why, the preferred way to evaluate the ecological influence of toxic compounds is mortality or bioassay experiments in general [7,31]. In recent years, aquatic herbicides are widely used for controlling undesirable weeds[23]. The use of herbicide to control aquatic weeds has applied in fish management where they used in aquatic habitats especially rice fields and some fish farms [34]. Herbicides are the most widely used toxic chemicals for various purposes in gardens and agriculture. Water often serves as the sink for these chemicals after their application in the different fields. These pollutants are often not entirely specific for their target organisms. Because of their universal presence in the environment, non-target species, such as aquatic biota, can be affected. A large amount of circumstantial evidence exists to link a decline in the population level [13] to impairments the development, disturbances in behavior [2], deformities [18], and various diseases in aquatic organisms [35].

Herbicide contamination of surface waters derived from agricultural properties is a problem of worldwide importance due to aquatic contamination by these products [26]. Developing countries have the adverse effects of these chemical compounds in ecosystem on fish production[27]. The application of herbicides has hazard effects on the fish beside the change of available plankton food [24]. The herbicide may produce an immense disruption of the ecological balance causing damage to non-target organisms including fish [26,6]. Anthropogenic activities that potentially affect fish lead to changes in their habitats. The responses of fish to such environmental challenges are ultimately reflected as overall alteration in metabolism. The responses are initially reversible, but prolonged exposure to environmental pollutants brings about permanent (pathological) changes in fish physiology. These alterations are reflected as reduced survival, growth [2], reproductive dysfunction [33], and immune suppression [19]. Several biochemical and physiological responses occur when a toxicant enters an organism, which may be an acclemation to the organism or may lead to toxicity [10,19,22]. There is widespread scientific consensus that herbicides have a hormone-like function and can thus interfere with the endocrine system of fish [20].

Roundup (Glyphosate 48%), a commercial formula containing an active ingredient, which is the acid equivalent of the isopropylamine salt of glyphosate (N-phosphonomethyl glycine) is a non-selective and post
emergent herbicide used for controlling aquatic weeds [1,4]. It enters the aquatic system through agricultural run-off or directly through careless application. It is widely used in the world due to its high efficiency. Its half-life in soil is 30 – 90 days. Many physiological functions in fish are affected by herbicide pollution.

The aim of the present study was to achieve the effects of short-term glyphosate exposure on serum GH levels and muscle protein content of tilapia (Oreochromis niloticus) as indicators of herbicide toxicity.

MATERIALS AND METHODS

Nile tilapia (Oreochromis niloticus) of both sexes with an average weight 57.61 ± 4.8 g and length 14.87 ± 1.7 cm were collected from a private farm and acclimated in laboratory conditions in declorinated tap water under a natural photoperiod (12h light – 12h dark) for 15 days. Water was oxygen saturated through constant aeration in a static system. Water quality was as follows: temperature 22 ± 0.5 °C, pH 7.6 ± 0.1, dissolved oxygen 7.3 ± 0.4 mg/L, non-ionized ammonia 0.007 ± 0.001 mg/L, nitrite 0.02 ± 0.005 mg/L, alkalinity 73 ± 5.5 mg/L CaCO3 and hardness 23 ± 1.2 mg/L. All water parameters were determined according to APHA [2]. Fish were fed once a day at 3% of total body weight with a commercial feed containing 40% crude protein. Feces and pellet residues were removed daily by suction. After the acclimation, fish were assigned to treatments into 12 glass aquaria (70 L each) with a density of 10 fish/aquarium as four groups (three replicates per treatment). The first group was kept as control (untreated fish). The other three groups (1, 2 & 3) were exposed for 96h to 1, 3, and 5 mg/L glyphosate herbicide respectively under controlled laboratory conditions. Pervious experiments carried out in our laboratory established 7 mg/L as the LC50 96h. Fish did not receive food during the experimental period. The herbicide was added to the water only at the beginning of the experiment. Mortality was controlled at 24, 48, 72 and 96 h after the start of the tests. Dead individuals were counted and removed immediately. Constant aeration and water quality were not changed throughout the experimental period and remained within the optimal values mentioned before. At the end of exposure period (96h), all control and exposed fish were sampled and blood was collected from the caudal vein, centrifuged at 3000 rpm for 15 minutes to obtain serum, and stored at -20 °C until analysis. GH levels were determined in serum of control and exposed fish at a private medical laboratory. Muscles were removed and placed in ice and stored at -20 °C. Tissue protein was determined by Kjeldahl method suggested by McChenzie and Wallace[23]. One –way analysis of variance (ANOVA) and Dun-Can’s multiple range tests were used. Data (n = 10) were expressed as mean ± standard deviation (SD) and mean differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

It investigated the changes in serum GH levels and muscle protein content. Results of the present work are shown in tables, 1 and 2.

Fish exposed to glyphosate concentrations of 3 and 5 mg/L showed rapid respiration and increased rate of gill cover movements, slow - down of reflexes and swimming movements, fish floated at the surface of water gasping for more oxygen and very rapid movements in various directions of the aquaria. The mucous secretion appeared to increase and accumulated on the gills. Control and group 1 (1 mg glyphosate/L) concentrations mortality were zero. Two aquaria in group 2 (3 mg glyphosate/L) have two died fish (one fish in each aquarium) at the end of the experiment. Four fishes were died in group 3 (5 mg/L) distributed in the three aquaria at 72 and 96 h (Table 1).

O. niloticus exposed to Roundup glyphosate herbicide exhibited a significant reduction (p<0.05) in serum GH levels compared to the control. The levels of GH decreased with all glyphosate concentrations showed a negative correlation with it. The average higher levels of GH (0.8 ng/ml) were recorded in the control group. While the average lower levels (0.3 ng/ml) were recorded for the group 3 that exposed to 5 mg/L glyphosate.

Muscle total protein content of O. niloticus decreased significantly (p<0.05) with increasing of glyphosate concentrations. The average higher (56.5 % dry weight) and lower (35.8% dry weight) muscle protein content were recorded in the control group and the group 3, respectively.

Discussion: In this study, we observed that the glyphosate herbicide even used at low-concentrations affected the growth of O niloticus, which is considered as a highly resistant fish species. In aquatic toxicology, if LC10 (mg/L) concentrations is lower than 1 mg/L, the chemical is highly toxic and if between 1-10 mg/L, then it is considered to be a moderately toxic [24]. Therefore we report glyphosate herbicide to be moderately toxic to Nile tilapia. The abnormal behavior of fish in the aquaria could be attributed to the effect of glyphosate on CNS and cardiovascular system [5]. There is wide spread scientific consensus that herbicide have a hormone-like function and can thus interfere with the endocrine system of fish [20].
Table 1: Cumulative mortality of fish, Oreochromis niloticus (n = 30 fish, each concentration) exposed to different concentrations of glyphosate herbicide

<table>
<thead>
<tr>
<th>Herbicide concentrations (mg/L)</th>
<th>GH levels (ng/ml)</th>
<th>Muscle protein content (% dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/L</td>
<td>1.0</td>
<td>48.6 ± 4.1</td>
</tr>
<tr>
<td>5 mg/L</td>
<td>0.5</td>
<td>42.6 ± 4.1</td>
</tr>
<tr>
<td>7 mg/L</td>
<td>0.3</td>
<td>35.8 ± 4.2</td>
</tr>
<tr>
<td>10 mg/L</td>
<td>0.3</td>
<td>28.6 ± 4.1</td>
</tr>
</tbody>
</table>

Table 2: Changes in serum GH levels and muscle protein content (dry weight) of O. niloticus exposed to different concentrations of Roundup a glyphosate herbicide under laboratory conditions

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Herbicide concentrations</th>
<th>GH levels (ng/ml)</th>
<th>Muscle protein content (% dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Without herbicide</td>
<td>0.8</td>
<td>56.5 ± 4.8</td>
</tr>
<tr>
<td>1</td>
<td>1 mg/L</td>
<td>0.6</td>
<td>48.4 ± 3.3</td>
</tr>
<tr>
<td>2</td>
<td>3 mg/L</td>
<td>0.5</td>
<td>42.6 ± 4.1</td>
</tr>
<tr>
<td>3</td>
<td>5 mg/L</td>
<td>0.3</td>
<td>35.8 ± 4.2</td>
</tr>
</tbody>
</table>

Wiegnd et al.,[14], observed that herbicides lead to physiological stress and decrease in relative growth rate. Marcia and Lee [21], recorded that sub lethal levels of herbicide induced behavioral and physiological effects on fish. Marcia et al., [22], indicated that the action of herbicides considered as endocrine-disrupting chemicals. John [15], found that herbicides are endocrine disrupting chemicals that can inhibit endogenous hormones. Katherine et al.,[16], recorded that herbicides lead to decrease in Triiodothyronine (T3) and Thyroxin (T4) that have direct effects on growth. Results of the present work confirm the observations of the previous authors. The decrease of serum GH levels in glyphosate exposed fish is an obvious indicator of the negative effect of herbicides on growth of fish and finally on fish production.

The decrease of muscle total protein in tilapia, O. niloticus indicated that glyphosate has toxic effects on immune system [29], and harmful effect on nutritive value in these fishes. Hussein et al., [12]. recorded that the decrease of total protein in O. niloticus treated with atrazine herbicide was mainly due to globulin which means toxic effects on immune system in this fish. The changes in total protein as affected by herbicide exposure can be explained by different theories: led to increase in protein amount in the urine [28], harmful effects on spleen and liver [23], and inhibitory effects on macrophyte communities as photosynthesis inhibitor that led to a decline in the invertebrate fauna which is essential as prey for fish [17]. Swietla and Zuk [22], recorded that herbicides act as inhibitor of the synthesis of nucleic acids and proteins. Some authors correlated a generalized hypoproteinemia in fish after toxicant stress with a disturbance in osmoregulation [11]. Marcia et al., [23], recorded that protein levels were enhanced in the liver and white muscles of herbicide exposed fish, whereas it increased in the plasma. They recorded that fish exposed to 1.0 mg/L of clomazone herbicide, showed a reduction in muscle protein from 64.6 to 46.85 mg/g tissue. They interpreted the protein reduction is due to physiological acclimation of the fish to overcome stress situation using protein catabolism to supply high energy demand. Also, Katherine et al., [16], recorded a significant reduction in protein of herbicide exposed fish. The present results are in agreement with the observations of the previous authors.

In conclusion, the present study suggested the urgent need to use the natural and non-pollutant substances to eradicate the undesirable weeds for protecting fish and man. Glyphosate herbicide can not be recommended for weed controlling in aquatic media. Serum GH hormone levels and muscle protein content may be a good early biomarkers of glyphosate toxicity.

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REFERENCES


