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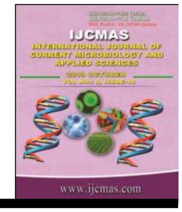

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Acute Toxicity of Glyphosate Herbicide on Nile Tilapia (*Oreochromis niloticus*)

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A B S T R A C T

The present study aimed to assess the acute toxicity effects of herbicide, glyphosate on Nile tilapia (*Oreochromis niloticus*). The bioassay experiments were performed in a static renewal regime with *Oreochromis niloticus* exposing to varying acute toxicity concentrations of glyphosate viz., 15.33, 30.67, 61.34, 122.68 and 245.36 mg/l for 96 hrs and the gill, liver and kidney tissues were dissected out. The standard histology protocol was followed to study the histological alterations. In the present study, 100 % mortality was observed in concentrations of 122.68 and 245.36 mg/l of glyphosate. The LC_{50} was determined to be 49.22 mg/l after 96 hrs of exposure. The histological alterations like lamellar fusion, hyperplasia and degenerated secondary lamellae were observed in the gill of fish exposed to glyphosate. Similarly, irregular nucleus, melanomacrophage formation and vacuole formation were observed in the liver of fish treated at different concentrations of glyphosate. The histological alterations like dilation of Bowman's space, glomerular shrinkage and disappearance of the shape of glomerulus was observed in the kidney exposed to glyphosate. The intensity of the histological alterations in gill, liver and kidney was found to depend on the concentration of the toxicant and duration of exposure. The histological alteration observed in the present toxicity study suggests that glyphosate can be a potential toxicant and hence the responsible use of the particular herbicide near the fish farm or in the area close to the aquatic environment should be practiced.

Introduction

The constant discharge of agricultural wastes into the aquatic environment has led to accumulation of the herbicides, pesticides, heavy metals and other variety of pollutants. Herbicides present in these wastes are washed down, carried by rains and flood to nearby aquatic environment. In the world today, glyphosate is the most widely used herbicide and its consumption has increased to about 95% in the period from the year 2000 to 2004 (Ali Sani and Muhammad, 2016; and Adedeji and Okocha, 2012). The consumption of glyphosate pesticide in India is 180 MT in 2001 – 2002 and 210 MT in 2005 – 06. About

866 MT of glyphosate was sold in 2014-15 in India, according to the Directorate of Plant Protection, Quarantine and Storage. Glyphosate is the best herbicide used for control of aquatic and semi aquatic weeds such as cattail, rushes, smartweeds, and floating-leaf plants like water lily and lotus. It is one of the established herbicide used worldwide because of its low persistence and it is a major pollutant of rivers and surface water. Some surfactants that are present in the formulation of glyphosate are toxic to aquatic organisms and hence are unsuitable for aquatic use (Okayi *et al.,* 2010).

Glyphosate showed a high water solubility varying from 10000 to15700 mgl-1 at 25^0C (USEPA, 1993). The half-life of glyphosate ranged from 7 to 14 days and has low vapor pressure which suggested that loss to the atmosphere from treated surfaces will be small (Giesy *et al.,* 2000). Fishes are very sensitive to a wide variety of agrochemicals including glyphosate herbicide that may arise mainly from the approved agricultural practices. Histological biomarkers provide powerful tools to detect and characterize the biological end points of toxicant and carcinogen exposure (Hinton *et al.,* 1992).

In ecotoxicological studies, histology is gaining importance for rapid evaluation of the toxic effect of pollutants and considered as an important tool for examining the effect in different organs and even tissue of the organisms (Latif *et al.,* 2013). Bawa *et al.,* (2017) conducted the acute toxicity experiment of glyphosate (Roundup ® 41% SL) on fingerlings of *Cyprinus carpio* and the calculated LC_{50} was 3.260 ppm and evaluated the histological and biochemical changes in liver of exposed fishes.

They observed that the liver of fishes exposed to glyphosate exhibited vacuolation of hepatocytes, pyknotic nuclei, degeneration of cytoplasm, and infiltration of leukocytes, necrosis and severe vasodilation in the treatments. Nile tilapia, *Oreochromis niloticus* has a vast potential for settlement to any complex environment conditions in lotic and lentic water bodies (Dwivedi *et al.,* 2016; Tiwari *et al.,* 2016). Knowledge on the population structure of this commercially exploited species is a prerequisite for a more detailed study on its biology and to manage them in fisheries.

Hence, the present study was conducted to determine the lethal concentration and the acute toxic effects of glyphosate herbicide on Nile tilapia (*O. niloticus*) with emphasis on the histological changes in the gills, liver and kidney tissues.

Materials and Methods

Two hundred adult fishes of Tilapia, *O. niloticus* with an average length of 17.0 ± 1.5 cm and weight of 100 ± 5.0 gm were procured from Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India. They were acclimatized to laboratory conditions in Fiberglass Reinforced Plastic (FRP) Tanks of 1000 L capacity for one month prior to exposure to glyphosate. Fishes were fed with commercial floating pellets and unconsumed feed were removed properly.

The experimental design was based on Static Renewal Test (SRT), Range Finding and Definitive Test (Acute Toxicity Test) described by USEPA, 2003. For each bioassay test, a series of five test concentrations of glyphosate and a control were used.

The acute toxicity test concentrations were selected based on the range finding test viz. 15.33, 30.67, 61.34, 122.68 and 245.36 mg/l. The physico chemical parameters (pH, DO and temperature) of test concentrations of glyphosate were analysed by the standard procedure of APHA (2012). After the acclimatization period, fishes were randomly selected and stocked at the rate of 10 fishes per plastic trough with 50 liter water for the five experimental runs and a control. A duplicate set was also maintained simultaneously.

Exposure medium was changed every 24 hrs to maintain the desired concentration of glyphosate. Mortality of fishes at each concentration was recorded during the experimental study. Then the numbers of dead fishes were fed in the probit software to determine the LC_{50} of the glyphosate on tilapia. At the end of the experiment (96 hrs), live fish samples were collected from control and the three concentrations (15.33, 30.67 & 61.34 mg/l), sacrificed and their gill, liver and kidney tissues were excised out and fixed in Bouins fixative for 24 hrs.

Later, the tissue samples were processed adopting the usual histological procedure by Humason, 1972. The tissues were washed with 70% ethanol and dehydrated through a graded series of ethanol. They were embedded in paraffin, sectioned at 4 – 5 µm thickness, stained with hematoxylin and eosin and examined using microscope. Also, light photomicrographs were taken to observe any changes in its structure. The morphological changes of the gill, liver and kidney sections noted in the experimental fish were compared with those of the control fish.

Results and Discussion

Glyphosate is one of the herbicide used for controlling annual and perennial grasses, broad-base leafed weeds, trees and other species. It is practically non-toxic to fish. However, roundup was more toxic to fish than was glyphosate. In the present study, the mean physicochemical parameters of test concentrations of glyphosate at different concentrations are given in Table 1. The temperature of each test concentrations varied from 24-27ºC. The pH and dissolved oxygen of each test concentrations were 7.1-7.6 and 5.1-5.8 mg/l, respectively.

The fish mortality at different concentrations of glyphosate on fish is presented in Table 2. The maximum mortality was observed in 245.36 mg/l and minimum mortality was observed at 15.33 mg/l concentration. The calculated mean LC_{50} value of glyphosate on *O. niloticus* at 24, 48, 72 & 96 hrs was 59.51, 55.95, 52.38 & 49.22 mg/l respectively. Nwani *et al.,* (2013) reported that the 96 hrs LC_{50} value of glyphosate in adults of *Tilapia zillii* was 211.80 mg/l. At the same time, Ali Sani and Muhammad (2016) reported that the 96 hrs LC_{50} value of glyphosate for juveniles of *Clarius gariepinus* was 0.0072 ml/l. Wannee *et al.,* (2003) found the 96 hrs LC_{50} value of glyphosate for young (1.69 + 0.31 g) and adult (16.87 + 3.87 g) Nile tilapia were 16.8 & 36.8 mg/l respectively. Ayoola (2008) reported that the 96 hrs LC_{50} value of glyphosate for *Clarius gariepinus* was 0.275 mg/l. The toxicity study of glyphosate herbicide on fishes are consistent with previous reports (Bawa *et al.,* 2017; Shiogiri *et al.,* 2012, 2010; Nwani *et al.,* 2010; Lushchak *et al.,* 2009; Langiano and Martinez, 2008 and Ayoola, 2008). In the present study, no adverse behavioural changes or any mortality were recorded in the control fish throughout the period of the experiment. The exposed fishes at higher concentrations (122.68 and 245.36 mg/l) became very weak and settled at the bottom and died before 24 hrs duration of exposure.

Histological studies

The histopathological changes in the gill, liver and kidney tissues of the control and experimental fishes were observed and the observations are presented in Figure 1-3.

Gill

No recognizable changes were observed in the gills of the control fishes. Each gill consisted of a primary lamellar filament and secondary lamellae (Fig.1A). Under light microscopic observations, the histological alterations like lamellar cell fusion and lamellar cell hyperplasia was observed at 15.33 and 30.67 mg/l of glyphosate exposure (Fig. 1B-C), whereas at the concentration of 30.67 mg/l of glyphosate (Fig 1D), fully degenerated gill lamellae were observed. As per previous researchers, Ayoola (2008) and Wannee *et al.*, (2003), it is evidenced that histological alteration in gill tissue could be used as bio-indicator for pesticide exposure in Tilapia (*Oreochromis niloticus*). Neskovic *et al.*, (1996) reported that the gills of *C.carpio* exposed to 5.0 mg/l glyphosate concentration showed epithelial hyperplasia and sub epithelial edema. Wannee *et al.*, (2003) reported that tilapia (*O. niloticus*) which exposed to glyphosate at the concentrations of 46.9 mg/l showed filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm in the gill at 96 hrs exposure. Similarly, histological changes like edema, fusion of lamellae irregular thickening of primary lamellae epithelium, epithelial lifting, blood congestion and lamellar aneurysm and necrosis of lamellae were observed in gills of Asian sea bass exposed to glyphosate (Thanomsit *et al.*, 2016). Hence, fish gills are sensitive organ easily affected by many toxicants even at low concentrations (Karlsson, 1993).

Liver

The histology of control fish liver revealed the typical parenchymatous appearance (Fig.2A). In light microscopic observation, the liver was divided into irregularly shaped lobules separated by the hepatopancreas and bile duct. The liver of fish was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus. In the present study, irregular shaped nucleus and death of hepatic cells were observed on 96 hrs exposure at 15.33 and 30.67 mg/l (Fig. 2B-C).

But, melanomacrophage formation was observed in the liver of fish exposed to glyphosate at 60.37 mg/l of 96 hrs of acute toxicity (Fig. 2D). Wannee *et al.*, (2003) reported that infiltration of leukocytes, increasing hepatocyte size with pyknotic nuclei, and presence of vacuoles in tilapia exposed to glyphosate at 46.9 mg/l.

Neskovic *et al.*, (1996) reported the congestion of few sinusoid and signs of early fibrosis in liver tissues of *C.carpio* exposed to 10.0 mg/l glyphosate concentration. Ayoola (2008) reported fatty degeneration, severe fat vacuolation, diffuse hepatic necrosis darkly stained specks of necrotic nuclei and infiltration of leukocytes in the liver tissues of Juvenile African Catfish (*Clarias gariepinus*) at 94 mg/l of glyphosate. Akinsorotan *et al.*, (2013) reported that vacuolation of hepatocytes and necrosis in the liver tissues of adult *Clarias gariepinus* exposed to 38.4 mg/l glyphosate.

Deivasigamani (2015) reported slightly vacuolated cells, fatty degeneration and necrosis in liver tissues of *C. carpio* exposed to 86 mg/l glyphosate. Stoyanova *et al.*, (2015) reported the changes in liver of *C. carpio* exposed to glyphosate and the liver of the exposed fish showed slightly vacuolated cells with fatty degeneration. Samanta *et al.*, (2016) reportedvacuoles, enlarged and pyknotic hepatocytes, excess fat deposition, inflammation of hepatocytes and enlarged acentric nuclei, vacuolation in the cytoplasm and increase in sinusoidal space in *Heteropneustes fossilis* when exposed to glyphosate-based herbicide.

Table.1 Mean physico-chemical parameters of the test concentrations (glyphosate) on *O. niloticus*

Conc. (mg/l)	Temperature (ºC)	pH	Dissolved oxygen (mg/l)
Control	24 ± 3	7.2 ± 0.3	5.1 ± 0.2
15.33	26 ± 2	7.6 ± 0.2	5.2 ± 0.3
30.67	27 ± 3	7.3 ± 0.3	5.8 ± 0.1
61.34	26 ± 1	7.2 ± 0.2	5.7 ± 0.2
122.68	25 ± 2	7.4 ± 0.4	5.6 ± 0.3
245.36	26 ± 2	7.1 ± 0.2	5.4 ± 0.3

Table.2 Rate of mortality of Nile tilapia on exposure to glyphosate

Exposed concentration (mg/l)	Fish mortality (%) during Experiment (hr)				% mortality
	24	**48**	**72**	**96**	
Control	0	0	0	0	0
15.33	0	0	0	0	0
30.67	20	20	30	30	30
61.34	30	40	40	50	50
122.68	40	60	60	60	60
245.36	100	100	100	100	100
Lethal Concentration (LC_{50} at 96 hrs)					**49.22mg/l**

Fig.1 Photomicrograph of gill of fish exposed to glyphosate

A. Control: PGL - Primary gill lamellae; SGL - Secondary gill lamellae; CC- Chloride cells
B. 15.33 mg/ : LF - Lamellar fusion
C. 30.67 mg/l : H - Hyperplasia; SSGL - Shortening of secondary gill lamellae
D. 61.34 mg/l : DSGL - Degenerated secondary gill lamellae

Fig.2 Photomicrograph of liver of fish exposed to glyphosate

A. Control: H - Hepatocytes
B. 15.33 mg/ : IRSN - Irregular shape of hepatocytes
C. 30.67 mg/l : MMP - Melanomacrophages
D. 61.34 mg/l : VF - Vacuole formation

Fig.3 Photomicrograph of kidney of fish exposed to glyphosate

A. Control: BS - Bowmans space; G - Glomerulus
B. 15.33 mg/ : DBS - Dilation of bowmans space
C. 30.67 mg/l SBC - Shrunken bowmans capsule
D. 61.34 mg/l : DSG - Disappearance of shape of some glomerulus

Kidney

No recognizable changes were observed in the kidney of the control fishes (Fig.3A). At the light microscopic observation, the renal corpuscle was composed of the glomerulus and Bowman's capsule.

Histological alterations like dilation of Bowman's space, glomarular shrinkage and disappearance in the shape of glomerulus were observed in kidney tissues of fish exposed to glyphosate (Fig.3B-D) at different concentrations (15.33, 30.67 and 61.34 mg/l) respectively. Samanta *et al.,* (2016) observed the histological alterations like loss of hematopoietic tissue, degenerative changes in glomeruli, proximal and distal convoluted tubule, and epithelial cell lining of the renal tubules in the kidney of *H. fossilis* exposed to glyphosate. Deivasigamani (2015) reported highly expanded renal tubules, separated epithelial lining from the tubular cells, loss of cellular integrity, dilation, oedema, hypertrophied nuclei of renal tubules, necrosis and pyknotic nuclei in kidney tissues of *C. carpio* exposed to 86 mg/l glyphosate.

Ayoola (2008) reported haematopoietic necrosis and severe pyknotic nuclei in the kidney tissues of Juvenile African Catfish (*Clarias gariepinus*) at 94 mg/l of glyphosate.

Wannee *et al.,* (2003) reported similar results such as dilation of Bowman's space and accumulation of hyaline droplets in the tubular epithelial cells of the first proximal tubule in tilapia exposed to glyphosate at 36 mg/l.

The present investigation suggested that acute toxic exposure to glyphosate leads to damages in the tissues of gill, liver and kidney of tilapia, O*reochromis niloticus*, confirming the possibility of glyphosate to be a toxicant. Therefore, the responsible use of glyphosate herbicide on/near fish farms or in area close to aquatic environment should be encouraged.

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