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## EFFECT OF GLYPHOSATE ROUNDUP ON OXYGEN CONSUMPTION IN FRESHWATER FISH *RASBORA DANICONIUS*

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### KEYWORDS

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## ABSTRACT

The toxicity of Roundup a glyphosate-based herbicide was determined for the freshwater fish *Rasbora daniconius*. Acute toxicity experiments for Glyphosate roundup (41%) was conducted using static bioassay for 24h, 48h, 72h and 96h on the freshwater fish *Rasbora daniconius*. The LC<sub>50</sub> value based on probit analysis was found to be 5.66ppm for 96h of Glyphosate roundup (41%). After determining the LC<sub>50</sub> concentrations for the freshwater fish *Rasbora daniconius*, one-tenth of the 96h LC<sub>50</sub> was taken as sub-lethal concentration for studies on oxygen consumption. The glyphosate roundup (41%) exposed fish showed behavior like surfacing phenomenon, irregular, erratic and darting swimming movements, hyper excitability, increased mucus secretion and loss of equilibrium before finally sinking to the bottom. In oxygen consumption studies a decrease in the respiratory rate observed as exposure period increase in both lethal and sub-lethal concentration compare to control due to toxicant induced stress avoidance and biotransformation.

## INTRODUCTION

Any change in the behavior and physiology of fish indicates the change in the water quality by the addition of hazardous substances in the aquatic ecosystem, since fish are the biological indicators of water quality. Hence, the present study was undertaken to evaluate the effect of glyphosate roundup (41%) on behavioral and oxygen uptake of the freshwater fish *R. daniconius*. Glyphosate roundup (41%) is the world's best selling herbicide, representing 60 per cent of global 'broad-spectrum' herbicide sales (Brausch *et al.*, 2007). The use of herbicides to control weeds are a part of agricultural management throughout the world. The long persistence of many herbicides in freshwater suggests that they are capable of producing adverse effects on fresh water animals particularly fish (Langiano *et al.*, 2008). It is most potentially harmful chemical reached unplanned manners into an environment and affect on the terrestrial and aquatic organisms such as fish (Ernest, 2004).

To determine the oxygen consumption is one of the indicators of the health status of the fish and help to assess the physiological state of fish species. Respiratory activity of a fish is often the first physiological response to be affected by the presence of chemical toxicant in the aquatic medium is the alterations in the respiratory activity. Herbicides in sub lethal concentrations present in the aquatic environment are too low to cause immediate death directly but may affect the functioning of the organisms, disrupt normal behavior and reduce the fitness of natural population (Modesto and Martinez, 2010). The changes in the respiratory activity of fish have been used by several investigators as indicators of response to environmental stress (Holden, 1973, Koundinya and Ramamurthi, 1980; Sarkar; 1999; Langiano and Martinez, 2008,). Study of oxygen consumption also helps in evaluating the susceptibility or resistance potentiality and also useful to correlate the behavior of the animal, which ultimately serve as predictors of functional disruptions of population. Hence the analysis of oxygen consumption can be used as a biodetector system to evaluate the basic damage inflicted on the animal which could either increase or decrease the oxygen uptake. Therefore an attempt has been made to study the effect of sub-lethal and lethal concentrations of 96h LC<sub>50</sub> of Glyphosate roundup (41%) on oxygen consumption and induced behavioral changes in fresh water fish *Rasbora daniconius*.

## MATERIALS AND METHODS

*Rasbora daniconius* is a commonly occurring freshwater fish available in the local ponds and rivers was selected for experimental studies. Bulk of sample of fishes, ranging in weight from 6-7 gms and measuring 5-7cm in length were collected from Godavari river at Kaigaon village of Aurangabad District. Fishes were acclimatized to the laboratory conditions for 1 week in glass aquaria. The fishes were fed with commercially available fish food. The level of the dissolved oxygen, PH, alkalinity and hardness were monitored and maintained constant. The tanks were continuously aerated with electrically operated aerator. Fishes of about the same size (6-7gms and 5-7cm) irrespective of sexes were selected for the experiment.

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Technical grades of Glyphosate roundup(41%) a herbicide was used in this investigation. 1ppm stock solution of herbicide Glyphosate roundup (41%) was prepared in glass distilled water (Langiano *et al.*, 2008). Batches of 10 healthy fishes were exposed to different concentrations of herbicide Glyphosate roundup (41%) to calculate the LC<sub>50</sub> value. It was found as 5.66mg/L for 96hrs using probit analysis method. Fish exposed to lethal and sub-lethal (1/10 of 96hrs LC<sub>50</sub> value) concentration of the herbicide Glyphosate roundup (41%) for behavioral and oxygen uptake study. Another group was maintained as control. For behavioral experiment 10 animals were exposed to lethal and sub lethal concentration of herbicide. At the end of each exposure period, observations were made on physical behavior and oxygen uptake. One tenth of the LC<sub>50</sub> (5.66 mg/l) was selected as a sub lethal concentration for the study. The control and Glyphosate roundup (41%) exposed fishes were kept under continuous observation during the experimental periods. The overall animal oxygen consumption was measured for lethal and sublethal concentrations as well as the controls by Winkler's method as modified by Strickland and Parsons. The oxygen consumption for lethal and sub lethal exposure were measured after 0h, 2h, 4h, 8h, 12h and 24h.

## RESULTS AND DISCUSSION

The acute toxicity observed 9.11 mg/l, 7.20 mg/L, 6.96 mg/L and 5.66 mg/L. for 24h, 48h, 72h and 96h respectively. The concentration of Glyphosate roundup(41%) herbicide that will bring about 50% mortality of the test organism in 96 h is referred to as 96 h LC<sub>50</sub>. The 96 h LC<sub>50</sub> of Glyphosate roundup (41%) herbicide observed in present study was 5.66 mg/L. This value (5.66mg/L) is the concentrations of the treatments required to bring about 50% mortality of *R. daniconius* within 96h period. (Table 1).The acute toxicity of Glyphosate roundup (41%) herbicide decrease with increase in time. Glyphosate roundup (41%) herbicide was highly toxic to

*R. daniconius*. The toxicity rate of the organism is dose-dependent. The findings of the present study is confirmed by the significantly different mortality rate obtained because of dosage given to the animals Rand and Pectrocelli (1985) and Omoregie (1991). The mortality pattern recorded corroborates with that reported by Rand and Pectrocelli1985; Folmar *et al.*,1979; Palanivelu *et al.*, 2005, Gluszczak, *et al.*, 2007; Barbieri, 2009; Ajani and Awogbade, 2012; Ada, *et al.*, 2012 Nikam and Shejule, 2015).

The result of the study revealed that fish exposed to the herbicide solutions showed respiratory difficulties, erratic swimming, loss of movement co-ordination, restlessness, loss of balance and increasing opercula ventilation and movement Similar results reported by Sarikaya and Yilmaz (2003) fish *Cyprinus carpio* exposed to 2,4-D (2,4-dichlorophenoxy acetic acid) herbicide. At higher concentration (9.11 mg/L) the fish showed erratic swimming, air gulping, loss of balance and excessive mucus secretion within 24 h. This occurs because it inhibits the AchE activity leading to accumulation of acetylcholine in the cholinergic synapses, leading to hyper stimulation (Marigaudar *et al.*, 2009).

Increase in mucous production on the skin and gills of the exposed animals is a well-known response of fish in cases of pollution of the aquatic environment (Bols *et al.*, 2001; Ada, *et al.*, 2012 Kodape *et al.*, 2012). Opercular movements increased initially in all exposure period but decreased later steadily. Mucus secretion in fish forms a barrier between the body and toxic media thereby probably reduces contact with the toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by Rao *et al.* (2003) and Parma DeCroux *et al.*, (2002) in *Prochilodus lineatus* under monocrotophos stress. The increased opercular gill movements observed initially may possibly compensate for increased physiological activity under stressful conditions (Shivkumar and David, 2004) Changes in the respiratory activity of fish have been used by several investigators as indicators of response to environmental stress

**Table 1: Percent mortality rate in *R. daniconius* against conc. Of Glyphosate roundup (41%) for 96h exposure.**

Concentration of Glyphosate roundup (41%)mg/L	%of Mortality	No. of exposedfish	Live fishes	Dead fishes
1.6	0	10	10	0
2.6	0	10	10	0
3.6	10%	10	09	1
4.6	70%	10	7	3
5.6	50%	10	5	5
6.6	70%	10	3	7
7.6	100%	10	0	10

**Table 2: The amount of oxygen Consumed in mg/g body weight/hr of the freshwater fish *R. daniconius* exposed to lethal and sub-lethal concentrations of Glyphosate roundup (41%) of 96h LC<sub>50</sub>**

Hours ofexposure	Control	Sub-Lethal (1/10 of 96h Lc <sub>50</sub> )	Lethal
0	0.65 ± 0.012	0.62 ± 0.011	0.68 ± 0.015
2	0.62 ± 0.013	0.58 ± 0.015	0.64 ± 0.011
4	0.60 ± 0.012	0.53 ± 0.012	0.57 ± 0.016
8	0.57 ± 0.011	0.49 ± 0.011	0.53 ± 0.012
12	0.55 ± 0.010	0.41 ± 0.013	0.50 ± 0.007
24	0.51 ± 0.011	0.38 ± 0.011	0.46 ± 0.006

Each value is mean of 5 individual observations

(Carpenter, 1930; Marigaudar *et al.*, 2009; Gopal and David, 2010; Jothinarendiran, 2012; Maharajan *et al.*, 2013; Ram Nayan Singh, 2014).

The present experiments also demonstrated that oxygen consumed by *R. daniconius* showed no linear relationship to exposure time when fish exposed to 1/10 sub-lethal concentration of 96h LC<sub>50</sub> of Glyphosate roundup (41%). The oxygen consumption rate was indeed increased in 2h and 4h of exposure period then decreased in 8h, 12h, and 24h exposure period compare to control fish (Table 2). This indicate fish *R. daniconius* is under stress of toxicant consume more oxygen to cope up the energy required for metabolic processes. Similar results have also been observed in different fish species for different chemical substances (Wu and Chen 2004; Barbieri, 2009). Oxygen consumed by *R. daniconius* showed linear relationship to exposure time when fish exposed to lethal concentration of 96h LC<sub>50</sub> of Glyphosate roundup (41%). The decrease in the consumption of oxygen is probably the result of alterations of energy metabolism (Olsen *et al.*, 2006).

These results clearly indicates that the Glyphosate roundup (41%) must be acting on the organized respiratory mechanism *i.e.* damaging epithelial cell layer of gills ultimately altering the elements involved in the respiration mechanism of the gills. The Glyphosate roundup (41%) must be acting on the enzyme sites of cells slowly in the lower concentration initially. If gills or membrane functions are destroyed due to xenobiotic chemicals or the membrane functions are disturbed by a change in permeability the oxygen uptake rate would rapidly decrease (Hartl *et al.*, 2001). Further, the metabolic rate in relation to respiration of fish could be increased under chemical stress (Chebbi and David, 2010; Palanivelu *et al.*, 2005; Barbieri 2009). A decrease in oxygen consumption was reported by several authors Vineetkumar, *et al.*, 2008; Anita Susan *et al.*, 2010; Gopal and David, 2010; Das and Gupta, 2012; Maharajan *et al.*, 2013; Ram Nayan Singh, 2014). The present study conclude that Glyphosate roundup (41%) herbicide is toxic to *R. daniconius*. The decline in oxygen consumption is result of onset of poisoning. The decline is due to damage of gills which reduces the efficiency of oxygen uptake. The respiratory rate of an organism is indicative of the physiological state and change in respiratory rate is an indicative of environmental stress.

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