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

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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_{50} value based on probit analysis was found to be 5.66ppm for 96h of Glyphosate roundup (41%). After determining the LC_{50} concentrations for the freshwater fish *Rasbora daniconius*, one-tenth of the 96h LC_{50} 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.

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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 (Ervnest,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 biodetectory 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 $LC_{50}$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.

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_{50} 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_{50}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_{50} (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_{50}. The 96 h LC_{50} 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, Glusczak, *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 Yýlmaz (2003) fish *Cyprinuscarpio* exposed to 2,4-D (2,4-dichlorophenoxyacetic 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 De Croux *et al.,* 2002) in *Prochiloduslineatus* 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 (Carpenter, 1930; Marigaudar *et al.,*2009; Gopal and David, 2010; Jothinarendiran, 2012; Maharajan *et al.,* 2013; Ram

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_{50}

Hours ofexposure	Control	Sub-Lethal (1/10 of 96h Lc_{50})	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

Nayan Singh, 2014).

The present experiments also demonstrated that oxygen consumed by *R. daniconius* showed no linear relationship to exposure time when fish exposed to1/10 sub-lethal concentration of 96h LC_{50} 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_{50} 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 (Hartlet 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.

REFERENCES

Ada, F. B., Ekpenyong, E. and Ayotunde, E. O.2012. Haematological, biological and behavioural changes in Oreochromisniloticus (Linne 1757) juveniles exposed to Paraquat herbicide. *J. Environ. Chem.* Ecotoxicol. **4(3):** 64-74.

Ajani, F. and Awogbade, A. A. 2012. Hematological Changes of the African Catfish Clariasgariepinus(Burchell,1822) JuvenilesInduced by Diuron, *British Biotechnology J.* **2(4):** 247-256.

Akinsorotan, A. M. 2014. Toxicity of dizensate (Glyphosate herbicide) on Clariasgariepinus fingerlings. *Advanced research in biological Science.* **2(1):** 1-5.

Barbieri, E., Quoy and Gaimard 2009. Effect of 2,4-D herbicide (2,4-dichlorophenoxyacetic acid)on oxygen consumption and ammonium excretion of juvenilesof Geophagusbrasiliensis (Quoy & Gaimard, 1824) (Osteichthyes, Cichlidae)Edison Barbieri Ecotoxicology (2009) **18:** 55-60.

Brausch, M. J. and Smith, N. P. 2007. Toxicity of three polyethoxy latedtal lowamine surfactant formulations to laboratory and field collected fairy shrimp,Thamnocephalusplatyurus. *Arch. Environ. Contam. Toxicol.* **52:** 217-221.

Bols, N. C., Brubacher, L., Ganassin, R. and Lee, L. E. J. 2001. Ecotoxicology and innate immunityof fish. *Dev. Comp. Immunol.* **25:** 853-873.

Carpenter, K. E. 1927. The lethal action of soluble metallic salts on fishes. *Brit. J. exp. Biol.* **4:** 378. (1930). *J. exp. Zool.* **56:** 407.

Chebbi, S. G. and David, M. 2010. Respiratory responses and behavioural anomalies of the carp Cyprinuscarpio under quinalphos intoxication in sublethal doses. *Science Asia.* **36:** 12-17.

Das, S. and Gupta, A. 2012. Effects of Cadmium Chloride on oxygen consumption and gill morphology of Indian flying barb, a teleost fish, Esomusdanricus. *J. Environmental Biology.* **33:** 1057-1061.

David, M., Shivakumar, H. B., Shivakumar, R., Mushigeri, S. B., Ganti, B. H. 2003. Toxicity evaluation of cypermethrin and its effect on oxygen consumption of the fresh water fish, Tilapia mossambica, Indian *J. Environ. Toxicol.* **13:** 99-102.

Ervnest, H. 2004. A Textbook of Modern Toxicology (3rd ed). *J. Wiley and Sons, inc, Hoboken, New Jersey.* p. 36.

Glusczak, L., dos Santos Miron, D., Moraes, B. S., Simões, R. R., Schetinger, M. R., Morsch, V. M. and Loro, V. L. 2009. Acute effects of glyphosate herbicide onmetabolic and enzymatic parameters of silver catfish (Rhamdiaquelen). *Comp. Biochem. Physiol. C.* **146:** 519-524.

Hartl, M. G. J., Hutchinson, S. and Hawkins, L. 2001. Organotin and osmoregulation: quantifying the effects of environmental concentrations of sediment-associated TBT and TPhT on the freshwater adapted European flounder, Platichthysflesus *L. J. Exp. Mar. Biol. Ecol.* **256:** 267-278.

Holden, A. V. 1973. Environmental Pollution by pesticides (Ed), CA Edwards Plenum Press, Chapter **6:** 542.

Jothinarendiran, N. 2012. Effect of Dimethoate Pesticide on Oxygen Consumption and Gill Histology of the Fish, Channa punctatus. Current Biotica. **5:** 500-507.

Maharajan, A., Usha, R., Paru Ruckmani, P. S., Vijay kumar B., Ganapiriya, S. V. and Kumarasamy, P. 2013. Sublethal effects of profenofoson oxygen consumption and gill histology of the Indian major carpCatlacatla(HAMILTON) Int. *J. Pure Appl. Zool.* **1(1):** 196-204.

Kodape, M., Tayade, D. N. and Akishor and Dabhadkar, D. 2012. Haematological and behavioural studied of freshwater fish, Channaorientalis exposed to novel derivative of 1,3,5-Triazine, *The Bioscan.* **7(1):** 165-169.

Marigoudar, S. R., Nazeerhmed, R. and David, M. 2009. Impact of cypermethrin on behavioural responses in the fresh water Teleost, Labeorohita (Hamilton). *World J. Zoology.* **4(1):** 19-23.

Langiano, V. C. and Martinez, C. B. 2008. Toxicity and effects of a glyphosate- based herbicide on the Neotropical fish Prochilobus lineatus Comp Biochem Physiol C. **147:** 222-231.

Modesto, K. A. and Martinez, C. B. R. 2010. Roundup causes oxidative stress in liver andinhibits acetylcholinesterase in muscle and brain of the fish Prochiloduslineatus. *Chemosphere.* **78:** 294-299.

Nikam, S. M. and. Shejule, K. B. 2015. Study of acute toxicity of bis (tributyltin) oxide (TBTO) on the freshwater fish,Nemacheilusbotia, from Nandur Madhmeshwar dam at Maharashtra, India *The Bioscan.* **10(2):** 517-519.

Olsen, R. E., Sundell, K., Mayhew, T. M., Myklebust, R. and Ring, E. 2006. Acute stress alters intestinal function of rainbow trout Oncorhynchus mykiss (Walbaum). *Aquaculture.* **250(1-2):** 480- 495. doi:10.1016/j.aquaculture.2005.03.014.

Omoregie, E., Ufodike, E. B. C. 1991. Acute toxicity of Gammalin 20

and Actellic 25EC to Oreochromisniloticus. *J. Aquatic Sci*. **6:** 13-17.

Palanivelu, V., Vijayavel, K., Ezhilarasi balasubramanian, S. and Balasubramanian, M. P. 2005. Impact of fertilizer (urea) on oxygen consumption and feeding the freshwater fish Oreochromis mossambicus. *Environ. Toxicol and Pharma*. **19:** 351-355.

Parma De Croux, M. J., A. Loteste and J. Cazenave 2002. Inhibition of plasma cholinesterase and acute toxicity of monocrotophos in Neotropical fish, Prochiloduslineatus (Pisces, Curimatidae). *Bull. Environ. Contam. Toxicol*. **69:** 356-362.

Ramamurthy, R., Nagaratnamma, R., Jayasundermma, B. and Rama Rao, P. 1987. Histopathological lesions in the gill of freshwater teleost, Cyprinuscarpioinduced by methyl parathion. *Matsya*.**13:** 144-147.

Ram Nayan Singh 2014. Effects of Dimethoate (EC 30%) on Gill Morphology, Oxygen Consumption and Serum Electrolyte Levels of Common Carp, *Cyprinus Carpiointernational J. Scientific Research in Environmental Sciences*. **2(6):** 192-198.

Rand, G. M. and Petrocelli, S. R. 1985. Introduction. In: Fundamentals of AquaticToxicology: Methods and Applications. Eds, Rand, G.M. and Petrocelli, S.R.Hemisphere Publishing Corporation, Washington. pp. 1-28.

Rao, J. V., Rani, S. C. H. Kavitha, P., Rao, R. N. Madhavendra, S. S 2003. Toxicity of Chlorpyrifos to the Fish Oreochromismossambicus. Bulletin of Environmental Contamination and Toxicology, Volume 70, Issue 5, pp 0985-0992

Sameer Gopal Chebbi and Muniswamy, David 2010. Quinalphos Induced Alterations in the Levels of Ions and Whole Animal Oxygen Consumption ofFreshwater Fish, Cyprinus Carpio (Linnaeus, 1758). *J. Veterinary Science & Technology*. **1(1):** 1-4.

Sarkar, S. K. 1999. Effects of two heavy metals (copper sulphate, cadmium sulphate) on the oxygen consumption of the fish Cyprinuscarpio (Linn.). *Uttar pradesh. J. Zool*. **19(1):** 13-16.

Sarikaya, R. and Yilmaz, M. 2003. Investigation of acute toxicity and the effect of 2,4-D (2,4-dichlorophenoxyacetic acid) herbicide on the behavior of the common carp (Cyprinuscarpio l.,1758; pisces, cyprinidae). *Chemosphere*. **52(1):** 195-201.

Shivakumar, R. and David, M. 2004. Toxicity of endosulfan to the fresh water fish *Cyprinus Carpio*. *Indian J. Ecol*. **31:** 27-29.

Susan Anita, T., Sobha, K. and Tilak, K. S. 2010. A study on acute toxicity, oxygen consumption and behavioral changes in three major carps, Labeorohita (ham), Catlacatla (ham) and Cirrhinusmrigala (ham) exposed to fenvalerate. *Bioresearch Bulle tin*.**1:** 33-40.

Vineet kumar, K. Patil and David, M. 2008. Behaviour and Respiratory Dysfunctionas an Index of Malathion Toxicity in the Freshwater Fish, Labeorohita(Hamilton). *Turkish J. Fisheries and Aquatic Sciences*. **8:** 233-237.

Wu, J. P. and Chen, H. C. 2004. Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation ofwhite shrimp (Litopenaeusvannamei) Chemosphere. **57:** 1591-1598.doi:10.1016/j.chemosphere. 2004.07.033