TOXICITY OF PARAQUAT ON FRESHWATER FINGERLINGS OF *Labeo rohita* (Hamilton)

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Abstract— Paraquat is registered and sold under different trade names in market over the last 64 years for agricultural to control unwanted plant and weeds. Paraquat has been banned in 32 highly acutely toxic and enter the body mainly by swallowing or through damaged skin, but also be enhanced thousands of death have accounted from ingestion, often suicide or dermal exposure to paraquat. The point of view concerning morphological changes acute and sub lethal toxicity of paraquat to fingerlings of labeo rohita mean weight of 6-8 g and length of 8-10cm were investigated under laboratory condition. Exposure paraquat shows in 96 hours LC50 was 25.71mg/L the toxicant leads to initial increase in the opercula ventilation rat which then decreased below the status by the end of 96 hours. Restlessness, immobilization, loss of balance and air gulping impaired equilibrium, erratic swimming, changes in orientation locomotion and convulsion were commonly observed before death during the acute bioassay. This results of the present study indicate the paraquat is highly toxic to the fingerlings of Labeo rohita. The use of paraquat should be strongly controlled and carefully monitored to eradicate from marketing areas and production areas to avoid the possible damage to environment and animals..

Index Terms— Paraquat, fingerlings of labeo rohita acute toxicity, Behavioral studies, environmental protection.

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1. Introduction

Generally agriculture is a main destination for chemicals sold, with 83% of all pesticides and herbicide ending up on farms. Of all different type of pesticide, herbicides are the most common and once again the heaviest user the agriculture industry. Now a day's using agricultural conventional products has heavy metals present in fertilizers Cd, Cr, Cu, manganese, molybdenum, nickel and zinc. The environmental sources arsenics are the pesticide, herbicides, and other agricultural products. Lead arsenate, in addition to being a component of industrial effluents, has been used as agricultural pesticide herbicide fungicide containing mercury contributes to environmental contamination. Eventually, many of these metals like toxin may accumulate in agricultural soils and pose a hazard to plant growth and animal nutrition.

The increasing human activities depend on the agriculture productions it releases of anthropogenic source including herbicide. On floods and usual run of could dissolve herbicide and shift them freshwater and seawater. Aquatic fishes may uptake herbicide from agricultural areas by different routes. Paraquat is sold under different trade names are Gramoxone, Crisquat, Dextrone X and Esgram in approximately 100 developed and developing countries. Hence it has been already banned in the European Union countries since 2007 (Dinisoliveria et al., 2008).

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Fishes generally produce detoxifying enzymes when exposed to toxicant like paraquat 2,4D, etc., but this may be hardly possible in acute exposures, which do not allow enough time for the fish to induce detoxifying enzymes as means of increasing immunity against the toxic effects of the chemical. The inability of the fish to detoxify the toxicant and excrete the resultant metabolites, besides direct damage by the toxicant to the epithelial cells of gills, possible destruction of liver (Omoregie et al., 1998) and internal asphyxiation (Duffus, 1980), may account for the rapid mortality recorded in acute lethal studies of this nature. Behavioral responses of fish exposed to sub lethal concentration of Dimethoate showed that they were under stress condition. During stress condition fish needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative source of energy is protein to meet the increased energy demand. The depletion of protein fraction in liver, muscle and gonad tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes (Malla Reddy and Bashamohideen, 1995).

Paraquat is a quaternary nitrogen herbicide that is sprayed on unwanted weeds and other vegetations before planting crops. It is a fast-acting, nonselective compound, which destroys tissues of green plants on contact and by translocation within the plant. Paraquat exerts its herbicidal activity by inhibiting reduction of NADP to NADPH during photosynthesis. This disruption leads to the formation of superoxide anion, singlet oxygen, and hydroxyl and peroxyl radicals. These reactive oxygen species (ROS) interact with the unsaturated lipids of membranes, resulting in the destruction of plant organelles, inevitably leading to cell death (Suntres 2002). It is produced commercially as a brownish concentrated liquid of the dichloride salt in 10–30% strength under the trade name of "Gramoxone" and for horticultural use as brown granules called "Weedol" at about 5% concentration (Singh et al 2003).

Paraquat poisoning has been widely reported worldwide, but only a few case reports are described in literature from India (Singh et al., 1999; Sandhu et al., 2003; Agarwal et al., 2006).

Paraquat involves cyclic reduction of oxidation reaction that release ROS and depletion of nicotinamide adenine dinucleotide phosphate hydrogen, photo degradation of paraquat results in the formation of N-methylisonicotinic acid, which further decomposes to yield methylamine-hydrochloride and carbon dioxide. In human, this poison has been reported to cause the failure of respiratory system, injury of central nervous system, pokinston's disease and cardiac disease. (Huang et al., 2013).

2. MATERIAL AND METHODS

2.1. Procurement and Rearing of Experimental Animal

The experimental animal Labeo rohita collected from the fish farm located at Puthur, Nagai District, Tamil Nadu - 10 km away from the university campus. The collected fish were transported to the laboratory with polythene bags filled half with water. About 25 fish were put in each bag and water was well aerated, using pressurized air from a cylinder. This mode of transit proved successful, since there was no mortality in all consignments throughout the course of this study.

The fish brought to the laboratory were acclimatized in large plastic trough for a fortnight before they were used for the experiment. The fish tanks (large plastic trough) were kept free from the fungal infection by washing with potassium permanganate solution. The fish were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in well-aerated tap water. Prior to experimentation they were acclimatized to experimental trough for one week. The fish measuring about 8-10 cm in length and 6-8 gm in weight were selected irrespective of their sex for the experiments. The fish were fed daily on oil less groundnut cake. The unused food was removed after 2 hours and water was renewed daily.

The test fish were critically screened for the signs of disease, stress, physical damage and mortality. The injured severely diseased, abnormal and dead individuals were discarded. Feeding was discontinued two days prior to the commencement of the toxicity experiments to reduce the additive effects of animal excreta in the test trough and feeding was restored at the sub lethal exposure periods (APHA, 1989).

2. Chemical for toxicity studies

In the present study the liquid formed Paraquat was used for the toxicity studies.

2.1. Designations of Paraquat

CAS number	: 4685-14-7
Chemical name	: 1, 1-dimethyl-4, 4 bipyridinium
Empirical formula	: C12H14N2
Molecular weight	: 186.3
Commercial herbicide name	: Gramoxone

2.2. Basic chemical and physical data of Paraquat

Form	: liquid
Colour	: dark-blue/green
Odor	: characteristic of pyridine bases
Boiling point	: approx 100 o C aqueous solution
Melting point	: not available
Flash point	: does not flash
Explosive properties	: non – explosive
Vapor pressure: not available	
Density	: 1.08 g/ml
Solubility	: soluble in/with water
PH-Value	: 6.5-7.5
Oxidizing properties	: non-oxidizing

2.3. Analytical procedure

Only healthy individuals collected from the same body of water were employed in all tests. The tests solutions were renewed every 24 hours to maintain the optimum dissolved oxygen level while conducting the experiments. Care was taken not to deviate from the modified main principles of bioassay techniques out lined by Sprague (1973) and recommended by APHA (1989).

2.4. Acute toxicity studies

Acute toxicity studies, popularly known as bioassay studies were conducted to determine the potency of the Paraquat. Static but renewal type of bioassay test was adopted in the present investigation to estimate the LC50 values of respective hours (Sprague, 1973). Experimental trough was covered with nylon net to prevent the escape of fish. Following the completion were well cleaned with 5 per cent nitric acid and subsequently dried to remove the herbicide from adhering to the walls of the experimental trough.

2.5. Screening test for exploratory test

The screening test was conducted to avoid delay and to save time and effort. The objective of this test is to obtain an approximate concentration of a substance likely to be hazardous to the test fish in their natural environment (Alabaster and Lioyd, 1982). A wide range of concentration viz., 175, 200, 225, 250, 270, 280 ppm of Paraquat was prepared from the stock solution. Ten fish were introduced in each plastic trough containing 10 liter of water with the required amount of Paraquat. The screening test was continued to assess the concentration at which all fish survived for 24 hrs and likewise the concentration at which most of the fish died simultaneously (Bansal et al., 1980).

2.6. Definitive test

Preliminary observation showed that beyond that 280 ppm of Paraquat, all the test fish were died and in 175 ppm all the fish were survived therefore the concentration of Paraquat falling between 175 and 280 ppm were prepared and test fish were introduced to a confined narrow rage concentration viz., 175, 200, 225, 250, 270, 280 ppm of Paraquat powder individual in the test medium, which showed no responses to stimulation and those opercula movements stopped were renewed quickly to avoid cannibalism among the fish. In all the tests, mortalities were recorded for 24, 48, 72 and 96 hours.

2.7. Calculation of LC50 values and regression lines

The recorded mortalities of Labeo rohita for 24, 48, 72 and 96 hrs exposure to Paraquat, were corrected for natural response by Abott's formula (Abott,
1925). The corrected mortality data was analyzed by following the method of Finney (1971) to determine the LC50 values (theoretical estimate of the concentration Lethal to 50 per cent of the test animals). The LC50 values were obtained by probit regression line, taking test concentration and corresponding percent mortalities on log concentration and probit scales respectively. Straight line (Regression line) was drawn between the points, which represent the survival percentage versus concentration (APHA, 1989). From the point at which the line intersects the 50 per cent survival line, a perpendicular line was drawn to the concentration ordinates which indicate the LC50 concentration of that particular period. By such graphical interpretation, the LC50 values and their fiducially limits (95% upper and lower confidence limit) were calculated.

2.8. Sublethal exposure to Paraquat

Sub lethal studies are helpful to assess the response of the test organism under water stress caused by herbicide Paraquat. According to Sprague (1973) one-tenth of the 96 hours of the LC50 values represents the lower sub lethal concentration. Hence, the one-tenth of the 96 hours LC50 (280 ppm) values of Paraquat were selected for the present investigation as sub lethal exposure concentration. The experimental fish were exposed to sub lethal concentration of Paraquat for the period of 30 days. The control and experimental fish were dissected at the end 30 days of exposure and the selected organs viz., gill, liver, kidney and muscle-were collected for protein profile studies.

2.9. Statistical analysis

The data of the present work were presented as mean ± standard deviation. Statistical analyses of the data were computed by SPSS (Version 10).

3.0. RESULTS

3.1. Acute toxicity studies

The acute toxicity studies of herbicide Paraquat on the freshwater fingerlings of Labeo rohita were estimated by the static bioassay method. The percentage mortality was recorded at 24, 48, 72 and 96 hrs. The recorded mortality data were subjected to probit analysis as described by Finney method (1971). The LC50 values upper and lower confidence limits, chi-square and regression results of Paraquat on fingerlings of Labeo rohita are given in the Table 1. The LC50 values of Paraquat at 24, 48,

72 and 96 hours of exposure periods were estimated at 28.13, 27.61, 26.22 and 25.71 mg/l respectively. The regression results were Y = 10.11 X –224.05; Y = 18.00 X –438.30; Y = 25.83 X 760.89 and Y = 31.25 X –932.25 for 24, 48, 72 and 96 hours. The goodness of fit was tested with the help of chi square (x2) test. It shows that the difference between observed and expected mortality were not significant and the differences only chance factor.

3.2. Behavioral responses

The sub lethal concentration (LC_{50}) of Paraquat for 24, 48, 72 and 96 hours and its 95 % confidence limits were determined from the data of the toxicity tests to get a basis of reference for analysis and determination of the mode of action for Paraquat toxicity on the test freshwater fingerlings of *Labeo rohita.* At sub-lethal concentrations of the toxicant exposed to *Labeo rohita* fingerlings showed similar symptoms in the first few hours of exposure but to a lesser extent. During the first few hours of the fish surfaced rarely but after 3 hours of exposure they surfaced frequently. The colour of the body turned pale and fins become more are less transparent..

4.0. DISCUSSION

Paraquat is the most highly acutely toxic herbicide to be marketed over the last 60 years. Yet it is one of the most widely used herbicides in the world, and in most countries where it is registered it can be used without restriction. It is used on more than 100 crops in about 100 countries. Gramoxone, manufactured by Syngenta, is the most common trade name for paraquat, but the herbicide is also sold under many different names by many different manufacturers. China is now the world's largest manufacturer of paraquat, producing more than 100,000 tonnes per year. Paraquat has been banned, or use disallowed, in 32 countries (including the countries of the Prepared by Meriel Watts PhD European Union), mainly for health reasons. But there has been strong industry resistance to including paraquat in the Rotterdam Conven tion on Prior Informed Consent and it remains outside the PIC list. Many international organisations, such as Rainforest Alliance, Fairtrade, Forest Stewardship Council, and food giants like Dole have voluntarily banned it from their production systems. Paraquat is a nonselective herbicide that has been shown to be highly toxic to animals and humans, and many cases of acute poisoning or death by Paraquat have been reported over the past years. (Fairshter and Wilson 1975; Haley 1979).

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Paraquat is highly acutely toxic and enters the body mainly by swallowing, or through damaged skin, but may also be inhaled. Thousands of deaths have occurred from ingestion (often suicide) or dermal exposure (mainly occupational) to paraquat. Paraquat is corrosive to the skin and once the skin is damaged it is easily absorbed into the body. One farmer died after just 3.5 hours spraying diluted paraquat with a leaking knapsack. Others have died from spilling the concentrate on their skin. Thousands more have suffered severe acute and chronic effects from occupational use. It represents a severe public health problem in many countries despite the fact that paraquat is considered safe by its manufacturers, who believe they have no responsibility for the suicides. Yet experience has shown that where paraquat is banned or restricted deaths from suicides drop dramatically. In developing countries paraquat is often applied under hazardous conditions that result in high dermal exposure. These conditions include high temperature and humidity, lack of protective clothing, leaking knapsack sprayers, lack of awareness of hazard, lack of control over the workplace, lack of facilities for washing, or medical treatment, and repeated exposure. In Malaysia women sprayers can spray herbicides, commonly paraquat, 262 days of the year. It was banned there in 2002 because of the unacceptable risk of adverse health effects, but industry pressure caused a reversal of the ban in 2006. As little as a teaspoon of concentrated paraquat can result in death. Death is by respiratory failure and may occur within a few days after poisoning or as long as a month later. There is no antidote. Paraquat damages the lungs, heart, kidneys, adrenal glands, central nervous system, liver, muscles and spleen, causing multi-organ failure, as well as damaging the skin and eyes.

4.1 Acute toxicity studies

Acute toxicity studies are useful to understand the level below which it may be considered ‘safe’ for the occurrence of a particular toxicant in the environment (Chan et al., 1986). The aim of the LC50 test is to provide on estimation of the acute toxicity of chemical compounds and provide some information about the intrinsic toxicity towards animals (Bendahou et al., 1997). The median lethal concentration (LC50) was calculate for a serious of time intervals and they were used for conducting the toxicity curve. The toxicity test and the application of LC50 values has gained acceptance among toxicologists and is gener
ally the most highly reliable test for assessing the potential adverse effects of aquatic life (Sprague, 1973).

Exposure to toxic substances may not result in immediate fish kills, but may affect fish populations by decreasing fecundity (number of eggs produced) reducing the viability of increasing the incidence of abnormalities and increasing natural mortality (Allan, 2000). Herbicides and insecticides used in agriculture have been responsible for a number of fish kills. Gramaxone like Endosulphans used in the cotton growing areas have been particularly problematic over the last decade (Napier et al., 1998). Atrazine at least concentration caused kidney damage in chronic exposed rainbow trout (Oulmi et al., 1995).Therefore the loss of fishes by the various direct and indirect format.

Surface water also can be contaminated directly by herbicide spray drift the travel and deposition of fine herbicide spray droplets away from their intended target. When we are spraying applied too close to water. Drift incidents can result in greater surface contamination than either runoff of leaching obvious acute effects such as fish kills can occur (Don et al., 1994).When croplands are treated some impacts of pesticides occur on non-target terrestrial and aquatic ecosystem (Surendra, 2010.

Gramoxone is a contact poison even in the plant kingdom. It could attack aquatic organisms especially those that flush water through their openings (mouth and anus) for the purpose of gaseous exchange. (Kori-Siakpere et al., 2007). Previous studies have investigated the toxicological effects of paraquat in fish Ogamba et al., 2011. However, the effects of paraquat on Labeo rohita have not been completely shown. Our

TABLE 1

TABLE: 1. LC_{50} VALUE AND REGRESSION EQUATION RESULTS FOR FINGERLINGS *LABEO ROHITA* TREATED WITH PARAQUAT EXPOSURE (96HRS)

Exposure hours	LC50 (mg/L)	LCL (mg/L)	UCL (mg/L)	Regression equation	Chi-square value (x2)
24	28.13	27.96	28.37	Y = 10.11 X – 224.05	2.834 (NS)
48	27.61	28.50	28.87	Y = 18.00 X – 438.30	3.110 (NS)
72	26.22	28.13	28.35	Y = 25.83 X – 760.89	3.054 (NS)
96	25.71	27.51	27.96	Y = 31.25 X – 932.25	4.418 (NS)

LC_{50} = 50 % Mortality, LCL = Lower Confidence Limit, UCL = Upper Confidence Limit, NS = Not significant.

results showed the 96 h LC50value are 25.71 mg/L similarly, Nwani et al., (2014) studied the 96 h LC50 value (i.e. 27.46 mg/L) was obtained from Clarias gariepinus exposed to paraquat, which suggests that this herbicide is highly toxic to fish. Ladipo (2011) Studied that the 96 h LC50 value of Paraquat dichloride was 60mg/L for Clarias gariepinus juveniles. The LC50 Value of this study for Clarias gariepinus juveniles is similar to the findings of Ayoola (2008).

There are several general references dealing with the toxicity of chemical, particularly herbicides to fish. Applecate et al. (1967) and Lawrence et al. (1965) both have studied extensive fish toxicity data. There has been relatively little investigation on the effect of herbicides on fish.. As the study of herbicides is becoming a more mature science and we have realized the possible through herbicide use, more emphasis and study will be placed on this aspect of the subject in developing new products.

4.2. Behavioral studies

Fish is a good sensitive indicator and can also be used as diagnostic tool because any change in its behavior indicates a rapid biological method to monitor aquatic pollution. Behavioral studies associated with exposure to fish to environmental contamination and pollutants have been reported in fish. These behaviors included the increased swimming and breathing rates, lethargic response and loss of equilibrium which in most cases were concentration dependent. (Chindah et al., 2004).

The behavior may be an adjustment of the internal homeostatic of the fish to the stress imposed by the toxicant (Edwards, 1973). The behavioral changes exhibited by fish exposed to toxicant elicit the potency and sensitivity of the fish to test chemical which can be used effectively as a biosensor of chemical stress. Loss of equilibrium, frequent surfacing, erratic swimming and gradual loss of activity have been reported in rainbow trout, C. gariepinus (Gabriel et al. 2002) and tilapia (Omoregie, 1998) exposed to various toxicants. The behavioral alteration may occur as a result of nervous impairment due to blockade of nervous transmission among the nervous system and various affecter sites (Fryday et al., 1996), failing organs and retarded physiological processes in fish body function. This may result in enzyme dysfunction and paralysis of respiratory muscle or depression of respiratory centre and disturbances in energy pathways leading to depletion of energy. (Cearley, 1971) Since aquatic organisms are in continuous contact with a polluted medium, hence breathing and feeding will be impaired due to the damages to respiratory and other organs of the body (Chindah et al., 2001).

Studies have shown that the mechanism of paraquat toxicity may also be attributed to its redox potential, which involves cyclic reduction-oxidation reactions that produce ROS and depletion of nicotinamide adenine dinucleotide phosphate hydrogen (Tsai et al., 2013) Photodegradation of paraquat results in the formation of N-methylisonicotinic acid, which further decomposes to yield methylamine-hydrochloride and carbon dioxide. In humans, paraquat poisoning has been reported to cause respiratory failure, severe central nervous system injury, and Pakinston's disease (Huang et al., 2013) In fish, paraquat has been reported to alter the activity of several enzymes. Thus, paraquat can affect the cardiac contraction, opercula ventilation, and embryonic development (Tortorelli et al., 1990). Therefore the food chain mostly ending with food style of human beings we are the prime responsibility to conserve further generation with selective food habit and creative awareness of avoiding indirect poison come back from environment which is we already seeded.

In the present study, the behavioral changes observed up to 96 hours during acute toxicity study of paraquat on L. rohita. Similar changes like loss of equilibrium, rapid Jerky movement, changes in orientation, locomotion and convulsion prior to death were observed on the paraquat treatment. These observations concordant with the behavior manifestation were recorded in Oreochromis niloticus exposed to butataf (Bath et al,. 1974; Chindah et al., 2001; Chindah et al., 2004; Fryday et al., 1996; Gabriel et al., 2010).

Similarly Nwani et al., 2015 studied physiological offects of paraquat in African freshwater catfish Clarias gariepinus this results strongly indicates that paraquat is highly toxic and paraguat should be strongly controlled and carefully monitored to avoid the damage done to the environment.

Similarly Nwani et al., (2015) studied the behavioural changes on paraquat intoxicated fish C.gariepius showed that the control specimens were not hyperactive and showed normal swimming patterns and fin movements throughout the exposure period. However, with increasing paraquat concentrations and exposure duration time, hyperactivity and jerky movements increased. In contrast, the swimming rate, fin movement, and equilibrium status decreased. Fish mortalities increased as the concentration of paraquat increased.

The Paraquat dichloride exerted toxic effect on the fish in the present study and toxicity increased with increased concentration. Abnormal behaviours such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercular acivities, surface to bottom movement, sudden quick movement and resting at the bottom observed in this study were similar to the observations of Omitoyin et. al., (2002).

Dangers of flushing toxic chemicals into the ecosystem through municipal sewage systems, one potentially divesting threat to wild fish populations comes from an unlikely source, estrogen. Male fish exposed to estrogen become feminized, producing egg protein normally synthesized by females. In female fish, estrogen often retards normal sexual maturation including egg production (Hogan, 2008). Therefore, expired pesticide herbicide formulations must be disposed carefully and care should be taken to avoid their discharge to water bodies and thus prevent lose to fish. Sub lethal pollution, which results in chronic stress conditions also have negative effect on aquatic life (Adedeji et al., 2008).

Not only can herbicide affect the food chain at any point through accidental contamination but they can also spread up through the food chain in multiplier effect, killing organisms unrelated to the targets. At the top of the food chain are humans, and we are as vulnerable as other animals to off-target poisoning. Hence, the present study it is concluded that the disposing of toxicants even in lesser quantity in to the water resources from agricultural soil may not produce immediate loss of fish living therein, but certainly bring about behavioral changes. Accumulation of herbicide in fish may be considered as an important warning signal for fish health and human consumption. So, precautions need to be taken in order to prevent future herbicide pollution, otherwise, these pollutions can be dangerous for fish and human health.

5.Conclusion

The results of this study revealed that herbicide Paraquat is toxic to fish organs and causes behavioural changes. Paraquat is widely used in the mistaken belief that it is harmless, safe and readily breaks down leaving no residues. Consequently, it is sprayed in public areas while people are present and by operators without protective clothing. The facts revealed in this study shows that Paraquat causes a range of defects and health problems to the test organism; Labio rohita. The herbicide has caused a host of acute and chronic effects on seeing thae behavioural changes. It could be concluded that Paraquat, has harmful effects on the physiology of fish which in turn affect the growth rate and reproduction and leads to deterioration of meat quality of the exposed fish to the point that it can be hazardous to humans at certain levels in water.

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