ABSTRACT

2,4-dichlorophenoxyacetic acid (2,4-D) is an herbicide commonly used in agriculture. The residues of 2,4-D is present in air, water, soil and edibles. It constitutes a real hazard for human and animal health as numerous accidents of poisoning deaths caused by this herbicide have been reported. The point of view concerning acute and sub-lethal toxicity of herbicide 2,4-D to fingerlings of Labeo rohita of mean weight of 6-8 g and standard length of 8-10 cm were investigated under laboratory condition. In 96 hours, LC$_{50}$ was 300 ppm the toxicant lead to initial increase in the opercula ventilation rate which then decreased below the status by the end of the 96 hours. Restlessness, immobilization, loss of balance and air gulping impaired equilibrium, erratic swimming, changes in orientation locomotion and convolution were commonly observed before death during the acute bioassay. Exposed cells of the fish gill had coagulation necrosis of the lamellae, per lamellae fibrosis and inflammations. The results from this study revealed that 2,4-D was very toxic to fingerlings of Labeo rohita under laboratory condition and its application should therefore, be restricted in the field.

KEYWORDS: 2, 4-D, Fingerlings of Labeo rohita, acute toxicity, Behavioral studies.

1.0. INTRODUCTION

Herbicide and related chemicals destroy the delicate balance between species that characterizes a functioning ecosystem. One of the important factors contaminating the natural habitat is agricultural herbicides. The increasing use of herbicides and pesticides in
agriculture including commercial and household production of vegetables for the control of pest and herb causes chemical pollution of aquatic environment. The chemical pollution causes potential health hazards to live stock, especially to fish, frogs, birds and mammals. However, the introduction of herbicides to the natural environment has also some negative effects, including unintentional intoxication of useful insects, fish, birds, mammals, and other inhabitants of aquatic and terrestrial biosensors (Senthil kumar, 2001).

2,4-Dichlorophenoxyacetic acid (2, 4-D) is a selective herbicide, with highest toxicity to broadleaf plants. 2,4- D is a common herbicide that is used around houses and gardens and also on golf courses, ball fields, parks, and in agriculture and forestry (Garabrant, 2002). Therefore, there is the need for studies to be carried out to determine the effect of the various chemicals on aquatic life, especially fish. Information emanating from such research is required to provide baseline data on herbicides produced in the developed world for developing and underdeveloped worlds (USEPA, 2000). Acute toxicity tests are carried out in the laboratory to determine the LC\textsubscript{50} that is concentration that will kill 50% of the fish or test organism under specified condition as observed by Lloyd. The period of exposure is usually 24, 48, 72, and 96 hours and the fish are not fed during the tests.

Each pesticides and herbicides exhibit different level of toxicity depending on the fish species. For example, Alexander et al., (1985) found the LC50 values of 2,4-D as 35mg/l for daphnia magna and 25mg/l for pimephales promelas (cyprinidae).The mortalities and LC\textsubscript{50} of fishes exposed to different pesticides vary. For instance in 96 h, LC\textsubscript{50} of Oreochromis niloticus when exposed to Paraquat was 3.56 mg L\textsuperscript{-1} while that of C. gariepinus exposed to diazinon was 6.03 mg L\textsuperscript{-1} (Okeke, 2004). Murty (1986) reported that in 96 h, LC\textsubscript{50} of endrin to various species of fish ranged from 0.7-2.1 mg L\textsuperscript{-1} under static condition while toxicity of 2,4-D depends on the type of formulation used. About 2,4-D products generally contain one or more inert ingredients. An inert ingredient is anything added to the product other than an active ingredient.

More than 60,000 of these chemicals are used in our day-to-day life in the form of fuel, industrial solvents, drugs, pesticide, fertilizers, food additives and consumer products. Besides these we are every year as many as 500-1000 new chemicals to the existing array of chemicals. Therefore need to search alternative resource instead of synthetic herbicide. Present study planed to identify the unsafe using herbicide 2,4-D which is available in market of throughout in India for sale to agricultural field as a name of herbicide. Our study not only
identified the dangerous chemical in agricultural field and also strongly creates awareness to avoid the conventional herbicide by used acute toxicity and behavioral studies.

2.0. 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).

2.2. Water Characteristics

In the present study chlorine free well water was used as test water and its character features were as follows:

- **pH**: 7.2 ± 0.2
- **Temperature**: 26 ± 1°C
- **Dissolved O₂**: 8.0 ± 10.0 ppm
- **Salinity**: 0.2 – 0.6 ppm
- **Alkalinity**: 250-260 mg/l as CaCO₃
- **Hardness**: 350-380 mg/l as CaCO₃
2.3. Chemical for toxicity studies
In the present study the powder formed 2, 4-Dichlorophenoxy acetic acid was used for the toxicity studies.

2.4. Designations of 2,4-Dichlorophenoxyacetic acid (2,4-D)

CAS. No. : 94-75-7
Registry Name : 2, 4-Dichlorophenoxyacetic acid
Chemical Name : 2, 4-Dichlorophenoxyacetic acid
Synonyms, Trade Name : 2, 4-D
Chemical Name (German) : 2, 4-Dichlorophenoxyessigsäure
Chemical Name (French) : Acid 2, 4-Dichlorophenoxyacétique
Appearance : Colorless, Crystalline powder with mostly odor

2.5. Basic chemical and physical data

Empirical formula : C₈C₆Cl₂O₃
Rel. Molecular Mass : 221.04g
Density : 1.563 g/ cm³
Boiling point : 160°C at 50 pa
Melting point : 140.5°C
Vapor pressure : < 10⁻⁵ pa
Solubility : In water : 0.55 g/L
In olive oil : 0.5 g/L, in benzene 6 g/L
In acetone : 850 g/L

2.6. 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 outlined by Sprague (1973) and recommended by APHA (1989).

2.7. Acute toxicity studies

Acute toxicity studies, popularly known as bioassay studies were conducted to determine the potency of the 2, 4-D. Static but renewal type of bioassay test was adopted in the present investigation to estimate the LC₅₀ values of respective hours (Sprague, 1973). The
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.

2.8. 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 Llloyd, 1982). A wide range of concentration viz., 100, 150, 200, 250, 300, 350 ppm of 2, 4-D was prepared from the stock solution. Ten fish were introduced in each plastic trough containing 10 liter of water with the required amount of 2,4-D. 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).

2.9. Definitive test
Preliminary observation showed that beyond that 300 ppm of 2,4-D, all the test fish were died and in 250 ppm all the fish were survived therefore the concentration of 2,4-D falling between 250 and 300 ppm were prepared and test fish were introduced to a confined narrow rage concentration viz., 250, 260, 270, 280, 290, 300 ppm of 2,4-D 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.

2.10. Calculation of LC$_{50}$ values and regression lines
The recorded mortalities of *Labeo rohita* for 24, 48, 72 and 96 hrs exposure to 2,4-D, 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 LC$_{50}$ values (theoretical estimate of the concentration Lethal to 50 per cent of the test animals). The LC$_{50}$ 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 LC$_{50}$ concentration of that particular period. By such graphical interpretation, the
LC$_{50}$ values and their fiducially limits (95% upper and lower confidence limit) were calculated.

### 2.11. Sublethal exposure to 2, 4-D

Sub lethal studies are helpful to assess the response of the test organism under water stress caused by herbicide 2, 4-D. According to Sprague (1973) one-tenth of the 96 hours of the LC$_{50}$ values represents the lower sub lethal concentration. Hence, the one-tenth of the 96 hours LC$_{50}$ (300 ppm) values of 2,4-D were selected for the present investigation as sub lethal exposure concentration. The experimental fish were exposed to sub lethal concentration of 2,4-D 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.

### 2.12. 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. Effects of sub-lethal concentrations

##### 3.1.1. Acute toxicity studies

The acute toxicity studies of herbicide 2, 4-D sodium salt 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 LC$_{50}$ values upper and lower confidence limits, chi-square and regression results of 2,4-D on fingerlings of *Labeo rohita* are given in the Table 1. The LC$_{50}$ values of 2,4-D at 24, 48, 72 and 96 hours of exposure periods were estimated at 30.15, 29.73, 29.25 and 28.74 ppm respectively. The regression results were $Y = 12.14X - 336.07; Y = 20.00X - 551.42; Y = 27.85X - 763.92$ and $Y = 34.28X - 934.28$ for 24, 48, 72 and 96 hours. The goodness of fit was tested with the help of chi square ($x^2$) test. It shows that the difference between observed and expected mortality were not significant and the differences only chance factor.

##### 3.1.2. Behavioral responses

The sub lethal concentration (LC$_{50}$) of 2,4-D 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 2, 4-D 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 color of the body turned pale and fins become more are less transparent.

### Table: 1. LC50 value and regression equation results for fingerlings *L. rohita* treated with 2,4-D sodium salt

<table>
<thead>
<tr>
<th>Exposure (hrs)</th>
<th>LC50 (ppm)</th>
<th>LCL (ppm)</th>
<th>UCL (ppm)</th>
<th>Regression equation</th>
<th>Chi-square value ($\chi^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>30.15</td>
<td>29.98</td>
<td>30.39</td>
<td>$Y = 12.14X – 336.07$</td>
<td>3.945(\text{NS})</td>
</tr>
<tr>
<td>48</td>
<td>29.73</td>
<td>29.60</td>
<td>29.88</td>
<td>$Y = 20.00X – 551.42$</td>
<td>4.222(\text{NS})</td>
</tr>
<tr>
<td>72</td>
<td>29.25</td>
<td>29.14</td>
<td>29.36</td>
<td>$Y = 27.85X – 763.92$</td>
<td>4.056(\text{NS})</td>
</tr>
<tr>
<td>96</td>
<td>28.74</td>
<td>28.52</td>
<td>28.97</td>
<td>$Y = 34.28X – 934.28$</td>
<td>5.420(\text{NS})</td>
</tr>
</tbody>
</table>

LC50 = 50 % Mortality, LCL = Lower Confidence Limit, UCL = Upper Confidence Limit, NS = Not significant.

### 4.0. DISCUSSION

The herbicide and pesticide, on reaching to aquatic systems, greatly influences the non target organisms such as fish and birds. The toxic effects of the chemicals may be physiological, biochemical and pathological in nature. The changes produced by these agents may be complex, damaging different organs, tissues or cells. Examinations of tissues from organisms after death may possibly reveal the causative agent. (Thenmozhi et al., 2011)

#### 4.1. Acute toxicity studies

The bio assessment of toxicity of the herbicides with reference to aquatic biota is playing a crucial role in establishing the toxicity guidelines of chemicals to non-target species. The toxicity evaluation with reference to aquatic fauna is a complex one since the synergistic or antagonistic interaction with chemicals modulates the toxic effects of chemicals on biota. The well known and generally accepted technique of evaluating the toxicity of a pesticide, is the determination of median lethal concentrations [LC50 values], where the toxicity of pesticides to an aquatic organism is assessed by the concentration of the toxic compound in water that will kill 50% of the animals exposed for a specific period of time.

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.,
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 median lethal concentration (LC$_{50}$) was calculated for a series of time intervals and they were used for conducting the toxicity curve. The toxicity test and the application of LC$_{50}$ values has gained acceptance among toxicologists and is generally the most highly reliable test for assessing the potential adverse effects of aquatic life (Sprague, 1973).

Sprague (1973) makes a plea for standardization of terminology and the use of detailed and accurate procedure with a view of rendering the bioassay results more meaningful reliable comparable and universally interpretable. One of the most important methods of determining the pollutant concentration that will not harm aquatic life is biological assay (or) bioassay. In the present investigation the term toxicity test is being used instead of bioassay.

The frequent uses of herbicide like pesticides for various industrial, agricultural and domestic purposes are veritable sources of pesticide introduction into the environment. These pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and after the physiological properties of water (Behalchandra et al., 2001). Agricultural pesticides are released into the atmosphere by the spray drift, post application volatilization and wind erosion of soil (Qiu et al., 2004). Ventura et al. (2008) reported that pesticides presents in aquatic environments could affect aquatic organisms in different ways. In India, more than 70% of the chemical formulations are employed in agricultural practices and to find their way to freshwater bodies, ultimately affect non-target organisms (Bhatnagar et al., 1992). The widespread use of herbicides has resulted in a steady increase in water pollution, evoking considerable damage of phytoplankton and zooplankton. Thus depleting essential sources of the food chain to the fish (Montanes et al., 1995). Fish and aquatic animals are exposed to pesticides in three primary ways (i) thermally direct absorption through the skin by Swimming in pesticide – contaminated waters (ii) breathing by direct uptake of pesticides through the gills during respiration and (iii) orally by drinking pesticide. Contaminated water or feeding on pesticide contaminated water or feeding on pesticide contaminated prey (Mathur and Singh, 2006).

Some soluble pesticides and herbicide are easily leached into streams and lakes (David, 2005). Mavura and Wangila (2004) investigated the extent of pollution of Lake with respect to organochlorine pesticide residues. They revealed that more contaminants are added to the lake during rainy season than dry season. Fish kills occur when pesticides are improperly
applied to or otherwise end up in bodies of water through either misapplication or drift (Mike, 2007). Accidental killing of fish due to the contamination of the aquatic environment remains among the most frequent poisoning cases. Every year, about 260 to 300 of such accidents are diagnosed (Helena and Zdenka, 2009).

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. 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).

The pesticide and herbicide residues were found to cause impairment in reproductive system and thyroid activity in aquatic fauna (Great and Mehrle, 1969). In case in Mozambique, pesticide has been burned on a site which has subsequently covered up with soil. Residues from the incineration had contaminated the surrounding soil, which local populations had discovered was toxic to fish and had been used by local populations to catch fish in the local river. The toxic soil would cause fish to die instantly, floating up to the surface, where they would be caught and consumed or sold on the local market (FAO and DINA, 2004). High DDT and dieldrin residues were reported in African, fish at levels that could potentially affect their reproduction have chronic toxic and behavioral effects and even drastically affect their population (Paul, 2005). Expired drugs and pharmaceuticals released in water lead to biological change in fish which leads to reproductive defects.

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 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). The effects of toxicants on the enzyme activity and protein content on fresh water fish have been observed by a number of investigators (Ramalingam and Ramalingam, 1982).
Surface water also can be contaminated directly by pesticide spray drift the travel and deposition of fine pesticide 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). The acute toxicity of glyphosate is considered to be low by the World Health Organization (WHO, 1994). However commercial glyphosate formulations are more acutely toxic than glyphosate.

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. Usually the work reported occurs as a brief note in conjunction with an herbicidal study of on aquatic weed problem. 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.

Acrolein is one of the herbicide it is also control aquatic weeds. Burdick et al. (1964) reported that acrolein is very toxic to fish. The chemical has 24 hours TLm³ of 46 PPb for fingerlings of brown trout (Salmo trutta L.) and 79 PPb for bluegills (Lepomis macrochirus R.). Chancellor and Ripper (1960) indicated that yellow perch, northern fathead minnow (Pimephales promelas R.) and pumpkin seed (Lepomis gibbosus L.) tolerated 300 ppm, although at 400 ppm 50% of the brown trout were killed. Frank et al. (1967) in comparing the residual effect of parquet, an ester of (2,4-dichlorophenoxy)acetic acid (2,4-D) dichlorobenil, and ferac in bond water and soil, found parquet was the least persistent in water it was not detected 12 days after application.

The data available from Van Vekenburg (1969) the 96 hrs LC₅₀ value of 2,4-D on striped bass was 70 mg/L; banded kill fish was 27 mg/L; Pumpkin seed was 65 mg/L; white perch was 40 mg/L; American eel was 300 mg/L; carp was 96.5 mg/L. According to Gabriel et al. (2010), the 96 hrs LC₅₀ value on Clarias gariepinus was 165.36 mg/L. The lower and upper confidence limit was 133.29 and 190.51 mg/L respectively. The differences may be due to a number of factors such as ability of fish to metabolize, biotransform and excrete wastes products. However, the hazards posed by the chemical to the fish in the environment may be decided by its persistence in the environment, mode of action and modifying influences of other...
environmental factors. This investigation reveals that the concentrations of the herbicide 2,4-D in this trial are toxic to fingerlings of *Labeo rohita*. The amount of toxicant present in the aquatic environment after application may exceed the recommended limit thereby causing physiological stress which may eventually head to death. Therefore the use of the herbicide in ponds, canals and agricultural field to control weed should be regulated to avoid or reduce to the minimum negative impact on the aquatic organism.

Again a close and careful comparison of LC$_{50}$ values of the present study with the earlier reports could bring into light that the LC$_{50}$ values also depend upon the physical and physiological nature of the fish. Further, the LC$_{50}$ values may be altered by various factors depending on the experimental condition. The findings of the present study are also clearly consistent with the statement that the median lethal concentrations differ from species to species for the same toxicant depending on the response of the animals (Zbinden and Flury-Roversi, 1981). Thus, the data on LC$_{50}$ values obtained for the test animal will be highly useful for the final evaluation of the extent of pollution of aquatic environment caused by agricultural chemicals, especially that of 2,4-D sodium salt. Further the acute toxicity test offers rapid and short term result to measure relative impact of the pesticide concentration on the organism at different time intervals. So this type of study may be useful to compare the sensitivity of various species of aquatic animals and potency of chemicals using LC$_{50}$ values. Thus, with the knowledge of LC$_{50}$ values obtained would be possible to establish limits and the levels of acceptability of toxic substances by the aquatic organism like fish.

**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 (Chindah *et al.*, 2004). These behaviors included the increased swimming and breathing rates, lethargic response and loss of equilibrium which in most cases were concentration dependent. The increase in TBF (tail beat frequency) and OBF (opercula beat frequency) may be associated with sudden response of the fish to the shock of exposure to the agro-chemical (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).

Fishes generally produce detoxifying enzymes when exposed to toxicant, 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 Dimehoate 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).

Mushigeri and David (2005) reported experimental the control fish behaved in natural manner *i.e.* they were active with their well-coordinated monuments. They were alert at the slightest disturbance, but in the toxic environment fish exhibited irregular erratic and darting swimming movements and loss of equilibrium which is due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyper stimulation. In the present study, the behavioral changes observed up to 96 hours during acute toxicity study of 2,4-D on *L. rohita*. They were the loss of equilibrium, rapid Jerky movement, changes in orientation, locomotion and convulsion prior to death. 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).

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, herbicide 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.

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.

5.0. ACKNOWLEDGEMENT

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