Determination of LC_{50} and Behavioural studies of freshwater fish *Catla catla* (Hamilton) Exposed to Ethion (50%EC)

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Abstract:

Farmers uses different type of pesticides for increasing crop production by preventing crop diseases, but in addition to the increasing usage, the concentration of pesticide used and frequency of application of pesticides is increasing globally. The aim of this study was to investigate Ethion 50% EC an organophosphate pesticide. toxicity and its effects on Behavioural characteristics of *Catla catla*. It is an insecticide, miticide and termiticide. For LC_{50} determination, Fish were acclimatized for 7days to 15days in laboratory conditions. The LC_{50} was calculated based on the fish mortality and the values of *Catla catla* exposed to Ethion (50%EC) for 24h 4.9 µg/l, for 48h 4.2 µg/l, for 72h 3.5 µg/l and for 96h 2.8 µg/l. The results of the present study indicated that Ethion is toxic to fish even for a short- term exposure and low dose.

Keywords: *Catla catla*, Ethion (50% EC), LC_{50}, Toxicity evaluation, behavioural studies.

I. Introduction:

The use of pesticides has become an inevitable feature of agriculture for pest control (Haque *et al.,* 2017). Pesticide contributes to enhancing crop production but at the same time pollutes the aquatic environment (Nair *et al.,* 2017). Pesticides from agricultural fields find their way into the natural water bodies through surface runoff and adversely affect the quality of water surfaces. Pesticides exposure causes toxicity in many aquatic organisms, and fish is the most common among them. Frequent pesticide exposures cause severe damage in any exposed organisms. Pesticides are present all over in environment and the mode of exposure to these toxicants in aquatic organisms are in different ways. Such as direct absorption through the skin, uptake of pesticides through the gills and drinking pesticides-contaminated water or feeding on prey.

Pesticides present in the environment with other similar compounds, may induce lethal or sub lethal effects in fish. The unrestricted, heavy use of synthetic pesticides results in a lethal effect on various non-target organisms in the aquatic environment and direct or indirect effect to users (Kumaresan V *et al.,* 2019). About two million tons of pesticides are currently utilized annually (Sharma *et al.,* 2019). Among the major groups of pesticides, organophosphorus is the widely used class of pesticides. Commonly used organophosphorus pesticides are malathion, ethion, methyl parathion, dichlorvos, trichlorfon, chlorpyrifos, diazinon, fenitrothion, quinalphos and phosphamidon etc. Many reports have indicated that ethion harms fish. Therefore, monitoring the impact of these pesticides is essential. Hence in the present study Ethion (50% EC) is exposed to the freshwater fish *Catla catla* to find out its toxicological effects.

II. Materials and methods

1.Test Chemical and stock and test solution

Ethion (50% EC) is an organophosphate strong insecticide, miticide and termiticide used on citrus, cattle and in the prevention of vector borne diseases. Its chemical name is S,S'-methylene bis(O,O-diethyl phosphorodithioate). It affects a neural enzyme called acetylcholinesterase and prevent it from working. Ethion is non-systemic with predominate contact action. It is an acetylcholine esterase inhibitor.

Preparation of stock solution:

The test pesticide Ethion stock solution was prepared by mixing 1g of the soluble liquid in 1 L of solvent. Test solutions of the required concentration were prepared by dilution the stock solutions for the range finding.

2.Test fish

The Indian major carp *Catla catla* (Hamilton) belongs to the family *Cyprinidae*. It is commonly called as Botcha in A.P. It is most valuable edible fish that can be found throughout India. *Catla* is a fish with a huge, broad, laterally compressed head, a large protruding lower jaw, and an upturned mouth lacking barbels. Eyes are large and located in front of the head. Lower lip of the *Catla* is very thick and the upper lip is absent. It has large greyish cycloid scales on its dorsal side and whitish on its belly. It is non predatory and its feeding is restricted to the surface and mid waters. Adults feed on zooplankton, but young ones on both zooplankton and phytoplankton. It grows very quickly to a length of 182 cm and 1 to 2 kg in one year under normal culture conditions. It is rich in proteins and is very suitable for human consumption. Hence, I selected *Catla catla* as my experimental fish.

3. Collection, acclimatization, and Acute Toxicity test of selected fish

The fingerlings of *Catla catla* were collected from the Kuchipudi fish form, Guntur (dt), A.P, with average body weight 7 ± 8g and size 7.5 ± 8.5cm. The fish were acclimatized to the laboratory conditions in large plastic tanks with sufficient dechlorinated ground water for 15 days at a room temperature of 28 ± 2^0C. The fish were fed with ground nut cake, rice bran once in two days, at the same time water was renewed and faecal matter and other waste products were siphoned off daily. The water in the tank was aerated continuously with aerators. Feeding was stopped one day prior to the experimentation.

During the experimental period, no food was given to the fish and the water was renewed daily. Experiments were conducted to select mortality range from 10% to 100% for 24, 48, 72 and 96 hours to determine LC_{50} values of Ethion exposed to the freshwater fish *Catla catla*. Two groups of fish, 10 individuals in each group. i). Control group (C), exposed to without toxicant, ii). Experimental Group, exposed to Ethion. All experiments were carried out in triplicate in static renewal system. The dead fish were removed from test chambers immediately after death; the data regarding the fish mortality was recorded from the tests at the end of each specific time period. The physic-chemical characteristics of water were determined by standard methods of APHA (2005). The lethal concentrations ensure death even before noticing the behavioural abnormalities.

4.Statistical Analysis:

The concentration-response data of each pesticide was analysed using the probit Analysis Method (Finney, 1971 method).

III. Results:

A. Determination of LC_{50}:

The result showed that Ethion was toxic to the test fish *Catla catla*. According to previous studies and toxicity of the pesticides was both time and concentration dependent, thus accounting for LC_{50} values obtained at different concentrations and times of exposure. The test result of the 96h LC_{50} of *Catla catla* exposed to Ethion obtained (Table no:4).

The data was computed according to Probit Analysis Method (Finney, 1971) and the LC_{50} values were determined. In present study 10%, 30%, 50%, 70% and 90% mortality of *Catla catla* for 24h values were obtained at 4.5µg/l, 4.7µg/L, 4.9µg/L, 5.1µg/L and 5.3µg/L; for 48h 3.8 µg/L, 4.0 µg/L, 4.2 µg/L, 4.4 µg/L and 4.6 µg/L; for 72h 3.1µg/L, 3.3µg/L, 3.5 µg/L, 3.7µg/L and 3.9 µg/L and for 96h 2.4 µg/L, 2.6 µg/L, 2.8 µg/L, 3.0 µg/L and 3.2 µg/L respectively exposed to Ethion. In the present study the LC_{50} values of *Catla catla* for 24h 4.9µg/l, for 48h 4.2µg/l, for 72h 3.5µg/l and for 96h 2.8 µg/l and the reported values were given in the Table 1, Table 2, Table 3 & Table 4 and concentration of toxicant verse percent mortality and probit mortality in Fig1 & Fig2 (24h), Fig 3 & Fig 4 (48h), Fig 5 & Fig 6 (72h) and Fig 7 & Fig 8 (96h).

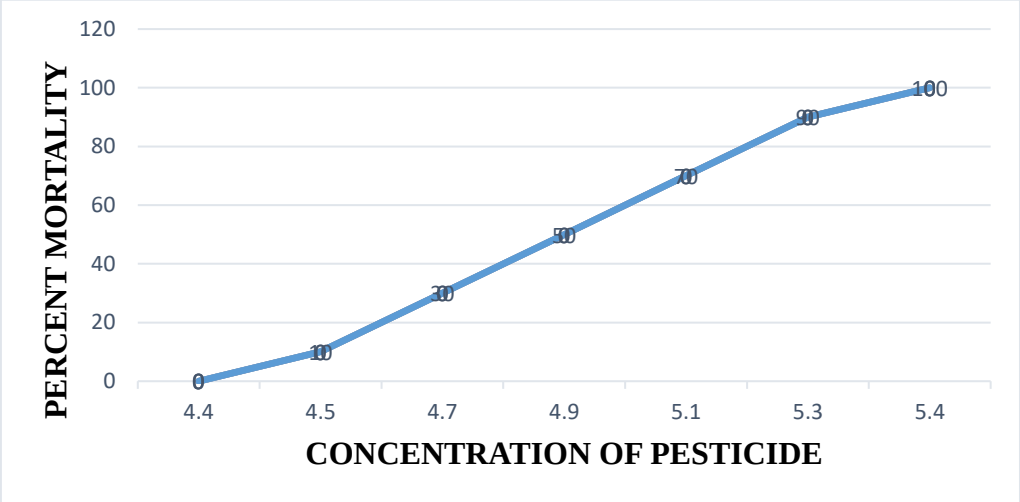
B. Behavioural studies:

Many changes were observed in the behaviour of *Catla catla* exposed to the test pesticide Ethion (50%EC). Fishes came to the surface of water much more frequently and tried to jump out of water. Moreover, treated fish exhibited increased opercular movement along with mucus secretion and progressively became sluggish and lethargic. Prior to death in contaminated medium, the fishes mostly showed abnormal swimming movements including loss of orientation and a tendency of muscular tetany. The toxic condition leads to the abnormal functioning of the body including loss of balance, moving in circular form. Fish in the treated group applied with highest concentration of the pesticide were lying laterally at bottom with loss of balance, swimming down in a spiral movement with jerks. Hypersensitivity and/or erratic, uncoordinated movements (when startled, fish will often swim in circles). Tremors, convulsions, coughing and finally death.

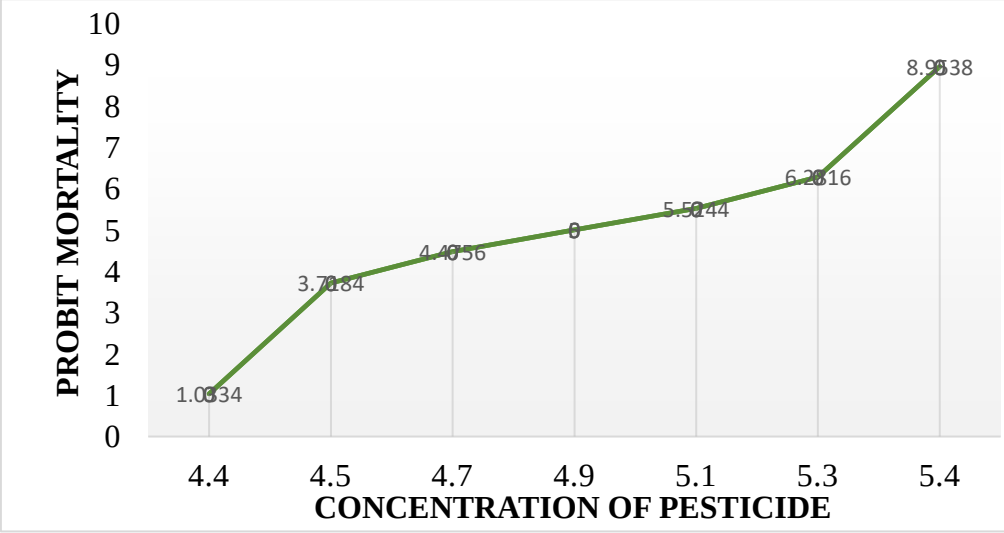
III. Table.1. Percent mortality and probit mortality of the freshwater fish *Catla catla* exposed to Ethion (50% EC) for 24h:

S. No.	Dose in µg/l	No. of fish exposed	No. of fish dead	% Mortality	Probit Value(Y)	LOG (100*DOSE) =X
1	4.5	10	1	10%	3.7184	2.6532
2	4.7	10	3	30%	4.4756	2.6720
3	**4.9**	**10**	**5**	**50%**	**5**	**2.6901**
4	5.1	10	7	70%	5.5244	2.7075
5	5.3	10	9	90%	6.2816	2.7242

III. Figure: 1. 24h dose response curve between percent mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC):



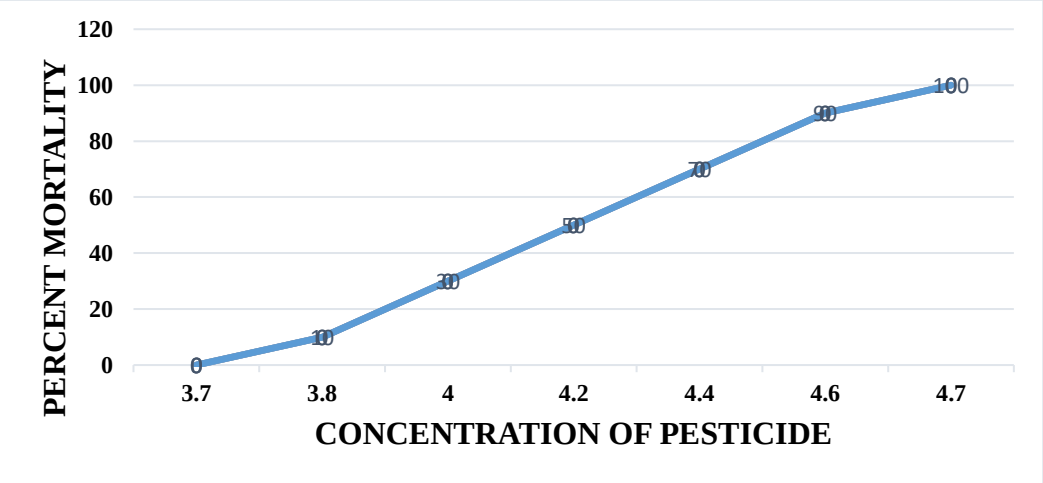
III. Figure: 2. 24h dose response curve between probit mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC):



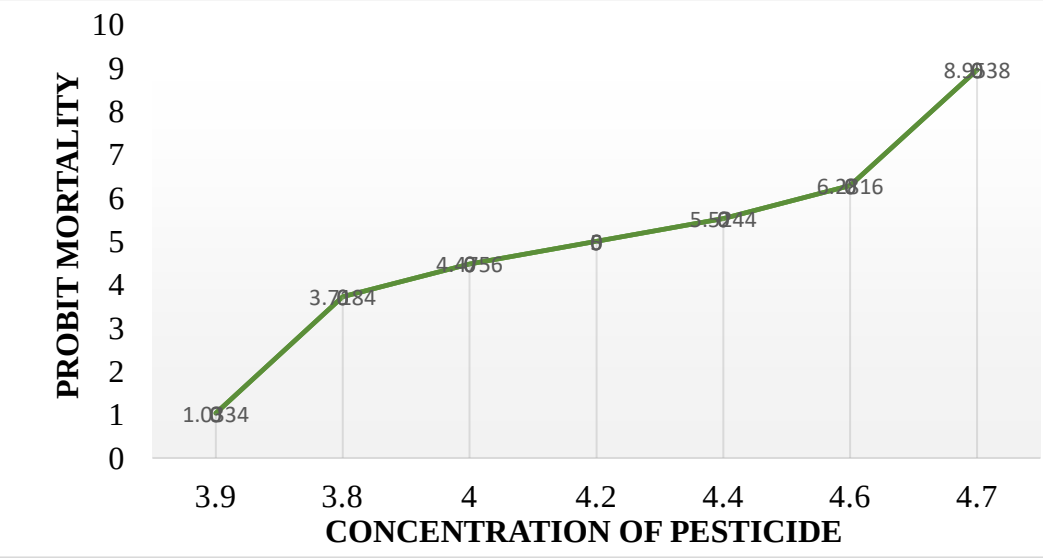
III. Table.2. Percent mortality and Probit mortality of the freshwater fish, *Catla catla* exposed to Ethion (50% EC) for 48h:

S. No.	Dose in µg/l	No. of fish exposed	No. of fish dead	% Mortality	Probit Value(Y)	LOG(100*DOSE) =X
1	3.8	10	1	30%	3.7184	2.5797
2	4.0	10	3	40%	4.4756	2.6020
3	**4.2**	**10**	**5**	**50%**	**5**	**2.6232**
4	4.4	10	7	60%	5.5244	2.6434
5	4.6	10	9	70%	6.2816	2.6627

III. Figure: 3. 48h dose response curve between percent mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC):



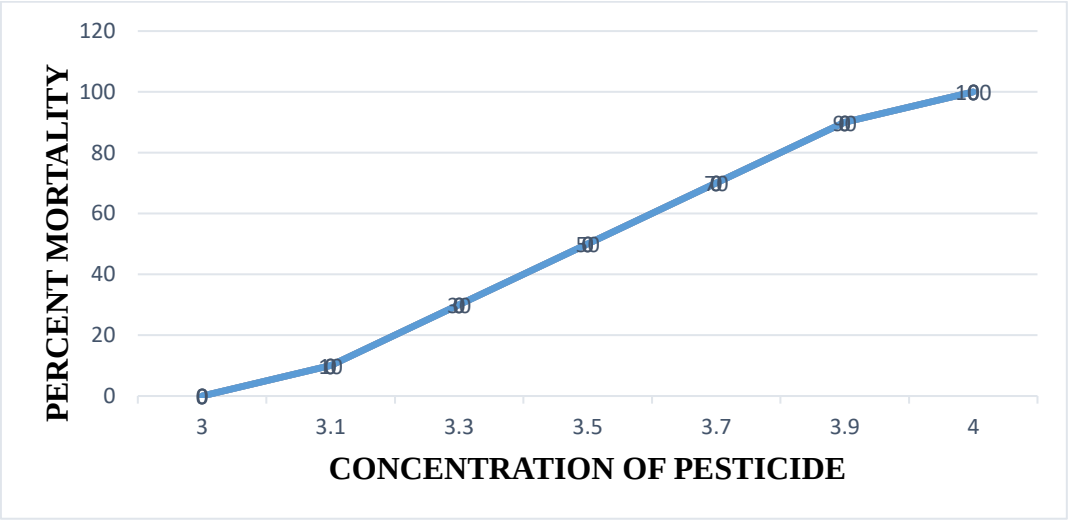
III. Figure: 4. 48h dose response curve between probit mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC):



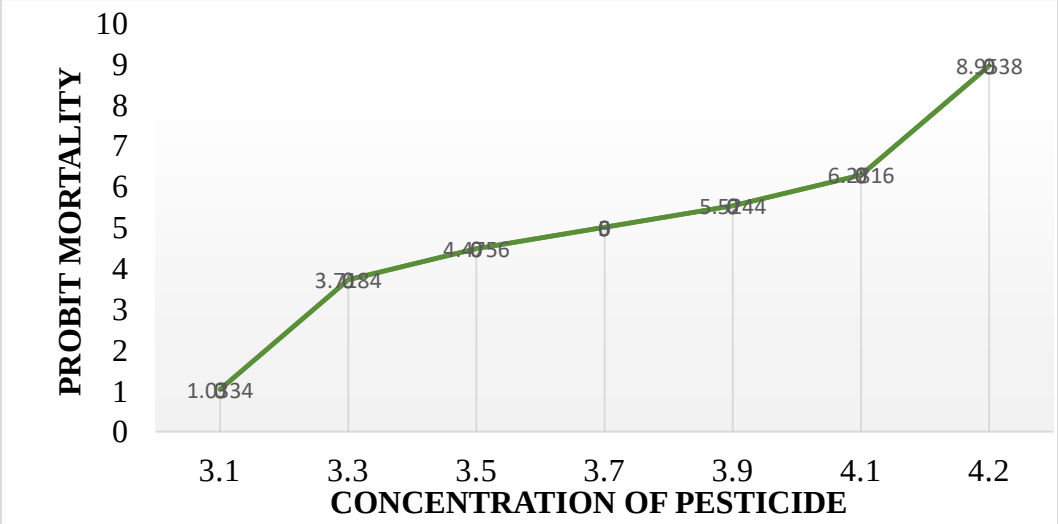
III. Table.3. Percent mortality and Probit mortality of the freshwater fish, *Catla catla* exposed to Ethion (50% EC) for 72h:

S. No.	Dose in µg/l	No. of fish exposed	No. of fish dead	% Mortality	Probit Value(Y)	LOG(100* DOSE) =X
1	3.1	10	1	10%	3.7184	2.4913
2	3.3	10	3	30%	4.4756	2.5185
3	**3.5**	**10**	**5**	**50%**	5	**2.5440**
4	3.7	10	7	70%	5.5244	2.5682
5	3.9	10	9	90%	6.2816	2.5910

III. Figure: 5. 72h dose response curve between percent mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC):



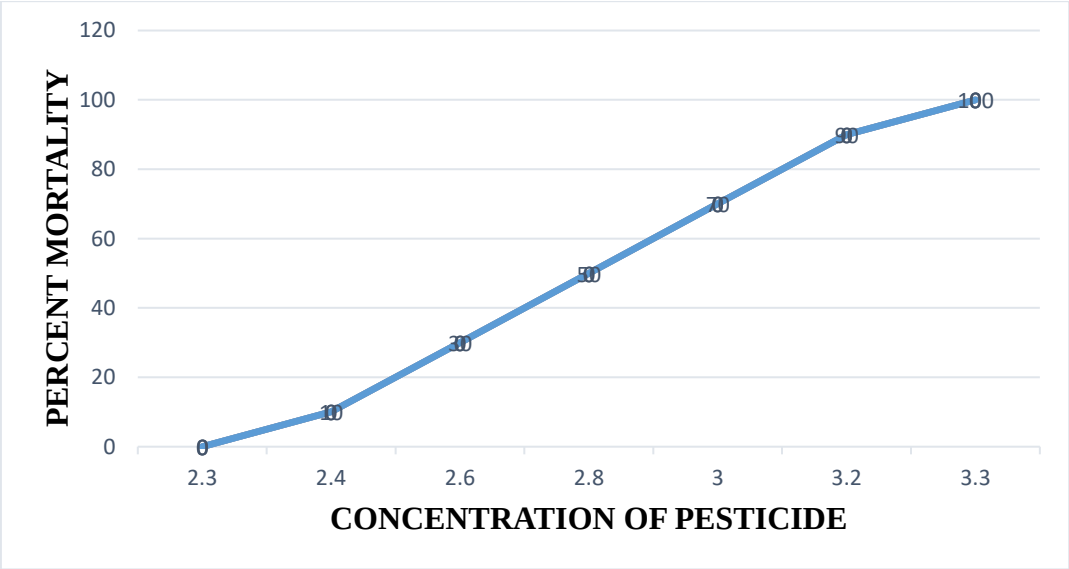
III. Figure: 6. 72h dose response curve between probit mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC):



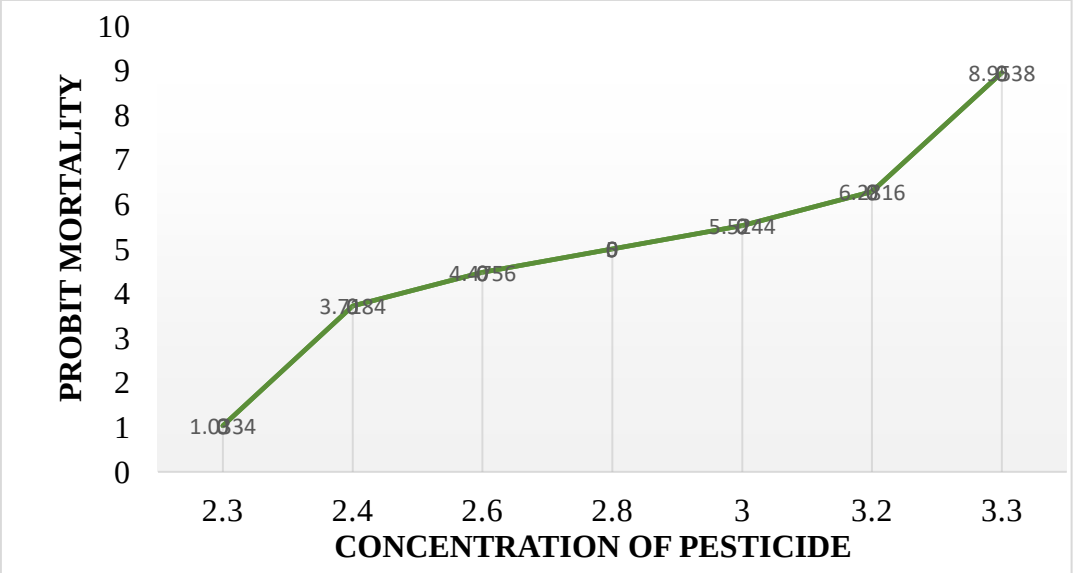
III. Table.4. Percent mortality and Probit mortality of the freshwater fish, *Catla catla* exposed to Ethion (50% EC) for 96h:

S. No.	Dose in µg/l	No. of fish exposed	No. of fish dead	% Mortality	Probit Value(Y)	LOG(100* DOSE) =X
1	2.4	10	1	10%	3.7184	2.3802
2	2.6	10	3	30%	4.4756	2.4149
3	**2.8**	**10**	**5**	**50%**	5	**2.4471**
4	3.0	10	7	70%	5.5244	2.4771
5	3.2	10	9	90%	6.2816	2.5051

III. Figure: 7. 96hrs dose response curve between percent mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC):



III. Figure:8. 96h dose response curve between probit mortality against toxicant concentration in freshwater fish, *Catla catla* exposed to Ethion (50% EC)



IV. Discussion:

Toxicity of pesticides, Heavy metals, other pharmaceutical chemical substances is influenced by physical factors like temperature, pH and biological factors like size, national status, species specificity and chronobiology of the animal (Lalitha V and V.Venkata Rathnamma, 2021). The present study was carried out to evaluate the acute toxicity and behavioural responses in freshwater fish *Catla catla* exposed to Ethion 50% EC. The results clearly indicated that exposure of fishes to pesticide resulted in increased mortality with increasing concentrations. In the present study observed that the fish *catla catla* is less resistant to Organophosphate pesticide Ethion (50%EC) and the LC_{50} values for 24hr, 48hr, 72hr and 96hrs were 4.9µg/l, 4.2µg/l, 3.5µg/l and 2.8µg/l respectively and the results were compared with other researchers. LC_{50} of an organophosphate pesticide Dimethoate to fresh water fish *Colisa fasciatus* for 24hr, 48hr, 72hr, 96hrs were found to be 22.15mg/l, 21.99mg/l, 21.74mg/l, and 21.65 mg/l respectively (Ram Naran Singh, 2013). Srivastava *et al*., (2010), observed 96h LC_{50} value in *Heteropneustes fossilis* was 100mg/l when exposed to Roger. These indicates that LC_{50} values were species specific, and different pesticides have different LC_{50} values.

The LC_{50} values of Acephate, an organophosphate for 24hr, 48hr, 72hr and 96hrs was 2000mg/l, 1800mg/l, 1650mg/l and 1550mg/l were estimated in the fresh water fish *Labeo rohita* (Lalitha Vinnakota *et al*.,2018). Glyphosate, determined LC_{50} values for 24, 48, 72 and 96hr were 3.757, 3.461, 3.15 and 3.043mg/l respectively when exposed to *Cirrhinus mrigala* (Rajeswari and Sunita 2021). Prabhanjan *et al*., (2023), experimented Deltamethrin exposures on *Pangasius hypophthalamus* and the LC_{50} value was 0.021mg/l at 96hr. The LC_{50} value of Ethion 50%EC to freshwater fish *Labeo rohita* was found to 1.2µg/l (Prasanna *et al*., 2020). The freshwater fishes like *Labeo rohita* and *Ctenopharyngodon idella* exposed to Fipronil (5%SC) insecticide, the LC_{50} values for 24hr (3.3, 2.7mg/l), 48hr (2.64, 2.3mg/l), 72hr (1.98, 1.9mg/l), and 96hr (1.5, 1.32mg/l) respectively Ch. Anithasmruthi *et al.,* (2022).

Ethion is considered more toxic to the test fish because in the present study observed LC_{50} value is 2.8µg/l for 96hr, which is lower when compared to *Labeo rohita* (100mg/l) exposed to Profenofos (Nagaraju B *et al.,* 2013) and *Cyprinus carpio* (62.4mg/l) Ismail *et al*., (2009). In addition, Nirmala K*, et al*., (2016)) reported that the LC_{50} values of fresh water fish *Labeo rohita* exposed to Flubendiamade (insecticide) were 24h(17mg/l), 48h (15mg/l), 72h (13mg/l), and 96h(11mg/l) respectively. The toxicity of a pesticide and heavy metals could

vary from species to species and this variation is due to size, age, health, physiological parameters of water and the differential tolerance of animals to exposure.

Behavioural traits are a functional reading of neural activity. Irritation occurred at the start of the exposure, which is consistent with the universal behavioural responses of cray fish to Ethion and hyperactivity may accelerate to death (Desouky *et al.,* 2013). Ethion's primary effect on liver is due to its metabolic activity, and it is an inhibitor of the neuro enzyme cholinesterase (ChE), thus the secondary damage is brain (ATSDR, 2017). Physiological variations due to inhibition of AChE can lead to a succession of behavioural changes that include impeded swimming performances, altered social behaviour, reduced foraging, and greater predation risks. The restlessness, hyperactivity with abrupt erratic swimming of *Catla catla* fingerlings, might have occurred due to the reduction of the acetylcholinesterase activity, similar changes were observed in *Heteropneustus fosilis* exposed to envoy 50 SC (Akter *et al.,* 2020). Their physiological alterations may potentially decrease their survival rate in the nature. Significantly lower acetylcholinesterase activities in fish due to pesticide exposure suggested the reasons of abrupt behaviour, increased oxygen consumption, and fish mortality at higher concentration of this organophosphate pesticide.

V. Conclusion:

The results of the present study showed that Ethion (50%EC) is more toxic to fish *Catla catla* which induced acute toxic effects in the form of behavioural changes in fish. The toxicity of Ethion on fish increased with increasing concentration and exposure time. It can be concluded that Ethion is toxic to fishes and its use should be in controlled manner to avoid its long-term harmful effects in the environment.

VI. Acknowledgments:

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VII. Conflicts of Interest:

The authors declare that they have no conflicts of interest

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