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Acute toxicity of Phenthoate (50% EC) on survival and behavioral pattern of freshwater fish *Labeo rohita* (Hamilton, 1822)

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Abstract
The present study deals with the bioaccumulation of Phenthoate and its effect on survival pattern and behavioral changes in freshwater fish *Labeo rohita.* The Pilot experiments were conducted to derive the LC_{50} concentrations. The LC_{50} values of Phenthoate were determined to be 3.0, 2.6, 2.3 and 2.1 mg/L for 24, 48, 72 and 96 h respectively. The percentage of mortality was calculated by following the method of Finney's probit analysis. During the course of experiment, the behavioral alterations were also observed. Hence, the present study revealed that, the LC_{50} value gradually decreased with the increase of exposure period and the mortality rate increased with increase in concentration of pesticide.

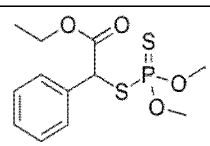
Keywords: Phenthoate, $LC_{50,}$ Mortality, Behavioral changes, *Labeo rohita*

1. Introduction
Environmental pollution resulting from industrial effluents and agricultural activities has become a global issue because of the extensive damage caused to the aquatic ecosystems and the disruption in the natural food chain, by several agricultural practices such as insecticidal and herbicidal application [1]. The increasing population has put a stress on resources, resulting in the excessive use of organophosphorus pesticides and fertilizers to meet the demand. These substances ultimately pollute the aquatic environment and cause severe damage to the aquatic life especially to the fish, prawn and crab species [2]. Among the different groups of pesticides organophosphates are being used commonly as insecticides due to their facilitation properties like less persistence and rapid biodegradability in nature [3, 4].
Phenthoate is a non-systemic organophosphorus insecticide, a cholinesterase inhibitor, moderately toxic to mammals with no residual activity. Phenthoate is formulated as emulsifiable concentrate (EC) of varying strengths. Phenthoate formulation has a board spectrum of effectiveness on crop pests especially against a wide range of chewing, piercing and sucking phytophagous insect pests on vegetables, rice, cotton, pulses etc. It has a strong pungent odor which acts as a repellent for adult moths due to which, egg laying can be prevented.
Acute toxicity of a pesticide refers to the chemical's ability to cause injury to an animal from a single exposure, generally of short duration. The acute toxicity test of the pesticides to fish has been widely used to acquire rapid estimates of the concentrations that cause direct, irreversible harm to test organisms [5]. The most common acute toxicity test is acute lethality and LC_{50} is customary to represent the lethality of a test species in terms of mortality and time. LC_{50} is the concentration of the chemical that results in the 50% death rate of the test organisms.
Labeo rohita is a major food fish and is a natural inhabitant of freshwater sections of the rivers which thrives well in all freshwaters below an altitude of approximately 549 meters. *L. rohita* is a bottom feeder and prefers to feed on plant matter including decaying vegetation and attains maturity towards the end of the second year. The spawning season of *L. rohita* generally coincides with the southwest monsoon and it takes place in flooded rivers. The fecundity varies from 226,000 to 2,794,000 depending upon the length and weight of the fish and weight of the ovary. The spawn of this fish is collected from rivers during monsoon and reared in tanks and lakes [6, 7]. Hence, the objective of the present work was to observe the behavioral changes and survival rates of freshwater fish *L. rohita* on exposure to pesticide, Phenthoate.

2. Materials and Methods
2.1 Acclimatization of fish
The healthy juveniles of fish *L. rohita* (length, 8.5 ± 0.5 cm; weight, 6.5 ± 0.5 gm) were obtained from local fish ponds at Buddam village in Guntur district in Andhra Pradesh, India. The experiment of acute toxicity of Phenthoate was conducted from March, 2013 to April 2013. Prior to conducting the experiment, the fish were acclimatized for a period of 10 to 15 days in dechlorinated tap water under laboratory conditions at room temperature. The tub containing fish was aerated with rich oxygen. Hygienic conditions were maintained by renewing water regularly and fish were daily fed with rice bran and fish pellets.

2.2 Estimation of physico-chemical parameters of water
The water used for experimentation was colorless, odorless and clear. The physico-chemical parameters of water such as temperature, pH, turbidity, total hardness, total suspended solids, biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen, sulphates and phosphates were estimated prior to the experiment according to the standard protocols of American Public Health Association (APHA) [8]. The estimated values are as follows: Temperature, 28 ± 2^0 C; pH, 7.12; Turbidity, 7.5 silica units; Total Hardness, 170 mgL^{-1}; Total Suspended Solids, 4 mgL^{-1}; BOD, 8-10 ppm; COD, Nil; DO, 5-6 mgL^{-1}, Sulphates (SO_4), trace amount and Phosphates, trace amount.

2.3 Phenthoate (Organophosphate)
The commercial grade formulation of Phenthoate 50% EC (Emulsifiable Concentrate), an organophosphorous pesticide is used as toxicant in the present experiment. The trade name is Phendal manufactured by Shanghai Tenglong Agrochem Co., Ltd. (Yangpu Building 24B, No. 2005, Yangpu Shanghai, China) was purchased from local pesticide market of Guntur, Andhra Pradesh, India. The characteristics of Phenthoate an organophosphorous pesticide are as follows:

IUPAC Name:	**(*RS*)-Ethyl [(dimethoxyphosphorothioyl) sulfanyl](phenyl)acetate**
Structural Formula:	
Molecular Formula:	$C_{12}H_{17}O_4PS_2$
Molar mass:	320.36 g·mol^{-1}
Appearance:	Colorless crystalline solid
Solubility in water:	11 mg/L (24 °C)

2.4 Acute exposure bioassay for Phenthoate
A common stock solution of Phenthoate was prepared by dissolving 1 gram (1000 mg) of pesticide in 100 mL of acetone and the required quantity of Phenthoate was drawn from the stock solution to maintain the standard concentration of 1 mg/L in the container.

The acclimatized fish were placed into separate container containing dechlorinated and aerated water. The Pilot experiments were conducted to derive the LC_{50} values.

Five batches of fish (10 fish in each batch) were exposed to 0.6, 1.2, 1.8, 2.4 and 3.0 mg/L for 24 hours. The mortality and behavioral responses of fish was observed from 1.8 to 3.0 mg/L of pesticide concentration. Later, two batches of 10 fish were again exposed to concentrations of 2.4 and 3.0 mg/L to evaluate the LC_{50} value for 24 hours. Then, the 50% of mortality was obtained at the concentration of 3.0 mg/L for 24 hours and the confirmation of acute toxicity (LC_{50}) was performed with three replicates.

To assess the LC_{50} value for 48 h, the fish were exposed to 0.52, 1.04, 1.56, 2.08 and 2.6 mg/L concentrations of organophosphate, Phenthoate. The fish mortality occurred from 1.04 to 2.6 mg/L concentration of Phenthoate. Then two batches of 10 fish were exposed to the concentration of 2.08 and 2.6 mg/L. The 50% of mortality was occurred at the concentration of 2.6 mg/L for 48 hours and the confirmation of LC_{50} value was done with three replicates.

To evaluate 72 h LC_{50} value, the acclimatized fish were exposed to 0.46, 0.92, 1.38, 1.84 and 2.3 mg/L concentrations of pesticide. The varied mortality of fish occurred from 0.92 to 2.76 mg/L concentration of toxicant. Then two batches of 10 fish were exposed to concentration of 1.84 and 2.3 mg/L. The 50% of mortality was obtained at the concentration of 2.3 mg/L and the confirmation of LC_{50} value for 72 h was done with three replicates.

The LC_{50} for 96 h was found by exposing acclimatized fish, *L. rohita* to 0.42, 0.84, 1.26, 1.68 and 2.1mg/L concentrations of pesticide, Phenthoate. The fish mortality began to occur from the concentration of 0.84 mg/L onwards. Then, three batches of 10 fish were exposed to the concentration of 1.26, 1.68 and 2.1 mg/L for 96 hours. During this exposure period 50% of mortality was observed at the concentration of 2.1 mg/L in the total fish and confirmation was done with three replicates. Finally, the median lethal concentration (LC_{50}) values of Phenthoate were found to be 3.0, 2.6, 2.3 and 2.1 mg/L for 24, 48, 72 and 96 h respectively.

During the whole experiment a control group was maintained with acetone for comparison. The percentage of mortality was calculated by following the probit analysis method [9]. The behavioral alterations in toxicant exposed fish were observed during the acute toxicity of Phenthoate.

2.5 Statistical analysis
The statistical analysis and graphs were obtained using Minitab 16.0 v statistical package.

3. Results
3.1 Determination of LC_{50}
The LC_{50} value was calculated as the concentration of test chemical which caused 50% mortality of total fish during the respective periods of exposure i.e., 24, 48, 72 and 96 h.

Then, the LC_{50} values of Phenthoate were evaluated and found to be 3.0, 2.5, 2.3 and 2.1 mg/L for 24, 48, 72 and 96 h respectively as shown in Table 1-4.

The mortality data was subjected to probit analysis and graphs were plotted between concentrations of Phenthoate and percent mortality of fish. The percent mortality gradually increased with the increase in concentration of pesticide (Figure 1-4).

During the experiment, the LC_{50} values were reduced as the exposure time increased along with Log concentration from 24 to 96 h (Table 5) and no mortality was observed in control group.

Table 1: Effect of Phenthoate on survival of *Labeo rohita* for 24 hours

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of Exposed	No. of Dead	Percent of Mortality	Probit Mortality
1	0.6	- 0.2218	10	0	0	--
2	1.2	0.0791	10	0	0	--
3	1.8	0.2550	10	1	10	3.72
4	2.4	0.3802	10	3	30	4.48
5	3.0	0.4771	10	5	50	5.00
6	3.6	0.5563	10	6	60	5.25
7	4.2	0.6232	10	7	70	5.52
8	4.8	0.6812	10	8	80	5.84
9	5.4	0.7323	10	9	90	6.28
10	6.0	0.7781	10	10	100	8.09

Table 2: Effect of Phenthoate on survival of *Labeo rohita* for 48 hours

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of Exposed	No. of Dead	Percent of Mortality	Probit Mortality
1	0.52	- 0.28.39	10	0	0	---
2	1.04	0.0170	10	1	10	3.72
3	2.08	0.3180	10	3	30	4.48
4	2.56	0.4082	10	4	40	4.75
5	2.60	0.4149	10	5	50	5.00
6	3.12	0.4941	10	6	60	5.25
7	3.67	0.5646	10	7	70	5.52
8	4.19	0.6222	10	8	80	5.84
9	4.71	0.6730	10	9	90	6.28
10	5.23	0.7185	10	10	100	8.09

Table 3: Effect of Phenthoate on survival of *Labeo rohita* for 72 hours

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of Exposed	No. of Dead	Percent of Mortality	Probit Mortality
1	0.46	- 0.3372	10	0	0	---
2	0.92	- 0.0362	10	1	10	3.72
3	1.38	0.1398	10	2	20	4.16
4	1.84	0.2648	10	4	40	4.75
5	2.30	0.3617	10	5	50	5.00
6	2.76	0.4409	10	6	60	5.25
7	3.22	0.5078	10	7	70	5.52
8	3.68	0.5658	10	8	80	5.84
9	4.14	0.6444	10	9	90	6.28
10	4.60	0.6627	10	10	100	8.09

Table 4: Effect of Phenthoate on survival of *Labeo rohita* for 96 hours

S. No.	Conc. of toxicant (mgl^{-1})	Log Conc.	No. of Exposed	No. of Dead	Percent of Mortality	Probit Mortality
1	0.42	- 0.3767	10	0	0	---
2	0.84	- 0.0757	10	1	10	3.72
3	1.26	0.1003	10	3	30	4.48
4	1.68	0.2041	10	4	40	4.75
5	2.1	0.3222	10	5	50	5.00
6	2.52	0.4014	10	6	60	5.25
7	2.94	0.4683	10	7	70	5.52
8	3.36	0.5263	10	8	80	5.84
9	3.78	0.5774	10	9	90	6.28
10	4.2	0.6232	10	10	100	8.09

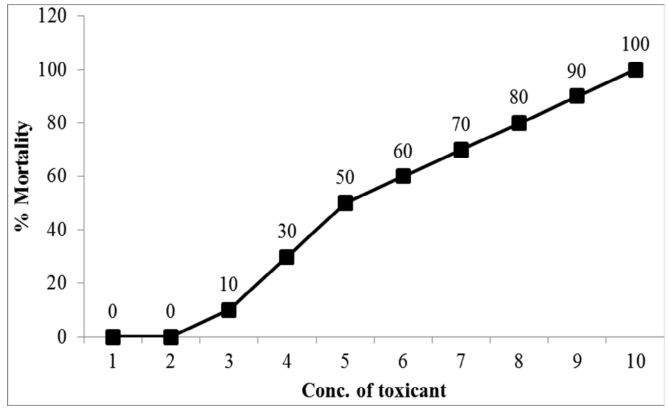


Fig 1: Relationship between the conc. of Phenthoate and mortality of *Labeo rohita* for 24 hours

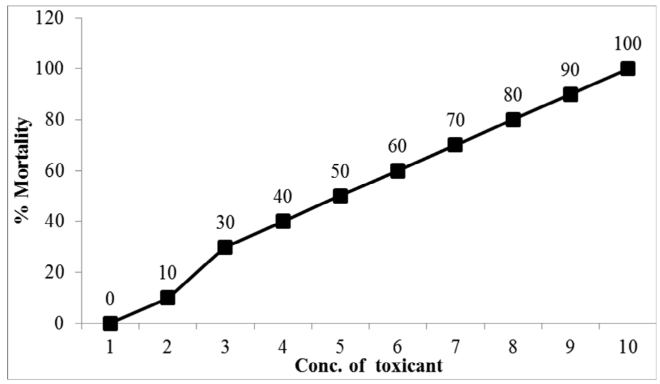


Fig 2: Relationship between the conc. of Phenthoate and mortality of *Labeo rohita* for 48 hours

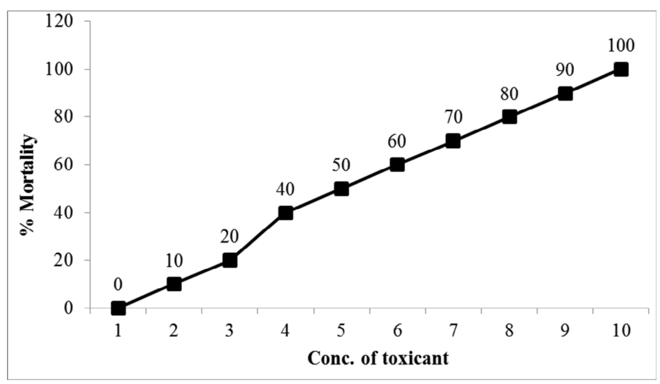


Fig 3: Relationship between the conc. of Phenthoate and mortality of *Labeo rohita* for 72 hours

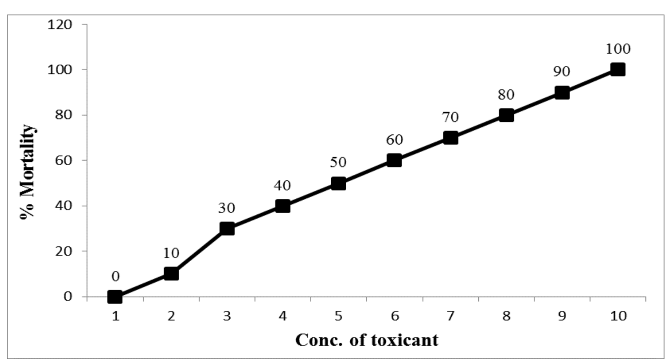


Fig 4: Relationship between the conc. of Phenthoate and mortality of *Labeo rohita* for 96 hours

Table 5: Estimated LC_{50} values and confidence limits of fish *Labeo rohita*

Time of Exposure	LC_{50} value (mgl^{-1})	Log Conc.	Percentile	Standard Error	95% Confidence Intervals(CI) UCL* - LCL*
24 h	3.0	0.4771	2.48755	0.289017	1.89590 - 3.11100
48 h	2.6	0.4149	2.29337	0.269723	1.72578 - 2.86855
72 h	2.3	0.3617	1.92727	0.216033	1.48643 - 2.39343
96 h	2.1	0.3222	1.74129	0 .202312	1.32713 - 2.17770

*UCL= Upper confidence limits, *LCL= Lower confidence limits

From the above results it is clearly indicating that the pesticide, Phenthoate can be rated as highly toxic (If LC_{50} is 1-50 mg/L) to the freshwater fish *L. rohita.*

3.2 Behavioral changes in fish due to acute toxicity

During the course of experiment, the fish have exhibited different behavioral alterations to the Phenthoate concentrations. In the initial stage, fish stopped swimming and remained in static position by sudden change in the surrounding water. Then rapid swimming with hyper excitation, jumping in the container at high concentration was noticed. Also vertical and downward swimming activities were observed followed by vertical hanging in the water and hitting to the container walls. Then the fish slowly became restless and excessive mucus formed all over the body. Surfacing frequency, gulping of surface water, opercular activities increased remarkably in exposed fishes. Loss of balance increased and body color of the fish became lighter. Defecation was increased and more fecal matter was found at the bottom of the circular tubs than control group fish. Finally fish was swimming at the bottom due to complete loss of balance and sank to the bottom with their ventral side facing upwards due to organophosphate, Phenthoate stress.

4. Discussion

In the present investigation, Phenthoate caused 100% mortality of *L. rohita* at 4.2 mg/L and 50% mortality at 2.1 mg/L during 96 h exposure and insecticidal toxicity influenced by factors like exposure time, temperature, dissolved oxygen, pH, concentration and water availability to the fish [10, 11]. Earlier studies revealed that the LC_{50} of a chemical for a species may vary under different environmental condition like time of exposure, size, and other impacts. Several reports were given for different LC_{50} values of various pesticides on freshwater fish [12, 13, 14].

Kumaravel [15] has reported monocrotopas caused 100% mortality of *L. rohita* at 0.0044 and 50% at 0.0036 ppm and also suggested that the lambda cyhalothrin caused mortality 100 % at 0.0029 and 50% at 0.0021mg/L. Intiyaz *et al.* [16] determined the 96 h LC_{50} value of Kethrin to *L. rohita* as 21.68 ppm. Das *et al.* [17] has studied the acute toxicity of neem in the fingerlings of Indian major carp *L. rohita* was found to be 2.36 ppm. Shukla [18] reported the LC_{50} value of dimethoate for *Colisa fasciatus* as 13.0 mgl^{-1} for 24 h, 11.4 mgl^{-1} for 48 h, 10.0 mgl^{-1} for 72 h and 9.3 mgl^{-1} for 96 h.

Although the observed acute toxicity (LC_{50}) value for 96 h is very low for Phenthoate (2.1 mgl^{-1}) but is highly toxic to *L. rohita* and considered to be less toxic in the field condition due to their less persistence and rapid biodegradability. Therefore the present data is useful to assess the potential risk of ecosystem according to Viran *et al.* [19].

In the present study, the impact of the pesticide could be observed by the behavioral changes like surfacing, erratic movement, increased mucous secretion, decreased opercular movement and loss of balance. Therefore, the present study can be taken as an indicator of aquatic pollution. Similar observations were made by Shivkumar *et al.* in *L. rohita* when exposed to endosulfan [20]. The erratic swimming of the treated fish indicates the loss of physiological equilibrium and the hyper-excitability of the fish invariably in the lethal and sub lethal exposure of chemical may be due to the inhibition of cholinesterase [21]. Abnormal swimming and loss of balance was caused by the deficiency in nervous and muscular coordination.

Opercular movement has been decreased with increase in toxicant concentration and accumulation of more fecal matter was observed in the container and similar results were observed by Imtiyaz *et al.* [16]. Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Behavioral changes of *L. rohita* have been studied for various chemicals by Marigouder *et al.* [22] and Pandey *et al.* [23]. The treated fishes also showed fading of their body color before death, these changes can be considered as symptoms of stress on account of the toxicological nature of the environment. Sweilum [24] reported that the abnormal movements were noticed in the fish *Channa gachua,* when applied different concentrations of dimethoate pesticides. The surfacing phenomenon of fish might be due to hydro toxic condition of the fish and these results are supported by Appa Rao *et al.* [25] and Charjan *et al.* [26]. The decrease in body weight could be due to excessive expenditure of more energy on metabolism in fish growth and it was proportionate to the concentration of the pesticides. Similar results were reported by Balasubramani *et al.* [27] and Cook *et al.* [28].

5. Conclusion

All the observations indicated the impact of Phenthoate toxicity and caused behavioral alterations, such as those observed in this study may result in severe physiological problems, ultimately leading to the death of fish. Therefore, the present investigation demonstrates a relation among pesticidal stress, behavioral disorders, survival and mortality rates. Thus, this study can be used as a tool for creating awareness among the local farmers so that the use of the highly toxic pesticide can be minimized.

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