TOXICITY EVALUATION AND BEHAVIOURAL STUDIES OF FRESHWATER FISH *LABEO ROHITA* EXPOSED TO ACEPHATE 95% SG (ORGANOPHOSPHATE)

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ABSTRACT:

Throughout the world organophosphate pesticides are widely employed in agriculture sectors because of its rapid degradation and less persistence. Blind use of these pesticides bound to affect the non-target organisms, fish being one of the most prominent among these. The aim of the present investigation was to determine the acute toxicity of Acephate 95% sg an organophosphate insecticide and as well as behavioural alterations in freshwater fish *Labeo rohita*, experimental fish were exposed to different concentrations of Acephate. The LC_{50} values of Acephate for 24hr, 48hr, 72hr and 96hr was found in 2000mg/l, 1800mg/l, 1650mg/l and 1550mg/l respectively and the LC50 values were calculated in Finney Probit Method. The effect of Acephate in Fish were showed uncontrolled behavior like mucus secretions, decreased agility, increased movement of opercula, rapid jerk movement, lightening of the body colour, erratic and irregular swimming were seen in exposure time of the toxicant.

Key words: Acephate, Insecticide, $LC_{50,}$ *Labeo rohita* and behavioural studies.

I.INTRODUCTION:

Pesticides are one of the most hazardous chemicals to the environment; various types of pesticides are used extensively in agricultural fields to protect the crops from injuries or damages caused by different types of pest. The improper management of pesticides in agriculture crops could result in contamination of water bodies. When pesticide reaches the aquatic environment, it may be present there for several days or weeks, depending upon its solubility, producing mass mortality, morphological, physiological and behavioural changes in the organisms. Karami-Mohajeri and Abdollahi, 2011 were reported that organophosphates (OS) and carbamate (CB) impair the metabolism of carbohydrates, fats and protein through the inhibition of AChE or affecting target organs directly.

To increasing awareness of the environmental hazards of pesticides necessitated the testing of toxicity of different aquatic organisms. The biological effects of pesticides and their mode of action are obtained from toxicity studies (Ambika and Selvisabhanayakam, 2012). Toxicity tests have been performed to evaluate the effect of toxicants on various aquatic organisms under laboratory conditions. Fishes are widely used to assess water quality of aquatic ecosystems because they serve as pollution bioindicators (Balistrieri LS *et al*., 2002). Toxicity is usually divided into 2 types, acute and chronic, based on the number of exposures to a poison and the time it takes for toxic symptoms to develop. Acute toxicity is due to short-term exposure and happens within a relatively short period of time, whereas chronic exposure is due to repeated or long-term exposure and happens over a longer period. Chronic low-level exposure to pesticides associated with serious health problems including metabolism impairment, neurotoxicity, carcinogenicity, reproductive and endocrine disruption as well as immune dysfunctions (Corsini, E *et al*., 2008 and Androutsopoulos, V.P *et al*., 2013). Hence, in the present investigation effect of Acephate 95% sg an organophosphate pesticide exposed to freshwater fish *Labeo rohita* to evaluate the toxicity and behavior study.

II. MATERIALS AND METHODS:

II.1. Test chemical:

The commercial grade formulations Acephate 95% SG an organophosphorous insecticide used for the control of stem bore, leaf folder and brown plant hoppers (BPH) on paddy. It was evaluated for against cotton sucking pest. It was pellet formations and manufactured

by M/S Rallis India limited. Trade name Hunk was purchased from local agro-chemical store. Acephate is slightly toxic to fish and amphibians. After high exposures, salamander hatchlings had decreased growth, activity, and feeding, increased muscle and spinal column deformities. It is a broad-spectrum insecticide and is highly toxic to bees and other beneficial insects (Christiansen, A. *et al*., 2011). The EPA classifies Acephate as a "possible human carcinogen". When animals were fed Acephate in their diets for two years, a greater number of them had liver or adrenal gland tumors. Reduction in the number of heamocytes might be a product of increased apoptosis, as Acephate is considered as an apoptotic inducer (Tripathi *et al*., 2007). Rajak *et al*., 2013 reported that Acephate shorten the developmental duration in a dipterans insect *Drosophila melanogaster*.

II.2. Experimental organism:

Labeo rohita one of the most important species of freshwater Indian major carp, easily found in all over north and central India it is also found in Godavari and Krishna rivers in south India. *Labeo rohita* is belongs to the family cyprinidae, and is commonly known as 'rohu'. It is rich in protein and is very suitable for human consumption. Hence, this carp has economically important edible fish and have a great commercial value.

II.3. Fish Collection and Acclimatization:

The freshwater fish *Labeo rohita* were collected from the fish hatcheries of Nandivelugu, Tenali mandal, Gunntur(dst), A.P, India which is 20km away from the university, brought to the laboratory and stored in large plastic tubs containing tap water. The average length and weight of the fishes (both sexes) were 8.2±1.5 cm and 10.4±2.0 gm respectively. At first fish were given prophylactic treatment by bathing them twice in 0.05% $KMno_4$ solution for 4-5 minutes to avoid dermal infections. The fish were acclimatized to laboratory conditions at 28±2ºC for 10days before exposure to pesticide. During this period the fish were fed with groundnut oil cake and rice bran and the medium is replaced daily. The supply of oxygen (O_2) into the water of aquariums was done by electrical aerators and the water was renewed daily. The fecal matter and other materials were siphoned off daily. All the precautions laid down by committee on toxicity tests to aquatic organisms APHA (1998) were followed.

II.4. Acute Toxicity Test:

To assess susceptibility and survival potential of the test organisms 24hr, 48hr, 72hr and 96hr LC_{50} tests have been conducted. The fingerling stage of fish is more reliable to conduct toxicity test of various waterborne toxicants (Dunham JB *et al.*, 2002 and Mebane CA *et al*., 2003). The test solution was prepared by dissolving the commercial grade of Acephate 95% SG in distilled water. During acute toxicity trails, the fish were subjected to 12-hr photo period and were not fed. Experiments were conducted to select mortality range 10% to 100% for 24, 48, 72 and 96 hours to determine LC_{50} values of Acephate in *Labeo rohita*. Pilot experiments were conducted using 1L bottles and one fish for each bottle was introduced to choose the mortality concentration, at which concentration the fish respond. For static renewal tests, 10L plastic tubs were used. For each test five concentrations were chosen with 10 fish in each concentration. 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. Static renewal tests were conducted to determine the LC_{50} values. The physic-chemical characteristics of water were determined by standard methods of APHA (1995). The lethal concentrations ensure death even before noticing the behavioural abnormalities. In the present study 24hr, 48hr, 72hr and 96hrs LC_{50} values were selected to study the morphological, physiological and behavioural alterations (As per the recommendations of committee on toxicity studies-Anon, 1975).

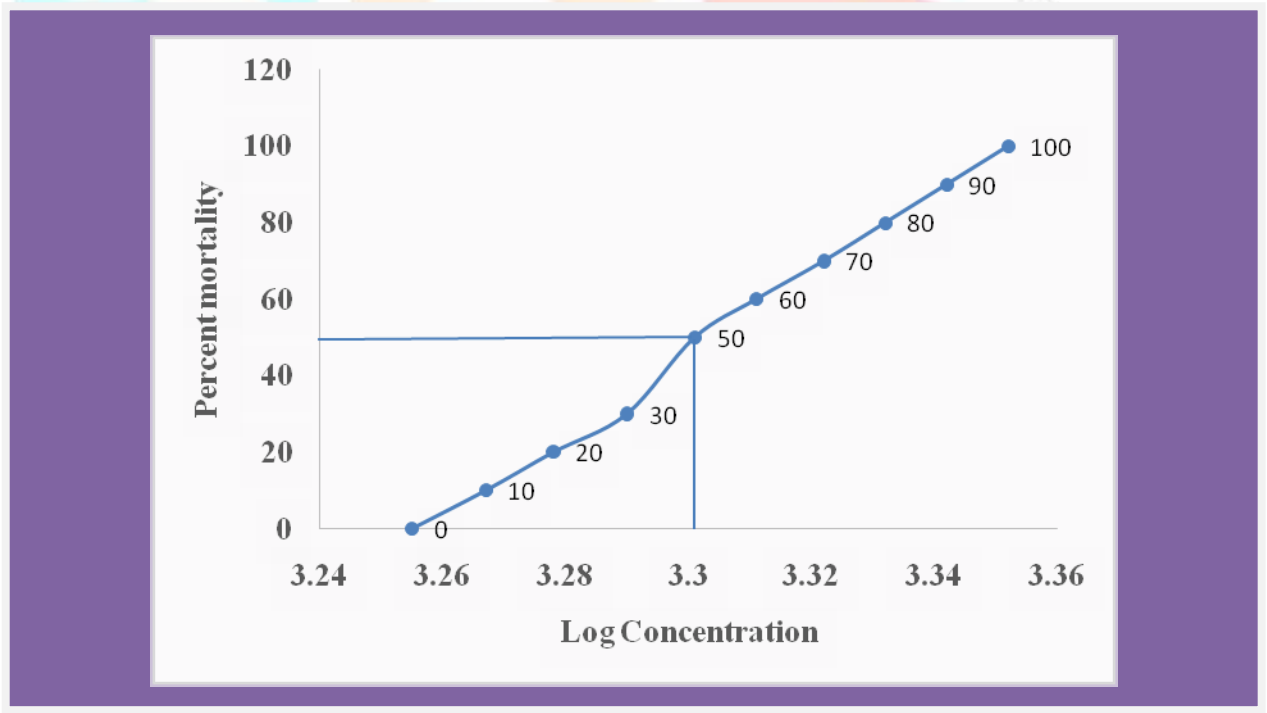
III. RESULTS AND DISCUSSIONS:

III.1.Determination of LC_{50} values:

The data was computed according to Probit Analysis Method (Finney, 1971) and the LC_{50} values were determined. In the present study Acephate caused 50% mortality to *Labeo rohita* for 24hr, 48hr, 72hr and 96hrs at 2000mg/l, 1800mg/l, 1650mg/l and 1550mg/l and the reported values were given in the Table.III.1,2,3,4 and Fig.III.1,2,3,4 respectively. The fish *Labeo rohita* were exposed to different concentrations of Acephate for 96hrs, showed 20 percent mortality at 1400mg/l, 30 percent mortality at 1450mg/l, 40 percent mortality at 1500mg/l, 50 percent mortality at 1550mg/l, 60 percent mortality at 1600mg/l, 70 percent mortality at 1650mg/l, 80 percent mortality at 1700mg/l, 90 percent mortality at 1750mg/l and 100 percent mortality at 1800mg/l were observed(III.Table.4). The graphical representation of percent mortality versus Log concentration and probit mortality versus Log concentration of Acephate showed in III.Fig.4 and III.Fig.5 respectively which were in agreement with the principle of Probit Analysis (Finney, 1971). The percent mortality gradually increased with the increased concentration of pesticide.

III.Table.1: Effect of Acephate on survival of *Labeo rohita* for 24 hours

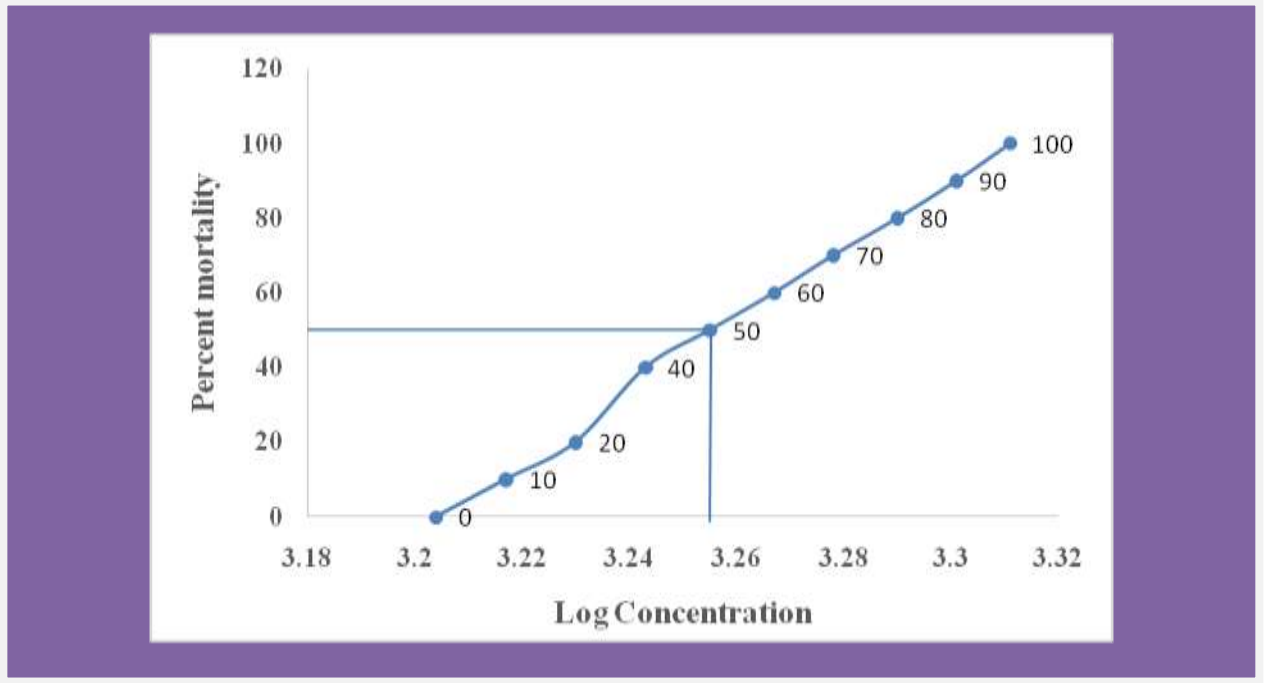
S. No.	Conc. Of toxicant(mg/l)	Log Conc.	No. of Exposed	No.of Dead	Percent Mortality	Probit Mortality
1	1800	3.255	10	0	0	.----
2	1850	3.267	10	1	10	3.72
3	1900	3.278	10	2	20	4.16
4	1950	3.290	10	3	30	4.48
5	2000	3.301	10	5	50	5.00
6	2050	3.311	10	6	60	5.25
7	2100	3.322	10	7	70	5.52
8	2150	3.332	10	8	80	5.84
9	2200	3.342	10	9	90	6.28
10	2250	3.352	10	10	100	8.09



III.Fig.1: Relationship between the Log conc. of Acephate and percent mortality of *Labeo rohita* for 24 hours.

III.Table.2: Effect of Acephate on survival of *Labeo rohita* for 48 hours

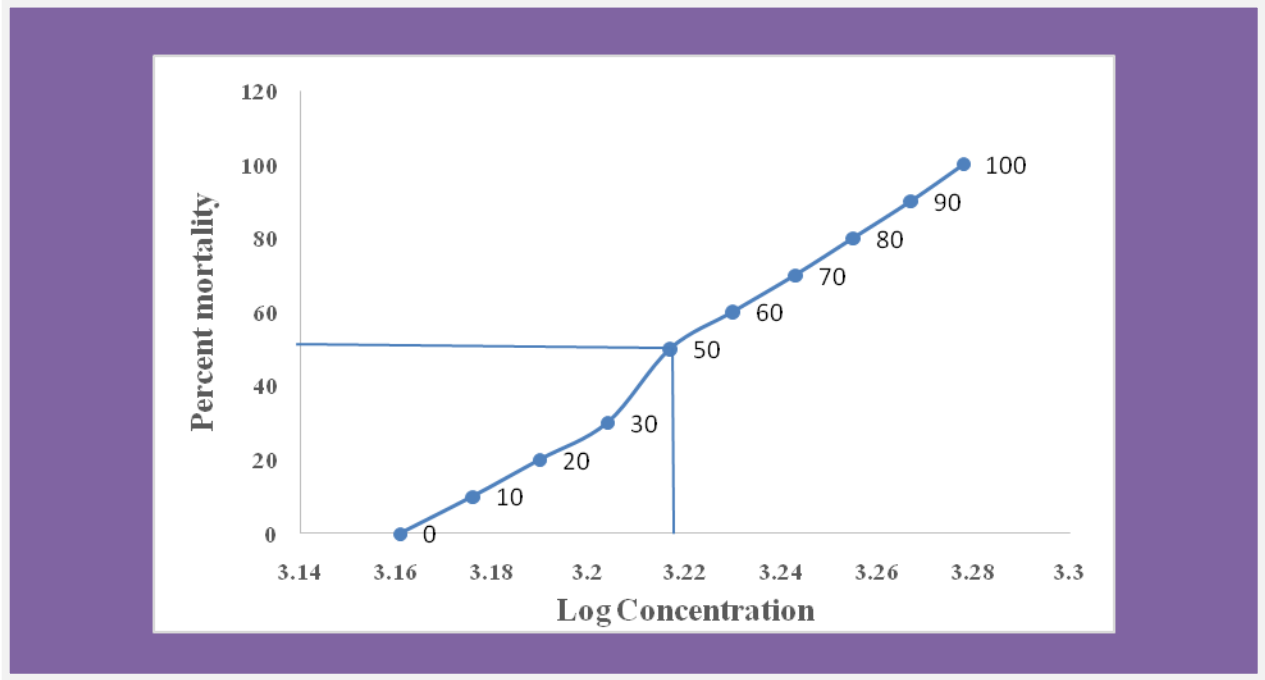
S No.	Conc. Of toxicant(mg/l)	Log Conc.	No. of Exposed	No.of Dead	Percent Mortality	Probit Mortality
1	1600	3.204	10	0	0	.----
2	1650	3.217	10	1	10	3.72
3	1700	3.230	10	2	20	4.16
4	1750	3.243	10	3	40	4.75
5	1800	3.255	10	5	50	5.00
6	1850	3.267	10	6	60	5.25
7	1900	3.278	10	7	70	5.52
8	1950	3.290	10	8	80	5.84
9	2000	3.301	10	9	90	6.28
10	2050	3.311	10	10	100	8.09



III.Fig.2: Relationship between the Log conc. of Acephate and percent mortality of *Labeo rohita* for 48 hours.

III.Table.3: Effect of Acephate on survival of *Labeo rohita* for 72 hours

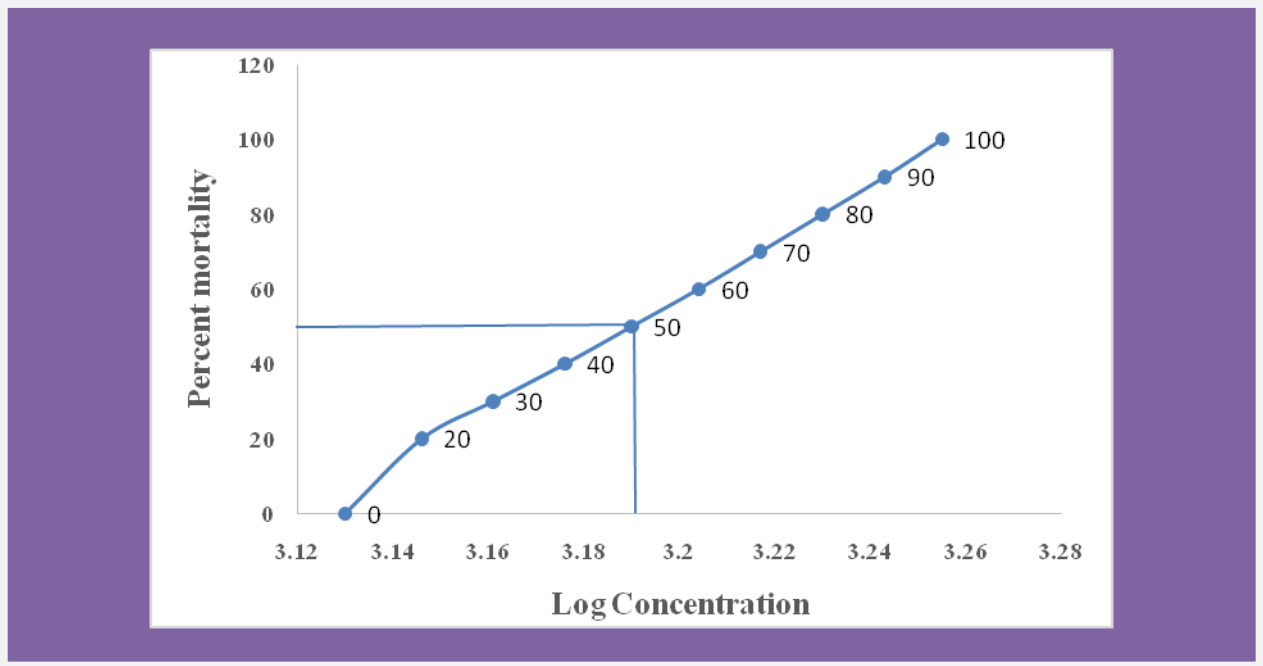
S No.	Conc. Of toxicant(mg/l)	Log Conc.	No. of Exposed	No.of Dead	Percent Mortality	Probit Mortality
1	1450	3.161	10	0	0	.----
2	1500	3.176	10	1	10	3.72
3	1550	3.190	10	2	20	4.16
4	1600	3.204	10	3	30	4.48
5	1650	3.217	10	5	50	5.00
6	1700	3.230	10	6	60	5.25
7	1750	3.243	10	7	70	5.52
8	1800	3.255	10	8	80	5.84
9	1850	3.267	10	9	90	6.28
10	1900	3.278	10	10	100	8.09



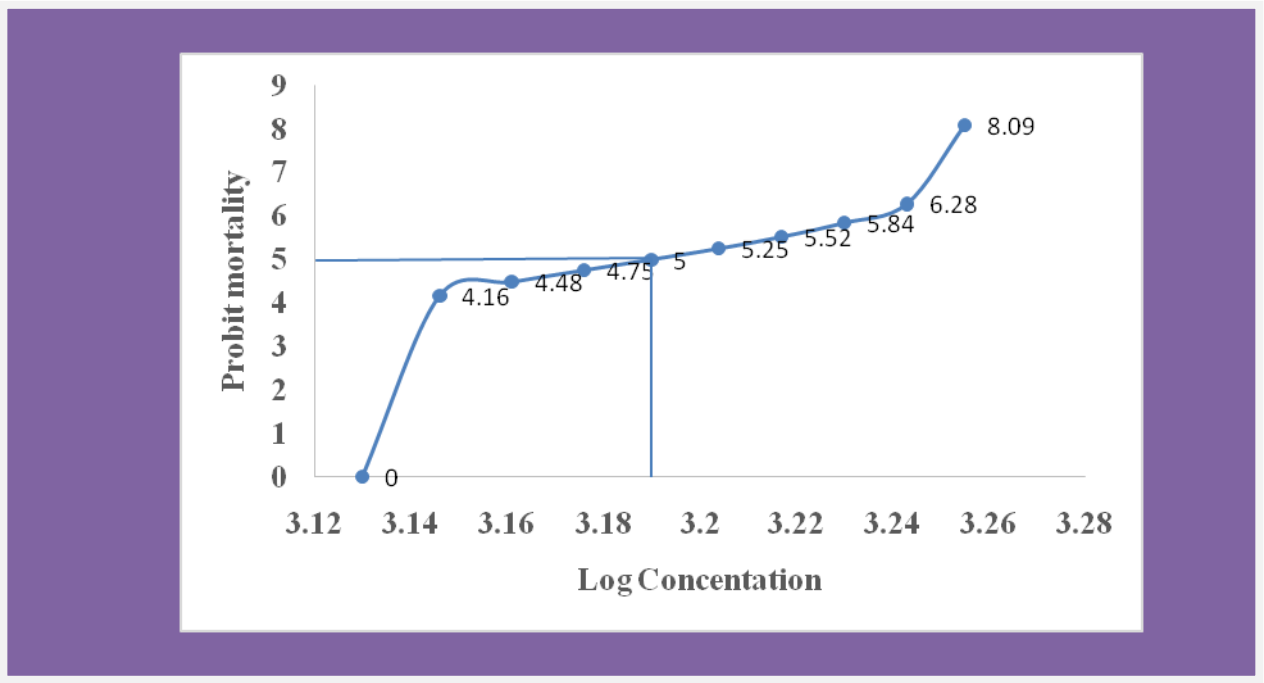
III.Fig.3: Relationship between the Log conc. of Acephate and percent mortality of *Labeo rohita* for 72 hours.

III.Table.4: Effect of Acephate on survival of *Labeo rohita* for 96 hours

S No.	Conc. Of toxicant(mg/l)	Log Conc.	No. of Exposed	No. of Dead	Percent Mortality	Probit Mortality
1	1350	3.130	10	0	0	.----
2	1400	3.146	10	1	20	4.16
3	1450	3.161	10	2	30	4.48
4	1500	3.176	10	3	40	4.75
5	1550	3.190	10	5	50	5.00
6	1600	3.204	10	6	60	5.25
7	1650	3.217	10	7	70	5.52
8	1700	3.230	10	8	80	5.84
9	1750	3.243	10	9	90	6.28
10	1800	3.255	10	10	100	8.09



III.Fig.4: Relationship between the Log conc. of Acephate and percent mortality of *Labeo rohita* for 96 hours.



III.Fig.5: Relationship between the Log conc. of Acephate and Probit mortality of *Labeo rohita* for 96 hours.

III.Table.5: Estimated LC50 values and confidence limits of fish *Labeo rohita* for 24hr, 48hr, 72hr and 96hrs.

S. No	Time of Exposure	LC50 value(mg/l)	Log Conc.	95%Confidence Intervals(CI)(UCL-LCL)
1.	24h	2000	3.301	1916.71 to 2133.29
2.	48h	1800	3.255	1716.71 to 1933.29
3.	72h	1650	3.217	1566.71 to 1783.29
4.	96h	1550	3.190	1466.71 to 1683.29

III.2.Discussion:

In the present scenario the motive of the farmers is single sided, to gain only maximum profit, ignoring their impact on the beneficial organisms, environment and human health. Lack of awareness among the farmers about these insecticides they use different management practices to kill insect-pests. Insecticide toxicity is influenced by physical factors like temperature and biological factors like size, national status, species specificity and chronobiology of the animal. The toxicity of a pesticide could vary from species to species and this variation is due to differential tolerance of animals to pesticide exposure. The 96hrs LC_{50} of Acephate for rainbow trout is >1,000mg/l; 2,050mg/l for bluegill fish; 1,725mg/l for large mouth black bass; 2,230mg/l for channel catfish; and 9950mg/l for gold fish (Worthing, C.R.(ed) 1987). The toxicity of Acephate to rainbow trout increased with increasing temperature (Morty, A.S. 1986). The lethal concentrations for 10min and 2hr were determined as 500mg L^{-1} and 300mg L^{-1}, respectively exposed to Acephate on *Paramecium caudatum* (J. Venkateswara Rao *et al.*, 2006). Jayashree J. Kulkarni and Madhav P. Bhilave, 2015 reporteed that the exposure of *Labeo rohita* to 10g/lit Acephate (75%sp) an organophosphate pesticide caused significant shifts in haematological and biochemical profile and exert significant effect on the level of total protein content in Intestine, Gill, Liver and Muscle at 24hr, 48hr, 72hr, 96hr exposure period of Acephate and also changes in haematological indices like RBCs and WBCs in *Labeo rohita.*

The 96hrs LC_{50} value of chlorantraniliprole exposed to freshwater fish *Labeo rohita* was found to 14.424mg/L^{-1}. The variations in the lethal concentration values are due its dependence upon various factors viz: sensitivity to the toxicant, its concentration and duration of exposure (Nagaraju Bantu and Venkata Rathnamma Vakita, 2013). According to PJ Gavit and RD Patil; 2016 the LC_{50} values of Acephate were 1762ppm, 1509ppm, 1281ppm and 1117ppm for 24hr, 48hr, 72hr and 96hrs respectively exposed to fish *Puntius sophore*. Depending upon the salinity of water Acephate exposed to white leg shrimp, *Litopenaeus vannamei*, 100% mortality occurred in the treatments of 101.25mg/l at 5.0% and 151.88mg/l at 20.0% for 24hrs, the mean LC_{50} values for 24, 48, 72 and 96hrs were 51.250, 38.007, 27.783, 18.247mg/L at 5.0% and 59.853, 43.390, 34.220, 27.337mg/L at 20.0% **(**Xiaodan wang *et al.*, 2013). The present study LC_{50} value of Acephate 95% SG exposed to the fish, *Labeo rohita* is agreed with the earlier reports and it is evidence from the above given LC_{50} values and it is moderately toxic to fish based on LC_{50} values.

III.3. Behavioural changes in fish due to acute toxicity:

Aim of the present investigation is to evaluate the acute toxicity of Acephate 95% SG and its toxicological effects on behavioural studies of Indian major carp *Labeo rohita*. During the experimental period, the behavioural changes were observed under the toxicant stress conditions carefully, and compared to control fish behaviour. Observations were also made on the changes in external morphology of the fish. Fish in toxic media exhibited passive drift, active upstream movement, loss of equilibrium, hyper excitability, moving in spiral fashion with jerky movements, convulsions, vertical movements and rapid flapping of the opercular movements with opened mouth and mucus secretions against toxicant also forms a barrier between the body and the toxic medium and minimize the irritating effect. Darting movements and hitting against the walls of test tanks, heamorrhage, deformities, and scoliosis were observed, finally settled to the bottom of the test tank. The symptoms are due to inhibition of AChE activity leading to accumulation of Acetylcholine in cholinergic synapses ensuing hyper stimulation and inhibition of AChE activity is a typical characteristic of organophophate compounds (Dembele K, *et al.*, 2000).

Decreased opercular movement probably helps in reducing absorption of pesticide through gills. Abnormal swimming and loss of balance was caused by the deficiency in nervous and muscular coordination which may be due accumulation of acetylcholine in synaptic and neuromuscular junctions observed by Rao JV *et al.*, 2005. The result showed a decreased survival rate with increased hours of the day with exposure period of organophosphate pesticide. My research work is co inside with those other researchers. 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 sublethal exposure of chemical may be due to the inhibition of cholinesterase (Anitha S. *et al.*, 2010). Decreased swimming behaviour and increased respiration rate were other effects of pesticides in the present study, found that contaminates such as pesticides disturb normal fish behavior after exposure.

IV. CONCLUSION:

Insecticide can be an important crop production tool to maximize the yield but heavy and indiscriminate use of chemicals also exposes farmers to serious health risks, result in negative consequences on non-target organisms. Authors suggest the farmers should be take careful precautions while using these pesticides to minimize their health problems and to maintain ecological balance. In the present study exposure to Acephate causes various effects on the survival and behavioural of *Labeo rohita* indicating of toxicity. The LC_{50} values of Acephate were 2000mg/l, 1800mg/l, 1650mg/l and 1550mg/l for 24hr, 48hr, 72hr and 96hrs respectively. Though the Acephate is moderately toxic to fish and its long term use affect fish and other non-targeted living organisms because of its bioaccumulation in to the organisms through food chain. The current observations indicate that the pesticide contamination affect the central nervous system of the fish due to the accumulation of toxicant.

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