



**TOXICITY EVALUATION AND HAEMATOLOGICAL STUDIES OF  
FLUBENDIAMIDE ON FRESHWATER FISH LABEO ROHITA**

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**ABSTRACT**

The acute toxicity flubendiamide (Insecticide) behavioural studies and haematological studies of labeo rohita exposed to flubendiamide (Insecticide) was observed for 24 hrs, 48 hrs, 72 hrs and 96 hrs. The freshwater fish labeo rohita were exposed to different concentrations of flubendiamide. The LC<sub>50</sub> values for 24hrs (17mg/lit), 48hrs (15 mg/lit), 72hrs (13mg/lit) and 96hrs (11mg/lit). The fish shows marked changes were observed in behavioural morphology and the Behavioural changes Viz: increased respiration rate, perpendicular swimming pattern were observed. Among morphological changes on the body, heavy mucous secretion, frequency of occurrence such changes were more pronounced in the beginnings of treatment and decreased. The response of fish to flubendiamide toxicant was both time and dose dependent. Haematological study revealed that haemoglobin (Hb) was decline Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) showed fluctuating pattern. The observed alterations were ultimately become the causative for affecting the general health status of the fish.

**KEYWORDS:** flubendiamide, labeo rohita, Toxicity evaluation, Behavioural and haematological changes.

**INTRODUCTION**

As the fishes are economically important non-target organisms, they are quite sensitive to a wide variety of toxicants and are used as pollution indicator in the water-quality management. Aquatic environment is plagued with different kinds of aquatic pollutants (Devi et al., 2008, Sachar and Raina, 2014). Insecticides are one such category of organic pollutants which play an important role in controlling different types of insect/ pests that cause damage to crop plants. Unfortunately, most of the insecticides are not biodegradable and tend to persist for years together in soil and water (Gaafar et al., 2010). There are a large number of pesticides currently used in agriculture belonging to a wide variety of chemical classes. Pesticide pollution in water affects the fish and other aquatic organisms, relatively sensitive changes in their surrounding environment (Ayas et al., 2007).

In the present study, an attempt has been made to analyze the toxicity of the flubendiamide (480 SC) (Suspension concentrate) on the freshwater fish labeo rohita. The result was expressed as lethal concentration in the case of aquatic organisms. Since some the fish of population may prove to be excessively susceptible and others may prove to be very resistant to the concentration of the toxicant that affects 50% of the population under consideration is expressed as LC<sub>50</sub> values, which is statistically calculated on the basis of the observed

percentage of mortality at different concentrations of the pesticides and haematological parameters (or) haematology also effects with the increased concentration in the aquatic media (MCV, MCH, MCHC).

**MATERIALS AND METHODS**

The common edible fish Labeo rohita were obtained from the local fish farm at kuchipudi, Guntur District of Andhra Pradesh, India .The fish were brought to the laboratory by using glass aquaria with high oxygen concentration and transferred in to the glass chambers for acclimatization. The length of the fish 6±8 cm, average body weight 6.5± 7.5 g in weight, irrespective of the sex were used in the experiment. The fish L. rohita were acclimatized to the laboratory conditions at 28±2oC. The fish were fed daily with commercial fish pellets and allowed to acclimate for 15 days. Fish were washed with 0.1% KMnO4 solution to avoid dermal infection. All the precautions laid down by (APHA et al., 1998) followed, for maintaining the fish. Water was renewed every day to provide freshwater, rich in oxygen. If mortality exceeds more than 5% during the acclimatization, the entire batch of fish was discarded. The experiments were conducted to determine the toxicity in different concentrations of the toxicant for 24, 48, 72, and 96 hrs in semi static system to calculate the LC50 values. The fish were exposed for 96hrs to lethal (96h LC50 of 11mg/L) and

sublethal concentration (1/10th 96h LC<sub>50</sub> of 1.1mg/L) of flubendiamide. 0.5ml of blood was taken directly by cardiac puncture with the help of heparinised needles using EDTA as an anticoagulant. Among blood parameters, Hb% was determined by using Sahli's haemoglobin meter (Dethloff *et al.*, 1999), Hct was determined by centrifugation method (Wintrobe, 1967).

## RESULT AND DISCUSSIONS

The result showed that flubendiamide was toxic to the fish than other pesticides according to previous studies and toxicity of the pesticides was both time and concentration dependent, thus accounting for LC<sub>50</sub> values obtained at different concentrations and times of exposure. The test result of the 96 hrs LC<sub>50</sub> of labeo rohita exposed to flubendiamide obtained (Table no:2). The 96 hrs LC<sub>50</sub> value of 11.008mg/L estimated by (Venkata Rathnamma *et al.*, 2013) for fish grass carp (*Ctenopharyngodon idella*) and Barbee GC, McClain WR, Lanka SK Stout MJ (2010), cry fish 951 microg L(-1) for the chlorantraniliprole pesticide respectively. In the present study, the LC<sub>50</sub> value of flubendiamide (fame) on the fish labeo rohita was found to be 11mg/L (Table no:2). In general, the toxicity varied with respect to species, size of fish and duration of exposure (Oh *et al.*, 1991; Dutta *et al.*, 1995). [Hassanein H. M.A., 2007], reported the 96h LC<sub>50</sub> value of a neem biopesticide (Triology) on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112ppm.

## BEHAVIOURAL STUDIES

A number of changes were observed in the behaviour of labeo rohita exposed to flubendiamide. Fishes came to the surface of water much more frequently and occasionally tried to jump out of water. Moreover, flubendiamide treated fish exhibited increased opercular movement, increased mucous 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 (convulsions) and at higher concentrations of insecticides resulting in death of the organism (Fukuto, 1990). The fish showed fading of their body colour when compared to controls. The percent mortality of labeo rohita after

exposure to various concentrations of flubendiamide for 24, 48, 72 and 96hrs has been depicted in (Table no:1).

Fish in the experimental 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. Thus the behavioural changes of the fish under insecticidal stress may have deleterious effects of making the fish fall an easy prey in their natural habitat and may affect the stability of the population reported by (Jayantha Rao, 1984). The results were correlated with (Venkata Rathnamma.V and nagaraju, 2013) chlorantraniliprole exposed to grass carp (*Ctenopharyngodon idella*) LC<sub>50</sub>(11.008mg/L<sup>-1</sup>) Barbee GC, McClain WR, Lanka SK Stout MJ (2010), cry fish 951 microg L(-1) for the chlorantraniliprole pesticide. [Hassanein H. M.A., 2007], reported the 96h LC<sub>50</sub> value of a neem biopesticide (Triology) on the grass carp fish, *Ctenopharyngodon idella* and was found to be 112ppm.

## HAEMATOLOGY

Blood parameters, generally, of fish are considered as suitable tool for evaluating the effects of chemicals. Past investigators have also identified changes in several haematological parameters as indicators of pollutants exposure specially metals (Cyriac *et al.*, 1989). The blood cell indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) seem to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with values of RBC count, hemoglobin concentration and packed cell volume. The values of blood cell indices were enhanced in common carp and other freshwater fish after the exposure of acute toxic level of pesticides (Rao, 2010).

The hematological parameters like RBC, WBC, Hb and Hematological indices like MCV, MCH, and MCHC are frequently used to assess the health status of fish. In the present study RBCs (Hb, MCH, MCHC) significantly decreased and MCV increased in *Labeo rohita*. Similar results were observed by Ko:pru:s *et al.*, 2006 in *Catla catla* treated with cypermethrin. Saxena K.K *et al.*, 2002 reported that the RBC count decrease may depend on age of animal, stress condition, sex, and availability of food in a particular medium in fish.

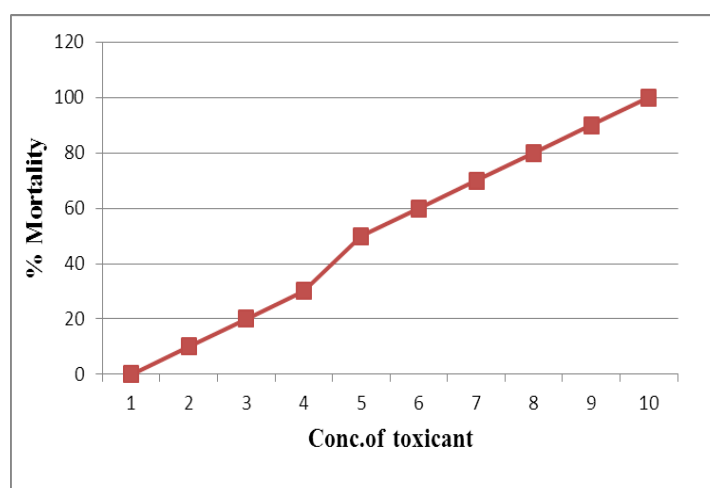
**Table 1. Number of dead specimens of labeo rohita and their percentage of mortality in different concentrations of flubendiamide at different time intervals.**

24 hrs			48hrs			72hrs			96hrs		
Con. of Pesticide in mg/lit	Log concentration	% mortality	Con. of pesticide in mg/lit	Log concentration	% mortality	Con. of pesticide in mg/lit	Log concentration	% mortality	Con. of pesticide in mg/lit	Log concentration	% mortality
14	1.146	10	12.5	1.096	10	11	1.041	10	8	0.903	10
15	1.176	20	13	1.113	20	12	1.079	20	9	0.954	20
16	1.204	30	14	1.146	30	12.5	1.096	30	10	1.000	30
17	1.230	50	15	1.176	50	13	1.113	50	11	1.041	50
18	1.255	60	16	1.204	60	14	1.146	60	12	1.079	60
19	1.278	70	17	1.230	70	15	1.176	70	12.5	1.096	70

20	1.301	80	18	1.255	80	16	1.204	80	13	1.113	80
21	1.322	90	19	1.278	90	17	1.230	90	14	1.146	90
22	1.342	100	20	1.301	100	18	1.255	100	15	1.176	100

**Table: 2 96 hrs lc50 values of flubendiamide on freshwater fish labeo rohita**

S.no	Toxicant conc.in mg/lit	Log. Conc	No.of fish exposed	No.of fish live	No.of fish dead	% Mortality	Probit mortality
1.	7	0.846	10	10	0	0	—
2.	8	0.904	10	9	1	10	3.72
3.	9	0.955	10	8	2	20	4.16
4.	10	1.000	10	7	3	30	4.48
5.	11	1.042	10	5	5	50	5.00
6.	12	1.071	10	4	6	60	5.25
7.	12.5	1.097	10	3	7	70	5.52
8.	13	1.114	10	2	8	80	5.84
9.	14	1.146	10	1	9	90	6.28
10.	15	1.177	10	0	10	100	—

**Figure.1: Relationship between the conc. of flubendiamide and % mortality of Labeo rohita for 96 hours.****Table: 3 Haematological Parameters of fish labeo rohita (Mean  $\pm$ S.D.) for various Concentrations of flubendiamide.**

INDICES	UNIT	CONTROL	24 Hrs	48 Hrs	72 Hrs	96 Hrs
RBC	$\times 10^6$ cm/mm	2.04 $\pm$ 0.06	1.85 $\pm$ 0.71	1.70 $\pm$ 0.07	1.19 $\pm$ 0.04	0.98 $\pm$ 0.01
Hb	g/dl	5.45 $\pm$ 0.87	4.72 $\pm$ 0.11	4.08 $\pm$ 0.91	3.6 $\pm$ 0.23	3.32 $\pm$ 0.19
MCV	Fl	121.5 $\pm$ 0.77	128.53 $\pm$ 0.85	135.11 $\pm$ 0.29	208.08 $\pm$ 0.16	259.51 $\pm$ 0.09
MCH	Pg	26.7 $\pm$ 0.44	25.35 $\pm$ 0.21	23.62 $\pm$ 0.16	32.27 $\pm$ 0.42	36.59 $\pm$ 0.51
MCHC	g/dl	22.6 $\pm$ 0.82	20.51 $\pm$ 0.80	18.44 $\pm$ 0.56	17.2 $\pm$ 0.50	15.77 $\pm$ 0.63

## CONCLUSION

In the present investigation the test species, Labeo rohita has shown differential toxicity level with the function of period. This shows that the more is the duration period the less is the concentration required. The observed percentage of mortality of Labeo rohita for flubendiamide in static tests continuous for different hours and different concentrations were shown in (Table no:1, 2).

Haematological parameters related to oxygen transport (RBC, Hb and Hct), and the calculated indices (MCV, MCH and MCHC) all exhibited marked differences between control and experimental groups in response to

insecticide flubendiamide in presently studied fish, Labeo rohita (Table no:3) confirm that Toxicity evaluation(LC<sub>50</sub>values),behavioural studies and haematological parameters are very sensitive indicators in fishes under toxicity of chemicals (presently flubendiamide). The alterations in the haematological parameters ultimately become the causative for affecting the general health status of the fish.

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