

Acute toxicity and effect of Flubendiamide (39.35% SC) on the oxygen consumption of the Fish, *Catla catla* (Hamilton)

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ABSTRACT

The fish *Catla catla* fingerlings were exposed to the test toxicant an insecticide Flubendiamide for 24, 48, 72 and 96 hrs and the LC₅₀ values were calculated and were reported to be 3.566, 3.456, 3.0221 and 2.892 mg⁵⁻¹ respectively for 24, 48, 72 and 96 hrs. After determination of LC₅₀ values the fish were exposed to sub-lethal concentration (1/10th of 96 hrs LC₅₀ value i.e., 0.289 mg⁵⁻¹) for 24, 48, 72, 96 hrs and 8 days and the changes in oxygen uptake of the Indian major carp *Catla catla* was studied. Fingerlings were exposed to sub-lethal (0.289 mg⁵⁻¹) and lethal (2.892 mg⁵⁻¹) concentrations of flubendiamide for 24 hrs, 48 hrs, 96 hrs and only to the sub-lethal concentration exposed for 8 days. In both sub-lethal and lethal concentrations for all exposed periods *Catla catla* showed an increase in oxygen consumption during the initial time of exposure and a gradual decrease during the subsequent periods of exposures. This initial increase was from 0-6 hours in 24, 48 and 96 hrs where as in 8 days exposed fish the initial increase is only up to 0-4 hours. Alterations in oxygen consumption may be due to respiratory distress as a consequence of impaired oxidative metabolism. In the present study it was observed that flubendiamide has altered respiratory metabolism in the test fish *Catla catla* which can be used as a bio-indicator for assessing pesticide induced alterations in the uptake of oxygen in the fish. Fish in the sub-lethal concentration were found under stress, but that was not fatal. The results obtained in all were discussed with the available literature.

Key words: Flubendiamide, *Catla catla*, LC₅₀ Values, and Oxygen consumption.

Introduction

After green revolution during sixties especially in India, pesticides emerged as knight armours for crops (Srivastava and Singh, 2014). Pesticides has credited with economic potential to enhance production of food and fibre and ameliorated in vector-borne diseases, the long-term use has caused effects on human health and the environment including aquatic ecosystem that evolved new branch of

aquatic toxicology (Igbedioh, 1991; Forget, 1993; Aktar *et al.*, 2009). Pesticides have been found to be highly toxic not only to fish but also to the other organisms which constitute the food chain. Agricultural run-off from near water bodies is the major cause of deposition of pesticides in aquatic ecosystem. The qualitative and quantitative chemical usage is of great concern ecologically. The discriminate use of chemicals is for the control of insect pests by elimination of target species whereas indiscrimi-

nate usage posed the problem on non-target organisms including man.

Wide and indiscriminate use of pesticides in modern agricultural practices throughout the world has indirectly created problem of pollution of aquatic ecosystems (Ganeshwade, 2012). These chemicals impair water quality which becomes unsuitable for all aquatic organisms due to their toxicity, persistence, bioaccumulation, and biomagnifications in food chain and ecological balance (Subramani Lavanya *et al.*, 2011). Fish accumulate these pollutants directly or indirectly from polluted waters and food chain (Jabeen *et al.*, 2016).

Mass mortality of fish due to pesticide exposure is rare, and results only from accidents or direct spraying of the water bodies. Fish are the most often tested aquatic organisms because they are the most conspicuous as predominant and are economically important to man because they are linked in the food chain. Evaluation of LC_{50} is the pioneer step in toxicological assessment of any chemical. It helps to select sub-lethal concentrations to carry out several toxicity tests. Therefore, this knowledge is essential for exploring impacts of any chemical on physical and physiological status of exposed organisms.

In India, flubendiamide, a rynoid pesticide is extensively used in rice and pulse crops to control pests. The excess pesticides used in the farms reach the water bodies by the surface run off and are known to cause ill effects on aquatic organisms. One of the indicators of the health status of a fish is its total oxygen consumption. It helps in evaluating the susceptibility or resistance potential useful to assess the physiological condition of an organism and to correlate the behaviour of the animal, which ultimately serve as predictors of functional disruptions of population. Hence, the analysis of total oxygen consumption can be used as a tool of bio-indicator system to evaluate the basic damage caused to the animal which could either decrease or increase the oxygen uptake (Maharajan *et al.*, 2013). In this viewpoint an attempt was made to study the effect of sub-lethal and lethal concentrations of flubendiamide on oxygen consumption of freshwater fish, *Catla catla* exposed for 24, 48, 96 hrs, and only sub-lethal concentration for 8 days.

Materials and Methods

The fingerlings of the test fish *Catla catla* of size 6-8

$\pm \frac{1}{2}$ cm and weight $6-7 \pm \frac{1}{2}$ gm were procured from local fish hatcheries of Nandivelugu, Tenali mandal, Guntur district, Andhra Pradesh. The fish were acclimated at $(28 \pm 2^\circ \text{C})$ in the laboratory conditions for two weeks. All the precautions laid down on recommendations of the toxicity tests to aquatic organisms were followed (Annon, 1975). Fish were regularly fed with rice bran and one day prior to the experimentation feeding was stopped. Experiments on the oxygen consumption of the fish *Catla catla* was carried out in a respiratory apparatus developed by Job (1955). Fish were exposed to sub-lethal ($1/10^{\text{th}}$ of 96 hrs LC_{50} value $0.289 \text{ mg}5^{-1}$) and lethal (96 hrs LC_{50} value $2.892 \text{ mg}5^{-1}$) concentrations of Flubendiamide for 24, 48, 72 and 96 hrs and only for sub-lethal concentration for 8 days. The samples for estimation of oxygen, consumption were taken from the respiratory chamber, at alternate hours of intervals for 24 hrs. The amount of dissolved oxygen consumption was calculated per gram body weight per hour. The dissolved oxygen content was estimated by modified Winkler's method as described by Golterman and Clymo (1969). The difference in the dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. Students, "t-test" was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant (Fisher, 1950).

Results

Toxicity

The calculated LC_{50} values for 24, 48, 72 and 96 hrs to the test fish *Catla catla* were presented in Table 1 and graphically represented in Figure 1. The LC_{50} values obtained were 3.566, 3.456, 3.0221 and $2.892 \text{ mg}5^{-1}$ respectively for 24, 48, 72 and 96 hrs. The LC_{50} values decreased with the increase in the period of exposure.

The toxicity may be influenced by exposure conditions, source and size of fish, formulation, and water quality. The ground water used for acclimatization of fish and experimental purpose was clear and unchlorinated. The hydrographical properties of water were estimated by the modified methods of Golterman and Clymo, (1969).

Oxygen Consumption

Determination of oxygen consumption by the fish is

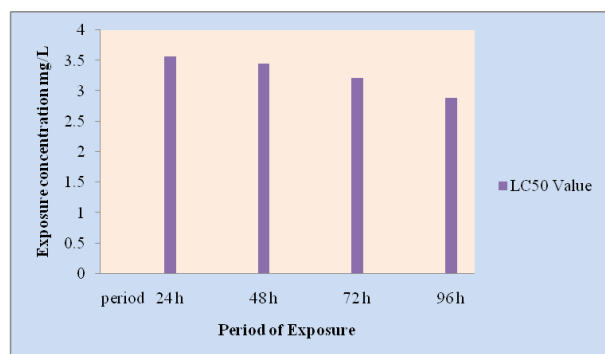


Fig. 1. Calculated LC₅₀ values for flubendiamide to the fish *Catla catla* under static exposure for 24, 48, 72 and 96 hrs.

an important indicator which reflects physiological state of animal and is useful for assessment of lethal effects (Tilak *et al.*, 2007). One of the indicators of the health status of a fish is its total oxygen consumption. It helps in evaluating the susceptibility or resistance potential useful to assess the physiological condition of an organism and to correlate the behavior of the animal, which ultimately serves as predictors of functional disruptions of population (Maharajan *et al.*, 2013). Oxygen consumption is an important physiological parameter which can be used as a useful measure of toxic effects of pesticides in sub-lethal and lethal concentrations because en-

ergy processes are indicators of overall physiological state of organism. Changes in the respiratory activity in fish have been used by several investigators as indicator of response to environmental stress (Ram Nayan Singh, 2014; Sudhasaravanan and Binukumari, 2015; Neelima *et al.*, 2016; Adnan Amin *et al.*, 2017).

Exposure to sub-lethal concentrations of toxicant is reported to increase respiratory activity, leading to increased ventilation and hence increased uptake of the toxicant. The comparative data of the whole animal oxygen consumption in control and experimental fish, *Catla catla* calculated per gram body weight per hour in sub-lethal and lethal concentrations of flubendiamide for 24, 48, and 96 hrs and 8 days were given in the Tables 2 and 3 and graphically represented in Figures 2 and 3.

The results of the experimental and control fish values are graphically represented in Figures 2 and 3 by taking hours of exposure on X axis and the amount of oxygen consumed per gram body weight per hour on Y axis. In sub-lethal concentrations of flubendiamide, it was observed that the fish showed increase in oxygen consumption during the initial time of exposure i.e., 0-6 hours in 24 and 48 hrs treated fish and 0-4 hours in 96 hrs and 8 days exposed fish and thereafter a gradual decrease was observed in the subsequent periods of exposure. The

Table 1. Calculated LC₅₀ values of flubendiamide to the fish *Catla catla* under static exposure for 24, 48, 72 and 96 hrs

S. No.	Test Fish	Exposure period	Concentration in mg/L	Log Conc.	No. of fish exposed	Percent mortality	Probit Mortality	LC ₅₀ Value
1	<i>Catla catla</i>	24 h	3.20	0.51	10	20	4.16	3.566
2			3.40	0.53	10	30	4.48	
3			3.60	0.56	10	60	5.25	
4			3.80	0.58	10	70	5.52	
5			4.00	0.60	10	80	5.84	
6		48 h	3.00	0.48	10	20	4.16	3.456
7			3.20	0.51	10	30	4.48	
8			3.40	0.53	10	50	5.00	
9			3.60	0.56	10	60	5.25	
10			3.80	0.58	10	70	5.52	
11		72 h	2.80	0.45	10	10	3.72	3.221
12			3.00	0.48	10	30	4.48	
13			3.20	0.51	10	50	5.00	
14			3.40	0.53	10	70	5.52	
15			3.60	0.56	10	80	5.84	
16		96 h	2.60	0.41	10	20	4.16	2.892
17			2.80	0.45	10	40	4.75	
18			3.00	0.48	10	60	5.25	
19			3.20	0.51	10	80	5.84	
20			3.40	0.53	10	90	6.28	

Table 2. Changes in oxygen consumption of the fish *Catla catla* under exposure to lethal and sub-lethal concentrations of flubendiamide for 24 and 48 hrs.

Hours	Control	24 Sub-lethal	24 Lethal	48 Sub-lethal	48 Lethal
0	0.795±0.0032	0.886±0.0038	0.874±0.0042	0.83±0.0040	0.846±0.0026
2	0.781±0.0045	1.436±0.0036	1.482±0.0046	1.428±0.0038	1.576±0.0048
4	0.770±0.0035	1.424±0.0042	1.454±0.0042	1.454±0.0042	1.592±0.0042
6	0.761±0.0041	1.406±0.0044	1.436±0.0040	1.474±0.0046	1.602±0.0044
8	0.754±0.0045	0.988±0.0044	0.922±0.0046	0.988±0.0046	0.932±0.0042
10	0.742±0.0043	0.83±0.0036	0.864±0.0044	0.892±0.0038	0.856±0.0038
12	0.736±0.0040	0.678±0.0040	0.688±0.0046	0.658±0.0036	0.626±0.0042
14	0.734±0.0044	0.548±0.0038	0.444±0.0038	0.454±0.0038	0.396±0.0038
16	0.732±0.0036	0.534±0.0036	0.42±0.0040	0.436±0.0044	0.364±0.0044
18	0.730±0.0042	0.515±0.0042	0.398±0.0042	0.388±0.0044	0.326±0.0046
20	0.725±0.0034	0.488±0.0034	0.378±0.0034	0.368±0.0046	0.292±0.0036
22	0.720±0.0044	0.466±0.0044	0.356±0.0044	0.356±0.0044	0.266±0.0044

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

Table 3. Changes in oxygen consumption of the fish *Catla catla* under exposure to lethal and sub-lethal concentrations of flubendiamide for 96 hrs and only for sub-lethal concentration for 8 days.

Hours	Control	96 h Sub -lethal	96 Lethal	8 d Sub-lethal
0	0.757±0.0032	0.829±0.0048	0.846±0.0026	0.456±0.0040
2	0.782±0.0046	0.846±0.0038	0.788±0.0046	0.460±0.0038
4	0.770±0.0035	0.857±0.0049	0.850±0.0042	0.388±0.0042
6	0.761±0.0041	0.634±0.0046	0.606±0.0040	0.407±0.0046
8	0.753±0.0046	0.784±0.0048	0.726±0.0046	0.394±0.0046
10	0.741±0.0044	0.594±0.0036	0.556±0.0034	0.386±0.0038
12	0.736±0.0042	0.564±0.0040	0.526±0.0036	0.401±0.0036
14	0.735±0.0045	0.456±0.0038	0.398±0.0038	0.378±0.0038
16	0.733±0.0034	0.439±0.0046	0.366±0.0040	0.356±0.0044
18	0.730±0.0042	0.288±0.0042	0.284±0.0042	0.309±0.0044
20	0.725±0.0034	0.266±0.0046	0.290±0.0034	0.298±0.0046
22	0.719±0.0044	0.256±0.0044	0.256±0.0044	0.276±0.0044

Results are mean values of four observations
Standard Deviation is indicated as (±)
Values are significant at $p < 0.05$

presence of sub-lethal concentration of toxicant in aquatic environment is inevitable. The toxicant stress on oxygen consumption along with depletion in oxygen in aquaculture practices makes fish less fit and reduces their growth due to lack of proper metabolism (Hyma Ranjani, 2015 and Bantu *et al.*, 2017). In lethal concentrations of flubendiamide, it was observed that a gradual increase in oxygen consumption during the initial time of exposure i.e., 0 to 6 hours in 24 and 48 hrs treated fish and 0 to 4 hours in 96 hrs and 8 days exposed fish and a gradual decline is observed during the later periods of exposure. In control fish the rate of oxygen consumption

gradually decreased and this might be due to starved conditions and the reduced metabolic rates of the starved fish (Anitha, 2015). From the present study it is clear that the flubendiamide affected oxygen consumption of *Catla catla* under all hours of exposure in both sub-lethal and lethal concentrations.

Discussion

Several authors have reported the effect of Rynoid pesticides on the oxygen consumption in fish. Mariya Dasu (2014) observed initial increase in oxy-

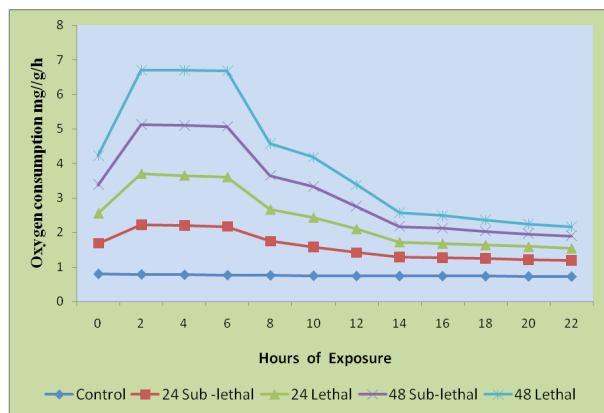


Fig. 2. Changes in oxygen consumption of the fish *Catla catla* under exposure to lethal and sub-lethal concentrations of flubendiamide for 24 and 48 hrs.

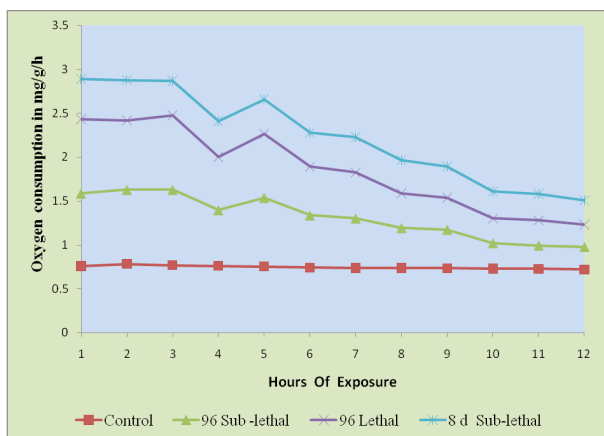


Fig. 3. Changes in oxygen consumption of the fish *Catla catla* under exposure to lethal and sub-lethal concentrations of flubendiamide for 96 hrs and sub-lethal concentration for 8 days.

gen consumption in *Labeo rohita* exposed to Thiocarb. Anitha (2015) also observed increase in oxygen consumption during the initial time of exposures, i.e. 1 to 6 hours and a gradual decrease was observed during the subsequent period of study in *Labeo rohita* exposed to Pyraclostrobin. In lethal concentrations the rate of oxygen consumption showed a decreasing trend from the beginning to the end. Bantu *et al.*, (2017) observed increase in oxygen consumption during the initial time of exposures i.e., 1 to 4 hours and a gradual decrease during the subsequent period in *Labeo rohita* exposed to sublethal concentrations of Indoxacarb for 24 hrs and 8 days. Oxygen consumption decreased in *Labeo* during exposure to lethal concentrations of indoxacarb for 24 hrs.

The initial increase in oxygen consumption in the present study is in agreement with Neelima *et al.*, (2016) in *Cyprinus carpio* exposed to cypermethrin. Jothinarendiran (2012) in *Channa punctatus* exposed to dimethoate, Bantu *et al.*, (2017) in *Labeo rohita* exposed to Indoxacarb, Tilak and Vijaya Kumar (2009) in *Channa punctatus* exposed to Quinaphos, Veeraiah (2001) in *Labeo rohita* exposed to Cypermethrin, Hyma Ranjani (2015) in *Catla catla* exposed to Glyphosate. The present work coincides with the reports of the same. The initial increase in oxygen uptake in sub-lethal concentration might be the reflection of an augmented physiological activity for elimination acting the chemical stress (Tilak and Vijaya kumar, 2009). Due to stress, muscular activity increases which results in an increased demand for oxygen. The increase in activity might boost up oxidative metabolism which results in increased supply of energy to combat the chemical stress (David *et al.*, 2003). Sree Veni and Veeraiah (2014) reported that due to stress there is increased respiratory activity resulting in increased ventilation and increased uptake of the toxicant in *Cirrhinus mrigala* exposed to cypermethrin. Several authors (Veenethkumar and David, 2008; Shereena *et al.*, 2009; Logaswamy and Remia, 2009) reported that alteration in whole animal oxygen consumption is due to the disturbance in oxidative metabolism in different species of fish exposed to pesticides. During the initial hours of exposure elevation in the rate of respiration could be explained in terms of acceleration of oxidative metabolism, as a result of sudden response to the toxic stimulus of the pesticide. With the onset of symptoms of poisoning, probably due to acclimatization to the chemical environment the rate decreased in the later periods of exposure. Similar observations were also made by Neelima *et al.* (2016) and Jothinarendiran (2012). Vani *et al.* (2020) also reported that there is an initial increase in oxygen consumption of fish *Cirrhinus mrigala* (Hamilton) with the effect of carbamate Cartap hydrochloride (50% SP) and gradually decreased. The results of the present study agree with above findings.

As the pesticides stimulate the peripheral nervous system, the activity of fish increases which requires more oxygen to fulfill the energy demand. This could be the reason for initial elevation in the rate of oxygen consumption (Rao, 1989). In sub-lethal medium, in the subsequent period of exposure the respiration rate of fish decreased which might be

due to acclimatization of the fish in the chemical environment (Rao, 1989 and Neelima *et al.*, 2016). Under toxic conditions, the oxygen intake decreases and a number of poisons become more toxic, so the amount of poison being exposed to the animal also increases. Fish breathe more rapidly and the amplitude of respiratory movements will increase (Vakita Venkata Rathnamma and Nagaraju Bantu, 2014). By triggering the process of detoxification, the fish might have overcome the pesticide toxicity. In the later period the decrease in oxygen consumption appears to be a protective measure to ensure that there is low intake of the toxic substances which agrees with the findings of Tilak and Vijaya Kumar (2009). Subsequent decrease in oxygen consumption may be due to increased entry of flubendiamide molecules or their accumulation in the body of fish as a function of time. In sub-lethal concentrations of the flubendiamide the decrease in oxygen consumption appears to be mainly due to lowering down of energy requirements which can be considered as adaptive and even strategic this is in accordance with findings of Tilak and Vardhan, (2002). Depletion in the oxygen consumption is due to disorganization of the respiratory action caused by rupture in the respiratory epithelium of the gill tissue and also secretion of mucus over the gill curtails the diffusion of oxygen (Neelima *et al.*, 2016).

Decreased oxygen consumption was observed by Maharajan *et al.*, (2013) in *Catla catla* exposed to Profenofos, by Vakita Venkata Rathnamma and Nagaraju Bantu (2014) in *Labeo rohita* exposed to Chlorantraniliprole, by Jipsa *et al.*, (2014) in *Tilapia mossambica* exposed to Cypermethrin, by Anthony Reddy (2015) in *Labeo rohita* exposed to Spinosad. Joshi and Kulkarni (2007) reported that *Garra mulllya* (Skyles) when exposed to Cypermethrin and Fenvalerate, oxygen consumption increased in the initial period in both lethal and sub lethal concentrations and thereafter decreased. They concluded that alteration in oxygen consumption increased and later decreased which is a bio-indicator for assessing the pesticide toxicity, which can be correlated with the present study.

Mucus secretion in fish forms a barrier between the body irritating effects, or to eliminate it through epidermal mucus. Similar observations were made by Rao *et al.* (2003) and Parma De Croux *et al.* (2002) in *Prochilodus lineatus* under monocrotophos stress. Opercular movements increased initially in all exposure periods but decreased later steadily in lethal

compared to sub lethal exposure periods. The increased opercular gill movements observed initially may possibly compensate for increased physiological activity under stressful conditions (Shivakumar and David, 2004). Gulping of air at the surface, swimming on the water surface, disrupted shoaling behaviour and easy predation was seen on the first day itself in lethal and sub-lethal exposure periods and continued the same more intensely, which is in accordance with the observations made by Ural and Simsek (2006). Gulping of air may help to avoid contact with the toxic medium. Surfacing phenomenon i.e., significant preference of upper layers the exposure period (Katja *et al.*, 2005). Finally fish sank to the bottom with the least opercular movements and died with their mouths open.

Under sub-lethal exposure, the fish bodies became lean towards the abdomen position compared to the control fish and they were found to be under stress, but this was not fatal. Leanness in fish indicates a reduced amount of dietary protein consumed by the fish under pesticide stress which is immediately utilized and not stored as body mass (Kalavathy *et al.*, 2001).

From the above results and discussion, it can be concluded that decrease in oxygen consumption in fish in response to the toxic stress is the cumulative effect of several stages at which the toxicant act. From the results obtained, it is clearly evident that flubendiamide affect the oxygen consumption of *Catla catla* in all exposed concentrations.

Conclusion

In conclusion, the analysis of data from the present investigation demonstrated that flubendiamide had a profound impact on respiration in *Catla catla* in both sub-lethal and lethal concentrations. Variation in the oxygen consumption in flubendiamide exposed fish was probably due to impaired oxidative metabolism and pesticide induced stress. Changes in gill architecture under flubendiamide stress would alter the diffusing capacity of gill with consequent hypoxic or anoxic conditions thus respiration may become a problematic task for the fish. These results suggest that the altered rates of respiration in *Catla catla* may also serve as a rapid biological monitor to assess the impact of pesticides such as flubendiamide on other biotic communities in the water body. This study also stresses the diligent use of pesticides to prevent environmental pollution.

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