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FIPRONIL 5% SC (Agadi SC) INDUCED EFFECTS ON OXYGEN CONSUMPTION IN FRESHWATER FISH *CATLA CATLA*

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ABSTRACT:

Fipronil is one of the major commercial insecticides in agricultural fields and it is playing an important role on non–target organisms especially to fishes. On exposure to toxicant stress, the physiological functioning of an organism gets disturbed. Hence, the present work evaluated the effect of the fipronil on the oxygen consumption of freshwater fish *Catla catla* and 96hrs LC_{50} was determined at 0.6 mg/L. $1/10^{th}$ of the 96h LC_{50} was taken as sublethal (0.06mg/l) concentration for oxygen consumption studies and it was carried out for a period of 24 hours, at regular intervals of 2 hours. In the lethal and sublethal concentration the rates of oxygen consumption was increased gradually in the initial time of exposures i.e. 1 to 6hrs after that it was suddenly falls down and the control fish oxygen consumption was also falls down 0hr to 24h. The experimental fish showed various types of swimming movements due to loss of equilibrium.

Key words: Fipronil, *Catla catla*, Oxygen consumption, Lethal and sublethal concentration.

I.INTRODUCTION:

Use of different types of pesticides is highly toxic to the aquatic animals due to agricultural runoff through different pathways. Fipronil is a broad-spectrum phenylpyrazole insecticide and it is used to control ants, beetles, cockroaches, ticks, termites, mole crickets, thrips, rootworms, weevils, and other insects. Fipronil directly acts on γ-aminobutyric acid chloride (GABA). It is a GABA receptor inhibitor (Qureshi *et al.,* 2016) and blocks GABAA-gated chloride channels in the central nervous system. Disruption of the GABA receptors by fipronil prevents the uptake of chloride ions resulting in excess neuronal stimulation and death of the target insect. Fipronil degrades rapidly in water when exposed to UV light to form fipronil-desulnyl. Under these conditions, fipronil has a half-life of 4 to 12 hours. Fipronil-amide is the primary residue formed from hydrolysis (Nigm and Crosby 2000). It has low toxicity to mammals but high toxicity to non-target organisms such as fish (Bo Zhang *et al.*, 2018).

Exposure of fish and other aquatic animals to pesticides and heavy metals depends on its Bioavailability, bioconcentration, biomagnifications, and persistence in the environment (Lalitha Vinnakota and Venkata Rathnamma, 2021). Fipronil accumulates in fish with a bioconcentration factor of 321 for whole fish, 164 for edible tissue, and 575 for nonedible tissue. Fish eliminated fipronil completely 14 days after being transferred to clean water. The primary metabolites in fish are fipronil-sulfone and fipronil sulfide (New pesticide fact sheet, 1996). To the best of our knowledge, this is the first study that attempts to explain how impairments in swimming behavior and due to the changes on neurotoxicity -AChE might affect the ability of fish to avoid contamination.

II. MATERIAL AND METHODS

i) Test fish:

The test fish *Catla catla* were acquired from a local commercial hatchery of Kuchipudi, Guntur District, AP, India. It is 20 km away from the Acharya Nagarjuna University. The fish were acclimatized to the laboratory conditions in adequate plastic tanks with unchlorinated ground water for two weeks at a room temperature of 30± 2°C. During the period of acclimatization fish were fed daily with fish meal on an average of 3% of their body weight. Feeding was stopped one day prior to the experimentation. All the precautions were taken and it was followed by APHA (2005). After lab acclimatization, the fish were transferred to the experimental tanks.

ii) Test pesticide:

The selected pesticide fipronil was obtained from local market (Guntur Bazar) for determine the toxicity study on behavioural changes and oxygen consumption in *Catla catla* fingerlings. The IUPAC name for fipronil is (±)-5-amino-1-(2,6-dichloro-α,α,αtriuoro-p-tolyl)- 4triuoromethylsulnylpyrazole-3-carbonitrile.

iii) Estimation of oxygen consumption

The lethal concentration (LC_{50} 0.6mg/L) of fipronil at 96 h was determined and present experiments were carried out to estimate the amount of oxygen consumption of *Catla catla* exposed to Fipronil by Winkler's method in lethal and sublethal concentration (1/10th of lethal) and it was carried out in glass jars for a period of 24 hours, at 2 hours of intervals. Care was taken to prevent the trapping of air bubbles. After one hour, water samples from the subsequent respiratory chamber were siphoned out into a bottle of known volume for evaluating the dissolved oxygen. The average O_2 consumption rate of the fish were analyzed using one way ANOVA Test ($p<0.05$).

III. RESULTS

Oxygen consumption:

In present investigation Fipronil is used to study the respiratory metabolism of *Catla catla*. The comparative data on the whole animal oxygen consumption of control and experimental fish, calculated per gram body weight in both lethal and sublethal concentrations of selected chemicals to the fish *Catla catla* was given in the Table: 1. The results of the experiments and control values are graphically represented by taking time on X-axis and the amount of oxygen consumed per gram body weight on Y-axis, Fig.1 showed significant decrease in oxygen consumption rate.

The results of oxygen consumption of *Catla catla* fish exposed to lethal and sublethal concentration of Fipronil against the controls were observed in the following exposure periods of 0h, 2h, 4h, 6h, 8h, 10h, 12h, 14h, 16h, 18h and 20h. The rates of oxygen consumption *Catla catla* were 0.897, 0.864, 0.839, 0.807, 0.779, 0.744, 0.713, 0.680, 0.648, 0.612 and 0.578 O_2/g/h in control; 0.875, 0.934, 1.046, 1.098, 0.874, 0.778, 0.705, 0.616, 0.524 and 0.402 O_2/g/h in lethal exposures; 0.882, 0.909, 0.889, 0.842, 0.758, 0.714, 0.672, 0.635, 0.594, 0.549 and 0.502 O_2/g/h in sublethal exposures respectively. The fish exposed to lethal concentrations, on the other hand, Fluctuations were recorded with relatively low rates of oxygen consumption and finally, it indicates that the O_2 consumption rate reduced progressively for every 2h intervals as compared to control.

In the present study when fish were exposed to lethal and sublethal concentration of fipronil, several behavioural changes were observed such as swimming at the surface of water. This surfacing phenomenon was more in fish exposed to lethal concentration and some extent high in sublethal concentration over the control fish.

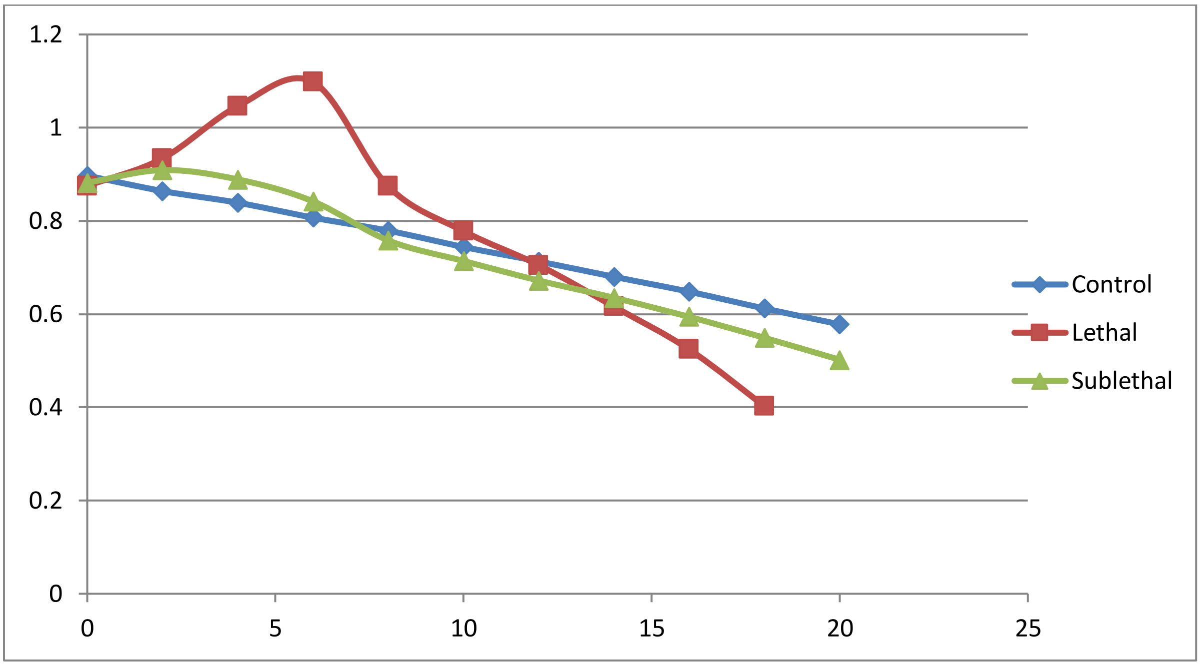
Table: IV.1: The amount of oxygen consumed in mg/gr body weight of the fish *Catla catla*) exposed to lethal and sublethal concentration of Fipronil.

Exposed period in hours	Control	Lethal	% Change	Sublethal	%Change
0	0.897±0.008	0.875± 0.005	-2.45	0.882± 0.006	-1.67
2	0.864±0.005	0.934±0.008	+8.10	0.909±0.003	+5.21
4	0.839±0.004	1.046±0.002	+24.67	0.889±0.004	+5.96
6	0.807±0.006	1.098±0.002	+36.06	0.842±0.005	+4.34
8	0.779±0.002	0.874±0.003	+12.19	0.758±0.002	-2.69
10	0.744±0.002	0.778±0.006	+4.57	0.714±0.006	-4.20
12	0.713±0.006	0.705±0.004	-1.12	0.672±0.003	-5.755
14	0.680±0.006	0.616±0.004	-10.39	0.635±0.004	-6.62
16	0.648±0.005	0.524±0.003	-19.13	0.594±0.007	-8.34
18	0.612±0.003	0.402±0.009	-34.31	0.549±0.002	-10.29
20	0.578±0.009	----------------	--------	0.502±0.003	-13.15

Values are the mean of five observations: Standard Deviation is indicated as (±),

Value are significant at $p < 0.05$

Fig: IV.1: The amount of oxygen consumed in mg/gr body weight of the fish *Catla catla* exposed to lethal and sublethal concentration of Fipronil.



Behavioural studies:

Pesticides disturb normal fish behavior so decreased swimming behaviour and increased respiration rate were found after exposure to pesticides (Lalitha vinnakota *et al.*, 2018). When exposed to lethal concentration, body surface acquired dark colour before their death which is one of the symptoms of toxicity. A film of mucus was observed on the gill and all over the body. γ-aminobutyric acid is one of the main neurotransmitters involved in the adaptation processes against the damage that hypoxia can cause to the brain. Fipronil, in environmentally relevant concentrations, affected swimming (distance moved) only in D. rerio, and 2,4-D affected only swimming (maximum speed) by *H. eques*. The enzymatic activity of AChE was altered (inhibited) only when D. rerio was exposed to fipronil, with no changes observed when fish are (Moreira *et al.*, 2021).

Fipronil impairs the brain GABAergic signaling of a hypoxia-tolerant fish *Oreochromis Niloticus (*Nile tilapia*)* during the transition from a normoxic to an acute hypoxic state (Priscila *et al.*, 2022). Hyperexcitation, loss of equilibrium, increased cough rate, flaring of gills, increase in production of mucus from the gills, darting movements, hitting against the walls of test tanks and curvature of spine. Anitha smruthi Ch *et al.*, 2022 has been reported toxicity evaluation and behavioural changes in fishes are very sensitive indicators under toxicity of chemicals.

IV. Discussion:

Oxygen consumption measurements provide whole animal stress and contaminant water quality and useful to assess the physiological state of an organism and it helps in evaluating the susceptibility and resistance potentiality. Oxygen uptake of fish is intimately connected with the extent of damage of gills and pesticide changes in the architecture of gill, altered diffusion due to the capacity of gill with consequent hypoxic/anoxic conditions and thus respiration might become problematic for the fish. Hence, in the present study laboured breathing was observed in the test fish *Catla catla* with concomitant gill damage, respiring through mouth was observed as an indication of respiratory distress/or hypoxic condition. Decrease in oxygen uptake might the interference of the toxicant with Haemoglobin content.

According to Dhamgaye *et al.*, 2020, that the oxygen consumption rate reduced progressively by 21% and 39% on 7th day and 65% and 82% on 28th day at 6.46 and 12.92 µgL-1 concentrations of fipronil .In the present study decreasing O2 consumption of *Catla catla* were observed with increasing concentration of fipronil and exposure period. A similar trend was reported earlier under fipronil toxicity in *Catla catla* (Anithasmruthi Ch, 2022) and λ-cyhalothrin toxicity in *Ctenopharyngodon idella* (Rajeswari *et al.*, 2020). The fluctuated response in respiration might attribute to reduction in gill permeability results decreased in consumption of oxygen.

During experimentation the control fish demanded more oxygen in the early hours later get settled and maintained a constant uptake of oxygen, while the exposed fish demanded more oxygen in the initial hours and later showed a decrease in the oxygen demand. In the present investigation oxygen consumption of the fish clearly showed that gradually decreases in the control fish throughout the study period whereas in the lethal concentration the rates of oxygen consumption is initially increases and suddenly falls down and died for 18h but in sub lethal concentrations the test fish *Catla catla* survives up to 20h.

Present study O_2 consumption of fish *Catla catla* exposed to fipronil. 0hr in control (0.897), in lethal (0.875) and sublethal concentration (0.882) and after 2h O_2 consumption in control (0.864), lethal (0.934) and sublethal (0.909) i.e. sudden changes in O_2 consumption levels were observed in both lethal and sublethal concentrations when compare with control values of the fish *Catla catla*. Meanwhile severe respiratory distress and rapid molecular movement noted and it leads to more intake of test pesticide fipronil which results in increased mucus secretion and decrease in oxygen uptake efficiency. Decreasing rate of respiration with increasing toxicants concentration was recorded in experimental fish (M. Ilavazhahanl *et al.*, 2017).

The toxicant stress in oxygen consumption along with depletion in oxygen in aquaculture practices make them less fit and reduction in growth due to lack of proper metabolism. The fish was in more stress during first hour and later they are showing signs of recovery. The recovery was evident as the toxicant exposure increased during 24h experiment. Comparison was made between the effects of sub-lethal and lethal concentrations of fipronil on *Catla catla* was decreased some extent increased i.e., 0-4h in sublethal and from 6-20h continuous decline was noted. Under lethal concentration of fipronil significant increase in oxygen consumption was found in the initial stages of exposure i.e., 1-6h in *Catla catla*. Hence, this fish was sensitive to fipronil toxic stress as a result more amount of oxygen is consumed. Fish in the sub lethal concentration were found under stress, but that was not fatal and the respiration rate of fish decreased in the subsequent period of exposure which is due to acclimatization of the fish in the toxicant environment and fluctuations in the oxygen consumption were noticed.

The consumption was more at initial periods later it was decreased. The reduced opercula activity has resulting in low oxygen consumption. When applied to water, fipronil varies greatly in its toxicity and potential to bioaccumulate in aquatic arthropods, depending on the species (Chaton *et al.,* 2001). In the present experiment surface feeder *Catla catla* showed difficult breathing activity in the lethal doses of fipronil due to the presence of more pesticide concentration in surface water and it is also supported by Anithasmruthi Ch, 2022 i.e. when comparison was made among two fishes namely *Catla Calta* and *Ctenopharyngodon idella*, *Catla Calta* showed severe behavioural activities. It is also an evident i.e. Fipronil was measured in surface water at concentrations of 0.829 to 5.290 µg/L in southwestern Louisiana during March through April, which corresponds to the timing of releases of rice field tail water. Results indicate that fipronil degradation products accumulate in riverbed sediment while the parent compound does not (USGS fact sheet, 2000).

The test fish *Catla catla* absorbed fipronil across the gill and diffused into the blood stream, resulting in toxic stress in the fish was suffered with lack of O_2 consumption. The changes in oxygen consumption progressed with the duration of exposure and concentration of pesticide and inhibition for metabolic activity disturb in the gills of test fish *Catla catla* at cellular level. It is supported by Rajeswari *et al.*, 2020 i.e. in lethal and sub-lethal concentration experimental fish *Ctenopharyngodon idella* exposed to λ-cyholothrin showed an increased tendency in oxygen consumption during the initial time of exposures i.e. 2 to 6 hours in lethal and fish showed gradual decrease in oxygen consumption from the starting period of exposure to till the end of the experiment in control and sublethal concentration.

The present work coincided with the results of the other researchers. The increased rate of oxygen might increase ventilation volume in order to compensate drop in oxygen due to reduction in permeability of gills. Either increase or decrease in the rate of oxygen consumption leads to vital tissue damages caused by lethal and sublethal doses of fipronil on fresh water fish *Catla catla*. In the present study, it is clear that an oxygen disturbance causes the metabolic disorders in fish which ultimately lead to the deterioration of the general health of the fish and finally it may fatal.

In the present study *Catla catla* exposed to fipronil the variation in oxygen consumption is an indicator of stress, which is frequently used to evaluate the changes in metabolism under environmental deterioration. Oxygen consumption of *Catla catla* under lethal and sublethal concentrations was compared with control. Since most of fish breathe in the water in which they live, any changes in the chemical properties in it might reflected in the animal's ventilator activity, particularly if the environmental factors affect exchange of respiratory gas. The behavioural changes such as signs of respiratory stress, loss of equilibrium and erratic swimming in the pesticide treated fish for the restrictive use of the pesticide in the environment (Sree Vidya and Sarala Nair 2019). Behavioral changes in fishes are very sensitive indicators under toxicity of chemicals by affecting the general health status of the fish (Prasanna *et al.*,2020).

In the current study low oxygen content in the medium of lethal and sublethal exposures not only cause for external stress and it also leads to internal stress. Low oxygen: nitrogen ratio in sublethal group is due to protein catabolism. The oxygen: nitrogen showed a decrease in sublethal concentrations of fipronil, indicating a shift towards protein primary metabolism corresponding to an alteration in energy utilization (Dhamgaye *et al.*, 2020). Gripp *et al.*, (2017) showed that exposure to fipronil and its metabolites at environmentally relevant concentrations can damage the antioxidant system and establish oxidative stress in tadpoles of the species *Eupemphix nattereri*, resulting in changes in their physiological conditions.

IV. CONCLUSION:

The accumulation of pesticide occurs in the organisms after pesticide reaches the aquatic environment result in the delayed mortality, reduced reproductive capacity, altered growth rate and reduced ability to withstand the changes in the environment. The effect can damage individual species and impact the natural biological communities and finally it leads to disturbs physiological functioning of an organism to toxicant stress. Any effect of pesticides in fish ultimately affects humans through the food chain because fish serves as a major food source of humans.

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