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TOXICITY EVALUATION AND BEHAVIOURAL STUDIES OF *CATLA CATLA* INDUCED TO FIPRONIL 5%SC (Agadi SC)

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ABSTRACT:

In addition to killing insects or weeds, Pesticides contaminate soil, water, turf, and other vegetation and also cause negative effects on a host of other organisms including birds, fish, beneficial insects, and non-target plants. Insecticides are generally the most acutely toxic class of pesticides. Fipronil 5%SC belongs to the phenylpyrazole group. It is a broad spectrum insecticide, toxic by contact and ingestion. Hence, the aim of the present study was to assess the effect of Fipronil 5%SC insecticide on freshwater fish *Catla catla*. The 96h LC_{50}value was found at 0.58mg/l, and observed the behavioral studies. Fipronil 5% SC disrupts the central nervous system by blocking GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels. For this reason, it causes hyperexcitation of exposed fish nerves and muscles.

Keywords: Fipronil 5%SC (Agadi SC), *Catla catla***,** LC_{50}, and Behavioral studies.

I.INTRODUCTION:

Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture to kill pests that damage crops. The use of chemical pesticides has become critically important to assure both quality and productivity of agricultural products [1](Tudi M *et al*., 2021) and to control household pests such as termites, fire ants, and mosquitoes [2](Matta J, 2020). However, pesticides also have negative effects on non-target organisms [3](Zaller JG and Brühl CA 2019), which might be considered beneficial organisms. Because chemical pesticides eventually flow into the surface water, it is expected these toxicants to affect aquatic environments more than terrestrial environments.

Although these negative effects could be acute (lethal) or chronic (sub-lethal) and vary depending on species [4](Conradie, S. R, *et al*., 2019).The toxicity evaluation and behavioral changes in fishes are very sensitive indicators under the toxicity of chemicals [5](Sharma, M *et al*.,2019). Present study *Catla catla* freshwater fish exposed to Fipronil 5%SC for 96hrs, to evaluate lethal concentration 50% mortality and behavioral changes and all the experiments made along with controls.

II. Materials and methods:

II.1. Test compound:

Fipronil 5% SC belongs to the phenylpyrazole group. It is a broad spectrum insecticide, toxic by contact and ingestion. It is also very effective for insects resistant or tolerant to pyrethroid, cyclodiene, organophosphorous and carbamate insecticide. Fipronil 5% SC is a modern insecticide of Phenylpyrazole group which controls the insect pests of rice, chilli, cabbage/cauliflower and sugarcane effectively. It has been proven to offer low dose, highly effective insect control against a broad range of economically important pests. Fipronil 5%SC is used to control ants, beetles, cockroaches, fleas, ticks, termites, mole crickets, trips, rootworms, weevils, and other insects. Neonicitinoids and Fipronil belong to a wide family of substances jointly referred to as the "systemic insecticides" due to their systemic properties, some carbamate and organophosphorus substances, however, can also act systemically [6](Simon-Delso N*et al.*, 2015).

II. 2.Animal selected:

Catla catla is an edible freshwater fish occurring abundantly in the freshwater bodies, rivers, lakes and ponds in India [7](Ramakrishna *et al*., 2013). Large population of fish consumers prefers these fishes, because of rich source of animal protein, tasty flesh and fewer bones. Besides its adaptability to the laboratory conditions and suitability to toxicity studies. Hence these fish were selected as the experimental animal for this investigation.

Biology of *Catla catla:*

Catla catla is a fish with large and broad head, a large protruding lower jaw, and upturned mouth. It has large, grayish scales on its dorsal side and whitish on its belly. It reaches up to 182cm (6.0 ft) in length and 38.6 kg (85 lb) in weight. *Catla catla* is a surface and midwater feeder. *Catla catla* are belongs to the family Cyprinidae, *Catla catla* is commonly known as *'boche*'. It is most valuable edible fish. *Catla catla* is the most famous Indian major carp, easily found rivers in South Asia. It is large omnivore and extensively used in aquaculture. The *Catla* occurs in rivers throughout much of northern and central and eastern India (Rohu fish farming information guide, 2015). Pakistan, Vietnam, Nepal and Myanmar, and has been introduced in to some of the rivers of peninsular India and Srilanka [8](Froese *et al*.,2013).This fish *Catla catla* 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. Procurement and maintenance of fish:

Healthy freshwater fish, *Catla catla* size 6±7 cm total length (TL) and 6.5±7.5 g body weight was collected from the Kuchipudi fish farm, Guntur District of A.P, India; fish were immediately transported in large plastic tanks with required aeration and brought to the laboratory. Then the fishes acclimatized to the laboratory conditions in large cement (200L) tanks with sufficient dechlorinated ground water for 15 days at room temperature 28±2^{0}C.During the acclimation period and subsequent periods of pesticides exposure, fish were held under a photoperiod of 12 h light and 12h dark. The fish were fed with fish meal, rice and commercial fish pellets once in two days, at the same time water was renewed every day rich in oxygen (aeration) and feeding was stopped one day prior to the experimentation.

All the precautions were laid by [9](APHA, 2005) were followed. As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of the animals, size and sex etc., precautions were taken throughout this investigation to control all these factors as far as possible. As a part of it, water from the same source has been used for maintenance of the fish. The size of the animals selected was also maintained strictly throughout the investigation.

II. 4.Studies on lethal toxicity:

The stock solution of the toxicant was prepared in distilled water and 1mg/l, concentration of Fipronil was taken, the control group was maintained for each experiment and added distilled water equal to the toxicant concentration which was used in the test. The Experiments were carried out to assess the lethal responses of Fipronil by the experimental animals. The acute toxicity (96hr LC_{50}) of test toxicants for the freshwater fish, *Catla catla* was determined in the laboratory using the static renewal method according to [10]OECD (1998). The containers of the test media were 15liters capacity; wherein for each test five containers were used and in each container 10 fish were introduced.

The fish were exposed to different concentrations of Fipronil pesticide with five replicates for each concentration. Another 10 fish were used per each concentration of the test toxicant, 10 fish were also maintained in separate container along with experimental group they were served as control. Water was renewed every day of test medium for every 24hr with respective concentrations of the Fipronil 5%SC without oxygen (aeration). The data on the mortality rate of the fish was recorded and the dead fish were removed. The toxicity tests were conducted to choose the mortality range from 10% for 4days (96hrs)in static renewal systems. Finney Probit analysis[11] (Finny, 1971) as recorded by [12]Roberts and Boyce (1972) was followed to calculate the median lethal concentration (LC_{50}) values and its 95% confidence limits. The mean values were derived following the method of Finney Probit Kill theory (1971).

III. Results and discussion:

Toxicity of pollutants to plants, animals, fish or wildlife can be evaluated simply by exposing a group of organisms under controlled conditions such as evaluation can be performed and is the indices of action. Pesticides can produce adverse effects in a biological system, seriously damaging its structure and function of living system finally leads to death of organism. Those adverse responses might be defined in terms of a measurement as acute toxicity. Pesticide application plays a crucial role in modern day agriculture. Leaching and mixing of chemicals from agricultural practices have a direct impact on the aquatic systems posing great threat to the aquatic life forms.

The present study *Catla catla* in static renewal bioassay are given in Table respectively. The reported LC_{50} values are given in Tables I, II, III, IV& Figure respectively. The results of the LC_{50} (Lethal Concentration) of the present study at 96hr, 72hr, 48hr and 24hrs were found 1, 0.87, 0.73 and 0.58mg for Fipronil 5%SC, results according to Finney Probit analysis the lower bound and upper bond 95% lethal confidence limits for Fipronil SC 0.58mggiven in Table no: V. The percent mortality and probit mortality increased with the increasing concentration of toxicant and the percent mortality. Changes in behavioral responses were noticed in fish exposed to FPN and found to affect the fish in dose and duration dependent pattern [13](Zabin SB *et al.*, 2018). The unrestricted, heavy use of synthetic pesticides results in a lethal effect on various non-target organisms in the aquatic environment and direct or indirect effect to users [14, 15](Al-Ghanim *et al.*, 2020; Kumaresan V *et al.*,2019).

In the present study observed that the fish *Catla catla*. Fipronil 5% SC to LC_{50} 0.58mg the results were compared with the other researchers and the result were compared with other researchers. The LC_{50} value of Ethion an organophosphate pesticide to freshwater fish was found to1.2µg/l [16](Prasanna *et al.*, 2020). The 96hr LC_{50} value of Carbamate pesticide pyraclostrobin was 6 ppm respectively to the freshwater fish *Ctenopharyngodon idella* [17](Katti Ravi babu *et al.*, 2021). (λ-Cyhalothrin of 5% EC formulation pyrethroid was used static renewal bioassay to evaluate 96 h LC50 in the freshwater fish *Ctenopharyngodon idella* and it was determined at 0.026 mg/l [18](G. Rajeswari *et al.*, 2020). The 96hr LC_{50} value of synthetic pyrethroid pesticide Bifethrin 10%EC for 96hr was observed as 2.2µg/l respectively to the freshwater fish *Ctenopharyngodon idella* [19](V. Chaitanyakumari *et al.*, 2021).

The mode of action of pesticides is not the same for all types of organisms like microorganisms, plants, invertebrates and vertebrates [20](LalithaVinnakota *et al.*, 2021). The fresh water fishes *Labeo rohita* and *Ctenopharyngodon idella* exposed to (Fipronil 5%SC insecticide)96hrs, to evaluate lethal concentration 50% mortality at 24hrs, 48hrs, 72hrs and 96hr were found to be 3.3 &2.7mg/l, 2.64 & 2.3, 1.98& 1.9 and 1.5 & 1.32 9 [21](Ch. Anithasmruthi *et al.*,2021). The freshwater fish *Labeo rohita* was exposed to flubendiamide (Insecticide) and the LC_{50} values for 24 hrs. (17 mg/lit), 48 hrs.(15 mg/lit), 72 hrs.(13 mg/lit), and 96 hrs. (11 mg/lit) [22](Nirmala K, *et al.*., 2016).

Table: I. The 24 hr acute toxicity of Fipronil on freshwater fish, *Catla catla* percent mortality and probit mortality:

S. no	Dose	Mortality	Probit value y	LOG(100*B1)	x*x	y*y	Xy
1	0.94	10	3.7184	1.973128	3.893234	13.8265	7.336879
2	0.98	30	4.4756	1.991226	3.964981	20.031	8.911931
3	1	50	5	2	4	25	10
4	1.2	70	5.5244	2.079181	4.322995	30.519	11.48623
5	1.6	90	6.2816	2.20412	4.858145	39.4585	13.8454
			25	10.24766	21.03935	128.835	51.58044

Table: II. The 48 hr acute toxicity of Fipronil on freshwater fish, *Catla catla* percent mortality and probit mortality:

S. no	Dose	Mortality	Probit value y	LOG (100*B1)	x*x	y*y	Xy
1	0.81	10	3.7184	1.908485	3.642315	13.8265	7.09651
2	0.85	30	4.4756	1.929419	3.722657	20.031	8.63530
3	0.87	50	5	1.939519	3.761735	25	9.69759
4	0.89	70	5.5244	1.94939	3.800121	30.519	10.7692
5	0.94	90	6.2816	1.973128	3.893234	39.4585	12.3944
			25	9.699941	18.82006	128.835	48.5930

Table: III. The 72 hr acute toxicity of Fipronil on freshwater fish, *Catla catla* percent mortality and probit mortality:

S. no	Dose	Mortality	Probit value y	LOG (100*B1)	x*x	y*y	Xy
1	0.67	10	3.7184	1.826075	3.33454	13.8265	6.79007
2	0.71	30	4.4756	1.851258	3.42715	20.031	8.28549
3	**0.73**	50	5	1.863323	3.47197	25	9.31661
4	0.75	70	5.5244	1.875061	3.51585	30.519	10.3585
5	0.79	90	6.2816	1.897627	3.60098	39.4585	11.9201
			25	9.313344	17.3505	128.835	46.6709

Table: IV. The 96 hr acute toxicity of Fipronil on freshwater fish, *Catla catla* percent mortality and probit mortality:

S. no	Dose	Mortality	Probit value y	LOG (100*B1)	x*x	y*y	Xy
1	0.52	10	3.718	1.716003	2.94466	13.826	6.38077
2	0.56	30	4.475	1.748188	3.05616	20.031	7.82419
3	**0.58**	50	5	1.763428	3.10967	25	8.81714
4	0.6	70	5.524	1.778151	3.16182	30.519	9.82321
5	0.64	90	6.281	1.80618	3.26228	39.458	11.3457
			25	8.811951	15.5346	128.83	44.1910

V. Pesticide concentration and Confidence Levels of 95% for exposed to Fipronil fish *Catla catla* at different exposed periods.

S.No	Time of Exposure (h)	Pesticide concentration (LC_{50})	Number of fish exposed	Percent mortality	95% confidence limits
1.	24	1.00	10	50	0.8038 to 1.4842
2.	48	0.87	10	50	0.8122 to 0.9318
3.	72	0.73	10	50	0.6745 to 0.7855
4.	96	0.58	10	50	0.5245 to 0.6355

II. BEHAVIOURAL CHANGES OF THE FISH IN TOXIC ENVIRONMENT:

The behavioral patterns of aquatic organisms that have been tested or investigated during the last hundred years of aquatic toxicity testing deals with the avoidance reactions, swimming and schooling behavior, level of swimming, predating behavior or escape from predator, agonistic and comfort behavior like coughing, chasing, nipping, biting, vacating, flicking, etc., and respiratory behavior like rate of opercular movement. Interestingly, the burgeoning literature in evolutionary ecotoxicology has shown that some fish populations having evolved under chronic pollution have divergent response to an experimental contamination, suggesting local adaptation to pollutants [23, 24,25](Gauff RPM *et al.*, 2022; Oziolor *et al.*, 2016; Brady *et al.*, 2017). For instance, killifish *Fundulus heteroclitus* from highly contaminated environments have evolved genetic-based physiological ability to cope with organic pollutants [26, 27](Lisa Jacquin *et al.*, 2020;Whitehead *et al.*, 2017). The divergence in behavior caused by pollution, but empirical evidence of behavioral local adaptation to pollution through genetic evolution and/or plasticity is still scarce [26](Lisa Jacquin *et al.*, 2020).

In the present investigation, during the course of exposure of fish to lethal concentration of Fipronil 5% SC for 96hr 0.58 mg/l, several behavioral changes were observed which, include erratic swimming movements and they appeared in distress. Hyper excitation, loss of equilibrium, increased cough rate, flaring of gills, increase in production of mucus from the gills, darting movements and hitting against the walls of test tanks were noticed in *Catla catla*. A film of mucus was also observed all over the body and also on the gills. Physiological stress has occurred in the form of neuronal excitation, which apparently has resulted in the continuous synthesis and destruction of neuro transmitters and enzymes [28](Fabiani C and Antollini SS 2019).

Present study *Catla catla* exposed Fipronil 5% SC and the observing were gulping air and swimming at the water surface (surfacing phenomenon) were observed also with mucus secretion on the body in the exposure periods. That fish in sub lethal concentration were found under stress but that was not fatal [29](S.K. Parveen *et al.*, 2021). Reported that the abnormal changes in the fish exposed to lethal concentration cypermethrin are time dependent [30](Lalitha .V *et al.*, 2018). Observed that the fish is exposed to cypermethrin, erratic swimming, hyper and hypoactive, imbalance in posture, increased surfacing activity, opercular movement, gradual loss in equilibrium, spreading of excess of mucus all over the surface of the body [21](Anithasmruthi ch *et al.* 2021).The variation in the LC_{50}values is due to its dependent upon various factors viz, sensitivity to the toxicants, its concentration and duration of exposure [16](Ch. Prasanna *et al.*, 2020). The toxic stress of pesticides has direct after on tissue chemical compounds [18, 11](G. Rajeswari *et al.*, 2020; V. Chaitanyakumari *et al.*, 2021).

The death may be the result of severe physiological stress at cellular level and changes in behavior of fish *Catla catla,* due to Fipronil 5% SC pesticide stress can be used as a biological indicator of pollution as biological early alarm system of the aquatic ecosystems. The abnormal behaviors observed in the fish might be caused by the neurotoxic effects and also by the irritation to the perceptive system of the body. Toxicants might damage nerve cell bodies, axons, and myelin sheaths and the biochemical level; they can alter the synthesis and release of neurotransmitters, which might be associated with behavioral changes. Organic chemicals as well as metals can affect neurotransmitters and behavior [31](Yanting Shi *et al.*, 2023). The deleterious effects of 4-NP on central nervous system as well as neuroendocrine homeostasis and cognitive functions [32](Salim S 2017).

Inhibition of Ach. E activity due to the exposure of 4-NP activity in Mytilus galloprovincialis [33](Vidal-Linan L *et al.*, 2015). So, the abnormal behavior shown by fish might be due to abnormal level of neurotransmitters [5](Sharma. M *et al*, 2019). Jumping to and from signify the avoidance reaction of the fishes to the toxicants. Fish avoid the area containing chemical so mostly fishes remain in the corners of the tank. The increase in surfacing and gulping of air from surface water after toxicant exposure could be an attempt of the animal to escape from the toxicant and to avoid breathing in the contaminated water. Secretion of excessive mucus is probably due to irritation of the skin due to direct contact with the toxicant. Mucus forms a layer between the body and toxicant to minimize irritating effect [34](Leal J *et al.*, 2017) and also inhibit the diffusion of oxygen during gaseous exchange.

IV. CONCLUSION:

To conclude the test species, *Catla catla* 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 mortality and probit mortality of *Catla catla* for Fipronil 5% SC in static tests continuous for different hours and different concentrations were shown in (Table No. 1). Control and experimental groups in response to insecticide Fipronil 5% SC in presently studied fish *Catla catla* (Table no.1) confirm that toxicity evaluation(LC_{50}values) and behavioral changes in fish are very sensitive indicators under toxicity of chemicals. The behavioral changes affecting the general health status of the fish.

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