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Research Article

TOXICITYY EVALUATION AND BEHAVIOURAL STUDIES OF CATLA CATLA INDUCED FIPRONIL 5%SC

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ABSTRACT

Environmental contamination from the use of pesticide ranges from water, air and soil pollution to alteration of the ecosystem resulting in detrimental effects to non-target organisms. Fipronil 5%SC one of the broad spectrum insecticide belongs to the phenylpyrazole chemical family. Fipronil's specificity towards insects is believed to due to its greater affinity to the GABA receptor in insects relative to mammals and its effect on glutamate-gated chloride (GluCl) channels, which do not exist in mammals. Because of its effectiveness on a large number of pests, Fipronil is used as the active ingredient in flea control products for pets and home roach traps as well as field pest control for corn, golf courses, and commercial turf. Its widespread use makes its specific effects the subject of considerable attention. The aim of the present study was to assess the effect of fipronilpesticide on common carp (*Catla catla*) for the 96-h LC_{50} value was found 0.23mg/l, observed the behavioural studies.

INTRODUCTION

Environmental contamination from the use of pesticide ranges from water, air and soil pollution to alteration of the ecosystem resulting in detrimental effects to non-target organisms. Evidence of pesticide threats to human health and trade-off between health and economic effects have been documented in several studies in the past (Antle and Pingali, 1994). The pesticide consumption in India is low (0.57 kg/ha) as compared to other countries like Japan (12 kg/ha), Taiwan (17 kg/ha) and West Germany (3 kg/ha), the pesticide residues in food especially vegetables in India are the highest in the world. This is mainly due to unregulated use of pesticides. The synthetic organic insecticides widely used in agriculture are general biocides having innate ability to cause injury to all living organisms as well as to the quality of environment. The presence of residues of these pesticides in food commodities and other components of the environment has proved toxic to human beings, domestic animals, birds, fish and other non-targeted fauna of the agro-ecosystem. Present study *Catla catla* fresh water fish exposed to Fipronil 5% SC insecticide exposed to 96hrs, to evaluate lethal concentration 50% mortality of the freshwater fish *Catla catla,* and all the experiments made along with controls.

MATERIALS AND METHODS

Animal selected

Catla catla is an edible freshwater fish occurring abundantly in the freshwater bodies, rivers, lakes and ponds in India (Ramakrishna *et al*., 2013). Large population of fish consumers prefers this fish, 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 this fish was selected as the experimental animal for this investigation. *Catla catla* is one of the three major carps produced and consumed in large quatity in India with global production of 1.35 million tons during 2006 (Anonymous, 2011).

Biology of Catla catla

Catla catla is belongs to the family Cyprinidae, and is commonly known as 'catla'. It is most valuable edible fish found all over India. It is the most famous Indian major carp, easily found all over north and central India. It is also found in Godavari and Krishna rivers in south India. 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.

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Procurement and maintenance of fish

Healthy freshwater fish, *Catla catla* (Hamilton) size 6±7 cm total length (TL) and 6.5±7.5 g body weight were 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 fish 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 hr light: 12hr 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. Then the fish were separated into the batch of having the length of the fish 6±7 and body size 6.5 7.5g were maintained in static water without any flow (Doudoroff *et al*., 1951). All the precautions were laid by (APHA *et al*., 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.

Pesticide Selected

Fipronil is a broad-spectrum insecticide that belongs to the phenylpyrazole chemical family. It disrupts the insect central nervous system by blocking GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels. This causes hyperexcitation of contaminated insects' nerves and muscles. Fipronil's specificity towards insects is believed to be due to its greater affinity to the GABA receptor in insects relative to mammals and its effect on GluCl channels, which do not exist in mammals, (Raymond-Delpech V *et.al*., (2005) Because of its effectiveness on a large number of pests, fipronil is used as the active ingredient in flea control products for pets and home roach traps as well as field pest control for corn, golf courses, and commercial turf. Its widespread use makes its specific effects the subject of considerable attention. This includes ongoing observations on possible off-target harm to humans or ecosystems as well as the monitoring of resistance development. *(*Maddison*, Jill E.et.al.,(2008)).*

Broad spectrum pesticides will kill insects indiscriminately, without regard to the species. These type of pesticides include most neonicotinoid, organophosphate, pyrethroid and carbamate insecticides and are identified on the labels of all commercial pesticides. Some broad spectrum pesticides, such as achlorpyrifos, can be effective to use in selectively targeting pests when used in moderation. It's important to consider the impact of using a broad spectrum pesticide on the natural enemies of beneficial insects. If more natural enemies survive, they will help control nuisance species later in the season and limit the amount of pesticides that need to be reapplied.

Fipronil has been shown to enter theaquatic environment from agricultural runoff or drift from aerial or ground based spraying applications where they may pose threat to nontarget organism including fishes (Gupta *et al*. 2012).

Studies on Lethal toxicity

The stock solution of the toxicant was prepared in 100% pure acetone and 0.1mg/L, concentration of Fipronil was taken, the control group were maintained for each experiment and added 100% pure quality of acetone equal to the toxicant concentration which was used in the test. Precaution was taken to minimize the acetone as solvent and 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 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 adding with pure acetone and 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% to 96% for 4days (96hrs)in static renewal systems. Finney Probit analysis (Finne.1971) as recorded by 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).

The data was subjected to the following statistical equations for at LC_{50} values.

$$\text{Log } LC_{50} = \frac{\text{Log } A + 50 - a}{b - a \text{ Log}^2}$$

Where:
A = Concentration of pesticide at 50% mortality
a = Percent kill just below 50% mortality
b = Percent kill just above 50% mortality

RESULTS AND DISCUSSION

Aquatic organisms are continually being exposed to various pollutants in the environment. 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 may be defined in terms of a measurement as acute toxicity. Pesticides are entering into aquatic ecosystem by agriculture runoff from land, impairing the quality of the water and making it unfavorable for aquatic life (Tilak *et al*., 2009).

Toxicity is relative property of a chemical which refers to its potential to have harmful effects on living organisms. It is a

function of the concentration of the toxicant and duration of exposure. The acute toxicity tests were conducted by earlier authors for Fipronil 5% SC pesticide for different species of fish. The toxicity tests provide a measure of the toxicity of compounds to a given species under specific environmental conditions (water quality, pH and temperature etc). (Ganeshwade *et al*., 2012), 96h LC_{50} value of Endosulfan to the fresh water fish *Channastriatus* as 0.0035ppm. (Nikam *et al.,* 2011), the 96h LC_{50} value of metasystox to the freshwater fish Nemacheilusbotia is 7.018 ppm.

Behavior of the animal can serve as the link between physiological and ecological processes; it may be used for studying environmental pollutant effects (Graham and Sloman, 2004). Lethal effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction and survival. Nwani *et al.,* (2010) reported that median lethal concentration of chlorpyrifos based pesticide. Termifos to African catfish *clariasgariepinus* were found to 0.861 mg l^{-1}. The LC_{50}values of chloranthraniliprole in the fish grass carp found to 11.008mg/l.0000

Short-term bioassay data are an 'early warning' in predicting acute poisoning in the field; they can be used to predict the toxicities of mixtures and they can also serve to prognoses effects in various physico-chemical conditions. Information generated from various toxicity tests can be of use in the management of pollution for different purpose like prediction of environmental damage of waste, comparison of various toxicants, animals or test conditions and regulation of waste discharge. Fish are adapted for aquatic respiration, during which they take water in, through the mouth and passed through gill chambers covered by the operculum. The flow of water is continuous for almost the whole of the respiratory cycle. In its passage, the water gives up oxygen to the blood and takes away the carbondioxide through diffusion. The process of oxygen is transported in the circulating fluid by haemoglobin present in the blood corpuscles.

In the present study the observed percent mortality along with exposure concentration of Fipronil 5%SC for 96hr to the fish, *Catla catla*in static renewal bioassay are given in TableII and Fig .I respectively. The reported LC_{50} values are given in Table II and Fig.2.respectively. The results of the LC_{50}(Lethal Concentration) of the present study at 96hr were found to be 0.23mg/l for Fipronil 5%SC, results according to Finney Probit analysis the lower bound and upper bond 95% lethal confidence limits for Fipronil 5%SC 0.23mg/l given in Table no:1. The percent mortality and probit mortality increased with the increasing concentration of toxicant. The percent mortality plotted against log concentration of Fipronil 5%SC gave dose response curve. The 96hr LC_{50} of Fipronil 5%SC was obtained by taking the mean LC_{50} derived from the percent and probit mortality curves.

In the present study observed that the fish *Catla catla* is less resistant to broad spectrum insecticide Fipronil 5%SC to LC_{50} 0.23 mg/l the results were compared with the other researcher, and the result were compared with other researchers. Mathivanan, (2004) have estimated LC_{50} value as 4ppm for organophosphate quinolphosexposed to *Oreochromismossambicus.* Synthetic pyrethroid lambda cyhalothrin for 24, 48, 72, 96 hr were 0.0026, 0.0024, 0.0022 and 0.0021 ppm, to fish *Labeo rohita* (Kumar. A and Sharma.B *et.al*., (2010)). The LC_{50} value of malathion an organophosphate pesticide to freshwater fish was found to 9.0 µl l^{-1}, Patil and David, (2008). Bilal Ahmad Bhat (2012) reported that the 96hr LC_{50} values of botanical pesticideKethrin and an Organophophate pesticide Dichlovos was found to be 21.68 ppm and 16.71 ppm respectively to the freshwater fish *Labeo rohita*.

Table 1 LC_{50} values with 95% confidence limits for Fipronil based on dissolved concentrations estimated according to (Finney Probit method, 1971)

S.No	Concentration	Exposed fish	%mortality	Log of toxicant concentration	95% Confidence limits
1	0.23mg/l	10	50	-0.6382	0.1900-0.2700

Table II The 96 hracute toxicity of Fipronil on freshwater fish, *Catla catla* percent mortality and probit mortality

S. No	Conc. mg/L	Log Conc.	No. of fish exposed(N)	No. of fish alive	No. of fish died	Percent mortality	Probit mortality
1	0.17	-0.7695	10	10	0	0	-
2	0.19	-0.7212	10	9	10	10	3.72
3	0.21	-0.6777	10	7	30	30	4.48
4	0.23	-0.6382	10	5	50	50	5.00
5	0.25	-0.6020	10	2	70	70	5.84
6	0.27	-0.5686	10	1	90	90	6.28
7	0.29	-0.5376	10	0	100	100	8.09

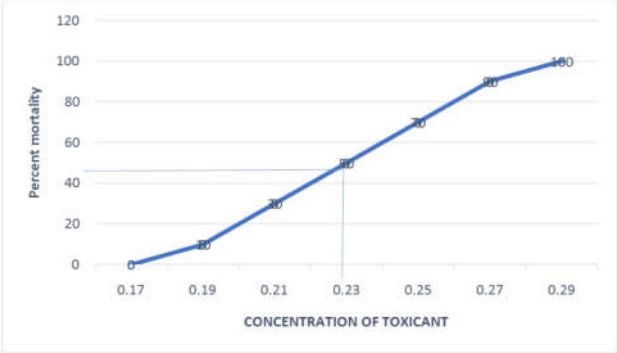

Figure 1 The graph showing dose response curve between percent mortality against concentration of toxicant in freshwater fish, *Catla catla*exposed to Fipronil

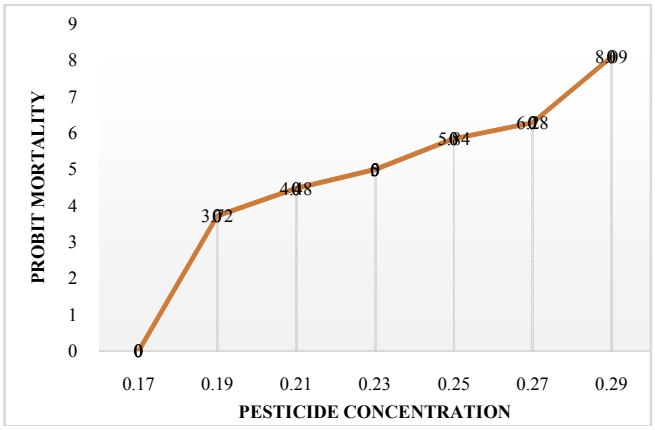

Figure 2 The graph showing dose response curve between probit mortality against Pesticide concentration in freshwater fish, *Catla catla* exposed to Fipronil

Behavioural Changes of the Fish in Toxic Environment

The behavioural 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 behaviour or escape from predator, agonistic and comfort behaviour like coughing, chasing, nipping, biting, vacating, flicking, etc., and respiratory behaviour like rate of opercular movement etc., (Veeraiah and Durga Prasad, 2001).

In the present investigation, during the course of exposure of fish to lethal concentration of Fipronil 5% SC for 96hr 0.23mg/l, several behavioural changes were observed which, include erratic swimming movements and they appeared to be 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* in the present study. 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 (Tilak *et al*., 2001).

Gulping air and swimming at the water surface (surfacing phenomenon) were observed also with mucus secretion on the body in the exposure periods. (Rao JV. 2006, Shivakumari.R, *et. al*., 2005) reported that fish in sub lethal concentration were found under stress but that was not fatal. David.M *et. al*., 2005 reported that the abnormal changes in the fish exposed to lethal concentration cypermethrin are time dependent. 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 (David.M, *et. al*., 2005).

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 (V.VenkataRatnamma and Nagaraju, 2013). The toxic stress of pesticides has direct after on tissue chemical compounds (Tilak and Yacobu, 2002). Chaudhary*, et.al,* (2000). 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.

CONCLUSION

In the present investigation 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 in static tests continuous for different hours and different concentrations were shown in (Table No. II). Control and experimental groups in response to insecticide Fipronil in presently studied fish *Catla catla* (Table no.II) confirm that Toxicity evaluation(LC_{50}values) and behavioural changes in fishes are very sensitive indicators under toxicity of chemicals. The behavioural changes affecting the general health status of the fish.

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