



TOXICITY EVALUATION AND BEHAVIOURAL STUDIES OF LABEO ROHITA AND CTENOPHARYNGDON IDELLA INDUCED FIPRONIL 5%SC

CH.ANITHA SMRUTHI, V.LALITHA, K. RAVIBABU and Dr. V.VENKATA RATHNAMMA

Department of Zoology & Aquaculture Acharya Nagarjuna University

ABSTRACT:

The economic importance of aquatic animals and pesticides for the betterment of the human beings is obviously known and tested. These pesticides behave as pollutants, when viewed for the ecosystem, environment and 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 fipronil pesticide on Indian major carp (*Labeorohita*) and Chinese major carp (*Ctenopharyngdonidella*) for the 96-h LC_{50} value were found 1.32 and 1.5mg/l, and observed the behavioural studies.

Keywords: Fipronil, *Labeorohita*, *Ctenopharyngdonidella*, LC_{50} , and Behavioural studies.

I.INTRODUCTION:

The use of chemical pesticides has become critically important to assure both quality and productivity of agricultural products [Oerke EC and Dehne HW,2004; Hayasaka D *et al.*,2012] and to control household pests such as termites, fireants, and mosquitoes [Drees BM,2014]. However, pesticides also have negative effects on non-target organisms [Clasen B *et al.*,2014; Krupke CH, and Long EY 2015], which may 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 [Laboy-Nieves EN, *et al.*,2009, Hayasaka D *et al.*,2012].

The majority of ecotoxicological studies neglect their sub-lethal effects [Shaw JR *et al.*,2008; Abbott LC *et al.*,2013] and focus on selected model species, such as daphnia *Daphnia magna* and zebrafish *Danio rerio* [Hayasaka D *et al.*,2012; Dai YJ *et al.*,2014]. Here, we present the results of experimental studies on the effects of fipronil (5-amino-1-[2, 6-dichloro4-(trifluoromethyl) phenyl]-4[(trifluoromethyl) sulfinyl]- 1H- pyrazole-3-carbonitrile).

Phenylpyrazoles (including fipronil) and neonicotinoids (including imidacloprid) are applied in a large scale, such as protecting plants from agricultural pests, controlling household pests, and controlling parasites on domesticated animals [Pisa LW *et al.*,2015]. Presently, they account for approximately one third of the world insecticide market [Simon-Delso N *et al.*,2015]. Although fipronil operate by disrupting neural transmission in the central nervous system of invertebrates [Simon-Delso N *et al.*,2015], This product has a different mode of action. Fipronil interferes with the passage of chloride ions by binding to a specific site within the gamma-aminobutyric acid (GABA) receptor, [Gunasekara AS *et al.*,2007;Mortensen SR *et al.*,2015]. Compared to other types of insecticides, fipronil are considered safer because of their low toxicity on fish and mammals. However, fipronil are very effective on arthropods in small concentrations [Overmyer JP *et al.*,2005]. Their increased use in recent years [Pisa LW *et al.*,2015; Simon-Delso N *et al.*,2015; Overmyer JP *et al.*,2005], moderate to high solubility [Raby M *et al.*,2018], and persistence in water [Tisler T *et al.*, 2009; McMahan RL *et al.*,2016] pose a serious concern regarding the potential adverse impacts on non-target aquatic invertebrates and vertebrates.

Fipronil in the environment has been reported in the U.S. and other parts of the world, and its environmental concentrations can be as high as 10.004 µg/L [Hayasaka D *et al.*, 2012, Mize SV *et al.*,2008; Ruby A *et al.*,2013]. Many of these studies reported the detection of fipronil and imidacloprid, or one or more of their degradation products, in aquatic environments exceeding their chronic levels of the U.S. EPA aquatic life benchmark for invertebrates (0.01 µg/L for insecticides) [USEPA, 2017].

In recent years, many studies have investigated the potential adverse effects of these insecticides on non-target organisms; however, the majority of these studies have focused on a limited number of commercially beneficial terrestrial invertebrates or on selected model organisms in aquatic ecosystems. For example, Pisa, *et al.*, 2015 found that, of 376 papers reviewed, the majority focused on the effects of fipronil and neonicotinoids on honeybees, and very few studied aquatic invertebrates, particularly marine species.

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. 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 objective of this paper is to highlight how to reduce the impact of pesticides on aquatic animals, as to save the bio diversity and aquatic environment so as to reduce the harmful effects on animals and human beings, as well as to save the economy of nation, which is important for development of country. Thus, these compounds do not usually present as serious problem as the earlier insecticides. Ideally pesticides should be highly selective, destroying target organisms while leaving non- target organisms unharmed (Ernest Hodgson, 2010).

The toxicity evaluation and behavioral changes in fishes are very sensitive indicators under the toxicity of chemicals (Anithasmruthich *et al.*, 2018). Present study *Labeo rohita* and *Ctenopharyngdon idella* fresh water fishes exposed to (Fipronil 5%SC insecticide) 96hrs, to evaluate lethal concentration 50% mortality of the freshwater fish *Labeo rohita* and *Ctenopharyngdon idella* all the experiments made along with controls.

II. Materials and methods:

II. 1. Animal selected:

Labeo rohita and *Ctenopharyngdon Idella* is an edible freshwater fishes occurring abundantly in the freshwater bodies, rivers, lakes and ponds in India (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.

II. 2. Biology of *Labeo rohita* and *Ctenopharyngdon idella*:

Labeo rohita and *Ctenopharyngdon idella* are belongs to the family Cyprinidae,. *Labeo rohita* is commonly known as 'rohu'. It is most valuable edible fish. *Labeo rohita* is the most famous Indian major carp, easily found rivers in South Asia. It is large omnivore and extensively used in aquaculture. The rohu 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 (Dahunukar, 2010 and Froese *et al.*, 2013). This fish *Labeo rohita* 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.

Ctenopharyngdon Idella is commonly called grass carp. It is a native Chinese freshwater fish with a broad distribution from the catchment area of the pearl River in southern china to that of the Heilongjiang River in northern china. It inhabits lakes, rivers and reservoirs. Grass carp is a basically herbivorous fish that naturally feeds on certain aquatic weeds. It is one of the largest numbers of family Cyprinidae.

II. 3. Procurement and maintenance of fish:

Healthy freshwater fish, *Labeo rohita* and *Ctenopharyngodon idella* (Hamilton) 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 $28\pm 2^{\circ}\text{C}$. 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.

II. 4. 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 *Vet.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, *et.al.*, 2008).

Broad spectrum pesticides will kill insects indiscriminately, without regard to the species. These types 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 the aquatic 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).

II. Studies on Lethal toxicity:

The stock solution of the toxicant was prepared in 100% pure acetone and 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 LC₅₀) of test toxicants for the freshwater fish, *Ctenopharyngodon Idella* 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% for 4days (96hrs) in static renewal systems. Finney Probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate the median lethal concentration (LC₅₀) 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 LC₅₀ 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

III. Results and discussion:

Pesticides are entering into aquatic ecosystem by agriculture runoff from land, impairing the quality of the water and making it unfavorable for aquatic life (Tilaket *et al.*, 2009). 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 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.

Behavior of the animal can serve as the link between physiological and ecological processes; it might 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.

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 carbon dioxide through diffusion. The process of oxygen is transported in the circulating fluid by haemoglobin present in the blood corpuscles.

Analysis of biochemical parameters could help to identify target organs of toxicity as well as general health status of animals and there is a need for information from the physiological angle. Incidentally, a great deal of attention has been focused to evaluate the hazardous effects of various pesticides on physiology of many non-target organisms (McKim and Lein, 2001; Al-Kahtani, 2011; Christelle Adam-Guillermin, 2012), concluded that changes in physiological and behavioral activity might serve as indicators of estimating the level of sub-lethal effects of pollutants. The effects of pesticides on fish are extensively studied (Scott, 2004; Surendra Kumar, 2010; Caceres *et al.*, 2010; Sandal and Yilmaz, 2011).

The nominal 96-h LC₅₀ of Fipronil for brown shrimp was 0.12 µg/L (0.06–0.24). This suggests brown shrimp is one of the most sensitive crustaceans to fipronil among all aquatic invertebrates studied so far. This 96-h LC₅₀ for brown shrimp is less than that for estuarine mysid shrimp *Neomysis americana* LC₅₀ = 0.14 µg/L Gan, *et al.*, 2012. Other sensitive marine invertebrates include estuarine grass shrimp *Palaemonetes pugio* with 96-h LC₅₀ of 0.32 µg/L [Baines D *et al.*, 2017], estuarine copepod *Amphiascustenuiremis* with 96-h LC₅₀ of 6.8 µg/L [Chandler GT *et al.*, 2004],

Fipronil is known to cause lethal and sub-lethal effects on non-target invertebrates in both aquatic and terrestrial ecosystems (Pisa, L. W. *et al.*, 2015). The acute toxicity was evaluated and 96 hr LC_{50} was 3.0 mg/L. Further, two sub-lethal concentrations (0.5 mg/L and 1.0 mg/L) were selected for the assessment of behavioural toxicity and protein metabolism investigations. The duration of exposure selected for each sublethal concentration was 7 and 14 days. Changes in behavioural responses were noticed in fish exposed to FPN and found to affect the fish in dose and duration dependent pattern (Zabin SB *et al.*, 2018).

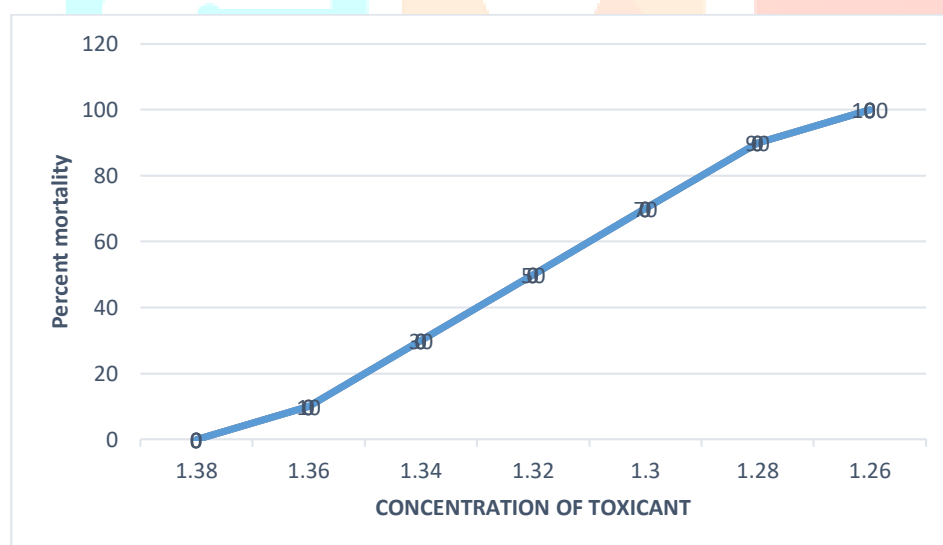
The unrestricted, heavy use of synthetic pesticides results in a lethal effect on various non-target organisms in the aquatic environment and direct or indirect effect to users (Al-Ghanim *et al.*, 2020; Sathyamoorthi A *et al.*, 2019; Kumaresan V *et al.*, 2019). Toxicity is relative property of a chemical 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 freshwater fish *Channa striatus* as 0.0035ppm. The 96h LC_{50} value of metasyntax to the freshwater fish *Nemacheilus botia* is 7.018 ppm (Nikam *et al.*, 2011).

The present *Labeo rohita* and *Ctenopharyngodon idella* in static renewal bioassay are given in Table I and II, Fig 1, II, III and IV 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 1.32 and 1.5mg/l for Fipronil 5%SC results according to Finney Probit analysis. The percent mortality and probit mortality increased with the increasing concentration of toxicant and the percent mortality plotted against log concentration of Fipronil 5%SC gave dose response curve.

In the present study observed that the fish *Labeo rohita* and *Ctenopharyngodon idella*. Fipronil 5%SC to LC_{50} 1.32 and 1.5 mg/l the results were compared with the other researcher, and the result were compared with other researchers. 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. *et al.*, (2010)). The LC_{50} value of malathion an organophosphate pesticide to freshwater fish was found to $9.0 \mu l l^{-1}$, Patil and David, (2008). Bilal Ahmad Bhat (2012) the 96hr LC_{50} values of botanical pesticide Kethrin and an Organophosphate pesticide Dichlofos was 21.68 ppm and 16.71 ppm respectively to the freshwater fish *Labeo rohita*.

Table: I. The 96 hr acute toxicity of Fipronil on freshwater fish, *Labeo rohita* 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	1.38	0.1398	10	10	0	0	-
2	1.36	0.1335	10	9	10	10	3.72
3	1.34	0.1271	10	7	30	30	4.48
4	1.32	0.1205	10	5	50	50	5.00
5	1.30	0.1139	10	2	70	70	5.84
6	1.28	0.1072	10	1	90	90	6.28
7	1.26	0.1003	10	0	100	100	8.09



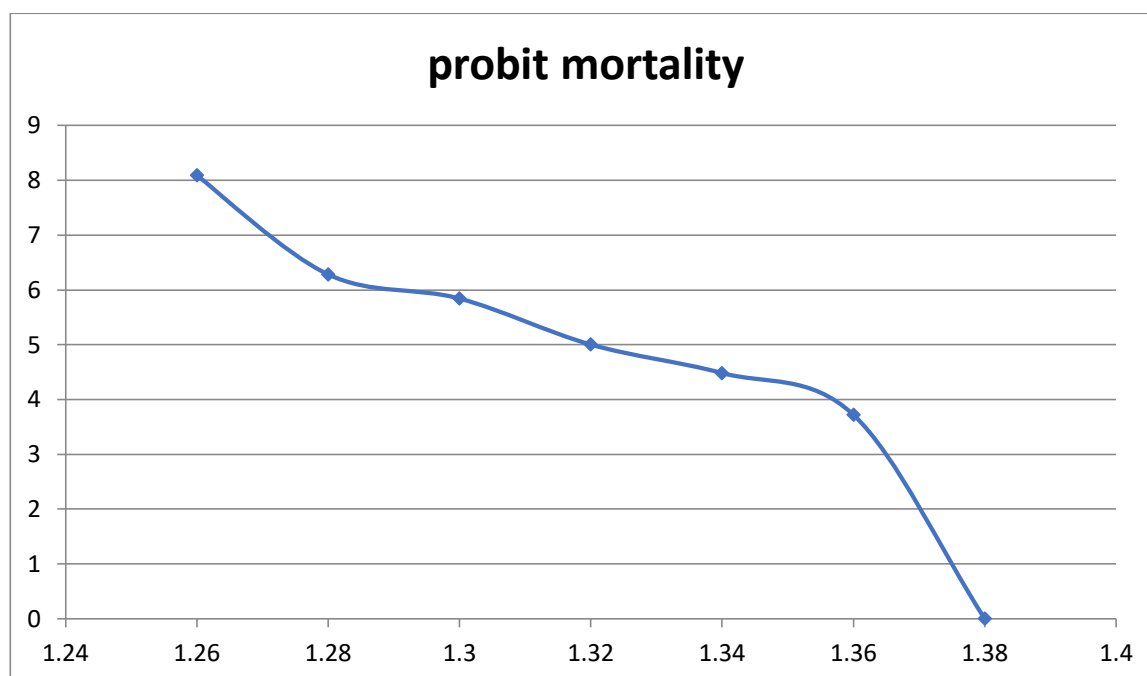
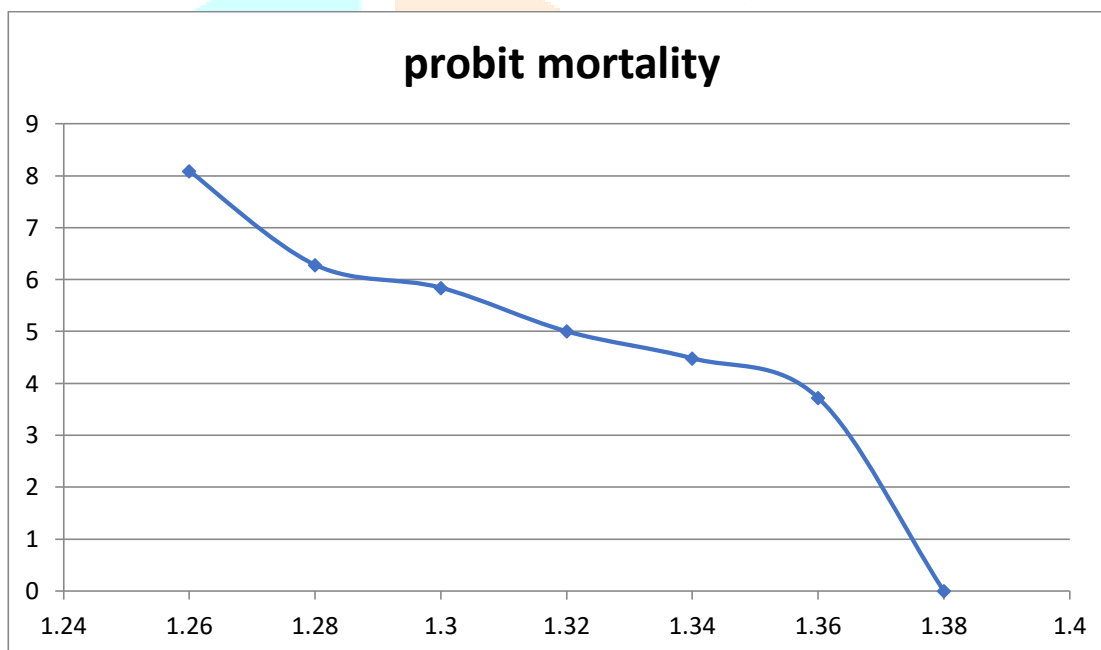
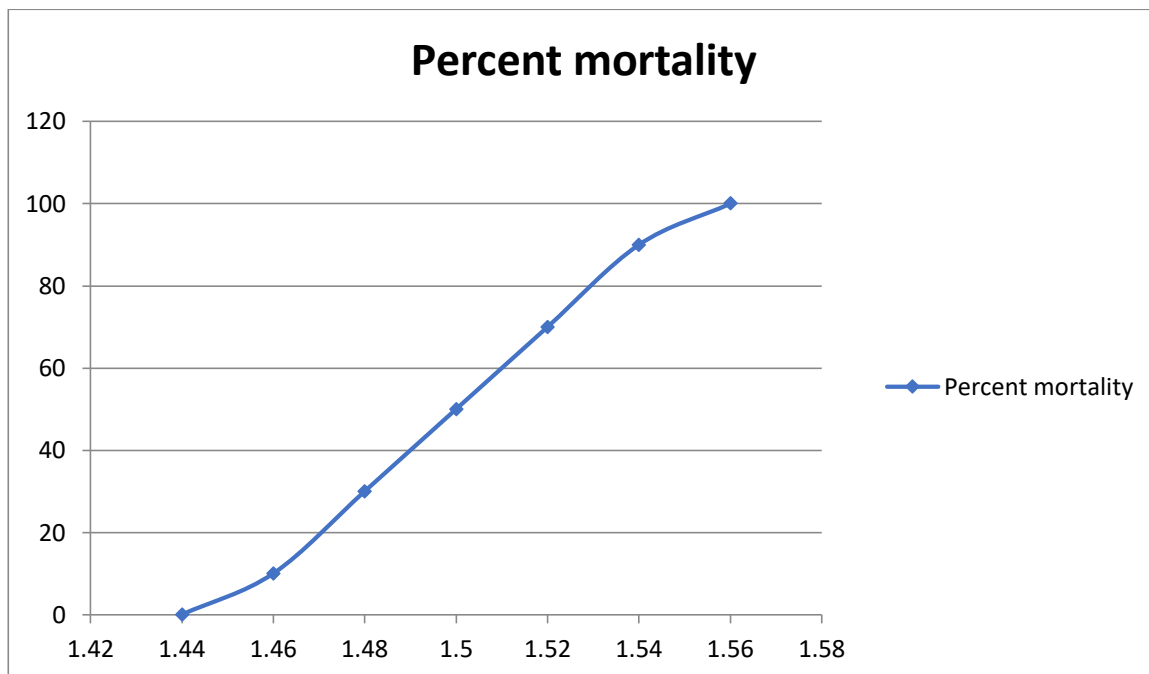


Table: II. The 96 hr acute toxicity of Fipronil on freshwater fish, *Ctenopharyngodon idella* percent mortality and probit mortality:

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	1.44	0.1583	10	10	0	0	-
2	1.46	0.1643	10	9	10	10	3.72
3	1.48	0.1702	10	7	30	30	4.48
4	1.5	0.1760	10	5	50	50	5.00
5	1.52	0.1818	10	2	70	70	5.84
6	1.54	0.1875	10	1	90	90	6.28
7	1.56	0.1931	10	0	100	100	8.09



II. 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).

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 (Bélanger-Deschênes *et al.*, 2013; Ozioloret *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 (Reid *et al.*

al., 2016; 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. For instance, brown bullhead fish *Ameiurus nebulosus* from a polluted river had a higher aggressiveness than fish from an unpolluted river, but only F₀ fish collected in the field were tested (Breckels and Neff, 2010).

In the present investigation, during the course of exposure of fishes to lethal concentration of Fipronil 5% SC for 96hr 1.32 and 1.5mg/l, several behavioural 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 *Labeo rohita* and *Ctenopharyngodon idella*. 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; Anitha, 2015; Nirmala, 2015).

Present study *Labeo rohita* and *Ctenopharyngodon idella* 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. (Rao JV. 2006, Shivakumari.R, *et al.*, 2005) 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 LC₅₀ 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 *Labeo rohita* and *Ctenopharyngodon idella*, 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. The deleterious effects of 4-NP on central nervous system as well as neuroendocrine homeostasis and cognitive functions (Jie X *et al.*, 2013). Inhibition of AChE activity due to the exposure of 4-NP activity in *Mytilus galloprovincialis* (Vidal-Linan L *et al.*, 2015). So, the abnormal behavior shown by fish might be due to

abnormal level of neurotransmitters. These changes occur much earlier than mortality. 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 (Rao JV, 2006) and also inhibit the diffusion of oxygen during gaseous exchange.

IV. CONCLUSION:

In the present investigation the test species, *Labeo rohita* and *Ctenopharyngodon idella* 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 *Labeo rohita* and *Ctenopharyngodon idella* for Fipronil in static tests continuous for different hours and different concentrations were shown in Table No. I and II. Control and experimental groups in response to insecticide Fipronil in presently studied fish *Labeo rohita* and *Ctenopharyngodon idella* confirm that Toxicity evaluation (LC₅₀ 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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