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ACUTE TOXICITY DETERMINATION OF CYFLUTHRIN TECHNICAL GRADE AND 10% WP, TO THE FISH CATLA CATLA, AS A BIOMARKER STUDY

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Abstract

The acute toxicity studies are conducted for the fish *Catla catla*, (one of the major carp and also cultivable one) in the laboratory in vivo, for the size range 3-5 cm length and 3-5 gm weight using Cyfluthrin, a synthetic pyrethroid with a cyanogroup, categorized as type II for both technical grade as well as its 10% WP which is the commercial formulation and the farmers use in agricultural practices and also for domestic purpose to maintain hygienic conditions. The toxicity tests are done by employing both the static renewal (SR) and continuous flow through system (CFTS). The static renewal values for 24, 48, 72 and 96 for technical grade Cyfluthrin are 5.994 µg/L, 5.812 µg/L, 5.593 µg/L, and 5.193 µg/L, respectively, similarly for 10% WP, it is 3.045 µg/L, 2.696µg/L, 2.596 µg/L, and 2.496 µg/L respectively for 24, 48, 72 and 96 h. Similarly for CFTS, in technical grade, the values are 3.087 µg/L, 2.950 µg/L, 2.384 µg/L, 2.260 µg/L for 24, 48, 72 and 96 h respectively and 10% WP the values are 1.933 µg/L, 1.757 µg/L, 1.603 µg/L, 1.365 µg/L respectively for 24, 48, 72 and 96 hrs. It is less toxic than group II synthetic pyrethroid with cyanogroup (Deltamethrin, Cypermethrin and Fenvalerate) but also toxic and its commercial formulation is also sufficiently toxic for the fish. The results of the present toxicant, Cyfluthrin and its formulation are going to be discussed with the work carried earlier and a comparison with others. The data is useful for consideration of permissible concentrations limits of the environmental policy and planning

Key Words: *Catla catla*, Cyfluthrin, 10% WP, synthetic pyrethroid, cyanogroup, Type II, Acute toxicity, static renewal (SR), continuous flowthrough system (CFTS).

INTRODUCTION

Toxicity is the effect of any chemical either on target organisms (pests), or non-target organisms, fishes, especially (in the aquatic environment, the fish is a good model of study). As the effect, the concentration will be the factor for its action adversely, which is time (duration) or concentration (level) of its presence in the environment, air/water. As the earth's subdivision, the hydrosphere is the largest, the first evolved vertebrates with closed circulatory system, the poikilothermic nektonic fish when evaluated for its toxic action, the concentrations are referred as either chromic or acute levels and such actions are either chronic or acute toxicity effects and the same are also either sub-lethal or lethal in terms of mortality respectively.

For any of the above to measure of the toxicity evaluation needs to follow certain norms and finally one can arrive to such concentrations that can be either chronic or acute to have its effect.

We know the population, is ever increasing and need for the required mouthful to be available by controlling the insect pests or weeds to increase the yield especially agricultural produce and to certain extent even aquaculture practices too. We have after revolution of independence, the green, blue and white to provide the required food for the human beings and cattle.

First and foremost is the spraying of chemicals to combat pests (target organism) and when these find their presence in any aquatic body (non-target organism) got affected.

Isra Mahamood *et al.* (2016) in their study report mentioned that a sufficient amount of pesticides are sprayed for mitigation of the damage caused by insect pests, but the effects they cause as toxic nature, surpassed. Rajamohan *et al.* (2020) reported on the effect of biota (flora and fauna), while presuming that several pesticides are going to be formulated and also are going to have impacts, on bio-diversity. This is what Kelly Macnamara (2021) and Tang *et al.* (2021) who also warned in the similar alarming lines that the globe is facing and going to face the pesticidal residues problem in the environment. After the book of silent spring, by Rachel Carson (1962) doors are opened to have the awareness of the chemicals particularly, the pesticides that are going to have 'havoc' impacts in the environment. After that the toxicity evaluation started pick-up in the research frontlines to study, the toxic action of the toxicants and pesticides are no doubt the toxicants.

The pioneering work of Doudroff *et al.* (1951) brought into the limelight of experiments that are carried in the laboratory for any toxicants as experimental protocols. Later Anon (1975), American Public Health Association in the revised additions of 20, 21 & 22 (1998, 2005 and 2012) and finally by Organisation for Economic Cooperation and Development (OECD, 2019) who all methods of toxicity evaluation.

The evaluation of a drastic effect as lethal (death/mortality) is so designed to determine the dose/concentrations of the chemical, toxicants (as all toxicants are pollutants whereas all the pollutants are not toxicants), and the time must be such that must have/had the criteria as effect (the death/mortality). It is nothing but to measure the response of the testing organism or groups of organisms. They are referred as 'Bio-assays'. It is defined by OECD (2019) 'as a test' which is designed to evaluate the relative potency of a chemical by comparing its effect on the living organisms, with that of standard preparation. By evaluation of such one can have a scientific basis, to be accepted by the scientific community, apart from that it must be easy and economical to have a realistic and sensitive aspect to measure the effect and only such data should be useful for the risk assessment.

They also reported about the mention of the candidate species for testing, size range and the physico-chemical parameters of the water that was used for the test. By taking all into the consideration the cultivable fish species *Catla catla*, with a new generation synthetic pyrethroid group chemical, Cyfluthrin is selected for the study in the prevailing water conditions as mentioned in the material methods.

American Public Health association in three different years of 2012, 2008 and 1998 recommended four different methods of the evaluation of the toxicity viz., static, static renewal, recirculation and continuous flow through systems. But in the present study both static renewal (SR) and continuous flow through system (CFTS) are both followed.

MATERIAL AND METHODS

Test species: *Catla catla*, the fresh water major carp organism selected for the study is from fish farms where it is cultured along with others. It has a length of 3-5 cm and also weight of 3 to 5 gms, considered as fingerlings.

Prior to the experimentation, the test organisms are made to acclimatize the laboratory conditions of the testing laboratory in the plastic tubs which had the ground water (pumped) and feeding them also for about two weeks of the temperature 28±2^{o}C that is prevailing. The feeding was with groundnut cake only prior to the experimentation of the specific durations and then it was stopped all that was also given by the recommendations of APHA (2012, 2008 and 1995) as well as by OECD (2019), but while in the process if the mortality exceeded 5%, the entire fish brought for the test as a batch was discarded and only acclimatized healthy fish are selected for the experiment.

Toxicants for the tests are from

The technical grade Cyfluthrin is obtained from the manufacturers, a multinational company by name Bayer House, Central Avenue, Hiranandani Estate, 400 607, Thane (W), Maharashtra, India, and its formulation 10% wettable powder (WP) is purchased from the locally available pesticide sellers that has the product of similar Bayer company which had made the technical grade.

Stock Solutions Preparation

By using acetone (Analytic grade) as a solvent stock as well as working test solutions of different concentrations are made and further diluted with water only and are taken as in $\mu g/L^{-1}$. The toxicants are dissolved in acetone and further working one are prepared only of the required quantity. For each set of experiment, the fish and water without the toxicant (but using only the highest quantity of the solvent) as control which was also kept. The dilution factor avoids so that it has reduction of the solvent acetone (OECD, 2019).

Test Conditions

The physico-chemical parameters of the water used to acclimatize and same water is used for experimentation. They are viz., Turbidity – 8 silica units, Electrical conductivity at 28°C – 816 Micro ohms/cm, pH at 28°C – 8.1, Alkalinity: Phenolphthalein – Nil, Alkalinity: Methyl orange – 472, Total Hardness (as $CaCO_3$) – 232, Carbonate Hardness (as $CaCO_3$) - 232, Carbonate Hardness (as $CaCO_3$) – 232, Non-Carbonate Hardness (as $CaCO_3$) - Nil, Calcium Hardness (as $CaCO_3$) – 52, Magnesium Hardness – 40, Nitrite Nitrogen (as N) – Nil, Sulphate (as SO_4^{2-}) – Trace, Chloride (as Cl^-) – 40, Fluoride (as F^-) 1.8, Iron (as Fe) – Nil, Dissolved Oxygen – 8-10 ppm, Temperature - 28±2°C. All the evaluating aspects of procedure and precautions given by the American Public Health Association [1998, 2005 & 2012 and OECD (2019)] for conducting the same to determine the LC_{50} values for 24, 48, 72 and 96 hrs are followed. First pilot experiments are conducted to infer of range of the toxicant that is effective. In each set of experimentation 10 litres is the maximum of the media. For this 24 litre capacity containers are only used for SR and for CFTS a flow rate of 4 litres in 60 minutes that was made by using poly-ethylene drip sets with regulators from a container of 24 litre capacity (@ 4 litres per hour) – i.e., fresh test solution of the same concentration is prepared for every 6 hrs in the reservoir.

The two methods of toxicity evaluation to determine the concentration of the toxic action of Cyfluthrin by taking technical grade as well as 10% WP, while in the experimentation mortality is recorded. As per the recommendations of the APHA (1998, 2005, 2012) the dead fish are removed as and when it was resulted. For both as toxicants, (TG and 10% WP a comparative study is made to determine the lethal concentrations for 24, 48, 72 and 96 hrs).

To calculate the LC_{50} value probit analysis by Finney (1971) that was recommended by Roberts and Boyce (1972) was employed. From tables of Fisher and Yates (1938), Probit values are drawn apart from using 1.96 as a normal variant is also used.

The data obtained so, was by statistical equation of the following:

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

Where:

A : Concentration of pesticide at 50% Mortality.
a = Per cent kill just < 50% mortality
b = Per cent kill just > 50% mortality

By using the following all the other parameters are also arranged.

$$S = \frac{\frac{LC_{84}}{LC_{50}} + \frac{LC_{50}}{LC_{16}}}{\text{Divided by two (whole)}}$$

Confidential

$$\text{Limits as F} = \text{antilog} \frac{277 \log S}{\pi N} = S^{2.77} / \sqrt{N}$$

Where N is 10, (the number of animals that are tested in the present case) and 100-A and 100-B, A=84, B=16, whose expected effects are between.

Upper confidence limit = LC_{50}X f (Calculated as earlier)
Lower confidence limit = LC_{50} / f (Calculated as earlier)

Further, the data is also processed by the Probit analysis and the computer generated as the output is taken which had given not only the LC_{50} values but also confidential limits on upper and lower (fiducial intervals) apart from equation of the regression and Rf values, Finney (1952) method and finally proved that is a good fit.

RESULTS

Table 1 is appended that infer the LC_{50} values for 24, 48, 72 and 96 hrs for both the methods that are employed as well as the toxicants selected.

Having the statistical validity, the data are also presented as figures 1 to 16 for 24, 48, 72 and 96 hrs of the toxicants in the two different types of the tests and proved along with the data and found that not only a good fit but yielded other aspects also.

The data of the present result can be inferred as following:

(1) The 10% WP, the commercial formulation that was used by all farmers in agricultural practices is also sufficiently toxic, when compared with technical grade (98%).

(2) The possible explanation, that can be for the toxic action, the ingredients that are mixed in it sufficiently impart effect also, and as a sort of cumulative or additive way.

(3) When the range of the sensitivity of the two, viz., technical grade and 10% WP varied 0.2 ml of the solvent of the solute (toxicant) for technical whereas 0.1 ml of the solvent of the solute (toxicant) for WP.

(4) The static renewal test of the method values for the determined duration of 24, 48, 72 and 96 hrs are higher to CFTS values method, by the fish, all that culminate and had more toxic action whereas in the later test method, which almost simulate the natural conditions and because of it only lower values.

(5) The earlier studies resulted that the toxicity is specific to different fish also duration of the exposure dependent even and the present study it is not an exception. The values for the fish *Catla catla* with the two toxicants, viz., technical grade and static renewal for 24, 48, 72 and 96 h are for the first time reported by these two methods with Cyfluthrin.

DISCUSSION

The toxicity studies for the different synthetic pyrethroids were mentioned review articles of different authors in different times too Some of such reports were by Ahrar Khan *et al* (2012), Prusty *et al* (2015) and Sana Ullah *et al* (2019).

As per the data provided by http://epagov/ppp00001/science/efed_databases description.htm#ecotoxicity, the chromic data of toxicity for Cyfluthrin and other synthetic pyrethroids is appended in the table 2, for some different fish. The data that is generated, clearly infers that there are very limited studied in the toxicity of evaluation of the toxicant presently studied.

Thethys and Selvam (2013), in their study mentioned that the synthetic pyrethroid residue concentrations when exposed to birds, fish and bees (the information is appended in the table 3 and the Cyfluthrin is toxic to fish.

In the insecticide fact Sheet (1994) it was mentioned that like all other synthetic pyrethroids including the present tested toxicant are neurotoxic which cause hyper excitation of the central nervous system that can lead to convolutions and finally the terminal point death. At ion level it causes abnormal flow of sodium and potassium influx and outflux that cause repetition of discharges or it may block the nerve impulses. The important aspect that was mentioned in the report was that Cyfulthrin affects the calcium concentration apart from inhibiting the enzyme for its transport that finally increased the amount of, neuro-transmitter acetylcholine increased at the synoptinimal (SNC) junction. The report also had a mention of the cause of the convolutions due to inhibition of two inhibitors (gamma aminobutyric acid and benzodiazepine) and either of one is the causative aspect for the effect.

While mentioning that the present tested toxicant was highly toxic to fish, sheep head minnow 4.05 ppb, Rainbow trout 0.58 ppb blue gill fish 1.5 ppb and even in such low concentrations the organisms got affected. Taking this concept in view, Prusty *et al*. (2015); Ahrar Khan (2012) and Sana Ullah *et al.* (2019) elaborated the effects of synthetic pyrethroids.

At the prevailing concentration of the test method whatever is applied, according to Prusty *et al* (2015), the toxicant being the stomach poison blocks the channels of sodium of fibres of nerve fibers (Central nervous system, sympathetic and parasympathic – CNS and their respective PNS), which result in the transmission of the nerve impulse not in a normal way to complete the 'action potential' for which it is intended. The chemicals of the type I synthetic pyrethroids, not having a cyanogroup (Permethrin, Bifenthrin and Allothrin etc.) differ in the closure of the gates (sodium and potassium) whereas the type II with cyanogroup (Deltamethrin, Cypermethrin Cyfluthrin and Fenvalerate) modify the opening of the sodium gate (influx of Na^+ ions) which according to Soderlund (2010).

Even Narahasi (1986) and Bradbury and Coats (1989 a&b) mentioned that while sodium gate is opened drastically for influx by type II than type I, the toxic action prevail. Apart from this, they inhibit GABA and Ca^+ $^+$ binding site being inhibited which all result in delay in the nerve transmission as a result, the impulse too and finally cause the death of the target as well as non-target organisms.

Ahrar Khan *et al* (2012), while quoting Casida (1980) in their review article mentioned that type II group in which the Cyfluthrin also as a member, due to hypersensitive action and blocks the influx and outflux of the ions in the nerve cells of CNS, ANS, and PNS.

Sana Ullah *et al* (2019) about the biomarkers in the toxicity evaluation opined that due to its environmental representation, especially in the aquatic environment studies of different types can be as the indices of toxicity. They can be reproductive and endocrine disruptive toxicity, bimolecular toxicity, oxidative stress, neurotoxicity. The changes as alterations are due to the inhibition of AChE activity and developmental toxicity and all cumulative they effect the organism in its acute as well as chronic toxic levels. In their report, they mentioned that the environmental fate, microbial degradation, photo-degradation, volatilization and hydrolysis by any means of which they change and the persistence depends on it. The synthetic pyrethroids effect the fish because they do not have enzyme hydrolase and have only oxidative reaction whereas in mammals they have the enzyme hydrolase (Esterase) and do have oxidative reactions as well, hence only toxic to the fish. The authors illustrated the mechanism of action, including for Cyfluthrin, the present tested toxicant where neuro-toxic action prevailed Table 2 and 3 that are appended showed that the present toxicant is toxic. The article by Sana Ullah (2019) had

a reference of use of the Cyfluthrin in the most populous countries China, Mexico, Ghana, Brazil, Australia, USA, France apart from India.

Mayada *et al* (2021) reported that the use of the synthetic pyrethroids was on raise and over 100 times more poisonous for fish due to their increase of sensitivity of the toxic action that was acting as agents. In the present study, the sensitivity range of the technical grade is 0.2 ml whereas for 10% WP, it is only 0.1 ml.

The insecticides including pyrethroids due to intensive farming use them and due to by surface runoff and surface drainage sometimes combined both find their way to the aquatic environment do damage the fish stocks when concentrations are not monitored (Kalawati Kumar, 2020).

Abdul Bashir *et al* (2020) while reporting the toxicity of three pyrethroids of which one belong to cyhalothrin same as the present one belonging group of the fish *Poecilia reticulata* which is also commonly called as 'guppy' fish. The LC_{50} value was 81.83 µg/L of size range 2.13±0.27 cm and had made an imbalance of trophic structure in the water body, hence the pesticide usage must be monitored and regulated.

In *Danio rerio* (zebra fish) because of the toxic effect of the synthetic pyrethroid fenvalerate type II exposure of $1/10^{th}$ of 96h LC_{50} value had an impact on the enzymes that were important for growth of the fish. The present studied fish *Catla catla* is the cultivable one such exposures are not recommended (Ghanim *et al* 2020).

Anilava Kaviraja and Abhik Gupta (2014), reported in the fishes certain biomarkers of study for the synthetic pyrethroids. They included the toxicity evaluation, haematological, hyperglycemia, enzymes of energy, that resulted a stress of oxidative nature, disturbances of metabolism of nitrogen and also AChE. The genomics study effects and some other aspects (genomics, protenomics metabolimics) all and the specificity in the sodium channel interactions which were the biomarkers of the study.

Barbara and Michael (2020) reported that the toxic action was due to modulation of chloride chemicals and had a modulation effect of not only on it but also on calcium and potassium channels. All unilaterally cause the death and the same is true even in the present study.

Sandhya Kadiru (2018) studied the effect of Cyfluthrin in Zebra fish (*Danio rerio*). For embryos of the fish, the 96 h LC_{50} value was 3.443 µg/L (static test). But the present study is of both static renewal and continuous flow through system and the fish is different.

According to the earlier reports of Sana Ullah *et al*. (2019) and Prusty *et al*. (2015) pyrethroids effect the fish more because they do not have the enzyme hydrolase when compared with birds and mammals which have, the same. Much work is on the four synthetic pyrethroids of group II, Deltamethrin, Cypermethrin, Fenvalerate and Cyhalothrin but absolutely no mention of the work Cyfluthrin, in the report of the present studied toxicant.

However, by performing only static tests in the fish, *Gambusia affinis*, Mohammad Ghouse (2019) reported the values as 31.32 µg/L, 28.97 µg/L, 26.69 µg/L, and 24.73 µg/L, for 24, 48, 72 and 96 hrs respectively. The fish size was 2.3 to 3.2 cms and 0.7 and 1.5 gm as weight and the present studied size and weight, methodology and organism all are different.

Elke *et al*. (2018) in the different mode of study, one of the isomer of Cyfulthrin (beta) in the fish, rainbow trout the toxicokinetic and toxicodynamic (TKTD) aspect, the killing rate happened at 0,.01965 µg/L and can tolerate upto 2.159 µg/L concentrations such type of studies are not practiced in the countries like India when the data will be quite useful for prescribing the tolerance limits, in monitoring the pesticide pollution.

Chloe *et al*. (2017) while in a study on the fish, *Primephales promelas* (fathead minnow) and *Danio rerio* (zebra fish) reported both individual as well as mixture toxicity of two pesticides, phastebuprim and Cyfluthrin. The study provided the data that for Cyfluthrin, the present studied toxicant 5.127 µg/L for *Primephales promelas* and 15.643 µg/L for *Danio rerio.*

When the mixture is tested it is nearly 7 times lower in the toxic concentration. However, the present study is individual toxic study not the mixture study. Daniel *et al.* (2012) studied the effect of Cyfluthrin (β isomer) and reported for the fish *Bryconamerican iheringi* as 4.2 µg/L and 5.6 µg/L respectively for 24 and 48 hours LC_{50} values. Even though the size is the same of the present study, the fish and methodology are different.

Elif and Sedat (2011) in a comparative study of the two pyrethroids (Cyfluthrin and tetramethrin) in the fish, *Cyprinus carpio* reported that for Cyfluthrin 96 hrs LC_{50} values was 21.5 µg/L (toxicity test method was static, size range of the fish 16.4) and cannot be compared with the present study.

Aylin *et al*. (2009) reported that in the carp, *Cyprinus carpio* (19.7 ± 3.2 cm length and weight 100.9 ± 46.4 g) the 48 h LC_{50} value was 10 µg/L. In a cross reference, they mentioned that for *Oncorhynchus mykiss*, rainbow trout 0.68 µg/L 1.5 µg/L *Lepomis macrochinus* bluegill 22 µg/L in carp *Cyprinus carpio* 3.2 µg/L in *Leuciscus indicus* (golden orfe) as per the report of Benli (2005).

In the study of the fish *Oreochromis niloticus* the static test resulted 25.82 µg/L and 21 µg/L for 48, and 72 respectively. The toxicity values differ with size and hydrographical conditions of the prevailing test media (Benli, 2005) and temperature.

Selvi and Sarikaya (2007) reported for *Pochilia reticulate* (guppy fish), the LC_{50} values 8.07 µg/L in static method for the size of the fish, which they have selected. According to their opinion due to the lipo-philicity of pyrethroids have high absorption through gills that resulted the toxic action and the fish lack the enzyme hydrolyse and all resulted, cumulatively the toxic action. The same may be true even in the present study.

According to Bayer Safety Data Sheet (2021), EPA, Pesticide fact sheet No.164 (1987) and Lewis *et al*. (2016), the Cyfluthrin is toxic to fish.

Sana Ullah *et al*. (2019) in their review article mentioned the toxicity values of other members of the type II synthetic pyrethroids, Deltamethrin, Cypermethrin, Fenvalerate and Cyhalothrin, but the work of the toxicant present tested is not mentioned. But as a member of the type II, when the data is compared the present toxicant is also toxic (As per tables 2 & 3).

A report of the λ-cyhalothrin another example of the group I to neotropical one *Brycon amazonicus* fish by Moraes *et al* (2013) and 96h value that was reported as 6.5 µg/L, along with other pyrethroids deltamethrin and cypermethrin. The toxic gradation was deltamethrin followed by λ-cyhalothrin and followed by cypermethrin (Other two toxicants belong to type II synthetic pyrethroids).

Brander *et al* (2012) as already was mentioned earlier in their report too, on the permethrin and Bifenthrin type I synthetic pyrethroids of anti-estrogenic activity in the fish *Menedia beryllina*. Both the studies are antagonistic and fish behaves differently for the two toxicants thereby the toxic aspects show differences. Both are type I synthetic pyrethroids but the difference in the metabolism showed variation in toxic effects.

Velisek *et al* (2009) reported a study based on the toxicity value only, alterations in the haematological as well as biochemical aspects in the fish *Onchorynchus mykiss*. The 96 h LC_{50} value for Bifenthrin was 1.47 µg/L and finally concluded that the lethal toxicity values vary due to temperature. The toxic action was due to culmination of several biomarker studies viz., haematological, biochemical and histopathological aspects which ever in the present study sounds good for the explanation in the tested fish.

Coats and Jeffery (1979) reported for four synthetic pyrethroids one is permethrin (the type I synthetic pyrethroid) to the fish Rainbow trout and others as type II, and concluded that type I is less toxic than type II but not be same for all species and also in different hydrographical conditions.

The static renewal values for 24, 48, 72 and 96 h in the *Cyprinus carpio* was reported by Balakrishna Naik *et al* (2018) as 4.2 ppm, 3.15 ppm, 3.01 ppm and 2.70 ppm for technical grade permethrin group I synthetic pyrethroid. Similarly in CFTS, the said values were 3.73 ppm, 3.09 ppm, 2.07 ppm and 2.04 ppm respectively as of the determination of specific durations. Hence, CFTS values are low when compared with SR as in the present study.

Sambasiva Rao *et al*. (2022) studied and reported for *Labeo rohita* using Bifenthrin another type I synthetic pyrethroid 24, 48, 72 and 96 hrs LC_{50} values as 0.525 µg/L 0.412 µg/L, 0.324 µg/L and 0.282 µg/L respectively for technical grade in static renewal system and for 10% EC, 0.260 µg/L, 0.208 µg/L, 0.613 µg/L and 0.139 µg/L respectively. Similarly, in CFTS method 0.419 µg/L, 0.362 µg/L, 0.282 µg/L and 0.211 µg/L for technical grade in 24, 48, 72 and 96 hrs respectively and for 10% EC 0.211 µg/L, 0.180 µg/L, 0.138 µg/L and 0.11 µg/L respectively.

Satyanarayana *et al* (2018) reported the toxicity study as determination of LC_{50} values 24, 48, 72 and 96 hrs of permethrin [another member of pyrethroid] for technical grade and for 25% EC to the fish *Ctenopharyngodon idella*, in static renewal as well as in CFTS. The same trend was observed as in the present study.
Of all the above aspects, the toxicants both technical grade and 10% WP are toxic.

CONCLUSION

The pyrethroids are synthesized from the flower extracts of the plant *Chrysanthemum cinerariaefolium*. They are toxic to fish and less toxic to mammals and birds where the concentration both in lethal and sublethal, they cause damage to all the fish. The pesticide selected in the present study which was introduced into body of the fish tested and others through aquatic medium initiated response at the threshold dose and increase in the intensity as the doses and exposure time when are increased. The knowledge gained from dose-response studies in target species can be used to formulate the permissible limits in the environments. However not much data is available for the present studied toxicant.

With the knowledge of LC_{50} value it would be possible for having the limits of tolerance of concentrations (permissible concentrations) to establish tolerable limits and safe concentrations for the toxicants and also for aquatic biota apart this, one can protect the aquatic environment and its associated fauna. No doubt chemicals of the pesticide nature cause a loss to the fish stocks as mortality increases but also the fish becomes inconsumable. The present study on the Cyfluthrin toxicity towards *Catla catla* is helpful to involve the biomonitoring and to evaluate the extent of aquatic pollution.

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Table 1. 24, 48,72, 96h LC_{50} values and Regression equation of Cyfluthrin Technical Grade and 10% WP in Static Renewal (SR) and Continuous Flow Through System (CFTS) in the freshwater fish *Catla catla*.

Duration	LC_{50} (µg/L)				Regression Equation			
	Static Renewal (SR)		CFTS		Static Renewal (SR)		CFTS	
	Technical	10% WP	Technical	10% WP	Technical	10% WP	Technical	10% WP
24 hrs	5.994 (5.867-6.123)*	3.045 (2.941-3.153)*	3.087 (2.904-3.283)*	1.933 (1.861-2.008)*	y=42.586x + -28.118 (42.586)[1] (-28.118)[2] (R2=0.9940)[3]	y=24.188x + -6.697 (24.188)[1] (-6.697)[2] (R2=0.9877)[3]	y=13.742x + -1.727 (13.742)[1] (-1.727)[2] (R2=0.9701)[3]	y=23.305x + -1.674 (23.305)[1] (-1.674)[2] (R2=0.9816)[3]
48 hrs	5.812 (5.690-5.937)*	2.696 (2.603-2.793)*	2.905 (2.783-3.032)*	1.757 (1.684-1.833)*	y=43.238x + -28.050 (43.238)[1] (-28.050)[2] (R^2=0.9901)[3]	y=24.033x + -5.352 (24.033)[1] (-5.352)[2] (R^2=0.9707)[3]	y=21.484x + -4.953 (21.484)[1] (-4.953)[2] (R^2=0.9871)[3]	y=20.719x + -0.068 (20.719)[1] (-0.068)[2] (R^2=0.9810)[3]
72 hrs	5.593 (5.407-5.786)*	2.596 (2.533-2.661)*	2.384 (2.201-2.581)*	1.603 (1.542-1.667)*	y=24.926x + -13.636 (24.926) [1] (-13.636) [2] (R^2=0.9707) [3]	y=36.888x + -10.284 (36.888) [1] (-10.284) [2] (R^2=0.9939) [3]	y=10.596x + 1.004 (10.596) [1] (1.004) [2] (R^2=0.9695) [3]	y=23.741x + 0.131 (23.741) [1] (0.131) [2] (R^2=0.9877) [3]
96 hrs	5.193 (5.066-5.322)*	2.496 (2.403-2.593)*	2.260 (2.115-2.414)*	1.365 (1.293-1.442)*	y=36.888x + -21.388 (36.888) [1] (-21.388) [2] (R^2=0.9939) [3]	y=22.245x + -3.837 (22.245) [1] (-3.837) [2] (R^2=0.9706) [3]	y=13.394x + 0.257 (13.394) [1] (0.257) [2] (R^2=0.9817) [3]	y=16.006x + 2.839 (16.006) [1] (2.839) [2] (R^2=0.9653) [3]

* values in the parentheses are 95% Fiducial Confidence Intervals.

1. value in the parentheses is Slope. 2. value in the parentheses is Intercept. 3. value in the parentheses is R^2.

Table 2
Chronic toxicity data for Cyfluthrin
Source: USEPA OPP Ecotoxicity Database

Chemical	Species	Ppb
Tefluthrin	Fathead minnow	0.00397 C
Farpropathrin	Fathead minnow	0.013 S
Deltamethrin	Fathead minnow	0.017 C
Deltamethrin	Fathead minnow	0.022 C
Cyfluthrin	**Sheepshead minnow**	**0.0247 S**
Tralomethrin	Fathead minnow	0.027 S
Lambda-Cyhalothrin	Fathead minnow	0.031 S
Fluvalinate	Fathead minnow	0.033 S
Fluvalinate	Sheepshead minnow	0.036
Cypermethrin	Fathead minnow	0.06 S
Tralomethrin (HAG 107)	Fathead minnow	0.088 S
Fenvalerate	Fathead minnow	0.09 C
Cypermethrin	Fathead minnow	0.14 C
Cefluthrin	Fathead minnow	0.14 C
Tralomethrin	Fathead minnow	0.18 C
Cefluthrin	Sheepshead minnow	0.27 S
Resmethrin	Fathead minnow	0.3 S
Permethrin	Fathead minnow	0.3 S
Resmethrin	Rainbow trout	0.32 S
Etoferprox	Rainbow trout	0.67 S
D-Phenothrin (Sumithrin)	Rainbow trout	1.1 S
Pyrethroid	Fathead minnow	1.9 C
Prallethrin	Rainbow trout	3 S
Resmethrin	Sheepshead minnow	7.05 C
Pemmethrin	Sheepshead minnow	10 S
Etolenprox MTI-500	Zebra fish	23 S

Source: http://www.epa.gov/ppp00001/science/efed_databasesdescription.htm# ecotoxicity.

Table 3
Acute effects of Pyrethroids and Pyrethroid formulations on non-target organisms

Pyrethroids	Birds (mg pyrethroids / kg body weight)	Fish	Bees
Allethrin	2030	Toxic	-
s-Bioallethrin (Esbiol)	680	Highly toxic	-
Resmethrin	-	Toxic	Highly toxic
Bioresmethrin	-	Highly toxic	Highly toxic
Tetramethrin	>1000	Highly toxic	Toxic
Permethrin	>13500	Highly toxic	Highly toxic
Fenvalerate	9932	Highly toxic	-
d-Phenothrin	>2500	Toxic	Toxic
Cypermethrin	-	Extremely toxic	Toxic
Esfenvalerate	-	Highly toxic	-
Bifenthrin	>2150	Toxic	-
Fenpropathrin	1089	Toxic	-
Refluthrin	4190	Highly toxic	-
Cyfluthrin	**4450**	**Toxic**	**Toxic**
Fluvalinate	>5620	Toxic	Non-toxic
Tralomethrin	7716	Extremely toxic	Highly toxic
Deltamethrin	>4640	Toxic	Highly toxic
Cyhalothrin	>5000	Highly toxic	-
Kadethrin	-	Toxic	Toxic
Alphacypermethrin	-	Toxic	Toxic
Lambda-cyhalothrin	>3950	Toxic	Toxic

Source: Thatheys. A.J. and A. De bor Borah Gnan Selvam (2013). Synthetic Pyrethroids Toxicity and Biodegradation. *Applied Ecology and Env. Sciences*, **1(3)**: 33-36.

Fig. 1. Graphical representation of 24h LC_{50} value in Static renewal for Technical grade of Cyfluthrin to the fish *Catla catla*

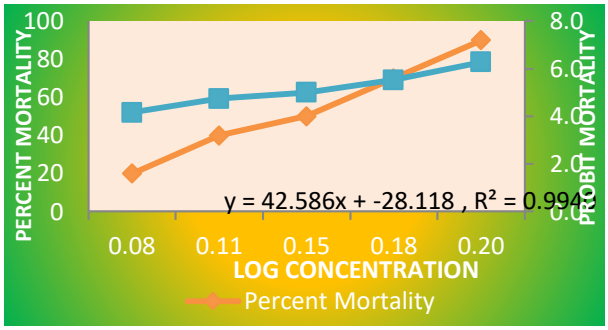

Fig. 2. Graphical representation of 48h LC_{50} value in Static renewal for Technical grade of Cyfluthrin to the fish *Catla catla*

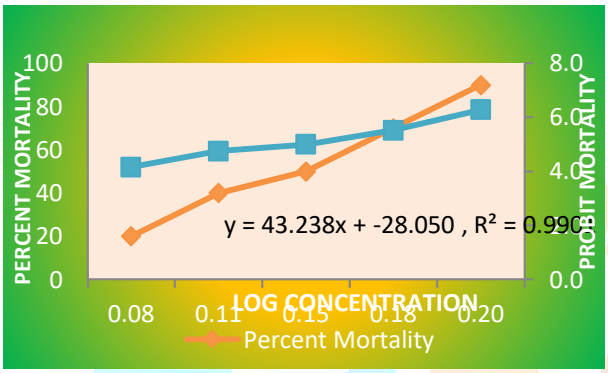

Fig. 3. Graphical representation of 72h LC_{50} value in Static renewal for Technical grade of Cyfluthrin to the fish *Catla catla*

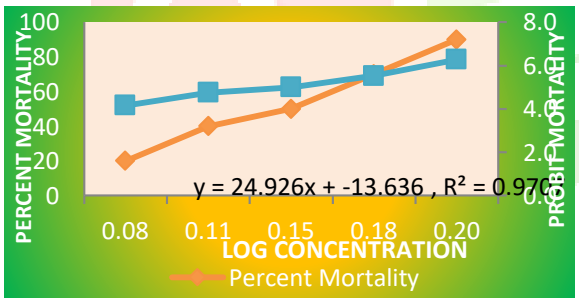

Fig. 4. Graphical representation of 96h LC_{50} value in Static renewal for Technical grade of Cyfluthrin to the fish *Catla catla*

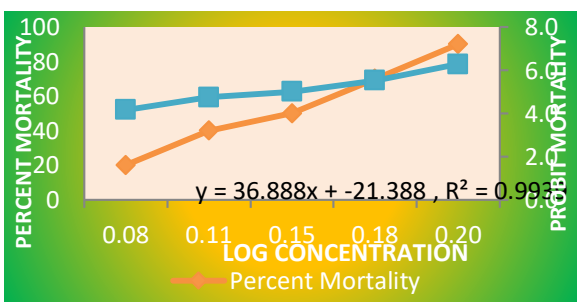

Fig. 5. Graphical representation of 24h LC_{50} value in Static renewal for 10% wettable of Cyfluthrin to the fish *Catla catla*

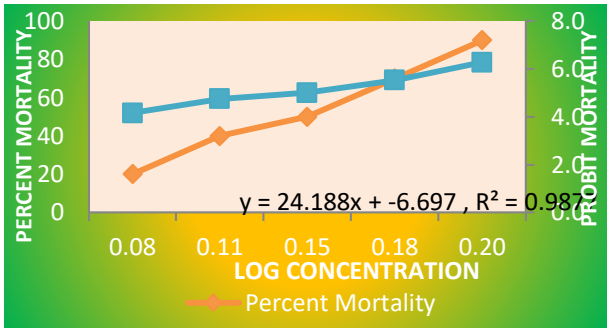

Fig. 6. Graphical representation of 48h LC_{50} value in Static renewal for 10% wettable of Cyfluthrin to the fish *Catla catla*

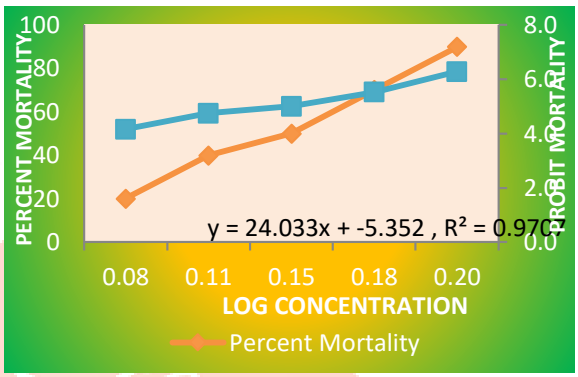

Fig.7. Graphical representation of 72h LC_{50} value in Static renewal for 10% wettable of Cyfluthrin to the fish *Catla catla*

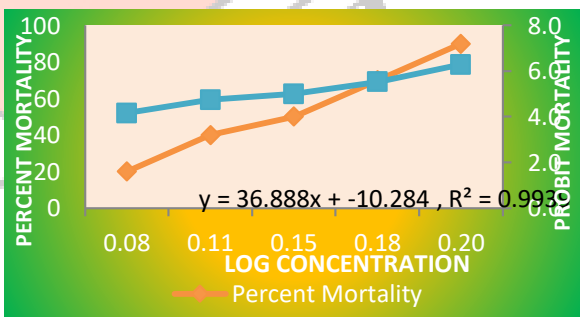

Fig. 8. Graphical representation of 96h LC_{50} value in Static renewal for 10% wettable of Cyfluthrin to the fish *Catla catla*

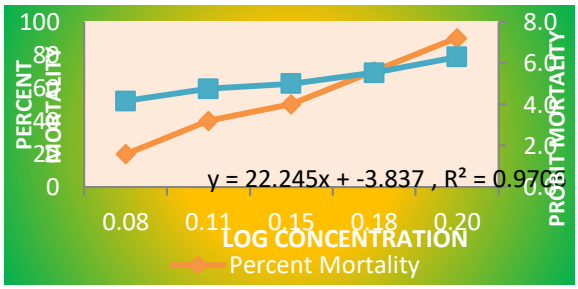

Fig. 9. Graphical representation of 24h LC_{50} value in Continuous Flow through system for Technical grade of Cyfluthrin to the fish *Catla catla*

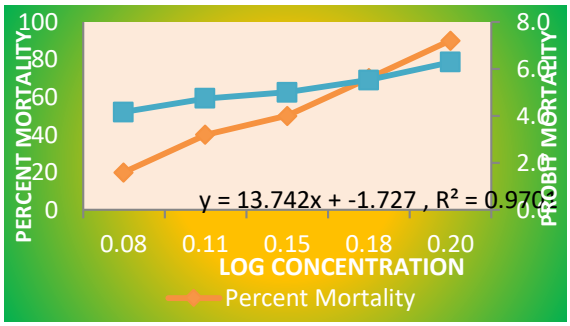

Fig. 10. Graphical representation of 48h LC_{50} value in Continuous Flow through system for Technical grade of Cyfluthrin to the fish *Catla Catla*

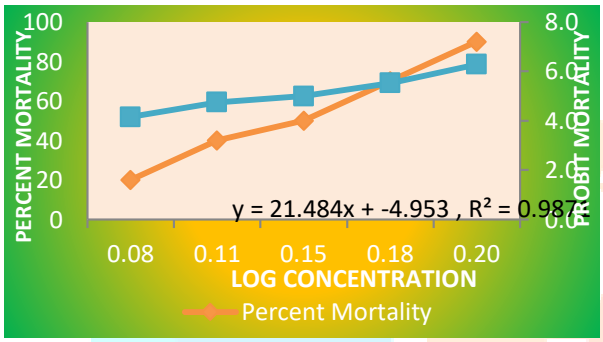

Fig. 11. Graphical representation of 72h LC_{50} value in Continuous Flow through system for Technical grade of Cyfluthrin to the fish *Catla Catla*

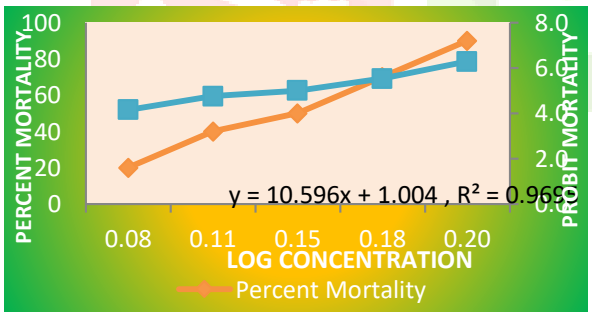

Fig. 12. Graphical representation of 96h LC_{50} value in Continuous Flow through system for Technical grade of Cyfluthrin to the fish *Catla catla*

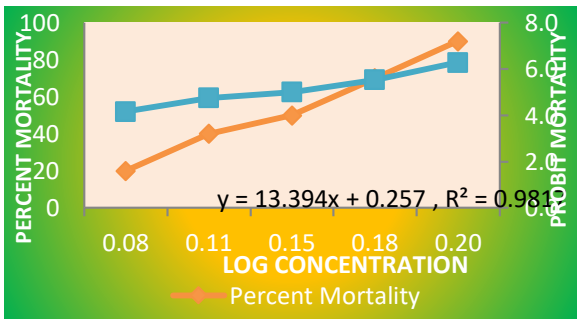

Fig. 13. Graphical representation of 24h LC_{50} value in Continuous Flow through system for 10% wettable of Cyfluthrin to the fish *Catla catla*

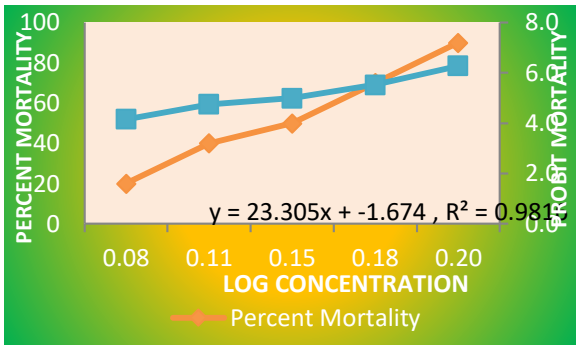

Fig. 14. Graphical representation of 48h LC_{50} value in Continuous Flow through system for 10% wettable of Cyfluthrin to the fish *Catla catla*

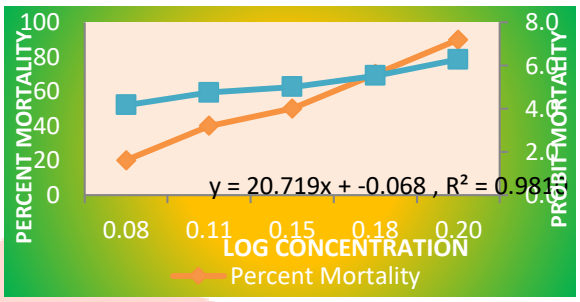

Fig. 15. Graphical representation of 72h LC_{50} value in Continuous Flow through system for 10% wettable of Cyfluthrin to the fish *Catla catla*

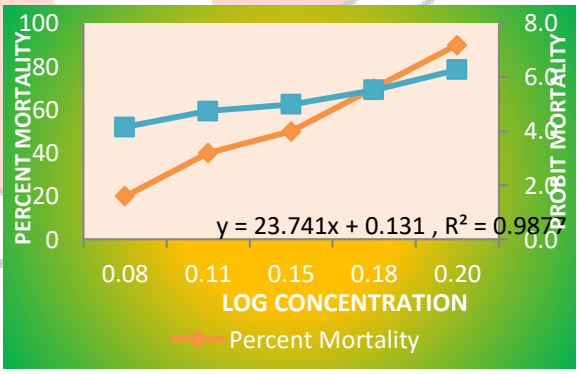

Fig. 16. Graphical representation of 96h LC_{50} value in Continuous Flow through system for 10% wettable of Cyfluthrin to the fish *Catla catla*