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ACUTE TOXICITY DETERMINATION OF TECHNICAL GRADE AND 10% EC OF BIFENTHRIN, A SYNTHETIC PYRETHROID OF NON-CYANOGROUP (TYPE-I) TO THE FISH *LABEO ROHITA* (HAMILTON)

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Abstract

The acute toxicity studies are conducted for the fish *Labeo rohita*, (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 Bifenthrin, a synthetic pyrethroid of a non-cyanogroup, categorized as type I. For both technical grade as well as its 10% EC 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 bifenthrin are 0.525µg/L, 0.412 µg/L, 0.324 µg/L, and 0.282 µg/L, respectively, similarly for 10% EC it is 0.260 µg/L, 0.208 µg/L, 0.163 µg/L, and 0.139 µg/L respectively for 24, 48, 72 and 96 h. Similarly for CFTS, in technical grade, the values are 0.419 µg/L, 0.362 µg/L, 0.282 µg/L, 0.211 µg/L for 24, 48, 72 and 96 h respectively and 10% EC the values are 0.211 µg/L, 0.180 µg/L, 0.138 µg/L, 0.111 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) and this type I without a cyanogroup is also toxic and its commercial formulation is also sufficiently toxic for the fish. The results of the present toxicant, bifenthrin 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: *Labeo rohita*, Bifenthrin, 10% EC, synthetic pyrethroid, non-cyanogroup, Type I, Acute toxicity, static renewal, continuous flowthrough system (CFTS).

INTRODUCTION

No doubt, the pesticides that are used benefit the crop damage or any produce of fishery, piggery and to maintain the public health in order to have hygiene conditions. The use and in excessive quantities also indiscrimination all by any means transport them to the aquatic environment and there all of them are the repository residues. These in terms as well as of concentrations, they cause pollution of pesticides and as such effect at two levels either acute or chronic. The acute concentrations will be such that all the ambient organisms may die which result in a loss of biodiversity and may be a threat to the biota. In other way the concentration will be such that not causing the death referred as chronic, a delayed effect but continuously harmful to them.

The global use of these pesticides according to Isra Mahamood *et al.* (2016) estimated that about 5.2 billion pounds as sprayings and such use mitigate the damage caused by the insect pests but risks they pose have surpassed as harmful ones either immediately (Acute) or in the long run (Chronic).

After the book reading of 'silent spring' by Rachel Carson (1962), only of the aftermath awareness the catalysing approach opened, the new era of the environment that really 'silenced' the field of the toxicity and toxicology to open the eyes and ecotoxicology prevailed from then after 1970's.

According to Macneale *et al.* (2010) echoed that the pesticides effect the primary producers, consumers (primary-secondary-tertiary) and decomposers. In that the nektonic organism may be primary or secondary or tertiary consumers, especially the fish get effected as they are more in number among the vertebrates in the aquatic environment. Here target organism and non-target organisms terms apply insects as pests the targeted one but the others the non-targeted group.

It requires to analyse the toxicity concentrations whether they are either acute or chronic causing the harmful effects, hence referred as the toxicants. This toxicity evaluation, first to have tests as recommended by Doudroff *et al.* (1951), Anon (1975), American Public Health Association (1998, 2005 and 2012) and latest by OECD (2019).

According to the test guidelines No.203 Organisation for Economic Cooperation and Development (OECD, 2019), the fish that is to be tested exposed to the specific test chemical (in the present study *Labeo rohita* and Bifenthrin respectively), under either any test (here in the present study static renewal test or continuous flow through test, (CFTS) until mortalities and visible observable alterations in their behaviour that can be noted and to have that the concentrations that must kill at least 50% of the exposed the organisms which can be referred as lethal concentration noted as LC_{50}. In that, it must be of an accuracy, precision apart from the limit of both detection and determination, and must be of working range and finally must be specific for the chemical and the test organism. While selecting the test species that must be readily available easy to maintain in the laboratory and must have a historical use either in culture or cultivable ones, disease free as well as healthy. The OECD (2019) recommended only 9 days as a acclimatization duration before it is finally going to have the testing. The temperature and oxygen saturation should be 80/% of the air, and feeding either 3 times per week or daily until 24-48 hours before the organisms are made to have to the test.

They are 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 *Labeo rohita*, with a new generation synthetic pyrethroid group chemical Bifenthrin is selected for the study in the prevailing water conditions as appended in the table 1.

However, taking into guidelines of APHA (1998, 2005 and 2012) the following four methods of toxicity testing are most familiar, which the reports of earlier studies had basing on that only followed. They are: (1) Static test 2) Static renewal test (SR) (3) Recirculation test (4) and Continuous flow through test (CFTS).

Hence, in the present study, the toxicant Bifenthrin is selected and also EC formulation of 10% of it and tested against the fish *Labeo rohita*. The toxicity tests are performed by employing static renewal and continuous flow through system (CFTS) to determine the LC_{50} values for 24, 48, 72 and 96 hrs.

MATERIAL AND METHODS

Test species**:** The fresh water fish *Labeo rohita* are brought from a local fish farm where along with other major carps cultured. The fish size in length and weight ranges from 30-50 mm and 3-5 gm respectively and are the fingerlings.

Initially they are acclimatized to the laboratory conditions in the tubs of the plastic material which contained the ground water and were fed for 15 days at the room temperature of 28+2^{o}C. In that period they were made to have feeding with cake made of groundnut only. But while experimenting in the toxicity durations of 24, 48, 72 and 96 hrs, they were not fed and also two days of the prior to the experimentation. All the precautions that were there as recommendations of APHA (1998, 2005 and 2012 and OECD 2019) are followed during the entire toxicity evaluation procedure of the experimentation. During such procedure if the mortality exceed 5% the entire batch of the fish are discarded and such acclimated fish are only selected.

Procurement of the technical grade and the commercial grade

The technical grade (98% pure) was supplied by M/s. Kalyani Industries, (Agrochemical Suppliers, 1202/1204, 12th Floor B-wing, Kailash Business park Ghotopur power, India) and the 10% EC is purchased locally that was available in the market of the same company.

Preparation of stock solution

The stock solutions are made with acetone and the working concentrations and further diluted with water only and are taken as in mgL^{-1}. The toxicants are dissolved in acetone and further working solutions are made of the required quantity. For each set of the experiment, fish and water without the toxicant (but using the highest quantity of the solvent only) as control was also kept. The dilution factor avoids to minimise the solvent acetone.

Test Conditions

In the Table 1 which is appended provide the information of the existing physico-chemical parameters of the water used for the fish to acclamatise and also for conducting the toxicity experiments. All the evaluating aspects and precautions laid down 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 only followed. The Pilot experiments are first conducted, to choose/select the concentrations to know the toxicity range in terms of the effective one. In each exposure of the toxicant 10 fish are taken for each of the test and they are in water of 10 litres. For the continuous flow through system, reservoirs of 24 liters capacity are used and the test a flow rate 4 litres in 60 minutes was made by using the poly-ethylene drip sets with regulators from a container of 24 liter capacity (@ 4 litres per hour) and for every 6 hours fresh test solutions were prepared in the reservoirs.

The two types of experiments that are only for to determine the toxicity of both the Bifenthrin technical grade and 10% EC also in various concentrations to determine the fish deaths (mortality) during experimentation. The data on the fish deaths i.e., the mortality rate of fish was recorded. As per the recommendations of the APHA (1998, 2005, 2012) the dead fish are removed as and when it resulted. The experiments are conducted for the durations of 24, 48, 72, 96 hrs by employing both static renewal as well as continuous flow through system which are selected for both the toxicants.

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

The data was subjected to the following statistical equations for arriving 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 = Per cent kill just below 50% mortality
b = Per cent kill just above 50% mortality

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

$$S = \frac{\dfrac{LC_{84}}{LC_{50}} + \dfrac{LC_{50}}{LC_{16}}}{2}$$

$$F = \text{antilog} \frac{(277 \log S)}{\pi N} = S^{2.77} / \sqrt{\bar{N}}$$

Where N is the number of animals tested whose expected effects are between 16 and 84%mortality.

Upper confidence limit = LC_{50}X f
Lower confidence limit = LC_{50} / f

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

RESULTS

The sensitivity range for the technical grade is 0.2 ml of the toxicant dissolved in the solvent whereas for 10% EC it is 0.1 ml. The toxicity values for both the toxicants in static renewal and continuous flow through system for 24, 48, 72 and 96 hrs is appended in the table 2, along with regression equation, fiducial 95% confidential limits, slope, intercept and R^2 values also. The graphs are also appended as Fig. 1, 2, 3 and 4 are the resulted one from probit Vs concentration and also concentrations Vs percent kills for 24, 48, 72 and 96 hrs in static renewal and continuous flow through systems, respectively.

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

(1) The 10% EC, the commercial formulation that was used by all the 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 was due to the ingredients that are mixed in it are 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% EC 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 EC.
(4) The static renewal test method values for determined duration 24, 48, 72 and 96 hrs are higher to the continuous flow through system method. In the former test method, absorption of the toxicant both to the surfaces of the containers and the test organism and accumulation of the excretory wastes all culminate and had more toxic action whereas in later test method, which almost simulate the natural conditions, hence lower.
(5) The toxicity values are species specific and also duration of the time interval exposure dependent even, the present study there is no exception. The values for the fish *Labeo rohita* 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.

DISCUSSION

The toxicity studies of Bifenthrin, the source from USEPA OPP as ecotoxicity data base as compared to other synthetic pyrethroids is appended as table 3. The data projected that Bifenthrin, the values for different freshwater and fish is very limited of studies and the factor of sensitivity cannot be generated as with other synthetic pyrethroids. (Source: http://www.epa.gov./opp.OOD/science/efed/database_dewcriotion.htm/ecotoxicity).

Thathyas and Selvam (2013) reported on the acute toxicity of the synthetic pyrethroids formulations that were used to birds, fish and Bees which was presented as table 4. According to the information that mentioned it was toxic to the fish.

National Pesticide information Centre (NPIC document, 2011) mentioned that the half life period of the synthetic pyrethroid in the soil ranges from 106-147 days and was considerable in the environment without any photo or microbial degradation.

It was also mentioned in the report that for Oncorhynchus mykiss (rainbow trout) and Lepomis macrochinus (bluegill sunfish), the LC_{50} values for 96 h ranged from 0.10 and 0.18 ppb respectively.

It can be concentrated 0.0037 µg/L in the fish Pimephales promealas (fat head minnows) as the days increase from 127 to 254 and the factor of bioconcentration increases by 7000 due to the exposure. In the fish, Dorosoma cepedianum (Gizzard shed), when the sediment adsorption of 7750 mg/L, of the 8^{th} day LC_{50} value was at 0.0207 µg/L.

The toxicity studies for the different synthetic pyrethroids were given in their respective review articles by Sana Ullah *et al.* (2019), Saumya Biswas *et al.* (2019), Prusty *et al.* (2015) and Ahrar Khan *et al.* (2012).

At the prevailing concentration of the test method whatever is applied, according to Prusty *et al.* (2015), the toxicant being the contact poison as well as stomach poison blocks the sodium channels of the nerve fibers (Central nervous system, Para and synthetic – 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 present studied toxicant belongs to the type I synthetic pyrethroid without a cyanogroup (Permethrin, Bifenthrin and Cyhalothrin etc.) differ in the closure of the gates (sodium and potassium) whereas the type II with cyanogroup (Deltamethrin, Cypermethrin and Fenvalerate) modify the opening of the sodium gate (influx of Na^+ ions) which according to Soderland (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 as well as the Ca^{++} ions binding and all culminate the nerve transmission delayed 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 I synthetic pyrethroids to which the present toxicant also belongs, due to hypersensitive action and blocks the influx and outflux of the ions in the nerve cells of CNS, ANS, and PNS and thus impart the toxic action.

Sana Ullah *et al.*(2019) about the biomarkers in the toxicity evaluation opined that due to the presence of the synthetic pyrethroids in the environment, 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. Behavioural in consistencies and alterations inhibition of AChE activity and developmental toxicity and all cumulative the 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 the 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 Bifenthrin, the present tested toxicant. The article had a reference of use of the Bifenthrin in the most populous countries China, Mexico, Ghana, Brazil, Australia, USA, France apart from India. They made a cross reference of Frank *et al.* (2018), Tu *et al.* (2016), Crago and Schlenk (2015), Bessel *et al.* (2011), Jim *et al.* (2009), Brander *et al.* (2016), Bertotto *et al.* (2018), Forsgreen *et al.* (2013), Brander *et al.* (2012) and Velisek *et al.* (2009a, b) in the different biomarkers of the toxicity only to fish.

Sana Ullah *et al.* (2022) reported that Bifenthrin induced the toxic effect in the fish Ctenopharyngodon idella at 6 µg/L and mentioned the work related to Cyprinus carpio 2.08 µg/L and 0.80 µg/L for Tilapia by Velisek *et al.* (2009a) respectively. The combination of Reactive oxygen species (ROS) and Lipid peroxidation (LPO) were the biomarker studies which caused the toxic effect accord to their study. The same might be true even in the present study of the fish *Labeo rohita*.

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% EC, it is 0.1 ml. By mentioning a cross reference of Brander (2016), in their review article reported that Bifenthrin, to the fish Menidia beryline due to metabolic disruption of endocrine signals resulted the toxic effect at 0.5, 5.0 and 50 mg/L by exposing to 14 and 20 days respectively. Mayada *et al.* (2021) in the fish Oreochromis niloticus reported but Bifenthrin intoxication was due to the acute concentration of 6.81 µg/L level at 96 h exposure and physiological, neurological as well as behavioural induces the toxic action that impaired the inflammatory responses all culminate the toxic action.

Saha and Saha (2021) reported a study of the fish Clarias batracus using Bifenthrin as the toxicant. The static renewal tests were conducted and LC_{50} values are determined for 24, 48, 72 and 96 h. They reported the values as 5.093, 4.659, 3.693 and 3.464 respectively. The fish is a cat fish, adult, resistant carnivorous whereas the present studied one Juvenile size of not carnivorous fish only. The fish showed indices of the toxicity of having behavioural alterations and the authors not mentioned the reasons of the toxicity.

Bano *et al.* (2021) in the fish *Labeo rohita*, the present studied one due to the exposure of the Bifenthrin and chlorpyrifos mixture had an impact on erythrocytes that damaged cells and DNA of erythrocytes which finally resulted the death of the fish.

Saha *et al.* (2021) reported on the Asian gigantic cat fish Heteropneustes fossilis and determined the toxicity values due to the exposure of Bifenthrin for 24, 48, 72 and 96 hrs, which was 4.87, 4.47, 3.54 and 3.40 µg/L by employing static renewal tests only. The fish is carnivorous and the present studied one is not but also a lot of size difference also exists for the reason of variation in the LC_{50} values. The morphological effect of the variations of the opercular movements that resulted as alteration/reduction in oxygen intake which finally caused the death of the fish and might be the same even in the present study.

Kalawati Kumari (2020) in her review article, about the pesticides effect the fishes and also mentioned that, insecticides including the 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.

Abdul Bashir *et al.* (2020) while reporting the toxicity of the three pyrethroids of which one belong to type I of synthetic pyrethroid, cyhalothrin to the fish Poecilia reticulata the guppy fish. The LC_{50} value was 81.83 µg/L for the size range 2.13±0.27 cm and had made an imbalance of trophic structure in the water body and pesticide usage must be monitored and regulated.

Ghanim *et al.* (2020) reported that in the zebra fish Danio rerio due to 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 *Labeo rohita* is an important cultivable fish and such exposure of the chemical is not recommended.

Saha and Saha (2020) reported on the fish Oreochromis mossambicus also the 96 h LC_{50} value as 2.423 µg/L for Bifenthrin the type of I synthetic pyrethroid. It is strongly toxic to fish and the death was due to many factors and finally proved that it was low in toxic effect at lower temperature whereas in warmer waters it was more toxic.

Tamzin *et al.* (2019) reported the toxicity of Bifenthrin to the fish Ochorhyuchus mykiss (rainbow trout) and the 96h LC_{50} value was 6.2 µg/L and the biochemical alterations of proteins resulted the death of the organism.

Jaroslava *et al.* (2019) reported on the acute toxicity of the two pyrethroids – cypermethrin and deltamethrin commercial formulations only that had impact on the fish physiology.

Dereck *et al.* (2018) reported on the pyrethroid Bifenthrin, had a mixture of toxic action both of type II and I. The wave of depolarization was varied that caused the toxic action for not to have normal for the survival. On the similar lines, Anitha *et al.* (2012) reported the toxicity and neuronal aspects in the fish Catla catla which had effect on GABA receptors and the 96 h value as 0.23 mg/L. This supports the toxic action of the Bifenthrin, the present toxicant action for causing the death of the fish as in the present study. Similar observation was also reported by Dhruv Kumar and Manta Kumari (2018), in the fish Channa punctata and Heteropneustes fossils using the toxicant lambda cyhalothrin the type II synthetic pyrethroid.

Kathetine *et al.* (2017) had a interesting finding using Amphipods as testing organisms and the toxicity was caused by Bifenthrin that was reported in urban storm water of wetlands. The LC_{50} value for Austrochilotonia species that was reported as 1.09 µg/L for 96h and could trace the toxicant due to contamination.

Humanaz *et al.* (2017) reported the pesticide mixture toxicity which contain Bifenthrin as one of them, apart from chlorpyrifos (organophosphate) and Endosulfan (an organo chlorine) to the fish, Catla catla, Cirrhinus mrigala and *Labeo rohita*. The method of the toxicity test was static bioassay and the 96h LC_{50} values were 1.09, 1.81, 1.63 and 1.90, 2.78 and 2.38 µg/L respectively. Catla was more sensitive followed by *Labeo rohita*, the present tested fish and Cirrhinus was more resistant. The effect of the enzyme superoxide dismutase activity that was altered responsible for the toxic action. The toxic action shown by the synthetic pyrethroid Bifenthrin is significant for all the three major carps which are cultivated in polyculture.

Renu Chaudhari and Kamal Kumar Saxena (2016) exposed the fish Channa punctatus using bioallethrin another synthetic pyrethroid of type I. The erythrocytes of the fish showed morphological alterations that was responsible for the action of the toxic nature to the fish, at sublethal concentrations that are determined by the static bioassay.

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, oxidative stress factors, enzymes of nitrogen of metabolism, Acetyl Cholinesterase (AChE) activity, gene expression, Genotoxic effects, omic aspects (genomics, protenomics metabolimics) all and the specificity in the sodium channel interactions which were the biomarkers of the study. In the present study, the toxicity evaluation was done as a study of biomarker using Bifenthrin as toxicant in the fish *Labeo rohita*.

Moraes *et al.* (2013) reported on the toxicity of the λ-cyhalothrin another synthetic pyrethroid of type II to the neotrophical fish Brycon amazonicus and the 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.

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

Velisek *et al.* (2009b) reported a study based on the toxicity value only, that had 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 the culmination of several biomarker studies viz., haematological, biochemical and histopathological aspects which even 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 concluded that type I is less toxic than type II but not be same for all species and also in different hydrographical conditions.

WHO (2011) mentioned some of the guidelines as specifications and evaluations for Public Health Pesticides for Bifenthrin in their report and the information pertains the toxicity evaluation and the toxic to the fish tested.

Balakrishna Naik *et al.* (2018) reported on the toxicity of the permethrin the synthetic pyrethroid of type I (which the present toxicant also belongs) to the fish Cyprinus carpio. The static renewal LC_{50} values for 24 hrs, 48 hrs, 72 hrs, and 96 hrs were 4.2 µg/L, 3.75 µg/L, 3.01µg/L and 2.70 µg/L respectively for technical grade and 6.3 µg/L, 5.3 µg/L, 4.43 µg/L, and 3.72 µg/L, for 25% EC. For continuous flow through system, for technical grade 3.73 µg/L, 3.09 µg/L,, 2.07 µg/L, and 2.04 µg/L, for 24, 48, 72 and 96 hrs respectively, whereas for 25% EC it was 5.32 µg/L, 4.36 µg/L, 3.54 µg/L and 2.73 µg/L respectively. The toxicity was due to combinations of several aspects of biomarker studies as other effects.

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 type I synthetic pyrethroid] for technical grade and for 25% EC in static renewal as well as continuous flow through system (CFTS). The values for the fish Ctenopharyngodon idella that are reported as 0.261 µg/L, 0.108 µg/L, 0.114 µg/L, and 0.078 µg/L, in static renewal test for technical grade for 24, 48, 72 and 96 hrs respectively and for 25% EC, it was 0.523 µg/L, 0.332 µg/L, 0.220 µg/L, and 0.156 µg/L, respectively. Similarly in CFTS for technical grade 0.229 µg/L, 0.131 µg/L, 0.083 µg/L, and 0.021 µg/L, for technical grade respectively for the 24, 48, 72 and 96 hrs and for 25% EC 0.376 µg/L, 0.248 µg/L, 0.141 µg/L, and 0.122 µg/L. In the present study with Bifenthrin, for the fish *Labeo rohita* are in the range as above and proved that Bifenthrin is also toxic to fish.

Of all the above aspects, the toxicants both technical grade and 10% EC are toxic.

CONCLUSION

Pyrethroids are the synthetic derivatives of pyrethrin’s, which are the toxic components contained in the flowers of Chrysanthemum cinerariae folium. Although the synthetic pyrethroids are less persistent and less toxic to mammals and birds but, they are highly toxic to a number of non-target organisms even at very low concentrations especially the fish due to the presence of cyano group in the toxicant tested belonging to type II. For this reason, these organisms are extremely sensitive to neurotoxic effects of pyrethroids when they reach surface water-courses. Since their transport, the toxicity of pyrethroid pesticides has been studied in various fish species. In fresh water, the presence or absence of fish has been widely used as a biological indicator of the degree of pollution. The data obtained on the concentration of the selected pollutants which are lethal to fish provide the necessary information, apart from identifying a safe concentration limit above which fish are likely to be killed. Thus, 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 are also increased. The knowledge gained from dose-response studies in the target species can be used to set standards for human exposure and the amount of the chemical residue that is allowed in the environments.

With the knowledge of the LC_{50} value it would be possible to establish the tolerable limits and safe concentrations of the toxicants for the aquatic biota and one can protect the aquatic environment and its associated fauna. No doubt pesticides can create a great loss by fish mortalities on one hand and on the other hand rendering them unfit for human consumption. If consumed, these fish can cause health hazard a situation for those who consume must aware that in these fish as reported. The present study on the Bifenthrin toxicity towards *Labeo rohita* is helpful to involve the biomonitoring to evaluate the extent of aquatic pollution and its impact on the non-target aquatic fauna including fish. In the present study of the fish, the selected test animal, is less susceptible to toxic action of the synthetic pyrethroids in general and Bifenthrin in particular and the present data can be a tool for documentation of toxic action by the xenobiotic chemicals.

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Table 1
Physical and Chemical properties of water used for the present study

S. No.	Water Characteristic / Parameter	Quantity
1	Turbidity	8 silica units
2	Electrical conductivity at 28°C	816Micro ohms/cm
3	pH at 28°C	8.1
4a*	Alkalinity: Phenolphthalein	Nil
B*	Alkalinity: Methyl orange	472
5*	Total Hardness (as $CaCO_3$)	232
6*	Carbonate Hardness (as $CaCO_3$)	232
7*	Non Carbonate Hardness (as $CaCO_3$)	Nil
8*	Calcium Hardness (as $CaCO_3$)	52
9*	Magnesium Hardness	40
10*	Nitrite Nitrogen (as N)	Nil
11*	Sulphate (as SO_4^{2-})	Trace
12*	Chloride (as Cl^-)	40
13*	Fluoride (as F^-)	1.8
14*	Iron (as Fe)	Nil
15	Dissolved Oxygen	8–10ppm
16	Temperature	28±2°C

* The sample water used for experiments was clear, colorless and odorless.
* The following results were in mg/l

Table 2. 24, 48, 72, 96hrs LC_{50} values, 95% fiducial confidential intervals, Regression equation, Slope, Intercept and R^2 values in Static Renewal (SR) and Continuous Flow Through System (CFTS) for Technical Grade (TG) and 10% E.C. of Bifenthrin to the freshwater fish *Labeo rohita*.

Duration	LC_{50} (µg/L)				Regression Equation			
	Static Renewal		CFTS		Static Renewal		CFTS	
	Technical	10% E.C.	Technical	10% E.C.	Technical	10% E.C.	Technical	10% E.C.
24 hrs	0.525 (0.511-0.539)*	0.260 (0.253-0.266)*	0.419 (0.406-0.432)*	0.211 (0.205-0.218)*	y=33.588x + 14.409 (33.588)[1] (14.409)[2] (R2=0.9816)[3]	y=36.888x + 26.604 (36.888)[1] (26.604)[2] (R2=0.9939)[3]	y=29.760x + 16.242 (29.760)[1] (16.242)[2] (R2=0.9937)[3]	y=27.167x + 23.339 (27.167)[1] (23.339)[2] (R2=0.9486)[3]
48 hrs	0.412 (0.397-0.427)*	0.208 (0.200-0.217)*	0.362 (0.344-0.380)*	0.180 (0.173-0.188)*	y=24.202x + 14.335 (24.202)[1] (14.335)[2] (R^2=0.9814)[3]	y=20.003x + 18.632 (20.003)[1] (18.632)[2] (R^2=0.9888)[3]	y=17.067x + 12.541 (17.067)[1] (12.541)[2] (R^2=0.9818)[3]	y=22.005x + 21.380 (22.005)[1] (21.380)[2] (R^2=0.9608)[3]
72 hrs	0.324 (0.310-0.339)*	0.163 (0.154-0.172)*	0.282 (0.268-0.297)*	0.138 (0.129-0.147)*	y=19.438x + 14.513 (19.438)[1] (14.513)[2] (R^2=0.9804)[3]	y=15.264x + 17.023 (15.264)[1] (17.023)[2] (R^2=0.8981)[3]	y=17.041x + 14.371 (17.041)[1] (14.371)[2] (R^2=0.9897)[3]	y=13.290x + 16.432 (13.290)[1] (16.432)[2] (R^2=0.9894)[3]
96 hrs	0.282 (0.268-0.297)*	0.139 (0.132-0.147)*	0.211 (0.197-0.225)*	0.111 (0.100-0.123)*	y=17.041x + 14.371 (17.041)[1] (14.371)[2] (R^2=0.9897)[3]	y=16.267x + 18.943 (16.267)[1] (18.943)[2] (R^2=0.9376)[3]	y=13.328x + 14.018 (13.328)[1] (14.018)[2] (R^2=0.9720)[3]	y=8.177x + 12.805 (8.177)[1] (12.805)[2] (R^2=0.9761)[3]

* 95% Fiducial Confidence Intervals.

1. Slope.
2. Intercept
3. R^2

} all are in the parentheses.

Table 3
Chronic toxicity data for synthetic pyrethroids
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 4

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: Thathyas. 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 System for Technical Grade of Bifenthrin to the fish *Labeo rohita.*

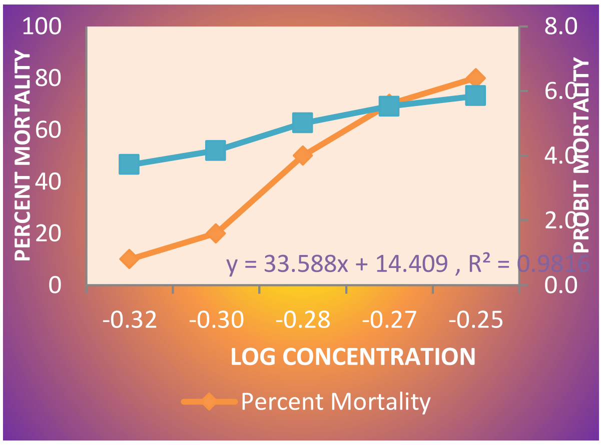

Fig. 2. Graphical representation of 24h LC_{50} value in Static Renewal System for 10% E.C. *of* Bifenthrin to the fish *Labeo rohita.*

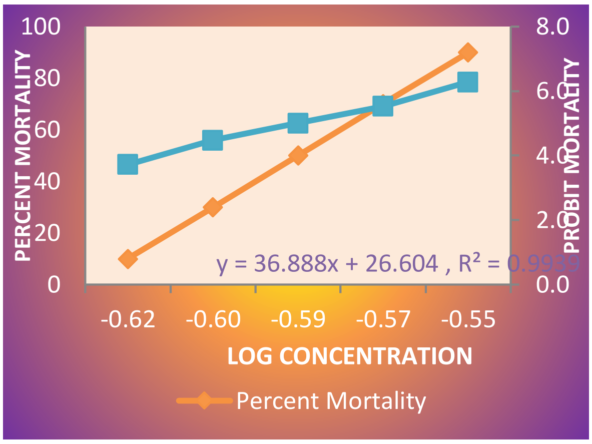

Fig. 3. Graphical representation of 24h LC_{50} value in Continuous Flow through system for Technical Grade of Bifenthrin to the fish *Labeo rohita.*

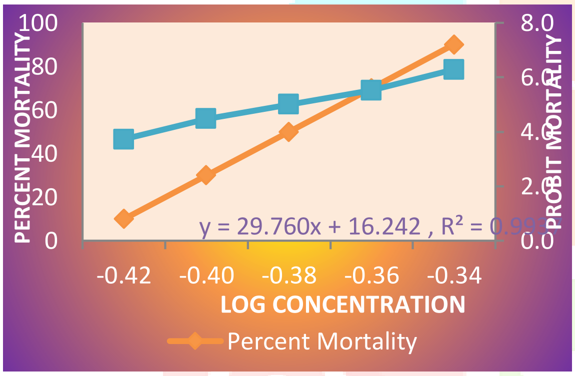

Fig.4. Graphical representation of 24h LC_{50} value in Continuous Flow through system for 10% E.C. of Bifenthrin to the fish *Labeo rohita.*

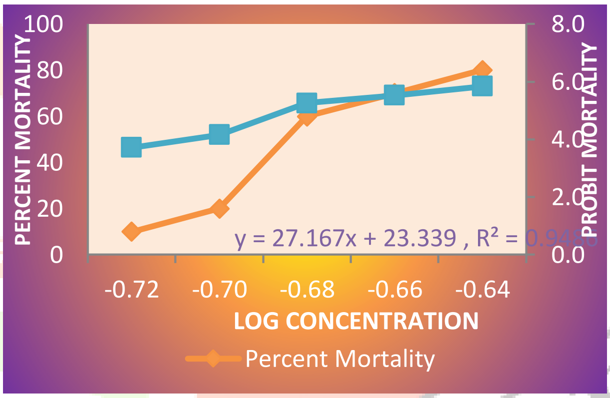

Fig. 5. Graphical representation of 48h LC_{50} value in Static Renewal System for Technical Grade Grade of Bifenthrin to the fish *Labeo rohita.*

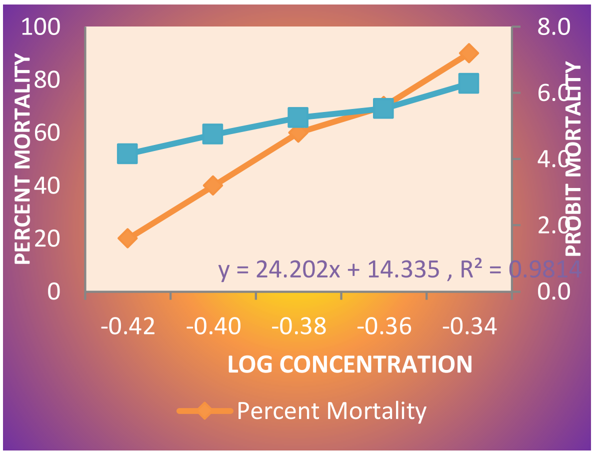

Fig. 6. Graphical representation of 48h LC_{50} value in Static Renewal System for 10% E.C. of Bifenthrin to the fish *Labeo rohita.*

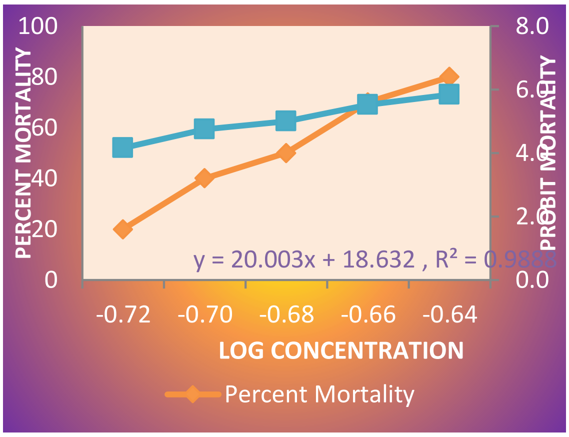

Fig. 7. Graphical representation of 48h LC_{50} value in Continuous Flow through system for Technical Grade of Bifenthrin to the fish *Labeo rohita.*

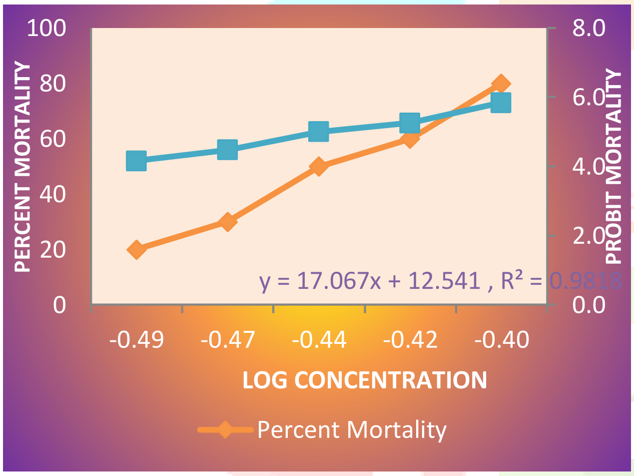

Fig. 8. Graphical representation of 48h LC_{50} value in Continuous Flow through system for 10% E.C. of Bifenthrin to the fish *Labeo rohita.*

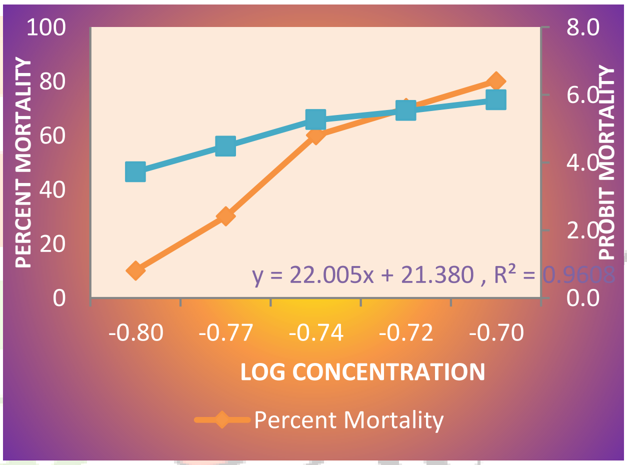

Fig. 9. Graphical representation of 72h LC_{50} value in Static Renewal System for Technical Grade Grade of Bifenthrin to the fish *Labeo rohita.*

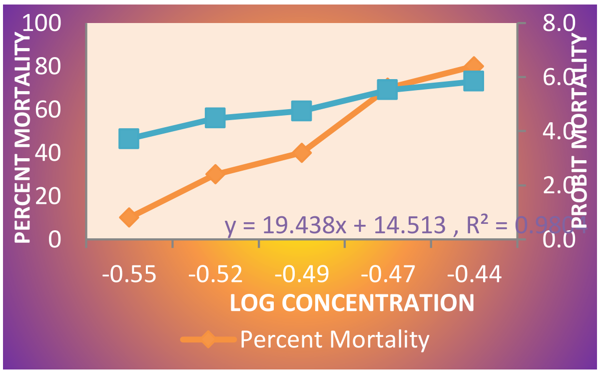

Fig. 10. Graphical representation of 72h LC_{50} value in Static Renewal System for 10% E.C. of Bifenthrin to the fish *Labeo rohita.*

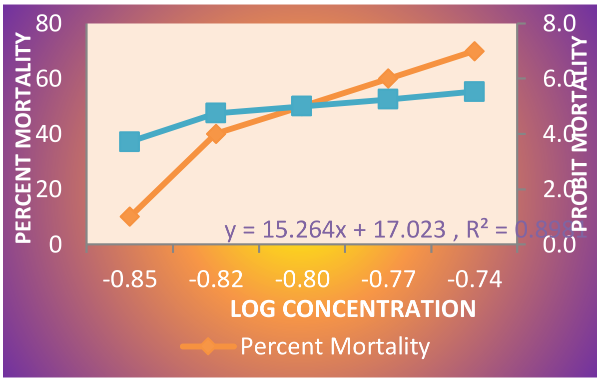

Fig. 11. Graphical representation of 72h LC_{50} value in Continuous Flow through system for Technical Grade of Bifenthrin to the fish *Labeo rohita.*

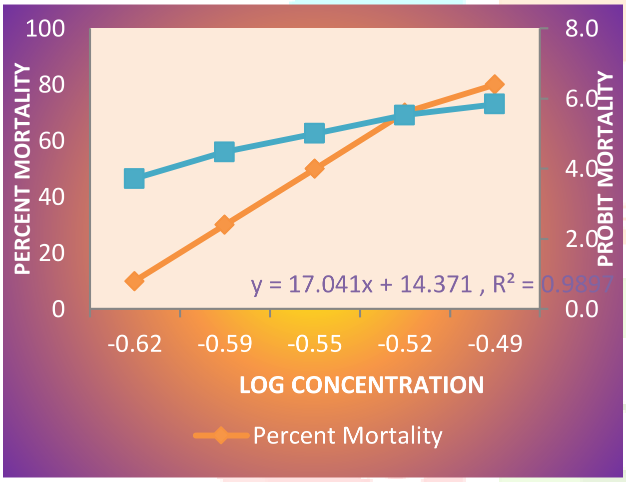

Fig. 12. Graphical representation of 72h LC_{50} value in Continuous Flow through system for 10% E.C. of Bifenthrin to the fish *Labeo rohita.*

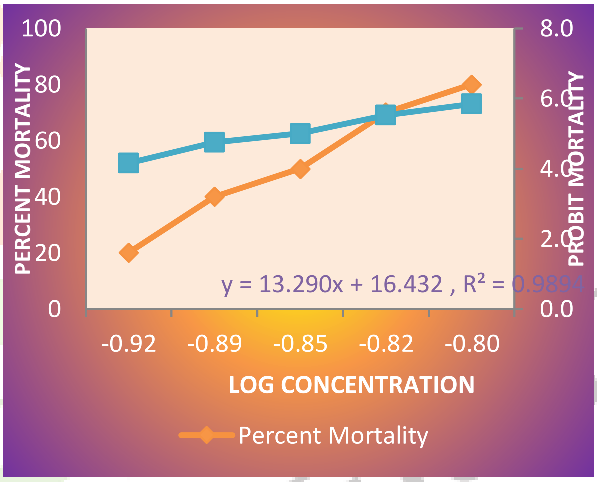

Fig. 13. Graphical representation of 96h LC_{50} value in Static Renewal System for Technical Grade of Bifenthrin to the fish *Labeo rohita.*

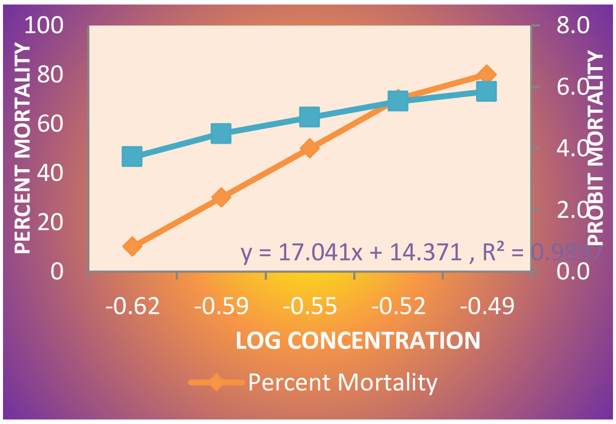

Fig. 14. Graphical representation of 96h LC_{50} value in Static Renewal System for 10% E.C. of Bifenthrin to the fish *Labeo rohita.*

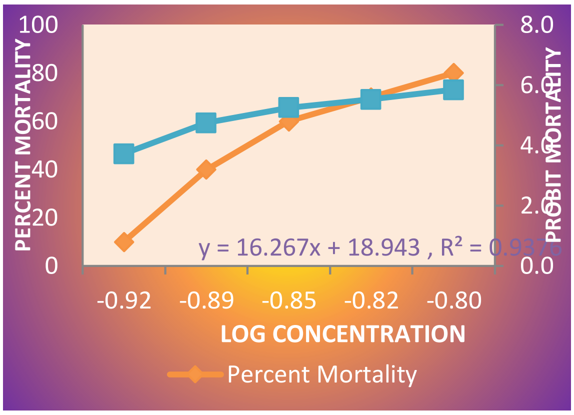

Fig. 15. Graphical representation of 96h LC_{50} value in Continuous Flow through system for Technical Grade of Bifenthrin to the fish *Labeo rohita.*

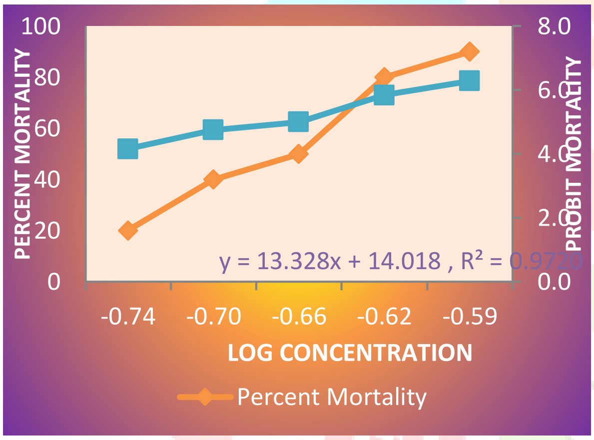

Fig. 16. Graphical representation of 96h LC_{50} value in Continuous Flow through system for 10% E.C. of Bifenthrin to the fish *Labeo rohita.*