TOXICITY OF PERMETHRIN, A SYNTHETIC PYRETHROID TO THE FRESH WATER FISH *CTENOPHARYNGODON IDELLA* (GRASS CARP)

S. Satyanarayana*, G. SrinivasaRao and N. GopalaRao
Department of Zoology and Aquaculture,
AcharyaNagarjuna University, Nagarjunanagar-522 510, A.P., India.

ABSTRACT

Toxicity of a synthetic pyrethroid permethrin, the technical grade (TG) and 25% EC is determined by employing both the static and continuous flow through systemsin the fresh water fish *Ctenopharyngodon idella* (grass carp). The LC_{50} values for 24, 48, 72 and 96 hours of the technical grade are 0.261 µg/L, 0.168 µg/L, 0.114 µg/L and 0.078ug/L respectively and for 25% EC, 0.523 µg/L, 0.332 µg/L, 0.220 µg/L and 0.156 µg/L respectively in the static system. Similarly in the continuous flow through systems (CFTS) the values of technical grade (TG) for 24, 48, 72 and 96 hrs are 0.229 µg/L, 0.131 µg/L, 0.083 µg/L and 0.021 respectively and for 25% EC 0.376µg/L, 0.248 µg/L, 0.141 µg/L and 0.122 µg/L respectively. The static values are higher to CFTS values and 25% EC the commercial formulation is also sufficiently toxic in action and these are duration and concentration reliant. The morphological changes of the experimented fish, in their behavior, that are observed while exposing them to both the toxicants which are also reported here that can be served as indices or indicators of the toxic stress.

Keywords: *Ctenopharyngodon idella*, synthetic pyrethroid, type I, Permethrin, Technical Grade (TG), Emulsifiable Concentration (EC), Static and Continuous flow through systems.

INTRODUCTION

Boon and bane are the two words which aptly suit to the usage of the pesticides in their application, the former is for the control of the pests whereas the later is for their effect on the non-target organisms. It is a fact, in fact not an illusion to think about it being and been the problem of not only locally but globally, that came to a reality of realisation especially after the advent of the 'Silent Spring', a book by Rachel Carson (1962). Latif *et al.* (2013) in their study earlier reported that more than 200 types of the organic pesticides are being used extensively to protect the production of the produce, either terrestrial or even aquatic nature.

Among them, Agnieszka *et al*. (2018) reported that there 42 (Forty two) types of chemicals known as fourth generation pesticides the synthetic pyrethroids which are being divided into type I and II, without a cyano group or with a cyano group respectively. According to the same people these chemicals are sufficiently more in quantity of its usage and being 2250 times more toxic to insects than to mammals of course toxic even to fish. Because of these chemicals usage along with the organochlorines, organophosphates and carbamates, globally, its consumption is being sufficiently alarming in India (Agarwal *et al*. 2010). The aquatic environment is the ultimate sink when released into the surroundings (Kaushik *et al*., 2015) and the act of the defilement envisage the potential toxicity which may be either acute or chronic (Maund *et al*., 2012).
In the aquatic environment, the largest subdivisions of the earth, Hydrosphere where various animal groups are inhabited and the fishes especially, having the more migratory habit, cold blooded, poiklothermic nektonic and gill respiring organisms are sensitive to these chemicals and are the biomarkers of the study in assessing the toxicant effects. (Anilava Kaviraj and Abhik Gupta 2014). Sana Ullah and Zorriehzara (2015), and Murthy *et al*., (2013) in their review articles mentioned how the fish are affected by the pesticides in their respective appraisals. Prusty *et al*. (2015), Hasibur Rehman *et al*. (2014) and Ahrar Khan *et al.* (2014) in their evaluation as review articles on synthetic pyrethroids reported that the aquatic organisms are affected by these 4^{th} generation chemicals, the synthetic pyrethroids. The analysis of their reports as reviews in the assessment also include the permethrin, a synthetic pyrethroid of type I and its effect on the aquatic organisms including the fish. Due to the paucity of the information that is available on the fish *Ctenopharyngodon idella*, the grass carp, the present study is aimed to study the effects of the toxicant. Hence, the main object of the study is to determine the acute toxicity of permethrin technical grade (TG) and 25% EC to the fish to evaluate its potential level of toxic action while observing the changes on the behavior of the fish an ethological concept in the laboratory while exposing to both the toxicants showing its reaction, serving as the indices of the toxic stress..

MATERIALS AND METHODS

The freshwater fish *Ctenopharyngodon idella* (both sexes, length 3-5 cm, weight 4-5 g) have been used as the test organisms for the present investigation. Healthy and active fish were obtained from the local fish farm, at Nandivelugu, a rural area of Guntur district, A.P, India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at room temperature 28±1°C and 12-12 h dark and light cycle. Water was renewed every day. During the period of acclimatization, the fish were fed (ad libitum) with groundnut oil cake and rice bran. Feeding was stopped one day prior to the acute toxicity test. All the precautions recommended by APHA on toxicity tests to the aquatic organisms (APHA, 1995, 2005 and 2012) were followed. If mortality exceeded 5% in any batch of the fish during acclimatization, the entire batch of that fish were discarded. Permethrin technical grade (TG) was supplied by Sudharshan Chemical Industries, Pune and 25% EC was purchased that is also formulated by the same company available from the local market of Guntur, A.P., India.

Physical & Chemical properties of water used for the present experiments are (in mg/L):Turbidity - 8 silica units, Electrical conductivity at 28ºC-8.16 Micro ohms/cm, pH at 28ºC-8.2.**Alkalinity**: Phenolphthalein-Nil, Methyl orange as $CaCO_3$-472, Total Hardness-320, CalciumHardness-80, Magnesium Hardness-40, Nitrite nitrogen (as N)- Nil, Sulphate (As SO_4) - Trace, Chloride (as Cl^-) - 40, Fluoride (as F^-) - I.S, Iron (as Fe) -Nil, Dissolved Oxygen - 8-10 ppm, Temperature - 28±2°C.

Toxicity Test: Experiments were conducted to determine the acute toxicity of the Permethrin in various concentrations of technical and commercial grade formulation of 25%EC with five replicates for each concentration along with the control group. The control groups were added with the equal quantity of acetone that was used for the preparation of the highest working solutions of both the toxicants. The concentrations of the test chemical used in short term definitive tests were in between the lowest concentration at which there was no mortality and the highest concentration at which 100 per cent mortality that was resulted. The data on the mortality rate of fish was recorded for 24h, 48h, 72h and 96 hours. The number of dead fish at each concentration are noted, but took a precaution while experimentation to scoop the dead fish immediately. The toxic tests were conducted to choose the mortality that range from 10% to 90% for the entire duration of the experiments. Finney's probit analysis (Finney, 1971) as recommended by Roberts and Boyce (1972) was followed to calculate the LC_{50} values. The respective values for the mortality as values of probit mortality were taken from the Fisher and Yates (1938). For the determination of the 95% confidence limits, LC_{50} values and a normal variate of 1.96 were taken into consideration.

Further, the data is also inspected and, graphically verified by plotting both the probit verses the log concentration and also percent mortality and log concentration by Finney (1952) using the excel software package. The computer generated output is taken which has also given not only the LC_{50} values, but also lower and upper limits, regression equations, slope and R^2 values and is behold that it is good fit.

RESULTS AND DISCUSSION

The LC_{50} values of both the toxicants to the fish in the static test and continuous flow through system (CFTS) along with upper and lower limits and regression are presentedas the table 1.

Balakrishna Naik *et al.* (2018) reported the toxicity of permethrin and 25% EC to the fish *Cyprinus carpio* and the two toxicants technical grade and 25% EC were both toxic. EPA (2009) mentioned that permethrin which was extensively used is highly toxic though not extremely toxic or least toxic to fish. Hence the above mentioned as references also reiterates the same opinion.

Ibrahim *et al*. (2013) too reported the LC_{50} values in the mediterranian fish, *Solea sengengaiensis.*

Hasibur Rahman*et al*. (2014) while systematically reviewing the pyrethroid toxicity mentioned that due to its low volatility in the air and low polarity in water in their usage plants had advantage when applied for pest control. Being lipophilic, adsorption on the soil as well hydrophilic, absorption also occurs andthis high insecticidal property make them to have more use in application and are all biodegradable. They are divided into two main groups without or with a cyano-group as type I and II respectively. Type I Allethrin, permethrin and type II cypermethrin Fenvalerate, Deltamethrin etc are the examples. The mechanism of action of permethrin which was mentioned in their report because it is hypersensitive to stimuli received from sense organs and sends a 'train of impulses' that can block the sodium channels from outside to inside of the nerve cells in this way the effect was affected. Apart from this, the aquatic organism, especially the invertebrates and fish in majority with a range of less of than 1 ppb concentrations were toxic. Deltamethrin (Type II) of the synthetic pyrethroids is the most toxic, Allethrin (type I) least toxic and Fenvalerate, Cypermethrin (type II) and Permethrin (type I) are intermediately toxic to aquatic organisms All the above opinions are found to be true in the present study results and also in the earlier reports. Srivastava *et al.,* (2016) while reviewing the pesticides toxicity to fish, used the term 'necessary evil' by them in terms of effects of the chemicals and suggested the avoidance in usage and also the judicious rationalism of the application of them into the target sites.

In the fish *Cyprinus carpio* while exposing them to the two different chemicals, Biosal (Phytopesticide) and Permethrin Imrana *et al*. (2009) reported the toxicity values. The 24 hour LC_{50} value is 35.37 ppm (35 µg/L), whereas in the present study had a different methodology that is static, whereas in the present one is also with static apart from the continuous flow through simulating the natural conditions which have resulted the lower values. The study mentioned about the reported value of 24 h LC_{50}, to the fish *Cyprinus carpio,* and also *Atlantic solmon* as 9 µg/L. In the fish *Oreochromis niloticus* LC_{50} value for 72 hours is 54 µg/L, that was also mentioned as the study of Sophinska *et al*., (1995) and 14 days LC_{50} value of permethrin to carps was 11 µg/L, where in the long duration exposure resulted lower concentrations of lethal and can be inferred as from the present study where the values decreased from 24 hours to 96 hours. The toxicity is species specific and duration dependent. The same opinions were expressed in the review articles of Sana and Zorriehzahra (2015) and Murthy *et al*., (2013) also, where in the toxicity of permethrin of the present study along with the other synthetic pyrethroids same is also holds good.

The type I pesticides of pyrethroids induce presynaptic repetitive discharges and type II cause delay in the release of Neurotransmitter causing membrane depolarization. This might be the cause of the toxic action of pesticide, permethrin in the fish. (Prusty et al 2015). The majority of the studies of toxicity evaluation in the fish do not mentioned about the toxic action of mechanism as mentioned by them and in the present study the same might be the reason. Omer Saylor (2016) reported in the fish *Pseudorasbora parva*, that the 96 hr LC_{50} value as 88.252 µg/L employed static method which is different of the methodology of the present study. The toxicant had a variable sensitivity and the report was for invasive species whereas the present studied one is cultivable species.

Reza and Gholamrenza (2012) had a comparative study of the toxicity of the permethrin 25% EC and Monocrotophos 36% to the fish *Cyprinus carpio* of juvenile size group. 56.89 mg/L was the value reported for 96 hrs by the static method and in the present study it is lower for the tested fish that was studied. Even though the definite cause of the death of toxic action is not mentioned in the report but cautioned that being toxic to the aquatic environment, they opined that it should not be contaminated and loaded with pesticides like permethrin and monocrotophos.

Maund *et al*. (2012) in their review of Ecotoxicology of the synthetic pyrethroids mentioned that permethrin range of LC_{50} value was 1.5 – 246 µg/L to the different fish and hinted as unpublished report of compliance service International, Rochester. The death of the fish was due to the disturbance of the endocrine system as the causative mechanism which might also be the cause of death even in the present study.

Agnieszka *et al.* (2018) cautioned about the adverse effects on human beings, due to contamination especially with the three synthetic pyrethroids, permethrin, deltamethrin and alpha cypermethrin and suggested all the three pesticides must be cautiously used. Anilava Kaviraj and Abhik Gupta (2014) opined that the synthetic pyrethroid toxicity studies must be precluded to envisage the directions of the different bio-markers of study in the different fish and the present study of the fish *Ctenopharyngodon idella* would be one among them.

Thatheyus and Selvam (2013) in their report of the synthetic pyrethroids toxicity and biodegradation mentioned that permethrin is highly toxic though not extremely toxic as cypermethrin and cyhalothrin of type II synthetic pyrethroids.

Rice *et al*. (1997) studied and reported thepermethrin toxicity along with other toxicants to the fish *Oryzias latipes* (Old Japanese madaka). Among the studied toxicants, permethrin is the most toxic chemical when compared with chloropyriphos (organo phosphate) 2-4 Dinitrophenol (Fungicide), Strychnine (Alkaloid) and phenol (polar-Narcotic). The values that were reported as 0.024 µg/L for 24 h and 0.011 µg/L for 48 hr. for permethrin in static method. The report also mentioned that 48 h static LC_{50} values of adult pulp fish *Cyprenodon macularius* was 0.05 mg/L, for rainbow trout *Ochorynchus mykiss* was 0.06 mg/L and for western mosquito fish, *Gambusia affinis* was 0.097 mg/L.

They also referred another study and mentioned that the 48h static values as 0.0174 and 0.0321 mg/L for *Onchorynchus mykiss* and for *Pimephales promelas* respectively. According to another study report mentioned by them also to the adult *Oryzias latipes,* the 48h LC_{50} value was 0.06 mg/L. Even though the variation of concentrations with the present study can be due to the species specificity and specific water characteristics that was used in the experiments, the same mechanism of the toxic action that also prevails in the present study. By taking all the data for different fish, it can be used in the direction of formulation of the permissible limits against the standard water characteristics in environmental policy and planning.

Sapana Devi and Gupta (2014) made a comparative study of the type I and II synthetic pyrethroid. Permethrin and Deltamethrin respectively to the fish *Anabas testudineus* , while aiming at the sub lethal effects on growth and feeding reported the LC_{50} values of 24, 48, 72 and 96 hrs as 2.07 mg l^{-1}, 1.41 mg l^{-1}, 1.02 mg l^{-1} and 0.93 mg l^{-1} respectively. The fish has a bimodal way of respiration which resulted to have higher values that can be considered as a resistance in the toxic stress whereas the present studied fish, *Ctenopharyngodon idella* is purely an aquatic respiring one, (one way) that resulted the lesser values due to the toxic stress.

The fish in their study they reported that growth and feeding inhibition was due to the toxic stress, while having bimodal respiration, which can also be attributed with the present studied one because it is cultivable. The report by the same authors mentioned that due to copper intoxication in the fish *Cyprinus carpio* wherein the energy being spent for sustenance rather than to the growth and cessation of feeding due to the hormonal imbalance. The same can be possible even in the present study of experimentation. In the year 2013, using the data available SAGE [http://www.sage pesticides.qc.ca] permethrin was categorized as extremely toxic chemical, which the result of the present study too affirm the same. To combat that better to select pesticides that have the lower risk, control (reduce) the run off as well as the soil erosion by crop rotation and better to have a proper pond management.

Showing the resistance or biodegradability of the toxicant which are the other two important characteristics contributes for the toxicity. To prove this, when a comparative study is attempted and reported by Tiwari and Ansari (2014) by selecting an organochlorine, endosulphan an organophosphate chloropyriphos and a synthetic pyrethroid as toxicantson the fish and finally confirmed it as correct. In the fish *Danierio* (Zebra fish), the reported 96h LC_{50} values for the three toxicants were 0.10, 0.16 and 0.13 µg/L, for endosulphan, chloropyriphos and permethrin respectively and the order toxicity was endosulphan, followed by permethrin and more resistant was chloropyriphos.

According to Melissa Kaplan (2014) report, the use of the synthetic pyrethroids was alarming and when transported into the aquatic environment being carcinogenic and also due to endocrine disruption harm the non-target organisms like fish and the present studied fish is a candidate as one of the cultivable in the aquaculture practices when it is in sub-lethal concentrations can harm them. This was also reiterated by the report of the extoxnet (1993) wherein the permethrin is mentioned as toxic to fish.

Robert *et al.* (1983) reported that the permethrin is toxic to fat head minnows and snails and the range of no effect concentration were between 0.66 and 1.4 µg.

According to Zitko *et al.* (1977) the lethal concentration of Permethrin for Juvenile *Altantic salmon* was 0.9 µg/L and for *Cyprinus carpio* fry was 3.5 µg/L which showed that the fish might be more resistant to Permethrin, as compared to *Atlantic salmon* that is nearly four times more sensitive to the toxicant.

Zitko *et al.* (1979) also reported that the synthetic Pyrethroids have higher toxicity to fishes during exposure. LC_{50} of Permethrin after 72h was 54 µg/L to the fish *Oreochromis niloticus.* AI–Akil *et al.* (1995) reported the LC_{50} for Permethrin against *Oreochromis nilotics* and was far greater than that of *Cyprinus carpio* which may be due to the different levels of tolerance among the different species.

Baker *et al.* (2003) reported that the acute toxicity of Permethrin on guppies *Poecilia reticulate* and the 48h LC_{50} value was estimated as 245.71g/l. The report gave the differential toxicity also to the fish and was in the range of 0.05-97.01g/l.

These findings can be concluded that the Permethrin that was studied is a highly toxic synthetic Pyrethroid pesticide widely used in agriculture and vector control operations as well as in aquaculture. A special attention is drawn to its heavy use in the mosquito control programs of domestic nature which necessitates in the depth study of the acute and chronic toxicity to fish and the other non-target species which have to be undertaken. In addition the potential risk from Permethrin metabolites should also be investigated to get a more complete picture in terms of toxicity.

The mechanism of the lethal action in the fish involves numerous physiological systems including the nervous and respiratory and renal ion regulation and the cumulative action of all of them resulted, the toxic effect (Haya, (1989) & Miyamoto, J. (1976). The very high toxicity of Permethrin to fish might be due to the nature of having with a lower capacity to hydrolyse the toxicant according to Glickman and Lech (1981) study opinion.

Drago *et al.* (2014) reported on the acute Permethrin neurotoxicity. The study mentioned that the type I synthetic pyrethroid which is used on agricultural crops, is highly toxic to animals particularly the fish. The toxicant is being neurotoxic and its main mechanism of action is axonal sodium channel depolarization which causes repetitive nerve impulses. At higher concentration, the pyrethroid, Permethrin causing both *in vivo* and *in vitro* studies increase the levels of acetyl choline and acetyl cholinesterase as well as monoamine oxidase and ATP enzymes all of them were inhibited and also opined that inhibition of GABA receptors producing the excitability and also the convulsions (Prusty *et al* 2015 & Ponepal *et al*., 2010).

According to Johansson *et al.* (2006), the Permethrin is not only toxic to fish but also to frogs. They opined that the negative effects on the growth of the tadpoles during the process of metamorphosis due to the toxic action The study also revealed that the exposure at lower pesticide concentration did not caused an increase in mortality or impaired growth but in higher concentration the effect was observed. According to the review of the report by Vishnu Vardhan (2016) which revealed that the Permethrin not only toxic to most of the warm blooded animals but also to the cold blooded fish. Its toxic action is due to neurotoxic nature which first influence neuron membranes by drawing out sodium channel enactment.

Parent *et al.* (2011) studied and reported the effects of the synthetic pyrethroid insecticide, Permethrin on two estuarine fish species. The fish studied includes Juvenile red drum *Sciaenops ocellatus* and adult mumichhog *Fundulus heteroclitus.* The juveniles are more sensitive than adults. The 96h LC_{50} values of the two fish, tested were 8 and 23 µg/L respectively. They hinted upon not to spray the Permethrin pesticide near the areas of the estuaries.

Kumaraguru and Beamish (1981) reported lethal toxicity of rainbow trout *Salmo gairdneri* Permethrin (NRDC-143).The 96h LC_{50} values varied 0.62 and 6.43 µg/L between 5º and 20ºC. The temperature and size variation of the fish showed difference in the toxicity.

The toxicity is species specific and also the hydrographical conditions of the laboratory that were tested and reported vary and influenced the toxicity.

However, the present study work when compared to the reports of earlier researchers apart from being toxic the static values are higher to continuous flow through system (CFTS) and 25% EC is also sufficiently toxic with other ingredients as 75% causing additive/cumulative/synergistic/effect attributed to the toxic nature.

The studies on the toxicity that were in the literature survey mostly are cold water fish and very few are warm water and the present studied one is for first time among the warm waters of cultivable species (CCME,2006).

BEHAVIOURAL ALTERATIONS

During experimentation, while observing the mortality in 24, 48, 72 and 96 hrs the fish *Ctenopharyngodon idella* behaved abnormally when compared to the fish in the control medium without the toxicant. Mucous secretions on the skin and gills prone to fungal infections, gulping of oxygen with more opercular movements, suffocating with respiratory distress to have a hyper ventilation irregular abrupt erratic jerky and darting movements, a surfacing phenomenon to meet the demand for more oxygen and in long duration of exposure the equilibrium was lost, drowning to the bottom, slime deposition increased and the pale skin color were all observed.

Such findings were similar to the earlier reports of Omer Saylor (2016) in the fish *pseudorasbora parva* induced by permethrin, in the fish *Labeo rohita* induced by Cypermethrin, Khatun *et al.* (2014) and Anitha *et al.* (2010 & 12) in the three major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* induced by Fenvalerate, Yasser Said (2007) in the fish *Oreochromis niloticus* due to intoxification of Deltamethrin, and also by Srinivasa Rao *et al.* (2018) in the fish *Ctenopharyngodon idella* by the Deltamethrin, Balakrishna naik *et al.,* (2018) in the fish *Cyprinus carpio* by Permethrin. Sopinska *et al*. (1995) for permethrin to *Cyprinus carpio* and Velisek *et al.* (2011) cypermethrin as the toxicant in the fish *Oncorynchus mykiss*, Sapna Devi and Gupta (2014) in the fish *Anabas testudenius* for both permethrin and cypermethrin as toxicants.

Not only with synthetic pyrethroids of type I and II mentioned as above even with the three other classes of compounds, organochlorines, organophosphates and carbamates in different fish with different chemicals, such patterns of behaviour were observed that were summarized in review articles of Sana Ullah and Zorriehzara (2015) and Murthy *et al.* (2013).
These observations are due to endocrine disruptions regulated by toxic stress that preclude the toxicity (Scott *et al*. 2011) and such alterations are depicted as biomarkers of the study by Anilava Kaviraj and Abhik Gupta (2014).

Conclusions

Permethrin, the present toxicant of the synthetic pyrethroid of type I studied, due to indiscriminate usage when transported into the aquatic environment depending on the concentration the result may be either acute toxicity, the sudden death and or in sub lethal concentrations delayed effect. The behavior alterations are the signs of toxic action that may lead to a respiratory distress, or haemotological changes, or the biochemical changes in the vital organs of the fish and the tissue deposition of lipophilic nature being absorbed all might added as in the present study resulting the death of fish. Formulations are causing cumulative or additive toxicity as in the present study hence it needs to have a strict quality control to recommend the safe pesticides for use in the agriculture as well as in the aquaculture practices.

REFERENCES

[1] Agarwal, A., Ravi, S. and P.B. Sarma 2010. Water Pollution with special reference to pesticide contamination in India. *J. Water Resource and Protection*. **2**: 432-438.

[2] Agnieszka Chrustek, lgpHolynskaIwan Inga Dziemhowska, Joanna Bougusiewicz, MarcinWroblewski, Anna Cwynar and DorotaOleszewskaStoniana 2018. *Current research on the safety of pyrethroid used on Insecticides. Medicina.***54**: 61-76 pp.

[3] AI, Akil A.S, Shamsi M.J.K. AI and Hyafer, A.V.M. 1995. LC_{50} for Permethrin against *Oreochromisniloticus*versus *Cyprinuscarpio. Journal of King Sand University Sciences,* 7: 235-243.

[4] Anilava Kaviraj and Abhik Gupta 2014. Biomarkers of type II Synthetic pyrethroid pesticides in freshwater fish. *Biomedical Research International*. Article ID 928063. 7 page.

[5] Anita Susan, Sobha, K. and Tilak, K.S. 2010. Studies on biochemical changes in the tissue of *Labeorohita*and *Cirrhinusmrigala*exposed to Fenvalerate, a synthetic Pyrethroid. *J. Toxicol.Environ. Health Sci.,* **2(5):** 53-62.

[6] Anita Susan, T, Sobha, K. and Tilak, K.S. 2012. Toxicity and Histopathological changes in the three Indian major carps, *Catlacatla*(Hamilton*), Labeorohita*(Hamilton)and*Cirrhinusmrigala*(Hamilton). *International Journal of Plant Animal and Environmental Science*, **2(1):** 1-15 pp.

[7] APHA, AWWA and WEF. 2012. Standard methods for the examination of water and waste waler, 22nd Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA.

[8] APHA, AWWA and WEF. 2005. Standard methods for the examination of water and waste waler, 21st Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA.

[9] APHA, AWWA and WEF. 1998. Standard methods for the examination of water and waste waler, 20th Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA.

[10] Ahrar Khan, L. Ahmmad and M.Z. Khan 2012. Haemato biochemical changes induced by pyrethroid insecticides in avian, fish and Mammalian species. *International Journal of Agriculture and Biology.***14**: 834-842.

[11] BalakrishnaNaik, R., N. GopalaRao and G. SrinivasaRao 2018. Toxicity and Behavioural changes in the fresh water. Common carp *Cyprinuscarpio* exposed to permethrin. *Asian Journal of Microbiol. Biotechnology. Env. Science*. **20(4):** 1200-1208.

[12] Baker, Erkoc, Selvi, Kocak 2003. Investigation on acute toxicity of Permethrin on guppies *Poecilia reticulate. Chemosphere*. **51(6**): 469-74.

[13] CCME (Canadian Council of Ministers of the Environment) 2006. A Protocol for the Derivation of Environmental and Human Health Soil Quality Guidelines Supporting technical document. CCME, Winnipeg, pp.1-57.

[14] Drago Bonny, Namrata S. Shah, Samir H. Shah. 2014. Acute Permethrin neurotoxicity: Variable presentations high index of suspicion. *Toxicology Reports*.(1) pp.1026-1028.

[15] EPA 2009. Permethrin Facts, EPA 738. F-09-001.

[16] Extoxnet, The Extension Toxicology Network 1993.A pesticide information project of cooperative Extension officers of Cornell University, Michigan State University, Oregon State University and University of California.

[17] Finney, D.J. 1952. Probit Analysis, 2nd edition, Journal of the institute of Acturies. 78(3): 388-390.

[18] Finney D.J 1971. Probit Analysis, Third Edition, Cambridge University Press, London.303 pp.

[19] Fisher, R.A. and Yates, F. 1938. Statistical tables for biological, agricultural and medical research (3rded.), London: Oliver & Boyd, pp.26-27.

[20] Glickman, A.H., A.A.R. Hamid, D.E. Rickert and J.J. Lech 1981. Elimination and metabolism of permethrin isomers by rainbow trout. *Toxicol. Appl. Pharmacol*. **57**: 88-98.

[21] Hasibur Rehman, Al Thbiani, Shalini Saggu, Zahid Khorshid, Abbar Anand Mohan Abid, Ansari A. 2014. Systematic review on pyrethroid toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology Studies.* **2(6**): 60-70.

[22] Haya, K. 1989. Toxicity of pyrethroid insecticides to fish. *Environ. Toxicol.Chem*. **8**: 381-391.

[23] Ibrahim, A. EI-Elaimy, M.F.F. Baryomy, A.G. Kalief, G.M. Radriguez and J.M. Mancera 2013. Pyrethrid Neurotoxic impacts on medieterranean fish Stress and growth and metabolism. *Egypt Journal Expt. Bio*. **9(1):** 61-71.

[24] Imrana, M.S., M.A. Kazmi, O.B. Lazmi and SNOH, Naqvi 2009. Toxicity of Biosol synthetic pyrethroid against common carp. *Pakistan J. Zool*. **41(3)**: 235-238.

[25] Johansson Markers, Henna Piha, Henrick Kylin and Juha and Juha Merila 2006. Toxicity of six pesticides to common frog (*Ranatemporaria*) tadpoles. *Environmental toxicology and chemistry*. **25(12),** 3164-3170.

[26] KaushikMondal, B. Karnakar and Salma Haque 2015. A Review on effects of pyrethroid pesticides on freshwater fish behavior and fish reproduction. *Journal of global sciences*. **4(6):** 2594-2598.

[27] Khatun, M.A., Rahman, M.R. and Islam. M.S. 2014. Effects of cypermethrin and diazinon on haematology of *Labeorohita* (Hamilton).*Int. Jour. Develop. Res*., **4(5):** 953-957.

[28] Kumaraguru, A.K,, Beamish, F.W.H. 1981. Lethal toxicity of permethrin (NRDC-143) to rainbow trout, *Salmogairdneri*, in relation to body weight and water temperature. *Water Res.* **15:**503–505.

[29] Latif A, Ali, M, Sayyed, A.H,,Iqbal, F, Usman, K, Rauf, M, Kaoser, R. 2013. Effect of copper sulphate and lead nitrate, administered alone or in combination, on the histology of liver and kidney of *Labeorohita. Pak. J. Zoo.***45**: 913-920.

[30] Melissa Kaplan's Herp 2014. Pyrethroids: Not as safe as you think. Melissa Kaplan's Herp Care Collection. *www.anapsid.org/pyrethroids.html*.

[31] Miyamoto, J. 1976. Degradation metabolism and toxicity of synthetic pyrethroid. *Environmental Health Perspectives*, **14,** 15-28.

[32] Maund, S.J., P.J. Campbell, J.M., Gidings, M.J. Hamr K. Henry, E.D. Pilling, J.S. Warington and J.R. Wheeler 2012. Ecotoxicology of Synthetic Pyrethroids. *Current Chem.***34**: 137-166.

[33] Murthy, K.S., Kiran, B.R., Venkateshwarlu, M. 2013. A review on toxicity of pesticides in fish. *International Journal of Open Scientific Research*, **1(1):** 15-36.

[34] Omer Saylar 2016.Toxic effects of permethrin on pseudorashoraparva. *Journal of Environmental Biology*. **37**: 1247-1253.

[35] Parent, L.M, Delorenzo, M.E, Fulton, M.H. 2011. Effects of synthetic pyrethroid insecticide Permethrin on two estuarine fish species. *Journal of Environmental Science Health*, **46(7**): 615-622.

[36] PonepalAlinaPaunesau Maria Crisitiana, Octavian DRACHICI and Alexandra Gabriel Marinescu 2010. Changes of some physiological parameters in several fish species by the action of Talstar insecticide, a synthetic Pyrethroid. Analde University, Biological publication print ISSN: 1224-5119. Tom XVII (1): pp-175-179

[37] Prusty, A.K., Meena, D.K., Mohapatra, S., Panikar, P. Das, P. Gupta, S.K. and Behara, B.K. 2015. Synthetic Pyrethroids type II and fresh water fish culture perils and mitigation: A Review, *International Aquatic Research*. **7**: 163-191.

[38] Rachel Carson (1962). Silent spring, 200 page, Houghton Mifflin-USA. (originally published in 1962 and republished in 2002).

[39] Reza, D. and Gholamreza, A. 2011. Comparative study on the acute Toxicity of synthetic pyrethroidpermethrin 25% and Monocrotophos and Neem Based Pesticide Neem Gold EC 0.03% to Juvenile *Cyprinuscarpio* (Linn.) *J. Biol. Environ. Science.***6(16):** 105-108.

[40] Rice, P.J., Drewes, C.D., Klubertanz, T.M., Bradbury, S.P., Coats, J.R. 1997. Acute toxicity and behavioral effects of chlorpyrifos, permethrin, phenol, strychnine, and 2,4-dinitrophenol to 30-day-old Japanese medeka (*Oryziaslatipes*). *Environ. Toxicol. Chem.***16:** 696–704.

[41] Roberts, M. and Boyce, C.B.C. 1972. Methods in Microbiology (7-A Ed), Norris J.R. and Ribbows, D.W. Academic Press, New York. 479.

[42] Roberts, L., Spehar, Danny, K.T. and B.R. Nordling 1983. Toxicity of synthetic pyrethroid – permethrin to the fish fat head minnow and snails. *Aquatic Toxicology*.**3(2)**: 171-182.

[43] Sapana Devi, M. and Gupta, M. 2014. Acute toxicity of deltamethrin and permethrin and their sublethal effects on growth and feeding in *Anabas testudineus. Int. Research J. of Biological Sciences*. **3(4):** 18-22.

[44] Sana Ullah, Mohammad Jallil Zorriehzahra 2015. Ecotoxicology: A Review of Pesticide Induced Toxicity in Fish. *Advances in Animal and Veterinary Sciences*. **3(1):** 41-57.

[45] Scott, G.R. and Sloman, K.A. 2004. The effect of environmental pollutants on complex fish behaviour integrating behavioural and physiological indicators of toxicity. *Aquatic toxicology*. **68**: 369-392.

[46] Sophinska A Lutinka H and Guz L 1995. Influence of Permethrin on the fish Cyprinus carpio. Medical Wet, 51:747-750.

[47] SrinivasaRao, G., S. Satyanarayana, R. BalakrishnaNaik and N.GopalaRao 2018. Toxicity and effect of technical grade and 11% EC of deltamethrin to the fish *Ctenopharyngodon idella* (Grass carp). *IJCT*. **6**: 10-17.

[48] Srivastava, P., A. Singh and A.K. Pandey 2016. Pesticides Toxicity in Fishes: Biochemical, Physiological and Genotoxic aspects. *Biochemical Cell. Arch*. **16(2):** 199-218.

[49] Thatheyus, A.J. andA. De bor Borah GnanSelvam 2013. Synthetic Pyrethroids: Toxicity and Biodegradation. *Applied ecology and Env. Sciences*, **1(3):** 33-36.

[50] Tiwari Priyanka, AnsariBadreAlam 2014. Comparative Study of Acute Toxicities of Endosulfan, Chlorpyrifos and Permethrin to Zebrafish, Daniorerio (Cyprinidae). *Sch. Acad. J. Biosci.*, **2(7):** 404-409.

[51] Velisek Joseph, AlzbetaStara and ZdenkaSvobodova 2011. The effects of pyrethroid and Triazine pesticides on fish physiology. Chapter 17 Pesticides in the modern world pests control and pesticides exposure and Toxicity Assessment. pp 377-402.

[52] Vishnuvardhan 2016. A short review on Permethrin. *Research and Reviews Journal of Pharmacology and toxicological studies* (*RRJPTS).***4(3):** 94-100.

[53] Yasser Said El-Sayed and T.T. Saad 2007. Subacuteintoxification of a Deltamethrin Based preparation Boutox 5% EC in MonosexNile Tilapia. *Oreochromisniloticus* L. *Basic and Clinical Pharmacology & Toxicology*. **102**: 293-299.

[54] Zitko, V. Carson, W.G. and Metcalfe, C.D. 1977. Lethal threshold of Permethrin for Juvenile Altantic salmon and *Cyprinuscarpio*. *Bull Environ. Contam. Toxicol*, **18**: 35-41.

[55] Zitko, V. Carson, W.G. and Metcalfe, C.D. 1979. Acute toxicity effects of Synthetic Pyrethroids among different fish species. *Bull Environ. Contam. Toxicol*, **21**: 338-334.

Table 1. The LC_{50} values 24, 48, 72 and 96 hrs Regression equation of permethrin Technical grade (TG) and 25% EC in static and continuous flow through system (CFTS) to the fish *Ctenopharyngodon idella*.

LC_{50} (µg/L)					**Regression Equation**			
	Static		**CFTS**		**Static**		**CFTS**	
Hr.	**Technical**	**25% E.C.**	**Technical**	**25% E.C.**	**Technical**	**25% E.C.**	**Technical**	**25% E.C.**
24h	**0.261 (0.252-0.270)***	**0.523 (0.510-0.535)***	**0.229 (0.222-0.236)***	**0.376 (0.361-0.390)***	**y=24.725x + 19.427 (24.725)[1] (19.427)[2] (R^2=0.9834)[3]**	**y=38.686x + 15.898 (38.686)[1] (15.898)[2] (R^2=0.9824)[3]**	**y=27.805x + 22.807 (27.805)[1] (22.807)[2] (R^2=0.9365)[3]**	**y=22.969x + 14.768 (22.969)[1] (14.768)[2] (R^2=0.9702)[3]**
48h	**0.168 (0.161-0.175)***	**0.332 (0.318-0.345)***	**0.131 (0.124-0.138)***	**0.248 (0.233-0.264)***	**y=20.519x + 20.910 (20.519) [1] (20.910) [2] (R^2=0.9689) [3]**	**y=24.846x + 15.470 (21.846) [1] (15.470) [2] (R^2=0.9841) [3]**	**y=15.811x + 18.963 (15.811) [1] (18.963) [2] (R^2=0.9903) [3]**	**y=14.580x + 13.825 (14.580) [1] (13.825) [2] (R^2=0.992) [3]**
72h	**0.114 (0.107-0.121)***	**0.220 (0.208-0.233)***	**0.083 (0.075-0.091)***	**0.141 (0.120-0.166)***	**y=14.692x + 18.884 (14.692) [1] (18.884) [2] (R^2=0.9875) [3]**	**y=16.202x + 15.650 (16.202) [1] (15.650) [2] (R^2=0.9847) [3]**	**y=9.639x + 15.434 (9.639) [1] (15.434) [2] (R^2=0.9807) [3]**	**y=5.136x + 9.369 (5.136) [1] (9.369) [2] (R^2=0.9831) [3]**
96h	**0.078 (0.071-0.086)***	**0.156 (0.144-0.169)***	**0.021 (0.015-0.029)***	**0.122 (0.108-0.137)***	**y=9.2324x + 15.208 (9.232) [1] (15.208) [2] (R^2=0.9518) [3]**	**y=11.169x + 14.018 (11.169) [1] (14.018) [2] (R^2=0.9867) [3]**	**y=2.711x + 9.538 (2.711) [1] (9.538) [2] (R^2=0.9356) [3]**	**y=7.477x + 11.829 (7.477) [1] (11.829) [2] (R^2=0.9824) [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.