Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(6): 194-199

Received: 21-09-2018
Accepted: 23-10-2018

Parvaiz Ahmad Ganie
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India

Dar Jaffer Yousuf
ICAR-Central Soil Salinity Research Institute, Karnal, Haryana, India

Raja Aadil Hussain Bhat
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India

Irfan Ahmad Bhat
College of Fisheries Science, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

Kishor Kunal
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India

PK Pandey
College of Fisheries, Central Agricultural University, Lembucherra, Agartala, Tripura, India

Debajit Sarma
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India

Correspondence
Parvaiz Ahmad Ganie
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, Uttarakhand, India

Investigation of acute toxicity and behavioral response of Indian major carp, *Cirrhinus mrigala* (Hamilton, 1822) in response to Cypermethrin (25% EC)

Parvaiz Ahmad Ganie, Dar Jaffer Yousuf, Raja Aadil Hussain Bhat, Irfan Ahmad Bhat, Kishor Kunal, PK Pandey and Debajit Sarma

Abstract
Environmental protection has fascinated the attention of the varied section of people globally thereby creating global issue amongst researchers and scientists working in this area. Pesticides are toxic to aquatic biota which form important components of the food chain such as fishes. Fishes are important sources of protein in nation-state's diet. So a detailed understanding of the pollutant effect on fishes would be rewarding for fish conservation and fishery development. Cypermethrin, a synthetic pyrethroid pesticide and potential toxic pollutant contaminating aquatic ecosystems, was investigated in the present study for acute toxicity. Indian major carp (*Cirrhinus mrigala*) were selected for the bioassay experiments. Experimental fish were subjected to different concentrations of cypermethrin ranging from 0.40 to 6.40µg/l for 96 hrs in test containers. The static renewal test method of acute toxicity test was used. Water temperature was regulated at 26.8 ± 3° C. In addition, behavioral changes such as loss of schooling behaviour, swimming near the water surface, hyper activity, erratic movements, seizures, loss of buoyancy, darting movements and hitting against the walls of test tanks at each cypermethrin concentration were observed for the individual fish. Data acquired from the cypermethrin acute toxicity tests were evaluated using the arithmetic method of Karber. The 96 hr LC50 value for *Cirrhinus mrigala* was estimated as 4.57µg/l. The possible variation in the LC 50 values can be attributed to toxicant sensitivity, its concentration and duration of exposure.

Keywords: *Cirrhinus mrigala*, LC50, Static renewal bioassay, Cypermethrin, Toxicity

1. Introduction
Environmental stressors and the risks associated with them have always been an intrinsic part of society. Aquatic ecosystems are specifically sensitive to exposure to toxic contaminants. Pollutants either individually or in combination may have sub-lethal effects at the cellular, organ and individual level. Recently, many new broad-spectrum pesticides have been developed that have the potential for widespread use in the environment. In large-scale applications of these pesticides by methods such as crop dusting, orchard and forest spraying or mosquito control, some inevitably enter the aquatic environment [1]. Synthetic pyrethroid insecticides are extensively used in place of organochlorine, organophosphorus and carbamate insecticides to control pests. These insecticides are more likely to be toxic to fish and other aquatic organisms [2-7]. Several larvicides and adulticides, including resmethrin and permethrin, were evaluated for toxicity to measure the effects of mosquito control pesticides on non-target organisms [8]. Many products containing cypermethrin are classified as 'restricted use pesticides' by the United States Environmental Protection Agency due to cypermethrin toxicity to fish. Cypermethrin is classified as a toxicity class II (moderately toxic) chemical, whilst others are designated as toxicity class III (slightly toxic) [9] Non-target organisms such as aquatic invertebrates and fish are extremely sensitive to the neurotoxic effects of these insecticides [10, 11]. Toxic effects of pyrethroids on non-target organisms have been reviewed and reported to be in the microgram toxicity range [4] In both the laboratory and field, absorption of pyrethroids substantially reduces toxicity. Cypermethrin has been classified 'immobile' by the United States Environmental Protection Agency [9] therefore, in the field most of the affected organisms show rapid recovery. At comparable concentrations, pyrethroids have been reported to be up to 1000 times more toxic to fish than to mammals and

birds [12-13]. The hypersensitivity of fish to pyrethroid intoxication is partly due to species-specific differences in pyrethroid breakdown, but primarily to the increased sensitivity of the piscine nervous system to these pesticides [5]. Among the pyrethroids, One such type II pyrethroid, cypermethrin is a widely used pesticide and is among the most effective pyrethroid preparations [14] The underlying mechanism of its effectiveness in fish is similar to that of other pyrethroids containing-cyano- 3-phenoxybenzyl groups. They do so by blocking the sodium channels of nerve filaments, thereby prolonging their depolarisation phase; moreover, they also affect the GABA receptors in the nerve filaments [15, 16]. Cypermethrin, a synthetic pyrethroid, is used to control ectoparasitic infestations in livestock such as cattle, sheep, poultry and other animals. Lately, the compound has been used as a chemotherapeutic agent for controlling ectoparasitic infestations (*Lepeophtheirus salmonis* and *Caligus elongatus*) in marine cage culture of Atlantic salmon, *Salmo salar* [17-21]. Cypermethrin is very toxic for fish (in laboratory tests 96 hr LC50 were generally within the range of 0.4-2.8 µg/l), and in aquatic invertebrates LC50 in the range of 0.01-5 µg/l [22, 23]. Fish sensitivity to pyrethroids may be elucidated by their relatively slow metabolism and elimination of these compounds [15] reported that the half-lives for the exclusion of several pyrethroids by rainbow trout are all longer than 48 h, while elimination half-lives for birds and mammals range from 6 to 12 h.

This study investigates the toxic effects of cypermethrin (25% EC) on Indian carp (*Cirrhinus mrigala*), the standard test species pursuant to [24] and [25] by the determination of 96-hr LC50 values and evaluates behavioral disorders of the fish exposed to different concentrations of the toxicant. This species is commonly found in India, Pakistan, Bangladesh, and other countries. It fulfills most of the requirements of a model species, including availability, throughout the year.

2. Materials and methods

2.1 Test animals

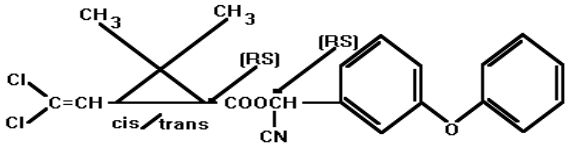
The live specimens of freshwater fish *Cirrhinus mrigala* (Hamilton), family: Cyprinidae and order: Cypriniformes with an average body weight of (7.0 ± 2.0) g at the juvenile life stage, were procured from Shramjeevi Carp Seed Center, Raigad, Maharashtra. Fish were maintained in large holding tanks of 500 L capacity and acclimatized under aerated conditions for 21 days at wet laboratory complex, CIFE, Seven Bungalow Campus. During this period fish were fed with a formulated diet (>35% protein) twice a day. The photoperiod was maintained as per normal day and night approximately 12 hours, and every effort was made to provide an optimal condition for fish; no mortality occurred during this period. All the fish used for any one set of experiment belonged to the same population. Only healthy and adult animals of almost same size were used for experiments, irrespective of sex

2.2 Test chemical

Before exposure, quality of water was tested according to the **[24]**. The pesticide used for the study was commercial grade formulation of a synthetic pyrethroid insecticide, manufactured by Gharda Chemicals Ltd, Maharashtra, India. having 25% EC (w/w) cypermethrin [(RS)-α-cyano-3-phenoxybenzyl(1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2 dimethylcyclopropanecarboxylate] technical grade as active ingredient.

2.3 Test chemical structure

Chemical formula: $C_{22}H_{19}O_3NC_{l2}$



Cypermethrin (8 isomers)

2.4. Physical and chemical properties

Technical cypermethrin varies from a viscous, yellow liquid to a semi-solid crystalline mass at ambient temperatures. Cypermethrin is highly stable to light and at temperatures below 220°C. It is resistant to acidic rather than alkaline media with optimum stability at pH 4. Cypermethrin hydrolyzes under alkaline conditions in a similar way to simple aliphatic esters. Dilute aqueous solutions are subject to photolysis, which occurs at a moderate rate.

Cypermethrin is available in commercial grade as 10% E.C. and 25 % E.C. with trade names Ammo, Avocado, Barricade, CCN 52, Cymbush, Folcord, Imperator, Kafil super, Polytron, Ripcord, Stockade, Cyperkill, Ustaad, Cypermethrin, etc.

2.5 Acute toxicity bioassay

The acute toxicity bioassay was conducted in a static renewal system to determine the 96-hour LC50 value of cypermethrin. Test concentrations were determined on the basis of the range-finding assay, where the lowest value was selected as the highest concentration at which 0% mortality occurred and the highest value as the lowest concentration at which 100% mortality occurred. The test specimens were exposed to seven different nominal concentrations (0.4, 1.4, 2.4, 3.4, 3.50, 4.4, 5.4, and 6.4) µg/L, along with one control (without test chemical).Ten (10) fish were kept in each test concentration in 80 L of water (100-L plastic tub) with complete replacement of test water after every 24 hours. The mean fish-loading ratio in the experimental tanks was 0.875 ± 0.12 g fish/L. No crowding stress was observed during experimentation. Mortality of fish from cypermethrin exposure was recorded up to 96 hours at every 24-hour interval (Table 1). The experiment was repeated thrice, following the recommendations of [24]. Feeding was stopped 24 hours before exposure and fish were also not fed during the experimentation period, as recommended by [26, 27]. No mortality was observed in the control group. For the determination of the LC 50 value of cypermethrin, the arithmetic method of Karber, as reported by [28] was considered (Table 2 and fig 1).

2.6 Physicochemical properties of test water

Physicochemical parameters were measured before fish loading and during experimentation. The temperature of the test water varied from 26.8 ± 3°C and pH value ranged from 7.70 ± 0.80. The dissolved oxygen (DO) ranged from 7.26 ± 1.13 mgL^{-1} and total hardness from 234.66 ± 11.20 mgL^{-1} while as nitrate-N ranged from 0.013±0.031 mgL^{-1} during the experiment.

3. Results

3.1 Acute toxicity

Total mortality observed in different treated groups at 96 hours of exposure is presented in Table 2. An increase in

number of mortalities, with an increase in the concentration of the insecticide, was observed. The 96-hour LC 50 value of Cypermethrin was found to be 4.57 μgL^{-1}.

3.2 Behavioral effects

Fish exposed to Cypermethrin exhibited behavioral abnormalities, such as hyperactivity, random swimming, and loss of equilibrium, increased surface activity. Opercular movements increased initially in all exposure periods but decreased later steadily. At the beginning of the exposure, the initial 30 minutes was a period of hyperactivity, during which fish become restless. During the course of the experiment, they tried to avoid the test water for some time by swimming fast, jumping, darting, hanging vertically in the air and other random movements in treated groups. At higher test concentrations, fish expressed erratic swimming with jerky movements; along with hyperexcitation. They also secreted excessive amounts of mucus, which covered the buccal cavity, body, and gills. Under such conditions, the efficiency of oxygen uptake decreased considerably, which was manifested as enhanced breathing rate along with more frequent visits to surface water for gulping fresh air. Eventually, there was a loss of balance, exhaustion, and lethargy owing to respiratory incumbency. Finally their entire activity decreased, and they settled down at the base of water aquaria and died. Fishes of the control group were free from such behavioral changes.

Table 1: Data on fish survival at different test concentrations and sampling intervals in *C.mrigala*

Concentration (µg/L)	Number of alive fish at different time intervals (hours)				% survival at 96 hours		% mortality at 96 hours	
	24	48	72	96				
0	30	30	30	30	100		0	
0.4	30	30	30	30	100		0	
1.4	30	30	30	30	100		0	
2.4	30	30	30	28	93.24		6.76	
3.4	30	30	30	24	79.92		20.08	
4.4	30	29	26	18	59.94		40.06	
5.4	28	20	17	11	36.63		63.37	
6.4	22	14	4	0	0		100	

Table 2: Determination of LC50 value of Cypermethrin in *C. Mrigala* at 96 hours

Concentration (µg/L)	Concentration difference	Number of alive fish	Number of dead fish	Mean death	Mean death * Concentration difference
0	0	30	0	0	0
0.4	0.4	30	0	0	0
1.4	1	30	0	0	0
2.4	1	28	2	1	1
3.4	1	24	6	4	4
4.4	1	18	12	9	9
5.4	1	11	19	16.5	16.5
6.4	1	0	30	24.5	24.5
SUM(∑)					**55**

$$LC_{50} = LC_{100} - \frac{\sum(\text{Mean death} * \text{Conc. Difference})}{\text{No. of fish taken}} = 6.4 - (55/30) = 4.5667 = 4.57\ \mu g/L$$

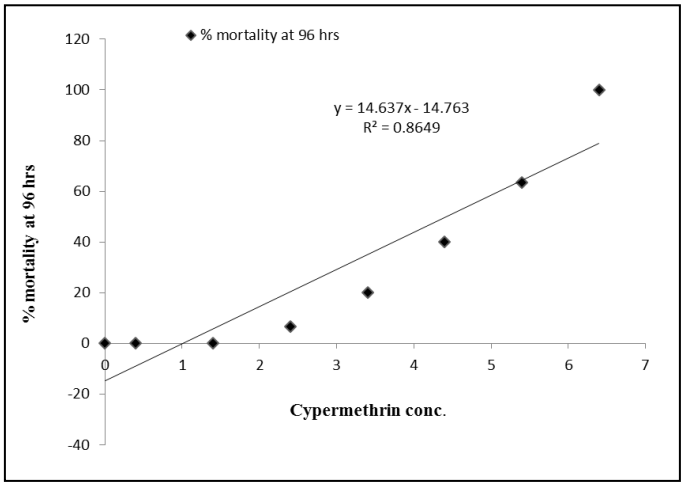


Fig 1: Graphical representation of LC 50 96 hrs of *C. mrigala* in response to Cypermethrin

4. Discussion

The acute test for a long time has been a major component in toxicity testing. In which acute chemical toxicity is determined as a 96hr LC 50 value however, the environmental significance of the death of individuals after short-term exposure to high concentration is questionable. In contrast to this our result depicts that cypermethrin is very toxic even at low concentration 4.57 µg L^{-1}96 hr LC 50

Lethality in the present study is almost similar to the other reported studies on the same or similar compounds carried out on fish species. The 96 hr LC 50 for cypermethrin in few studies reported are 0.139 mg L^{-1} in *Labeo rohita* [29]. 063 mg L^{-1} in *Clarias gariepinus* [30], 41.786µg L^{-1}in *Oncorhynchus mykiss* [31], 4.0 µg L^{-1} in *Labeo rohita* [32], 5.13 µg L^{-1} in *Cirrhinus mrigala* [33] 0.00050 ml/l. in *Channa punctatus* [34], 0.00022 ml/l in *Heteropneustes fossilis* [35]. The difference in LC 50 values in different species can be attributed to the differential ability of the fish species to withstand and metabolize the cypermethrin toxicant. Also the variation may be due to species, size, age and sex of test animals, the condition of the animal, water temperature, water quality, and duration of exposure and pesticide formulation. Therefore, several LC50 values may exist for the same pesticide for even same species of fish. The acute toxicity treatments exhibited strong negative effects on survival as toxicant concentration increases. This suggests dose-dependent Survival and concentration graded lethality (Table 1), the varying degree of

mortality reported in this study is consistent with the report of [36] who reported the differences in an organisms biological adjustment and behavior response to change in water chemistry.

The behavioural study gives direct responses of the fish to the pesticides and related chemicals. [37] And [38] commented that the behavioural activities of an organism represent the final integrated results of a diversified biochemical and physiological processes. Changed behavioral responses can be taken as an index of the stress sensed by the fish exposed to cypermethrin, by which they try to lower the additional entry of cypermethrin present in the medium or minimize damage to their body tissues. Similar behavioral changes were also observed in guppy fish *Poecilia reticulata*, after exposure to cypermethrin [39, 40] and permethrin [41] *Channa punctatus* while exposed to mercuric chloride, profenofos and malathion [42, 43] hexavalent chromium [44], and cypermethrin and λ-cyhalothrin [45]. Fish of control group were free from such behavioural changes, thereby indicating cypermethrin to be responsible for above-altered behavior and fish mortality.

Increase in the opercular movement was initially observed but later decreased with the advancement of exposure period. They slowly became lethargic, restless, and secreted excess mucus all over the body. Recurrently some of the fish were hyper excited resulting in erratic movements. Excess secretion of mucous in fish forms a non-specific response against toxicants, thereby probably reducing toxicant contact. It also forms an obstruction between the body and the toxic medium, so as to minimize its irritating effect, or to absorb it through epidermal mucus. Similar observations were made by [46] and [47]. In the toxic environment fish exhibited irregular, erratic, darting swimming movements and loss of equilibrium followed by hanging vertically in the water. The above symptoms are due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ensuing hyper stimulation. And inhibition of AChE activity is a typical characteristic of organophosphate compounds [48-49].

The surfacing phenomenon of fish observed under cypermethrin exposure might be due to the hypoxic condition of the fish as reported by [50]. The increased surfacing during the initial periods of exposure to cypermethrin concentrations suggests an elevated rate of metabolism. Variations in ventilation rate and surfacing frequencies are the general symptoms observedin the fish after exposure to the pesticide and these activities help the fish to avoid contact with poison and fight against stress. The preceding behavioral abnormalities of the fish and subsequent death imply that the toxic effect is mediated through the disturbed nervous/cellular enzyme system affecting the respiratory function and nervous system, which involves control of almost all vital activities [51-53, 42].

5. Conclusion

In this study the atypical changes in the fish exposed to a lethal concentration of cypermethrin were time-dependent. The results of present work revealed that cypermethrin is indeed highly toxic to young *Cirrhinus mrigala.* The toxicity of cypermethrin on fish increased with increasing concentration and exposure time. Behavioral characteristics are obviously sensitive indicators of toxicant's effect. It is necessary, however, to select behavioral indices of monitoring that relate to the behavior of the organism in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in the natural system. Hence this type of study can be valuable to compare the sensitivity of the various species of aquatic animals and potency of chemicals using LC 50 values and to derive safe environmental concentration, by which there is minimum lethality and stress to the animals.

6. Acknowledgment

The authors are indebted to, Director ICAR-Central Institute of Fisheries Education, Mumbai, India, for providing support and necessary facilities for carrying out this experiment.

7. Conflict of interest

The authors declare that there is no conflict of interest.

8. References

1. Environment Agency. Pesticides in the Aquatic Environment. Update of the Report of the National Rivers Authority, National Centre for Toxic and Persistent Substances; Water Quality, 1997, 26.
2. Elliot M. Synthetic Pyrethroids. American Chemical Society, Washington, 1977, 1-28.
3. Casida JE, Gammon DW, Glickman AH, Lawrence LJ. Mechanism of pyrethroid insecticides. Annual Reviews in Pharmacology and Toxicology. 1983; 23:413-418.
4. Smith TM, Stratton GM. Effects of synthetic pyrethroid insecticides on nontarget organisms. Residue Reviews. 1986; 97:93-119.
5. Moore A, Waring CP. The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). Aquatic Toxicology. 2001; 52:1-12.
6. Polat H, Erkoc FU, Viran R, Kocak O. Investigation of acute toxicity of beta-cypermethrin on guppies, *Poecilia reticulata.* Chemosphere. 2002; 49:39-44.
7. Ko pru cu K, Aydin R. The toxic effects of pyrethroid deltamethrin on common carp (*Cyprinus carpio* L.) embryos and larvae. Pesticide Biochemistry and Physiology; 2004; 80:47-53.
8. United States Environmental Protection Agency. Pesticide Fact Sheet Number 199: Cypermethrin. Office of Pesticides and Toxic Substances, Washington, DC, 1989, 2-9.
9. Milam CD, Farris JL, Wilhide JD. Evaluating mosquito control pesticides for effect on the target and nontarget organisms. Archives of Environment Contamination Toxicology. 2000; 39:324-328.
10. Reddy ATV, Ayyanna K, Yellamma K. The sensitivity of brain cholinesterase to cypermethrin toxicity in freshwater teleost *Tilapia mossambica*. Biochemistry International. 1991; 23(5):959-962.
11. Philip GH, Reddy PM, Sridevi G. Cypermethrin-induced *in vivo* alterations in the carbohydrate metabolism of freshwater fish, *Labeo rohita*. Ecotoxicology Environment Safety. 1995; 31:173-178.
12. Edwards R, Millburn P, Hutson DH. Comparative toxicity of cis-cypermethrin in rainbow trout, frog, mouse and quail. Toxicology and Applied Pharmacology. 1986; 84:512-522.
13. Bradbury SP, Coats JR. Comparative toxicology of the pyrethroid insecticides. Rev. Environmental. Contamination and Toxicology. 1989; 108:133-177.
14. Bradbury SP, Coats JR. Comparative toxicology of the pyrethroid insecticides. Bulletin of Environmental Contamination and Toxicology; 1989a; 108:134-177.

15. Bradbury SP, Coats JR. Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. Environmental Toxicology and Chemistry. 1989b; 8:373-380.
16. Hayes AW. Principles and Methods of Toxicology. Raven Press, New York; 1994, 1468.
17. Richards RH. Diseases of farmed fish: Salmonids. Veterinary Records. 1983; 112:124-126.
18. Roth M, Richards RH, Sommerville C. Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. Journal of Fish Diseases. 1993; 16:1-26.
19. Hart JL, Thacker JRM, Braidwood JC, Fraser NR, Mattews JE. Novel cypermethrin formulation for the control of sea lice on salmon (*Salmo salar*). Veterinary Records. 1997; 140:179-181.
20. Boxaspen K, Holm JC. The development of pyrethrum-based treatments against the ectoparasitic salmon lice *Lepeophtheirus salmonis* in sea cage rearing of Atlantic salmon Salmo salar L. Aquaculture Research. 2001; 32:701-707.
21. Treasurer JW, Wadsworth SL. Interspecific comparison of experimental and natural routes of *Lepeophtheirus salmonis* and *Caligus elongatus* challenge and consequences for the distribution of chalimus on salmonidsand therapeutant screening. Aquaculture Research. 2004; 35:773-783.
22. Sthephanson RR. Aquatic toxicology of cypermethrin.I. Acute toxicity to some freshwater fish and invertebrates in laboratory tests. Aquatic Toxicology; 1982; 2:175-185.
23. Sarkar B, Chatterjee A, Adhikari S, Ayyappan S. Carbofuran- and cypermethrin-induced histopathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery. Journal of Applied Ichthyology. 2005; 21:131-135.
24. APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater, Washington, DC, 1998.
25. OECD (Organisation for Economic Co-operation and Development). OECD Guidelines for Testing of Chemicals. OECD, Paris, 1993.
26. Ward GS, Parish PR. Toxicity tests. In: Manuals of methods in aquatic environment research, part 6. FAO Fish Technical paper. 1982; 185:1-23.
27. Reish DL, Oshida PS. Short-term bioassay. In: Manuals of methods in aquatic environment research, part 6. FAO Fish Technical Paper; 1987; 246:1-62.
28. Dede EB, Kaglo HD. Aqua-toxicological effects of water-soluble fractions (WSF) of diesel fuel on *Oreochromis niloticus* fingerlings. Journal of Applied Science and Environmental Management. 2001; 5:93-96.
29. Das BK, Mukherjee SC. Toxicity of cypermethrin in Labeo rohita fingerlings: biochemical, enzymatic and hematological consequences. Comparative Biochemistry and Physiology; 2003; 134:109-121.
30. Ayoola SO, Ajani EK. Histopathological Effects of Cypermethrin on Juvenile African Catfish (*Clarias gariepinus*). World Journal of Biological Resources. 2008; 1:1-14.
31. Küçükbay FZ, Dörücü M, Yazlak H. Acute Toxicity of Synthetic Pyrethroid Cypermethrin to the Young Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792).Asian Journal of Chemistry. 2009; 21(3):1801-1806.
32. Marigoudar SR, Ahmed RN, David. Cypermethrin induced respiratory and behavioral responses of the freshwater teleost, *Labeo rohita* (Hamilton).Veterinarski arhiv. 2009; 79(6):583-590.
33. Veni SMS, Veeraiah K. Toxicity effect of cypermethrin (10% EC) to the freshwater fish *Cirrhinus mrigala* (Hamilton).International Journal of Pure and Applied Zoology. 2014; 2(4):365-372.
34. Zahra K, Yadav S, Tanya, Jyoti, Deeksha, Sandeep, Deepti. Assessment of acute toxicity of cypermethrin and its mitigation by green tea extract in fresh water fishes, *Channa punctatus.* IAJPS. 2016; 3(4):374-378.
35. Singh A, Zahra K. LC 50 assessment of cypermethrin in *Heteropneustes fossilis*: Probit analysis. International Journal of Fisheries and Aquatic Studies. 2017; 5(5):126-130.
36. David M, Philip GH. Accumulation of fenvalerate and induced alteration in Lactate andSuccinate dehydrogenizes activity in *Labeo rohita*. Journal of Ecophysiological and Occupational Health. 2005; 5(3, 4):207-211.
37. Radhaiah V, Girija M, Rao KJ. Changes in selected biochemical parameters in the kidney and blood of the fish, *Tilapia mossambica* (Peters), exposed to heptachlor. Bulletin of Environment Contamination and Toxicology. 1987; 39:1006-1011.
38. Warner RE, Peterson KK, Burgman L. Behavioral pathology in fish. A quantitative study of sub-lethal pesticide toxication. Journal of Applied Ecology. 1966; 3:223-242.
39. Kakko I, Toimela T, Tahti H. The synaptosomal membrane-bound ATPase as a target for the neurotoxic effects of pyrethroids, permethrin and cypermethrin. Chemosphere. 2003; 51(6):475-480.
40. Li X, Ping X, Xiumei S, Zhenbin W, Liqiang X. Toxicity of cypermethrin on growth, pigments, and superoxide dismutase of *Scenedesmus obliquus*. Ecotoxicology and Environmental Safety. 2005; 60(2):188-192.
41. Baser S, Erkoc F, Selvi M, Kocak O. Investigation of acute toxicity of permethrin on guppies *Poecilia reticulata.* Chemosphere. 2003; 51(6):469-474.
42. Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK, Verma MS, *et al*. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety. 2005; 61:114-120.
43. Pandey AK, Nagpure NS, Sunil P, Trivedi Kumar R, Kushwaha B, Lakra WS *et al*. Investigation of acute toxicity and behavioral changes in *Channa punctatus* (Bloch) due to organophosphate pesticide profenofos. Drug and Chemical Toxicology. 2011; 34(4):424-428.
44. Mishra AK, Mohanty B. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). Environmental Toxicology and Pharmacology. 2008; 26:136-141.
45. Kumar A, Sharma B, Pandey RS. Preliminary evaluation of the acute toxicity of cypermethrin and λ-cyhalothrin to *Channa punctatus*. Bulletins of Environment Contamination Toxicology. 2007; 79:613-616.
46. Tripathi PK, Srivastava VK. Toxic effects of Dimethoate (organophosphate) on metabolism and enzyme system of freshwater teleost fish *Channa punctatus*. Asian Fisheries Society, Manila, Philippines, Asian Fisheries Science.

2003; 16:349-359.
47. Rao JV. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus.* Pesticide Biochemistry and Physiology. 2006; 86:78-84.
48. Dembele K, Haubruge E, Gaspar C. Concentration effects of selected insecticides on brain acetylcholine in the common carp *Cyprinus carpio*. Ecotoxicology and Environmental Safety; 2000; 45:49-54.
49. Timchalk C, Nolan RJ, Mendrala AL, Dittenber DA, Brzak KA, Mattsson JL *et al*. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans", Toxicology Science. 2000; 66(1):34-53.
50. Radhaiah V, Jayantha RK. Behavioral response of fish, *Tilapia mossambica* exposed to fenvalerate. Environment Ecology. 1988; 6(2):2-23.
51. Desaiah D, Koch RB. Inhibition of ATPase activity in channel catfish brain by ketone and its reduction product. Bulletins of Environment Contamination Toxicology. 1975a; 13:153-158.
52. Desaiah D, Koch RB. Toxaphene inhibition on ATPase activity in catfish, *Ictalurus punctatus*, tissues. Bulletins of Environment Contamination Toxicology. 1975b; 13:238-244.
53. Bansal SK, Murthy RC, Chandra SV. The effects of somedivalent metals on cardiac and branchial Ca^{2+} ATPase in a freshwater fish Saccobranchus fossils. Ecotoxicology and Environmental Safety. 1985; 9:373-377.