



International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2021; 9(1): 414-419

© 2021 IJFAS

www.fisheriesjournal.com

Received: 14-11-2020

Accepted: 26-12-2020

Clara Bindu F

Unit of Toxicology, Department
of Zoology, Bharathiar
University, Coimbatore, Tamil
Nadu, India

Anila P Ashokan

Unit of Toxicology, Department
of Zoology, Bharathiar
University, Coimbatore, Tamil
Nadu, India

Sutha J

Unit of Toxicology, Department
of Zoology, Bharathiar
University, Coimbatore, Tamil
Nadu, India

Dr. Ramesh M

Professor, Unit of Toxicology,
Department of Zoology,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Corresponding Author:

Dr. Ramesh M

Professor, Unit of Toxicology,
Department of Zoology,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Sub-lethal toxicity of alphamethrin on biochemical indices in a freshwater fish, *Cyprinus carpio*

Clara Bindu F, Anila P Ashokan, Sutha J and Dr. Ramesh M

DOI: <https://doi.org/10.22271/fish.2021.v9.i1e.2428>

Abstract

The focus of the study is to evaluate the effects of a pyrethroid pesticide, alphamethrin on freshwater fish *Cyprinus carpio*. The LC₅₀ of alphamethrin for 96 h was determined as 6 µg/L. For sub-lethal study, 1/10th (0.6 µg/L-Treatment I) and 1/5th (1.2 µg/L-Treatment II) of 96 h LC₅₀ concentration were exposed to *C. carpio* for 35 days. At each interval period, biochemical responses such as plasma glucose, protein and muscle glycogen were determined. During the study period, plasma glucose level was significantly increased with decrease in plasma protein in both the treatment groups. The muscle glycogen content in the fishes treated with 0.6 µg/L and 1.2 µg/L of alphamethrin were also observed to decrease significantly. The overall study concludes that alphamethrin at sublethal concentration affects the biochemical indices of *Cyprinus carpio*. The changes in these biochemical indices can be effectively used to monitor the alphamethrin pesticide in the aquatic environment.

Keywords: Pesticide, synthetic pyrethroids, sub-lethal toxicity, freshwater fish, glycogen

1. Introduction

Pollution has been regarded as one of the most severe menaces to the environment, including heavy metals, pesticides, sewage, oil, etc. in both freshwater and marine environment^[1]. Over the world-wide ranges, pyrethroids pesticides have a high biological action and low rapid toxicity compare to the other pesticides (organochlorine and organophosphate)^[2]. However, the concentration of residues in aquatic environments has increased with the wide application of pyrethroids pesticides, threatening the health of the aquatic animals^[3,4]. With the restriction of organophosphorus pesticides, synthetic pyrethroids have begun to occupy the market, accounting for 38% of the global pesticide market^[5]. Of these pyrethroid insecticides, alphamethrin is extensively used for a wide range of crops in agriculture because it causes high toxic effect on a broad spectrum of insect pests. Alphamethrin is commonly and effectively used on cotton, cereals, pulses, vegetables, fruits, oilseeds, tea, tobacco and other crops. Although alphamethrin is considered safe for human beings and mammals, long-term and large-scale use of it poses a potential hazard to both natural environment and non-target organisms.

The synthetic pyrethroid insecticide alphamethrin is not only used in agricultural practices during crop production, but also in public health programs and as an ectoparasiticide in animals. This account for 30% of global pesticide consumption^[6]. Alphamethrin have been reported to damage vital organs of various target and non-target species^[7], also reduces reproductive ability^[8] in fishes. Sarikaya^[9] has reported that the 96 hr LC₅₀ value for Nile tilapia was found to be 5.99 µg/L showing that it is a highly toxic synthetic pyrethroid pesticide. The seepage of this insecticide into water bodies, enter the food chain and leads to its accumulation in the tissues of the aquatic organisms of higher tropic levels like fish. The toxicity of alphamethrin in fish can be due to rapid absorption through gills and, the lacking of enzymatic system to hydrolyse these pesticides^[10]. Alphamethrin was found to cause changes in reproductive and oxidative metabolism of *Lymnaea acuminata*^[11] which may be due to inhibition of sodium channels of cells.

Fish accumulate various fold higher concentration of chemical pesticide residues than the surrounding water in aquatic environment. Severe contamination of aquatic environment by chemical pesticides can cause chronic and acute poisoning of fish and other organisms^[12-14].

The accumulated pesticide damage skeletal system, various vital organs of fish and cause biochemical alterations in fish. The pesticide hazard to aquatic organism is further increased by biomagnification of the synthetic pesticides from water by aquatic organisms [15]. The pesticide causing neurotoxicity, biochemical changes, hormonal alteration, reproductive toxicity, developmental abnormalities. The low solubility of synthetic pesticides greatly contributes to their high concentration in various fish and finally bioaccumulation in human body on consumption of these biopesticide contaminated fish. Despite their low availability, pesticides exhibit high toxicity to animals and humans their presence in food raises various safety issues [16].

Blood is an excellent medium of intercellular transport. It acts as a pathophysiological reflector of the health status of animals exposed to the toxicant and other conditions [17, 18]. In general, biochemical parameters are extensively used as sensitive biomarkers for detecting the potential adverse effects of different contaminants [19-21]. Among them, glucose and total protein are commonly used as strong indicators of toxic stress, induced by environmental pollutants, and general health and nutritional status of fish. Proteins play a vital role in architecture, physiology, and metabolism. During stressful condition, fish typically use their body protein, converting it into energy to cope with the energy demand. In this sense, proteins are considered an alternative source of energy, along with glucose, to meet the increased energy demands of fish under stress conditions [22]. Another parameter utilized to measure stressful levels of pollutants, which also serves as an indicator of stress in fish is muscle glycogen [23-25]. Biochemical descriptions of fish and other aquatic organisms under pollution stress serve as important biomarkers in aquatic ecosystem control [26, 27].

The common carp *Cyprinus carpio* is the most extensively cultivated fish throughout the world. The fish is also a very good model for ecotoxicological research because of its availability throughout the season and easy acclimatization to laboratory conditions. Hence, the objective of the present study was to investigate the changes in biochemical indices such as plasma protein, glucose and muscle glycogen in *Cyprinus carpio* exposed to sublethal concentration of alphamethrin.

2. Materials and Methods

2.1 Chemicals and reagents

Chemicals of analytical grade (> 98%) were purchased from Loba Chemie Pvt. Ltd, Mumbai, India (reagents and chemicals).

2.2 Animal and Maintenance

Fingerlings of *Cyprinus carpio* (4.3 ± 0.4 g and 6.7 ± 0.6 cm) were acquired from the Tamil Nadu Fisheries Development Corporation, Aliyar, Tamil Nadu, India. Fingerlings cultured from the same brood stock were collected. In the laboratory, the fish were stocked in a large cement tank (1000 L capacity) before the commencement of the experiment. The water quality parameters of tap water were determined according to APHA [28] and are as follows: temperature $26.6 \pm 1.5^\circ\text{C}$, pH 7.0 ± 0.1 , dissolved oxygen 7.1 ± 0.02 mg/L, and total hardness 17.5 ± 0.4 mg/L. During the acclimation period, the fish stock was maintained under natural photoperiods and ambient temperature. The fish were fed ad libitum with rice bran and ground nut oil cake in dough form once in day before the water was replaced. The water was renewed daily

for the removal of excess feed and faecal matter. All experiments were performed in compliance with relevant laws and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3 Determination of LC₅₀ and Sublethal toxicity study

In the present study, preliminary toxicity tests were carried out to find out median lethal tolerance limit of fish *Cyprinus carpio* to alphamethrin for 96 h. Various concentration of alphamethrin (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 µg/L) along with control (toxicant free) were maintained and was recorded at the end of 24, 48, 72 and 96 h. Based on the mortality/survival of fish the 96 h LC₅₀ for alphamethrin was found to be 6 µg/L.

For sublethal toxicity, three glass aquaria of 100 L capacity were taken and filled with 100 L of water and 50 fishes were transferred into each glass tanks. One tank served as control (toxicant free) and for the sublethal toxicity studies, 1/10th (0.6 µg/L-Treatment I) and 1/5th (1.2 µg/L-Treatment II) of 96 h LC₅₀ concentration of alphamethrin were studied for a period of 35 days with 7 days intervals. Four replicates were maintained for the treated and control groups. Water was changed daily to avoid faecal and feed accumulation.

2.4 Collection of blood and tissues

At the end of every 7 days intervals (7th, 14th, 21st, 28th and 35th day), 10 fishes from each group were euthanized and the blood were collected by cardiac puncture and expelled immediately into separate heparinized plastic vials kept on ice. The collected blood was centrifuged at 10,000 rpm for 10 mins at 4°C to separate the plasma, which was used for the estimation of plasma glucose and plasma protein. Simultaneously, the muscles were dissected, homogenized with 1 mL of 0.1 M Tris-HCl buffers (pH 7.5) in a Teflon homogenizer, and then centrifuged at 1000 rpm at 4°C for 15 min. The supernatant was used to determine the glycogen content in the muscles.

2.5 Estimation of plasma glucose and protein

Plasma glucose was estimated by O-Toluidine method [29]. To 0.1 mL of plasma, 5.0 mL of O-Toluidine reagent was added, mixed well and kept in water bath for 10 min. The samples cooled under tap water and the optical density (OD) of the test samples were measured at 630 nm and expressed as mg/100 mL. Plasma protein was estimated according to the method of Lowry *et al.* [30]. To 0.10 mL of plasma, 0.90 mL of distilled water was added, mixed well and treated with 5 mL of solution C [50 mL of solution A (2% sodium carbonate in 0.1 N NaOH) and 1 mL of solution B (500 mg of copper sulphate in 1% sodium potassium tartarate solution)]. The mixture was allowed to stand for room temperature for 10 min. To this, 0.5 mL of Folin-phenol reagent was added and the colour intensity was read at 720 nm. The protein contents were expressed as µg/mL.

2.6 Estimation of Glycogen

Glycogen content in the muscles was estimated using Anthrone method [31]. To 1 mL of the supernatant, 5 mL of ethanol was added and kept in refrigerator overnight for complete precipitation of glycogen. The contents were centrifuges at 2500 rpm for 15 min and the residue was dissolved in 1 mL of distilled water. To this 1 mL of glucose standard and 10 mL of anthrone reagent was added. The mixture was kept in a boiling water bath for 15 min, cooled

down and the colour intensity was measured at 620 nm. The presence of glycogen was expressed mg of glycogen/g wet wt. of tissue.

2.7 Statistical analysis

All values were expressed as mean \pm S.E. One-way ANOVA (analysis of variance) followed by Duncan multiple range test (DMRT) was employed to determine the significance of the samples between control and alphamethrin treated groups were evaluated by. Different alphabets represent the significance levels at $p < 0.05$.

3. Results and Discussion

Chemicals such as pesticides and fertilizers accumulate in soil or water bodies. Exposure to over permissible limits of these chemicals is associated with mortalities, various diseases, and

diverse genetic alterations and disorders in non-target organisms including humans, terrestrial, and aquatic animals [32]. For this reason, biomarkers are considered valuable tools to evaluate the general health of aquatic organisms and ecosystems [33]. Among the biomarkers, glucose and protein levels are considered as important stress indicators of toxic stress causing xenobiotics in the environment [34].

Changes in glucose level of fish *Cyprinus carpio* treated with Treatment I and II of alphamethrin for 35 days are given in Fig. 1. During the above treatment period glucose level was elevated significantly ($p < 0.05$) both in Treatment I and II. In Treatment I, the increase during the study period remained more or less the same with a percent decrease of 57.68, 61.89, 54.26, 40.34 and 47.59 at each exposure period. In Treatment 2, it was noted a percent increase of 54.28, 34.26, 47.02, 26.98, and 21.33 on each sampling period.

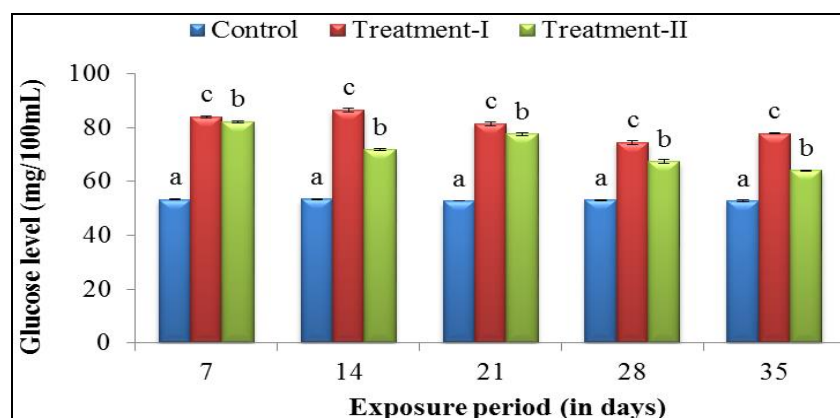


Fig 1: Glucose level of *Cyprinus carpio* exposed to different sublethal concentrations (0.6 $\mu\text{g/L}$ – Treatment I; 1.2 $\mu\text{g/L}$ – Treatment II) of alphamethrin for 35 days. All the values are expressed as means \pm SE. The values are indicated by different letters are significantly different from one another ($P < 0.05$) according to DMRT.

Blood glucose level is a widely employed biomarker in toxicological and chemical risk assessment studies. The glucose levels of fish *Cyprinus carpio* treated with alphamethrin significantly ($p < 0.05$) increased in both the sublethal concentration due to the effected gluconeogenesis or glycogenolysis. *Cyprinus carpio* exposure to deltamethrin lead to hyperglycemia [35] and cypermethrin caused hyperglycemia in some freshwater fish species [36]. Ullah *et al.* [37] observed an increase in the blood glucose level was observed in the silver carp (*Hypophthalmichthys molitrix*) treated with 2 $\mu\text{g/L}$ of deltamethrin. Under stressed condition, the release of glucocorticoids and catecholamine

(hyperglycemic hormones) degrades the glycogen and glucose, which further leaks out in the blood resulting in hyperglycemia [38].

The data on muscle glycogen level of fish *Cyprinus carpio* treated with sub lethal concentrations (Treatment I and II) of alphamethrin are presented in Fig. 2. The muscle glycogen content showed a significant ($p < 0.05$) decreasing trend during the study period in both treatments. In Treatment I, the glycogen level recorded a percent decrease of 36.07, 41.21, 41.65, 49.55 and 66.69 on 7, 14, 21, 28 and 35th day. In Treatment II, the glycogen level showed a percent decrease of 19.36, 53.41, 43.23, 55.45 and 57.67 on each sampling period.

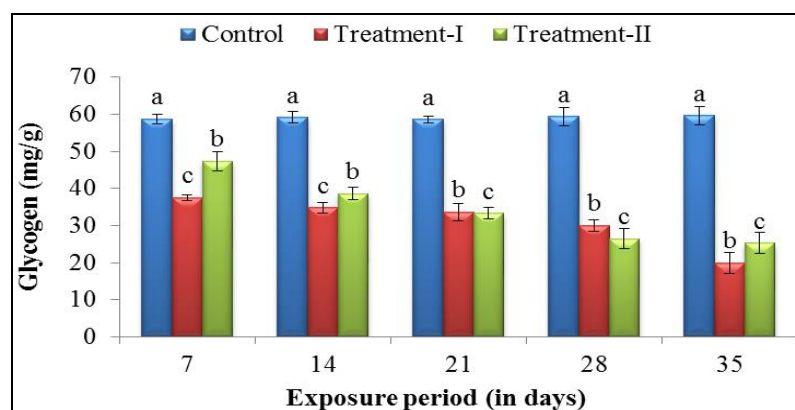


Fig 2: Glycogen level of *Cyprinus carpio* exposed to different sublethal concentrations (0.6 $\mu\text{g/L}$ – Treatment I; 1.2 $\mu\text{g/L}$ – Treatment II) of alphamethrin for 35 days. All the values are expressed as means \pm SE. The values are indicated by different letters are significantly different from one another ($P < 0.05$) according to DMRT.

Glycogen is the main storage source of energy in all organisms and hence their levels in the fishes that have been exposed to various toxic chemicals indicate the health of these fishes [23]. The muscle glycogen levels in the *Cyprinus carpio* treated with alphamethrin showed a significant ($p < 0.05$) decrease in both the concentration at the end of each interval period might be due to a hypoxic state which is accompanied with glycogen reserve depletion [39]. Similarly, Tripathi and Singh [11] observed a significant decrease in glycogen content of about 37% in hepatopancreas and 46% in ovotestis after 96 h exposure to 12 $\mu\text{g/L}$ of alphamethrin. In general, a reduction in glycogen level and increased glucose level showing enhanced glycogenolysis and reduction of glycolytic pathway

[40]. The reduction in glycogen level in deltamethrin and permethrin treated fish *Anabas testudineus* indicates the use of glycogen to cope of with the stress [24].

Fig. 3 represents the data on changes on protein content of *Cyprinus carpio* treated with sub lethal concentrations (Treatment I and II) of alphamethrin for a period of 35 days. The plasma protein of the alphamethrin treated fish was found to decrease throughout the study period, showing 15.08, 35.62, 34.51, 36.42 and 39.63% and 12.28, 26.94, 26.33, 38.65 and 59.63% at each sampling period in Treatment I and II respectively. Statistical analysis found the values to be significantly ($P < 0.05$).

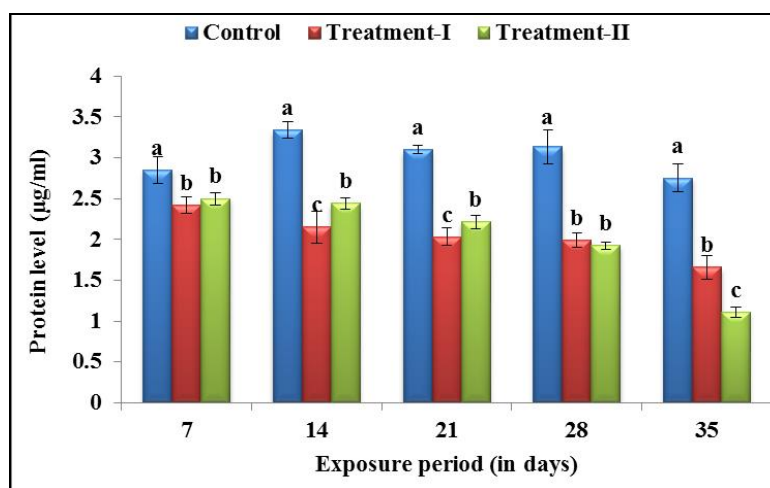


Fig 3: Protein levels of *Cyprinus carpio* exposed to sublethal concentrations of alphamethrin for 35 days. All the values are expressed as means \pm SE. The values are indicated by different letters are significantly different from one another ($P < 0.05$) according to DMRT.

Proteins perform different key functions in fish, such as making the functional and structural cell components, nitrogenous metabolism source, and an energy source under chronic stress. In the present study, the protein contents of *Cyprinus carpio* was found to decrease significantly ($p < 0.05$) with respect to the exposure days might be associated with protein degradation to fulfill the increased demand of energy for metabolic purposes or reduced protein synthesis under stress. An exposure of 12 $\mu\text{g/L}$ of alphamethrin for 96 h in the snails observed a significant decrease in the total protein levels to 63% in hepatopancreas and 60% in ovotestis [11]. A decrease in the protein content through gluconeogenesis was noted in *Acipenser nudiiventris* when treated with atrazine [41]. Similarly, decrease in protein content was noted in gill and muscle of catfish, *Clarias batrachus* exposed to alphamethrin [42] due to the utilization of protein in gluconeogenesis pathway. Narra *et al.* [43] reported that the reduction in plasma protein in chlorpyrifos treated fish *Clarias batrachus* indicate the breakdown of protein to meet energy demand caused by chlorpyrifos.

4. Conclusion

The present study indicates that alphamethrin has significant effect on plasma protein, glucose and glycogen contents in muscle of the fish, *Cyprinus carpio*. This study helps to establish the safe limits and effects of alphamethrin on freshwater fishes with a dose dependency.

5. References

1. Sapana DM, Gupta A. Sublethal toxicity of commercial formulations of deltamethrin and permethrin on selected

biochemical constituents and enzyme activities in liver and muscle tissues of *Anabas testudineus*. Pesticide Biochemistry and Physiology 2014;115:48-52.

2. Kong Y, Li M, Shan X, Wang G, Han G. Effects of deltamethrin subacute exposure in snakehead fish, *Channa argus*: Biochemicals, antioxidants and immune responses. Ecotoxicology and Environmental Safety 2021;209:111821
3. Siegler K, Phillips BM, Anderson BS, Voorhees JP, Tjeerdema RS. Temporal and spatial trends in sediment contaminants associated with toxicity in California watersheds. Environmental Pollution 2015;206:1-6.
4. Awoyemi OM, Kumar N, Schmitt C, Subbish S, Crago J. Behavioral, molecular and physiological responses of embryo-larval zebra fish exposed to types I and II pyrethroids. Chemosphere 2019;219:526-537.
5. Chen L, Xu P, Diao J, Di S, Li R, Zhou Z. Distribution, metabolism and toxic effects of beta-cypermethrin in lizards (*Eremias argus*) following oral administration. Journal of Hazardous Materials 2016;306:87-94.
6. Prama MJ, Lotegtec A, Compana M, Bacchetta C. Changes of hematological parameter in *Prochilodus jineatus* (Pisces: Prochilodontidae) exposed to sublethal concentration of cypermethrin. Journal of Environmental Biology 2007;28:147-149.
7. Joshi N, Dharmata, Sahu AP. Histopathological changes in the liver of *Heteropneustes fossilis* exposed to cypermethrin. Journal of Environmental Biology 2007;28:35-37.
8. Singh PK, Ansari BA. Effect of neem-based formulations (Nimbecidine and Ultineem) on the sparing success of

- zebrafish *Danio rerio* (Cyprinidae). Bioscan Research 2010;5:669-672.
9. Sarikaya R. Investigation of acute toxicity of alpha-cypermethrin on adult Nile tilapia (*Oreochromis niloticus* L.). Turkish Journal of Fisheries and Aquatic Sciences 2009;9:85-89.
 10. Viran R, Erkoç FÜ, Polat H, Koçak O. Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotoxicology and Environmental Safety 2003;55(1):82-85.
 11. Tripathi PK, Singh A. Toxic effects of cypermethrin and alphamethrin on reproduction and oxidative metabolism of the freshwater snail, *Lymnaea acuminata*. Ecotoxicology and Environmental Safety 2004;58:227-235.
 12. Saravanan M, Kim JY, Hur KJ, Ramesh M, Hur JH. Responses of the freshwater fish *Cyprinus carpio* exposed to different concentrations of butachlor and oxadiazon. Biocatalysis and Agricultural Biotechnology 2017;11:275-281.
 13. Nataraj B, Hemalatha D, Rangasamy B, Maharajan K, Ramesh M. Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeo rohita* exposed to organophosphorus pesticide profenofos. Biocatalysis and Agricultural Biotechnology 2017;12:185-190.
 14. Maharajan K, Muthulakshmi S, Karthik C, Nataraj B, Nambirajan K, Hemalatha D *et al.* Pyriproxyfen induced impairment of reproductive endocrine homeostasis and gonadal histopathology in zebrafish (*Danio rerio*) by altered expression of hypothalamus-pituitary-gonadal (HPG) axis genes. Science of The Total Environment. 2020;735:139496.
 15. Al-Ghanim KA, Mahboob S, Vijayaraghavan P, Al-Misned FA, Kim YO, Kim HJ. Sub-lethal effect of synthetic pyrethroid pesticide on metabolic enzymes and protein profile of non-target zebra fish, *Danio rerio*. Saudi Journal of Biological Sciences 2020;27(1):441-447.
 16. Caulibaly K, Smith JS. Effect of pH and cooking temperature on the stability of organophosphorus pesticides in beef muscle. Journal of Agricultural and Food Chemistry 1994;42:2035-2039.
 17. Marijani E, Nasimolo J, Kigadye E, Joselin Benoit Gnonlofin G, Okoth S. Sex-related differences in hematological parameters and organosomatic indices of *Oreochromis niloticus* exposed to aflatoxin B1 diet 2017;4268926.
 18. Ramesh M, Thilagavathi T, Rathika R, Poopal RK. Antioxidant status, biochemical, and hematological responses in a cultivable fish *Cirrhinus mrigala* exposed to an aquaculture antibiotic Sulfamethazine. Aquaculture 2018;491:10-19.
 19. Kroon F, Streten C, Harries S. A protocol for identifying suitable biomarkers to assess fish health: A systematic review. PloS one 2017;12(4):e0174762
 20. Osman AGM, Abouelfadi KY, Abd EI Reheem AEI, Mahmoud UM, Kloas W, Moustafa M. Blood biomarkers in Nile tilapia *Oreochromis niloticus niloticus* and African catfish *Clarias gariepinus* to evaluate water quality of the river Nile. Journal of Fisheries Science.com 2018.
 21. Sayed AH, Soliman HAM. Modulatory effects of green tea extract against the hepatotoxic effects of 4-nonylphenol in catfish (*Clarias gariepinus*). Ecotoxicology and Environmental Safety 2018;149:159-165.
 22. Hemalatha D, Muthukumar A, Rangasamy B, Nataraj B, Ramesh M. Impact of sublethal concentration of a fungicide propiconazole on certain health biomarkers of Indian major carp *Labeo rohita*. Biocatalysis and Agricultural Biotechnology 2016;8:321-327.
 23. Saravanan M, Karthika S, Malarvizhi A, Ramesh M. Ecotoxicological impacts of clofibric acid and diclofenac in common carp (*Cyprinus carpio*) fingerlings: hematological, biochemical, ionoregulatory and enzymological responses. Journal of Hazardous Materials 2011;195:188-194.
 24. Devi MS, Gupta A. Sublethal toxicity of commercial formulations of deltamethrin and permethrin on selected biochemical constituents and enzyme activities in liver and muscle tissues of *Anabas testudineus*. Pesticide Biochemistry and Physiology 2014;115:48-52.
 25. Oliveira JM, Losano NF, Condessa SS, de Freitas RMP, Cardoso SA, Freitas MB *et al.* Exposure to deltamethrin induces oxidative stress and decreases of energy reserve in tissues of the Neotropical fruit-eating bat *Artibeus lituratus*. Ecotoxicology and Environmental Safety 2018;148:684-692.
 26. Al-Asghar NA, Abdel-Warith AA, Younis EM, Allam HY. Haematological and biochemical parameters and tissue accumulations of cadmium in *Oreochromis niloticus* exposed to various concentrations of cadmium chloride. Saudi Journal of Biological Sciences 2015;22:543-550.
 27. Abdel-Warith AWA, Younis ESMI, Al-Asghar NA, Rady AM, Allam HY. Bioaccumulation of lead nitrate in tissues and its effects on hematological and biochemical parameters of *Clarias gariepinus*. Saudi Journal of Biological Sciences 2020;27:840-845.
 28. APHA. Standard methods for the examination of water and wastewater 2005;21:258-259.
 29. Cooper GR, McDaniel V. Estimation of blood glucose by O-Toluidine reagent. Standard Method of Clinical Chemistry 1970, 159.
 30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 1951;193:265-275.
 31. Carrol NN, Langley RW, Roe JH. Determination of polysaccharide content by anthrone reagent. Journal of Biological Chemistry 1956;220:583-593.
 32. Ullah S, Begum M, Ahmad S, Dhama K. Genotoxic effect of endosulfan at sublethal concentrations in Mori (*Cirrhinus mrigala*) fish using single cell gel electrophoresis (comet) assay. International Journal of Pharmacology 2016;12:169-176.
 33. Arrighetti F, Ambrosio E, Astiz M, Capítulo AR, Lavariás S. Differential response between histological and biochemical biomarkers in the apple snail *Pomacea canaliculata* (Gasteropoda: Amulliariidae) exposed to cypermethrin. Aquatic Toxicology 2018;194:140-151.
 34. Bhoi SS, Patole SS. Biochemical changes in fresh water fish *Channa marulius* exposed to sub lethal concentrations of cypermethrin and fenvalerate. International Journal Life Sciences 2019;13:14-18.
 35. David M, Mushigeri SB, Shivakumar R, Philip GH. Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: alterations in protein

- metabolic profiles. *Chemosphere* 2004;56(4):347-352.
36. Das BK, Mukherjee SC. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. *Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology* 2003;134(1):109-121.
 37. Ullah S, Li Z, Hasan Z, Khan SU, Fahad S. Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (*Labeo rohita*, Hamilton) at acute concentration. *Ecotoxicology and Environmental Safety* 2018;161:270-280.
 38. Uddin M, Alim M, Islam S, Rashid H, Shahjahan M. Temperature changes alter the acute toxicity responses of cypermethrin in Zebrafish. *Progressive Agriculture* 2018;29:64-70.
 39. Srivastava P, Singh A, Pandey AK. Pesticides toxicity in fishes: Biochemical, physiological and genotoxic aspects. *Biochemical and Cellular Archives* 2016;16(2):199-218.
 40. Narra MR. Single and cartel effect of pesticides on biochemical and haematological status of *Clarias batrachus*: A long-term monitoring. *Chemosphere* 2016;144:966-974.
 41. Naji M, Yousefi Jourdehi Y, Hosseinzadeh Sahafi H. Impacts of Atrazine on some blood and biochemical indices in farmed *Acipenser nudiventris*. *Survey in Fisheries Sciences* 2019;5(2):19-27.
 42. Tripathi G, Bandooni N. Impact of alphamethrin on antioxidant defense (catalase) and protein profile of catfish. *The Environmentalist* 2011;31(1):54-58.
 43. Narra MR, Rajender K, Reddy RR, Rao JV, Begum G. The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*. *Chemosphere* 2015;132:172-178.