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IMPACT OF ORGANOPHOSPHATE PESTICIDE ROGOR ON BIOCHEMICAL PARAMETERS OF FRESHWATER CATFISH *Clarias magur* (HAMILTON, 1822)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pesticides are not only dangerous to the environment, but also hazardous to a person's health. Studies on pesticides have clearly established their link to cancer, Alzheimer's Disease, ADHD, birth defects and many more. Rogor is one of the organophosphates extensively used in agricultural practice. It is highly soluble in water for which it can easily enter into the nearby water sources and affect aquatic organisms. In the present study we have planned to observe the effects of rogor in some biochemical parameters in freshwater cat fish *Clarias magur*. Based on the LC_{50}, we used two sub-lethal concentrations of Rogor - $1/5^{th}$ (i.e; 13 mg/L) and $1/10^{th}$ (i.e; 6.5mg/L) in our experiment. After 7 consecutive days of treatment, we have observed a dose dependent decrease in protein, glycogen and lipid content in both liver and muscle of the treated fishes. The amount of protein, glycogen and lipid was decreases with the increasing concentration of the rogor and the results were significant at 0.1 (P<0.1). The amount of blood glucose and total blood cholesterol was also decreases with increasing concentration of rogor.

Keywords: Pesticides; rogor; *Clarias magur*; biochemical.

1. INTRODUCTION

Pesticides are used worldwide mainly to control or to manage agricultural pest. According to the National Institute of Environmental Health Sciences, a pesticide is any substance that is used to destroy, repel, control, or prevent plants and animals considered to be pests [1]. The most commonly applied pesticides are fungicides, herbicides, insecticides, rodenticides, bactericides and so forth; each of which is intended to be effective against specific pests. Over 98% of sprayed insecticides and

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95% of herbicides reach a destination besides their target species, as they are sprayed across the entire agricultural fields [2]. Runoff can carry pesticides into aquatic environments while wind can carry them to grazing areas, human settlements and undeveloped areas, potentially affecting other species. Because of the widespread use of these chemicals in agricultural system, people are exposed to low levels of pesticide residues through their diets. The primary pesticide consuming countries include China, USA, Argentina, India, Japan, Canada, Brazil, France, Italy and Thailand [3]. Studies by the UK government showed that pesticide concentrations exceeded those allowable limits for drinking water in some samples of river water and groundwater [4].

Fishes are very sensitive to a wide variety of toxicants in water. Various experiments have been conducted to test the genotoxic effects, biochemical alterations, histopathological effects and its bioaccumulation in different species of freshwater fishes due to toxicant contamination [5-7]. The chemical composition of protein and lipid is traditionally used as an indicator of the nutritional value as well as the physiological condition of fish and its habitat [8,9]. A number of workers have reported decline in protein level of various organs and tissues under toxic stress of various chemical. A progressive decrease in the protein content of the liver of fresh water fish *Puntius ticto* with increasing concentration of two sublethal doses of Dimethoate was reported by Ganeshwade [10]. In another study Verma et al. observed significantly decrease total protein in blood serum in the fishes *Clarias batrachus* exposed to pesticides Endocel and Rogor [11]. Prakriti et al also observed an alteration in free amino acids and protein content in *Clarias batrachus* after treated with Endocel and Rogor [12]. Normal levels of protein in different tissues and serum are essential for the metabolic harmony of the organism. Protein profile of cells and tissues gives an indication of physiological status of an animal and there exists a dynamic equilibrium between the synthetic and degradation pathways.

Begum and Vijayaraghavan observed a gradual decrease in muscle glycogen and an increase in lactate contents in rogor treated *Clarius batrachus* [13].

The blood glucose level can be an indicator of biological stress caused by pollutants such as pesticides and metals [14]. Oluah and Chineke observed significantly decrease liver glycogen, liver protein and serum glucose concentrations with increasing λ-cyhalothrin test concentration in the African catfish *Clarias gariepinus* [15]. In the same experiment they also observed significantly increase serum cholesterol level. A reduction in hepatic glycogen indicating impairment in the carbohydrate metabolism was reported in *Anabas testudineus* treated with acute lethal and sublethal concentrations of furadan [16]. A reduction in glycogen content of liver and muscle of *Channa punctatus* exposed to endosulfan was reported by Sastry and Siddiqui [17]. Kalita and Khatun observed a dose dependent decrease in total RBC and an increase in total WBC in rogor treated *Clarius magur* [18].

The liver in animals is greatly affected by the pesticidal contaminants present in their environment. In a study Kalita and Phukan reported hydropic changes, infiltration of leukocyte cells in portal vein and blood spilling in the liver tissues of rogor treated *Clarius magur* [19]. Treatment with endrin also produced acute pathological changes in the liver of *Channa punctatus* [20]. Vacuolar degeneration of the cytoplasm, localized necrosis and hypertrophy of hepatic cells were reported in *Heteropneustes fossilis*, when treated with the pesticide chlordecone [21].

Among different classes of pesticides, organophosphates are most commonly used because of their high insecticidal property. Rogor (Dimethoate) is one of the organophosphates extensively used in agricultural practice. Rogor is highly soluble in water for which it can easily enter into the nearby water sources and affect aquatic organisms. Once absorbed, organophosphate molecules bind to an enzyme acetylcholinesterase making the enzyme inactive. This leads to an overabundance of acetylcholine within synapses and neuromuscular junctions, that may lead to disorder of many normal functions [22].

With a thorough historical overview on the context of various experiments, we have made an effort to investigate the influence of organophosphate pesticide Rogor on biochemical changes in liver and muscles, and changes in blood glucose and blood cholesterol level in freshwater catfish *Clarias magur.* Catfishes are attracting attention of the pisciculturists owing to their high production potential from paddy fields and stagnant shallow ponds [23]. *Clarias magur* is one of the commonly reared cat fishes in Asian countries and is an edible fish found in ponds. The fish is popular for its tasty flesh, rapid growth and high market price.

2. MATERIALS AND METHODS

2.1 Collection and Maintenance of Experimental Fishes

We collected the freshwater catfish *Clarias magur* from local market of Guwahati. Three earthen pots each with capacity of 20 liters covered with mosquito net, were used to culture the experimental fishes. Tap water containing pH 7.2±0.3 and dissolved oxygen 7.9mg/L±0.2 was used to culture the fishes. Fishes were treated with 0.1% $KMnO_4$ for five minutes to get rid of any dermal infection. The fish weighing 100±10 Gms were selected for the works. Fishes were maintained at temperature of 27±1°C in our laboratory conditions for acclimatization. They were allowed to acclimatize for 15 days. During this period, the fishes were fed twice a day with the commercial fish food, mosquito larvae and chironomous larvae to avoid their starvation. Twenty-four hours before starting the experiment, the food was stopped to clear off the alimentary canal.

2.2 Chemical used in the Experiment

The organophosphorus pesticide Rogor (dimethoate 300g/liter or 30% EC), is collected from local market. Dimethoate [IUPAC Name- O, Odimethyl S-(N-methylcarbamoylmethyl) phosphoro-dithioate] is a systemic insecticide widely used for controlling insect pests of fruits, vegetables and crop plants [24]. It was first patented and introduced in the 1950's by American Cyanamid. Dimethoate shows moderate persistence in water. It irreversibly inhibits acetyl cholinesterase enzyme which is essential for Central Nervous System function and works primarily as nerve poison. It is available in aerosol spray; dust, emulsifiable concentrate, and ultra-low volume concentrate formulations. It acts by contact, ingestion, inhalation and dermal absorption.

2.3 Determination of Sub-lethal Concentration and Treatment Doses

The LC_{50} value (lethal concentration) of Rogor for *Clarias magur* is 65mg/liter for 96 hrs. [13]. Based on this value, we used two sub-lethal concentrations of Rogor - $1/5^{th}$ (i.e; 13 mg/L) and $1/10^{th}$ (i.e; 6.5mg/L) of LC_{50} value.

2.4 Sample Collection

Treatments were carried out in two different dose levels for 7 consecutive days. After 24 hours of the last exposure period, blood samples were obtained by severance of caudal peduncle and collected in Eppendorf tubes containing EDTA anticoagulant. Fishes were then sacrificed to isolate liver and muscle tissues for biochemical parameters.

2.5 Methods for Biochemical Parameters

For estimation of total protein, glycogen and lipids from liver and muscle, 1Gm of each tissue was transferred into the homogenizer containing 10%TCA; and then processed to prepare tissue extract. From tissue extract, protein content was estimated by Folin phenol reagent method [25]; Glycogen content was analyzed by using Anthrone reagent method [26]. Gravimetric method was used to estimate total lipid contents. To estimate total Cholesterol in blood, standard kit (ROBOniK) was used; and for blood glucose, Glucometer (Dr. Morepen, GlucoOne) was used.

2.6 Statistical Analysis

For the experimental parameters, the data obtained were statistically analyzed by using mean ± S.D (Standard Deviation). One-way ANOVA test was used to derive significant difference between means through SPSS.

3. RESULTS

In the present experiment, we observed dose dependent decrease in protein (Table 1 and Fig. 1.) and glycogen (Table 2 and Fig. 2.) content in both liver and muscle of the treated fishes. The amount of protein and glycogen was decreases with the increasing concentration of the rogor. The results were significant at 0.1 ($P<0.1$). Total lipid content (Table 3 and Fig. 3) in liver and muscle was also decreases in treated fishes. The decrease amount was dose dependent and the results were significant at 0.05 ($P<0.05$). Blood glucose and total blood cholesterol (Table 4 and Fig. 4.) in treated fishes were also decreases with the increasing concentration of the rogor. The results were significant at 0.1($P<0.1$).

3.1 Liver Glycogen

Each value is mean ±SD of six observations (+ indicates increase over control, - indicates decrease over control, results are significant at 0.1 ($P<0.1$).

Table 1. Total protein in liver and muscle in control and rogor treated *C. magur.*

Experimental groups	Liver protein (mg/Gm)	Muscle protein (mg/Gm)
Control	120.18±6.98	128.89±3.72
6.5mg/L	98.35±3.87	107.12±5.96
13mg/L	77.64±5.21	92.19±6.00

Each value is mean ±SD of six observations (+ indicates increase over control, - indicates decrease over control, results are significant at 0.1 ($P<0.1$).

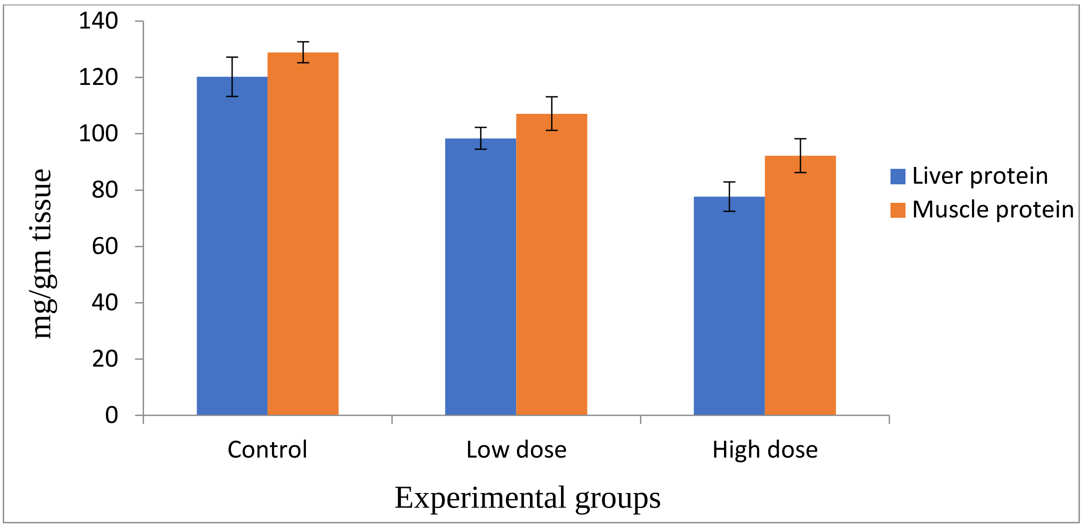

Fig. 1. Total protein content in liver and muscle of control and rogor treated *C. magur*.

Table 2. Total glycogen in liver and muscle in control and rogor treated *C. magur*

Experimental groups	Liver glycogen (mg/Gm)	Muscle glycogen (mg/Gm)
Control	38.01 ± 3.61	31.22 ± 3.30
6.5mg/L	24.27 ± 2.42	18.99 ± 2.35
13mg/L	10.87 ± 1.56	10.24 ± 2.22

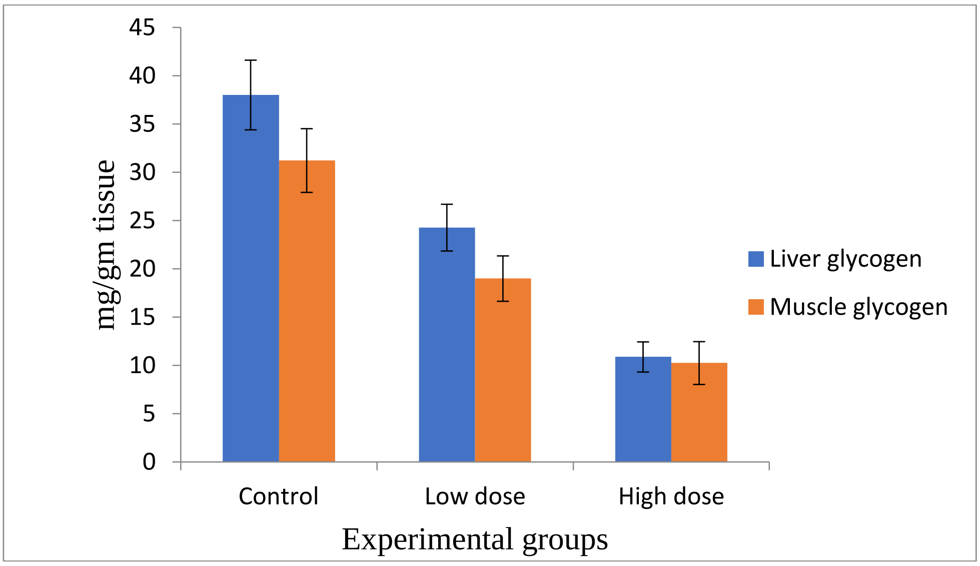

Fig. 2. Total glycogen content in liver and muscle of control and rogor treated *C. magur*

Table 3. Total lipid in liver and muscle in control and rogor treated *C. magur*

Experimental groups	Liver lipid (mg/Gm)	Muscle lipid (mg/Gm)
Control	0.073±0.005	0.085±0.008
6.5mg/L	0.067±0.008	0.079±0.006
13mg/L	0.055±0.005	0.07±0.006

Each value is mean ±SD of six observations (+ indicates increase over control, - indicates decrease over control, results are significant at 0.05 (P<0.05)

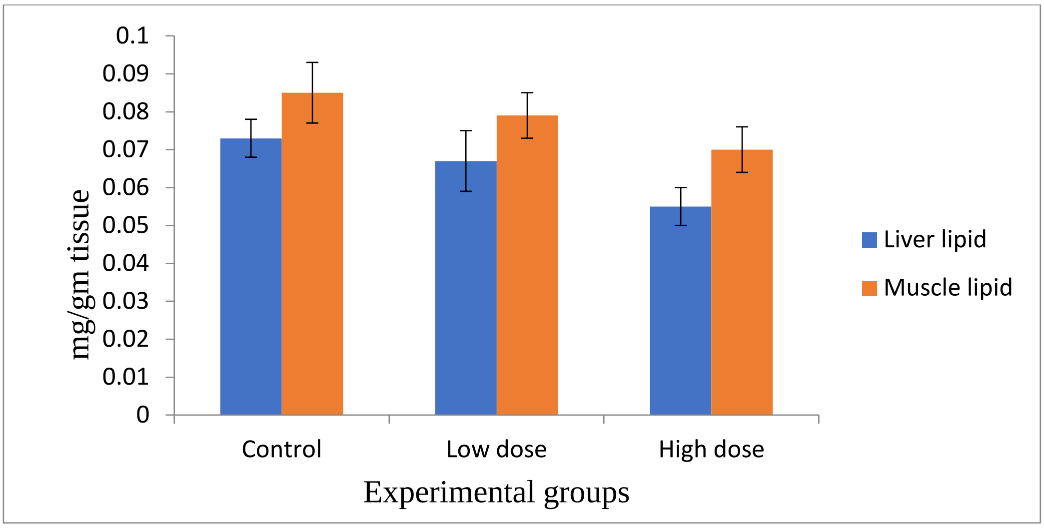

Fig. 3. Total lipid content in liver and muscle of control and rogor treated *C. magur*

Table 4. Blood glucose and Total cholesterol in blood in control and rogor treated *C. magur*

Experimental groups	Blood glucose (mg/dl)	Total cholesterol in blood (mg/dl)
Control	108.67±6.87	187.20±3.44
6.5mg/L	92.83±3.40	168.51±3.32
13mg/L	78.67±2.88	157.61±4.02

Each value is mean ±SD of six observations (+ indicates increase over control, - indicates decrease over control, results are significant at 0.1 (P<0.1)

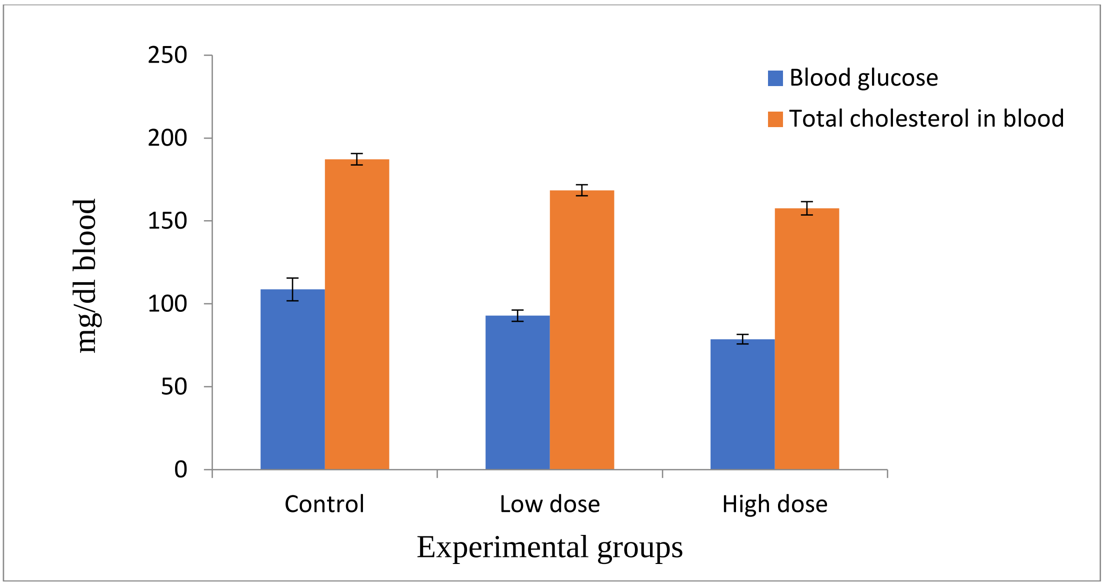

Fig. 4. Blood glucose and Total cholesterol level in blood of control and rogor treated *C.*

4. DISCUSSIONS

The current study provides an idea to assess health status of fish and the serious ecological consequences of pollutants in freshwater communities. In our present work, we observed significant dose dependent decrease of protein content in both liver and muscle of rogor treated fishes. The decrease of total protein may be due to the inhibition of RNA synthesis disturbing the protein metabolism or this may be due to liver damage where most protein synthesis usually occurs. These results were agreed with that of Sing and

Sarma, who reported drastic decrease in the protein content of gill, brain, muscle, liver, kidney and heart of the *Clarias batrachus* after treated with carbosulfan [27]. Under conditions of stress many organisms will mobilize proteins as an energy source via oxidation of amino acids. The depletion in total protein content may be due to augmented proteolysis and possible utilization of their product for metabolic purposes as reported by Ravinder and Suryanarayana [28]. Fish liver is an excellent organ for the study of toxicity effects, due to its role in the animal metabolism. Ganeshwade reported a progressive decrease in the protein and glycogen content in the liver of *Puntius ticto* with increasing concentration of Dimethoate [10]. The toxicity of dimethoate showed a direct correlation with the concentration and time exposure. Liver is an important organ involved in metabolic processes and in the detoxification and xenobiotics [29]. The chemical composition of protein and lipids is traditionally used as an indicator of the nutritional value as well as the physiological condition of fish and its habitat [30,31].

In the present study the amount of glycogen was decreases with the increasing concentration of the rogor and the results were significant at 0.1 ($P<0.1$). Decrease in liver glycogen content may be due to toxic stress. During stress an organism needs sufficient energy which is supplied from reserved glycogen. Glycogen is stored in the organism mainly in the liver and muscles in the form of carbohydrate. A reduction in glycogen content of liver and muscle of *Channa punctatus* exposed to endosulfan was reported by Sastry and Siddiqui [32]. In a study, Vijayaram et al. reported a decrease liver glycogen in the fish *Anabas testudineus* exposed to sublethal concentration of pulp and paper mill effluent [33]. The effect of exposure of the European eel, *Anguilla anguilla* to the sublethal doses of lindane showed a decrease in muscle glycogen level [34].

In fish, lipids play important physiological roles providing energy, essential fatty acids and fat-soluble nutrients for its normal growth and development. In this study, we observed a dose dependent significant decrease in total lipid content in liver and muscle of the treated fishes. The decreased in lipid content in both liver and muscle tissues suggested that the lipid have been directed to meet the metabolic demand for extra energy needed to mitigated the toxic stress. Our results were supported by the findings of Rao et al. [35], who reported a decrease in total lipid content in the fish *Channa punctatus* after exposure of carbaryl and fenthoate, in both combined and individual application.

The blood glucose level can be an indicator of biological stress caused by pollutants such as pesticides and metals [36]. In our experiment we observed a dose dependent decrease in blood glucose level. Carbohydrates are the primary and immediate source of energy. Thus, its decrease clearly demarcates the action of the chemical as the manifestation of stress to the test fishes. Our result was consisted with the findings of Oluah and Chineke [15], who observed significantly decrease liver glycogen, liver protein and serum glucose concentrations with increasing λ-cyhalothrin test concentration in the african catfish *Clarias gariepinus*.

We also observed a dose dependent significant decrease in total cholesterol content in blood of the treated fishes, that might be due to the impaired metabolism of liver. This result is consisted with the work of Gill et al. [37], who reported a decrease in blood cholesterol in endosulfan treated fishes. Ceron et al. also reported a decrease in plasma cholesterol and triglyceride contents during 96 hrs treatment with sublethal concentration of diazinon in the fish *Anguilla Anguilla* [38].

Thus, the results of the present study indicate one significant manifestation of the toxic response of the fish under the stress of the pesticide exposure.

5. CONCLUSION

Pesticides are often considered as a quick and easy solution for controlling insect pests in agriculture. If the credits of pesticides include enhanced economic potential in terms of increased production of food, then their debits have resulted in serious health implications to man and environment. Pesticides have contaminated almost every part of our environment including soil, air, and water across the world, that poses significant risks to the environment and non-target organisms ranging from beneficial soil microorganisms, to insects, plants, fish, birds, mammals etc. Evidences suggest that some of these chemicals pose a potential risk to humans and other life forms. The world-wide deaths and chronic diseases due to pesticide poisoning is about 1 million per year. Highly toxic pesticides are still in widespread use that constitute a substantial challenge to human health. The importance of education and training among agricultural workers should be taken as a major vehicle to ensure a safe use of pesticides. The best way to reduce pesticide contamination is to do a safer use of pesticides and possible implementation of biological pest control methods.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. NIEHS. Pesticides; 2021. Retrieved:https://www.niehs.nih.gov/health/topics/agents/pesticides/index.cfm on 25.03.2022
2. Miller GT. Sustaining the Earth: An Integrated Approach. Thomson/Brooks/Cole. 2004;211–216.
   ISBN 978-0-534-40088-0.
3. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, Kohli SK, Yadav P, Bali AS, Parihar RD. Worldwide pesticide usage and its impacts on ecosystem. SN Appl. Sci. 2019; 1:1446.
   DOI: 10.1007/s42452-019-1485-1.
4. Bingham, S. Pesticides in rivers and ground water Archived 2009-03-02 at the Wayback Machine. Environment Agency, UK; 2007.
5. George AD, Akinrotimi OA, Nwokoma UK. Haematological changes in African catfish (Clarias gariepinus) Exposed to Mixture of Atrazine and Metolachlor in the laboratory. J. of Fisheries Sciences. 2017;11(3):048-054.
6. Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK, Lakra WS. Mutagenic and genotoxic effects of carbosulphan in freshwater fish C. punctatus using micronuclear assay and alkaline single-cell gel electrophoresis. Food chem. Toxicol. 2010;48:202-208.
7. Soundararajan M, Veeraiyan G. Effect of heavy metal arsenic on haematological parameters of freshwater fish, Tilapia mossambica. Int. J. Modn. Res. Revs. 2014;2(3):132-135.
   ISSN-2347-8314
8. Moghaddam HN, Mesgaran MD, Najafabadi HJ, Najafabadi RJ. Determination of chemical composition, mineral content and protein quality of Iranian Kilka fish meal. Int. J. Poult. Sci. 2007;6:354-361.
9. Aberoumad A, Pourshafi K. Chemical and proximate composition properties in different fish species obtained from Iran. World J. Fish mar. Sci. 2010; 2:237-239.
10. Ganeshwade RM. Biochemical Changes Induced by Dimethoate in the Liver of Fresh Water Fish Puntius ticto (HAM), Biological Forum-An International Journal. 2011;3(2):65-68.
    ISSN: 0975-1130
11. Verma P, Mishra BB, Rani P. Influence of Endocel and Rogor on serum free amino acid and total protein level in Clarias batrachus (Linn.). Journal of Environmental Biology. 2015;36:639-643.
12. Prakriti V, Mishra BB, Rani P. Influence of Endocel and Rogor on serum free amino acid and total protein level in Clarias batrachus (Linn.). Journal of Environmental Biology; 2016.
    ISSN: 2394-0379.
13. Begum G, Vijayaraghavan S. Organophosphate Insecticide Rogor on Some Biochemical Aspects of Clarias batrachus (Linnaeus). Environmental Research. 1999;80(1):80-83.
14. Silbergeld EK. Blood glucose: a sensitive indicator of environmental stress in fish. Bull. Environ. Contam. Toxicol. 1974;11(1):20 – 24.
15. Oluah NS, Chineke AC. Alterations in the biochemical parameters of the african catfish clarias gariepinus [Burchell] exposed to sublethal concentrations of lambda-cyhalothrin. Annals of Environmental Science. 2014;8:1-7.
16. Bakthavalsalam R, Reddy S. Changes in the content of glycogen and its metabolites during acute exposure of Anabas testudineus (Bloch) to furadan. J. Bio. sci. 1982;4(1):19 – 24.
17. Sastry KV, Siddiqui AA. Chronic toxic effects of the carbamate pesticide sevin on carbohydrate metabolism in a freshwater snakehead fish, Channa punctatus. Toxicol. Lett. 1983;14 (1-2):123 – 130.

18. Kalita E, Khatun N. Haematological and genotoxic effects induced by organophosphate insecticide “Rogor” in freshwater catfish Clarias magur (Hamilton, 1822). International Journal of Scientific Research. 2018;7(8):49-50, ISSN: 2277-8179
19. Kalita E, Phukan S. A study on the effect of Rogor on Liver, Kidney and Intestine of Freshwater Catfish Clarias magur. In the proceedings of International Conference on Recent Trends in Agriculture, Food Science, Forestry, Horticulture, Aquaculture, Animal Sciences and Climate Change (AFHABEC-2018). Edited by, Dr. Govind Chandra Mishra, Published by Krishi Sanskriti Publications; 2018.
    ISBN: 978-93-85822-64-3, pp 30-33
20. Sastry KV, Sharma SK. The effect of endrin on the histopathological changes in the liver of Channa punctatus. Bull. Environ. Contam. Toxicol. 1978;20:674 – 677
21. Shrivastava SM, Shrivastava VK. Toxicological effects of carbaryl on testicular morphology, gonadotropin, alkaline and acid phosphatase, total lipid and testosterone levels in Mus musculus . Poll. Res. 1998;17(3):215 – 218.
22. Robb EL, Baker MB. Organophosphate Toxicity; 2021.
    Retrieved:https://www.ncbi.nlm.nih.gov/books/NBK470430/
23. Bagchi P, Chatterjee S, Ray A, Deb C. Effect of quinalphos, organophosphorus insecticide, on testicular steroidogenesis in fish, Clarias batrachus. Environ, Contam. Toxicol. 1990;44:871-875.
24. NIH. Dimethoate. National Library of Medicine resources; 2019.
    Retrieved:https://pubchem.ncbi.nlm.nih.gov/compound/Dimethoate
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin-phenol reagent. J. Biol. Chem., 1951;193:265.
26. Zwaan A. De, Zandee DI. The utilization of glycogen and accumulation of some intermediates during anaerobiosis in Mytilus edulis L. Comparative biochemistry and physiology. 1972;43(1):47-54.
27. Singh RK, Sharma B. Carbofuran induced biochemical changes in Clarias batrachus, Pesticide. Science. 1998;53:285–290.
28. Ravinder V, Suryanarayana N. Decis induced biochemical alterations in a freshwater catfish, Clarias batrachus, Ind. J. Comp. Ani. Physiol. 1985;6:5–12.
29. Yang J, Chen H. Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. Zoological Studies. 2003;42(3):455461.
30. Moghaddam HN, Mesgaran MD, Najafabadi HJ, Najafabadi RJ. Determination of chemical composition, mineral content and protein quality of Iranian Kilka fish meal. Int. J. Poult. Sci. 2007;6:354-361.
31. Aberoumad A, Pourshafi K. Chemical and proximate composition properties in different fish species obtained from Iran. World J. Fish mar. Sci. 2010;2:237-239.
32. Sastry KV, Siddiqui AA. Chronic toxic effects of the carbamate pesticide sevin on carbohydrate metabolism in a freshwater snakehead fish, Channa punctatus. Toxicol. Lett. 1983;14 (1-2):123 – 130.
33. Vijayaram K, Loganathan P, Janarthanan S. Liver dysfunction in fish Anabas testudineus exposed to sublethal levels of pulp and paper mill effluent. Environ. Ecol. 1991;9(1):272 – 275.
34. Ferrando MD, Andreu-Moliner E. Effects of lindane on fish carbohydrate metabolism. Ecotoxicol. Environ. Saf. 1991;22(1):17 – 23.
35. Rao KR, Rao KS, Sahib IK, Rao KV. Combined action of carbaryl and phenthoate on a freshwater fish (*Channa punctatus bloch*). Ecotoxicology and environmental safety; 1985. PMID 3936694
36. Silbergeld EK. Blood glucose: a sensitive indicator of environmental stress in fish. Bull. Environ. Contam. Toxicol. 1974;11(1):20 – 24.
37. Gill TS, Pande J, Tewari H. Effects of endosulfan on the blood and organ chemistry of freshwater fish, Barbus conchonius Hamilton. Ecotoxicology and Environmental Safety. 1991;21(1):80-9.
38. Ceron JJ, Sancho E, Ferrando MD, Gutierrez C, Andreu E. Metabolic effects of diazinon on the European eel Anguilla Anguilla. Journal of Environmental Science and Health, Part B. 1996;31:1029-1040.