Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(1): 831 – 840 (2025)
www.ejabf.journals.ekb.eg

Sub-Lethal Effects of Profex Super on Hematological, Biochemical, and Histopathological Responses in *Clarias magur* (Hamilton, 1822)

Dr. Evarani Kalita[1*], Sujata Songhalee Gogoi[2], Lupamudra Borah[3], Papori Deka[1]

[1]Assistant Professor, Department of Zoology, Handique Girls' College, Guwahati, India
[2]MSc student, Department of Zoology, Handique Girls' College, Guwahati, India
[3]Research Scholar, Department of Zoology, Cotton University, Panbazar, Guwahati-01, India

[*]Corresponding Author: evaranaikalita123@gmail.com

ARTICLE INFO

Article History:
Received: Nov. 9, 2024
Accepted: Jan. 4, 2025
Online: Jan. 26, 2025

Keywords:
Clarias magur,
Profex super,
Hematological,
Biochemical,
Histopathological

ABSTRACT

Pesticides pose a threat to the environment and can be harmful to human health. Freshwater fishes are negatively impacted by the pesticides that runoff from agricultural activities. The present study aimed to evaluate the impact of the commonly used organophosphate pesticide, Profex Super, on the freshwater catfish *Clarias magur*. To study the potential hazards of Profex Super, the fishes were collected and acclimatized for a week, grouped, and exposed to experimental doses of 0.05 and 0.1µl/ L for 24 and 48 hours for each of the doses to determine the changes in hematological, biochemical, and histopathological responses. The LC50 value of Profex was found as 1µl/ L in *Clarias magur*. The study indicated a significant decrease in hemoglobin, RBCs, and hematocrit levels, accompanied by a dramatic increase in white blood cells. The histological study of the Profex-treated fishes revealed various alterations in important organs, such as the kidney and liver. In the liver of the treated fishes, distortion of the central vein and portal vein along with lymphocyte infiltration was observed in the 0.1µl/ L treated groups. Degenerative changes were also observed in the kidney tissues at the dose of 0.1µl/ L. A significant decrease in liver protein and blood glucose levels was observed in Profex-treated fish at 0.1µl/ L for both durations. However, it was more pronounced during the 48-hour treatment period. The current study's findings showed that exposure to the organophosphate Profex Super led to changes in hematological and biochemical profiles, as well as histopathological responses in the freshwater catfish, *Clarias magur*.

INTRODUCTION

The contamination of aquatic environments with pesticides is on the rise as a result of the widespread use in agriculture and fish farming. This pollution can have detrimental effects on the survival of aquatic organisms, particularly fish, due to the presence of varying chemical compositions in the water. The use of pesticides in agriculture is unavoidable, but their effects on non-target organisms outweigh their impact on the target pests. The toxicity of different pesticides varies depending on their types.

Fishes are highly sensitive to any changes in the physical, chemical, and biological characteristics of aquatic environments **(Obinna *et al.*, 2019)**. Toxic chemicals in the water are known to be harmful to fish survival. As a result, fish are considered to be highly sensitive biological indicators of any negative changes in the aquatic ecosystem. Despite the positive outcomes of using pesticides in agriculture, their environmental use often leads to adverse environmental and public health effects **(Ahmed *et al.*, 2024)**. Profex Super insecticide is a potent solution, combining profenofos (40%) and cypermethrin (4% EC), effectively tackling a broad spectrum of pests. Profenofos (PFF) is highly toxic to various organisms, including insects, pollinators, birds, mammals, and invertebrates, affecting ecosystem functioning **(Ishchaya *et al.*, 1993; Kumar & Chapman, 2001; Abass *et al.*, 2007; Raj *et al.*, 2024)**. In a study conducted by **Latha and Mohan (2018)**, *Glossogobius giuris* exposed to different sub-lethal concentrations of malathion showed decreased levels in RBC, Hb%, hematocrit (PCV) levels, and mean corpuscular hemoglobin concentration (MCHC), along with increased WBC, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Once the toxicants enter the bloodstream, they will reach and accumulate in the internal organs. Numerous studies have quantified contaminants in different fish organs to evaluate environmental quality **(Begum *et al.*, 2004; Majnoni *et al.*, 2013; Bharti & Rasool, 2021)**. They seek causal relationships with fish health, and based on these, the liver is likely to be the best choice, followed by the kidney and gills. **Devi and Mishra (2013)** and **Chamarthi *et al.* (2014)** observed leukocyte infiltration in the liver of *Channa punctatus* and *Cyprinus carpio*, respectively, under exposure to chlorpyrifos and quinalphos. **Al-Mamoori *et al.* (2014)** also observed the same histophatological changes, as well as vasodilatation in small blood vessels and necrosis in *Cyprinus carpio* liver after acute and chronic exposure to 0.05, 0.1, 0.25mg/ L chlorfos. The kidney in fish receives the largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution **(Hinton & Laurén, 1990; Ortiz *et al.*, 2003; Velmurugan, 2007)**. Following exposure of fish to toxic agents such as pesticides, histological alterations in the fish kidney were found at the level of the tubular epithelium and glomerulus **(Ortiz *et al.*, 2003; Thophon *et al.*, 2003)**. The protein and lipid composition of fish is typically used to determine its nutritional value, physiological condition, and habitat. Several studies have shown that exposure to certain chemicals can lead to a decrease in protein levels in the organs and tissues of fish **(Das & Mukherjee, 2000; Verma *et al.*, 2015; Kalita *et al.*, 2022)**. Therefore, this study aimed to examine the effects of profex super insecticide on various hematological (total RBCs, WBCs, hemoglobin and hematocrit), biochemical (total liver protein and blood glucose), and histopathological responses (liver and kidney tissues) in the freshwater catfish *Clarias magur*.

MATERIALS AND METHODS

A survey was conducted using questionnaires and interviews to gather information on the use of pesticides by the local farmers in the Garukhuti Village (Nitai Pukhuri) of Sivasagar district. Based on the survey report, we selected the pesticide Profex Super to carry out the present work.

Healthy freshwater catfish, *Clarias magur*, with an average body weight of 80±5g, were obtained from the local market for experimental purposes. At first, the specimens were treated with a 0.01% KMnO4 solution for 2 minutes and were then transferred to three separate earthen pots, each filled with 20 liters of dechlorinated tap water. They were acclimatized for 10 days in laboratory conditions. The water was aerated constantly and replaced daily. The fish were fed commercial fish food daily. The physicochemical characteristics of the experimental water were measured every alternate day.

The organophosphate pesticide Profex Super was obtained from the local market of Demow, where 80% of the population mainly uses it to control the vegetable pest population. Based on the LC_{50} value (1µl/ L of water), two different doses of the chemical were selected to carry out the present work, i.e., 1/10th (i.e 0.1µl/ L) and 1/20th, (i.e., 0.05µl/ L). The fish were divided into three groups. The first group served as the control, while the other two groups were the experimental groups for both doses. Each group was treated for periods of 24 and 48 hours.

For hematological parameters, blood samples were collected in Eppendorf tubes containing EDTA anticoagulant by severing the caudal peduncle. Total RBCs and WBCs were counted using an improved Neubauer hemocytometer **(Shah & Altindag, 2004)**. Hemoglobin (Hb) levels were measured using Sahli's hemoglobinometer. Hematocrit (% or volume fraction) was calculated by the hemoglobin concentration of the blood. Moreover, the total liver protein was measured using the Lowry method **(Lowry *et al.*, 1951)**. The blood glucose level was determined using a glucometer. Standard eosin-hematoxylin staining procedure was conducted for histopathological examination of liver and kidney tissues. To identify any nuclear abnormalities, micronucleus tests were conducted on blood using the Leishman stain. The data were statistically analyzed using the mean ± S.E. (Standard Error). The one-way ANOVA test was performed to determine significant differences between means using SPSS.

RESULTS

In the current study, the field survey uncovered the widespread misuse of Profex Super by local farmers who did not follow the recommended doses. In the experiment involving the chemical in *Clarias magur*, fluctuations were observed in hematological parameters depending on the dosage. The total amount of WBCs increased during both treatment periods with increasing the concentration of the Profex (Table 1). Results were significant (*P*< 0.001) except in the group treated with 0.05µl/ L for 24 hours. In contrast, the total amount of RBCs decreased during both treatment periods with increasing the concentration of the chemical, and the results were significant (*P*< 0.001), except in the group treated with 0.05µl/ L for 24 hours. There was also a significant (*P*< ,0.001) reduction in total hemoglobin except in the group treated with 0.05µl/ L for 24 hours (Table 1). However, the hematocrit level decreased significantly (*P*< 0.001) in all treated groups.

Table 1. Total count of RBCs, WBCs, hemoglobin, and hematocrit in Profex Super treated *C. magur*

Groups	Haemoglobin (gm/dl)	RBC (106cells/µl)	WBC (103cells/µl)	Hematocrit (%)
Control	16.40± 0.250	3.731 ± 0.314	54.17 ± 0.038	80.36 ± 1.212
0.05µl/L for 24 hours	17.63 ± 0.222[b]	3.217 ± 0.043[b]	54.77 ± 0.097[b]	86.35 ± 1.081[a]
0.05µl/L for 48 hours	13.96 ± 0.276[a]	2.830 ± 0.025[a]	55.31 ± 0.072[a]	68.53 ± 1.33[a]
0.1µl/L for 24 hours	13.70 ± 0.152[a]	2.889 ± 0.023[a]	55.43 ± 0.104[a]	67.27 ± 0.740[a]
0.1µl/L for 48 hours	10.10 ± 0.846[a]	2.001 ± 0.085[a]	56.11 ± 0.098[a]	49.81 ± 1.673[a]

Each value is mean ±SE of six observations (+ indicates an increase over control; - indicates a decrease over control; superscript a indicates significance at 0.001 (*P*<0.001); superscript b indicates no significance.

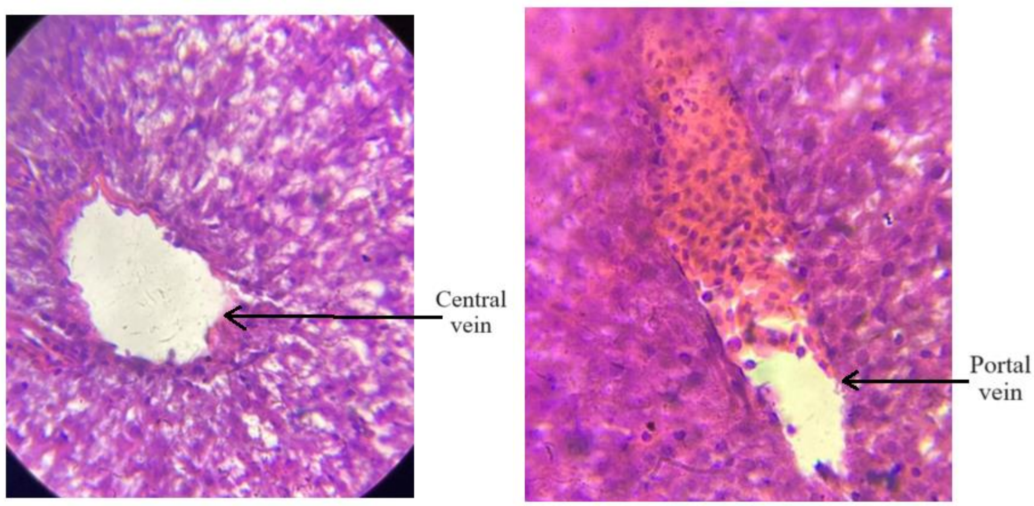
In terms of biochemical parameters, a significant dose-dependent decrease in blood glucose level was observed, except in the group 0.5µl/ L for 24 hours (Table 2). Although liver protein content was decreased in all the treated groups, it was significant (*P*< 0.001) only at the dose level of 0.1µl/ L for both 24 hours and 48 hours duration (Table 2).

Table 2. Blood glucose and liver protein in profex super treated *C. magur*

Groups	Protein (gm/gm of tissue)	Blood Sugar (mg/dl)
Control	0.650 ± 0.005	49.50 ± 0.427
0.05µl/L for 24 hours	0.646 ± 0.006[b]	48 ± 0.364[b]
0.05µl/L for 48 hours	0.611 ± 0.011[b]	46 ± 0.726[a]
0.1µl/L for 24 hours	0.578 ± 0.011[a]	37.50 ± 0.424[a]
0.1µl/L for 48 hours	0.513 ± 0.016[a]	26.83 ± 0.791[a]

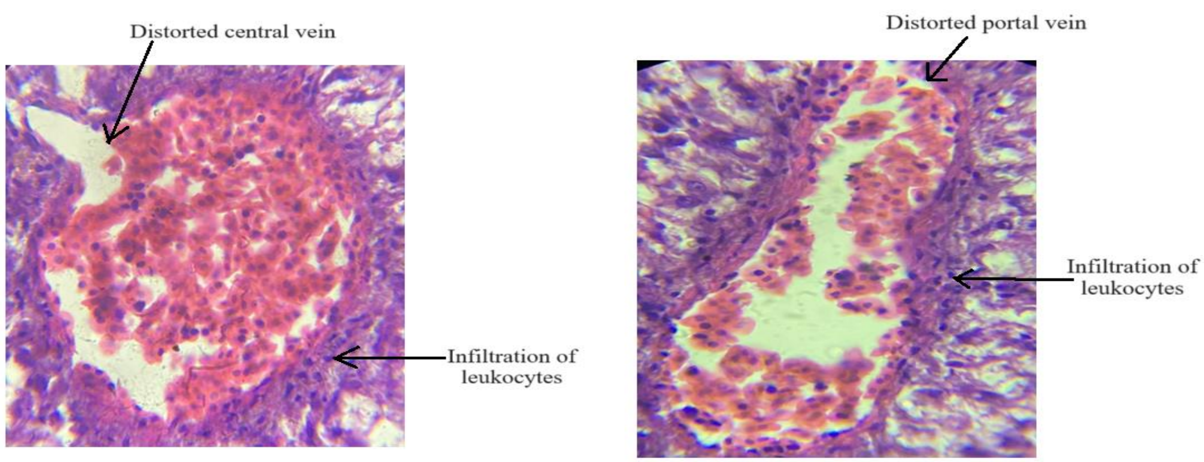
Each value is mean ±SE of six observations (+ indicates increase over control; - indicates decrease over control; superscript a indicates significance at 0.001 (*P*<0.001); superscript b indicates no significance.

In the liver of the treated fishes, distorted central and portal veins along with the infiltration of lymphocytes (Fig. 2A, B) were observed in all the treated groups, except 0.05µl/ L for 24hrs, when compared with the control group (Fig. 1A, B). Histopathological examination of the kidney sections from the fishes treated with 0.1µl/ L for 24hrs and 48hrs showed distorted Bowman's capsule (Fig. 3B) when compared to control (Fig. 3A).



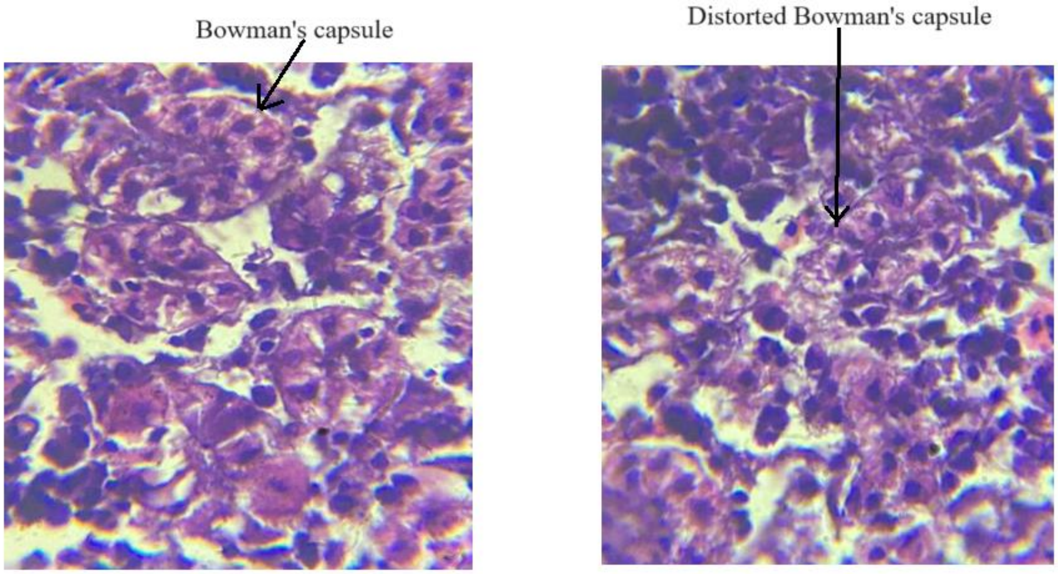
(A) **(B)**

Fig. 1. Photomicrographs of the liver section of control fishes showing: (A) Normal central and (B) Portal vein (X100)



(A) **(B)**

Fig. 2. Photomicrographs of the liver sections of the fishes treated with 0.1µl/L for 24hrs showing: (A) Distorted central and (B) Portal vein with infiltration of leukocytes (X100)



(A) **(B)**

Fig. 3. (A) Photomicrographs of the kidney sections of the control and (B) treated fishes with 0.1µl/ L for 24hrs showing normal and distorted Bowman's capsule, respectively (X100)

DISCUSSION

The result of the present investigation reveals that the entire physiology of the fish was affected under stress due to the exposure to Profex. Profex Super introduced hematological disturbance followed by metabolic disorders in the fish, which ultimately led to the deterioration of the general health of the fish.

In the present investigation, *C. magur* showed considerable alteration in different blood parameters after exposure to sublethal concentrations of 0.5 and 0.1µl/ L of profex super at different durations of treatments. Fish exposed to different sub-lethal concentrations of profex showed decreased levels in total RBCs, hematocrit (PCV), and hemoglobin concentration along with an increase in total WBCs. The effects were more pronounced at the concentrations of 0.1µl/ L of Profex, when treated for 48 hours.

A significant decrease in the total count of RBCs, hematocrit values, and hemoglobin percentage indicates the presence of anemia associated with erythropenia. The anemia may result from the suppression of red blood cell production and an increase in their destruction, as demonstrated by the histopathological study of the kidney. Hemoglobin reduction may be caused by THE toxic effects of Profex on its synthesis, inhibiting the pathway by affecting enzyme activity involved in Hb synthesis. Similar findings were reported in *Labeo rohita* exposed to lethal and sublethal concentrations of profenofos **(Zenebehagos *et al.*, 2017)**, in the Nile tilapia, *Oreochromis niloticus* **(Khan, 2019)**, and in *Ctenopharyngodon idella* **(El-bouhy *et al.*, 2023)**.

In the current study, the total count of THE white blood cells increased in a dose-dependent manner at all the exposure periods to Profex Super. However, the effect was more pronounced at the concentrations of 0.1µl/ L of Profex, when treated for 48 hours. An increase in the WBC count may be due to the direct stimulation of the immune system by a toxic substance or induced tissue damage. The increase in WBCs count in treated fish indicates toxemia, impairing the defence mechanism and resulting in leukocytosis to cope with the situation. Similar results were reported by **Ramesh and Saravanan (2008)** in *Cyprinus carpio* exposed to chlorpyrifos. Enhanced WBC count in *Ctenopharyngodon idella* has been also reported by **El-bouhy *et al.* (2023)** when exposed to profenofos.

Fishes exposed to Profex Super showed a significant decrease in blood glucose and liver protein levels. The decrease in blood glucose may be due to its enhanced utilization as an immediate source to meet energy demands under the stress of the chemical. Due to stress, fish make necessary adjustments and utilize stored energy to provide an immediate energy to the body's defence mechanisms and to protect all body systems from the harmful effects of pesticides. Similar effects were observed by **Kole *et al.* (2022)** in *B. gonionotus* as a result of sumithion exposure.

The protein content in the liver of *C. magur* is decreased upon increasing the concentrations of Profex Super. It indicates that the tissue protein undergoes proteolysis. The decrease in proteins might be partly due to their utilization in cell repair and tissue organization. In a similar study, **Zenebehagos *et al.* (2023)** reported similar effects on total protein in *Ctenopharyngodon idella* exposed to profenos.

In the present study, histopathological observations indicate that Profex Super caused tissue changes in the liver and kidney of fish. Changes in the liver may occur because it is the primary site for detoxification. As a result, toxicants are expected to reach the liver in large quantities for detoxification and elimination. Histological alterations in the kidney indicate nephrotoxicity caused by the tested compound and its metabolites, as kidneys are the primary route for eliminating most of the organophosphorus compound. Similarly, numerous liver and kidney alterations were found in *Cyprinus carpio* **(Rahman *et al.*, 2020)** and *Egretta alba* **(Taha, 2022)**, as a result of profenos exposure.

CONCLUSION

The present work summarized the effects of Profex Super on the hematological, biochemical parameters, and histopathology of the liver and kidney of *Clarias magur*. Pesticides have various harmful effects on different aspects of fish, leading to significant losses in sustainable aquaculture production. Pesticides not only harm fish but also pose a serious health risk to consumers as they bio-accumulate in fish tissues. Although pesticides were initially used to increase agricultural productivity and benefit human life, this report demonstrates that their substantial adverse effects on nature require their minimized use for the benefit of ecosystems and food production.

ACKNOWLEDGEMENTS

The authors acknowledge the financial assistance from the Department of Science and Technology (DST), Govt. of India, under DST-CURIE (WISE KIRAN Division) scheme for women P.G. Colleges (Ref. No. DST/CURIE-PG/2022/88(G)) and the Department of Zoology, Handique Girls' College for the laboratory facilities.

COMPETING INTERESTS

There is no conflict of interest as we don't intend to use them for litigation but for advancing knowledge. Also, the research was not funded by any agency; instead, it was funded by the personal efforts of the authors.

AUTHORS CONTRIBUTIONS

The authors confirm contribution to the paper as follows:

- EK: Experimental design, interpretation of data, and manuscript writing
- SSG: Field survey, animal handling and treatment, and hematological experiments
- LB: Histopathological experiments and statistical analysis
- PD: Biochemical experiments

REFERENCES

Abass, K.; Reponen, P.; Jalonen, J. and Pelkonen, O. (2007). In vitro metabolism and interaction of profenofos by human, mouse and rat liver preparations. Pestic. Biochem. Physiol., 87:238–247.

Ahmad, M.F.; Ahmad, F.A.; Abdulrahman, A.; Zeyaullah, M.A.; Muzammil, A.K.; Saati, A.A.; Wahab, S.; Elbendary, E.Y.; Kambal, N.; Abdelrahman, M.H. and Hussain, S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. Heliyon, 10(7), e29128. https://doi.org/10.1016/j.heliyon.2024.e29128.

Al-Mamoori, A.M.J.; Al-Zubaidy, F.M.; Al-Rezzaq, A.J.A.; Hadi, M.A. and Yass, M.J. (2014). Biomarkers of Chlorfos toxicity in Common Carp Cyprinus carpio. J. Environ. Sci. Toxicol. Food Technol., 8(1):109–112.

Begum, G. (2004). Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish Clarias batrachus (Linn) and recovery response. Aquat. Toxicol., 66(1):83–92. https://doi.org/10.1016/j.aquatox.2003.08.002.

Bharti, S. and Rasool, F. (2021). Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on *Channa punctatus* (Bloch) fish. Toxicol. Rep., 8:443–455.

Cengiz, E.I. (2006). Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environ. Toxicol. Pharmacol., 22:200–204. https://doi.org/10.1016/j.etap.2006.03.006.

Chamarthi, R.R.; Bangeppagari, M.; Gooty, J.M.; Mandala, S.; Tirado, J.O. and Marigoudar, S.R. (2014). Histopathological alterations in the gill, liver and brain of *Cyprinus carpio* on exposure to Quinalphos. Am. J. Life Sci., 2(4):211–216.

Das, B.K. and Mukherjee, S.C. (2000). Chronic toxic effects of quinalphos on some biochemical parameters in *Labeo rohita* (Ham.). Toxicol. Lett., 114(1–3):11–18. https://doi.org/10.1016/s0378-4274(99)00185-x.

Devi, Y. and Mishra, A. (2013). Study of behavioural and morphological anomalies of fry fish of fresh water teleost, *Channa punctatus* under chlorpyrifos intoxication. Int. J. Pharmacol. Biol. Sci., 4(1):865–874.

El-Bouhy, Z.M.; Mohamed, F.A.S.; Elashhab, M.W.A. and El-Houseiny, W. (2023). Toxicity bioassay and sub-lethal effects of profenofos-based insecticide on behavior, biochemical, hematological, and histopathological responses in Grass carp (*Ctenopharyngodon idella*). Ecotoxicol., 32(2):196–210. https://doi.org/10.1007/s10646-023-02628-9.

Hinton, D.E. and Laurén, D.J. (1990). Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In: McCarthy, J.F.; Shugart, L.R., Eds. Biomarkers of Environmental Contamination. Boca Raton: Lewis Publishers. p. 51–65.

Ischaaya, I. (1993). Insect detoxifying enzymes: their importance in pesticide synergism and resistance. Arch. Insect Biochem. Physiol., 22:263–276.

Kalita, E.; Devi, K.; Sultana, N.; Bayan, J. and Tamuli, H. (2022). Impact of organophosphate pesticide rogor on biochemical parameters of freshwater catfish *Clarias magur* (Hamilton, 1822). Uttar Pradesh J. Zool., 43(8):31–38.

Khan, M.P. (2019). Effects of profenofos, an organophosphate pesticide, on the hematological parameters of nile tilapia (*Oreochromis niloticus*). Master Thesis, Department of Fisheries Management, Bangladesh Agricultural University.

Kole, K.; Islam, R.; Elisia, C.; Nahid, N.; Sultana, N.; Haque, R.; Salam, S. and Mohammod, G. (2022). Toxicological effect of sumithion pesticide on the hematological parameters and its recovery pattern using probiotic in *Barbonymus gonionotus*. Toxicol. Rep., 9:230–237. https://doi.org/10.1016/j.toxrep.2022.02.004.

Kumar, A. and Chapman, J.C. (2001). Profenofos residues in wild fish from cotton-growing areas of New South Wales, Australia. J. Environ. Qual., 30:740–750.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193(1):265–275. https://doi.org/10.1016/S0021-9258(19)52451-6.

Majnoni, F.; Rezaei, M.; Mansouri, B. and Hamidian, A.H. (2013). Metal concentrations in tissues of common carp, *Cyprinus carpio*, and silver carp, *Hypophthalmichthys molitrix* from the Zarivar Wetland in Western Iran. Arch. Pol. Fish, 21:11–18. https://doi.org/10.2478/aopf-2-13-0002.

Mohan, M.R. and Latha, N. (2018). Hematological response of freshwater fish *Glossogobius giuris* from river Cauvery exposed to malathion pesticide. Multilogic in Sci., 8(26):80–83.

Obinna, O.; Eyo, J. and Emmanuel, E.O. (2019). Role of Fish as Bioindicators: A Review. IRE Journals, 2(11):15.

Ortiz, J.B.; De Canales, M.L.G. and Sarasquete, C. (2003). Histopathological changes induced by lindane (ϒ-HCH) in various organs of fishes. Sci. Mar., 67(1):53–61.

Rahman, A.N.A.; Mohamed, A.A.R.; Mohammed, H.H.; Elseddawy, N.M.; Salem, G.A. and El-Ghareeb, W.R. (2020). The ameliorative role of geranium (*Pelargonium graveolens*) essential oil against hepatorenal toxicity, immunosuppression, and oxidative stress of profenofos in common carp, *Cyprinus carpio* (L.). Aquaculture, 517:734777.

Raj, A.; Kumar, A. and Khare, P. (2024). The looming threat of profenofos organophosphate and microbes in action for their sustainable degradation. Environ. Sci. Pollut. Res., 31:14367–14387. https://doi.org/10.1007/s11356-024-32159-7.

Ramesh, M. and Saravanan, M. (2008). Haematological and biochemical responses in a freshwater fish, *Cyprinus carpio* exposed to Chlorpyrifos. Int. J. Integr. Biol., 3(1):80–83.

Shah, S.L. and Altindag, A. (2004). Hematological parameters of Tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull. Environ. Contam. Toxicol., 73:911–918. https://doi.org/10.1007/s00128-004-0513-y.

Taha, A. (2022). Assessment of non-target toxicity of profenofos insecticide on the aquatic bird; the white egret *Egretta alba*. Egyptian J. Aquat. Biol. Fish, 26(2):263–276.

Velmurugan, B.; Selvanayagam, M.; Cengiz, E.I. and Unlu, E. (2007). The effects of monocrotophos to different tissues of freshwater fish *Cirrhinus mrigala*. Bull. Environ. Contam. Toxicol., 78:450–454. https://doi.org/10.1007/s00128-007-9190-y.

Verma, P.; Mishra, B.B. and Rani, P. (2015). Influence of Endocel and Rogor on serum free amino acid and total protein level in *Clarias batrachus* (Linn.). J. Environ. Biol., 36:639–643.

Zenebehagos, Z.; Chaitanya, K.; Krishnan, G.K.G.; Teka, Z. and Mulugeta, M. (2017). Toxic effect of profenofos on blood parameters in the freshwater fish, *Labeo rohita* (Hamilton). Innovat. Int. J. Med. Pharmaceut. Sci., 2(2):14–18.