



Sub-Lethal Effects of Endosulfan+Chlorpyrifos Mixture on Biochemical Parameters and Blood Genotoxicity Markers of Two Cyprinidae Fish Species *Catla catla* and *Labeo rohita*

Huma Naz^{1*}, Sajid Abdullah², Fariha Latif³, Farzana Abbaasi⁴, Naila Hedayat⁵, Tanveer Ahmed^{6*}, Khalid Abbas² and Muhammad Adeel Hassan⁷

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

²Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

³Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan

⁴Department of Zoology, The Islamic University of Bahawalpur, Bahawalpur, Pakistan

⁵Department of Botany, University of Education, Bank Road Campus, Lahore, Pakistan

⁶Department of Life Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan

⁷Department of Parasitology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

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Authors' Contribution

HN executed the research work. SA planned the research work. FL helped in lab work. FA and NH helped in writing the manuscript. TA and MAH performed statistical analysis. KA guided as member of supervisory committee.

Key words

Fish, Enzymes, Mixture toxicity, DNA damage, Chronic, Insecticides

ABSTRACT

The current study was performed to check the sub-lethal effects of endosulfan+chlorpyrifos mixture on biochemical parameters viz. peroxidase (POx), superoxide dismutase (SOD), catalase (CAT) and Phase-II glutathione (GST) in hepatic, neural, nephron, bronchial, cardiac and muscle tissue of *Catla catla* and *Labeo rohita*. The geno-toxic effects were studied in terms of DNA damage and nuclear abnormalities in the RBCs of both fish species. The fish was exposed to the mixture for 60 days and the fish sample was taken after the 15-day period. The negative control (NC) fish were kept in water having no insecticides. The results showed that during the first two samplings, an increase in activities of POx, SOD, and GST in all tissues of both fishes exposed to the insecticides mixture was noted as compared to the control. The trend of SOD level in organs of both fish species was noted as hepatic>neural>nephron>bronchial>cardiac>muscle tissue. The POx and GST levels in *L. rohita* and *C. catla* were observed as: hepatic>neural>bronchial>nephrotic>cardiac>muscle tissues. CAT activity was increased in the bronchial, hepatic, and nephrotic tissues of both fishes while it was reduced in cardiac, neural, and muscle tissues. The result of DNA damage showed that GDI and DN were higher during the first 15 days after that damage was lower. The *L. rohita* showed higher MN and NAs during the first 15 days of exposure after that damage was lower. However, *C. catla* showed an increase in the formation of MN and NAs throughout the exposure period.

INTRODUCTION

In the last few years, the application of agrochemicals like insecticides has been increased in the agricultural

sector for improving the yield and quality of the crops (Doruchowski *et al.*, 2017) with less work and time but this has negative consequences for the environment (Ullah, 2015). This practice has increased and becomes a necessary evil, especially in developing countries and those countries where agriculture is expected to be the backbone of the economy (Doruchowski *et al.*, 2017). After application, these pesticides ultimately enter into the water bodies in significant quantities through contaminated water,

* Corresponding author: dr.humanaz98@gmail.com, tanvirahmeduaf@gmail.com
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Abbreviations

E, Endosulfan; C, Chlorpyrifos; GDI, Genetic Damage index; DN, Damaged nuclei; MN, Micronuclei; BIN, Binucleated nuclei; DEN, Dashed nuclei; BN, Blebbled nuclei; DN, Dumb nuclei; NN, Notched nuclei

agricultural and urban run-off, bottom sediments, waste, atmospheric fall-out by rain and municipal water treatment, etc. (Kumari, 2020). Extensive use of these chemicals resulted in water pollution which poses a serious threat to freshwater biodiversity due to their ability to bioaccumulate and induce toxicity (Cui *et al.*, 2015). Now, the assessment of toxicity associated with pesticides and their harmful effects on non-target aquatic animals has been a matter of worldwide concern (Matozzo *et al.*, 2018).

The most commonly used insecticides in agriculture are organochlorine and organophosphate (Ullah *et al.*, 2015, 2016). The metabolites of these insecticides finally enter the water bodies (Das and Mukherjee, 2003). Among the organophosphate, a widely used insecticide is chlorpyrifos with a long half-life and high stability (Wu *et al.*, 2016). Exposure to pesticides to aquatic organisms especially fish led to toxic impacts, such as alteration in the acetylcholinesterase activity of *Cyprinus carpio* and behavior of *Labeo rohita* as well as impairment in *Gobio cypris rarus* embryos and larvae development (Wang *et al.*, 2015; Mustafa *et al.*, 2014; Zhu *et al.*, 2014). Endosulfan belongs to organochlorine insecticide also broadly used throughout the world and can affect aquatic life through its bio-magnification and disturb homeostasis and metabolic activities and also induce damage to DNA (Indirabai *et al.*, 2010; Adhikari *et al.*, 2006; Ullah, 2015). It also induced the formation of reactive oxygen species (ROS) (Ghaffar *et al.*, 2015) causing oxidative stress in aquatic organisms, especially fish, by modulation of antioxidant systems in fish (Shao *et al.*, 2012). The organisms kept balance in the formation and elimination of ROS with the help of an antioxidant defense system including superoxide dismutase (SOD), peroxidase (POx), catalase (CAT), and glutathione-S-transferase (GST) (Kilili *et al.*, 2004; Valavanidis *et al.*, 2006). Against oxidative stress, the first line of defense is SOD which transfers the free oxygen radicals into hydrogen peroxide and molecular oxygen, that is further converted into H₂O and O₂ by POx and CAT enzymes (Zheng *et al.*, 2016; Cheng *et al.*, 2018). GST is a Phase-II enzyme present almost in all species and plays a role in the detoxification of toxicants (Hayes *et al.*, 2005).

The unnecessary production of ROS may also cause DNA damage like oxidation and breakage of DNA strands (Oruc *et al.*, 2014). The toxicological and safety effects evaluation of pesticides is necessary due to their deleterious effects such as cancer, chromosomal aberrations, gonadotoxicity, infertility, and fetal malformations (Ahmad *et al.*, 2012). The alkaline single-cell gel electrophoresis assay also famous as the comet assay identify the DNA damage in term of alkali-labile sites, strand breaks, and delayed-repair sites (Ng and Romano, 2013). The other most promising test is micronucleus (MN) which is

associated with nuclear abnormalities (NAs) and has also been used in the field of ecotoxicology to detect abnormalities at the chromosomal level (Bolognesi and Hayashi, 2011). Both these simple tests are widely applied due to their high sensitivity and statistical power to assess the genotoxic impacts.

Another major problem in environmental risk evaluation is that aquatic bodies contain different insecticides in a mixture form rather than a single chemical (Schreiner *et al.*, 2016). Fish are good specimen which is not only used to assess the quality of aquatic system but their physiological systems are also used as valuable biomarkers to detect pollution. In this context, the present study provides more information about the effects of organophosphate and organochlorine pesticides on the enzymes and DNA functioning in non-target organisms the fish.

MATERIALS AND METHODS

Experimental specimens and sub-lethal trail

The two fish species, *Catla catla* and *Labeo rohita* (90-day old) from the Cyprinidae family were got from the fish seed Hatchery, Faisalabad. Both fishes were, live transferred to the Toxicology laboratory at the Fisheries Research Farm of UAF. Fishes were acclimatized to the laboratory environment by keeping them in the rectangular cemented tanks for 14 weeks. The experiment was conducted with 20 specimens of both species with equal weight and size, separately, kept in a 100-L aquarium facilitated with an oxygen pump. The control fish were kept in water having no insecticide.

Fishes were kept in 1/3rd of LC₅₀ of endosulfan+chlorpyrifos mixture, separately, for 60 days. The LC₅₀ (96 h) concentration of the mixture for *L. rohita* and *C. catla* was calculated as 1.95±0.02 and 1.35±0.01 µg/L⁻¹, respectively (Naz *et al.*, 2019a, b). The sampling of fish (n=5) were done after 15, 30, 45 and 60 days interval labeled as D1, D2, D3, and D4, respectively. In the sub-lethal trial, no mortality was observed. During the trial, water pH (7), temperature (28°C), and total hardness (220 mg/L⁻¹) were also kept constant. Fish of positive control (PC) were injected with a dose of cyclophosphamide at 20 µg/g⁻¹ of body weight to study the blood genotoxic markers.

Preparation of insecticides solutions

The clean water having no insecticide was used for control. The stock-I solution was made by mixing 1g of technical grade endosulfan (97% purity) and chlorpyrifos (98% purity), separately, in 95% analytical grade methanol (100ml). The stock-II solution, E+C mixture of insecticide of required ratio (1:1) were made in deionized water.

Tissue enzymes markers

After the sub-lethal trial, activities of enzymes viz. superoxide dismutase SOD, CAT and POx, and phase-II GST were assessed in cardiac, bronchial, muscle, nephritic, neural and hepatic tissues of both fishes. The homogenates of tissues were ready according to the procedure given by Zia *et al.* (2007). Giannopolitis and Ries (1977) protocol was used to analyze the SOD activity. Chance and Mehaly (1977) procedure was adopted to quantify the CAT and POx activities. Mannervik (1985) method was followed to calculate the GST activity.

Blood genotoxic markers

Comet/ SCGE assay

The blood was collected from the caudal vein of the fish and treated according to Singh *et al.* (1988). According to Jose *et al.* (2011) the damaged DNA was evaluated. The length of the tail was used to classify five types of damaged DNA known as comets. The following formula was applied to quantify the DNA damage:

$$\text{Damaged cell (\%)} = \frac{\text{Types II} + \text{III} + \text{IV}}{\text{Type 0} + \text{Type I} + \text{Type II} + \text{Type III} + \text{Type IV}} \times 100$$

Micronucleus test

The slides for micronuclei were prepared according to Barsiene *et al.* (2004) method. Fenech *et al.* (2003) procedure was followed to score the micronuclei and other nuclear anomalies in the blood of fishes. To compute MN frequency, the following formula was used:

$$\text{MN\%} = \frac{\text{Number of cells containing micronucleus}}{\text{Total number of cells counted}} \times 100$$

Data analyses

The obtained data was statistically analyzed through the 8.1 version of statistics software. The ANOVA (a linear model) under CRD was applied to data to see the differences among tissues for enzyme activities followed by the student Newman-Keul test for mean comparison. The data obtained from the comet assay and MN test was analyzed through a non-parametric Mann-Whitney U-test. The significance level was set as $p > 0.05$.

RESULTS AND DISCUSSION

Tissue enzymes markers

The results of this study showed that E+C-exposed fish species showed significant change in antioxidant enzymes viz. SOD, POD, GST and CAT as compared to control group. The change in antioxidant enzyme activities may be a response against oxidative stress due to free

radicals. During the first two samplings (D1 and D2) an increase in activity of SOD, POx, and GST in all tissues of both fishes exposed to the E+C mixture was noted as compared to the control. However, a decline was noted in D3 and D4 sampling. The trend of SOD level in both fish species was noted as: hepatic > neural > nephron > bronchial > cardiac > muscle tissue. The POx and GST level in *L. rohita* and *C. catla* was observed as: hepatic > neural > bronchial > nephrotic > cardiac > muscle tissues. In the present study, CAT activity was initially (D1 and D2 sampling) increased in the bronchial, hepatic, and nephrotic tissues of both fishes. While it was decreased in neural, cardiac, and muscle tissues of E+C mixture exposed fishes throughout the experiment (Fig. 1). Similar findings were observed by Naz *et al.* (2022) for *Cirrhinus mrigala* when exposed to three different binary mixtures of insecticides viz. endosulfan, chlorpyrifos and bifenthrin. According to Webb *et al.* (2005) changes in enzyme activity, as well as a reduction, reveal that contaminants have shown reaction inside the body of fish. Several parameters, including species of fish toxicant dose, and period of exposure influence the duration and amplitude of these reactions (Piazza *et al.*, 2015). Several authors had reported specie and dose-specific responses of enzymes, due to persistent organic pollutants (either increase or decrease in enzyme activity) (Lu *et al.*, 2013; Koenig *et al.*, 2012). Antioxidant enzymes work in the defense mechanism of the fish body. These act to defend fish against oxidative stress while a decrease in their activity disrupt the redox status of the cell. Reactive oxygen species (ROS) are induced by POP exposure to cells. Enzymes get back to normal activity when ROS got removed from the fish bodies (Stara *et al.*, 2012; Ural, 2013). Various organs are under the effect by pesticide (Limon-Pacheco and Gonsebatt, 2009) like the liver, heart, stomach, intestine, spleen, kidney, gallbladder, muscle, swim-bladder, brain, operculum, gills, vertebra and gonads; however, all these are not commonly used but they also could serve as valuable evidence in terms of ecotoxicology (Jovicic *et al.*, 2014).

The metabolites of endosulfan are more persistent and toxic as compared to the original form (Awasthi *et al.*, 2000). According to Salvo *et al.* (2012), activity of antioxidant enzymes (SOD, CAT, GST, and GPx) in the liver of *Cyprinus carpio* were considerably altered by sub-lethal endosulfan exposure. The activities of SOD and GST in cardiac tissues were dramatically increased after exposure to endosulfan (Jalili *et al.*, 2007).

Organophosphate pesticides (OP) use two pathways to induce ROS. The first choice is oxidation-reduction cycle, which is catalyzed by cytochrome P 450S. The chemical link $-P=O$, which was changed

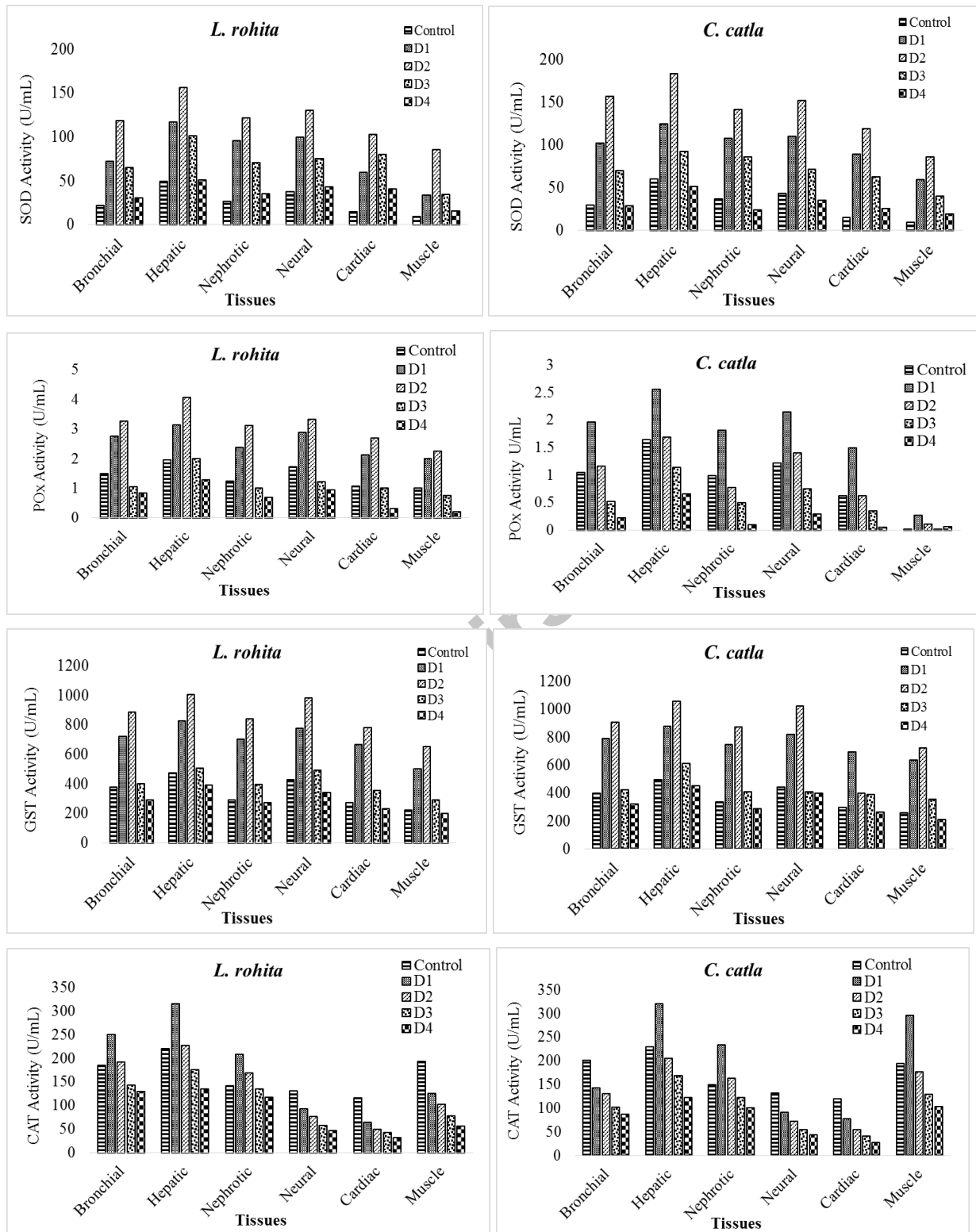


Fig. 1. Activity of antioxidant enzymes in different tissues of *C. catla* and *L. rohita*. Samples of tissues were taken after 15 days (D1), 30 days (D2), 45 days (D3) and 60 days (D4).

from the $-P=S$ or was previously present in organophosphate insecticides, may easily gain an electron and transfer into the oxygen molecule to form superoxide anion, which can subsequently be produced other ROS such as hydroxyl ion (Kovacic, 2003). Secondly, these ROS are limited by antioxidant enzyme otherwise, these causes an excessive accumulation of ROS. Plasma membrane and organelle enzyme activities as well as nerve conductance are interrupted by OP (Karaoz *et al.*, 2002). Similarly, many researchers reported the fluctuation in GST, POx, SOD, and CAT activities in different fish species exposed to insecticides (Wang *et al.*, 2009; Abdullah *et al.*, 2018; Ozok, 2020; Deb and Das, 2021; Naz *et al.*, 2021). The chlorpyrifos+endosulfan mixture induced fluctuations in GST, SOD, CAT, and POx activities in various organs (liver, brain, gills, heart, kidney, and muscle) of fish, *Labeo rohita* (Naz *et al.*, 2019a) and *Catla catla* (Naz *et al.*, 2021). According to Usman *et al.* (2020) decline in CAT activity in various tissues depends on the type of toxicants and exposure duration. Siddique *et al.* (2020, 2021) observed an initial rise in GST activity of *L. rohita* up to 28 days after that it decreased up to 56 days.

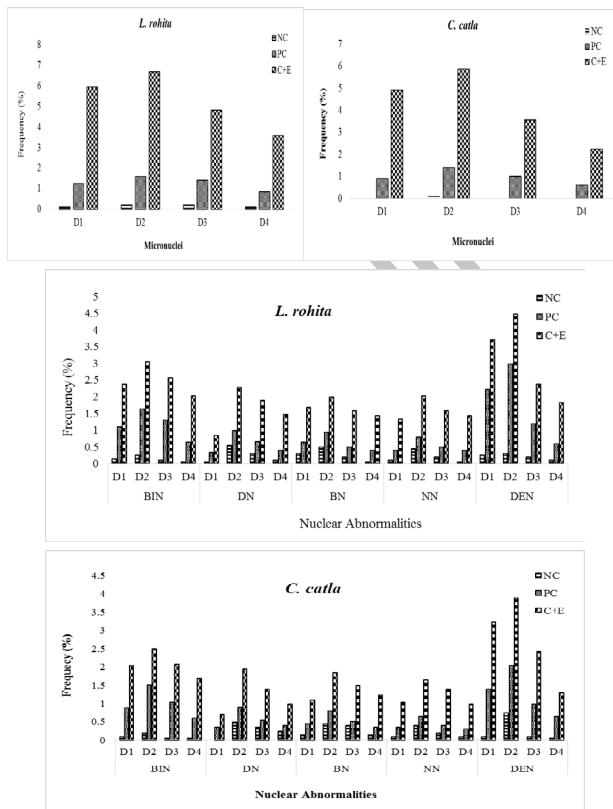


Fig. 2. Micronuclei and nuclear anomalies in RBC of *C. catla* and *L. rohita*.

Blood genotoxicity markers

Results showed that *L. rohita* had higher MN and NAs in RBCs during the first sampling (D1) of exposure to the E+C mixture after that damage was lower throughout the experiment. However, *C. catla* showed an increase in the formation of MN and NAs in RBCs throughout the exposure period. The result of geno-toxicity showed that GDI and DN were higher during the first sampling (D1) after that damage was lower (Figs. 2, 3). The fish, *Cirrhina mrigala* showed exposure depended on changes in DNA damage, MN, and NAs during chronic exposure to three different mixtures of insecticides (Naz *et al.*, 2022). In aquatic environments, the SCGE/CA is a frequently applied method for detecting geno-toxicity (Frenzilli *et al.*, 2009). This assay offers the benefit of identifying individual cells with damaged DNA (Buschini *et al.*, 2004; Lee and Steinert, 2003). According to Lee and Steinert (2003), interactions between DNA molecules and contaminants can appear in a variety of ways, including DNA damage caused by ROS, DNA repair inhibition, compound action directly on DNA, and metabolites' interaction with DNA. ROS has been found to cause cellular and DNA damage at levels above normal (Cadet *et al.*, 2003).

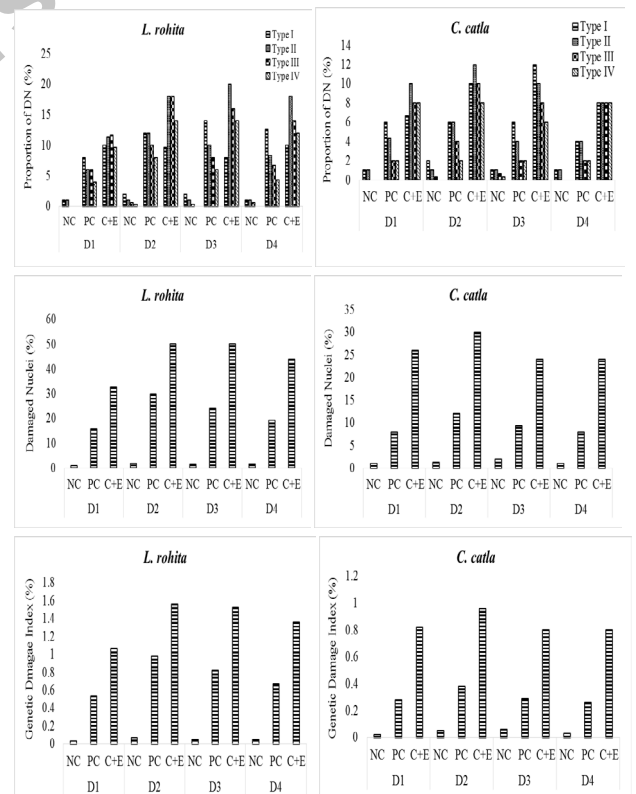


Fig. 3. Percentage of comet types, damaged nuclei and genetic damage index in RBC of *C. catla* and *L. rohita*.

With the oxidative potential of hydroxyl radical and indiscriminate reactivity with cellular constituents like lipids in cell membranes, DNA, and enzyme proteins, the hydroxyl radical is the most significant free radical of biological and toxicological importance, with a lifetime of a few nanoseconds (Jackson and Loeb, 2001). Another method of DNA damage caused by pesticide exposure is the presence of heavy metals like chromium, iron, cadmium, nickel, copper, zinc, lead, and manganese in these pesticides (Hayat *et al.*, 2007). Through Fenton-like processes, these metal cations affect the polyanionic DNA (Ercal *et al.*, 2001). Fenech and Ferguson (2001) reported the ability of live organisms to build and control particular enzyme systems for restoring DNA damage. Two electrophilic groups, alkyl and phosphoryl groups that are produced by the metabolism of organophosphate are ideal targets for nucleophilic attack. Through the phosphorylation process, this could interact with DNA (Ali *et al.*, 2009). Several researchers successfully applied to quantify the insecticides induced DNA damage in terms of damaged nuclei and GDI in various fish species viz., *Catla catla* (Naz *et al.*, 2019b), *Labeo rohita* (Nataraj *et al.*, 2020), *Cyprinus carpio* (Hemalatha *et al.*, 2020), silver carp (Ullah *et al.*, 2019) and *Cyprinus carpio* (Ambreen and Javed, 2019).

The toxicity of aneugenic and clastogenic aquatic contaminants is evaluated by the use of MN frequency in fish erythrocytes (Udroiu, 2006; Ferraro *et al.*, 2004). Tubulin polymerization failure may be linked to nuclear changes such as BL and LB (Vardavas *et al.*, 2016). Furthermore, NAs are produced as a result of complications in the development of mitotic fuse due to the chemical's aneugenic activity (de-Campos Ventura *et al.*, 2008). As in previous research, the signification formation of NAs frequencies in fishes was noted after exposure to QP-containing pesticides (Sadiqul *et al.*, 2016), carbosulfan, glyphosate, atrazine (Nwani *et al.*, 2014) and formalin (Mert *et al.*, 2015). Similarly, MN and NAs formation in erythrocytes of insecticides exposed fish species by using micronucleus test were also recorded by many authors (Mitkovska and Chassovnikarvo, 2020; Naz *et al.*, 2021; Davico *et al.*, 2020).

CONCLUSION

The findings of the current study suggest that the fish enzyme activities, nuclear anomalies, and comet assay are valuable potent diagnostic tools for monitoring insecticide toxicity in the aquatic environments. However, this study suggests that extensive use of insecticides should be minimized or applied under strict environmental regulations. Farmers should adopt another strategy like

biological control to kill pests instead of insecticides.

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IRB approval and ethical statement

The present study was approved by the Institutional Biosafety and Bioethics Committee of University of Agriculture, Faisalabad and was conducted following institutional guidelines for ethical conduct.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Abdullah, S., Mateen, A., Abbas, K., Naz, H., Hassan, W. and Anum, S., 2018. Changes in glutathione S-transferase activity in fish *Channa striata* Exposed to different aquatic pollutants (heavy metals and pesticides mixture). *Pakistan J. Zool.*, **13**(Suppl.): 42-47.
- Adhikari, S., Sarkar, B., Chattopadhyay, A., Chattopadhyay, D., Sarkar, S. and Ayyappan, S., 2006. Effect of cypermethrin on breeding performances of a freshwater fish, *Labeo rohita* (Hamilton). *Chem. Ecol.*, **22**: 211-218. <https://doi.org/10.1080/02757540600682062>
- Ahmad, L., Khan, A. and Khan, M.Z., 2012. Pyrethroid-induced reproductive toxico-pathology in non-target species. *Pak. Vet. J.*, **32**: 1-9.
- Ali, D., Nagpure, N.S., Kumar, S., Kumar, R., Kushwaha, B. and Lakra, W.S., 2009. Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Fd. Chem. Toxicol.*, **47**: 650-656.
- Ambreen, F. and Javed, M., 2019. Nuclear damage in peripheral erythrocytes of *Cyprinus carpio* exposed to binary mixture of pesticides. *J. Zoo Biol.*, **2**: 39-47. <https://doi.org/10.33687/zoobiol.002.01.1506>
- Awasthi, N., Ahuja, R. and Kumar, A., 2000. Factors influencing the degradation of soil-applied endosulfan isomers. *Soil Biol. Biochem.*, **32**: 1697-1705. [https://doi.org/10.1016/S0038-0717\(00\)00087-0](https://doi.org/10.1016/S0038-0717(00)00087-0)
- Barsiene, J., Lazutka, J., Syvokiene, J., Dedonyte, V., Rybakovas, A., Bjornstad, A. and Andersen, O.K., 2004. Analysis of micronuclei in blue mussels and fish from the Baltic and north seas. *Environ. Toxicol.*, **19**: 365-371. <https://doi.org/10.1002/>

- tox.20031
- Bolognesi, C. and Hayashi, M., 2011. Micronucleus assay in aquatic animals. *Mutagenesis*, **26**: 205-213. <https://doi.org/10.1093/mutage/geq073>
- Buschini, A., Carboni, P., Furlini, M., Poli, P., and Rossi, C. 2004. Sodium hypochlorite-, chlorine dioxide- and peracetic acid-induced genotoxicity detected by the comet assay and *Saccharomyces cerevisiae* D7 tests. *Mutagenesis*, **19**: 157-162. <https://doi.org/10.1093/mutage/geh012>.
- Cadet, J., Douki, T., Gasparutto, D. and Ravanat, J.L., 2003. Oxidative damage to DNA: Formation, measurement and biochemical feature. *Mut. Res.*, **531**: 5-23.
- Chance, M. and Mehaly, A.C., 1977. Assay of catalase and peroxidase. *Methods Enzymol.*, **2**: 764-817.
- Cheng, C.H., Guo, Z.X., Luo, S.W. and Wang, A.L., 2018. Effects of high temperature on biochemical parameters, oxidative stress, DNA damage and apoptosis of pufferfish (*Takifugu obscurus*). *Ecotoxicol. Environ. Safe.*, **150**: 190-198. <https://doi.org/10.1016/j.ecoenv.2017.12.045>
- Cui, L.L., Ge, J., Zhu, Y.D., Yang, Y.Y. and Wang, J., 2015. Concentrations, bioaccumulation, and human health risk assessment of organochlorine pesticides and heavy metals in edible fish from Wuhan, China. *Environ. Sci. Pollut. Res.*, **22**: 15866-15879. <https://doi.org/10.1007/s11356-015-4752-8>
- Das, B.K. and Mukherjee, S.C., 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: Biochemical, enzymatic and haematological consequences. *Comp. Biochem. Physiol.*, **134**: 109-121. [https://doi.org/10.1016/S1532-0456\(02\)00219-3](https://doi.org/10.1016/S1532-0456(02)00219-3)
- Davico, C.E., Loteste, A., Parma, M.J., Poletta, G. and Simoniello, M.F., 2020. Stress oxidative and genotoxicity in *Prochilodus lineatus* (Valenciennes, 1836) exposed to commercial formulation of insecticide cypermethrin. *Drug Chem. Toxicol.*, **43**: 79-84. <https://doi.org/10.1080/01480545.2018.1497643>
- Deb, N. and Das, S., 2021. Acetylcholine esterase and antioxidant responses in freshwater teleost, *Channa punctata* exposed to chlorpyrifos and urea. *Comp. Biochem. Physiol. C Toxicol. Pharm.*, **240**: 108912. <https://doi.org/10.1016/j.cbpc.2020.108912>
- De-Campos-Ventura, B., De-Angelis, D.D.F. and Marin-Morales, M.A., 2008. Mutagenic and genotoxic effects of the Atrazine herbicide in *Oreochromis niloticus* (Perciformes, Cichlidae) detected by the micronuclei test and the comet assay. *Pestic. Biochem. Physiol.*, **90**: 42-51. <https://doi.org/10.1016/j.pestbp.2007.07.009>
- Doruchowski, G., Swiechowski, W., Masny, S., Maciesiak, A., Tartanus, M., Bryk, H. and Hołownicki, R., 2017. Low-drift nozzles vs. standard nozzles for pesticide application in the biological efficacy trials of pesticides in apple pest and disease control. *Sci. Total Environ.*, **575**: 1239-1246. <https://doi.org/10.1016/j.scitotenv.2016.09.200>
- Ercal, N., Gurer-Orhan, H. and Aykin-Burns, N., 2001. Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem.*, **1**: 529-539.
- Fenech, M. and Ferguson, L.R., 2001. Vitamins/minerals and genomic stability in humans. *Mut. Res.*, **475**: 1-6.
- Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S. and Zeiger, E., 2003. Human project: Detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte culture. *Mut. Res.*, **534**: 65-75. [https://doi.org/10.1016/S1383-5718\(02\)00249-8](https://doi.org/10.1016/S1383-5718(02)00249-8)
- Ferraro, M.V.M., Fenocchio, A.S., Mantovani, M.S., De-Oliveira, R.C. and Cestari, M.M., 2004. Mutagenic effects of tributyltin and inorganic lead (Pb II) on the fish *H. malabaricus* as evaluated using the comet assay and the piscine micronucleus and chromosome aberration tests. *Genet. mol. Biol.*, **27**: 103-107. <https://doi.org/10.1590/S1415-47572004000100017>
- Frenzilli, G., Nigro, M. and Lyons, B.P., 2009. The comet assay for the evaluation of genotoxic impact in aquatic environments. *Mut. Res.*, **681**: 80-92. <https://doi.org/10.1016/j.mrrev.2008.03.001>
- Ghaffar, A., Riaz, H., Ahrar, K. and Abbas, R.Z., 2015. Hemato-biochemical and genetic damage caused by triazophos in freshwater fish *Labeo rohita*. *Int. J. Pharm. biol. Sci.*, **17**: 637-642. <https://doi.org/10.17957/IJAB/17.3.14.1016>
- Giannopolitis, C.N. and Ries, S.K., 1977. Superoxide dismutase occurrence in higher plants. *Plant Physiol.*, **59**: 309-314. <https://doi.org/10.1104/pp.59.2.309>
- Hayat, S., Javed, M. and Razzaq, S., 2007. Growth performance of metal stressed major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system. *Pak. Vet. J.*, **27**: 8-12.
- Hayes, J.D., Flanagan, J.U. and Jowsey, I.R., 2005. Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.*, **45**: 51-88. <https://doi.org/10.1146>

- annurev.pharmtox.45.120403.095857
- Hemalatha, D., Nataraj, B., Rangasamy, B., Maharajan, K. and Rames, M., 2020. Exploring the sublethal genotoxic effects of class II organophosphorus insecticide quinalphos on freshwater fish, *Cyprinus carpio*. *J. Ocean. Limnol.*, **39**: 661-670.
- Indirabai, W.P.S., Tharani, G.G. and Seetha, P., 2010. Impact of sublethal concentration of endosulfan on biochemicals and histology of organ tissues of freshwater fish, *Labeo rohita* (Hamilton, 1822). *Bioscan*, **5**: 215-218.
- Jackson, A.L. and Loeb, L.A., 2001. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. *Mut. Res.*, **477**: 7-21. [https://doi.org/10.1016/S0027-5107\(01\)00091-4](https://doi.org/10.1016/S0027-5107(01)00091-4)
- Jalili, S.H., Ilkhanipour, M., Heydari, R., Farshid, A.A. and Salehi, S., 2007. The effects of vitamin E on endosulfan induced oxidative stress in rat heart. *Pak. J. Nut.*, **6**: 375-380. <https://doi.org/10.3923/pjn.2007.375.380>
- Jose, S., Jayesh, P., Mohandas, A., Philip, R. and Singh, I.S.B., 2011. Application of primary haemocyte culture of *Penaeus monodon* in the assessment of cytotoxicity and genotoxicity of heavy metals and pesticides. *Mar. Environ. Res.*, **71**: 169-177. <https://doi.org/10.1016/j.marenvres.2010.12.008>
- Jovicic, K., Nikolic, D.M., Visnjic-Jeftic, Z., Dikanovic, V., Skoric, S., Stefanovic, S.M., Ienhardt, M., Hegedis, A., Krpo-Cetkovic, J. and Jaric, I., 2014. Mapping differential elemental accumulation in fish tissues: Assessment of metal and trace element concentrations in wels catfish (*Silurus glanis*) from the Danube River by ICP-MS. *Environ. Sci. Pollut. Res.*, **22**: 3820-3827. <https://doi.org/10.1007/s11356-014-3636-7>
- Karaoz, E., Gultekin, F., Akdogan, M., Oncu, M. and Gokcimen, A., 2002. Protective role of melatonin and combination of vitamin C and vitamin E on lung toxicity induced by chlorpyrifos-ethyl in rats. *Exp. Toxicol. Pathol.*, **54**: 97-108. <https://doi.org/10.1078/0940-2993-00236>
- Kilili, K.G., Atamassova, N., Vardanyan, A., Clatot, N., Al-Subarea, K., Kanellopoulos, P.N., Makris, A.M., Kampranis, S.C., 2004. Differential roles of tau class glutathione S-transferases in oxidative stress. *J. Biol. Chem.*, **279**: 24540-24551. <https://doi.org/10.1074/jbc.M309882200>
- Koenig, S., Fernandez, P. and Sole, M., 2012. Differences in cytochrome P450 enzyme activities between fish and crustacea: Relationship with the bioaccumulation patterns of polychlorobiphenyls (PCBs). *Aquat. Toxicol.*, **108**: 11-17. <https://doi.org/10.1016/j.aquatox.2011.10.016>
- Kovacic, P., 2003. Mechanism of organophosphates (nerve gases and pesticides) and antidotes: Electron transfer and oxidative stress. *Curr. med. Chem.*, **10**: 2705-2709. <https://doi.org/10.2174/0929867033456314>
- Kumari, K., 2020. Pesticides toxicity in fishes: A review. *J. Ent. Zool. Stud.*, **8**: 1640-1642.
- Lee, R.F. and Steinert, S., 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mut. Res.*, **54**: 43-64. [https://doi.org/10.1016/S1383-5742\(03\)00017-6](https://doi.org/10.1016/S1383-5742(03)00017-6)
- Limon-Pacheco, J. and Gonshebbat, M.E., 2009. The role of antioxidants and antioxidant related enzymes in protective responses to environmentally induced oxidative stress. *Mut. Res.*, **674**: 137-147. <https://doi.org/10.1016/j.mrgentox.2008.09.015>
- Lu, Y., Zhang, A., Li, C., Zhang, P., Su, X., Li, Y., Mu, C. and Li, T., 2013. The link between selenium binding protein from *Sinonovacula constricta* and environmental pollutions exposure. *Fish Shellfish Immunol.*, **35**: 271-277. <https://doi.org/10.1016/j.fsi.2013.04.040>
- Mannervik, B., 1985. The isozymes of glutathione transferase. *Adv. Enzymol. Relat. Areas mol. Biol.*, **57**: 357-417. <https://doi.org/10.1002/9780470123034.ch5>
- Matozzo, V., Fabrello, J., Masiero, L., Ferraccioli, F., Finos, L., Pastore, P., Gangi, I.M.D. and Bogialli, S., 2018. Ecotoxicological risk assessment for the herbicide glyphosate to non-target aquatic species: A case study with the mussel *Mytilus galloprovincialis*. *Environ. Pollut.*, **233**: 623-632. <https://doi.org/10.1016/j.envpol.2017.10.100>
- Mert, R., Benli, A.C.K. and Arslan, G., 2015. Determination of histological and genotoxic effects of formalin on Nile tilapia (*Oreochromis niloticus* L.). *Aquacult. Res.*, **46**: 2798-2807. <https://doi.org/10.1111/are.12434>
- Mitkovska, V. and Chassovnikarova, T., 2020. Chlorpyrifos levels within permitted limits induce nuclear abnormalities and DNA damage in the erythrocytes of the common carp. *Environ. Sci. Pollut. Res.*, **27**: 7166-7176. <https://doi.org/10.1007/s11356-019-07408-9>
- Mustafa, G., Mahboob, S., Al-Ghanim, K.A., Sultana, S., Al-Balawi, H.F.A., Sultana, T., Al-Misned, F. and Ahmed, Z., 2014. Acute toxicity I: Effect of profenofos and triazophos (organophosphates) and carbofuran and carbaryl (carbamates) to *Labeo rohita*. *Toxicol. Environ. Chem.*, **96**: 466-473.

- <https://doi.org/10.1080/02772248.2014.952517>
- Nataraj, B., Hemalatha, D., Rangasamy, B., Maharajan, K. and Rames, M., 2017. Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeo rohita* exposed to organophosphorus pesticide profenofos. *Biocatal. Agric. Biotechnol.*, **12**: 185-190. <https://doi.org/10.1016/j.bcab.2017.09.006>
- Naz, H., Abdullah, S., Abbas, K., Hassan, W., Batool, Perveen, S., Maalik, S. and Mushtaq, S., 2019a. Toxic effect of insecticides mixtures on antioxidant enzymes in different organs of fish, *Labeo rohita*. *Pakistan J. Zool.*, **51**: 1355-1361. <https://doi.org/10.17582/journal.pjz/2019.51.4.1355.1361>
- Naz, H., Abdullah, S., Abbas, K., Tariq, M.R., Shafique, L. and Nazeer, G., 2019b. Comet assay: Quantification of damaged DNA in *Catla catla* exposed to endosulfan+chlorpyrifos. *Punjab Univ. J. Zool.*, **34**: 85-88. <https://doi.org/10.17582/journal.pujz/2019.34.1.85.88>
- Naz, H., Abdullah, S., Ahmed, T., Abbas, K. and Ijaz, M.U., 2021. Regression analysis for predicting the duration dependent response of oxidative stress dynamics and nuclear abnormalities in *Catla catla* exposed to chlorpyrifos and endosulfan. *J. Anim. Plant Sci.*, **31**: 1167-1173. <https://doi.org/10.36899/JAPS.2021.4.0314>
- Naz, H., Abdullah, S., Ahmed, T., Abbas, K., Ijaz, M.U., Kumar, S., Hassan, M.A. and Shah, S.Q.A., 2022. Toxicity, oxidative stress and geno-toxicity: Lethal and sub-lethal effects of three different insecticides mixtures on *Cirrhina mrigala*. *J. Anim. Pl. Sci.*, **32**: 256-265.
- Ng, W.K. and Romano, N., 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Rev. aquat. Sci.*, **5**: 220-254. <https://doi.org/10.1111/raq.12014>
- Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B., Kumar, P. and Lakra, W.S., 2014. Induction of micronuclei and nuclear lesions in *Channa punctatus* following exposure to carbosulfan, glyphosate and atrazine. *Drug. Chem. Toxicol.*, **37**: 370-377. <https://doi.org/10.3109/01480545.2013.866138>
- Oruc, E.O., Sevgiler, Y. and Uner, N., 2004. Tissue-specific oxidative stress responses in fish exposed to 2, 4-D and azinphosmethyl. *Comp. Biochem. Physiol.*, **137**: 43-51. <https://doi.org/10.1016/j.cca.2003.11.006>
- Ozok, N., 2020. Effects of cypermethrin on antioxidant enzymes and lipid peroxidation of Lake Van fish (*Alburnus tarichi*). *Drug Chem. Toxicol.*, **48**: 51-63.
56. <https://doi.org/10.1080/01480545.2019.1660363>
- Piazza, Y., Pandolfi, M., Da-Cuna, R., Genovese, G. and Nostro, F., 2015. Endosulfan affects GnRH cells in sexually differentiated juveniles of the perciform *Cichlasoma dimerus*. *Ecotoxicol. Environ. Saf.*, **116**: 150-159. <https://doi.org/10.1016/j.ecoenv.2015.03.013>
- Sadiqul, I.M., Ferdous, Z., Nannu, M.T.A., Mostakim, G.M. and Rahman, M.K., 2016. Acute exposure to a quinalphos containing insecticide (convoy) causes genetic damage and nuclear changes in peripheral erythrocytes of silver barb, *Barbonymus gonionotus*. *Environ. Pollut.*, **219**: 949-956. <https://doi.org/10.1016/j.envpol.2016.09.066>
- Salvo, L.M. Bainy, A.C.D., Ventura, E.C., Marques, M.R.F., Silva, J.R.M.C., Klemz, C. and De-Assis, H.C.S., 2012. Assessment of the sublethal toxicity of organochlorine pesticide endosulfan in juvenile common carp (*Cyprinus carpio*). *J. environ. Sci. Hlth.*, **47**: 1652-1658. <https://doi.org/10.1080/10934529.2012.687236>
- Schreiner, V.C., Szocs, E., Bhowmik, A.K., Vijver, M.G. and Schafer, R.B., 2016. Pesticide mixtures in streams of several European countries and the USA. *Sci. Total Environ.*, **573**: 680-689. <https://doi.org/10.1016/j.scitotenv.2016.08.163>
- Shao, B., Zhu, L.S., Dong, M., Wang, J., Wang, J.H., Xie, H., Zhang, Q.M., Du, Z.Q. and Zhu, S.Y., 2012. DNA damage and oxidative stress induced by endosulfan exposure in zebra fish (*Danio rerio*). *Ecotoxicology*, **21**: 1533-1540. <https://doi.org/10.1007/s10646-012-0907-2>
- Siddique, Q., Abdullah, S., Naz, H., Abbas, K. and Shafique, L., 2020. Changes in glutathione s-transferase activity and total protein contents of *Labeo rohita*. *Punjab Univ. J. Zool.*, **35**: 25-29. <https://doi.org/10.17582/journal.pujz/2020.35.1.25.28>
- Siddique, Q., Abdullah, S., Naz, H., Abbas, K., Shafique, L. and Liu, Q., 2021. Sub-lethal effects of chlorpyrifos on glutathione S-transferase activity and total protein contents of fish, *Labeo rohita*. *Pakistan J. Zool.*, **54**: 1467-1470. <https://doi.org/10.17582/journal.pjz/20180806160859>
- Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L., 1988. A simple technique for quantization of low levels of DNA damage in individual cells. *Exp. Cell Res.*, **175**: 184-191. [https://doi.org/10.1016/0014-4827\(88\)90265-0](https://doi.org/10.1016/0014-4827(88)90265-0)
- Stara, A., Machova, J. and Velisek, J., 2012. Effect of chronic exposure to simazine on oxidative stress

- and antioxidant response in common carp (*Cyprinus carpio* L.). *Environ. Toxicol. Pharmacol.*, **33**: 334-343. <https://doi.org/10.1016/j.etap.2011.12.019>
- Udroiu, I., 2006. The micronucleus test in piscine erythrocytes. *Aquat. Toxicol.*, **79**: 201-204. <https://doi.org/10.1016/j.aquatox.2006.06.013>
- Ullah, R., Zuberi, A., Naeem, M. and Ullah, S., 2015. Toxicity to hematology and morphology of liver, brain and gills during acute exposure of mahseer (*Tor putitora*) to cypermethrin. *Int. J. Agric. Biol.*, **17**: 199-204.
- Ullah, S., 2015. *Protective role of vitamin C against cypermethrin induced toxicity in Labeo rohita (Ham.): Biochemical aspects*. M.Phil. thesis Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan.
- Ullah, S., Begum, M., Dhama, K., Ahmad, S., Hassan, S. and Alam, I., 2016. Malathion induced DNA damage in fresh water fish, *Labeo rohita* (Hamilton, 1822) using alkaline single cell gel electrophoresis. *Asian J. Anim. Vet. Adv.*, **11**: 98-105. <https://doi.org/10.3923/ajava.2016.98.105>
- Ullah, S., Li, Z., Zain-Ul-Arifien, M., Khan, S.U. and Fahad, S., 2019. Multiple biomarkers based appraisal of deltamethrin induced toxicity in silver carp (*Hypophthalmichthys molitrix*). *Chemosphere*, **214**: 519-533. <https://doi.org/10.1016/j.chemosphere.2018.09.145>
- Ural, M.S., 2013. Chlorpyrifos-induced changes in oxidant/antioxidant status and haematological parameters of *Cyprinus carpio carpio*: Ameliorative effect of lycopene. *Chemosphere*, **90**: 2059-2064. <https://doi.org/10.1016/j.chemosphere.2012.12.006>
- Usman, T., Abdullah, S., Naz, H., Abbas, K., Shafique, L. and Siddique, Q., 2020. Acute toxic effect of technical grade insecticides on behavior, catalase activity and total protein contents of fish, *Ctenopharyngodon idella*. *Pakistan J. Zool.*, **52**: 2023-2026. <https://dx.doi.org/10.17582/journal.pjz/20181103091108>
- Valavanidis, A., Vlahogianni, T., Dassenakis, M. and Scoullas, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol. environ. Saf.*, **64**: 178-189. <https://doi.org/10.1016/j.ecoenv.2005.03.013>
- Vardavas, A.I., Stivaktakis, P.D., Tzatzarakis, M.N., Fragkiadaki, P., Vasilaki, F., Tzardi, M., Datseri, G., Tsiaoussis, J., Alegakis, A.K., Tsitsimpikou, C., Rakitskii, V.N., Carvalho, F. and Tsatsakis, A.M., 2016. Long-term exposure to cypermethrin and piperonyl butoxide cause liver and kidney inflammation and induce genotoxicity in New Zealand white male rabbits. *Fd Chem. Toxicol.*, **94**: 250-259. <https://doi.org/10.1016/j.fct.2016.06.016>
- Wang, C., Lua, G., Cuib, J. and Wang, P., 2009. Sublethal effects of pesticide mixtures on selected biomarkers of *Carassius auratus*. *Environ. Toxicol. Pharmacol.*, **28**: 414-419. <https://doi.org/10.1016/j.etap.2009.07.005>
- Wang, Y., Chen, C., Zhao, X., Wang, Q. and Qian, Y., 2015. Assessing joint toxicity of four organophosphate and carbamate insecticides in common carp (*Cyprinus carpio*) using acetylcholinesterase activity as an endpoint. *Pestic. Biochem. Physiol.*, **122**: 81-85. <https://doi.org/10.1016/j.pestbp.2014.12.017>
- Webb, D., Gangnon, M.M. and Rose, T., 2005. Metabolic enzyme activities in black bream, *Acanthopagrus butcheri* from the swan canning estuary, Western Australia. *Comp. Biochem. Physiol.*, **141**: 356-365. <https://doi.org/10.1016/j.cbpc.2005.07.010>
- Wu, L., Hu, M., Li, Z., Song, Y., Yu, C., Zhang, H., Yu, A., Ma, Q. and Wang, Z., 2016. Dynamic microwave-assisted extraction combined with continuous-flow microextraction for determination of pesticides in vegetables. *Fd. Chem.*, **192**: 596-602. <https://doi.org/10.1016/j.foodchem.2015.07.055>
- Zheng, J.L., Zeng, L., Shen, B., Xu, M.Y., Zhu, A.Y. and Wu, C.W., 2016. Antioxidant defenses at transcriptional and enzymatic levels and gene expression of Nrf2-Keap1 signaling molecules in response to acute zinc exposure in the spleen of the large yellow croaker *Pseudosciaena crocea*. *Fish Shellfish Immun.*, **52**: 1-8. <https://doi.org/10.1016/j.fsi.2016.02.031>
- Zhu, B., Gong, Y.X., Liu, L., Li, D.L., Wang, Y., Ling, F. and Wang, G.X., 2014. Toxic effects of triazophos on rare minnow (*Gobiocypris rarus*) embryos and larvae. *Chemosphere*, **108**: 46-54. <https://doi.org/10.1016/j.chemosphere.2014.03.036>
- Zia, M.A., Rahman, K., Saeed, M.K. and Anjum, F., 2007. Thermal characterization of hyperproduced glucose oxidase from *Aspergillus niger* BCG-5 mutant strain. *Int. Confer. Compl. Med. Engin.*, pp. 1950-1955. <https://doi.org/10.1109/ICCME.2007.4382088>