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Acute Insecticides Mixture Induced Oxidative Stress, DNA Damage and Nuclear Abnormalities in Three Economically Important Freshwater Species *Catla catla*, *Cirrhina mrigala,* and *Labeo rohita*

(Campuran Racun Insektisida Akut Tekanan Oksidatif, Kerosakan DNA dan Keabnormalan Nuklear dalam Tiga Spesies Air Tawar Penting Secara Ekonomi *Catla catla, Cirrhina mrigala* dan *Labeo rohita*)

HUMA NAZ[1,*], SAJID ABDULLAH[2], TANVEER AHMED[3], NAJEEB-UR-REHMAN[1], BASHARAT ALI[4], KOUSAR AZIZ[5], MUHAMMAD ADEEL HASSAN[6], NIMRA ZAHID[1], WARISHA SAIF[1], MUHAMMAD AHMAD[1], MAHNOOR CHAUDARY[7], RASHID IQBAL[8,9], ABEER HASHEM[10], GRACIELA DOLORES AVILA-QUEZADA[11], KHALID F. ALMUTAIRI[12] & ELSAYED FATHI ABD_ALLAH[12]

[1]*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*
[2]*Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan*
[3]*Department of Life Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan*
[4]*Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan*
[5]*Department of Zoology, University of Education, Faisalabad Campus, Lahore, Pakistan*
[6]*Department of Parasitology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*
[7]*Department of Zoology, Bahauddin Zakariya University, Multan, Pakistan*
[8]*Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan*
[9]*Department of Life Sciences, Western Caspian University, Baku, Azerbaijan*
[10]*Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box. 2455, Riyadh 11451, Saudi Arabia*
[11]*Facultad de Ciencias Agrotecnológicas, Universidad Autónoma de Chihuahua, 31350, Chihuahua, Chihuahua, México*
[12]*Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia*

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ABSTRACT

Insecticides are the most significant pollutants that negatively affect the aquatic ecosystem globally. They are extensively applied in various industries and agriculture to manage the pests and weeds. The freshwater ecosystems are especially vulnerable to these insecticides because insecticides enter into them through leaching, drifting, runoff and drainage. Insecticides can adversely affect the aquatic animals including fish. Therefore, the experiment was conducted to evaluate the acute toxic effect of bifenthrin(B), chlorpyrifos(C) and endosulfan(E) mixtures on antioxidant enzymes (SOD, CAT, Pox, and GST) activities and genotoxic potential in three fish species *Cirrhina mrigala, Labeo rohita,* and *Catla catla* exposed for 4 days. Results demonstrated that the CAT activity increased in gills (G), liver (L), and kidney (K) of three fish species exposed tertiary mixture while it was decreased in brain (B), heart (H) and muscle (M) of fish. Comparison among three fish species showed that there was minor difference among fish species for CAT activity. Exposure of insecticides mixture caused a significant increase in GST, POx, and SOD activities in all selected organs of three species of fish. GST activity was maximum in B of fish followed by the L, M, K, G, and H. The POx activity in organs of three fish species followed the trend: L>B>G>K>H>M. The SOD activity in organs of fish followed the trend: L>B>K>G>H>M. DNA damage in terms of micronuclei (MN), nuclear abnormalities (BN, DN, BLN, NN, and DEN), genetic damage index (GDI) and % damaged nuclei (DN) in peripheral erythrocytes of three fishes increased significantly as a result of pesticides exposure with increasing duration as 96>72>48>24-h. The highest damage in DNA (DN and GDI), NA and MN were observed in erythrocytes of *C. catla* followed by that of *C. mrigala* and *L. rohita*. As a conclusion, antioxidant activities and DNA damage of different fish species based on their physiological differences may be useful biomarker for evaluation of aquatic pollution.

Keywords: Acute; CAT; fish; genotoxicity; GST; organs; POx; SOD; toxicants

ABSTRACT

Racun serangga adalah bahan pencemar paling ketara yang memberi kesan negatif kepada ekosistem akuatik di seluruh dunia. Ia digunakan secara meluas dalam pelbagai industri dan pertanian untuk menguruskan perosak dan rumpai. Ekosistem air tawar amat terdedah kepada racun serangga ini kerana ia masuk ke dalamnya melalui larut lesap, air hanyut, air larian dan saliran. Insektisida boleh memberi kesan buruk kepada haiwan akuatik termasuk ikan. Oleh itu, uji kaji ini dijalankan untuk menilai kesan toksik akut campuran bifenthrin(B), chlorpyrifos(C) dan endosulfan(E) kepada aktiviti enzim antioksidan (SOD, CAT, POx dan GST) serta potensi genoketoksikan dalam tiga spesies ikan *Cirrhina mrigala, Labeo rohita* dan *Catla catla* yang terdedah selama 4 hari. Keputusan menunjukkan bahawa aktiviti CAT meningkat dalam insang (G), hati (L) dan buah pinggang (K) tiga spesies ikan terdedah kepada campuran tertier manakala ia berkurangan dalam otak (B), jantung (H) dan otot (M) ikan. Perbandingan antara tiga spesies ikan ini menunjukkan terdapat perbezaan kecil antara spesies ikan untuk aktiviti CAT. Pendedahan campuran racun serangga menyebabkan peningkatan ketara dalam aktiviti GST, POx dan SOD dalam semua organ terpilih bagi tiga spesies ikan ini. Aktiviti GST adalah maksimum dalam B ikan diikuti oleh L, M, K, G dan H. Aktiviti POx dalam organ tiga spesies ikan mengikut trend: L>B>G>K>H>M. Aktiviti SOD dalam organ ikan mengikut trend: L>B>K>G>H>M. Kerosakan DNA dari segi mikronukleus (MN), keabnormalan nuklear (BN, DN, BLN, NN dan DEN), indeks kerosakan genetik (GDI) dan % nukleus rosak (DN) dalam eritrosit periferi tiga ekor ikan meningkat dengan ketara akibat pendedahan racun perosak dengan peningkatan tempoh 96>72>48>24-jam. Kerosakan tertinggi dalam DNA (DN dan GDI), NA dan MN diperhatikan dalam eritrosit *C. catla* diikuti oleh *C. mrigala* dan *L. rohita*. Sebagai kesimpulan, aktiviti antioksidan dan kerosakan DNA spesies ikan yang berbeza berdasarkan perbezaan fisiologi mereka mungkin penanda bio berguna untuk penilaian pencemaran akuatik.

Kata kunci: Akut; CAT; ikan; genoketoksikan; GST; organ; POx; SOD; ketoksikan

INTRODUCTION

From last two decades, application of synthetic chemicals as insecticides has been increased in agriculture (Pereira, Fernandes & Martinez 2013). Three major groups of insecticides include organochlorines, organophosphates, and pyrethroids are severally affecting the target as well as non-target organisms. Researcher has paid much attention on ecological and biological aspects of contamination, and toxicity caused by these insecticides on non-target organism inhibiting in water bodies and their negative effects on these animals (John & Shaike 2015). The chemicals that are used in agriculture can go to the waterway and end up in the aquatic habitat. Fish are the most vulnerable to these chemicals and may absorb through skin, gills, and contaminated foods (Sunanda et al. 2016). Sub-lethal and lethal effects of insecticides vary between the species, sex and life stage of the fish (Majumder & Kaviraj 2018). Exposure of insecticides caused morphological, haematological, biochemical, histopathological, histopathological, neurobehavioural, and developmental changes, while lethal dose caused high mortalities in fish and other aquatic animals (Sunanda et al. 2016).

Insecticides toxicity is linked with the formation of reactive oxygen species (ROS) which can induce oxidative stress in aquatic animals (Oropesa, Garcia-Cambero & Soler 2008). Fish tissues have a defensive mechanism to overcome the detrimental effects of ROS (Tejada et al. 2007). The first antioxidant enzyme is superoxide dismutases (SOD), which convert the superoxide (O^{-2}) into hydrogen peroxide (H_2O_2). Catalase (CAT) is a peroxisomal enzyme; detoxify the H_2O_2 into O_2 and water. Peroxidase (POx) is also very important enzyme responsible for metabolism of variety of peroxides such as H_2O_2 into water and oxygen. Another very important enzyme is glutathione-S-transferase (GST), plays a role in biotransformation of many xenobiotics and converts them into glutathione conjugate that can easily excrete from the fish body (Modesto & Martinez 2010). Oxidative stress occurs when ROS concentration exceed the capacity of antioxidant enzyme to remove them and can disturb the structural integrity and activities of these enzymes (Pereira, Fernandes & Martinez 2013). To assess the acute impacts of pesticides biochemical tests are the good choice, diagnose toxic impact of these pesticides on target tissues and also evaluate the health status of fish (Banaee et al. 2008).

Other than antioxidant enzymes, a wide range of biomarker assay such as comet assay (Ng & Romano 2013) and micronuclei (MN) test are commonly used to detect the genotoxic effects of pollutants (Bolognesi & Hayashi 2011). For genotoxic study such as nuclear abnormalities in fish blood have gained much attention because their erythrocytes are nucleated (Costa et al. 2011). Nucleated cells play a crucial role because they contain nucleus which has capacity to go through the cell division process which leave them vulnerable to chromosomal damage. The MN test is applied to examine the genotoxicity of toxicant which refers to its capacity to harm the genetic material, by observing the micronuclei in the cell's cytoplasm. The responses of these biomarkers can vary significantly according to type of toxicant and species of the animal. The different fish species showed various degree of sensitivity to the same insecticides because of variation in their metabolic reactions and physiological response to the chemical such as toxicity of binary mixture of four pyrethroids and two organophosphates to zebra fish (Zhang et al. 2010) and similarly, pyrethroids and organophosphates to *O. niloticus* (Amin et al. 2020).

The former used more than one insecticide or a combination of insecticides to improve crop yield and for the effective control of pests. The negligent use and handling of these toxicants results in three kinds of pollution including land, air, and water (Khan et al. 2020). The Indian major carps such as *C. mrigala*, *L. rohita*, and *C. catla* are the freshwater native and most consumable fish species of Pakistan. According to Yaseen et al. (2024), these fish species are being adversely affected by the aquatic toxicants which fall into waterbodies via agriculture run-off and sewage wastes resulting in cause different alterations in histological, biochemical, and hematological parameters of fish. Therefore, comparative studies are required to detect the effects of pesticides on different species of fish. Therefore, the present work was conducted to assess the comparative response of three fish species against pesticides mixture toxicity.

MATERIALS AND METHODS

FISH SAMPLING AND ACCLIMATIZATION

The experimental fish including *L. rohita, C. mrigala,* and *C. catla* were procured from the fish seed hatchery, Faisalabad, Punjab Pakistan and transport live to the wet laboratory of Fisheries Research Farm, University of Agriculture Faisalabad, Pakistan. One gram of technical grade bifenthrin (B), chlorpyrifos (C), and endosulfan (E) were dissolved to prepare stock-I solution, separately, in 100 mL of methanol. While insecticides mixture (1:1:1) solution of required concentrations were prepared by further dilutions of stock-I in deionized water. After two weeks of acclimatization to laboratory environment, each fish species was exposed insecticide mixture for 4-day separately to LC_{50} concentration. The basic parameters as pH (7.5), temperature (30 ºC), DO (5.00 mgL^{-1}) and total hardness (250 mgL^{-1}) were kept constant. The 96-h LC_{50} was determined as 1.09 μgL^{-1} (*C. catla*), 1.81 μgL^{-1} (*C. mrigala*) and 1.63 μgL^{-1} (*L. rohita*) by Naz et al. (2017). A group of fish (n=10) for negative control (NC) was kept in aquarium having pesticides free water. In positive control (PC) group, cyclophosphamide was used. Cyclophosphamide has been widely used for positive control to induce genomic instability (Cavas & Ergen 2005). To measure the oxidative stress and genotoxic status, three biological replicates were sacrificed at 24-, 48-, 72- and 96-h.

OXIDATIVE STRESS EVALUATION

The tissues *viz.* heart (H), muscle (M), gills (G), liver (L), brain (B), and kidney (K) of each fish species were separated to measure the antioxidant enzymes *viz.* catalase (CAT), superoxide dismutase (SOD), peroxidase (POx), and glutathione S-transferase (GST). The unit (U/mL) of enzyme express as the level of an enzyme activity in a solution, where one unit is defines as the quantity of the enzyme that capable of converting of one micromole of substrate into product per minute under particular conditions.

SOD Assay Giannopolitis and Ries (1977)'s method was used to measure the activity of SOD. To each cuvette, 0.016 mL of riboflavin, 0.05 mL of enzyme extract, and 1 mL of buffer was added. The cuvettes were all put in a light box with a 30-watt lightbulb. The cuvettes were moved into the spectrophotometer after 12 min, where NBT (0.033 mL) and NACN/EDTA (0.067 mL) were introduced to mixture of reaction. After 20 s, absorbance (A_{560} nm) was recorded. The one milliliter of buffer was utilized as a blank. The SOD activity was calculated by assessing the percentage inhibition of NBT.

$$SOD\ Activity = \frac{Blank\ (Abs) - Sample\ (Abs)\ X\ 100}{Blank\ (Abs)}$$

CAT Assay Assay of CAT was performed by following the procedure of Chance and Mehaly (1955). In order to prepare the buffer substrate solution, 10 mM H_2O_2 (0442 mL) was dissolved in 60 Mm sodium phosphate buffer. A cuvette, 1.95 mL of buffered substrate solution combined with 0.05 mL of enzyme extract and was placed inside a spectrophotometer, which was adjusted to zero at wavelength of 240 nm. Three min was the reaction time, and the readings of absorbance were noted after 1-min interval.

$$CAT\ Activity = \frac{\Delta A/mintxdilutionx2\ \text{mL}}{0.04M - 1cm - 1x0.05\ \text{mL}}$$

POx Assay POx assay was performed according to the protocol of Chance and Mehaly (1955). For POx assay, 3 mL of buffer substrate solution was prepared by adding guaiacol (750 µL), H_2O_2 (0.3 mL) and phosphate buffer (47 mL, pH 6.5, 0.2 M) in a cuvette. Additionally, the 60 µL of enzyme extract was also added and put it into spectrophotometer. After 3 min of reaction, the absorbance was observed at 470 nm. The phosphate buffer was used as blank.

$$POx\ Activity = \frac{\Delta A/3}{26.6_x60/3000}$$

GST Assay Activity of GST was calculated according to the method of Mannervik (1985). Briefly, to prepare the buffer substrate solution, mix well 980 µL of PBS (pH 6.5), 10 µL of CDNB (100 mM) and 10 µL of 100mM of glutathione in a beaker. A plastic cuvette (1.5 mL) was filled with 900 µL of substrate solution and incubate it at room temperature for 5 min. Subsequently, in the cuvette enzyme sample (100 µL) was added and recorded the absorbance at A_{340} nm with the help of spectrophotometer. The PBS (100 µL) was used as a blank.

$$GST\ activity = \frac{\left(Adjusted \frac{\Delta 340}{min}\right)}{0.0096\ \mu M - \frac{1}{cm}} \times \left(\frac{1.0\ mL}{0.1\ mL}\right) \times \begin{matrix} any\ sample \\ dilution \end{matrix}$$

Comet Assay Blood was collected from caudal vein of each fish species with the help of needle attached to a syringe. Blood was immediately poured into anticoagulant-coated tubes (Kousar & Javed 2015). Comet assay was performed according to Singh et al. (1988) which involved three steps including slide preparation, lysis, electrophoresis and staining.

Slide Preparation One mL of phosphate buffer saline was added into each fish blood sample. In 200 µL of 1.7% agarose of low-melting-point, diluted 60 µL sample was mixed well. 0.5% agarose having normal melting point was applied uniformly on the slide with 110 µL of the mixture. Slides were covered with a cover slip and allowed to solidify in refrigerator for 10 min. After 10 min, a third layer of 0.8% agarose 90 µL with low-melting-point was applied after carefully removed the glass cover slips.

Lysis Once the gel had solidified, carefully removed the cover-slips and the slides were placed in cold lysing solution (10 mM Tris, pH 10, 2.5 M NaCl and 100 mM EDTA) with 1% Triton X-100 and 10% DMSO was just added fresh before use). The slides were kept in refrigerator for 1 h at 4 °C.

Unwinding of DNA The slides were arranged side by side in a comet assay tank (CSL-COM 20; Cleaver, UK). The fresh electrophoresis solution, (EDTA (200 mM), NaOH (1N) and pH >13.5) was added into tank until it reached a level of about 0.25 cm above the slides. For unwinding, slides were kept in the tank for 20 min.

Electrophoresis The same solution was used to perform the electrophoresis which was carried out for 25 min at 300 mA and 25V.

Neutralization Using the Tris buffer (pH 7.5; 0.4 M), the slides were neutralized. The 75 µL ethidium bromide (20 μgmL^{-1}) was used to stain DNA and Epi-Fluorescence microscope (N-400M, American Scope; UK) was used for examine the slides. Total 150 cells (50 per replicate) were scored. Broken DNA migrated out of the nucleus that resembled comet was classified into five types according to length of comets tail as complete damage (Type IV), high level damage (Type III), medium level damage (Type II), low level damage (Type I) and undamaged (Type 0). CometScore (TriTek Corp., Sumerduck, USA) was used to measure the length of comet tail (Jose et al. 2011).

$$GDI = \frac{(TypeI) + 2(TypeII) + 3(TypeIII) + 4(TypeIV)}{Type0 + TypeI + TypeII + TypeIII + TypeIV}$$

MICRONUCLEUS ASSAY

On slide, a drop of blood was smeared instantly and fixed in methanol immediately for 10 min and air dried. Finally, with Wright-Giemsa stain (Barsiene et al. 2004), the slides were stained for 8 min. A binocular microscope was used according to the criteria described by Fenech et al. (2003) for nuclear abnormalities (NA) and scoring of micronuclei (MN) performed (per 1,000 cells) on coded slides.

$$MN\% = \frac{Number\ of\ cell\ containing\ MN}{Total\ no.\ of\ cell\ counted} x\ 100$$

STATISTICAL ANALYSES

Each experiment was performed with three replicates both in term of biological and technical replicates. The normality assumption of the data was verified by using Shapiro-Wilk test. Data obtained from antioxidant enzyme activities was statistically analyzed using a linear model (ANOVA) under factorial design however, for comparison of means and to check the statistical differences among different treatments Tukey's/Student Newnan-Keul tests was applied. Data obtained from DNA damage and nuclear abnormalities were expressed as mean (±SE) and analyzed by non-parametric Mann-Whitney U-test.

RESULTS

OXIDATIVE STRESS STUDIES

The overall mean showed that CAT activity increased in gills (242.14±18.0 UmL^{-1}), liver (282.28±13.25 UmL^{-1}) and kidney (187.29±14.93 UmL^{-1}) of three fish species exposed tertiary mixture when compared to control gills (184.27±14.32 UmL^{-1}), liver (233.09±7.17 UmL^{-1}) and kidney (141.48±6.69 UmL^{-1}), respectively. while it was decreased in brain, heart, and muscle of fish. Comparison among three fish species showed that there was statistically ($P<0.05$) minor difference among fish species for CAT activity. However, *C. catla* was the most sensitive for CAT activity with mean value of 168.15±010.3 UmL^{-1} followed by *L. rohita* (166.93±9.61 UmL^{-1}) and *C. mrigala* (160.65±9.17 UmL^{-1}) (Figure 1). Exposure of insecticides mixture significantly enhanced the GST, SOD, and POx activity in all selected organs of three species of fish. GST activity was maximum in B followed by the L, M, K, G, and H (Figure 2). Figure 3 shows that the activity of POx in three fish species followed the order: L>B>G>K>H>M. The highest SOD activity was noted in L followed by B, K, G, H and M (Figure 4).

DNA DAMAGE

DNA damage in terms of micronuclei (MN), nuclear abnormalities (BN, DN, BLN, NN, and DEN), genetic damage index (GDI) and % damaged nuclei (DN) in peripheral erythrocytes of three fishes increased significantly due to pesticides exposure with increasing duration as 96>72>48>24-h (Figure 2-5). The *C. catla*

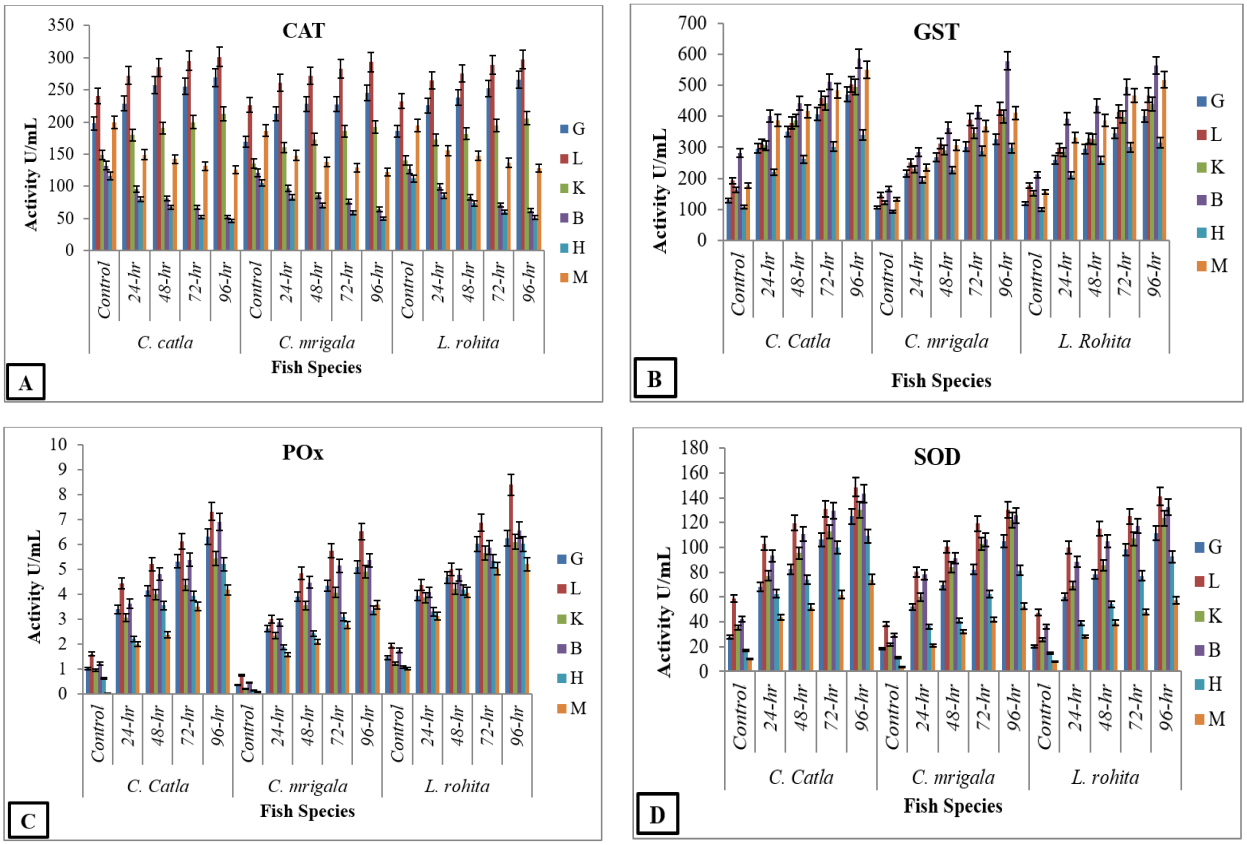

FIGURE 1. Species- and organ-specific alteration in (A) CAT, (B) GST, (C) POx, and (D) SOD enzymes activities after exposing to insecticides mixture at different hour of exposure

showed maximum DN (%) of 25.83±8.51 followed by *L. rohita* 22.75±3.77, and *C. mrigala* (19.92±4.26). The GDI index in RBCs of fish followed the pattern as: *C. catla* (0.82±0.22) > *L. rohita* (0.75±0.15) > *C. mrigala* (0.64±0.14). Similar trend was observed for MN frequency (%) as highest value was observed in *C. catla* (3.14±0.84) than *L. rohita* (2.74±0.81) and *C. mrigala* (2.56±0.78). The NAs in RBCs were lowest in *C. mrigala* (7.90±3.70) and highest in *C. catla* (10.26±3.64) and *L. rohita* (8.36±3.53).

DISCUSSION

OXIDATIVE STRESS STUDIES

In present work activities of SOD, POx, and GST increased in all observed organs of three fish species in a duration-dependent manner while CAT level decreased in brain, heart and muscle of fish. Acute exposure of endosulfan and chlorpyrifos increased SOD, POx, GST, and CAT in brain, liver, heart, gills, kidney, and muscle of *L. rohita* (Naz et al. 2019). Abdullah et al. (2018) reported increased the GST level in liver, gills, kidney and muscle of *Channa striata* exposed to endosulfan and deltamethrin mixture. Naz et al. (2017) noted that time-dependent increased in SOD activity in liver of *L. rohita, C. catla,* and *C. mrigala* exposed to acute bifenthrin+chlorpyrifos+endosulfan mixture. Acute exposure of malathion caused increased in activity of GST, SOD, and CAT in liver, muscle and gill of *L. rohita* in duration-dependent manner (Thenmozhi et al. 2011). Sharbidre, Metkari and Patode (2011) showed that acute toxic of methyl parathion and chlorpyrifos induced activity of CAT, GST, and SOD in liver, gills and brain of *Poecilia reticulata*. Kaur and Jindal (2017) observed the depletion in gills, kidney and liver of *Ctenopharyngodon idellus* exposed to chlorpyrifos (1.4- and 2.44- µg/L). Blahova et al. (2013) also noted the decline in CAT level in zebra fish due to chronic toxicity of atrazine. Activity of CAT and SOD significantly enhanced in *Persian sturgeon* exposed to endosulfan (Safari et al. 2016). Acute toxicity of methyl parathion altered the enzymes activities in liver and gills of *C. catla* (Abhijith, Ramesh & Poopal 2016). Diazinon significantly increased the CAT and SOD in liver, gill, kidney and muscle of *C. catla* (Reddy, Vineela & Kumar 2016). Diazinon and methyl parathion caused significant augmentation in level of GST, POD, and SOD in liver, gills, and muscle of *Oncorhynchcus mykiss* (Isik &

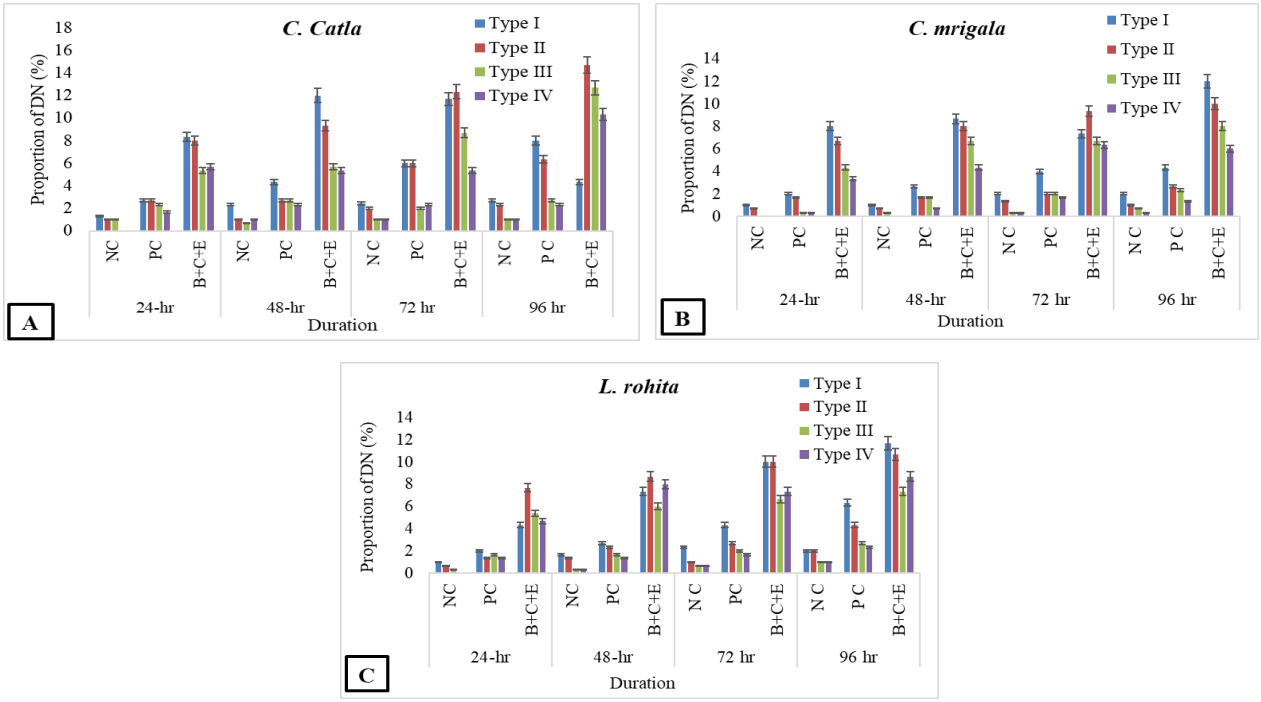

FIGURE 2. Percentage of different types of DNA damage in (A) *C. catla*, (B) *C. mrigala*, and (C) *L. rohita* induced by negative control (NC), positive control (PC), and insecticides mixture (B+C+E) at different hour of exposure

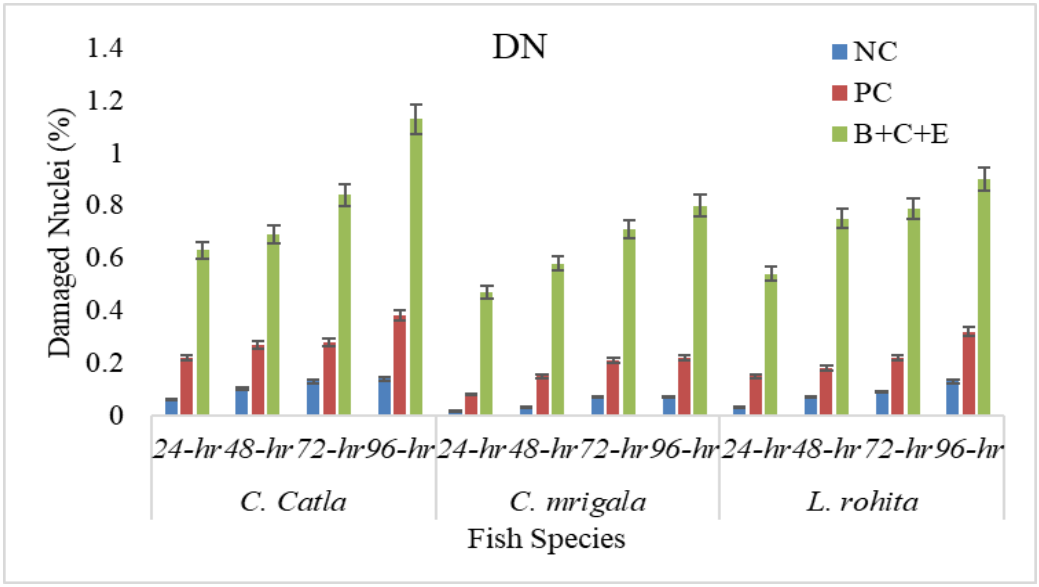

FIGURE 3. The effect of exposure duration to the specific damage to nuclei after insecticides mixture exposure for each species

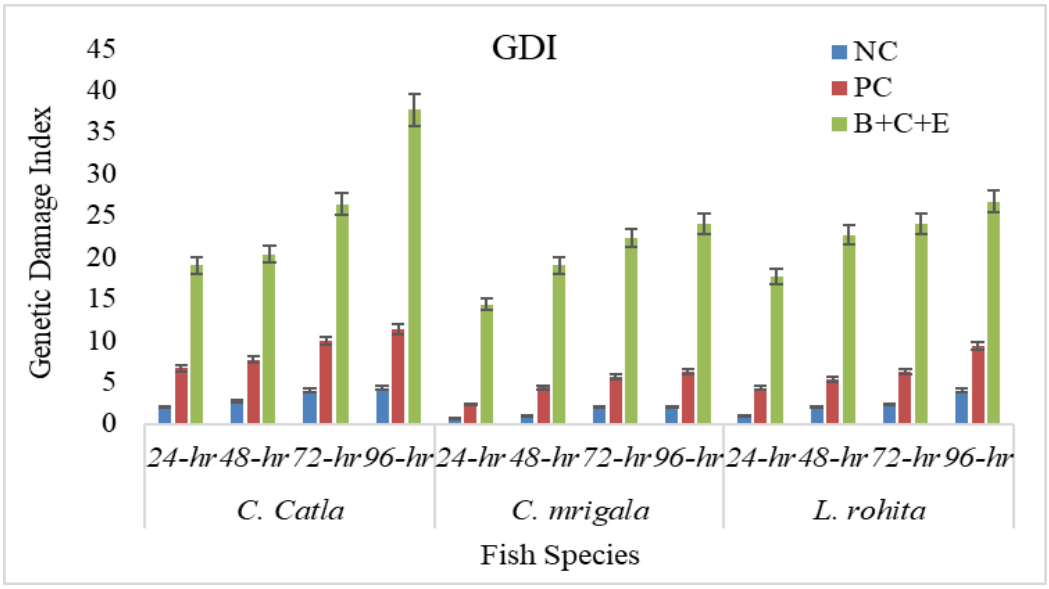

FIGURE 4. Specific genetic damage index (GDI) for each fish species at different duration of insecticides mixture exposure

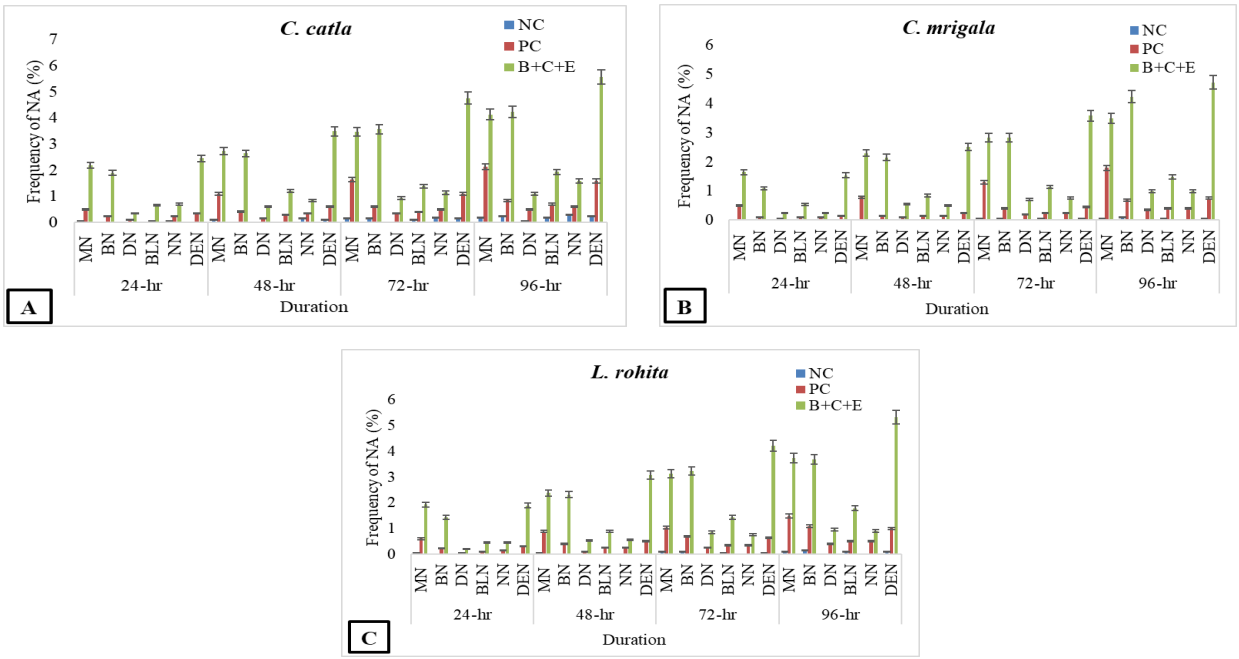

FIGURE 5. Insecticides mixture induced nuclear abnormalities in erythrocytes of (A) *C. catla*, (B) *C. mrigala*, and (C) *L. rohita*

Celik 2008). Vijayakumar et al. (2016) noted the increase in SOD and CAT level in gill, liver and kidney of *L. rohita* exposed to cypermethrin. Endosulfan has the ability to induced antioxidant enzyme activity in topmouth gudgeon *Pseudorasbora parva* (Wu & Ding 2016). Cypermethrin exposure caused induction in activity of POD and CAT of *L. rohita* (Dawar et al. 2016). Karmakar et al. (2016) recorded the increase in SOD and CAT level in liver, gill, and kidney of rohu exposed to malathion. Significant increase in SOD, CAT, and GPx activity in zebrafish exposed to mixture of imidacloprid, dichlorvos, and atrazine was noted (Shukla et al. 2017). Exposure of endosulfan increased the level of SOD, POx, and CAT in heart tissue (Jalili et al. 2007). Ullah et al. (2014) reported the cypermethrin induced CAT and POx level in muscle, brain, kidney, gills, and liver of *Tor putitora.*

In the present study, CAT, SOD, POx, and GST showed tissue- and species-specific response. Monteiro et al. (2006) also reported the tissue specific response of SOD, GST, and CAT in *Brycon cephalus* exposed to acute dose of methyl parathion. Zhang et al. (2004) reported increased POx activity due to dichlorophenol in liver of *Carassius auratus*. Ahmad et al. (2000) observed the tissue- and species-specific changes in antioxidants systems of fish. Time-dependent increased in CAT activity in gill, liver, and kidney of *L. rohita* exposed to malathion was also reported by Patil and David (2013).

Comparison among three fish showed that *C. catla* had higher activity of antioxidant enzymes than that of *C. mrigala* and *L. rohita*. The difference in toxicity to the different fish species might be due to differences in the absorption of pesticides, their accumulation, biotransformation, and excretion (Omitoyin, Ajani & Fajinmi 2006). Several other factors which influence these responses include species, exposure duration, and toxicant concentration (Piazza et al. 2015).

DNA DAMAGE

With the duration of exposure, DN, GDI, MN, and NA in erythrocytes of three fishes significantly differed as 24<48<72<96-h. Duration-dependent induction in DNA damage in red blood cells (RBCs) of goldfish exposed to monocrotophos for 24, 48, 96, and 168 h was recorded by Zhao et al. (2015). Endosulfan exposure significantly induced DNA damage in zebrafish (Shao et al. 2012). Acute exposure of cypermethrin significantly induced GDI in *L. rohita* erythrocytes (Gadhia, Prajapati & Gadhia 2016). After exposure to dimethoate, induction of micronucleus and DNA damage were increased in *C. Punctatus* as the exposure time increased (Ali et al. 2014). Ambreen et al. (2018) also reported the increased percentage of DN and GDI in RBCs of *Ctenopharyngodon idella* exposed to chlorpyrifos and endosulfan. Nwani et al. (2010) observed the damage in DNA and MN frequencies in RBCs of *C. punctatus* exposed to carbosulfan. Time-dependent increased in MN in erythrocytes of *L. rohita* exposed to tannery industry effluent was also confirmed by Walia et al. (2015). Naqvi, Shoaib and Ali (2016) confirmed the micronucleus formation in RBCs of *Oreochromis mossambicus* exposed to different pesticides (cypermethrin, malathion, chlorpyrifos, and buctriland lambda-cyhalothrin). Nan et al. (2015) recorded the increase in formation of MN and three comet parameters of *Misgurnus anguillicaudatus* as the treatment time increased. Dar et al. (2015) also confirmed the MN induction in RBCs of *Carassius carassius* due to endosulfan exposure.

Ansoar-Rodriguez et al. (2015) also confirmed the formation of micronucleus and comet parameters like primary DNA damage in *Oreochromis niloticus* erythrocytes exposed to insecticide imidacloprid. Exposure of lambda-cyhalothrin induced the formation of MN and other anomalies (notched, lobed, blebbed and bi-nucleated cells) in erythrocytes of mosquitofish (Muranli & Güner 2011). Time-response relationship in MN induction in blood cells of *Cirrhinus mrigala* exposed to acute chlorpyrifos was observed by Bhatnagar, Yadav and Cheema (2016). Wu and Ding (2016) also confirmed the induction of micronuclei frequencies in peripheral erythrocytes of to topmouth gudgeon (*Pseudorasbora parva*) after endosulfan. Significantly duration-dependent increasesd in MN and other anomalies frequencies (deformed nucleus, nuclear buds, binucleated cells) in RBCs of *C. catla* were observed by Anbumani, Mary and Kumar (2015).

CONCLUSION

It was concluded that acute exposure of bifenthrin, chlorpyrifos, and endosulfan mixture significantly altered the enzymatic profiles of gills, liver, and heart of *C. mrigala*, *C. catla*, and *L. rohita*. Significant effect on DNA integrity based on comet assay conducted on these fish species were observed after hours of exposure. The existence of insecticides in aquatic bodies is dangerous to these species and affect the survival of fish. These parameters can be used as a good biomarker for evaluating the toxic effect of insecticides in aquatic environment.

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*Corresponding author; email: dr.humanaz98@gmail.com