Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com
NLM ID:101629416
OPEN ACCESS
Zoology

Therapeutic Potential of Thymoquinone Against Malathion Induced Hepato-Toxicity in Fresh Water Major Carp *Labeo rohita* (Hamilton, 1822)

Preeti Tirkey[1], Raghuveer Kumar Gupta[1], Somenath Ghosh[1], Dr. Malabika Sikdar[1*]

[1]Department of Zoology, Dr. Harisingh Gour University, Sagar-470003, Madhya-Pradesh

DOI: https://doi.org/10.36347/sajb.2025.v13i10.003 | **Received:** 13.07.2025 | **Accepted:** 22.09.2025 | **Published:** 10.10.2025

***Corresponding author:** Dr. Malabika Sikdar
Department of Zoology, Dr. Harisingh Gour University, Sagar-470003, Madhya-Pradesh

Abstract | **Original Research Article**

Wide use of the pesticide increases the possibility of causing undesirable toxicity in aquatic organisms. Pesticides cause toxicity in the environment *via* agricultural- run-offs and vector spray which are commonly used all over the world for crop protection from various pests. Malathionis is a widely used pesticide leads negative effects on the physiology of aquatic organisms principally in fishes. This study examines the curative effects of thymoquinone (a bioactive compound of *Nigella sativa)* on malathion induced toxicity in the Livers of *Labeo rohita*. Further study reveals its anti-oxidants activities in the Livers of *Labeo rohita*. For achieving this, mature *Labeo rohita* was taken and randomly divided into four groups (N=5/group) where one group was kept as control and three were experimental. Second group was exposed with malathion (M), 1 µg/L for 30 days, third group was treated with thymoquinone (TQ; 5mg/kg of body weight) alone treated group, fourth group was exposed with malathion followed by thymoquinone supplementation. After completion of experiments, all fish were sacrificed, and Livers dissected out from the body for further study analysis. To find the toxic effect of malathion on Livers, alteration in various biochemical indices including lipid peroxidation (LPO), nitrite content (NO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) along with histopathological changes were measured. The ultra-structure of Livers displays recovery in thymoquinone treated group which can be seen under the Scanning Electron Microscopy (SEM). Malathion treated group show damage in Livers as well as augmented amount of mucus on the Liver's lamella, necrosis, and hyperplasia due to the malathion-induced toxicity. Malathione exposure also lowered anti-oxidants enzyme activity indicate by low SOD, CAT, GPx and GR ($p<0.05$) activities in Livers. Therefore, the findings corroborate the fact that malathion causes damage in Livers as well as increased oxidative stress. Moreover, this study also observed the ameliorative effect of thymoquinone on Livers which possibly overcome malathion induced toxicity in fish.

Keywords: Malathion, Livers, thymoquinone, hyperplasia, necrosis, *Labeo rohita*.

INTRODUCTION

Rapid industrial expansion and technical progressions render massive effects on developing industries resulting in increased environmental degradation by hazardous pollutants [1]. Pollutants negatively affect the environment and survival of organisms. Several kinds of pesticides (insecticides, fungicides, herbicides, and bactericides) and their classes like organophosphorus, pyrethroids, and organochlorine act as pollutants which are used in agricultural fields, industries and homes from ancient times, based on the different object species as well as their effectiveness against them. One such extensively employed organophosphate is Malathion (O, O-dimethyl-S-1, 2-bis methoxycarbonyl ethyl phosphorothioate). It is anticipated to improve food production and offer protection from insects, pests and disease vectors. It also causes harmful impacts on environmental factors that are associated with detrimental consequences on birds, mice, earthworms, reptiles, insects and aquatic animals including fishes [2]. According to studies, researchers all over the world are working to illuminate the effect of malathion intoxication in many species of freshwater fishes. It can harm fish even at low concentrations by masking their growth as well as physical activities. For illustration, malathion is reported to causebehavioural abnormalities in *L. rohita* fingerlings and air-breathing fish (*Anabas testudineus*; [3, 4]). Furthermore, from behavioural changes, malathion has been found to

prompt oxidative stress and respiratory dysfunction in *L. rohita* [5]. *L. rohita* is a freshwater fish species which is commonly known as Rohu. It is found throughout the Asia, more notably in India, Pakistan, Sri Lanka, and Bangladesh. *L. rohita* is used as model organisms for scrutinizing the fundamental mechanisms triggering cellular damage, defense against free radicals, tissue wounds and physiological alterations like ageing, several diseases, disorders, and genotoxic effects [6-8]. Several organs of fish are mainly used for estimating the biochemical and physiological noxious endpoints of chemicals in risk assessment studies, in the brain, blood peripheral erythrocytes, kidneys, gills, and Livers. The Livers are the key organs which are directly in contact with the target of pollutants, the detoxification epicentre, and the organ with the highest potential for evidently indicating physiological and pathological alterations, even at the tiny scale and in response to exceedingly low concentrations of pollutants. Since ancient times, medicinal plants have shown various health benefits in terms of reducing adverse environmental effects. Numerous conventional herbs are also simply available, considered safe, and are very useful for the prevention of many illnesses. One such traditional medicinal plant *N. sativa*, commonly known as black cumin or black seed belongs to the family Ranunculaceae [9]. The medicinal value of *N. sativa* is pertaining to its quinone constituent i.e., TQ. TQ has a variety of pharmacological properties like anti- inflammatory, anticancer, antibacterial, hepato-protective, fungicidal and nephro-protective. Thus the present study was undertaken to investigate the curative effect of TQ on malathion-induced toxicity on haematological parameters and Livers physiology of *L. rohita*, which holds a significant place in aquatic ecosystem.

MATERIALS AND METHODS

Experimental animals:

L. rohita (25±0.5 gram), with an average length (8.5±0.5 cm), procured from the fisheries farm located in Ratona, Sagar (M.P.) India was used for the present study. Fish received a preventive treatment upon arrival i.e., submerging them 2 to 3 times in 0.05% (w/v) potassium permanganate ($KMnO_4$) to treat any bacterial or fungal infection. *L. rohita* were acclimatized to the laboratory conditions for 15 days, before the onset of the experiments. Fish were kept in 100-liter semi-static glass aquaria. The aquaria were filled with UV-sterilized Millipore water (Millipore Direct-Q 3UV system); dechlorinated tap water with a pH of 7±0.06, a salinity of 15%, a temperature of 25-27 ºC, and dissolved oxygen content of 7-8 gm/L. Normal diet *ad libitum* was provided to all groups during the entire experiment [10]. To perform the experiment, mature *L. rohita* were taken and randomly divided into four groups (n=7/group) where Group - 1 was kept as control (C), Group - 2 was exposed with malathion (dissolved in tween-20) (M), 2 µg/L for 30 days, Group - 3 was treated with thymoquinone (TQ) alone i.e., 5 mg/kg body weight/day for30 days, and Group -4 was exposed with M(2 µl/L) followed by TQ i.e., M+TQ supplementation for 30 days. For validation of the study, this experiment was repeated thrice.

Test chemical:

Commercial grade Malathion (50% EC) was purchased from the local market Sagar (M.P.), India, because it is most widely employed in the fields as a pesticide. Thymoquinone (≥98%), Cat. no: 274666, Analytical grade, MW: 164.20 g/mol was purchased from Sigma Aldrich.

Dissection and tissue collection:

After completion of the experiment, fish were anaesthetized using tricaine methane-sulphonate (MS-222, Merck). For histological and biochemical analysis, Livers were excised by removing the operculum from both treated as well as normal fish. Then, it was washed in 0.9% saline, soaked in filter paper and processed for cellular, histological, and biochemical analyses.

Blood collection and sample preparation:

After the anesthetization of fish, blood was collected from the caudal veins. All blood samples were collected in anticoagulant (K3-EDTA) containing tubes for the haematological study. The haematological analyses from the blood samples were performed on the same day. Four aliquots were used to determine the red blood cell (RBC) counts, haematocrit (Ht) levels, white blood cell (WBC) counts, and haemoglobin (Hb) concentrations from the control and all experimental groups. The RBC and WBC counts were performed by using a haemo-cytometer. The Hb concentrations were estimated by using Drabkin's reagent at 540 nm, and Ht levels were estimated by using a micro haematocrit centrifugation method. The erythrocyte indices [mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC)] were measured from the Ht, RBC and Hb data with standard formulas [11-13].

Histo-morphological study of Livers:

A total of 3 fish from each group were selected for the histomorphic study of Livers. Fish were anesthetized and sacrificed; Livers were collected, weighed and immediately transferred into Bouin's fluid fixative for 24-48 hours. The Livers were preserved and proceeded in ascending series of alcohol, xylene and fixed in wax. For staining, 5 µm thick sections of Livers were cut by rotary microtome Leica (RM2125), deparaffinized in xylene, dehydrated in a graded series of alcohol and stained with hematoxylin and eosin [28].

Scanning electron microscopy of Livers:

SEM was employed for histological samples. Livers slides (4 µm thick) were employed for the SEM analysis. To dissolve the embedded wax (mounting medium), glass slides were dipped in xylene for 10 to 15 min., rinsed with trichloro trifluoroethane ($C_2Cl_2F_3$), and then dried using solvent evaporation. Slides then

received a gold (Au) surface coating that conducts electricity [14]. Images were taken in each group at various magnifications, including low and high via using SEM (FEI Nova Nano SEM 450) [15, 16].

Antioxidant enzyme activity measurement:

For the measuring antioxidant activity, four *L. rohita* from each group were taken, Livers were removed, weighed, instantly frozen, and kept at -80 °C for further analysis. Next, Livers were homogenized with a tissue homogenizer (Remi, Mumbai, India), using 0.02 M Tris-Cl (w/v) at pH 7.4 and then centrifuged at 15,000 rpm for 20 min with cooling centrifuged. The supernatant was separated and frozen for antioxidant enzyme analysis [17]. Aggregate protein concentration was assessed in the supernatant using Bovine serum albumin (BSA) as standardby Lowry's method [23]. The whole method was executed as published elsewhere [17-19].

Estimation of Malondialdehyde (MDA) assay

To estimate MDA, 50 µl of supernatant of Livers were incubated for 30 min at RT in 1 ml of 0.5 M Tris-maleate buffer (pH-5.5). Following the addition of 1.5 ml of 0.8% thiobarbituric acid, there action mixture was heated in a water bath using a tight condenser for 10 min. The reaction mixture was then quickly chilled before being agitated ferociously (3:1 w/v) with n-butanol and pyridine reagent. Then, 1 ml of 1N NaOH was added, and the absorbance was taken at 548 nm. MDA concentration was calculated with an extinction coefficient of 0.152 and represented as nmol MDA/mgprotein [20].

Measurement of Nitrosative stress

Nitrosative stress was estimated by assessment of Livers nitrite levels. Concentration of nitrite in Livers was measured using the Griess reagent (0.1% naphthyl ethylenediamine dihydrochloride + 1% sulphanilamide). Equal volumes of Griess reagent and Livers supernatant were added and incubated for 5 min in the dark and absorbance was taken at 540 nm. Nitrite levels were considered using a standard curve prepared by sodium nitrite and represented as nmol µM/mg protein following the protocol as published elsewhere [21].

Super Oxide Dismutase (SOD) Assay:

A reaction mixture containing 27 ml of 100 mM phosphate buffer (pH 7.8), 1 ml of 2.25 mM nitro blue tetrazolium, 1 ml of 1 M sodium carbonate, 1.5 ml of 200 mM l-methionine,and 3 mM ethylene diamine tetra acetic acid (EDTA) wasused for measuring SOD activity. Livers homogenate of 50 µl was mixed in 1.35 ml of reaction mixture, and then 200 µl of 60 µM riboflavin was poured to start the reaction and provided lighting of 20 Watt fluorescent lamp. The blue color was developed and SOD activity was measured at 560 nm. Livers homogenate was added in the same quantity, in the second reaction mixture, then subjected in dark, served as the blank as previously done [22, 23].

Catalase (CAT)assay:

For estimating CAT activity in Livers, a reaction mixture comprising, 1µmol of H_2O_2 in 0.05 M Sodium Phosphate buffer at pH 7 then 50 µl Livers sample was added. Afteradding H_2O_2 in Sodium-Phosphate buffer, the reaction was started at 25°C. Optical density was observed at 240 nm on a UV-Visible spectrophotometer. OD was documented by using a UV-visible spectrophotometer at 240 nm. Extinction coefficient 0.0436 mM/cm and unit/ml were used to calculate the activity [24]. The catalase activity is expressed in U/mg protein.

Glutathione peroxidase (GPx) assay

Liver samples (50µl) were added in the reaction mixture o f30 mM of potassium phosphate buffer, 10 mM of sodium azide,4 mM reduced glutathione, 2.5 mM of H_2O_2 and 6 ml of double distilled water. The reaction mixture was incubated at 37 ºC for 4 min. After incubation, 0.5 ml of 10% (w/v) tri- choloacetic acid (TCA) was added for protein precipitation. After that, the reaction mixture was centrifuged at 3000 rpm for 5 min. at room temperature. The centrifugation pellet was discarded and 1 ml of 3 M di-potassium hydrogen phosphate was added to 1 ml of supernatant along with 1 ml of Elman's reagent was added to the reaction mixture, mixed well, and kept for 3 min. After that, the absorbance was taken at 412 nm [25, 26].

Glutathione reductase (GR) Assay:

For estimating GRactivity,20 µl of Liver sample was added in the reaction mixture containing 0.2 M of phosphate buffer pH 7.4, 0.2 mM ETDA, 1 ml of oxidized glutathione and 0.2 mM of NADPH. The oxidation of NADPH was decreased in OD per min. at 340 nm for 3 min. using a spectrophotometer and µmole NADPH produced per min. at 30 ºC was defined as a unit of GR. The results were expressed at unit/mg protein [27].

Statistical analysis:

All statistical analyses were performed using one-way ANOVA followed by Bonferroni post hoc- test. Student's *t*- test was used for comparison between control and all experimental groups *viz*., M, TQ and M+TQ. All the experiments were repeated thrice and the data was repreented as Mean ± SEM. The P values were considered to be significant at $P < 0.05$ levels.

RESULTS

Histological analysis of Livers:

The exposure with malathion in *L. rohita* shows Livers with the abnormal micro-architecture of Glission's Capsules vacuolation, degradation of hepatic tissues and dilation of hepatic artery suggesting tissue injury. As compared to the control group the thymoquinone alone group shows the normal micro-architecture of Glission's Capsules and non-dilation of hepatic artery. In this group, the structures of hepatocytes show normal structure and central artery was having

normal diameter in comparison with the control group. Co-administration group (M+TQ) reveals the normal micro-architecture of Glission's Capsules and non-dilation of hepatic artery along with the repair of damages caused by the malathion along with thymoquinone (Fig. 1)

Scanning electron microscopic analysis of Livers induced by malathion-induced toxicity:

In SEM analysis, it was observed that malathion exposed group shows the damages in the hepatocytes, vacuolation of tissues and distorted structure of Glisson's Capsule can also be observed in malathion exposed group. Interspaces between two-Glisson's Capsules are more spaced in malathion- exposed group as compared to the control group. In thymoquinone alone, exposed group Livers show normal alignment and Glisson's Capsules, when compared to the control group. In malathion and thymoquinone exposed group shows a significant restoration of the damage caused by malathion in the Liver as compared to the control group (Fig. 2).

Antioxidant activity in Livers of *L.rohita*

Lipid peroxidation levels in Livers:

Malathion exposure shows an increased level of MDA in Livers as compared to the control group, while malathion exposure followed by thymoquinone supplementation shows low MDA levels in M+TQ group but not up to the control group. Thymoquinone alone supplementation in the Liver non- significantly decreased the MDA levels in the Livers of TQ group as compared to the control group (Fig. 3).

Nitrite content in Livers:

Malathion exposure shows an increased level of nitritecontent in Livers as compared to the control group. Co- supplementation of thymoquinone decreases the nitritecontent levels in Livers restoring the damages caused by the malathion in M + TQ group. Thymoquinone supplementation alone shows a non-significant enhancement of nitrite content levels in Livers as compared to the control group (Fig. 4).

SOD activity in Livers:

Malathion exposure shows a significant decrease in SOD activity in Livers as compared to the control and thymoquinone alone treated group. Malathion exposure followed by thymoquinone administration increases the SOD activity in Livers of M+TQ group as compared to the malathion-exposed group but not up to the control group (Fig. 5).

Catalase activity in Livers:

Malathion exposure significantly lowered the catalase activity in Livers in contrast to the control group and thymoquinone alone administrated group. Malathion exposure followed by thymoquinone supplementation restored the catalase activityin Livers of M+TQ group as compared to malathion malathion- exposed group (Fig. 6).

GPx activity in Livers:

Malathion exposure shows significantly reduced GPx activities in the Livers of fishes as compared to control groups. Co-administration of malathion and thymoquinone reveals a significant increase in the GPx activities, which was triggered by the malathion in M+TQ group as compared to the malathion-exposed group but not up to the control group. Thymoqunione alone supplementation shows a non-significant enhancement in GPx activities as compared to the control group (Fig. 7).

GR activity in Livers:

Malathion exposure significantly lowered the GR activity in Livers as compared to the control and as well as thymoquinone alone treated group. Malathion exposure followed by thymoquinone administration exhibits the elevation in GR activity in M+TQ group as compared to the malathion group (Fig. 8).

Haematological analysis:

The effects of malathion, thymoquinone, and its combination on the haematological parameters of *L. rohita* were noted. The malathion exposure was significantly lessened the RBC counts, Hb concentrations, Ht levels, and erythrocyte indices. Simultaneous administration of Melathion and thymoquinone induced a noticeable normalization of the haematological parameters when compared to the malathion alone treated groups (Fig. 9A-10B).

DISCUSSION

In this study, we investigated the effect of malathion (a pesticide) on the Livers functions as well as on blood parameters. It is observed that malathion significantly reduced red blood cell counts, hemoglobin levels, hematocrit and anti- oxidant activities in fish. In addition, this study also investigated the effect of malathion-induced toxicity on oxidative stress along with assessing the curative effect of thymoquinone in the Livers of *L. rohita*. The pesticide toxicityis linked to both the active as well as passive substance and the transporters of the pollutants [29]. The isomers of pesticides may vary, which makes their precise toxicity to be different. Most of the time, studies are carried out to examine and contrast the toxicity of various pesticides. Pesticides with a single isomer are more harmful than those with a combination of several isomers [30]. Fish toxicity is also influenced by temperature, health, size, weight, and age [31]. With the toxins present in the surrounding water, there is a high risk that the typical Liver architecture will change as a result of the Liver's constant contact with environmental water, changing its structure and integrity. A linear increase of oxidative stress is seen when *L. rohita* was exposed and treated with malathion pesticide toxicity. The increase in the events of antioxidant enzymes indicated an effort of the

fish to remove oxyradicals. The increased level of these enzymes, i.e., increased level of GST, detoxify and eliminate toxic xenobiotics and ROS. A very identical result has been reported by authors [32, 33]. in *Macrobrachium malcolmsonii* after heavy metals exposure, [20, 34] in Torputitora and [35] in *L. rohita* after exposure to Cypermethrin, and in *L. rohita* after exposure to endosulfan [36, 37]. In the present study, the damage caused by malathion in the Liver's structure was restored after treatment with thymoquinone. The Livers show lesions and mucus in the Liver filament, and distortions in the primary and secondary lamella were clearly visible in malathion exposure group. The Livers ultra-structural reactions during the current investigation not only showed the organism's decline but also its adaptation to the polluted environment. Malathion exposure also induced blood toxicity indicated by low RBC, WBC count, haematocrit level, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. Blood is an admirable indicator of toxic stress and haematological analysis in fish is commonly used to measure the toxic stress and functional positions of animals. We observed that oxidative stress triggered blood toxicity in malathion-exposed groups whereas thymoquinone supplementation lowered its toxicity indicated by high RBC, WBC and platelet counts in fishes. Hence, thymoquinone acts as a potent antioxidant which significantly lowers oxidative and nitrosative stress indicated by low LPO and NO levels in Livers of *L. rohita*. The outcomes of this study exhibit that the haematological parameters as RBCs, WBCs, platelets count and antioxidant enzyme activities were significantly higher in the malathion-exposed exposed followed by the thymoquinone-supplemented group than the alone malathion exposed group.

Moreover, oxidative stress is one of the potential reasons, leading to tissues injury. It is commonly believed that oxidative stress induces toxic effects [38]. Reactive oxygen species (ROS) damage membranes at increased levels as observed in the current study, whichmakes them leaky and ultimately leads to necrosis and cell death or apoptosis. However, the oxidative stress induced is observed through ROS-induced alterations of cellular components including proteins, lipids, DNA, and high or low molecular mass antioxidants [39]. The ROS is made up of various enzymes, including SOD, GR, GSH-Px, GST, and others. These enzymes constitute a high molecular mass antioxidant group, which operates on low molecular mass antioxidants while the system is at rest to keep the amount of ROS constant. The larger creation of ROS during the current investigation than its removal is what caused the increase in ROS level, which is why antioxidant enzyme activities were also shown to increase concurrently. High ROS levels may be associated with damage in the structure of the Livers shown in scanning electron microscopic and histological images. LPO is considered highly deleterious and it is attacked by free hydroxide radical (itself produced from H_2O_2 through the Fenton reaction [40], resulting of oxidative damage to tissues or organs [41]. LPO involves the direct reaction of lipids and oxygen, for forming free intermediate radicals and producing semi-stable peroxides. The mechanism of pathological free radicals leads to lipid peroxidation along with phospholipids degradation and loss of integrity of the membrane, an important factor of toxicity induction [42]. The results of the current study are in correspondence with the results of the earlier studies on different fish species exposed to different pesticides including cypermethrin [43] endosulfan [36, 37, 44] and sensor [45]. There was a time- dependent increase in SOD activity, which is correlated with increasing superoxide anion generation. In order to shield the cell from oxidative damage brought on by superoxides, SOD catalyzes the conversion of superoxide anion radicals to H_2O_2 and molecular oxygen [45] GR plays a significant role in cellular antioxidant protection. It catalyzes the oxidized glutathione conversion back to its reduced state [38]. On its prominent role as an antioxidant-protecting component, it is used as a potential biomarker in animals under oxidative stress development.

In the current study, the activity of GR increased which is in positive correlation with the increase in GSH. The results obtained are in congruence with previous studies on Channa punctatus exposed to atrazine [31], GSH has a central role in modulating oxidative stress induced LPO, working as a reducing substrate in oxidative reactions [2]. According to some researchers, GSH provides secondary protection against induced oxidative stress by sustaining a reduced state of the cell. The time-dependent increase in the GSH in the current study might be the primary protective response of Rohu against malathion-mediated time-dependent increase in oxidative stress. GSH-Px and GST are GSH-dependent enzymes. Both play a key role in protecting the tissues against oxidative stress [46]. GST catalyzes the conjugates of xenobiotics to tripeptide glutathione [12]. The activities of GSH-Px and GST increased time-dependently with a timely increase in the GSH, which shows a positive correlation between both these enzymes and GSH. The activities of these enzymes increased to resist malathion- induced toxicity and to protect the system from oxidative stress [47]. The increase in the activities of GST and GSH-Px observed in the current study conforms with some previous studies [35], while some [32-35] reported a reduction in the activity of GST observed no change in the activity of GST. In teleosts, zinc sulfate exposure resulted in the presence of mucous droplets, the coarse texture of the Liver filaments, and the blebbed epithelium of secondary lamellae [47]. Histological sections show that dis- orientation in the primary and secondary Liver lamella was caused by malathion-induced toxicity in the treated group as compared to the control group and recovery of primary and secondary Livers lamella can be seen in the thymoquinone- treated group alone. Malathion along

with thymoquinone groups shows significant recovery due to thymoquinone treated group when compared with the control group.

CONCLUSION

The present study revealed that malathion as a pesticide causes a negative impact on the Livers and blood parameters ultimately affecting the growth parameters of the *L. rohita*. Prolonged use of this pesticide also increases oxidative stress and causes severe damage to the primary organ i.e., the Livers of the fish which is the most important organ for the survival and fitness of the fish. Histological studies also show that malathion damages the primary and secondary lamella of the Livers as prolonged exposure to pesticides along with induced blood toxicity, and restoration and repair can be seen after the administration of thymoquinone. SEM results also indicate that after malathion administration, Livers show damage in the Livers and repair can be seen in the thymoquinone co- administered group. In conclusion, it is evident from the present study that TQ (A derivative of plant *N. sativa*), bearing several antioxidant properties has the potential to reduce the malathion-induced toxicity via restoration of the damages and as well as decrease the level of oxidative enzymes.

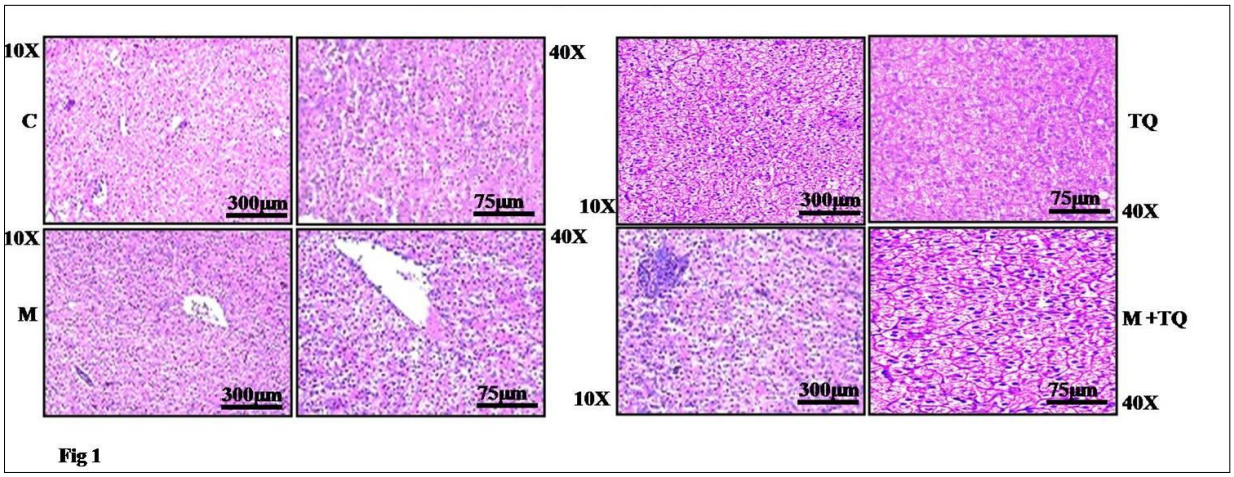

Fig. 1: Showing normal hepatocytes in Control (C) groups. Malathion (M) show damages in hepatocytes. Thymoquinone (TQ) groups show normal of hepatic cells as resembled to control. Malathion + Thymoquinone (M+TQ) groups show significant amelioration as compared to Malathion treated group

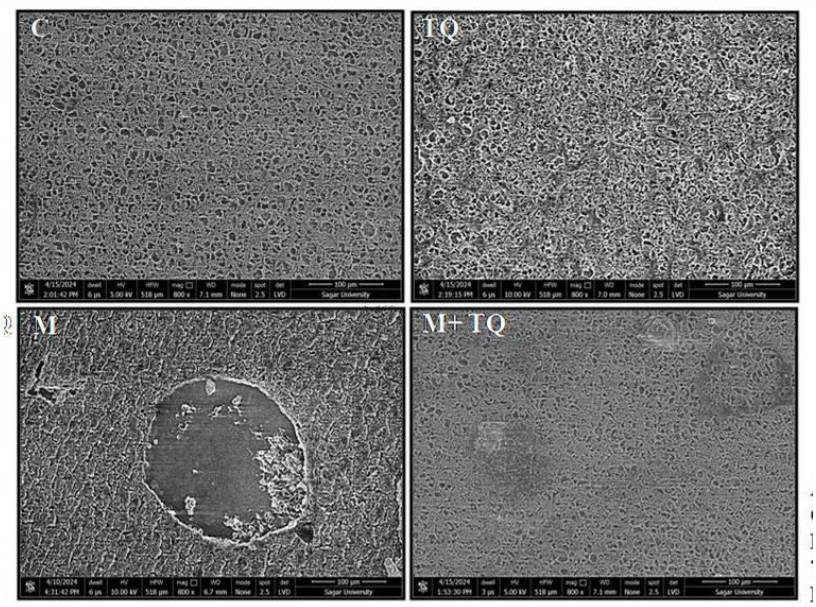

Fig. 2: Scanning Electron micrographs showing four groups control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Image showing normal hepatocytices in control group (C). Degeneration of hepatocytics (M) group. In (TQ) group image showing hepatocytes in normal condition as compared to normal In (M+TQ) groups shows the minimal damages as well as repair of hepatocytes as compared to control group.

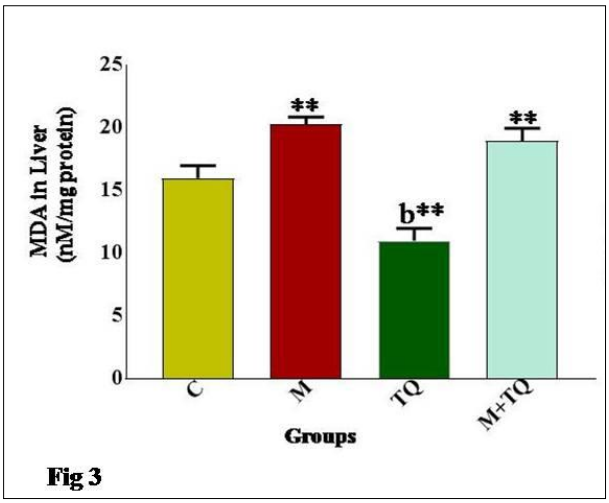

Fig. 3: Histogram showing MDA levels in liver of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

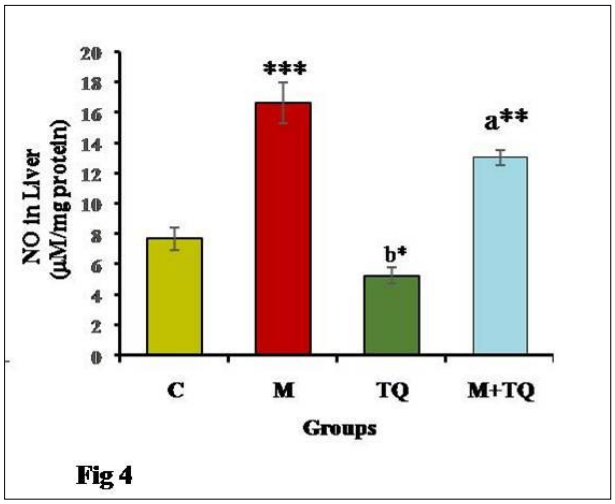

Fig. 4: Histogram showing Nitrate content levels in liver of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

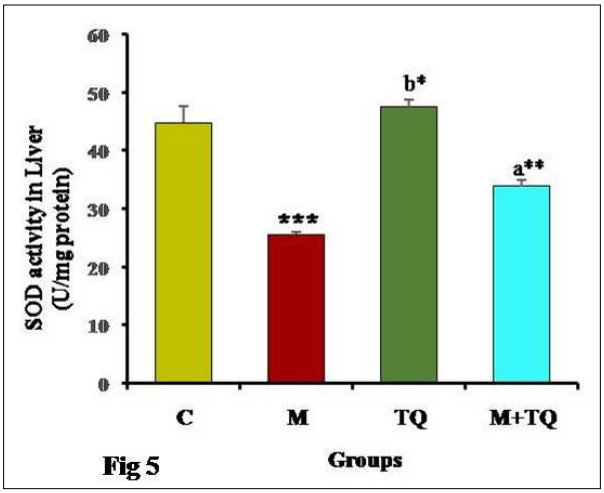

Fig. 5: Histogram showing SOD activity levels in liver of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

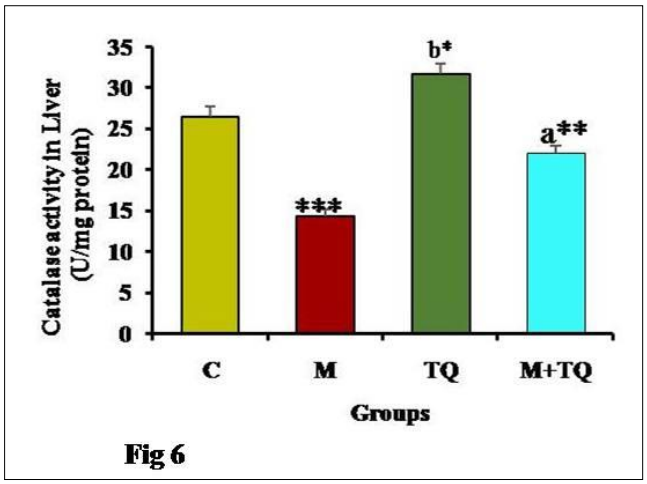

Fig. 6: Histogram showing Catalase (CAT) activity levels in liver of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

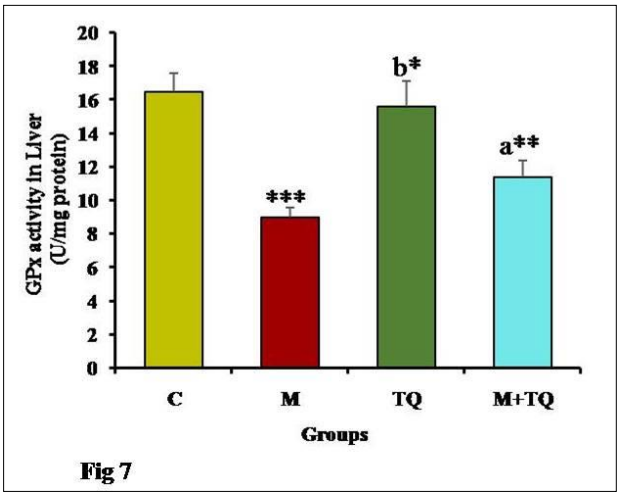

Fig. 7: Histogram showing GPx activity levels in liver of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

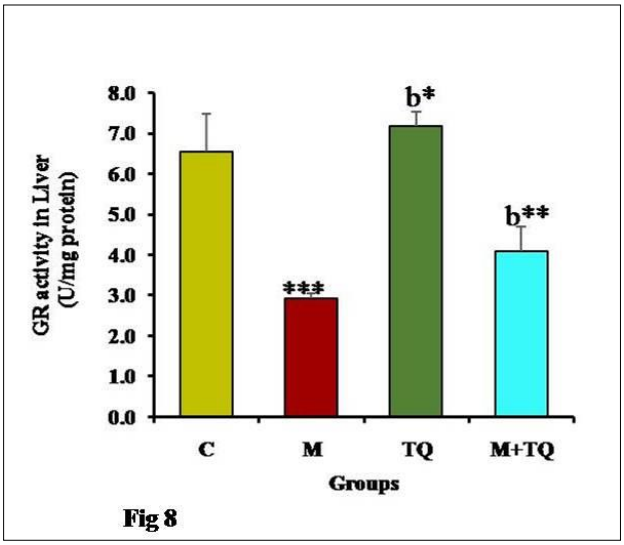

Fig. 8: Histogram showing GR activity levels in liver of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

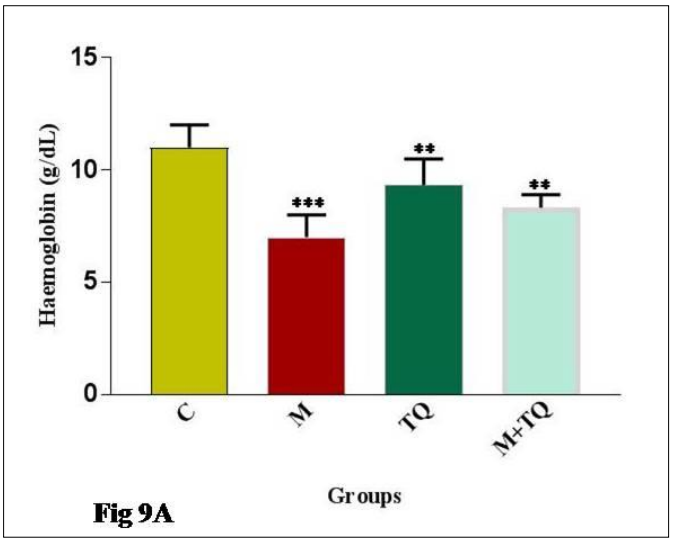

Fig. 9: A Histogram showing Haemoglobin levels in blood of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

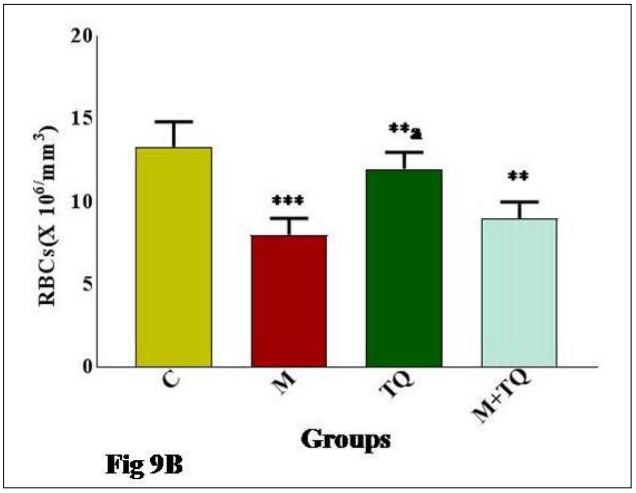

Fig. 9: B Histogram showing RBC Count in blood of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

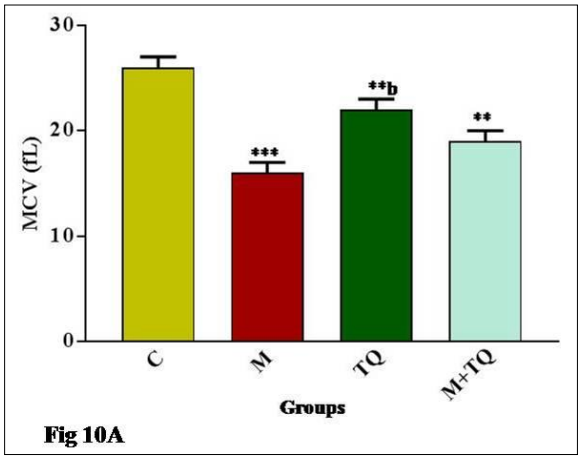

Fig. 10: A Histogram showing MCV level of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

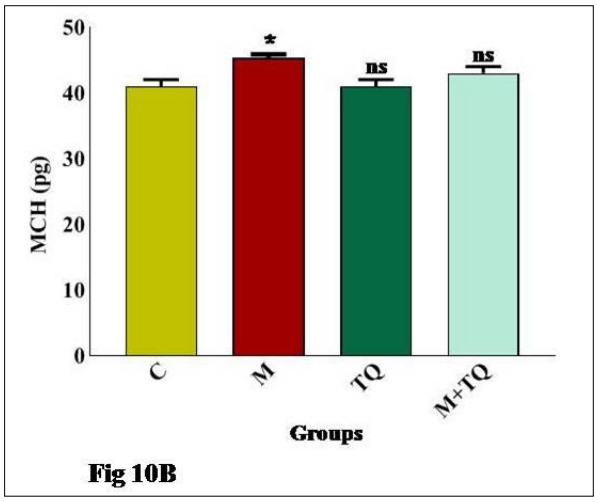

Fig. 10: B Histogram showing MCH levelx of *L. rohita.* Control (C), Malathion (M), Thymoquinone (TQ) and Malathion + Thymoquinone (M + TQ). Data represented as Mean ± SEM. Control *vs* other groups **p* <0 .05, ** *p* < 0.01, * *p* < 0.001 & a p < 0.05 b *p* < 0.01 comparison among groups**

Acknowledgements:

The authors wish to thank the Sophisticated Instrumental Center Facility, DST-FIST sponsored, Department of Zoology, for providing an infrastructure facility at Dr. Harisingh Gour Central University, Sagar, M.P (India). This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical permission:

The authors confirm that the experiment's animals were treated in accordance with international norms regarding the use of animals as research subjects. The permission was taken from an institutional committee of Dr. Harisingh Gour Vishwavidyalaya in the Department of Pharmaceutical Sciences vide no. 379/CPCSEA/IAEC/2022/08.

Ethical approval:

All applicable institutional and/or national guidelines are followed for the care and use of experimental animals.

Data availability statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of interest statement:

The authors declare that they have no known competing financial interests that could have looked to influence the work reported in this article.

Funding:

The authors declare that no funds, grants or other support were received during the preparation of this manuscript.

Credit authorship contribution statement:

PT: Conceptualization, Methodology, Formal analysis, Investigation, Writing –original draft. **RKG:** Conceptualization, Methodology. **SG:** Writing - original draft, Review & editing and data analysis. **MS:** Review & editing, Visualization, and Supervision.

REFERENCES

1. Azam Z, Sapra L, Baghel K, Sinha N, Gupta RK, Soni V, et al. *Cissus quadrangularis* (Hadjod) inhibits RANKL- induced osteoclastogenesis and augments bone health in an estrogen-deficient preclinical model of osteoporosis via modulating the host osteo-immune system. Cells. 2023; 12 (2):216.
2. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, et al. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology. 2000; 148 (2-3):187- 197.
3. Baghel K, Azam Z, Srivastava R. Dietary restriction- induced alterations on estrogen receptor alpha expression in regulating fertility in male *Coturnix coturnix japonica*: Relevance of *Withania somnifera* in modulation of inflammation and oxidative stress in testis. Am J Reprod Immunol; c2024 .p. 1-15.https://doi.org/10.1111/aji.13816
4. Baghel K, Azam Z, Srivastava R, Gupta N, Kango N. Withaferin-A attenuates diabetes mellitus-induced male reproductive dysfunction mediated by ERα in brain and testes of Swiss albino mice. Sci Rep. 2023; 13 (1): 17-25.
5. Baghel K, Niranjan MK, Srivastava R. Water and food restriction decreases immune reactivity of oestrogen receptor alpha and antioxidant activity in testes of sexually mature *Coturnix coturnix japonica*. J Anim Physiol Anim Nutr (Berl). 2020; 104 (6):1738-1747. https://doi.org/10.1111/jpn.13394
6. Baghel K, Niranjan MK, Srivastava R. *Withania somnifera* inhibits photo refractoriness which

triggers neuronal apoptosis in both pre-optic and paraventricular hypothalamic area of *Coturnix coturnix* japonica: involvement of oxidative stress induced p53 dependent Caspase-3 mediated low immune reativity. Photochem Photobiol Sci; c2023 .p. 1-14.
7. Baghel K, Srivastava R. Effect of estrogen and stress on estrogen receptor1 in the HPG axis of immature male *Gallus gallus domesticus*: Involvement of anti-oxidant system. Theriogenology; c2020. https://doi.org/10.1016/j.theriogenology.2020.05.048
8. Baghel K, Srivastava R. Photoperiod dependent expression of estrogen receptor alpha in testes of Japanese quail: Involvement of *Withania somnifera* in apoptosis amelioration. Biochem Biophys Res Commun; c2020. https://doi.org/10.1016/j.bbrc.2020.10.064
9. Baghel K, Srivastava R. Stress and steroid interaction modulates expression of estrogen receptor alpha in the brain, pituitary, and testes of immature *Gallus gallus domesticus.* Stress; c 2021. https://doi.org/10.1080/10253890.2021.1965119
10. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicabsle to acrylamide gels. Anal Biochem;c 1971. https://doi.org/10.1016/0003- 2697(71)90370-8
11. Behnke U. Methods of enzymatic analysis. Food Nahrung; c1983. https://doi.org/10.1002/food.19830271027
12. Boyde A, Reid SA. A new method of scanning electron microscopy for imaging biological tissues. Nature. 1983. https://doi.org/10.1038/302522a0
13. Carlberg I, Mannervik B. Purification and characterization of the flavor enzyme glutathione reductase from rat liver. J Biol Chem. 1975; 250 (14):5475-5480.
14. Dabas A, Nagpure NS, Mishra RM, Kushwaha B, Kumar R, Kumar P. Investigation of cadmium-induced genotoxicity and oxidative stress response in Indianmajor carp, Labeo rohita. Hum Ecol Risk Assess Int J. 2014; 20 (2): 510-526.
15. Dutta HM, Nassar SST, Munshi JSD, Richmonds C. Behavioral changes in an air-breathing fish, Anabas testudineus, exposed to malathion. Bull Environ Contam Toxicol. 1994; 52: 80-86.
16. Farah MA, Ateeq B, Ali MN, Sabir R, Ahmad W. Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere. 2004; 55 (2):257-265.
17. Hayes JD, Strange RC. Invited commentary potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. Free Radic Res. 1995; 22 (3): 193-207.
18. Hermes-Lima M, Carreiro C, Moreira DC, Polcheira C, Machado DP, Campos ÉG. Glutathione status and antioxidant enzymes in a crocodilian species from the swamps of the Brazilian Pantanal. Comp Biochem Physiol A Mol Integr Physiol. 2012; 163 (2): 189-198.
19. Huculeci R, Dinu D, Staicu AC, Munteanu MC, Costache M, Dinischiotu A. Malathion-induced alteration of the antioxidant defence system in kidney, Liver, and intestineof *Carassius auratus gibelio*. Environ Toxicol. 2009; 24 (6): 523–30.
20. Kabila V, Bhavan PS, Geraldine P. The fresh water prawn *Macrobrachium malcolmsonii*- an accumulator of nickel? J Environ Biol. 1999; 20 (4):307–12.
21. Kaur R, Dua A. Fish scales as indicators of wastewater toxicity from an international water channel Tung Dhab drain. Environ Monit Assess. 2012; 184: 2729-2740.
22. Kubrak OI, Atamaniuk TM, Husak VV, Drohomyretska IZ, Storey JM, Storey KB, et al. Oxidative stress responses in blood and Livers of *Carassius auratus* exposed to the mancozeb-containing carbamate fungicide Tattoo. Ecotoxicol Environ Saf. 2012; 85: 37-43.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J BiolChem.; c1951.
24. Luque B, Lacasa L, Ballesteros F, Luque J. Horizontal visibility graphs: Exact results for random time series. Phys Rev E. 2009; 80 (4):46103.
25. Lushchak VI. Environmentally induced oxidative stress in aquatic animals. Aqua Toxicol. 2011; 101 (1):13-30.
26. Lushchak V, Kubrak OI, Storey JM, Storey KB, Lushchak VI. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere. 2009; 76 (7):932-937.
27. Maksymiv IV, Husak VV, Mosiichuk NM, Matviishyn TM, Sluchyk IY, Storey JM, et al. Hepatotoxicity of herbicide Sencor in gold fish may result from induction of mild oxidative stress. Pestic Biochem Physiol. 2015; 122: 67-75.
28. Miller TG, Mackay WC. Relationship of secreted mucus to copper and acid toxicity in rainbow trout. Bull Environ Contam Toxicol. 1982, 28 (1).
29. Min EY, Kang JC. Effect of waterborne benomyl on the hematological and antioxidant parameters of the Nile tilapia, *Oreochromis niloticus*. Pestic Biochem Physiol. 2008; 92 (3): 138–43.
30. Nahar M, Jat D. Long-term exposure of alcohol induced behavioral impairments and oxidative stress in the brain mitochondria and synaptosomes of adult zebrafish. Zebra fish. 2021; 18 (2):110-124.
31. Nwani CD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch). Int J Environ Res Public Health. 2010; 7 (8):3298–312.
32. Pandey KB, Rizvi SI. Protective effect of resveratrol on markers of oxidative stress in human erythrocytes subjected to in vitro oxidative insult. Phytother Res. 2010; 24 (S1): 4.
33. Patil VK, David M. Behavioural and respiratory

dysfunction in the freshwater fish, *Labeo rohita* (Hamilton) under malathion intoxication. J Basic Clin Physiol Pharmacol. 2008; 19 (2): 167-176.
34. Patil VK, David M. Behavioral and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). Turk J Fish Aquat Sci. 2010; 10: 233-239.
35. Pradhan MK, Kumar R, Srivastava SK. Low dose toxicity of cypermethrin on protein profile and enzyme activity in freshwater fish, *Heteropneustes fossilis*. Zool Sci. 2013; 30 (1): 45–50.
36. Reddy P, Pillai AK. Toxic effect of malathion on branchial morphology of a freshwater fish *Tilapia mossambica*. Curr Sci. 1979; 48 (13): 596-598.
37. Reyes JL, Luque J, Lacasa L. Apollonian complex networks. J Phys A Math Theor. 2009; 42 (48): 485102.
38. Roy A, Bhattacharya J, Tudu BK, Bandyopadhyay R, Chatterjee M, Hazra DK, et al. An electronic tongue prototype for mineral water taste assessment. Sensors Actuators B Chem. 2008; 131 (1): 286-291.
39. Sabeena Farvin KHS, Andersen LL, Nielsen HH, Jacobsen C. Antioxidant activity of Cod (*Gadus morhua*) protein hydrolysates: In vitro assays and evaluation in 5% fish oil-in-water emulsion. Food Chem. 2014; 149: 326–34.
40. Shaw BJ, Handy RD. Dietary copper exposure and recovery in Nile tilapia, *Oreochromis niloticus*. Aquat Toxicol. 2006; 76 (2):111–21.
41. Sharma B, Singh S. Toxicological effects of carbofuran on certain hematological parameters of *Clarias batrachus*. Bull Environ Contam Toxicol.1991; 47 (6): 902-908.
42. Singh S, Srivastava AK. Response of *Channa punctatus* to repeated exposures of Malathion. J Environ Biol. 1999; 20 (1): 1–6.
43. Sinha AK, Nayan R, Kanungo J, Das BK. Nitric oxide modulation causes oxidative stress and affects immune responses in fish. Curr Sci. 2004; 87 (5): 601-607.
44. Sinha AK, Kanungo J. Photo induced lipid peroxidation and its modulation by antioxidantvitamins in thebrain of a freshwater catfish, *Clarias batrachus*. Photochem Photobiol Sci. 2005; 4 (6): 548-553.
45. Van-Gossum H, De-Braekeleer K, Stoks R. Inter-sexual differences in phenoloxidase activity in a damselfly: immune investment or plumage synthesis? Biol J Linn Soc. 2007; 91 (3): 583-588.
46. Webb MR, Trentham DR. Analysis of the reaction between nucleotide analogues and calcium binding proteins by fluorescence stopped flow. Biochemistry. 1981; 20 (7): 1828-1836.
47. Xu Q, Zhao Q, Peng C. Apoptosis of mouse Leydig cells induced by di-(2-ethylhexyl) phthalate involves oxidative stress. Toxicology. 2010; 271 (1–2): 20-26.