Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2025; 14(3): 356-362
Received: 10-04-2025
Accepted: 15-05-2025

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Bifenthrin-induced alterations in liver and brain biochemical markers of zebrafish, *Danio rerio*

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DOI: https://www.doi.org/10.22271/phyto.2025.v14.i3e.15386

Abstract
Bifenthrin is a synthetic pyrethroid that affects the enzyme system in living things and causes oxidative stress by producing free radicals and changing oxygen or antioxidant free radicals. Synthetic pyrethroids are the source of pollution and are utilized globally for household, agricultural, and other applications. It has detrimental effects on humans, insects, and aquatic life in many ways. The purpose of the current study was to examine how exposure to Bifenthrin affected the antioxidant enzymes Catalase (CAT), Reduced Glutathione (GSH), and Lipid Peroxidation (LPO) in the liver and brain of *Danio rerio* during the course of 5,10,15 and 20 days length of exposure. Adult fish were given four different concentrations of bifenthrin for this study: 0.47, 0.93, 1.39, and 1.86µg/l (20%, 40%, 60%, and 80% of the sublethal dose of 96-h LC_{50}). After being exposed to Bifenthrin, Zebrafish, *Danio rerio*, showed oxidative stress in their liver and brains, as evidenced by a marked dose- and time-dependent decrease in the activity of the enzymes CAT and GSH and an increase in LPO.

Keywords: Bifenthrin, liver, brain, zebrafish, oxidative stress

Introduction
Rapid industrialization and fast growth in human population causes different kinds of pollution like air, water and soil. Water is the most precious natural resources that exist on earth. The most precious resource of nature on the planet is water. It is a crucial component of the life support system, and the preservation of health is profoundly affected by its quality. Industrial waste, home sewage, drainage, chemical fertilizers, and certain types of pesticides used widely in agricultural practices for food production are the main contributors of contamination of water [1]. At present, various categories of pesticides such as organophosphates, organochlorins, carbamates, synthetic pyrethroid and natural products are used at present in agriculture to control pests. In the 1970s, the synthetic counterpart of the decorative plant *Chrysanthemum cinerariaefolium* was used to create synthetic pyrethroids, that are synthetic forms of naturally occurring pyrethrins.
Synthetic pyrethroids originally used in agriculture to preserve fruits, vegetables, cotton, grains, cereals, and ornamental. However, they were also used for a number of other applications, including insecticides, lice shampoos, repellents for insects like mosquitoes and for the management of ectoparasites like lice, tick bugs etc [2].
Type-I pyrethroids like bifenthrin are commonly used in agricultural fields to control pests and public health applications to control of vectors and ectoparasites [3]. Due to moderately hazardous it is allowed by WHO for public uses [4]. Due to excellent insecticidal property and rapid degradation in agricultural field, pyrethroids are dominant insecticide over the worldwide markets [5]. Bifenthrin is generally more demanding due to their very high insecticidal property, low cost, and low toxic for mammalian and birds [6]. Extremely persistent in the environment, bifenthrin has been found in floor wipes, house dust, urban regions, agricultural fields, and human and animal body tissues from all over the world [7-9]. Bifenthrin was the most found pesticide in the samples of stream and wetland bed sediments of USA [7,10].
The widespread and increasing use of Bifenthrin has raised significant concerns about its environmental persistence and its toxic effects on non-target aquatic organisms [11]. Earlier studies have evaluated various toxicological endpoints and biomarkers to investigate Bifenthrin-induced toxicities, such as oxidative stress, developmental abnormalities, neurotoxicity, behavioural disturbances, endocrine disruption, and immune system impairment in pregnant mice [12-17]. Toxicological effects of Bifenthrin have also been investigated in several model fish species, including fathead minnows [18] and Zebrafish [19,20]. However, research on Bifenthrin-induced toxicities in fish remains limited compared to other pyrethroids

like cypermethrin and deltamethrin, with the majority of existing studies focused primarily on Zebrafish.

Pesticides can cause various physiological and biochemical alterations in fish and other aquatic organisms by affecting the activity of several enzymes. Dimethoate is reported to exhibit toxicity toward adult Zebrafish, embryos, and fingerlings, leading to a notable decline in fecundity, embryo viability, hatchability, and fingerling survival [21].

Bifenthrin has the potential to induce oxidative stress in aquatic organisms, as all aerobic species rely on molecular oxygen for metabolic processes, which can inadvertently generate reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) [22]. Due to their high reactivity, these ROS can damage essential biomolecules, including lipids, proteins, carbohydrates, and nucleic acids [23]. Antioxidants play a crucial role in safeguarding biological systems by counteracting harmful oxidative reactions, preventing DNA damage, excessive oxidation, and cell death [24]. According to the World Health Organization (WHO), around 3 million cases of pesticide poisoning occur each year, resulting in approximately 22,000 deaths globally [25].

Catalase (CAT) is a widely occurring enzyme present in nearly all oxygen-exposed organisms, where it plays a vital role in breaking down hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Additionally, it is capable of oxidizing various toxic substances, including formaldehyde, formic acid, phenol, and alcohols. Reduced glutathione (GSH), an antioxidant contributes in shielding cells against reactive oxygen species (ROS), including peroxidase and free radicals. In live cells, the glutathione defence enzyme system detoxifies and gets rid of xenobiotics, which results in the production of compounds that are readily soluble in water and are quickly removed from the body. Lipid peroxidation (LPO) is a widely recognized process used to assess cellular damage in both plants and animals. It serves as a key indicator of oxidative stress within cells and tissues. The peroxidation of lipids caused by free radicals is considered a primary mechanism underlying cellular damage and destruction [26].

The present study aimed to evaluate the toxic effects of sub-lethal concentrations of Bifenthrin on catalase (CAT) activity, reduced glutathione (GSH) levels, and lipid peroxidation (LPO) in the liver and brain tissues of Zebrafish (*Danio rerio*) as they are officially recommended as model species for toxicological research by the Organization for Economic Cooperation and Development (OECD) [27].

Materials and Methods

Collection and Maintenance of Zebrafish

Zebrafish were collected from the local ponds of Gorakhpur, stocked and acclimatized for a month before rearing them in glass aquaria containing de-chlorinated water under the laboratory conditions. The water of the aquarium was aerated continuously using diffusers connected to a mechanical air compressor. The pH was maintained between 6.6 and 8.5 and temperature of the water was maintained between 25 ± 2℃. The fishes were fed alternately with brine shrimps, spirulina granules, tubifex worm, blood worm and other fish food purchased from local markets twice a day [28].

Biochemical Studies: The objective of the biochemical analysis was to measure alterations in the liver and brain of Zebrafish at various doses and exposure times. Therefore, mature adult Zebrafish weighing approximately 1 gram in weight and 3.4 - 4 cm in length were procured from stock aquarium and exposed to four different doses of Bifenthrin 20%, 40%, 60% and 80% of the 96-h LC_{50} value i.e., 0.47, 0.93, 1.39 and 1.86 µg/l respectively [29]. Each group, total four as per doses, with fifty fishes each was exposed to the pesticide for 20 days continuously. The water in the aquaria was replaced daily with fresh addition of bifenthrin during the experiment. A group of control fishes was maintained with in aquaria with water without bifenthrin. Required number of fishes were removed from control as well as exposed groups after 5, 10, 15 and 20 days for biochemical analysis of their brain and liver.

The activity of enzyme catalase and level glutathione was evaluated in tissue homogenate of brain and liver samples by following the well-established previously reported methods. Catalase (CAT) activity was determined by the method of Sinha [30]. The activity of CAT was expressed as units/mg protein (µmol of H_2O_2 consumed/min/mg protein). Reduced Glutathione (GSH) content in the both the tissue homogenate was estimated according to the method of Paglia *et al* and expressed as GSH mg/mg protein [31]. Thiobarbituric acid reacting substances (TBARS) and the colour reaction for malondialdehyde (MDA) were used to quantify the amounts of LPO in the liver and brain in accordance with the methods described by Placer *et al*. A standard calibration curve plotted using 1,1,3,3' tetra-methoxypropane was used to measure the MDA concentration and the results were presented as µM of MDA formed/30 min/mg protein [32].

The significance of the data was tested using two-way analysis of variance (ANOVA). The means (n=6) ± standard deviation (SD) are used to represent all the data, and a difference was deemed significant at $P<0.05$.

Results and Discussion

On exposure of Bifenthrin, the Zebrafish displayed abnormal behaviour such as restlessness, sudden and jerky movements, increase in opercular movements accompanied with surface to bottom movements and loss of balance.

The liver and brain of Zebrafish administered to different doses of Bifenthrin (0.47, 0.93, .39, and 1.86 µg/l) and times of exposure (5, 10, 15, and 20 days) exhibited significant ($p<0.05$) alterations in CAT activity, GSH, and LPO levels.

Table 1 displays the impact of Bifenthrin exposures on CAT activity in the liver of Zebrafish. A significant ($p<0.05$) decrease in catalase activity with respect to control was observed in the animals exposed to different doses of bifenthrin. The catalase activity was estimated to be 144.98±0.12 (93%), 138.11±0.32 (88%), 129.52±0.37 (83%) and 125.13±0.20 (80%) µM H_2O_2 utilized/min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively after exposure for 5 days and 115.75±0.26 (73%), 107.98±0.19 (67%), 98.43±0.20 (63%) and 87.10±0.50 (55%) µM H_2O_2 utilized/min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively after exposure for 20 days in comparison to control indicating the effect to be dose and time dependent.

Table 1: Effect of Bifenthrin on CAT activity (µM H_2O_2 utilized/min/mg protein) in the liver of Zebrafish*

Concentrations(µg/l)	Exposure period (days)			
	5	10	15	20
Control (0.00)	156.23±0.21 (100%)	155.01±0.30 (100%)	155.23±0.35 (100%)	157.06±0.50 (100%)
20% (0.47 µg/l)	144.98±0.12 (93%)	137.24±0.43 (88%)	130.74±0.23 (84%)	115.75±0.26 (73%)
40% (0.93µg/l)	138.11±0.32 (88%)	128.97±0.11 (83%)	121.96±0.11 (78%)	107.98±0.19 (67%)
60% (1.39 µg/l)	129.52±0.37 (83%)	120.50±0.16 (78%)	117.15±0.34 (75%)	98.43±0.20 (63%)
80% (1.86 µg/l)	125.13±0.20 (80%)	112.29±0.16 (72%)	108.51±0.18 (70%)	87.10±0.50 (55%)

ANOVA computation Summary					
Origin of Variances	**Degree of freedom**	**Sum of squares**	**Variance**	**F-values**	**Sign. level**
Operational Variation	3	1708.44	569.48	13.63	P<0.05
Change due to Concentrations	4	5325.61	1331.40	31.88	P<0.05
Combined Effect of all Interactions	12	501.12	41.76		
Total	19	1735.17			

The mean (n = 6) plus standard deviation (SD) is represented by the values.
Data were significant at P<0.05 (two-way ANOVA).
Numbers enclosed in parenthesis represent the percentage change, rounded to the closest value, with respect to the control value, which is assumed to be 100%.
*The exposure concentrations used were 20%, 40%, 60% and 80% (0.47, 0.93, 1.39 and 1.86µg/l) of 96-h LC_{50} value.

Table 2 displays the impact of Bifenthrin exposures on GSH levels in the liver of Zebrafish. A significant reduction in GSH level from 3.95±0.06 (93%), 3.17±0.03 (74%), 2.74±0.12 (64%) and 2.23±0.15 (52%) mg/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 5 days and 2.77±0.04 (64%), 2.23±0.07 (51%), 1.95±0.03 (45%) and 1.42±0.02 (33%) mg/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 20 days with respect to control indicates that the reduction was dose and time dependent.

Table 2: Effect of Bifenthrin on GSH level (GSH mg/mg protein) in the liver of Zebrafish*

Concentrations(µg/l)	Exposure period (days)			
	5	10	15	20
Control (0.00)	4.26±0.10 (100%)	4.41±0.07 (100%)	4.34±0.08 (100%)	4.35±0.08 (100%)
20% (0.47 µg/l)	3.95±0.06 (93%)	3.67±0.05 (83%)	3.35±0.05 (77%)	2.77±0.04 (64%)
40% (0.93µg/l)	3.17±0.03 (74%)	3.24±0.02 (73%)	3.02±0.03 (69%)	2.23±0.07 (51%)
60% (1.39 µg/l)	2.74±0.12 (64%)	2.71±0.02 (61%)	2.56±0.02 (59%)	1.95±0.03 (45%)
80% (1.86 µg/l)	2.23±0.15 (52%)	2.17±0.02 (49%)	2.10±0.03 (48%)	1.42±0.02 (33%)

ANOVA computation Summary					
Origin of Variances	**Degree of freedom**	**Sum of squares**	**Variance**	**F-values**	**Sign. level**
Operational Variation	3	1.69	0.56	11.98	P<0.05
Change due to Concentrations	4	13.14	3.28	69.56	P<0.05
Combined Effect of all Interactions	12	0.56	0.04		
Total	19	15.41			

The mean (n = 6) plus standard deviation (SD) is represented by the values.
Data were significant at P<0.05 (two-way ANOVA).
Numbers enclosed in parenthesis represent the percentage change, rounded to the closest value, with respect to the control value, which is assumed to be 100%.
*The exposure concentrations used were 20%, 40%, 60% and 80% (0.47, 0.93, 1.39 and 1.86µg/l) of 96-h LC_{50} value.

Similarly, dose and time dependent increase in the level of LPO as shown in table 3 was observed in the liver of the animal. 15.09±0.11 (105%), 16.25±0.13 (113%), 17.44±0.17 (122%) and 19.15±0.12 (134%) µM of MDA formed/30 min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 5 days and 21.99±0.07 (134%), 23.00±0.04 (140%), 24.13±0.14 (147%) and 25.00±0.33 (152%) µM of MDA formed/30 min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 20 days with respect to control.

Table 3: Effect of Bifenthrin on LPO level (µM of MDA formed/30 min/mg protein) in the liver of Zebrafish*

Concentrations(µg/l)	Exposure period (days)			
	5	10	15	20
Control (0.00)	14.31±0.12 (100%)	14.75±0.13 (100%)	15.44±0.11 (100%)	16.46±0.61 (100%)
20% (0.47 µg/l)	15.09±0.11 (105%)	15.93±0.17 (108%)	17.97±0.08 (116%)	21.99±0.07 (134%)
40% (0.93µg/l)	16.25±0.13 (113%)	17.95±0.20 (121%)	19.00±0.04 (123%)	23.00±0.04 (140%)
60% (1.39 µg/l)	17.44±0.17 (122%)	19.00±0.04 (129%)	20.22±0.10 (131%)	24.13±0.14 (147%)
80% (1.86 µg/l)	19.15±0.12 (134%)	20.06±0.09 (136%)	21.50±0.19 (139%)	25.00±0.33 (152%)

ANOVA computation Summary					
Origin of Variances	**Degree of freedom**	**Sum of squares**	**Variance**	**F- values**	**Sign. level**
Operational Variation	3	90.51	30.17	38.01	P<0.05
Change due to Concentrations	4	90.73	22.68	28.59	P<0.05
Combined Effect of all Interactions	12	9.51	0.79		
Total	19	190.76			

The mean (n = 6) plus standard deviation (SD) is represented by the values.
-data were significant at P<0.05 (two-way ANOVA).
Numbers enclosed in parenthesis represent the percentage change, rounded to the closest value, with respect to the control value, which is assumed to be 100%.
*The exposure concentrations used were 20%, 40%, 60% and 80% (0.47, 0.93, 1.39 and 1.86µg/l) of 96-h LC_{50} value

Alterations observed in the brain of the Zebrafish on exposure to different doses of bifenthrin on CAT activity, GSH level and LPO level is displayed in table 4, 5 and 6.

Table 4 displays the impact of Bifenthrin exposures on CAT activity in the brain of Zebrafish. A significant ($p<0.05$) decrease in catalase activity with respect to control was observed in the animals exposed to different doses of bifenthrin. The catalase activity was estimated to be 137.41±0.65 (90%), 125.64±0.33 (82%), 117.10±0.32 (77%) and 105.57±0.30 (69%) µM H_2O_2 utilized/min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively after exposure for 5 days and 115.75±0.26 (73%), 95.23±0.13 (63%), 85.31±0.19 (57%) and 79.12±0.18 (53%) µM H_2O_2 utilized/min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively after exposure for 20 days in comparison to control indicating the effect to be dose and time dependent.

Table 4: Effect of Bifenthrin on CAT activity (µM H_2O_2 utilized/min/mg protein) in the brain of Zebrafish*

Concentrations(µg/l)	Exposure period (days)			
	5	10	15	20
Control (0.00)	152.61±0.40 (100%)	154.01±0.32 (100%)	153.28±0.32 (100%)	149.94±1.30 (100%)
20% (0.47 µg/l)	137.41±0.65 (90%)	136.98±0.39 (89%)	110.65±0.26 (72%)	100.53±0.15 (67%)
40% (0.93µg/l)	125.64±0.33 (82%)	125.09±0.23 (81%)	102.28±0.15 (67%)	95.23±0.13 (63%)
60% (1.39 µg/l)	117.10±0.32 (77%)	115.89±0.22 (75%)	96.26±0.14 (63%)	85.31±0.19 (57%)
80% (1.86 µg/l)	105.57±0.30 (69%)	105.84±0.26 (68%)	87.88±0.19 (57%)	79.12±0.18 (53%)

ANOVA computation Summary					
Origin of Variances	**Degree of freedom**	**Sum of squares**	**Variance**	**F-values**	**Sign. level**
Operational Variation	3	2487.25	829.08	17.81	P<0.05
Change due to Concentrations	4	7924.41	1981.10	42.55	P<0.05
Combined Effect of all Interactions	12	558.61	46.55		
Total	19	10970.28			

The mean (n = 6) plus standard deviation (SD) is represented by the values.
Data were significant at P<0.05 (two-way ANOVA).
Numbers enclosed in parenthesis represent the percentage change, rounded to the closest value, with respect to the control value, which is assumed to be 100%.
*The exposure concentrations used were 20%, 40%, 60% and 80% (0.47, 0.93, 1.39 and 1.86µg/l) of 96-h LC_{50} value.

Table 5 displays the impact of Bifenthrin exposures on GSH levels in the brain of Zebrafish. A significant reduction in GSH level from 4.57±0.12 (80%), 3.85±0.10 (68%), 3.20±0.06 (56%) and 2.88±0.09 (51%) mg/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 5 days and 3.10±0.01 (53%), 2.94±0.02 (50%), 2.46±0.02 (42%) and 1.87±0.05 (32%) mg/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 20 days with respect to control indicates that the reduction was dose and time dependent.

Table 5: Effect of Bifenthrin on GSH level (GSH mg/mg protein) in the brain of Zebrafish*

Concentrations(µg/l)	Exposure period (days)			
	5	10	15	20
Control (0.00)	5.69±0.17 (100%)	5.52±0.08 (100%)	5.74±0.21 (100%)	5.83±0.22 (100%)
20% (0.47 µg/l)	4.57±0.12 (80%)	4.19±0.03 (76%)	3.55±0.04 (61%)	3.10±0.01 (53%)
40% (0.93µg/l)	3.85±0.10 (68%)	3.63±0.02 (65%)	3.09±0.02 (54%)	2.94±0.02 (50%)
60% (1.39 µg/l)	3.20±0.06 (56%)	3.00±0.03 (54%)	2.86±0.04 (50%)	2.46±0.02 (42%)
80% (1.86 µg/l)	2.88±0.09 (51%)	2.31±0.06 (42%)	2.32±0.06 (40%)	1.87±0.05 (32%)

ANOVA computation Summary					
Origin of Variances	**Degree of freedom**	**Sum of squares**	**Variance**	**F-values**	**Sign. level**
Operational Variation	3	1.71	0.57	6.89	P<0.05
Change due to Concentrations	4	26.36	6.59	79.62	P<0.05
Combined Effect of all Interactions	12	0.99	0.08		
Total	19	29.07			

The mean (n = 6) plus standard deviation (SD) is represented by the values.
Data were significant at P<0.05 (two-way ANOVA).
Numbers enclosed in parenthesis represent the percentage change, rounded to the closest value, with respect to the control value, which is assumed to be 100%.
*The exposure concentrations used were 20%, 40%, 60% and 80% (0.47, 0.93, 1.39 and 1.86µg/l) of 96-h LC_{50} value.

Similarly dose and time dependent increase in the level of LPO as shown in table 6 was observed in the brain of the animal. 17.04±0.10 (111%), 18.06±0.05 (117%), 19.98±0.15 (130%) and 21.29±0.13 (138%) µM of MDA formed/30 min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 5 days and 24.44±0.14 (139%), 25.69±0.32 (146%), 26.63±0.21 (152%) and 27.96±0.28 (159%) µM of MDA formed/30 min/mg protein on exposure to the 0.47, 0.93, 1.39 and 1.86µg/l of bifenthrin respectively for 20 days with respect to control.

Table 6: Effect of Bifenthrin on LPO activity (µM of MDA formed/30 min/mg protein) in the brain of Zebrafish*

Concentrations(µg/l)	Exposure period (days)			
	5	10	15	20
Control (0.00)	15.39±0.15 (100%)	16.31±0.10 (100%)	16.47±0.14 (100%)	17.55±0.52 (100%)
20% (0.47 µg/l)	17.04±0.10 (111%)	18.72±0.20 (115%)	20.77±0.20 (126%)	24.44±0.14 (139%)
40% (0.93µg/l)	18.06±0.05 (117%)	20.30±0.14 (124%)	22.63±0.21 (137%)	25.69±0.32 (146%)
60% (1.39 µg/l)	19.98±0.15 (130%)	21.80±0.24 (133%)	23.80±0.20 (144%)	26.63±0.21 (152%)
80% (1.86 µg/l)	21.29±0.13 (138%)	23.06±0.06 (141%)	25.63±0.21 (156%)	27.96±0.28 (159%)
ANOVA computation Summary				
Origin of Variances	**Degree of freedom**	**Sum of squares**	**Variance**	**F-values**	**Sign. level**
Operational Variation	3	102.41	34.13	33.06	P<0.05
Change due to Concentrations	4	152.44	38.11	36.91	P<0.05
Combined Effect of all Interactions	12	12.39	1.03		
Total	19	267.24			

The mean (n = 6) plus standard deviation (SD) is represented by the values.
Data were significant at P<0.05 (two-way ANOVA).
Numbers enclosed in parenthesis represent the percentage change, rounded to the closest value, with respect to the control value, which is assumed to be 100%.
*The exposure concentrations used were 20%, 40%, 60% and 80% (0.47, 0.93, 1.39 and 1.86µg/l) of 96-h LC_{50} value

Bifenthrin is used to control pests in cotton, fruits, vegetable crops. It is used in public health and animal husbandry and fish production to control ecto-parasites [33]. It has been utilised for managing indoor pests owing to its minimal toxicity to animals. Synthetic pyrethroid is one of the most prevalent pollutants in the freshwater aquatic system due to its widespread use [34]. Synthetic pyrethroids contain chlorine atoms in a vinyl side chain of the compound. In addition to altering the calcium channels and gamma-amino butyric acid receptors in nerve filaments, they also block sodium channels by prolonging their depolarisation phase [35].

All pesticides can induce oxidative stress, which can change antioxidants or enzyme systems that scavenge free oxygen radicals and produce free radicals. Depletion of antioxidants, excessive buildup of reactive oxygen species (ROS), or both can lead to disturbance in the delicate balance between oxidants and antioxidants resulting into a condition known as oxidative stress [36]. Like all aerobic animals, aquatic animals have several cellular defence pathways to control the amount of ROS and guard against the negative effects of free radicals under normal metabolic setting [37]. The defence system includes enzymatic (CAT) and non-enzymatic antioxidants such as reduced glutathione (GSH) and its precursor that either by repairing the damaging effect of free radicals or by scavenging the free radicals counteract the adverse effects of the free radicals [36]. Industrial pollutants are reported to result into oxidative stress in fishes exposed to them [39]. Failure in neutralizing the free radicals by the antioxidant defence system of a living organism results in damage to the vital biomolecules. Catalase (CAT) is a common enzyme found almost in all the organisms that are exposed to oxygen. It catalyses the decomposition of H_2O_2 to O_2 and H_2O. Therefore, CAT is considered as the primary enzyme that eliminates the ROS formed in the hepatic cell during bio-transformation of xenobiotics. The activity of this enzyme however, gradually decreased significantly with time on exposure to Bifenthrin in the present study. Ullah *et al* reported that "Bifenthrin has capability to induce stress through enhanced ROS production and subsequently results in damage to other components of the cell or tissues including proteins, enzymes, DNA etc" [22]. The liver plays an important role in intermediate metabolism of an individual as well as in the metabolism of xenobiotics and is considered as a good indicator of the health status of fish. The liver is important in oxidative stress as it exhibits more antioxidant defence. Decreased CAT activity might be due to the influx of superoxide radicals generated in the fish due to bifenthrin as reported by various workers [40, 41]. The dose-dependent decrease in the activity of catalase observed during the present study has also been reported by other investigators. The decrease in CAT activity in brain and liver of Zebrafish exposed to Bifenthrin with respect to control may be due to oxy-radical production. A time dependent decrease in the CAT activity was also observed by Rosety *et al.*, in sea bream gills exposed to malathion [42]. Wielgomas and Krechniak, have reported the alteration in CAT activity in wistar rat liver after exposure to alpha-cypermethrin and chloropyriphos [43]. Naveed *et al.*, reported the decrease in CAT activity in the tissues of brain, liver and kidney of *Channa punctatus* exposed to triazophos [44]. Singh and Ansari reported decrease in CAT activity in brain and liver of Zebrafish exposed to cypermethrin [41]. Tripathi and Bandooni, also, observed a decrease in CAT activity in the brain, gill, liver and skeletal muscles of *Channa punctatus* (Bloch). The increase or decrease of enzyme activity is related to the intensity of cellular damage [46].

Glutathione (GSH) is nonenzymatic antioxidant that protects the cells from the ROS such as free radicals and peroxides by scavenging them and by transforming the xenobiotics to the water-soluble products so that they can be rapidly excreted from the body of the organism. The reduced glutathione (GSH) antioxidant system is the protective mechanism of cells and also a key factor in the development of immune response by immune cells. Reduction in the level of reduced glutathione might increase the risk of the oxidative stress[47]. A significant decline in GSH level in brain and liver under the present experimental study may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from Bifenthrin. The decrease in the level of GSH is correlated with oxidation of glutathione peroxidases that occurs due to increase in oxidative stress and detoxification process via oxidation of toxicants. Sharma and Ansari, have also reported the significant concentration and time dependent decrease in the level of GSH [37]. Rao observed the decreased level of GSH in the gill, liver, brain and muscle of *Oreochromis mossambicus* exposed to organophosphate insecticide [46]. Farombi *et al.*, observed the reduction in GSH level in kidney, gill and heart of African catfish, *Clarias gariepinus* treated with butachlor [48]. The decreased GSH level in liver and brain of treated fish indicates that more protection against the ROS is required.

Evaluation of lipid peroxidation is a major an important biomarker of the oxidative stress and cause of liver damage [40]. The complicated process of lipid peroxidation is brought on by free radicals reacting with cellular membranes, which are abundant in polyunsaturated fatty acids. Increased lipid peroxidation following pesticide exposure indicates that oxidative cell damage brought on by free radicals may be a factor in the toxicity of pesticides. The measured levels of LPO in the liver and brain of *Danio rerio* after 20 days of exposure to Bifenthrin during the current study indicate that ROS production is elevated, which may be related to the metabolism of Bifenthrin causing membrane lipids in both types of tissues to peroxide. According to Sayeed *et al*., increased LPO in *Channa punctatu*s exposed to deltamethrin was the result of oxidative stress and causes several degenerations [49]. Impairment of enzymatic antioxidant system may be the reason that favours accumulation of free radicals that may be responsible for increased LPO on bifenthrin exposure. Elevation in the level of LPO was observed in the liver, gill, muscle and brain of organo-phosphorous insecticide intoxicated *Oreochromis mossambicus* in comparison to control [46]. Agrahari *et al*., also observed an increase in the total lipid content in liver, gill, muscle and brain of *Channa punctatus* exposed to monocrotophos [49].

Conclusion

According to the current study, bifenthrin damages aquatic creature's antioxidant defence mechanisms, particularly those of fish. It is best to prevent such harmful compounds from contaminating the environment. other investigation is required to examine the impact of other pesticides and insecticides on the fish's antioxidant enzyme status in the lab and in the field prior to using such markers in the present situation.

Acknowledgement

The authors wish to acknowledge the authorities of Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, for providing facilities to conduct this study.

References

1. Maruthanayagam, G. and Sharmila. Haematobiochemical variations induced by the pesticide, Monocrotophos in *Cyprinus carpio*, during the exposure and recovery periods. Nat Environ. Poll. Tech. 2004;3:491-494.
2. Brander SM, Gabler MK, Fowler NL, Connon RE, Schlenk D. Pyrethroids pesticides as endocrine disruptors: molecular mechanisms in vertebrates with a focus on fishes. Environ Sci Technol. 2016a;50:8977-8992.
3. Syed F, Awasthi KK, Chandravanshi LP, Verma R, Rajawat NK, Khanna VK, *et al*. Bifenthrin-induced neurotoxicity in rats: involvement of oxidative stress. Toxicol Res (Camb). 2018;7(1):48-58.
4. Wang X, Gao X, He B, Jin Y, F Z. Cis-Bifenthrin causes immunotoxicity in murine macropahges. Chemosphere. 2017;168:1375-82.
5. Hénault-Ethier L. Health and environmental impacts of pyrethroid insecticides: what we know, what we don't know and what we should do about it. Executive summary and scientific literature review. Prepared for Équiterre. Montreal; 2015. 68 p.
6. Gray L, Florez SD, Barreiro AM, Vadillo-Sánchez J, González-Olvera G, Lenhart A, *et al*. Experimental evaluation of the impact of household aerosolized insecticides on pyrethroid resistant Aedes aegypti. Sci Rep. 2018;8:12535.
7. Hladik ML, Kuivila KM. Pyrethroid insecticides in bed sediments from urban and agricultural streams across the United States. J Environ Monit. 2012;14(7):1838-4.
8. Hall LW, Anderson RD. Spatial analysis of Bifenthrin sediment concentrations in California water bodies from 2001 to 2010: identification of toxic and non-toxic areas. Hum Ecol Risk Assess Int J. 2014;20(2):497-509.
9. Yang Y, Wu N, Wang C. Toxicity of the pyrethroid Bifenthrin insecticide. Environ Chem Lett. 2018;16(4):1377-91.
10. Jeppe KJ, Kellar CR, Marshall S, Colombo V, Sinclair GM, Pettigrove V. Bifenthrin causes toxicity in urban stormwater wetlands: field and laboratory assessment using *Austrochiltonia* (Amphipoda). Environ Sci Technol. 2017;51(12):7254-62.
11. Phillips BM, Anderson BS, Hunt JW, Siegler K, Voorhees JP, Tjeerdema RS, *et al*. Pyrethroid and organophosphate pesticide-associated toxicity in two coastal watersheds (California, USA). Environ Toxicol Chem. 2012;31(7):1595-603.
12. Jin Y, Pan X, Cao L, Ma B, Fu Z. Embryonic exposure to cis-Bifenthrin enantioselectively induces the transcription of genes related to oxidative stress, apoptosis and immunotoxicity in Zebrafish (*Danio rerio*). Fish Shellfish Immun. 2013a;34(2):717-23.
13. Syed F, John PJ, Soni I. Neuro developmental consequences of gestational and lactational exposure to pyrethroids in rats. Environ Toxicol. 2016;31(12):1761-70.
14. Jin Y, Wang J, Pan X, Miao W, Lin X, Wang L, *et al*. Enantioselective disruption of the endocrine system by Cis-Bifenthrin in the male mice. Environ Toxicol. 2015;30(7):746-54.
15. Scollon EJ, Starr JM, Crofton KM, Wolansky MJ, DeVito MJ, Hughes MF. Correlation of tissue concentrations of the pyrethroid Bifenthrin with neurotoxicity in the rat. Toxicology. 2011;290(1):1-6.
16. Lu X. Enantioselective effect of Bifenthrin on antioxidant enzyme gene expression and stress protein response in PC12 cells. J Appl Toxicol. 2013;33(7):586-92.
17. Zhao M, Chen F, Wang C, Zhang Q, Gan J, Liu W. Integrative assessment of enantioselectivity in endocrine disruption and immunotoxicity of synthetic pyrethroids. Environ Pollut. 2010;158(5):1968-73.
18. Beggel S, Connon R, Werner I, Geist J. Changes in gene transcription and whole organism responses in larval fathead minnow (*Pimephales promelas*) following short-term exposure to the synthetic pyrethroid Bifenthrin. Aquat Toxicol. 2011;105(1-2):180-8.
19. Jin Y, Wang J, Sun X, Ye Y, Xu M, Wang J, *et al*. Exposure of maternal mice to cis-Bifenthrin enantioselectively disrupts the transcription of genes related to testosterone synthesis in male offspring. Reprod Toxicol. 2013b;42:156-63.
20. Bertotto LB, Richards J, Gan J, Volz DC, Schlenk D. Effects of Bifenthrin exposure on the estrogenic and dopaminergic pathways in Zebrafish embryos and juveniles. Environ Toxicol Chem. 2017;37(1):236-46.
21. Ansari S, Ansari BA. Embryo and fingerling toxicity of Dimethoate and effect on fecundity, viability and survival of Zebrafish, *Danio rerio* (Cyprinidae). World J Fish Marine Sci. 2011;3(2):167-73.

22. Ullah S, Zuberi A, Alagawany M, Farag MR, Dadar M, Karthik K, *et al*. Cypermethrin induced toxicities in fish and adverse health outcomes: its prevention and control measure adaptation. J Environ Manag. 2018;206:863-71.
23. Hermes-Lima M. Oxygen in biology and biochemistry: role of free radicals. In: Story, KB (Ed.) Functional metabolism: Regulation and Adaption. Wiley-liss, Hokoben, NJ, pp: 2004;319-368.
24. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 2001;73:239-44.
25. Amin KA, Hashem KS. Deltamethrin induced oxidative stress and biochemical changes in tissues and blood of catfish (Clarias garipinus): antioxidant defense and role of alphatocopherol. Vet Res. 2012;8:45-52.
26. Yarsan E, Tanyuksel M, Celik S, Aydin A. Effects of aldicarb and malathion on lipid peroxidation. Bull Environ Contam Toxicol. 1999;63:575-81.
27. Organization of Economic Cooperation and Development (OECD). Guidelines for testing of chemicals, Guideline 2010. "Fish, Early-life stage Toxicity Test." Adopted July 17,1992. 1992.
28. Ansari BA, Kumar K. A record of Zebrafish *Danio rerio* (Cyprinidae) from Uttar Pradesh with notes on sexual dimorphism. J Adv Zool. 1882;3(1):88-9.
29. Singh CB, Ansari BA, Kushwaha VB. Toxicity of an organophosphate Ethion, a synthetic pyrethroid Bifenthrin and neem-based formulation Neem care on Zebrafish, *Danio rerio*. CIBTech Journal of Zoology. 2024;13:342-8.
30. Sinha AK. Colorimetric assay of Catalase. Anal Biochem. 1972;47:389-94.
31. Paglia DE, Valentine WN, Dahlgren JG. Effects of low-level lead exposure on Pyrimidine 5' - nucleotidase and other erythrocyte enzymes. Possible role of pyrimidine 5'- nucleotidase in the pathogenesis of lead induced anemia. J Clinical Investig. 1975;56:1164-9.
32. Placer ZA, Cushman I, Johnson BC. Estimation of product of lipid peroxidation (Malonyldialdehyde) in biochemical systems. Anal Biochem. 1966;16:359-64.
33. Treasurer JW, Wadsworth SL. Interspecific comparison of experimental and natural routes of Lepeophtheirus salmonis and Caligus elongatus challenge and consequences for distribution of chalimus on salmonids and therapeutant screening. Aqua Res. 2004;35:773-83.
34. Carriquiriborde P, Diaz J, Mugni H, Bonetto C, Ronco AE. Impact of cypermethrin on stream fish populations under field-use in biotech-soybean production. Chemosp. 2007;68:613-21.
35. Burr SA, Ray DE. Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. Toxicol Sci. 2004;77:341-6.
36. Scandalios JG. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defences. Braz J Med Biol Res. 2005;38:995-1014.
37. Sharma DK, Ansari BA. Effects of Deltamethrin on CAT, LPO and GSH in Tissues of Zebrafish *Danio rerio*. Res J Environ Toxicol. 2013;7(1):38-46.
38. Lima PL, Benassi JC, Pedrosa RC, Dal Magro J, Oliveira TB, Filho D. Time-course variations of DNA damage and biomarkers of oxidative stress in tilapia (Oreochromis niloticus) exposed to effluents from a swim industry. Arch Environ Contam Toxicol. 2006;50:23-30.
39. Patlolla AK, Bornes C, Yedjov C, Velma VR, Tchounwou PB. Oxidative stress, DNA Damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. Environ Toxicol. 2009;24(1):66-73.
40. Singh CB, Ansari BA. Effect of Cypermethrin on CAT, LPO and GSH in the liver and Brain of Zebrafish, *Danio rerio*. 2017;10(35):7470-8.
41. Rosety M, Rosety-Rodriguez M, Ordonez FJ, Rosety I. Time course variations of antioxidant enzyme activities and histopathology of gilthead seabream gills exposed to malathion. Histol Histopathol. 2005;20:1017-20.
42. Wielgomas B, Krechniak J. Effect of alpha-cypermethrin and chlorpyrifos in a 28-days study on free radical parameters and cholinesterase activity in Wistar rats. Polish J Environ Stud. 2007;16:91-5.
43. Naveed A, Janaiah C, Adilabad AP. Effect of triazophos on protein metabolism in the fish, Channa punctatus (Bloch). Curr Res J Biol Sci. 2011;3:124-8.
44. Tripathi G, Bandooni N. Impact of alphamethrin on antioxidant defense (catalase) and protein profile of a catfish. Environmentalist. 2013;31:54-8.
45. Rao JV. Toxic effect of novel organophosphorous insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, Oreochromis mossambicus. Pesticide Biochemistry and Physiology. 2006;86:78-84.
46. Farombi EO, Ajimoko YR, Adelowo OA. Effect of Butachlor on antioxidant enzyme status and lipid peroxidation in freshwater African catfish, Clarias gariepinus. Int J Environ Res Public Health. 2008;5:423-7.
47. Serdar O. The effect of dimethoate pesticide in some biochemical biomarkers in Gammarus pulex. Environ Sci Pollut Res. 2019;26:21905-14.
48. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin S. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, Channa punctatus (Bloch). Ecotoxicol Environ Safety. 2003;56:295-301.
49. Agrahari S, Gopal SK, Pandey KC. Biomarkers of monocrotophos in a freshwater fish, Channa punctatus (Bloch). J Environ Biol. 2006;27:453-7.