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Full Length Article

Hemato-biochemical and Genetic Damage Caused by Triazophos in Fresh Water Fish, *Labeo rohita*

Abdul Ghaffar[1], Riaz Hussain[2], Ahrar Khan[3*] and Rao Zahid Abbas[2]
[1]*Department of Life Sciences (Zoology) The Islamia University of Bahawalpur-63100, The Islamia University of Bahawalpur-63100, Pakistan*
[2]*University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur-63100, Pakistan*
[3]*Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad-38040, Pakistan*
[*]For correspondence: ahrar1122@uaf.edu.pk

Abstract

Extensive use of pesticides in agriculture sector represents a major proportion of pollutants and poses serious threats to both environment and aquatic organisms. In the present study micronucleus assay and nuclear abnormalities were used as a biomarker to assess the cyto-genotoxic potential of different concentrations of triazophos insecticide in fresh water fish (*Labeo rohita* L.). For this purpose 16 fresh water fish of same age and weight were kept into four equal groups (A-D) having four each. All the fish were kept in aquaria with 75 L water capacity for six days for acclimatization. Various sub-lethal concentrations of organophosphate insecticide triazophos (0.010, 0.015 and 0.200 ppm) were tested. For hematological and cyto-genotoxic studies blood samples were collected from each fish after 48, 72 and 96 h of post-treatment. Duplicate thin blood smear was made from fresh blood of each fish. Results revealed that total erythrocyte count, pack cell volume, hemoglobin concentration, serum total proteins, mean corpuscular hemoglobin concentration and mean corpuscular volume values were significantly ($P<0.05$) decreased, which indicated that fish were suffering from microcytic hypochromic anemia. Lymphocyte and monocyte values were also significantly decreased while leukocyte count was significantly increased. The results obtained by micronucleus assay showed significantly a higher frequency of erythrocyte with micronuclei, blebbed nuclei, lobed, notched, heteropicnotic nuclei, binucleated and pear shape erythrocyte. The serum analysis showed that the concentration of different enzymes and lipid peroxidation products were significantly increased in exposed fish. It can be concluded that triazophos poses adverse hemato-biochemical and DNA damage effects in aquatic organisms.

Keywords: Triazophos; Fish; Blood; Serum; Micronuclei; Nuclear changes

Introduction

Organic pollutants, different pesticides, pharmaceuticals and heavy metals are constantly dispersed into environment, which have become a major threats to marine life (Jacquet *et al.,* 2011; Sharaf *et al.,* 2013; Yang *et al*., 2013; Kousar and Javed, 2014; Mahboob *et al*., 2014). Over 80% of aquatic pollution originates from agricultural, industrial and urban activities (Munaron *et al.,* 2012; Rasool *et al.,* 2013). Pesticides are frequently used in agriculture for consumption of phytosanitary products and pests eradication and ultimately contaminate water resources through various processes, including spray drift, run-offs and leaching (Ali *et al.,* 2014a). Huge numbers of synthetic compounds are responsible for multiple and adverse effects on the variety of life such as human population, aquatic organisms, organ function, ecosystem levels, reproductive status and biodiversity (Barranger *et al.,* 2013; Praveena *et al.,* 2013; Saleemi *et al.,* 2014). Among different water and other environmental contaminants, which pose mutagenic and carcinogenic effects the pesticides are considered as problematic one (Ahmad *et al.,* 2012). Extensive application of organophosphates due to their rapid biodegradability and non-persistent nature poses countless abnormalities to different animals, fish, human beings, ecosystems and reduction of many beneficial microorganisms (Naveed *et al.,* 2010; Chishti *et al.,* 2013). In addition, occupational exposure to pesticides poses major threats including adverse reproduction, congenital anomalies and genotoxic effects (Hussain *et al.,* 2011; Ahmad *et al.,* 2012). Due to less toxic and low persistence than many other compounds these pesticides are routinely employed in the field of agriculture, livestock sector, public health management and medicine (Deka *et al.,* 2012; Hundekari *et al.,* 2013; Hussain *et al.,* 2014). Frequent and extensive use of organophosphorus insecticides results in accumulation of these chemicals in natural water systems and variety of daily consumable food materials including vegetables and grains as a the major sources of contact (Schipper *et al.,* 2008; Edwards *et al.,* 2013; Rivadeneira *et al.,* 2013).

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Triazophos (O, O-diethyl O-1phenyl-1 H-1, 2, 4-triazol-3-yl phosphorothioate) is an important member of organophosphorus pesticides that is extensively applied to protect different food and cereal crops (Jain *et al.,* 2010, Smita *et al.,* 2011). Previously various reports are available regarding the hepatotoxic, nephrotoxic and oxidative stress in rats (Jain *et al.,* 2010), histopathological changes in different organs and immune competent cells due to triazophos (Voccia *et al.,* 1999; Ambali *et al.,* 2011) and degenerative changes in spleen, kidneys and liver in fish (Jain *et al.,* 2010; Naveed *et al.,* 2010).

One of the most important environmental concerns about the pesticides contamination is the bioaccumulation in the ecosystem and subsequent propagation through the trophic chain (Galhano *et al.,* 2011). Fish respond to toxic chemicals similar to other higher vertebrates are also considered the best biomarker for early assessment of substances which are potentially harmful to humans. The morphological and nuclear changes in erythrocytes reveal all the physical and chemical abnormalities in different organisms when they are exposed to different synthetic groups of pesticides/insecticides (Ambali *et al.,* 2011; Hussain *et al.,* 2012). Mutagenic changes are frequent and have been well-investigated in several species of marine organisms (Akcha *et al.,* 2012; Dallas *et al.,* 2013). Exposing species to genotoxicants may result in irreversible and reversible DNA abnormalities such as DNA base modifications, single or double-strand breaks and DNA adducts. These nuclear abnormalities can be rapidly defeated by DNA repair mechanisms (Mateuca *et al.,* 2006). Moreover, irreversible changes in DNA can result in anxiety during cell division ultimately leading to aneuploidy and variations in nuclear DNA contents. In addition the blood cells have been shown to reveal all the physical and chemical changes when exposed to various (Akcha *et al.,* 2012; Lewis and Ford, 2012; Hussain *et al.,* 2014).

Among the different mutagenic biomarkers, the micronucleus (MN) assay is considered the most important reliable, rapid, easier and useful technique for routine biomonitoring programs, to evaluate the pollutant-induced stress syndrome and to assess the genotoxic potential of different environmental pollutants (Schipper *et al.,* 2008; Bolognesi and Hayashi, 2011). Moreover, previously it is reported that micronucleus assay is a sensitive biomarker to detect genotoxic potential/damage caused by different industrial discharges and environmental pollutants in aquatic species (De Lemos *et al.,* 2008).

Assessment of toxicological and safety effects of pesticides is crucial because these chemicals pose adverse effects including infertility, cancer, gonadotoxic effects, fetal malformations and chromosomal aberrations (Ahmad *et al.,* 2012). The adverse effects of triazophos on different hematological parameters and different visceral organs of birds have been reported (Ghaffar *et al.,* 2014). However, the information is limited in fish species exposed to sub-lethal concentrations of triazophos (Naveed *et al.,* 2010; Chishti *et al.,* 2011). Previously no information is available in accessible literature about the mutagenic and various nuclear abnormalities induced by triazophos in exposed fish. The objective of this study was to determine the extent and basis of changes produced by triazophos in economically important freshwater fish species, *Labeo rohita.*

Materials and Methods

Experimental Fish and Triazophos Concentrations

A total of 16 healthy fresh water fish *Labeo rohita* (Rahu) of uniform size, same age and weight (250-300 g) were procured from the local fish breeding center in District Bahawalpur, Pakistan. All the fish were kept in laboratory of Department of Life Sciences (Zoology), The Islamia University of Bahawalpur. The fish were kept in aquaria having about 75 L water. All the aquaria were carefully washed and cleaned with chlorinated water before filling and releasing the experimental fish. For acclimatization purpose the fish were kept without any treatment for a period of five days. All the fish were kept in glass aquaria under ambient temperature (16 to 22^{o}C). The experimental treatments were carried out for 96 h. After five days of acclimatization periods, all the fish were randomly allocated to four different groups (A-D) having 4 fish each. Triazophos (95%technical grade) was purchased from Ali Akbar Enterprises Pakistan. Different concentrations (0.010, 0.015 and 0.200 ppm) of insecticides were made and all the experimental fish were exposed in four aquaria (A-D) for 96 h under similar conditions.

Blood Collection and Serum Separation

Blood sample about 1.5 mL from each fish was collected after 48, 72 and 96 h of post treatment with and without anticoagulant (EDTA; 1 mg/mL) and serum was separated. The blood samples were used for erythrocyte, pack cell volume, hemoglobin concentration (Ghaffar *et al.,* 2014), mean corpuscular volume, mean corpuscular hemoglobin concentration, leukocyte count, lymphocyte and monocyte values (Islam *et al.,* 2013).

Micronuclei and Nuclear Changes

For different nuclear abnormalities in erythrocyte of fish duplicate thin blood smears were made from each fish. All the smears were air dried, fixed in methanol and stained with Wright-Giemsa stain for 3–4 min. The frequency of micronuclei and different nuclear abnormalities was studied using oil immersion lens with the help of light microscope (Nikon, Tokyo, Japan) at the Department of Pathology, University of Agriculture, Faisalabad, Pakistan. A total of 1500 erythrocytes/fish/smear were observed (Hussain *et al.,* 2012).

Serum Biochemistry

Serum enzymes (ALT, AST and ALP) were determined by spectrophotometrically using commercially available kits (Ahmad *et al.,* 2013). The lipid peroxidation product serum malondialdehyde was analyzed spectrophotometrically (Ali *et al.,* 2014b).

Statistical Analysis

The data thus collected in this experiment were subjected to statistical analysis. Mean ± SE values of hematological and cellular abnormalities were computed by analysis of variance and different group means were compared by Duncan's multiple range test.

Results

Hematological and Serum Biochemical Parameters

The results revealed that erythrocyte count, pack cell volume, hemoglobin concentration, serum total proteins, mean corpuscular hemoglobin concentration, mean corpuscular volume, lymphocyte and monocyte was significantly reduced in fish exposed to higher levels of triazophos (0.015 and 0.20 ppm) when compared to untreated fish (Table 1). The values of total leukocyte count were significantly increased in fish treated with higher levels of triazophos (0.015 and 0.200 ppm) as compared to fish of control group. Serum alanine aminotransferase (ALT) in group D after 72 h and in groups C-D after 96 h of treatment increased significantly as compared to control group. The values of serum AST and ALP in fish of group D after 48 h and in groups C–D after 72 and 96 h were significantly increased. Similarly serum MDA and NO values in group D after 48 h and in groups C–D after 72 and 96 h were significantly increased (Table 2).

Genotoxic and Cellular Abnormalities in Erythrocyte

The results on various nuclear and morphological changes in erythrocytes of fish are presented in Table 3. The results revealed that percentile rate of cells with micronuclei (Fig. 1), blebbed nuclei (Fig. 2), lobed nuclei (Fig. 2), pear shape erythrocyte (Fig. 3), binucleated erythrocyte (Fig. 3), notched nuclei, heteropicnotic nuclei and was significantly increased in fish exposed to higher levels of organophosphate insecticide (0.015 and 0.200 ppm) at 72 and 96 h of post treatment.

Discussion

Present study revealed that erythrocyte count, pack cell volume, hemoglobin concentration, serum total proteins, mean corpuscular hemoglobin concentration and mean corpuscular volume were significantly reduced, which indicated that fish were suffering from microcytic hypochromic anemia. The values of total leukocyte count increased significantly, while lymphocyte and monocyte reduced significantly in fish treated with higher levels of triazophos as compared to fish of control group.

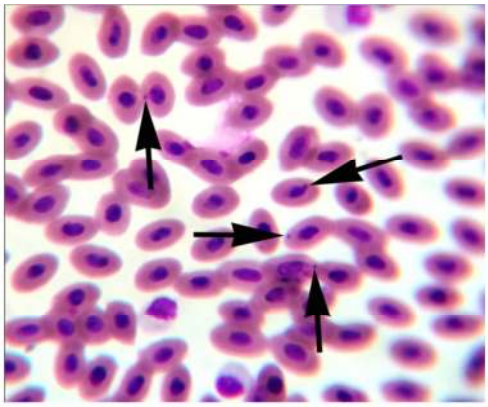

Fig. 1: Blood smear of triazophos treated fish showing micronuclei (arrows). x1000. Giemsa stain

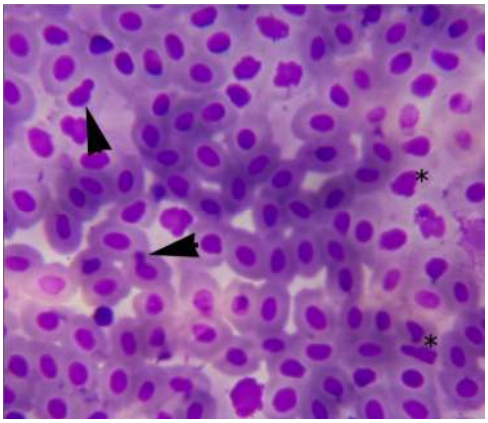

Fig. 2: Blood smear of triazophos treated fish showing lobed (arrow heads) and blebbed nucleated erythrocyte (*). ×1000. Giemsa stain

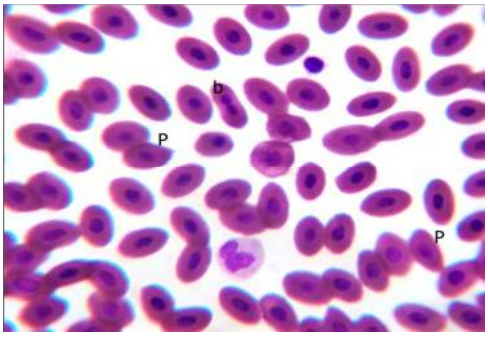

Fig. 3: Blood smear of triazophos treated fish showing binucleated erythrocyte (b) and pear shape erythrocyte (P). ×1000. Giemsa stain

Table 1: Various hematological parameters of fish (*Labeo rohita*) administered different levels of triazophos

Parameter	Groups			
	A	B	C	D
Erythrocyte (10^6/mm^3)				
48	3.78±0.05	3.61±0.01	3.31±0.03*	3.06±0.02*
72	3.74±0.02	3.53±0.02	3.27±0.02*	2.73±0.03*
96	3.79±0.01	3.50±0.01	3.25±0.04*	2.33±0.04*
Pack cell volume (%)				
48	31.13±0.76	28.14±0.27	22.65±0.63*	17.94±0.49*
72	31.17±0.67	25.92±0.24*	20.40±0.64*	17.49±0.65*
96	31.11±0.70	24.74±0.27*	18.58±0.65*	15.19±0.38*
Hemoglobin concentration (g/dL)				
48	7.98±0.09	7.55±0.26	6.67±0.02*	6.31±0.09*
72	8.09±0.07	7.30±0.22	6.27±0.05*	5.89±0.10*
96	8.03±0.10	6.95±0.08*	5.44±0.04*	4.67±0.24*
Mean corpuscular volume (fL)				
48	76.23±2.11	73.23±1.13	66.32±1.19*	62.23±2.22*
72	74.34±3.17	71.19±2.10	64.02±1.07*	60.03±1.14*
96	75.29±1.15	71.90±2.01	59.55±1.12*	58.02±1.21*
Mean corpuscular hemoglobin concentration (g/dL)				
48	22.21±0.88	18.85±0.21	15.43±0.25*	14.29±0.06*
72	21.65±0.64	17.82±0.21	13.80±0.20*	12.87±0.20*
96	22.41±0.43	17.42±0.08	13.49±0.19*	12.52±0.11*
Leukocyte counts (10^3/mm^3)				
48	15.03±0.13	17.15±0.32	20.75±0.67*	23.7±0.18*
72	15.61±0.23	17.72±0.17	22.15±0.65*	24.35±0.06*
96	15.59±0.08	18.4±0.31	23.35±0.35*	25.40±0.08*
Lymphocyte (%)				
48	20.67±0.65	18.16±0.07	16.18±0.05*	15.14±0.10*
72	20.42±0.39	17.85±0.42	14.91±0.24*	14.36±0.07*
96	20.24±0.69	17.69±0.21	13.99±0.16*	13.32±0.04*
Monocyte (%)				
48	4.23±0.04	4.01±0.05	3.8±0.05*	3.57±0.04*
72	4.18±0.02	3.91±0.02	3.54±0.02*	3.36±0.02*
96	4.19±0.01	3.84±0.02	3.34±0.03*	3.19±0.03*
Serum total proteins (g/dL)				
48	3.81±0.11	3.65±0.02	2.98±0.05*	2.72±0.05*
72	3.75±0.08	3.62±0.24	2.72±0.02*	2.47±0.02*
96	3.78±0.04	3.36±0.03	2.46±0.02*	2.32±0.01*

Values (mean ± SE) in rows bearing asterisk are significantly ($P \leq 0.05$) different from control group

Table 2: Serum biochemical changes observed in fresh water fish (*Labeo rohita*) given various doses of triazophos

Parameter	Groups			
	A	B	C	D
Alanine aminotransferase (IU/l)				
48	24.9±0.57	24.2±0.10	25.4±0.10	26.3±0.17
72	24.4±0.22	25.9±0.26	26.7±0.04	32.5±0.48*
96	25.1±0.23	26.6±0.04	32.2±0.80*	37.6±1.00*
Aspartate aminotransferase (IU/L)				
48	16.3±0.25	17.8±0.25	18.7±0.41	22.9±1.14*
72	16.4±0.30	19.4±0.34	23.2±0.24*	25.4±0.31*
96	15.8±0.29	19.4±0.40	25.2±0.17*	28.6±0.34*
Alkaline Phosphates (IU/L)				
48	34.3±0.44	36.7±0.86	37.5±0.34	46.1±0.63*
72	35.5±0.33	37.3±0.32	43.2±1.66*	52.7±1.51*
96	36.3±0.17	38.4±0.32	47.9±0.73*	67.0±3.75*
Malondialdehyde (nmol/mL)				
48	9.1±0.11	9.29±0.04	10.1±0.06	13.8±0.12*
72	9.2±0.05	10.3±0.04	11.5±0.28*	15.6±0.20*
96	9.4±0.26	10.8±0.17	14.8±0.23*	17.8±0.67*
Nitic oxide (µmol/L)				
48	36.0±0.53	37.4±0.37	38.2±0.16	44.2±1.11*
72	36.7±0.46	38.2±0.26	46.2±0.37*	48.6±0.44*
96	36.9±0.08	38.5±0.22	47.0±0.38*	50.5±0.47*

Values (mean±SE) in rows bearing asterisk are significantly ($P \leq 0.05$) different from control group

Alterations in different hematological parameters and blood chemistry of fish exposed to different pollutants can be used as the most suitable and reliable biomarkers to determine the physiological indices of stress in fish induced by various toxic substances (Karanthi *et al.,* 2004; Hussain *et al.,* 2014). The decreased values of erythrocyte could be due to toxic impacts of insecticide on blood producing tissues and on circulating erythrocytes. Reduced level of hemoglobin in this study may be due to inadequate iron supply. The lower values of hemoglobin could also be due to toxic impacts of triazophos on maturating erythrocytes (Karanthi *et al.,* 2004; Hussain *et al.,* 2011), while lower values of hematocrit could be attributed to increase destruction of erythrocyte (Auon *et al.,* 2014). Leukocytosis in present study is suggestive of the stress conditions and process of inflammation induced by triazophos and the sensitivity of the immune system. Decreased values of serum total proteins, monocyte and lymphocytes may be due to stress-induced deleterious effects of triazophos such as hepatic dysfunction, impaired protein synthesis and immunosuppressive impacts (Auon *et al.,* 2014). Abnormalities in these hematological parameters (decreased MCHC and MCV) could be related to adverse impacts of insecticide (Carvalho *et al.,* 2006). The lower levels of hematological parameters could also be related to decreased erythropoiesis, osmoregulatory dysfunction, deficiency of hemopoietin along with impaired production of hematopoietic progenitor cells (Salih, 2010; Hussain *et al.,* 2011).

Results of this study showed that the incidence of erythrocytes with micronuclei, notched, heteropicnotic nuclei and lobed nuclei was significantly higher in fish treated with higher levels of insecticide triazophos. In the published literature to the best of our knowledge, no report could be found about the frequency of these mutagenic impacts of triazophos in fish have not been studied. The formation of micronucleus and lobed nucleus erythrocyte in our study could also be due to higher production of caspase activated DNase leading to cleavage of cytoskeletal (vimentin, gelsolin and fodrin) and nuclear proteins as a result of oxidative stress to mitochondrion (Hussain *et al.,* 2014). These cytopathogenic alterations could be due to increased process of lipid peroxidation resulting to increased generation of intracellular reactive oxygen and nitrogenous species (Campos-Pereira *et al.,* 2012; Hussain *et al.,* 2012; 2014). Earlier studies have indicated that nuclear alterations in erythrocytes could be due to chromosomal abnormalities induced by variety of toxic substances (Hussain *et al.,* 2012). The lobed and blebbed nuclei erythrocyte in present study could be due to failure of tubulin polymerization, oxidation of mRNA and nitration of proteins which have impact on intracellular metabolism (Campos-Pereira *et al.,* 2012; Hussain *et al.,* 2014).

Table 3: Various nuclear changes observed in erythrocytes of fish (*Labeo rohita*) given various doses of Triazophos

Parameter	Groups			
	A	B	C	D
Cells with Micronuclei (%)				
48	0.15±0.04	0.20±0.01	0.77±0.04*	0.90±0.01*
72	0.18±0.02	0.22±0.01	1.05±0.02*	1.23±0.05*
96	0.20±0.01	0.23±0.01	1.69±0.03*	2.32±0.01*
Blebbed Nuclei (%)				
48	0.09±0.02	0.07±0.01	0.13±0.03*	0.42±0.02*
72	0.11±0.00	0.13±0.01	0.29±0.02*	0.62±0.02*
96	0.12±0.00	0.14±0.01	0.39±0.01*	0.75±0.01*
Lobed Nuclei (%)				
48	0.14±0.02	0.17±0.01	0.20±0.03*	0.46±0.02*
72	0.16±0.01	0.18±0.02	0.25±0.05*	0.75±0.03*
96	0.15±0.03	0.19±0.01	0.33±0.04*	0.88±0.03*
Notched nuclei (%)				
48	0.13±0.04	0.16±0.02	0.30±0.03*	0.51±0.03*
72	0.15±0.01	0.16±0.00	0.37±0.01*	0.64±0.02*
96	0.16±0.02	0.18±0.01	0.48±0.02*	0.76±0.01*
Binucleated erythrocytes (%)				
48	0.03±0.01	0.04±0.01	0.11±0.02*	0.25±0.03*
72	0.04±0.01	0.05±0.01	0.21±0.03*	0.36±0.01*
96	0.03±0.01	0.06±0.01	0.25±0.04*	0.42±0.01*
Heteropicnotic Nuclei (%)				
48	0.041±0.01	0.05±0.01	0.09±0.02	0.30±0.02*
72	0.04±0.01	0.05±0.01	0.12±0.01*	0.53±0.03*
96	0.04±0.02	0.05±0.02	0.13±0.07*	0.78±0.01*
Pear shape Erythrocyte (%)				
48	0.36±0.04	0.41±0.02	0.48±0.02*	0.56±0.01*
72	0.37±0.02	0.39±0.02	0.5±0.01*	0.69±0.01*
96	0.38±0.03	0.41±0.03	0.56±0.09*	0.80±0.03*

Values (mean ±SE) in rows bearing asterisk are significantly ($P \leq 0.05$) different from control group

The concentration of different serum enzymes including ALT, AST and ALP increased significantly in insecticide exposed fish. Previously no report could be found about the toxic effects of insecticides on similar serum enzymes. Increased concentrations of these enzymes could be related to increased lipid peroxidation products and synthesis of reactive oxygen species inducing serious damage to biological membranes and different other cytoplasmic contents. In this study insecticide induced higher levels of malondialdehyde and nitric oxide which might be related to induction of oxidative stress in association to impair intracellular metabolism and over synthesis of reactive oxygen species by NADPH oxidase which potentiate cell swelling and impaired mitochondrial function, since the organophosphate insecticides are well known to cause oxidative stress (Jain *et al.,* 2010; Mossalam *et al.,* 2011; Hussain *et al.,* 2013; Hundekari *et al.,* 2013). The possible cause of oxidative stress in this study is the activation of intracellular signaling molecules and N-methyl-D-aspartate receptors by insecticide intoxication. Numerous studies have reported that oxidative stress results due to increased synthesis of reactive oxygen/nitrogenous species, phosphorylation of JNK, ERK1/2 and $p38^{MAPK}$ by exogenous H_2O_2 and inactivation of mitogen-activated protein kinase (Czaja *et al.,* 2003; Clausen *et al.,* 2004).

Conclusion

The hematological changes, nuclear abnormalities and serum biochemical changes recorded in this study suggest the triazophos insecticide toxicity and its mutagenic potential in fresh water fish. As triazophos has rendered microcytic hypochromic anemia and genetic damage in the fish, resultantly decreased body weight gain of the fish will occur for which further studies are required. Moreover, when such treated fish comes in food chain, then definitely humans are vulnerable to adverse effects of such insecticides.

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