Pak. J. Agri. Sci., Vol. 54(3), 607-611; 2017
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: DOI: 10.21162/PAKJAS/17.5939
http://www.pakjas.com.pk

PESTICIDES MIXTURE TOXICITY; EFFECTS ON SUPEROXIDE DISMUTASE ACTIVITY IN INDIAN MAJOR CARPS

Huma Naz[1,*], Sajid Abdullah[1], Khalid Abbas[1] and Muhammad Anjum Zia[2]

[1]Department of Zoology, Wildlife & Fisheries, University of Agriculture Faisalabad -38040, Pakistan;
[2]Department of Biochemistry, University of Agriculture, Faisalabad -38040, Pakistan.
[*]Corresponding author's e-mail: **humanaz98@yahoo.com**

The current study was conducted to determine the tolerance limits of Indian major carps viz. *Catla catla, Cirrhina mrigala* and *Labeo rohita* against pesticides mixture (Bifenthrin + Chlorpyrifos + Endosulfan) and to see it effect on antioxidant enzyme like superoxide dismutase (SOD) activity in the liver of these species. The 96-hr LC_{50} and lethal concentration of pesticides mixture (BIF+CPF+END) for *C. catla, C. mrigala* and *L. rohita* was determined in the static bioassay methods as 1.09, 1.81 1.63 and 1.90, 2.78, 2.30 μgL^{-1}, respectively.*C. catla* exhibited higher sensitivity toward tertiary pesticides mixture followed by *L. rohita* and *C. mrigala*. The activity of SOD in the liver of all three fish species exposed to pesticides mixture was significantly ($p<0.05$) higher as compare to control fish. The SOD activity showed time dependent increasing trend in the liver of all three fish species.

Keywords: Acute toxicity, antioxidant enzyme, pesticides mixture, water pollution, aquatic ecosystem

INTRODUCTION

Due to rapid increase in the industrialization and human population, the pollution in aquatic ecosystem has become an universal phenomenon in the present day world (Belazutshi and Raghuprasad, 2008). The main sources of water pollution are industrial waste, domestic sewage, drainage and agriculture effluent which contain pesticides (Maruthanayagam and Sharmila, 2004). These pesticides are too toxic and have adverse effects on aquatic organisms and water quality (Barbieri and Ferreira, 2011). Acute toxicity refers to the toxicant's ability to cause harm to an organism from a single exposure, commonly of short period. Mostly the acute toxicity test of pesticides has been used for rapid estimation of the concentrations that cause direct and irreversible damage to the fish (Pandey *et al.,* 2005). Acute lethality and LC_{50} are most common acute toxicity tests which signify the lethality of test organisms in terms of mortality and exposure duration (Karra *et al.,* 2015). Pesticides are able to disturb the fish organs, nervous system, reproduction, hormone regulation, embryonic development and growth at non-lethal dose (Palma *et al*., 2008 and 2009a,b).

Most common environmental pollutant entering the aquatic ecosystem is endosulfan, an organochlorine pesticide causing mass mortality of fish. It is highly stable and accumulates in the food web (EJF, 2002; Pandey *et al.,* 2006). In the beginning of 1980's organophosphate (OP) pesticides has been extensively used in place of organochlorine (Svoboda *et al.,* 2001). Most preferable organophosphorus is chlorpyrifos, because of their low cumulative ability and lower persistence in the environment but has adverse effects on aquatic life (Sparling and Fellers, 2007). Organochlorine and organophosphorus pesticides have been fully replaced by synthetic pyrethroid (Tiwari and Ansari, 2014) due its high selectivity and short term persistence in the environment (Goulding *et al.,* 2013). Bifenthrin belong to pyrethroids pesticides those are extremely dangerous to fish because at the nerve cell ending it interfere with Na^+ channel gating as well as with other ions channels like Cl^- and Ca^{2+} (Burr and Ray, 2004).

Pesticide stimulated oxidative stress has become increasingly reported from the last few decades in toxicological research as a possible mechanism of toxicity (Ciccheti and Argentin, 2003; Sharma *et al.,* 2005). To save the biomolecules from the injurious effects of reactive oxygen species (ROS) resulting from the breakdown of different toxicants, fish possess an antioxidant defense system. Antioxidant defense system of fish consist of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) and glutathione reductase (GR) (Mate, 2000). First defensive mechanism in fish against oxidative stress is SOD; facilitate dismutation of superoxide radicals to H_2O_2. Therefore, this can be used as biomarker for ROS production in pesticide toxicity (Kohen and Nyska, 2002). Species-specific response to toxicants is of great importance, because it is a key feature estimating toxicity as well. A lot of information on freshwater organisms was able to document a few of these species-specific sensitivities to various contaminants (Pyle and Wood, 2008; Schjolden *et al.,* 2007).*Catla catla, Cirrhina*

mrigala and *Labeo rohita* are the common freshwater fishes of south-east Asian countries. Information regarding the 96-hr $LC_{50}$and lethal toxicity of pesticides mixture in *L. rohita*, *C. catla* and *C. mrigala* has not been reported till date. Therefore, this study was conducted to determine the 96-hr LC_{50} and lethal toxicity of pesticides mixture (Bifenthrin+Chlorpyrifos+Endosulfan) to Indian major carps and its effect on oxidative stress biomarker such as superoxide dismutase activity in the liver of three fish species.

MATERIALS AND METHODS

***Acute toxicity test*:** The experimental fish species (*Catla catla, Cirrhina mrigala* and *Labeo rohita*) were purchased from Fish Seed Hatchery, Faisalabad, Pakistan. The acute toxicity tests (96-hr LC_{50} and lethal concentration) were conducted in the wet laboratory at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. Before the start of the experiment, fish were kept in cemented tank for acclimatization for two weeks. During this period fish were fed with commercial feed at 3% wet body weight. All aquaria (70-L) were well aerated with an air pump and filled with dechlorinated tap water and stocked with fish. Stock solutions of pesticides viz. Bifenthrin (BIF), Chlorpyrifos (CPF) and Endosulfan (END) were made in methanol while pesticides mixture (Bifenthrin+Chlorpyrifos+Endosulfan) of required concentration were made by further dilutions in the deionized water. The acute toxicity tests were replicated three times. During experimental trails, water pH, temperature and total hardness were maintained at 7.5, 30°C and 250 mgL^{-1}, respectively. The physico-chemical parameters were monitored on daily basis by following standard methods (A.P.H.A. 1998). Mortality was recorded during 96-hr after the start of the tests and dead fish were removed immediately.

***Superoxide dismutase activity*:** After determination of 96-hr LC_{50} value of Bifenthrin + Chlorpyrifos + Endosulfan mixture sampling was done at interval of 24, 48, 72 and 96-hr to evaluate the superoxide dismutase activity in the liver of all three fish species.

***Preparation of homogenate*:** To remove the RBCs, the dissected organ i.e. liver was rinsed with phosphate buffer of 0.2 M (pH 6.5) and homogenized in cold buffer (1:4 w/v). Homogenate organ was centrifuged at 10,000 rpm for 15 minutes at 4°C. After this, clear supernatant was separated for enzyme assay while residue was discarded.

***Enzyme Assay*:** The activity of superoxide dismutase was determined by measuring its ability to inhibit the photoreduction of Nitrobluetetrazole (NBT) by following the method of Giannopolitis and Ries (1977). Reaction mixture contained 1 ml of 0.067 mM phosphate buffer (pH 7.8), 0.016 ml of riboflavin and 0.05 ml enzyme extract. This mixture was kept in light box with an internally mounted light bulb of 30 Watt for 12 minutes. After that 0.067 ml of EDTA/NaCN solution and 0.033 ml of NBT was added to the illuminated reaction mixture and absorbance was noted after 20s of reaction by spectrophotometerically at A_{560} nm against blank (buffer).

***Statistical analyses*:**The whole experiment was performed with three replicates. The 96-hr LC_{50} and lethal concentrations of pesticides mixture for all three fish species were determined by analyzing the fish mortality data through Probit Analysis method (Hamilton *et al*., 1977) at 95% confidence interval. Analysis of variance and comparison of means were performed by using Tukey's/Student Newnan-Keul tests to find-out statistical differences among different variables under study.

RESULTS AND DISCUSSION

***Acute toxicity test*:** The acute toxicity data with mean 96-hr LC_{50} and lethal concentration (μgL^{-1} ±SD) values, duration of exposure, concentration of pesticides in mixture, 95% lower and upper confidence interval limits with their calculated chi-square values are given in Table 1. The calculated chi-square (χ^2) with higher p-values (0.992-1.000) showed higher precision of all regression models. Results indicated that among all three fish species, *C. catla* showed

Table 1. The 96-hr acute toxicity of pesticides mixture (μgL^{-1}±SD) for Indian Major Carps.

Treatment	Mixture Ratio	Fish species	LC_{50} (μgL^{-1})	95% C.I. LCL-UCL	Lethal conc. (μgL^{-1})	95% C.I. LCL-UCL	Pearson Goodness of Fit Tests			Regression equation (Y=a+bx)
							χ^2	DF	p-value	
BIF + CPF + END	1:1:1	*C. catla*	1.09±0.01c	0.906-1.218	1.9±0.02c	1.693-2.305	1.043	8	0.998	Y= -2.90102+2.33028**x (0.433215)
		C. mrigala	1.81±0.02a	1.635-1.960	2.78±0.02a	2.532-3.266	2.392	10	0.992	Y= -3.90617+1.95165**x (0.343120)
		L. rohita	1.63±0.02b	1.477-1.741	2.30±0.03b	2.127-2.632	0.699	8	1.000	Y= -4.89785+2.78874**x (0.490041)

Means with the same letters (small) in a single column are statistically similar at p<0.05.BIF=Bifenthrin; CPF= Chlorpyrifos; END=Endosulfan; *C. catla = Catla catla*; *C. mrigala=Cirrhina mrigala*; *L. rohita=Labeo rohita*; C.I.= Confidence Interval (μgL^{-1}); LCL=Lower Confidence Limit (μgL^{-1}); UCL= Upper Confidence Limit (μgL^{-1}); Lethal Conc., Lethal Concentrations (μgL^{-1});χ^2 =Chi-Square; DF= Degree of Freedom; y=Dependent variable; x=Independent variable; value within bracket is the standard error; **= Significant at p<0.01

more sensitivity toward BIF+CPF+END mixture with mean 96-hr LC_{50} and lethal concentration of 1.09±0.01 and 1.9±0.02 μgL^{-1}, respectively. The deviance chi-square and p-value for *C. catla* was calculated as 1.043 and 0.998, respectively. The mean 96-hr LC_{50} and lethal concentrations of tertiary mixture of pesticides (BIF+CPF+END) were ranged from 1.63±0.02-2.30±0.03 for *L. rohita* while it was estimated as 1.81±0.02 and 2.78±0.02 μgL^{-1}, respectively for *C. mrigala*. It showed higher tolerance limit against tertiary mixture of pesticides.

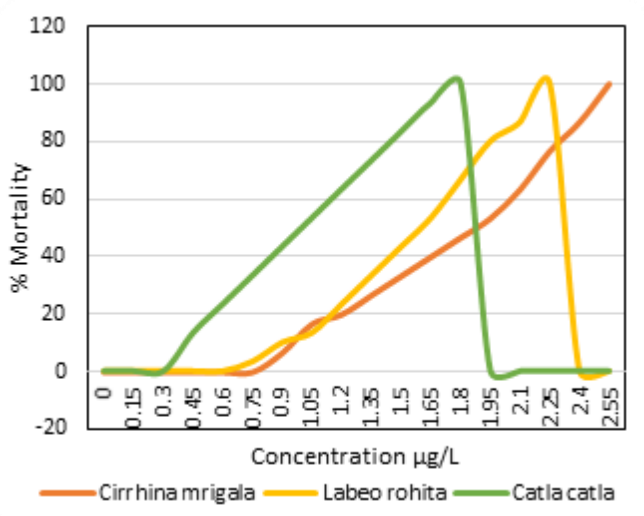

Figure 1. Relationship between the concentration of mixture and mortality of *C. mrigala*, *L. rohita* and *C. catla* during 96-hr acute toxicity.

The sensitivity of all fish species varied significantly under the exposure of tertiary mixture of pesticides (BIF+CPF+END). The sensitivity of three fish species toward pesticides mixture was increased in the following order: *C. catla*>*L. rohita*> *C. mrigala*. Our results are also supported by Ilyas and Javed (2013) who performed acute toxicity test with three fish species viz. *C. catla*, *C. mrigala* and *L. rohita* exposed to endosulfan. The 96-hr LC_{50} values of endosulfan for these major carps were calculated as 0.98, 1.06 and 2.15 μgL^{-1}, respectively which shows that the *C. catla* is more sensitive than other species. According to Al-Rudainy and Kadhim (2012), toxicant concentration and exposure duration are the main factors responsible for fish mortality. Ambreen and Javed (2015) reported the acute toxicity of pesticide mixture (CPF+END+BIF) to the fish, *Cyprinus carpio*and *Ctenopharyngodon idella.* The mean 96-hr LC_{50} and lethal concentrations of this mixture were 0.22±0.01 and 0.32±0.02 μgL^{-1}, respectively for *C. carpio* while the mean 96-hr LC_{50} and lethal concentrations were ranged from 2.16-7.49 μgL^{-1} and 3.86-13.86 μgL^{-1}, respectively for *C. idella.*

***Superoxide dismutase activity*:** In the present study, it was found that the activity of SOD in the liver of all three fish species exposed to tertiary mixture of pesticides was significantly ($p<0.05$) higher as compared to control (Table 1). Comparison among fish species showed that *C. catla* had higher SOD activity as compared to other species exposed to tertiary mixture (Table 2). Regression equation for enzyme activity is given in Table 3 which shows the dependence of SOD activity on exposure time was significantly positive with R^2 value upto 0.991, 0.976, and 0.99 for *C. catla*, *C. mrigala* and *L. rohita*, respectively. It

Table 2. The SOD activity (UmL^{-1}±SD) in liver of three fish species exposed to BIF+CPF+END mixture for different time intervals.

Exposure Time (hr)	Fish Species					
	C. catla		*C. mrigala*		*L. rohita*	
	Control	Treated	Control	Treated	Control	Treated
24	59.11±0.34a	103.20±0.49d	38.15±0.18a	80.35±4.59d	47.51±0.11a	99.96±0.25d
48	59.12±0.35a	119.75±0.48c	38.17±0.26a	100.42±6.40c	47.52±0.17a	115.21±0.34c
72	59.14±0.40a	131.08±0.28b	38.18±0.27a	119.17±10.23b	47.54±0.12a	125.05±0.45b
96	59.15±0.42a	148.67±0.65a	38.19±0.35a	130.05±7.12a	47.55±0.12a	140.05±0.29a

Means with similar letters in a single row are statistically similar at $p<0.05$.ANOVA followed by HSD Tukey test

Table 3. Comparison of means of SOD activity (UmL^{-1}±SD) in liver of three fish species exposed to BIF+CPF+END mixture for different time intervals.

Fish Species	Exposure Durations				*Overall Means
	24-hr	48-hr	72-hr	96-hr	
C. catla	103.20±0.49d	119.75±0.48c	131.08±0.28b	148.67±0.65a	125.68±19.13A
C. mrigala	80.35±4.59d	100.42±6.40c	119.17±10.23b	130.05±7.12a	107.50±21.85C
L. rohita	99.96±0.25d	115.21±0.34c	125.05±0.45b	140.05±0.29a	120.07±16.85B
Overall Means	94.50±12.36D	111.79±10.11C	125.10±5.96B	139.59±9.32A	

Means with similar letters in a single row and *column are statistically similar at $p<0.05$.ANOVA followed by HSD Tukey test

Table 4. Relationship between exposure time of BIF+CPF+END mixture and SOD activity (UmL^{-1}) in liver of three fish species.

Fish Species	Regression Equation	SE	r	R^2
C. catla	88.7 + 0.616 **Time (hr)	0.03451	0.995	0.991
C. mrigala	65.5 + 0.699 **Time (hr)	0.06355	0.988	0.976
L. rohita	87.5 + 0.542 **Time (hr)	0.03119	0.994	0.990

SE=Standard Error; r= Multiple Regression Coefficient; Coefficient of Determination; **=Highly significant at p<0.01

was observed that the SOD activity showed time dependent increasing trend in the liver of all three fish species. These findings are also supported by Ullah *et al.* (2016) who observed a time dependent elevation in superoxide dismutase activity in the liver of *L. rohita* exposed to endosulfan.

Alteration in enzymes activities and metabolic functions by increased reactive oxygen radicals is considered as the fish response to toxicant exposure (Oruc *et al.*, 2004; Isik and Celik, 2008). The induction in the SOD activity under oxidative stress considered as a first line of defense provide an important adaptation against toxicants produced stress (Gultekin *et al.*, 2000). In addition, liver is a key organ responsible for detoxification, metabolism and excretion of toxicants from the body (Hinton and Lauren, 1990). According to Mohamed (2009) the liver is also a target organ due to its large blood supply which causes noticeable toxicant exposure.

Sharbidre*et al.* (2011) reported the change in SOD responses in the liver of fish, *Poecilia reticulate* treated with methyl parathion (MP) and chlorpyrifos for 96 hours. Organophosphate insecticides (fenitrothion) significantly increased the SOD activity in *Oreochromis niloticus* L. fingerlings exposed to 96 hrs median lethal concentration (Abu-Zeid and Khalil, 2014). Suneetha (2014) reported stimulation in activity of SOD in the liver *L. rohita* exposed to two pesticides viz. organochlorine (endosulfan) and pyrethroid (fenvalerate).

***Conclusion*:**The present results suggest that the pesticides are highly toxic to aquatic animals when in combined form. The 96-hr LC_{50} and lethal responses of all three fish species to BIF+CPF+END mixture showed statistically significant differences. Among fish species *C. catla* was significantly more sensitive toward tertiary mixture. It was also concluded that the acute exposure of pesticides mixture alter the antioxidant activity in the liver of fish and these antioxidants activity may be used as biomarker of oxidative stress and for pollution monitoring.

REFERENCES

Abu-Zeid, E.H. and A.L.S.A. Khalil. 2014. Effects of acute fenitrothion insecticide exposure on DNA damage and oxidative stress biomarkers and health of Nile tilapia fingerlings, *Oreochromis niloticus*L. World J. Fish Mar. Sci. 64:361-370.

Al-Rudainy, A.J. and M.H. Kadhim. 2012. Hematological and neurotoxic effects of endosulfan pesticide on common carp *Cyprinuscarpio*. The Iraqi J. Vet. Med. 36:58-67.

Ambreen, F. and M. Javed. 2015. Assessment of acute toxicity of pesticides mixtures for *Cyprinuscarpio*and *Ctenopharyngodonidella*. Pak. J. Zool. 47:133-139.

A.P.H.A. 1998. Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association, New York.

Barbieri, E. and L.A.A. Ferreira. 2011. Effects of the organophosphate pesticide Folidol 600 on the freshwater fish, Nile tilapia (*Oreochromis niloticus*). Pestic. Biochem. Physiol. 99:209-214.

Belazutshi and S.G. Raghuprasad. 2008. Impact of pollution on fresh and marine water resources. J. Poll. Res. 27:461-466.

Burr, S.A. and D.E.Ray. 2004. Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. Toxicol. Sci. 77:341-346.

Ciccheti, R. and R.G. Argentin. 2003. The role of oxidative stress in the in vitro induction of micronuclei by pesticides in mouse lung fibroblasts. Mutagenesis 18:127-132.

E.J.F. 2002. End of the road for endosulfan: A call for action against a dangerous pesticide. Environmental Justice Foundation Ltd., London, UK.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutase occurrence in higher plants. Plant Physiol. 59:309-314.

Goulding, A.T., L.K. Shelley, P.S. Ross and C.J. Kennedy. 2013. Reduction in swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) following sublethal exposure to pyrethroid insecticides. Comp. Biochem. Physiol. Part C. 157:280-286.

Gultekin, F., M. Ozturk and M. Akdogan. 2000. Effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in vitro*). Arch. Toxicol. 74:533-538.

Hamilton, M. A., R.C. Russo and R.V. Thurston. 1977. Trimmed spearmen-Karber method for media lethal

concentration in toxicity bioassay. Environ. Sci. Technol. 11:714-719.

Hinton, D.E., P.C. Baumann, G.R. Gardner, W.E. Hawkins, J.D. Hendricks, R.A. Murchelano and M.S. Okihiro. 1990. Histopathologic biomarkers. Biochemical, physiological, and histological markers of anthropogenic stress. In: Hugget R., Kimerle R., Merles P. & Bergman H. (Eds), Biomarkers. Lewis Publishers, Boca Raton; pp.155-209.

Ilyas, R. and M. Javed. 2013. Acute toxicity of endosulfan to the fish species *Catlacatla*, *Cirrhinamrigala*and *Labeorohita.* Int. J. Agric. Biol. 15:149-152.

Isik, I. and I. Celik. 2008. Acute effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in various tissues of rainbow trout (*Oncorhynchus mykiss*). Pestic. Biochem. Physiol. 929:38-42.

Karra, S., P.V.V. Satish, K. Sunita and N.B. Jyothi. 2015. Studies on the biochemical responses in the tissues of freshwater fish *Labeorohita*exposed to the organophosphate, phenthoate. Int. J. Pharm. Sci. Rev. Res. 32:108-115.

Kohen, R. and A. Nysk. 2002. Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. ToxicolPathol. 30:620-650.

Maruthanayagam, C. and G. Sharmila. 2004. Haematobiochemical variations induced by the pesticide, monocrotophos in *Cyprinuscarpio*, during the exposure and recovery periods. Nat. Environ. Poll. Tech. 3:491-494.

Mate, J.M. 2000. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 153:83-104.

Mohamed, F.A.S. 2009. Histopathological studies on Tilapia zilliiand *Soleavulgaris*from Lake Qarun, Egypt. World J. Fish Mar. Sci. 1:29-39.

Oruc, E.O., Y. Sevgiler and N. Uner. 2004. Tissue-specific oxidative stress responses in fish exposed to 2, 4-D and azinphosmethyl. Comp. Biochem. Physiol. 137: 43-51.

Palma, P., V.L. Palma, R.M. Fernandes, A.M.V.M. Soares and I.R. Barbosa. 2009a. Endosulfansulphate interferes with reproduction, embryonic development and sex differentiation in *Daphnia magna*. Ecotoxicol. Environ. Saf. 72:344-350

Palma, P., V.L. Palma, C. Matos, R.M. Fernandes, A. Bohn, A.M.V.M. Soares and I.R. Barbosa. 2009b. Assessment of the pesticides atrazine endosulfansulphate and chlorpyrifos for juvenoid-related endocrine activity using *Daphnia magna*. Chemosphere 76:335-340

Palma, P., V.L. Palma, R.M. Fernandes, A.M.V.M. Soares and I.R. Barbosa. 2008. Acute toxicity of atrazine, endosulfansulphate and chlorpyrifos to *Vibrio fischeri*, *Thamnocephalusplatyurus* and *Daphnia magna*, relative to their concentrations in surface waters from the Alentejo region of Portugal. Bull. Environ. Contam. Toxicol. 81:485-489.

Pandey, S., N.S. Nagpure, K. Ravindra, S. Sharma, S.K. Srivastava and M.S. Verma. 2006. Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost *Channa punctatus* (Bloch) by alkaline single-cell gel electrophoresis. Ecotoxicol. Environ. Saf. 65:56-61.

Pandey, S., R. Kumar, S. Sharma, N.S. Nagpure, S.K. Srivastava, M.S. Verma. 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus* (Bloch). Ecotoxicol. Environ. Saf. 61:114-120.

Pyle, G. and C. Wood. 2008. Radiotracer studies on waterborne copper uptake, distribution, and toxicity in rainbow trout and yellow perch: A comparative analysis. Hum. Ecol. Risk Assess. 14:243-265.

Schjolden, J., J. Sorensen, G.E. Nilsson and A.B.S. Poleo. 2007. The toxicity of copper tocrucian carp (*Carassiuscarassius*) in soft water. Sci. Total Environ. 384:239-251.

Sharbidre, A.A., V. Metkari and P. Patode. 2011. Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, *Poecilia reticulate.*Pestic. Biochem. Physiol. 10:132-141.

Sharma, Y., S. Bashir, M. Irshad, S.D. Gupta and T.D. Dogra. 2005. Effects of acutedimethoate administration on antioxidant status of liver and brain of experimental rats. Toxicology 206:49-57.

Sparling, D.W. and G. Fellers. 2007.Comparative toxicity of chlorpyrifos,diazinon, malathion and their oxon derivatives to larval *Rana boylii*. Environ. Pollut. 147:535-539.

Suneetha, K. 2014. Toxic effect of endosulfan and fenvalerate on oxidative stress biomarkers and lipid peroxidation in freshwater fish *Labeorohita*(Hamilton). Int. J. Biol. Pharm. Res. 5:156-160.

Svoboda, M., V. Luskova, J. Drastichova and V. Zlabek. 2001. The effect ofdiazinon on haematological indices of common carp (*Cyprinuscarpio*L). Acta.Vet. Brno. 70:457-465.

Tiwari, P. and B.A. Ansari. 2014. Comparative study of acute toxicities of endosulfan, chlorpyrifos and permethrin to zebrafish, *Danio rerio*(Cyprinidae). Sch. Acad. J. Biosci. 2:404-409

Ullah, S., Z. Hasan and K. Dhama. 2016. Toxic effect of endosulfan on behavior, protein contents and antioxidant enzyme system in gills, brain, liver and muscle tissues of rohu, *Labeorohita*. Int. J. Pharmcol. 12:1-10.