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Research Article

Purification and Kinetic Characterization of Hepatic Catalase from Carnivore Fish, *Channa striata* Exposed to Agrochemicals (endosulfan+deltamethrin)

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FT performed the research and wrote the article. SA planned and supervised the study. HN helped in statistical anlyses. KA provided lab facilities for research. LS helped in writing the article.

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Antioxidant enzymes, Pesticides, Partial purification, Toxicants, Fish

Abstract | From last few years contamination of aquatic bodies has been increased due to extensive use of pesticides in agriculture. The exposure of aquatic animals to these pesticides results in acute and chronic health effects. Fresh water fish species such as *Channa striata* serve as important bio-indicators for aquatic contamination to access the changes caused by human activities effectively and reliable monitoring bio-system to recognize and predict hazardous effects of pollutants. Therefore, this study was done to evaluate the toxicity of endosulfan (END), deltamethrin (DM) mixture on hepatic catalase (CAT) activity of fish, *Channa striata* exposed to the sub-lethal concentration (1/3rd of LC_{50}) for 14 days. The CAT was partially purified by using ammonium sulphate precipitation technique. Results of partial purification demonstrated that the exposure of insecticides mixture decreased the hepatic CAT activity in fish (43.33±1.41 UmL^{-1}) as compare to control (50.33±1.41 Unit mL^{-1}). After desalting specific activity of hepatic CAT remain lowest in exposed fish as 134.30±0.71 Unit mg^{-1}in relation to control (148.02±0.41 Umg^{-1}). The fold purification of hepatic CAT from control and exposed *C. striata* was noted as 2.35±0.01 and 2.22±0.01, respectively. The percentage recovery of hepatic CAT from control and exposed *C. striata* was calculated as 81.77±1.41and 69.94±0.71, respectively. Results of kinetic characterization showed that optimum pH and temperature for hepatic CAT was noted as 7.5 and 25°C, respectively. It was concluded that the insecticides mixture exposure can alter the activity of antioxidant enzymes in fish. T
hese parameters can be used as a good biomarker to evaluate the aquatic pollution.

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Introduction

Pesticides and their metabolites released from industries and agricultural practices pose serious threat to aquatic life. Aquatic animals, particularly fishes are greatly susceptible to these toxic chemicals because they can amass in fish organ and transferred to humans through food chain by the process of bio-magnification.

The toxicity of pesticides to fish depends upon different factors such as biochemical processes of organisms and chemical nature of toxic compounds (Jaroli and Sharma, 2005). Different abiotic factors such as pH and temperature may alter the activities of enzymes (Ugolev and Kuzmina, 1993). Temperature is one of the important stressing factors for fish because it strongly affects the metabolic rate inpikilothrems (Clarke and Fraser, 2004).

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Endosulfanis a persistent organochlorine insecticide and poses severe threats to the aquatic animals (Tripathi and Verma, 2004) that include respiratory changes, physiological, biochemical and molecular alterations result in tissue damage (Dar *et al.*, 2015); for instance, it can also change the metabolism in fishes even lead to death in some cases (Tripathi *et al.*, 2004).

One of thesynthetic type two pyrethroid insecticides deltamethrin, has been largely used in agriculture and industrial sector and proved to be extremely toxic to aquatic animals including fish (Ural and Saglam, 2005; Koprucu *et al.*, 2006) under laboratory conditions.

Many classes of pesticides may provoke the formation of reactive oxygen species (ROS) and induce oxidative stress (Elia *et al.*, 2002; Sayeed *et al.*, 2003). To neutralize the toxic effect of ROS fishes have antioxidant defense enzymes like superoxide dismutase, catalase and glutathione peroxidase (Orbea *et al.*, 2002). These enzymes could save the fish's tissues from oxidative damage under normal conditions and can be used as sensitive biomarkers of oxidative stress in fish exposed to aquatic toxicants (Ahmad *et al.,* 2000). Most of the available literature deals with the effects of single pesticides to fish butthere is no information regarding to the effects of pesticides in mixture form. Therefore, current work was conducted to assess the effect of insecticides mixture (deltamethrin+endosulfan) on catalase activity in hepatic tissue of *Channa striata*.

Materials and Methods

Experimental fish and conditions

Carnivorous fish, *Channa striata* called "snakehead murrel", were collected from natural breeding grounds and shifted to the Toxicology laboratory at Fisheries Research Farm, University of Agriculture Faisalabad. *C.striata* were acclimatized to laboratory condition for 14days and then fish were moved to 100-L glass aquaria each containing a group of fish (n=10). The 96-hr LC_{50} of insecticides, deltamethrin (DM)+endosulfan (END) mixture for *C.striata* was 1.374 μgL^{-1} as calculated by Abdullah (2018). Fishes were exposed to the sub-lethal concentration (1/3rdof LC_{50}) of DM+END mixturefor 14 days.

Isolation and partial purification of CAT

For isolation of enzyme extract, hepatic tissues wereweighted and phosphate buffer (pH 6.5) added by the ratio of 4:1 (w/v). The sample was homogenized for 15 minutes by using pestle and mortar.Tissues homogenatewas filtered and centrifuged in refrigerator centrifugal machine at 10,000 rpm for 15 minutes.Both sediments and supernatants were separated for further analysis. The extracted CAT (crude) was then partially purified by ammonium sulphate precipitation using the method of Shin *et al.* (1993). Ammonium sulfate precipitationwas performed in two steps including Salting-in (60%) and Salting-out (80%) steps. Precipitated enzyme sample obtained through salting-out process in residue form was dialyzed against low ionic strength phosphate buffer (pH 7.4) with the help of dialysis bag. All of the obtained sample supernatants, sediments, and desalting sample were subjected to enzyme essay and protein contents estimation. Catalase activity was determined by following the method of Chance and Mehaly (1977) at 240 nm.

Estimation of protein contents

Protein contents of the sample were estimated by using Biuret method (Gornall *et al.*, 1949).

Kinetic characterization of CAT

Optimum pH was determined by assaying the purified CAT from hepatic tissues of wild *C.striata* at different pH ranging from 4-12 (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5 and 12.0).To obtain optimum temperature for purified hepatic CAT of *C.striata* was assaying at different temperatures ranging from 5-50^{o}C (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50) keeping the pH at which purified catalase showed highest activity (Nakamura *et al.*, 2000; Al-Bar, 2012).

Statistical analysis

Data obtained in this study were represented as Mean Standard Deviation (Mean±SD). ANOVA was applied to calculate statistical difference ($p<0.05$) in CAT activity between both control and exposed group (Steel *et al.*, 1997). MS excel was used to draw the graph.

Results

Activity of hepatic CAT

The result showed that lowest CAT activity was calculated in hepatic tissue to END+DM exposed *C. striata* (91.62±0.71 UmL^{-1}) in comparison to crude extract control(97.56±1.41 UmL^{-1}). Desalting result also confirmed that CAT activity was the lowest in END+DM exposed fish hepatic tissue as compared to controlled. The result also showed that enzyme activity decreased gradually from crude extract to desalted sample (Figure 1).

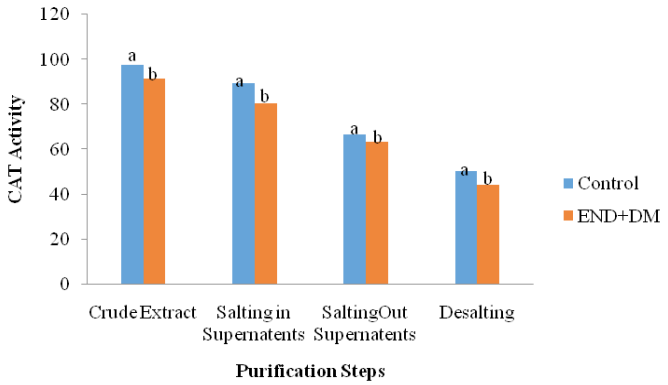

Figure 1: Partial Purification of hepatic CAT activity (Unit mL^{-1}) in *C. striata* exposed to END+DM

Specific activity of hepatic CAT

The lowest hepatic CAT specific activity was calculated inexposed *C. striata* (35.23±0.71 Unit mg^{-1}) as compared to control (37.52±1.41 Unit mg^{-1}). After desalting specific activity of hepatic CAT remain lower in exposed fish as 134.30±0.71 Unit mg^{-1} in relation to control (148.02±0.41 Unit mg^{-1}). Results also indicated that the activity and specific activity is antagonistic to each other at each step of partial purification of enzyme (Figure 2).

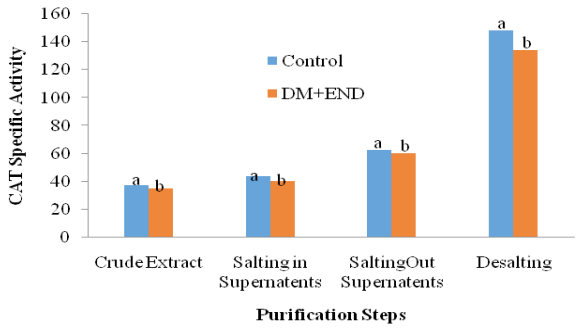

Figure 2: Partial Purification of hepatic CAT specific activity (Unit mg^{-1}) in *C. striata* exposed to END+DM

Total protein contents, Fold purification and Percent recovery

Total protein contents were significantly lower in hepatic tissues of END+DM exposed fish when compared with control**.** The highest fold purification was calculated in hepatic tissues of control *C. striata* (2.35±0.01) as compared to the exposed fish (2.22±0.01). Hepatic CAT in control *C.striata* had highest percent recovery as 81.77±1.41% as compared to stressed (69.94±0.71%). It was noted that total protein contents and percentage recovery decreased after every step of partial purification while fold purification was increased (Table 1).

Table 1: Partial purification of hepatic CAT in *C. striata* by using ammonium sulfate precipitation

Parameters	Precipitation Steps	Control	END+DM
Protein (mg mL^{-1})	Crude extract	2.65±0.01A[a]	2.60±0.00B[a]
	Salting in	2.03±0.01A[b]	1.98±0.01B[b]
	Salting out	1.06±0.01A[c]	1.05±0.01B[c]
	Desalting	0.34±0.01A[d]	0.33±0.03B[d]
Fold purification	Crude extract	1.00±0.02A[d]	1.00±0.02B[d]
	Salting in	1.18±0.01A[c]	1.15±0.01B[c]
	Salting out	1.42±0.03A[b]	1.25±0.01B[b]
	Desalting	2.35±0.01Aa	2.22±0.01B[a]
% recovery	Crude extract	100.00±2.12 A[a]	100.00±1.41 B[a]
	Salting in	91.84±1.14A[b]	87.55±0.71B[b]
	Salting out	85.96±0.71A[c]	84.89±1.12B[c]
	Desalting	81.77±1.41A[d]	69.94±0.71B[d]

Capital letters represent the significant (P<0.05) difference at different steps of purification within the same column while small lettered superscripts show the difference between treatments within the same row.

Kinetic characterization

The activity of partially purified hepatic CAT increased as pH and temperature increased. The optimum pH and temperature for hepatic CAT was noted as7.5 and 25°C, respectively (Figure 3-4).

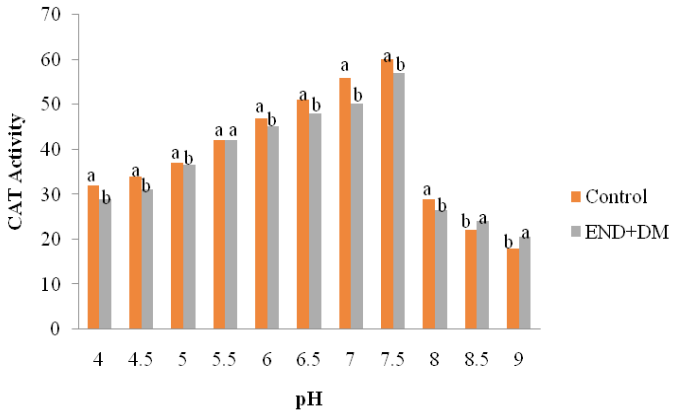

Figure 3: Effect of different pH on hepatic CAT activity (Unit/mL) in *C. striata* exposed to pesticides mixture

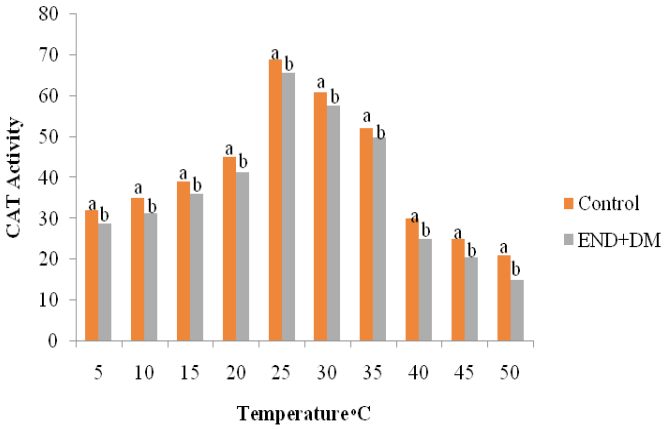

Figure 4: Effect of different temperature on hepatic CAT activity (Unit/mL) in *C. striata* exposed to pesticides mixture

Discussion

Results of present study showed that the CAT activity in hepatic tissue of exposed fish was decreased in contrast to control. Stara *et al.* (2013) reported the lower level of CAT in liver of *Cyprinuscarpio* exposed to zeta-cypermethrin in relation to control. Ahmed *et al.* (2016) reported higher liver CAT activity in control fish as compared to binary metal mixture treated *O. niloticus*. Salvo *et al.*(2012) recorded the decreased CAT activity in liver of common carp under sub-lethal exposure of endosulfan. Hamed (2016) also recorded the decreased liver CAT activity in the African catfish (*Clariasgariepinus*) under deltamethrin

exposure. These findings are also in accordance to Sayeed *et al.* (2003) who reported decline of hepatic CAT activity in *Channa punctatus* exposed to deltamethrin .Lihocin exposure gradually decreased CAT level in liver of *Catlacatla* (Vineela and Reddy, 2014).Thenmozhi *et al.* (2011) observed decreased CAT in the liver of *Labeorohita* exposed tomalathion. Significant decrease in liver CAT of *Channa punctatus* exposed to pyrethrioid insecticides was reported by Triphti and Sing (2013). Suneeth (2014) recorded significant inhibition in liver CAT of *Labeorohita* under exposure of endosulfan and fenvalerate when compared to control.Deltamethrin exposure caused depletion in hepatic CAT levels of tilapia (Abdelkhalek *et al.*, 2015). Depletion in hepatic CAT level of catfish upon exposure to malathion was reported by Hamed (2015). Cypermethrin decreased liver CAT in *Tor putitora* (Ullah, 2014). Dinu *et al.* (2010) recorded temporary inhibition in liver CAT activity of *Carassius auratus gibelio* exposed to deltamethrin. Rana *et al.* (2018) also reported the decreased CAT activity in C*hannas triata* exposed to sub-lethal dose of DM+END.

The existence of toxicants significantly affects the antioxidant enzymes (catalase) because toxicants can bind with site of catalase and inhibit its activity. The inhibition in catalase activity is may be due to direct binding of toxicants to thiol (-SH) groups of enzyme which consequently increased the ROS (Faheem *et al.*, 2012).

The unit of enzyme in one milligram of protein is known as specific activity of enzyme and it is an indicator of purification of enzyme. In present work, specific activity of CAT increased as from crude extract to dialysis. Specific activity in hepatic tissue of exposed fish decreased as compared to control. Ahmed *et al.* (2016) also recorded the decreased specific activity of purified hepatic CAT in stressed fish as compared to control.

In present study, it was noted that total protein contents and percentage recovery decreased after every step of partial purification while fold purification was increased. Some authors reported the similar results for percent recovery and fold purification of CAT in different fish species (Sarwar, 2013; Akram, 2014; Ahmed *et al.*, 2016; Rana *et al.*, 2018).

Optimum temperature and pH for hepatic catalase of *Channa striata* was noted as 25°C and 7.5, respectively. Ahmed *et al.* (2016) noted the optimum pH and temperature as found 7 and 25°C, respectively for purified CAT. Tariq (2013) obtained similar results in *C.mrigala,* Sarwar (2013) in *C.idella* and Akram (2014) in *C.catla.* Rana *et al.* (2018) also reported the maximum activity of purified CAT was recorded at pH 6.0 and 30°C temperature for *C. striata*.

Conclusion and Recommendations

This research work concluded that the insecticides mixture exposure could alter the antioxidant enzymes activity in fish. The biochemical technique such as partial purification is a simple and convenient method to purify the catalase and can be successfully used for evaluating the water pollution. It was also concluded that the other environmental factors such as pH and temperature could also affect the catalase activity in fish.

References

Abdelkhalek, N.K.M., Ghazy, E.W. and Abdel-Daim, M.M., 2015. Pharmacodynamic interaction of *spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: Impact on lipid peroxidation and oxidative stress. *Environ. Sci. Pollut. Res. Int.*, **22:** 3023-3031. https://doi.org/10.1007/s11356-014-3578-0

Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S. and Rezaie, A., 2004. Pesticides andoxidative stress: A Review. *Med. Sci. Monit.*, **6:** 141-147.

Ahmad, I., Hamid, T., Fatima, M., Chand, H.S., Jain, S.K., Athar, M. and Raisuddin, S., 2000. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. *Biochim. Biophys. Acta.*, **1523:** 37–48. https://doi.org/10.1016/S0304-4165(00)00098-2

Ahmed, T., Abdullah, S., Abbas, K., Zia, M.A., Nazish, N., Shah, S.Z.H. and Naqvi, S.M.A., 2016. Purification and partial characterization of liver catalase from control and Pb+Cd metal mixture stressed *Oreochromis niloticus. Pak. J. Agri. Sci.,* **53:** 431-437. https://doi.org/10.21162/PAKJAS/16.3256

Akhgari, M., Abdollahi, M., Kebryaeezadeh, A., Hosseini, R. and Sabzevari, O., 2003. Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Hum. Exp. Toxicol.*, **22:** 205-211. https://doi.org/10.1191/0960327103ht346oa

Akram, H., 2014. Purification and characterization of liver and kidney catalase enzyme in wild and farmed *Catlacatla*. M .Phil. Thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Al-Bar, O.A.M., 2012. Characterization of partially purified catalase from camel (*Camelus dromedarius*) liver. *Afr. J. Biotechnol.*,**11:** 9633-9640. https://doi.org/10.5897/AJB12.850

Abdullah, S., Mateen, A., Abbas, K., Naz, H., Hassan, W. and Anum, S., 2018. Changes in antioxidant enzyme (Glutathione S-transferase) activity in fish

Channa Striata exposed to the different aquatic pollutants (heavy metals and pesticides mixture). *Pak. J. Zool. Suppl. Ser.,* **13:** 42-47.

Chance, M. and Mehaly, A.C., 1997. Assay of catalase and peroxidase. *Methods Enzymol.,* **2:** 764-817.

Clarke, A. and Fraser, K.P.P., 2004. Why does metabolism scale with temperature? *Funct. Ecol.,* **18:** 243-251. https://doi.org/10.1111/j.0269-8463.2004.00841.x

Dar, S.B., Yousf, A.R., Balkhi, M.H., Ganai, F.A. and Bhat, F.A., 2015. Assessment of endosulfan induced genotoxicity and mutagenicity manifested by oxidative stress pathway in fresh water cyprinid fish crucian carp (*Carassius carassius*). *Chemosphere.,* **120:** 273-283. https://doi.org/10.1016/j.chemosphere.2014.07.031

Dinu, D., Marinescu, D., Munteanu, M.C., Staicu, A.C., Costache, M. and Dinischiotu, A., 2010. Modulatory effects of deltamethrin on antioxidant defense mechanisms and lipid peroxidation in *Carassius auratus gibelio* liver and intestine. *Arch. Environ. Contam. Toxicol.,* **58:** 757-764. https://doi.org/10.1007/s00244-009-9401-0

Elia, A.C., Waller, W.T. and Norton, S.J., 2002. Biochemical responses of Bluegill sunfish (*Lepomismacrochirus*, rafinesque) to atrazine induced oxidative stress. *Bull. Environ. Contam. Toxicol.,* **68:** 809-816. https://doi.org/10.1007/s00128-002-0027-4

Faheem, M., Sulehria, A.Q.K., Tariq, M., Khadija, I., Fiaz, A. and Saeed, M., 2012. Effect of sub-lethal dose of cadmium chloride on biochemical profile and catalase activity in fresh water fish *Oreochromis niloticus. Biologia Pak.,* **58:** 73-78.

Gamelin, F.X., Baquet, G., Berthoin, S., Thevenet, D., Nourry, C., Nottin, S. and Bosquet, L., 2009. Effect of high intensity intermittent training on heart rate variability in prepubescent children. *Eur. J. Appl. Physiol.,* **105:** 731-738. https://doi.org/10.1007/s00421-008-0955-8

Gornal, A.G., Bardwill, C.S. and David, M.M., 1994. Determination of serum proteins by means of biuret reaction. *J. Biol. Chem.,* **177**: 751-766.

Halliwell, B. and Gutteridge, J.M.C., 2007. Free Radicals in Biology and Medicine. Oxford University Press, Oxford.

Hamed, H.S., 2015. Vitamin E attenuated the oxidative stress and biochemical changes induced by endosulfan in female catfish (*Clarias gariepinus*). *World J. Fish. Marine Sci*., **7:** 313-324.

Hamed, H.S., 2016. Ameliorative effects of *Spirulina platensis* on deltamethrin-induced biochemical alterations and oxidative stress in the African catfish; *Clarias gariepinus. Open J. Marine Sci.,* **6:** 1-10. https://doi.org/10.4236/ojms.2016.61001

Jaroli, D.P. and Sharma, B.L., 2005. Effect of organophosphate insecticide on the organic constituents in liver of *Channa punctatus. Asian J. Exp. Sci.,* **19:** 121-129.

Koprucu, S.S., Koprucu, K. and Ural, M.S., 2006. Acute toxicity of the synthetic pyrethroid deltamethrin to fingerling European catfish, *Silurusglanis*. Bull. Environ. Contam. Toxicol., **76:** 59-65. https://doi.org/10.1007/s00128-005-0889-3

Martinez-Alvarez, R.M., Morales, A.E. and Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. *Rev. Fish Biol. Fish.,* **15:** 75–88. https://doi.org/10.1007/s11160-005-7846-4

Nakamura, K., Watanabe, M., Sasaki, Y. and Ikeda, T., 2000. Purification and characterization of liver catalase in a catalasemic beagle dog, comparison with normal dog liver catalase. *Int. J. Biochem. Cell. Biol.,* **32:** 89-98. https://doi.org/10.1016/S1357-2725(99)00110-7

Orbea, A., Ortiz-Zarragoitia, M., Sole, M., Porte, C. and Cajaraville, M.P., 2002. Antioxidantenzymes and peroxisome proliferation in relation to contaminant body burdens of PAHs and PCBs in bivalve molluscs, crabs and fish form the Urdaibai and Plentzia estuaries (Bay of Biscay). *Aquat. Toxicol.,* **58:** 75-98. https://doi.org/10.1016/S0166-445X(01)00226-0

Rana, A., Abdullah, S., Naz, H. and Abbas, K., 2018. Characterization of catalase form carnivorous fish *Channa striata*exposed to binary insecticides mixture (deltamethrin+endosulfan). *Pak. J. Agri. Res.,* **31:** 368-374. https://doi.org/10.17582/journal.pjar/2018/31.4.368.374

Ratushnyak, A. andreeva, M.G. and Trushin, M.V., 2005. Effects of type II pyrethroids on*Daphnia magna*: Dose and temperature dependences. *Riv. Biol.,* **98:** 349-357.

Salvo, L.M., Bainy, A.C.D., Ventura, E.C., Marques, M.R.F., Silva, J.R.M.C., Klemz, C.U. and Silva De-Assis, H.C., 2012. Assessment of the sublethal toxicity of organochlorine pesticide endosulfan in juvenile common carp (*Cyprinus carpio*) *J. Environ. Sci. Health.,***47:** 1652-1658. https://doi.org/10.1080/10934529.2012.687236

Sarwar, U., 2013. Purification and partial characterization of liver catalase enzyme from control and Pb+Cd metal mixture stressed grass carp, *Ctenopharyngodon idella*. M.Sc. Thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R. and Raisuddin, S., 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, (Bloch). *Ecotoxicol. Environ. Safe.,* **56:** 295- 301. https://doi.org/10.1016/S0147-6513(03)00009-5

Shin, K.S., Youn, H.D., Han, Y.H., Kang, S.O. And Hah, Y.C., 1993. Purification and characterization

of D-glucose oxidase from white-rot fungus *Pleurotusostreatus*. *Eur. J. Biochem.,* **215:**747-752. https://doi.org/10.1111/j.1432-1033.1993.tb18088.x

Stara, A. Steinbach, C., Wlasow, T., Gomulka, P., Ziemok, E., Machova, J. and Velisek. J., 2013. Effect of zeta-cypermethrin on common carp (*Cyprinuscarpio* L.). *Neuroendocrinol. Lett.,* **34:** 37-42.

Steel, R.G.D., Torrie, J.H. and Dickey, D., 1997. Principles and Procedure of Statistics. A biometrical approach 3rd Ed. McGraw Hill Book Co. Inc., New York, pp. 352-358.

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.

Tariq, M., 2013. Studies on the characterization of liver catalase in copper exposed *Cirrhinamrigala*. M.Sc. Thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan.

Thenmozhi, C., Vignesh, V., Thirumurugan, R. and Arun, S., 2011. Impacts of malathion on mortality and biochemical changes of fresh water fish *Labeorohita. Iran. J. Environ. Health. Sci. Eng.,* **8:** 325-332.

Tripathi, G. and Singh, H., 2013. Impact of alphamethrin on biochemical parameters of *Channa punctatus*. *J. Environ. Biol.,* **34:** 227-230.

Tripathi, G. and Verma, P., 2004. Endosulfan-mediated biochemical changes in the freshwater fish *Clariasbatrachus*. *Biom. Environ. Sci.,* **17:** 47-56.

Ugolev, A.M. and Kuzmina, V.V., 1993. Pishchevaritelnye protsessy I adaptatsii ryb (Digestive process and adaptations in fish), St.-petersburg, Gidrometeoizdat.

Ullah, R., Zuberi, A., Ullah, S., Ullah, E. and Dawar, F.U., 2014. Cypermethrine induced behavioral and biochemical changes in masheer, *Tor putitora*. *J. Toxicol. Sci.,* **39:** 829-836. https://doi.org/10.2131/jts.39.829

Ural, M.S. and Saglam, N., 2005. A study on the acute toxicity of pyrethroid deltamethrinon the fry rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Pestic. Biochem. Physiol.,* **83:**124-131. https://doi.org/10.1016/j.pestbp.2005.04.004

Vineela, D. and Reddy, S.J., 2014. Impact of lihocin on immunohaematological and antioxidant enzyme indices of carp fish. *Int. J. Pharm. Life Sci.,* **5:** 3517-3525.

Windisch, S.H., Kathover, R., Portner, H.O., Frickenhaus, S. and Lucassen, M., 2011. Thermal acclimation in Antarctic fish: transcriptomic profiling of metabolic pathways. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **301:** 1453-1466. https://doi.org/10.1152/ajpregu.00158.2011