Pak. J. Agri. Sci., Vol. 56(5), 1287-1291; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/20-7276
http://www.pakjas.com.pk

PARTIAL PURIFICATION AND KINETIC CHARACTERIZATION OF RENAL CATALASE FROM CARNIVOROUS FISH, *Channastriata* EXPOSED TO PESTICIDES MIXTURE,DELTAMETHRIN+ENDOSULFAN

Fatima Tufail[1], Sajid Abdullah[1], Huma Naz[2,*], Khalid Abbas[1], Tanveer Ahmed[3] and Muhammad Umar Ijaz[1]

Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad[1];Department of Zoology, Cholistan University of Veterinary and Animal Sciences Bahawalpur[2], Department of Life Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Kha[3], Pakistan.
[*]Corresponding author's e-mail: dr.humanaz98@gmail.com; humanaz@cuvas.edu.pk

The current work was accomplished to purify the renal catalase (CAT) from carnivorous fish, *Channa striata* exposed to sub-lethal dose (1/3rdof LC_{50}) of deltamethrin (DM)+endosulfan(END) mixture. Fish was kept under control and experimental conditions for 14-days. Water quality characteristics like temperature, total hardness and pH were kept stabilized as 30°C, 250 $mgL^{-1}$and 7.5, respectively. The biochemical technique Ammonium sulphate precipitation was used for partial purification of CAT. In Kinetic characterization optimum pH and temperature was determined for CAT. Spectrophotometer was used to analyze activity of enzyme. Results of isolation showed that activity of CAT was lowest in crude extract of DM+END exposed *C. striata* renal tissue as compared to control. Similarly, specific activity of CAT was also lowest in crude extract of exposed *C. striata* renal tissue as compared to control. As a result of partial purification of renal CAT, lowest specific activity was observed in exposed *C. striata* (80.94±0.00 Umg^{-1}) than control (88.97±1.41 Umg^{-1}).The fold purification of renal CAT from exposed and control fish was calculated as 1.24±0.00 and 1.28±0.01, respectively. The percentage recovery was about 58.07±1.41and 63.23±1.41 % for exposed and control *C. striata*, respectively. Kinetic characterization of CAT showed that the maximum activity was observed at pH 6.5 and temperature 30°C.
Keywords: Carnivorous fish, chronic exposure, insecticides mixture, catalase.

INTRODUCTION

Extensive use of pesticides in agriculture practices from last few years leads to increase in pollution of pesticides and their metabolites in aquatic ecosystems, raising serious environmental concerns (Fung and Mak, 2001).Aquatic animal's response to these pollutants frequently induces alterations at cellular and biochemical levels, primarily structural and functional changes in the cells, organs, physiology and behavior of the individual (Parvez and Raisuddin, 2005). So, aquatic animals are unavoidably exposed to a number of pesticides which are different in structure and toxicity. Several authors have been reported the impact of individual pesticides on fish (Joseph and Raj, 2011) while the data on toxicity of pesticides mixtures is comparatively less studied. Therefore, more research is required to understand their interactions on the living organisms and to assess the risks associated with them.

Among all the classes of pesticides, both organochlorine and pyrethroid insecticides are widely used, overlapping in soy-bean crops (Bacchetta *et al.*, 2014). Endosulfan is a chlorinated cyclodienes, reported as highly toxic to a broad range of aquatic organisms. Deltamethrinbelongs to class pyrethroids pesticides. The fish show several symptoms of stress when exposed to the deltamethrin (Datta and Kaviraj, 2003). Many classes of pesticides or their metabolites can stimulate the reactive oxygen species (ROS) formation and induce oxidative stress and cause oxidative damage in fish (Abdollahi *et al*., 2004;Velisek *et al*., 2012).

To overcome the toxicity of ROS, fish possessed an antioxidant defenses system includes superoxide dismutase, catalase and glutathione reductase (Orbea *et al*., 2002). Catalase is produced in peroxisome and disposed harmful free radicals, hydrogen peroxide (H_2O_2) (VijayaKumar *et al*., 2016).These antioxidants save the organs from oxidative injury under normal conditions. The antioxidants can serve as sensitive oxidative stress biomarkers in fish and can also detect aquatic toxicants (Ahmad *et al.*, 2000) and provide useful information about the eco-toxicological effects of pesticides. Some other factors (pH and temperature) may also cause the modifications in enzymes activities. Most important one is the temperature. It strongly affects the metabolic rate of fish (Clarke and Fraser, 2004). The current work was performed to elucidate the toxic effect of pesticides mixture on catalase activity in renal tissue of carnivorous fish, *Channa striata*.

MATERIALS AND METHODS

***Test Animal and Trail*:**Freshwater carnivorous fish, *Channa striata* were used for this experiment. C*hanna striata* were collected from natural breeding groundsandlive transfer to the wet laboratory at UAF. *Channa striata* were acclimatized to laboratory environment for few days and then transferred to 100-L glass aquaria. Each aquarium was filled with ten fishes. Abdullah *et al.* (2018) calculated the 96-hr LC_{50} value ($1.374 \mu g L^{-1}$) of deltamethrin(DM)+endosulfan(END) mixture for *C. striata*. Sub-lethal dose (1/3rdof LC_{50}) of DM+END was used to expose the fishes for 14-days. Water quality characteristics like temperature, total hardness and pH were kept stabilized as 30°C, 250 $mgL^{-1}$and 7.5, respectively.
***Isolation of tissue Enzyme Extract*:**Renal tissue was homogenizedinphosphate buffer of pH 6.5by the ratio of 4:1(w/v). Homogenate was filter and filtrate was centrifuged in refrigerator centrifugal machine at 10,000 rpm for 15 minutes.
***Partial Purification of CAT*:**Zia *et al*. (2007) method was applied to partially purify the crude CAT. The purification technique ammonium sulfate precipitation included Salting inland Salting outsteps.
***SaltingIn Method*:** Crude extract was saturated with ammonium sulfate (60%) by dissolving 42 g in 100 mLof enzyme andrefrigerated for 4 hrs at 4°C. After 4 hrs it wascentrifuged for 15 minutes at 10,000 rpmand4°C toobtain the supernatants and residues.
***Salting Out Method*:** The obtained supernatant was saturated up to 80% ammonium sulfate by adding 56 g/100 mL of enzyme and kept it at 4°C over night. The sample was centrifuged at 10,000 rpm for 15 minutes to obtain the supernatant and residue. One ml of phosphate buffer (pH 6.5) was added in residue.
***Residue Desalting*:**Precipitated enzyme sample obtained through salting out process was dialyzed against low ionic strength phosphate buffer (pH 7.4).
Enzyme Assay: Chance and Mehaly (1977) protocol was followed to check the CAT activity.
***Determination of Protein Contents*:** Biuret method proposed by Gornall *et al.* (1949) was used to evaluate the protein contents of sample.
Estimation of specific activity: Following formula was applied to calculate the specific activity of CAT:

$$SpecificActivity = \frac{\text{Activity of fraction}}{Proteincontentsoffraction}$$

***Fold Purification of CAT*:**

$$FoldPurification = \frac{\text{Specific activity of fraction}}{Specific\ activity\ of\ crude\ enzyme}$$

***Yield/Percentage Recovery of CAT*:**

$$PercentageRecovery = \frac{\text{Activity of fraction}}{ActivityofCrudeenzyme} \times 100$$

Kinetic Characterization of CAT

***Determination of Optimum pH and Temperature*:**To get the optimum pH purified CAT was tested against different pH ranging from 4-12. Optimum temperature was obtained by assaying the CAT against various ranges form 5-50°C by keeping the pH constant at which purified CAT had maximum activity.
***Data Analyses*:**Data obtained from this experiment analyzed by applying ANOVA to see the statistical differences (Steel *et al.*, 1997).MS excel was used to draw the graph.

RESULTS

***Activity of renal CAT*:** The inferences of this study showed that the crude CAT activity in renal tissue of DM+END exposed fish was decreased (125.33±0.71 U mL^{-1}) when compared with control (145.66±0.71 U mL^{-1}). However, after desalted CAT activity remained lower in exposed fish as 32.03±0.71U mL^{-1} in comparison of control (28.88±1.36U mL^{-1}).Figure 1 showed that at each step of purification the activity was decreased.

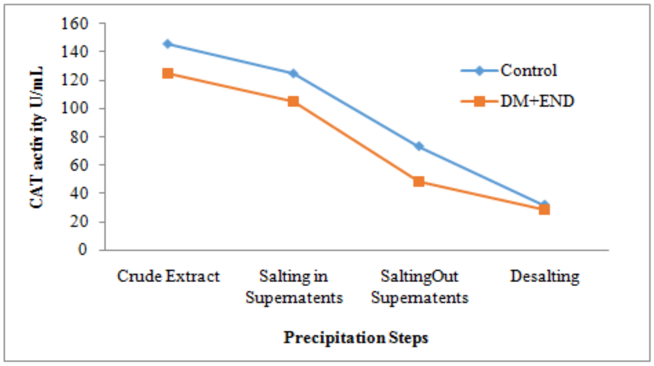

Figure 1. Partial purification of CAT from renal tissue of *C. striata*under control and exposed conditions.

***Specific activity of renalCAT*:** After partial purification specific activity was decreased in pesticides mixture exposed fish(80.94±0.00U mg^{-1}) as compared to control (88.97±1.41 U mg^{-1}).Figure 2 showed that specific activity increased at every step of partial purification.

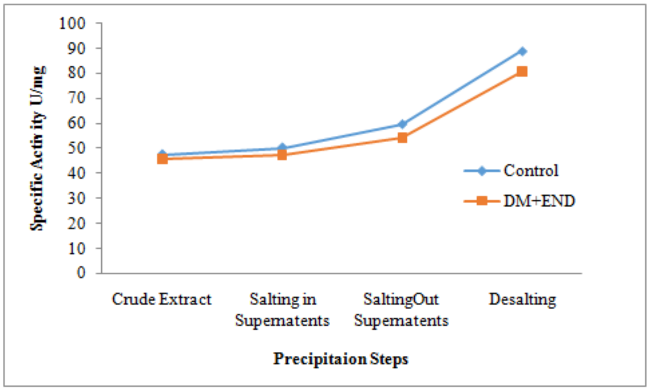

Table1. Partial purification of renalcatalase in *C. striata* by using ammonium sulfate precipitation

Precipitation Steps	Protein(mg mL^{-1})		Fold purification		% recovery	
	Control	DM+END	Control	DM+END	Control	DM+END
CrudeExtract	3.06±0.01A[a]	2.75±0.01A[b]	1.00±0.02D[a]	1.00±0.01D[a]	100.00±1.41A[a]	100.00±2.12A[b]
Salting In	2.48±0.01B[a]	2.24±0.03B[b]	1.05±0.01C[a]	1.03±0.03C[b]	85.65±2.83B[a]	84.30±1.41B[b]
Salting Out	1.23±0.01C[a]	0.88±0.01C[b]	1.18±0.01Ba	1.17±0.01B[b]	71.36±4.24C[a]	59.35±1.41C[b]
Desalting	0.36±0.01D[a]	0.35±0.01D[b]	1.28±0.01Aa	1.24±0.00A[b]	63.23±1.41D[a]	58.07±1.41D[b]

Small alphabet superscripts show the difference between treatments within the same row while capital alphabet shows significant ($P<$ 0.05) difference among different steps of purification within the same column.

Figure 2. Specific activity of renal CAT of *C. striata* at each step of partial purification.

CAT Fold purification and Yield/Percent recovery: This parameter identifies that how many folds an enzyme purified at every step of purification. It was noted that at last step highest fold purification of renal CAT was calculated as 1.28±0.01and 1.24±0.00 for control and exposed fish, respectively. The yield recovery was decreased at every step of purification and percentage recovery was about 58.07±1.41and 63.23±1.41 % for exposed and control fish, respectively (Table 1).

Kinetic Characterization

***Optimum pH and Temperature*:** Generally, activity of enzymes increased as the pH and temperature increased up to a certain limit. Similarly, in case of present study initially activity was increased, and maximum activity was observed at 6.5 pH and 30ºC temperature after that CAT activity decreased (Fig.3 and 4).

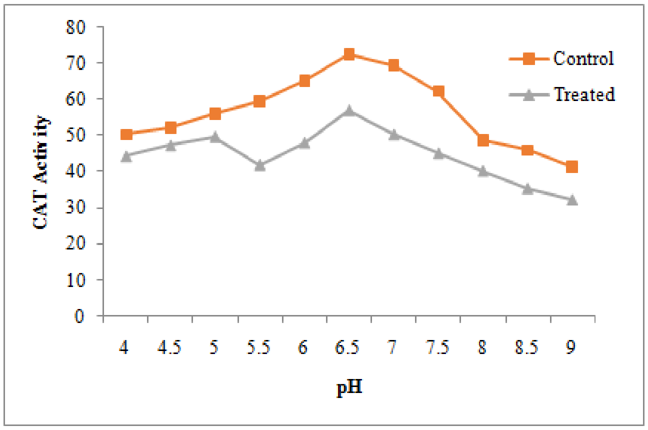

Figure 3. Effect of different pH on renal CAT activity (U/mL) of *C.striata* under control and exposed conditions.

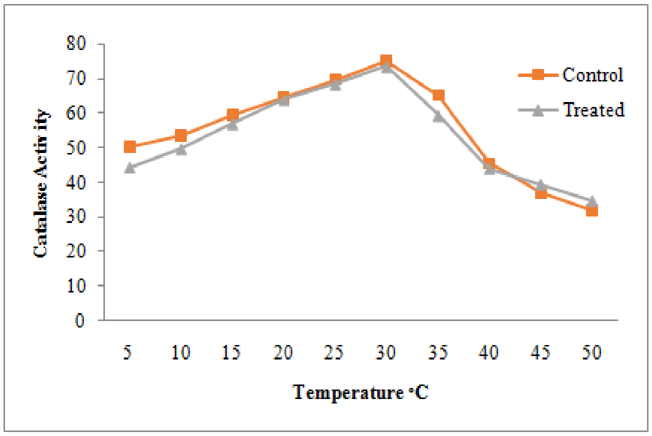

Figure4. Effect of temperature on renal CAT activity (U/mL) of *C.striata* under control and exposed conditions.

DISCUSSION

Catalase (CAT) is a peroxisomal antioxidant, which scavenges the hydrogen peroxide and transfers them into less reactive species (oxygen and water) thereby preventing lipid peroxidation. In present work renal CAT activity and specific activity in pesticides mixture exposed fish was lower than control. Similarly, deltamethrin exposure caused reduction in CAT level in fish (Atif*et al*., 2005; Sharma and Ansari, 2013). Exposure of deltamethrin caused inhibition in CAT activity in kidney tissues of *C. punctatus*(Sayeed *et al*., 2003).Vineela and Reddy (2014) also reported a gradual decrease in kidney CAT activity of *Catla catla* exposed to organochlorine pesticides (Lihocin).Low level of CAT was observed in kidney of African catfishunder sub-lethal dose of deltamethrin (Hamed, 2016). According toVasantharaja *et al.* (2012)sub-lethal dose of cypermethrin inhibited the CAT level in kidney of *Cirrhinus mrigala*. Tripathi and Singh (2013) also confirmed the decrease in CAT activity of *C. punctatus* due to toxicity of alphamethrin. Some other pyrethroids including cyfluthrin (Sepici-Dincel *et al*., 2009), deltamethrin (Yonar and Sakin, 2011), cypermethrin and λ-cyhalothrin (Kumar*et al.*, 2012), fenvalerate (Prusty *et al.*, 2013) and cypermethrin (Parthasarathy and Joseph, 2013) also decreased the CAT activity in different fish species.

Abdelkhalek *et al.* (2015) recorded a significant decrease in CAT inkidney tissue of tilapia fish upon exposure to deltamethrin. According to Suneeth (2014) exposure of endosulfan an organochlorine and fenvalerate a synthetic pyrethroid significantly reduced the CAT activity in *Labeo rohita* when compared to control. *Oreochromis niloticus* showed the inhibition in renal CAT activity when exposed to toxicants (Ahmed *et al.*, 2016).

The unit of catalase in one milligram of protein is known its specific activity and it is most important indication of CAT purification. In present study specific activity of CAT enhancedat every step of purification. Similarly, specific activity and fold purification of purified CAT from *Pyrobaculum calidifontis* was calculated as 23,500 U/mg of protein and 91, respectively (Amo *et al.*, 2002). Specific activity of CAT was observed as1011.84 Umg^{-1} for metal stressed fish (Ahmed *et al.*, 2016)

In present research optimum pH and temperature for renal CAT was observed as 6.5 and 30°C, respectively. Similarly, Ahmed *et al.* (2016) also noted the optimum pH and temperature for CAT purified from tilapia as 7.0 and 25°C, respectively. Vetrano *et al.* (2005) purified catalase form mammalian sources and noted that optimum pH lie in range of 7-9.

Conclusion**:** This work concluded that exposure of organic compounds can change the antioxidant enzymes response in fish. The catalase purification from renal tissue is convenient and simple technique and can be used for cross laboratory comparisons. Thus, catalase can serve as a valuable biomarker of aquatic pollution. This purified technique has successfully applied in all those areas where agriculture activities are intensive and water contamination has become a most important ecological concern. Results also concluded that the catalase activity changed in renal tissues of fish at different pH and temperature. This may suggest that catalase respond to pH and temperature may be a physiological adaptation under various environments.

REFERENCES

Abdelkhalek, N.K.M., E.W. Ghazy and M.M. Abdel-Daim. 2015. Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: Impact on lipid per-oxidation and oxidative Stress. Environ. Sci. Pollut. Res. Int. 22:3023-3031.

Abdollahi, M., A. Ranjbar, S. Shadnia, S. Nikfar and A. Rezaie. 2004. Pesticides and oxidative stress: A review. Med. Sci. Monit. 10:141-147.

Abdullah, S., A. Mateen, K. Abbas, H. Naz, W. Hassan and S. Anum. 2018. Changes in glutathione S-transferase activity in fish *Channa striata* exposed to differentaquatic pollutants (heavy metals and pesticides mixture). Pakistan J. Zool. Suppl. Ser. 13:42-47.

Ahmad, I., T. Hamid, M. Fatima, H.S. Chand, S.K. Jain, M. Athar and S. Raisuddin. 2000. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. Biochimica et Biophysica Acta. 1523:37-48.

Ahmed, T., S. Abdullah, K. Abbas and M.A. Zia. 2016.Catalase enzyme response to chronic Pb+Cdmetal mixture exposure, its purification andpartial characterization from the kidney offreshwater fish, *Oreochromis niloticus*. Pak. J. Zool. 48:1733-1740.

Amo, T., H. Atomi and T. Imanaka. 2002. Unique presence of manganese catalase in a hyperthermophilicarchecon, *Pyroaculumcalidifornis*VA1. J. Bacteriol. 184:3305-3312.

Atif, F., S. Parvez, S. Pandey M. Ali, M. Kaur, H.Rehman, H.A. Khan and S. Raisuddin. 2005. Modulatory effect of cadmium exposure on deltamethrin-induced oxidative stress in *Channa punctata* Bloch. Arch. Environ. Contam. Toxicol. 49:371-377.

Bacchettaa, C., A. Rossia, A. Aleb, M. Campanaa, M.J. Parmaa and J. Cazenave. 2014. Combined toxicological effects of pesticides: A fish multi-biomarker approach. Ecologi. Indicat. 36:532-538.

Chance, M. and A.C. Mehaly. 1997. Assay of catalase and peroxidase. Meth. Enzymolo. 2:764-817.

Clarke, A. and K.P.P. Fraser. 2004. Why does metabolism scale with temperature? Funct. Ecol.18: 243-251.

Datta, M. and A. Kaviraj. 2003. Acute toxicity of the synthetic pyrethroid deltamethrin to freshwater catfish *Clariasgariepinus*. Bullet. Environ. Contam. Toxicol. 70:296-299.

Fung, Y.S. and J.L.L. Mak. 2001. Determination of pesticides in drinking water by micellar electrokinetic capillary chromatography. Electrophoresis. 22:2260-2269.

Gornall, A.G., C.S. Bardwill and M.M. David. 1949. Determination of serum proteins by means of biuret reaction.J. Biol. Chem. 177:751-766.

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.

Joseph, B. and S.J. Raj. 2011. Impact of pesticide toxicity on selected biomarkers in fishes. Int. J. Zool. Res. 7:212-222.

Kumar, A., B. Sharma and R.S. Pandey. 2012. Alterations in nitrogen metabolism in freshwater fishes, *Channa punctatus* and *Clarias batrachus*, exposed to a commercial-grade λ-cyhalothrin, REEVA-5. Int. J. Exp. Pathol. 93:34-45.

Orbea, A., M. Ortiz-Zarragoitia, M. Sole, C. Porte and M.P. Cajaraville. 2002. Antioxidant enzymes 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.

Parthasarathy, R. and J. Joseph. 2011. Studies on the hepatic antioxidant defense system in λ cyhalothrin-induced oxidative stress in freshwater tilapia (*Oreochromis mossambicus*). AfricanJ. Environ. Sci. Technol. 45:530-534.

Parvez, S. and S. Raisuddin. 2005. Protein carbonyls: Novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). Environ. Toxicol. Pharmacol. 20:112-117.

Prusty, A.K., M.P.S. Kohli, NP. Sahu, A.K. Pal, N. Saharan, S. Mohapatra and S.K. Gupta. 2011. Effect of short term exposure of fenvalerate on biochemical and haematological responses in *Labeo rohita* (Hamilton) fingerlings. Pestic. Biochem. Physiol. 100:124-129.

Sayeed, I., S. Parvez, S. Pandey, B. Bin-Hafeez, R. Haqueand S. Raisuddin.2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, (Bloch). Ecotoxicol. Environ. Safe. 56:295-301.

Sepici-Dincel, A., A.C.K.Benli and M. Selvi, R. Sarikaya, D. Sahin, I.A. Ozkul and F. Erkoc. 2009. Sub-lethal cyfluthrin toxicity to carp (*Cyprinus carpio*L.) fingerlings: biochemical, hematological, histopathological alterations. Ecotoxicol. Environ. Safe. 72:1433-1439.

Sharma, D.K.and B.A. Ansari. 2013. Effects of deltamethrin on CAT, LPO and GSH in tissues of zebra fish *Danio rerio*. Res.J. Environ. Toxicol. 7:38-46.

Steel, R.G.D., J.H. Torrie and D.A. Dinkkey. 1997. Principles and Procedures of Statistics: A biometrical approach, 2nd ed. McGraw Hill Book Co., Singapore.

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.

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

Vasantharaja, C., K. Pugazhendy, M. Meenambal, S. Prabakaran, S. Venkatesan and C. Jayanthi. 2012. Protective role of *Cardiospermum halicacabum*a gainst the cypermethrin toxicity in the oxidative stress in the fresh water fish C*irrhinus mrigala* (Hamilton).Int. J. Rec. Sci. Res. 3:601-606.

Velisek, J., A. Stara, J. Machova and Z. Svobodova. 2012. Effects of long-term exposure to simazine in real concentration on common carp (*Cyprinus carpio* L.). Ecotoxicol. Environ. Safe. 76:79-86.

Vetrano, A.M., E.D. Heck, T.M. Marianos, V. Marianos, D.L. Laskin and J.D. Laskin. 2005. Characterization of the oxidase activity in mammalian catalase. J. Biol. Chem. 280:35372-35381.

Vijayakumar, A., N. Thirnavukkarasu, K. Jayachandran and M. Susiladevi. 2016. Attenuating properties of atropine against the cypermethrin toxicity in the oxidative stress in the fresh water fish *Labeo rohita (*Hamilton). Int. J. Mod. Res. Rev. 4:1088-1093.

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

Yonar, M.E.and F. Sakin. 2011. Ameliorative effect of lycopene on antioxidant status in *Cyprinus carpio* during pyrethroid deltamethrin exposure. Pestic. Biochem. Physiol. 99:226-231.

Zia, M.A., K. Rahman, M.K. Saeed and F. Anjum. 2007. Thermal characterization of hyperproduced glucose oxidase from *Aspergillus niger* BCG-5 mutant strain. ICME. Pp. 1950-1955.

[Received 24 Jun 2019; Accepted 3 Jul 2019; Published (online) 1 Sept 2020]