Studii şi Cercetări	Mai 2017	Biologie	26/2	84-89	Universitatea"Vasile Alecsandri" din Bacău

EFFECT OF AN INSECTICIDE CHLORANTRANILIPROLE ON BIOCHEMICAL CHARACTERISTICS OF SNAKEHEAD FISH, *CHANNA PUNCTATUS* (BLOCH, 1793)

Bantu Nagaraju, Zenebe Hagos, K.Ravi Babu, Gopala Krishnan, Krishna Chaitanya, Zenebe Teka, Kidu hintsa, Tesfay Weletnsae

Key words*: accumulate, food safety, Channa punctatus, declined, exposure*

INTRODUCTION

Pesticides were found to adversely affect a number of biological functions, thus causing harm to the non-target organisms and those compounds are known for this persistence in the environment and accumulation in the tissues for long periods for controlling the loss of produce due to pest attack and as a consequence of the demand for producing more food, there has been an increasing use of pesticides in developed countries(Rocío Inés Bonansea et al., 2016). Proteins are the most versatile biomolecules in living organisms, proteins are the main enzymes in a cell and regulate metabolism by selectively accelerating chemical reactions. They function as bio-catalysts, they conveyance and supply supplementary molecule oxygen, provide mechanical provision and defense mechanism against foreign substances, intercellular signaling and they transfer nerve impulses (Berg, et. al, 2005). They are the abundant macromolecules in a biological organization and are the byproducts of high molecular weight polypeptides. They not only assist as a fuel to produce energy but also play an energetic role in the structural and functional physiognomies of the living organism. Functionally, proteins show a prodigious diversity, establish a heterogeneous group, having diverse physiological roles and are involved in major physiological activities (Lehninger, 2013). Therefore, the valuation of the protein content can be measured as an analytical tool to determine the physiological levels of animals (Kapila and Ragathaman, 1999). The concentration of proteins in a tissue is a balance between the rate of their synthesis and degradation or catabolism (Schimke, 1974); the overall protein turnover in an animal is the dynamic equilibrium between these two (Grainde and Seglen, 1981).Hydrolysis of proteins is a quite common phenomenon wherein proteases split proteins stepwise into amino acids. Amino acids formed by protein degradation will also be utilized for energy production. Amino acids are vital intermediates in protein synthesis and degradation products appear in the form of different nitrogenous substances (Nagraju et al., 2013). Singh and Singh, 2006) reported that variations in lipids, phospholipids content in the *Heteropneustes fossilis* treated with endosulfan. The present investigation was intended to study the effect of Chlorantraniliprole on soluble, structural and total proteins, free amino acids, and total lipid content of fish, *Channa punctatus*.

MATERIAL AND METHODS

Procurement and maintenance of fish

The fresh water fish *Channa punctatus* size 12-13 cm and weight 18-20 g were brought from a local freshwater bodies located at Kuchipudi, Guntur district of Andhra Pradesh, India. The fish were fed daily with commercial fish pellets and acclimatized to the laboratory conditions at 28 + 2^{o}C for 15 days. During the acclimatization period daily fed with fish meal. If in any batch, mortality exceeds 5% during acclimatization, that entire batch of fish was discarded. The water used for acclimatization and conducting experiments was clear unchlorinated ground water.

The physical and chemical analyses of the water were carried out according to (APHA). The containers of the test media are of 15 liter capacity, where in each test five containers were used and each container consisted of ten fish. All the precautions were laid by APHA et al., (2005) were followed. Hence, in the present investigation, 96 hr LC50 and 1/10th of 96 hr LC50 were selected as lethal and sub lethal concentrations to study the behavioral responses and physiological alterations in experimental animal.

Physico-chemical analysis of water

Turbidity–8 Silica units, Electrical conductivity at 28^{o}C -816 micro ohms/cm, Alkalinity-1, Phenolphthalein-Nil, Methylorange-472, Total hardness (as CaCO3) - 232, Non carbonate hardness (as CaCO3)-Nil, Calcium hardness (As N) -Nil, Sulphate (as SO4) -Trace, Chloride (as Cl)- 40,

Fluoride (as Fl)- 1.8, Iron (as Fe) -Nil , Dissolved oxygen- 8-10 ppm, Temperature- 28±2oC. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA, 2005) were followed.

Estimation of soluble, structural and total proteins

The soluble, structural and the total proteins in the organs were estimated using the folin-phenol reagent method as described by Lowry, *et. al,* (1951). 1% homogenate (W/V) was prepared in ice-cold 0.25 M sucrose solution. For soluble and structural proteins 1.0 ml of the homogenate was taken and centrifuged at 3000 rpm for 10 minutes. The supernatant was separated and to both the supernatant and residue 3 ml of 10% trichloroacetic acid was added and again centrifuged at 3000 rpm.

The supernatants were discarded and the residues were taken for experimentation. For total proteins, 1 ml of homogenate was taken; to it 3 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm. Supernatant was discarded and the residue was taken for experimentation. All the three residues were dissolved in 5 ml of 0.1 N sodium hydroxide and to 1 ml of each of these solutions, 4 ml of reagent –D (mixture of 2% sodium carbonate and 0.5% copper sulphate in 50:1 ratio) was added. The samples were allowed to stay for 10 minutes, at the end of which 0.4 ml of folin-phenol reagent (Diluted with distilled water in 1:1 ratio before use) was added.

Finally, the optical density of the colour developed was measured using spectrophotometer at a wavelength of 600 nm. A mixture of 4 ml of reagent–D and 0.4 ml of folin-phenol reagent was used as blank. Bovine albumin was used for the preparation of protein standards. The protein content is expressed as mg/g wet wt of the tissue.

Estimation of free amino acids

Free amino acid levels in the tissues were estimated by the ninhydrin method as described by Moore and Stein (1954). Homogenates (4%) were prepared in cold phosphate extraction buffer (50 mmol, pH 7) and 2 ml of 15% TCA was added to 0.2 ml of the homogenate followed by centrifugation at 3000 rpm for 15 min. To the entire supernatant (2.2 ml), 2 ml of ninhydrin reagent was added and the contents were boiled for exactly 5 min.

The contents were cooled in ice-cold water and the volume was made up to 10 ml with distilled water and n-propanol in 1:1 ratio). The optical density of the colour developed was measured using spectrophotometer at a wavelength of 570 nm against a reagent blank. The free amino acid levels are expressed as µmol of tyrosine equivalents/g wet wt. of the tissue.

Estimation of lipids

Lipids were extracted as described by Floch et al. (1957), and estimated by the method of Barnes and Blackstock (1973). 50 mg of tissues were homogenised (5% w/v) in a waring blender in chloroform-methanol mixture (2:1). The homogenates were filtered through Whatman No.1 filter paper and the residue was rehomogenized as before and then filtered.

The non-lipid matter from pooled filtrate was removed by shaking vigorously with 0.88% KCL. 1 mL of filtrate was taken in a test tube and evaporated under nitrogen and 1 mL of concentrated H_2SO_4 was added and boiled for 10 min.For estimation of total lipid, 0.2 mL of solution was taken and 5 mL of vanillin reagent was added. The developed color was read in spectrophotometer at 520 nm against reagent blank.

RESULTS AND DISCUSSION

The data is presented on the levels of soluble, structural, total proteins, free amino acids and lipids in the organs of the fish *Channa punctatus* on exposure to lethal 24 hr and 1, 10, 20 and 30 days of sub lethal concentrations of Chlorantraniliprole. All results are presented in the tables from 1-4, a substantial decrease comparative to controls is seen in the soluble, structural and total proteins, amino acids and lipids of all the vital organs of fish, *Channa punctatus* at all the exposure periods in the lethal and sub lethal concentrations of Chlorantraniliprole. These protein levels also recorded a significant decrease in the organs of fish on day 1 and 10 on exposure to sub lethal concentration but on further exposure gradual reduction in the increase was observed at 20 and 30 day (Tables 1-2).

During the exposure periods, the levels of soluble, structural, total proteins, amino acids and lipids significantly decreased in the kidney, muscle and liver compared to control fish. The lowest decrease was observed in kidney (12.49%) at 24 h and maximum (121.78%) in liver at 96 h on exposure to the lethal concentrations. The similar observation was not the circumstance at sub lethal concentration, among the tissues of fish, the reduction in protein content was greater in liver than kidney and muscle exposed to the lethal and sub lethal concentrations of Chlorantraniliprole. The data presented in the table 4 , corresponding to the reduction in protein content a rapid increase in free amino acid levels in all the organs of fish at all the exposure periods in the lethal concentration of Chlorantraniliprole was observed. Also in under sub lethal concentration, however free amino acid levels was decreased, it is mainly more in the tissues of the fish exposed to lethal than the sub lethal concentration.

Table 1. Total protein content (mg/g wet wt) in the organs of fish, *Channa punctatus* on exposure to the lethal and sub lethal concentrations of Chlorantraniliprole

Organs	Control	Exposure periods							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	10	20	30
Kidney	131.78	115.32	99.76	85.72	61.44	102.51	97.26	95.79	108.42
SD ±	0.22	0.29	0.35	0.44	0.22	0.51	0.59	0.44	0.32
% Change	---	12.49	17.04	34.95	53.37	22.21	26.19	27.31	17.72
Muscle	146.87	121.39	113.45	98.77	82.36	119.43	114.89	128.22	131.92
SD ±	0.51	0.32	0.39	0.44	0.33	0.22	0.52	0.59	0.29
% Change	---	17.34	22.75	32.75	43.92	18.68	21.77	12.69	10.17
Liver	193.31	166.29	153.92	130.75	121.78	173.52	120.65	179.32	186.29
SD ±	0.01	0.22	0.32	0.35	0.51	0.52	0.44	0.22	0.29
% Change	---	13.97	25.39	32.36	56.11	10.23	0.3758	0.07.23	0.3.63

Table 2. Soluble protein content (mg/g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sub lethal concentrations of *Chlorantraniliprol*e

Organs	Control	Exposure period in days							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	10	20	30
Kidney	61.59	54.76	51.93	46.25	42.88	57.33	49.72	45.25	53.88
SD±	0.05	0.01	0.12	0.05	0.12	0.16	0.30	0.29	0.01
% Change	---	-11.08	-15.68	-24.90	-30.37	-6.91	-19.27	-26.53	12.51
Muscle	84.65	79.20	74.11	72.99	68.29	82.90	77.29	73.84	79.99
SD±	0.	0.61	0.29	0.45	0.31	0.41	0.20	0.45	0.21
% Change	---	-6.43	-12.45	-13.77	-19.32	-2.09	-8.69.	-12.77	-5.50
Liver	108.54	92.29	87.66	82.21	61.77	95.99	82.85	95.04	103.22
SD±	0.05	0.05	0.01	0.41	0.29	0.45	0.29	0.41	0.29
% Change	--	-14.97	-19.23	-23.75	-43.09	-11.56	-23.66	-12.43	-4.90

Table 3. Lipid content (mg/g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sub lethal concentrations of *Chlorantraniliprole*

Organs	Control	Exposure periods							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	10	20	30
Kidney	45.44	57.99	52.66	39.33	25.96	55.65	51.99	57.54	51.20
SD±	0.92	0.51	0.52	0.41	0.29	0.27	0.45	0.05	0.29
% Change	---	-27.61	-15.88	-13.44	-42.86	-22.46	-14.41	-26.64	-12.76
Muscle	34.32	29.57	27.65	25.22	21.33	28.43	29.98	21.76	19.66
SD±	1.06	0.44	0.39	0.31	0.25	0.46	0.42	0.49	0.51
% Change	---	-13.84	-19.43	-26.51	-37.84	-17.16	-12.64	-36.59	-42.71
Liver	27.54	21.96	19.72	18.99	16.44	24.75	21.97	15.65	12.78
SD ±	1.45	0.51	0.47	0.41	0.32	0.29	0.11	0.51	0.29
% Change	---	-10.43	-28.39	-31.04	-40.31	-10.14	-20.22	-43.17	-53.59

Table 4. Free amino acid levels (mg amino acid nitrogen / g wet wt.) in the organs of fish, *Channa punctatus* on exposure to the lethal and sub lethal concentrations of *Chlorantraniliprole*

Organs	Control	Exposure periods							
		Lethal (h)				Sub lethal (days)			
		24	48	72	96	1	10	20	30
Kidney	16.45	21.93	23.38	25.22	28.61	19.20	18.76	16.65	12.24
SD±	0.05	0.01	0.29	0.10	0.11	0.12	0.11	0.08	0.10
% Change	---	33.31	42.12	53.31	73.92	16.71	14.04	12.15	25.59
Muscle	18.45	21.96	24.78	26.21	28.92	20.55	23.62	11.49	9.85
SD±	0.01	0.05	0.11	0.12	0.14	0.12	0.08	0.01	0.05
% Change	---	19.02	34.30	42.05	56.74	11.38	28.02	37.72	46.61
Liver	33.18	29.27	25.75	28.53	22.98	21.34	24.32	27.93	19.28
SD±	0.05	0.10	0.12	0.29	0.12	0.22	0.11	0.01	0.0 5
% Change	---	11.78	22.39	14.01	30.74	35.68	26.70	15.82	41.89

In the present study, the toxic effects of chlorantraniliprole on the total protein content, free amino acid levels and lipids content in the tissues of the fish, *Channa punctatus* showed time dependent alterations. Total proteins, structural and soluble protein, free amino acid and lipid levels were exhausted in all the vital organs exposed to the lethal concentration of Chlorantraniliprole representative the breakdown of these proteins due to the severe pesticidal stress. Usually the breakdown of proteins dominates over synthesis under enhanced proteolytic activity (Harper, et. al, 1985). It is evident in the current study that the hypoprotenemia is related with the sudden raise free amino acid levels in the tissues of the fish exposed to the lethal concentrations. The maintenance of proteins in a highly organized state requires an active and incessant supply of energy. Similar observations were recorded with other pesticides in numerous fishes, as reported in *Cirrhinus mrigala* (Pechiammal and, Kiruthika, 2016) exposed to Rogor. Depletion of proteins in tissues may constitute a physiological mechanism and may play a vital role of compensatory mechanism below pesticidal stress, to distribute intermediates to the Kreb's cycle or to enhance osmolarity, by retaining free amino acid content in hemolymph, to compensate osmoregulatory problems encountered due to the seepage of ions and other indispensable molecules, during the pesticide stress (Rafat Yasmeen, 1986; Rajeshwari, 1986). The depletion of protein endorses increased proteolysis and possible utilization of the products of their degradation for metabolic purposes (Klassan, 1991). The reduction of protein level induces to diversification of energy to meet the imminent energy demands during the toxic stress (David, 1995).

The depletion of protein level induces to diversification of energy to meet the impending energy demand during toxic stress (Jagadeesan and Mathivanan, 1999). Under proteolysis, enhanced breakdown dominates over synthesis while in the case of anabolic process; increased synthesis dominates the protein breakdown (Harper, 1979). This is further corroborated through the increased levels of free amino acids in all the tissues. These amino acids might be fed into the TCA cycle as keto acids by way of transamination, since transaminase's are known to be elevated during pesticide intoxication (Jha and Verma, 2002). The increased levels of free amino acids might also be due to increased synthetic potentiality. This possibility might exist in the tissues of toxicant exposed fish.

It appears that protein degradation is in active phase over synthesis in the kidney, muscle and liver of fish at sub lethal concentration of toxicant as evidenced from the decrease in soluble, structural and total proteins with the significant increase in protease activity and amino acid levels. Similar reports were observed in Mus boodoja on exposure to BHC (Philip et. Al., 1988). But reduced decrease in soluble, structural and total proteins along with gradual rise in protease activity and free amino acid levels in the kidney, muscle and liver of fish at day 10 and 15 indicates the onset of acceleratory phase of protein synthesis over breakdown. The reduced decrease in structural proteins could be helpful to the animal to fortify its organs for developing resistance to the imposed sub lethal toxic stress; further the reduced magnitude of decrease in soluble protein fraction could indicate the synthesis of enzymes necessary for detoxification. Protein synthesis being an energetically expensive process, the increase in oxidative metabolism of the fish during sub lethal toxicant stress also strengthens the increase in its protein synthetic potentials. Degradation of proteins by proteolytic enzymes results in increased amino acid pool. Further, prevalence of pathological conditions in the organ systems of an animal may decrease protein synthetic acid pool. The above two factors could be responsible for the increase in free amino acid levels in the organs of fish exposed to the lethal concentration of toxicant. High concentrations of amino acids in tissues can lead to hyper amino acedemia which in turn can cause a number of side effects on the physiological conditions of the cell. The increase in the free amino acids in the organs of fish exposed to sublethal concentrations can be partly due to the increased proteolytic activity and partly due to certain transaminases reported to be indicators of protein degradation in salmonoids and liver intoxication in rainbow trout (Gingerich and Weber, 1976).

The slow increase in soluble protein in the fish exposed to the sub lethal stress could also support the elevation in these enzyme activities. The increase could be due to the stepwise induction of these enzymes greater and eater association of their oligomers (Kulkarni and Kulkarni, 1987). The increase in these enzyme activities could be helpful to the fish for structural reorganization of proteins and incorporation of keto acids into the TCA cycle to favour gluconeogenesis or energy production.

A decrease in the lipid content of the liver, muscle and kidney tissues exposed to chlorantraniliprole recommends that lipid might have been directed for energy production for other metabolic function in which these products play a vital role during toxicant stress condition. Lipids support as energy reserves to meet the metabolic requirement for more energy to mitigate toxic stress. Srinivas *et al.,* 1991 reported that decreased lipid content in *Tilapia mossambica* exposed to atrazine. Generally, the decreases in lipid contents in kidney, muscle and liver tissues were found to be increased with the time of exposure. Decline in the lipid levels may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol as reported by Mishra *et al.,* 2004. (Arockia and Milton, 2006) have shown decreasing trend of lipid content

in the brain, gill, kidney, liver and muscle tissues upon exposure to lannate in the fish *Oreochromis mossambicus.* Various authors studied similar reduction of lipids content in different tissues. Ram and Sathyanesan (1984) observed a reduced lipid content in the liver tissue of fish *Channa punctuatus* exposed to emisan.

CONCLUSION

A influence of chlorantraniliprole and its effect at cellular, molecular level and ultimately cause physiological and biochemical changes. The results of the current study obviously show the toxic nature of the toxicant on the biochemical parameters of the fish, *Channa punctuatus*. The changes in total soluble, structural proteins, free amino acids and lipid in the chlorantraniliprole treated fish will unusually affect the nutritive value of these fishes and all the metabolites studied are found to be sensitive changes in the normal indictors, which reflect changes in the normal activities of various functional systems.

ABSTRACT

Pesticides are mainly released into Lakes, ponds and rivers due to the runoff from agricultural fields. Pesticides are generally toxic to many non-target organisms such as fish. Fish, generally accumulate contaminants from aquatic environments and have been largely used in studies of food safety. The toxic effect of the chlorantraniliprole on biochemical characteristics (total protein, soluble and structural, free amino acids and lipid levels in muscle, kidney and liver) of the snake head fish *Channa punctatus* were estimated. The result shows declined levels of biochemical parameters during all the exposure periods when compared with control.

REFERENCES

1. APHA (2005) - . Standard Methods for the Examination of Water and Wastewater. 21st ed., American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington DC, USA;
2. AROCKIA, J.J. AND J.M.C. MITTON, 2006 - Effect of carbamate pesticide lannate (methomy1) on the biochemical components of the freshwater cichlid *Oreochromis mossambicus* (Peters). Ind. J. Environ Ecoplan., 12: 263-268;
3. BARNES, H. AND BLACKSTOCK, Z.J, 1973 - estimation of lipids in marine animals and tissues. Detailed investigation of the phosphovanilin method for total lipids. Journal of. Experimental Marine Biology and Ecology, 12, pp.103-118;
4. BERG, JM. TYMOCZKO, JL AND STRYER, L, 2005 - Biochemistry, fifth edition, W. H. Freeman and Company and Sumanas, Inc.;
5. BONANSEA R.I; MARINO D.J.; WUNDERLIN D.A.; AMÉ, M. V., 2016 - Tissue-specific bioconcentration of cypermethrin, chlorpyrifos and its mixture in a native fish (Jenynsia multidentata). Environ Toxicol Chem;
6. FOLCH, J.M., 1957 - A simple method for isolation and purification of total lipids from animal tissues. Journal of Biological and Chemistry, 226, pp. 497- 509;
7. GINGERICH, WH. WEBER, LJ., 1976 - Assessment of clinical procedures to evaluate liver intoxication in fish. Ecological Res. Ser. G.P.A. 600 P-118;
8. GRAINDE, B. SEGLEN, PO., 1981 - Effects of amino acid analogues on protein degradation in rat hepatocytes. *Biochemica*. *Ada. Biophysica. Acta*., 676, 43-50;
9. HARPER, HA. 1985 - In : Harper's Review of Biochemistry (eds.) D.W.Martin, P.A. Mayes and V.W.Rodwell,, Longe Medical Publications, MaruzenAsia, Singapore;
10. JAGADEESAN, G. MATHIVANAN, A., 1999 - Organic constituent changes induced by 3 different sub lethal concentrations of mercury and recovery in the liver tissues of Leaeo rohita fingerlings. Poll. Res. 18(1): 177-181;
11. JHA, BS. VERMA, BP., 2002 - Effect of pesticides mixture on protein content in the freshwater fish, Clarias batrachus. J.Ecotoxicol.Environ.Moint. 12(3): 177-180;
12. KAPILA MANOJ AND G. RAGATHAMAN, 1999 - Mercury, copper and cadium induced changes in total protein level in muscle tissue of an edible estuarine fish, Boleophthalmus ussumieri (CUV). J. Environ. Biol. 20(3): 231-234;
13. KLASSAN, CD, 1991 - Heavy metal and heavy antagonists. In: Gilman AG, Goodman LS and Gilman. Eds. Pharmacological Basis of Therapeutics. London, Baillie, Tindall. 1625;
14. KULKARNI, B.G. AND KULKARNI, R.C., 1987 - Effect of mercury exposure on enzymes in the clam, Ketelysia opima. Indian. I. Mar. Sci. 16: 256-266;
15. Lehninger principles of biochemistry, 2013 -. Albert L., David L. Nelson, and Michael M. Cox. New York: Worth Publishers;
16. LOWRY OH, ROSEBROUGH NJ, Farr AL and Randall RJ., 1951 - Protein measurement with the folin phenol reagent. J Biol Chem, 193: 265-275;
17. MISHRA, S.K., J. PADHI AND L. SAHOO, 2004 - Effect of malathion on lipid content of liver and muscles of *Anabas testudineus*. J. Appl. Zool. Res., 15: 81-82;

18. MOORE, S. STEIN, WH, 1954 - A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem, 211: 907-913;
19. NAGARAJU BANTU, VENKATA RATHNAMMA VAKITA, SOMAIAH KARRA., 2013 - Effect of Chlorantraniliprole on Biochemical and Certain Biomarkers in Various Tissues of Freshwater Fish *Labeo rohita*(Hamilton). Environment and Ecology Research 1(4): 205-215, 2013;
20. PECHIAMMAL.K AND KIRUTHIKA, K., 2016 - changes in biochemical parameters of freshwater fish, *cirrhinus mrigala* exposed to rogor (insecticide). world journal of pharmacy and pharmaceutical sciences, volume 5, issue 3, 723-728;
21. PHILIP, G.H; MALLA REDDY, P AND RAMAMURTHI., 1988 - Changes in protein metabolism in liver and kidney of Mus boodurga Gray after BHC feeding. Bull. Environ. Contam Toxicol. 41: 822-827;
22. RAFAT, Y., 1986 - Physiological responses of freshwater fish, Anabas scandens to the toxicity of endosulfan. Ph.D. Thesis, Osmania University, Hyderabad, India;
23. RAJESHWARI, K., 1986 - Effect of endosulfan toxicity on ion regulation in the freshwater field crab, Oziotelphusa senex senex (Fabricius). M. Phil. dissertation, S.V. University, Tirupathi, India;
24. RAM, R.H. AND A.G. SATHYANESAN, 1984 - Mercuric chloride induced changes in the protein lipid and cholesterol levels of the liver and ovary of fish, Channa punctatus. Environ. Ecol., 2: 113-117;
25. RICHMOND, W., 1973 - Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of lipid in serum. Clin. Chem., 19: 1350-1356;
26. SCHIMKE, RT., 1974 - In neuro sciences (Eds) F.O. Schmitt and F.G. worder, Cambridge, Massachusetts III, pp. 813-825;
27. SINGH, PB AND SINGH, V., 2006 - Impact of endosulfan on the profiles of phospholipids at sublethal concentration in the male *Heteropneustes fossilis* Bloch. Journal of Environmental Biology, 27(3), pp. 509-514;
28. SRINIVAS, T., T.A.V. PRASAD, G.M.D. RAFI AND D.C. REDDY, 1991 - Effect of atrazine on some aspects of lipid metabolism in freshwater fish. Biol. Inter., 23: 603-609.

AUTHORS` ADDRESS

BANTU NAGARAJU, ZENEBE HAGOS, GOPALA KRISHNAN, KRISHNA CHAITANYA, ZENEBE TEKA, KIDU HINTSA, TESFAY WELETNSAE - Department of Chemistry, College of Natural and computational sciences, Aksum University, Ethiopia, Eastern Africa, e-mail: nagaraju.bantu301@gmail.com;

K.RAVI BABU - Department of Zoology, Acharya Nagarjuna University, Nagarjuna nagar-522510, India.