

## RESEARCH ARTICLE

# Contaf (a systemic fungicide) induced histopathological changes in the target organs of Freshwater Teleost Fish, *Barbus carnaticus*

Vibhandik AM<sup>1\*</sup> and Wani GP<sup>2</sup>

<sup>1</sup>Swami Muktanand College Of Science, Department of Zoology, Yeola, 423401 Dist. Nasik. (MS) India

<sup>2</sup>Post Graduate and Research Department of Zoology. B.P. Arts, S.M.A. Science and K.K.C. Commerce College, Chalisgaon. 424117 Dist. Jalgaon (Maharashtra) India.

\*Corresponding author Email: [amvibhandik1966@gmail.com](mailto:amvibhandik1966@gmail.com)

Manuscript details:	ABSTRACT
<p>Available online on <a href="http://www.ijlsci.in">http://www.ijlsci.in</a></p> <p>ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print)</p> <p><b>Editor: Dr. Arvind Chavhan</b></p> <p><b>Cite this article as:</b> Vibhandik AM and Wani GP (2015) Contaf (a systemic fungicide) induced histopathological changes in the target organs of Freshwater Teleost Fish, <i>Barbus carnaticus</i>, <i>International J. of Life Sciences</i>, Special Issue A3: 32-36.</p> <p><b>Copyright:</b> © Author, This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p>	<p>The study deals with the effect of Contaf, (a systemic fungicide) on histological studies in <i>Barbus carnaticus</i>. LC 50 concentration of Contaf for <i>Barbus carnaticus</i> was found to be 5.9439 ppm for 24 h, 5.2142ppm for 48 h, 5.2469ppm for 72h and 5.2516 ppm for 96 h. and 1/10<sup>th</sup> of the corresponding LC50 values were treated as sublethal concentrations. The fish showed severe histological changes like severe necrosis i.e. local death of cells. Swelling, hypertrophy of cells, as the period of exposure go on increasing.</p> <p><b>Keywords:</b> <i>Barbus carnaticus</i>, Contaf, Histology.</p> <p><b>INTRODUCTION</b></p> <p>Pesticides are being used to control the population of the pests and to increase the crop yield. Indiscriminate use of pesticides in farm industry producing harmful effect on fish and other non target organisms of environment. Toxicants are known to changing the tissue organization, impair the normal structure and function of the body by inducing histopathological lesions. Many workers have reported toxicity induced by insecticides on various organs of fish (Ishihara and Tamura 1967; Mukherje and Bhattacharya, 1975; Dubale and Shah 1979; Dubale and Awasthi, 1982) Liver, kidney, ovary and testis are the worst organs affected by pesticides.</p> <p>The kidney as an organ for the excretion of waste materials from the body itself is exposed to harmful effects of the foreign materials including insecticides Liver is the main metabolic centre where detoxification and drug metabolism take place which makes it greatly vulnerable to changes by toxic substances. Since liver is the centre of metabolic activities and all toxic compounds are likely to be metabolized in this organ.</p> <p>The various hepatic enzymes are prone to toxic effects and can be used as indicators of sublethal exposure of fish to toxic metabolites. Liver is the largest gland of the body having several functions, it has no direct contact with the environmental pollutants dissolved in water but due to contact with</p>

blood it is indirectly affected. Fish exposed to sublethal concentrations of Contaf for different exposure periods showed considerable degree of alternations in the histology of gonads. The seminiferous tubules are normally of varying shapes and sizes. Each tubule has a definite thin fibrous wall which is not distinguishable after spawning phase. The testis of *Barbus carnaticus* showed significant changes on exposure of spermatogonic cells as well as inflammation of cells, contraction and vacuolization of tubules. It also affected ovary by disrupting the ovary and developing oocytes at various stages and cause breeding strength of fish.

Since there is dearth of information on histopathological studies of fish induced by insecticides an attempt has been made here to assess the histopathological lesions in the kidney, liver, ovary and testis of fish *Barbus carnaticus* induced by Contaf.

## MATERIALS AND METHODS

*Barbus carnaticus* (approx. wt. 100 g.) were collected from the Girna Dam, constructed on Girna river in Dist: Nasik, Maharashtra and acclimated to the laboratory conditions for a period of 15 days in a large tank of 1000 liter, previously washed with potassium permanganate and water temperature was  $26 \pm 35$  °C and pH 7.0 – 7.2 maintained in aquarium.

### Toxicity Assay :

Ten *Barbus carnaticus* were kept in a glass tank of dechlorinated tap water. The fish were treated with varying concentrations of Contaf. The  $1/10^{\text{th}}$  of the LC50 values were taken as sublethal concentrations for the 24, 48, 72 and 96 hours, respectively. To observe the histopathological changes in target organs of *Barbus carnaticus* a group of ten individuals exposed to different sublethal concentrations of Contaf for 24, 48, 72 and 96 hours, respectively. All individuals in control were maintained in pesticide free dechlorinated water in the separate tank. After exposure and completion of treatment, *Barbus carnaticus* were dissected liver, kidney, ovary and testis were removed and fixed for routine microtechnique procedure. Sections were cut (8  $\mu$ ) and stained with a haematoxyline and eosin.

## RESULTS AND DISCUSSION:

Histology of fish liver under control is given in (Fig. 1). The normal liver showed external structure of

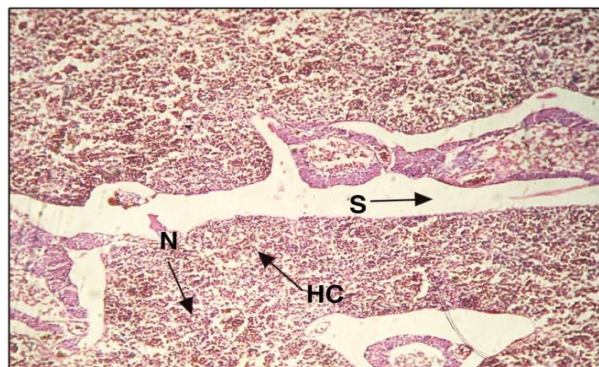
hepatic cells and connective tissue. Histopathology of experimental fish showed appearance of small vacuoles, degenerations of hepatocytes and necrosis of cells, proliferations of ducted cells (Fig.2). Hepatic cells are scattered and showed large vacuoles. In many places, necrosis is observed in liver at 96 hours stage. All these results of liver are in agreement with those of Saxena *et al*; (1989). They said that Malathion is more toxic than Carbaryl inhibition and the *de novo* synthesis of lipid and protein in the liver of *Ophiocephalus punctatus*.

Histology of fish kidney under control (Fig. 3) showed many nephrons and each nephron consists of two parts the glomerulus and urinary tubule, normal distinct glomerulus with proximal tubule, conducting tubule with sinus appeared in connective tissue, necrosis swelling in renal tubules etc. (Fig.4) As toxic products are eliminated through the kidney, kidney is susceptible to sublethal doses of Contaf. It might have caused degenerative changes in renal tubules and glomerulus i.e. necrosis in the proximal tubules and glomerulus of kidney. Degenerative changes in epithelial cells of collecting tubules of *Tilapia mossambica* exposed to Fenvalerate, has been reported by Radhaiah (1985). Shrinkage of glomerulus was reported in *Nemachelius denisoni* (Day) exposed to phosphomidan Rashatwar and Ilyas (1984). Similar results on fresh water teleosts were reported by Rao (2003) and Tilak *et al.* (2004). During the present investigation, changes were observed in the kidney of *Barbus carnaticus* exposed to different concentrations of Contaf. Histopathological changes were found in the glomerulus, renal tubules and haemopoietic tissue. A gradual increase in the damage was noticed and the severe histological lesions caused by physiological and biochemical disturbances in fish.

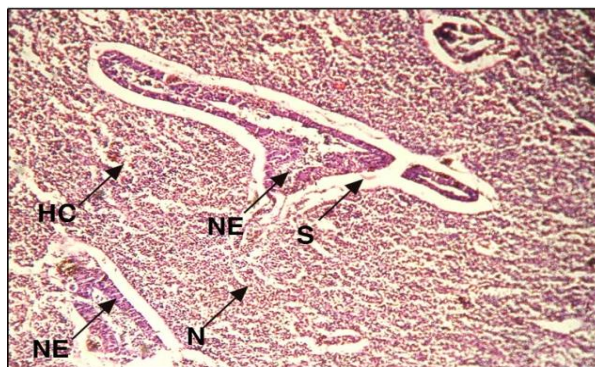
The ovary of normal *Barbus carnaticus* reveals that it is surrounded by an ovarian wall which is differentiated into an outer thin peritoneum a thicker tunica albuginea made up of connective tissue, muscle fibers and blood capillaris. The innermost layer is the germinal epithelium which joins with the tunica albuginea at several places and projects into the central lumen of the ovocoelin the form of finger like projections called *ovigerous lamellae* (Fig. 5). The histology of experimental fish ovary showed disrupted follicular epithelial cells. Nucleolus showed condensation of crescent shaped dark granules at one side. Degeneration of epithelial cells causes vacuolization, breakdown of germinal vesicles, many disrupted oogonia (Fig 6). Most of the workers have

shown that the fishes exposed to pesticides led to lowered steroid genesis Kapur *et al.*,1978. Stoppage of development of advanced oocyte stages and thus reducing the number of viable oocytes (Saxena and Garg, 1978; Yasuno *et al.*, 1980; Mani and Saxena, 1985). The increase in follicular atresia was obvious due to effect of pesticides on fish ovary. Both inhibited the growth of oocytes and raised incidences of follicular atresia were evident in ovary of *Channa orientalis* exposed to Nuvan Dimecron as have been

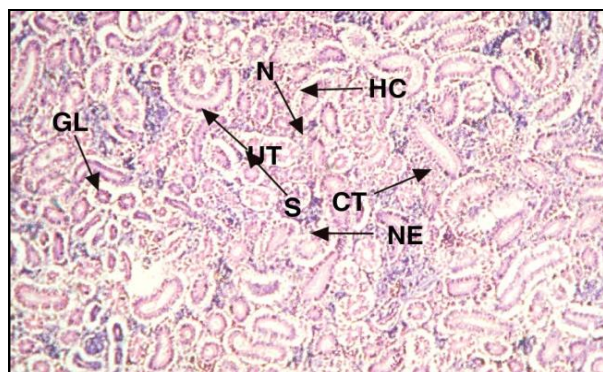
observed in the case of certain fishes (Mani and Saxena 1985; Ghosh 1986; Singh and Sahai 1986, Khillare and Wagh 1987; Patwardhan and Gaikwad 1990; Dutta *et al.*, 1994). The histological abnormalities in ovaries may be due to factors like ionizing radiations, electric current, parasitic infections. Xenobiotic toxicants, Sarojini and Victor (1985) and by variety of effluents and aquatic pollutants (Shukla *et al.*, 1984; Saxena and Garg,1978; Johnson *et al.*, 1998; Mc Comic *et al.*, 1989 Kumar *et al.*, 2000.)



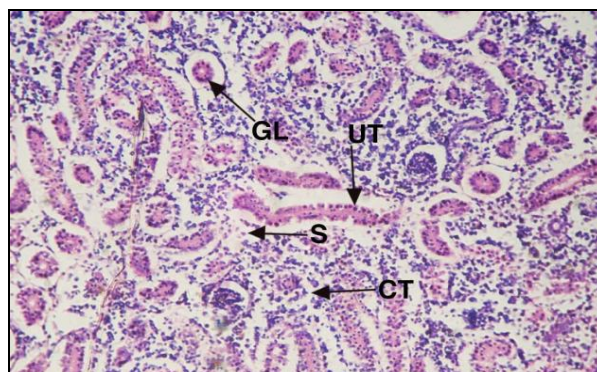
**Fig 1:** T.S. of liver from *B.carnaticus* (Control). HC- Hepatocyte, N-Nucleus, NC-Nucleolus, S-Sinusoid



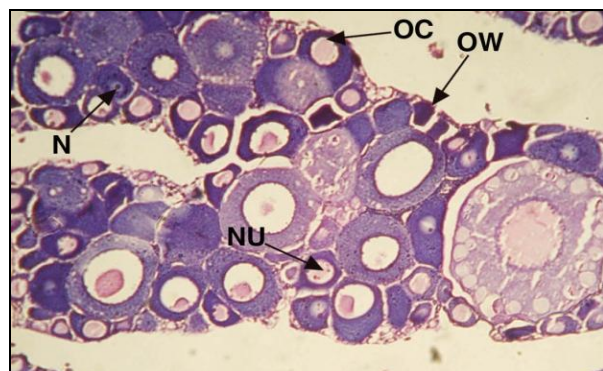
**Fig 2:** T.S. of liver from *B.carnaticus* (Exposed to Contaf for 96h). HC-hepatocyte,S-sinusoid, N- nucleus



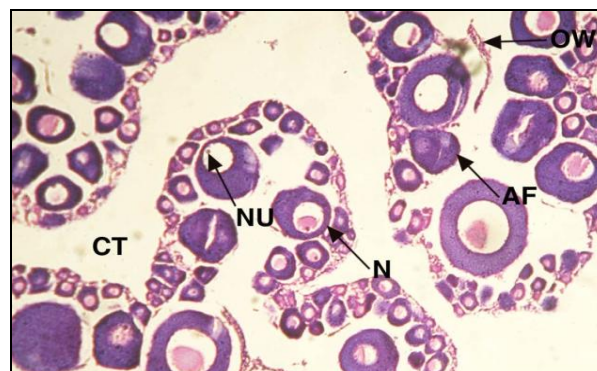
**Fig 3:** T.S. of kidney from *B.carnaticus* (Control). GL- Glomerulus, UT-Uriniferous Tubule, S-Sinus



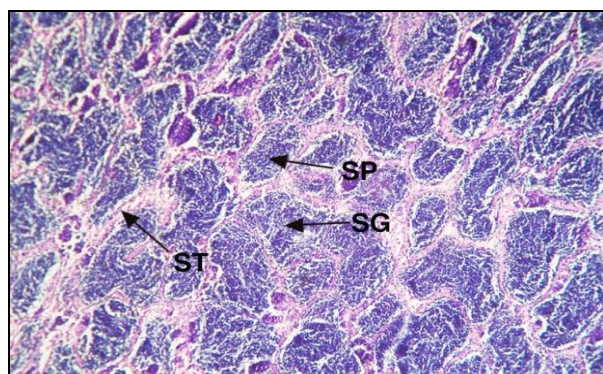
**Fig 4:** T.S. of kidney from *B.carnaticus* (Exposed to Contaf for 96h). GL-Glomerulus, UT-Uriniferous Tubule, S-Sinus.



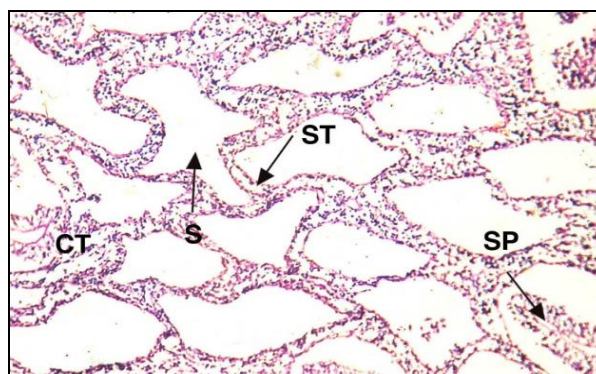
**Fig 5 :** T.S. of ovary from *B.carnaticus* (Control). OC-Oocyte, NU-Nucleolus,N-Nucleus, OW-Ovarian Wall.



**Fig 6:** T.S. of ovary from *B.carnaticus* (Exposed to Contaf for 96 h). OC-Oocyte, NU-Nucleolus, N- Nucleus, OW-Ovarian Wall, AF-Atretic Follicle.



**Fig 7:** T.S. of testis from *B. carnaticus* (control). SL-Seminiferous Lobule, SG- Spermatogonia, SP-Spermatids.



**Fig 8-**T.S. of testis from *B. carnaticus* (Exposed to Contaf for 96 h). SL-Seminiferous Lobule, SG-Spermatogonia, SP-Spermatids.

Almost all similar histopathological findings were reported by Hossain *et al.*, 2002 in the ovaries *Anabas testudineus*. The histological abnormalities in ovaries may be caused by several factors viz. ionizing radiations, electric current, parasitic infections, xenobiotic toxicants (Sarojini and Victor, 1985) and by a variety of effluents and aquatic pollutants (Shukla *et al.*, 1984; Saxena and Garg, 1978; Johnson *et al.*, 1988; Mc Comic *et al.*, 1989; Kumar *et al.*, 2000 ).

The histology of fish testis under control is given in (Fig.7). The normal testis showed healthy seminiferous tubules which is internally lined by tubular epithelium which gives rise to spermatocytes. Histopathology of experimented fish testis, showed disrupted seminiferous tubules and immature spermatogonia and general inflammatory response (Fig.8). Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants (Sokalet *et al.*, 1985 Ruby *et al.*, 1986 1987). Exposure of Contaf is responsible for histopathological damage of fish testis and vacuolization of tubular cells and distortion of seminiferous tubules, enlarged interstitium and haemorrhage in inter tubular area in albino rats exposed to pesticides have been reported. Dutta and Dikshith, 1973; Ghosh *et al.*, 1979; Baronia and Sahai 1993; Katti and Sathyanesan (1985) observed exposure dependent on concentration mediated changes in testis of *C. batrachus* treated with lead.

Kinnberg *et al.* (2000) have also documented concentration dependant effects on nonylphenol on testicular structure of the fish *Xiphophorus maculatus* and Zusti (2005) observed that the effect of fenthion on testes of *Glassogobiousgiuris*. They observed

reduction in size with spermatids and sperms in degenerating condition.

It is evident from results that Contaf is moderately toxic to *Barbus carnaticus*. The fish behaved normal in natural manner with coordinated movements. They were alert at the slightest disturbance but in the toxic environment and they showed irregular erratic and darting swimming movements and loss of equilibrium. The present investigation evidenced that Contaf is toxic and had profound effect on the behavior and histology of liver, kidney, ovary and testis of *Barbus carnaticus* in sublethal concentrations.

## REFERENCES

- Anees M A 1975 Acute toxicity of four organophosphorus insecticides to a fresh water teleost *C. punctatus* (Bloch) *Pak. J. Zool.*, 1: 135-141.
- Baronia k k and Sahai Y N 1993 DDT induced changes in the testis of albino rat, a histopathological study. *J. Environ. Biol.* 14, 153-156
- Bhattacharya S and Mukherjee S 1975 Toxic effects of endrin on hepatopancreas of the teleost fish *Clarias batrachus* (Linn). *Indian J. Exp. Biol.*, 13(2): 185-186.
- Dubale M S and Shah P 1979 Toxic effect of cadmium nitrate on the liver of *C. punctatus*. *Experientia*, 35: 643-644.
- Dubale M S and Awasthi M 1982 Biochemical changes in the liver and kidney of a cat fish *H. fossilis* exposed to dimethoate. *Comp. Physiol. Ecol.*, 7: 111-114.
- Dutta k k and Dikshith T S 1973 Histopathological changes in the testis and liver of rats repeatedly exposed to pesticides. *Exp. Path.* 8 363-370
- Dutta H M Nath A Adhikari S Roy P K Singh N K and Dutta Munshi J S 1994 Sublethal malathion induced changes in the ovary of an air breathing fish,

- Heteropneustesfossilis a histological study. *Hydrobiologia*, 294, 215-218
- Ghosh T K 1986 Comparative toxicological evaluation of two commonly used pesticides Ekalux(EC 25) and Roger (Diamethoate) on the ovarion recrudescence in a teleost , Sarotherodonmossambicus. Uttar Pradesh, *J. Zool.* 6(2).224-231
- Hossain Z M RahmanZand Mollah M F A 2002. Effect of dimecron 100 scwon*Anabas testudineus* ,Channapunctatus and Barbodesgonionotus. *Indan J. Fish*, 49 415-417.
- Ishihara Y. M. and Tamura O. 1967 Effect of pesticide on blood character and Oxygen consumption of Carp. *Bull Facul.Fich. Nagasaki Uni.* 24;65-68
- Johnson L L E Cartilar T K Colier B BMc Cain and U Varansi1998 Contaminant effects of ovarion development in English sole parophrysvetuler from Puget sound . *Con. J. Fish, Aquat. Sci.* 45. 2133-2146.
- KapurKKKaur and H S Toor 1978 The effect of fenitrothion on reproduction of a teleost fish. *Cyprinscarpiocommunis* (Linn) A biochemical study .*BullEnvironContam. Toxicol.*, 20 438-442.
- Katti S R and A G Sathyanesan 1985 Chronic effects of lead and cadmium on the testis of the catfish *Clariasbatrachus*, *Environ Ecology*, 3 596-598
- Khillare T K and S B Wagh 1987 Deveolopmental abnormalities induced by the pesticides in the fish *Barbusstigma* (Ham) *Indian J. Applied and Pure Biol.* 2(2). 73-76
- KoteswaraRao D 2003 Toxicity and effects of chlorpyrifos on freshwater fish *Catla catla* (Hamilton), *Ctenopharyn godonidella* (Valenciennes) and *Channapunctatus* (Bloch). Ph.D. thesis submitted to NagarjunaUniversity , Guntur. A.P. India.
- Kinnberg K B Korsgaard and P Bjerregrd 2000 Concentration dependent effects of nonylphenol on testis structure in adult platyfish *Xiphophorus maculates*, *Mar Environ. Res.* 50 169-173
- Kumar M Trivedi S P , Banerjee I and Soni A. (2000) Influence of anionic surfactant, linear alkyl benzene sulphonate on ovarian phosphatase activity in *Heteropneustesfossils* (Bloch). *Himalayan J. Environ. Zool.*, 14, 53-58.
- Mani K and Saxena P K 1985 Effect of safe concentrations of some pesticides on ovarian recrudescence in the feresh water murrel*Channapunctatus* (Bloch). A quantitative study. *Ecotoxicol Environ. Safety* 9 241-249
- McComic J H G N Stokes and R OHermantz 1989 Oocyte atresia and reproductive success in fathedminowsPmephaiesprometar exposed to acidified hard water environments. *Arch EnvironContam. Toxicol.*18. 204-207.
- Nigam S K B C Lakhad A B Karnik K N Thakore D K Bhatt K ArvindBabu and S K Kashyap1979 Effects of hexachlorocyclohexane feeding on testicular tissues of pure impreddswiss mice. *Bull. Environ. Contam.Toxicol.* 23 431-437
- Ptwardhan S A and Gaikwad S A 1990 Effect of sumithion on gonads of mosquito fish *Gambusiaaaffinis* (Baird and Girard ) In : *Trends in Ecotoxicology* (Eds R.C. Dalelaet al., ) pp 235-240. The Academy of Environmental Biology ,Lucknow.
- Radhaiah V 1985 Effect of heptachlor on the kidney of freshwater fish *Tilapia mossambica* (Peters) M. Phil. Dissertation.S.V.University, Tirupathi, India.
- Rashatwar s sand Ilyas I C 1984 Effect of phosphomidon in a freshwater teleost fish *Nemacheilusdenisoni* (Day). *Histopathological and biochemical Studies J Environ. Biol.* 5 (1), 1-18.
- Ruby S M D R Idler and P S Q Ying 1986 The effect of sublethal cyanide exposure on plasma vitellogenesis levels in rainbiow trout, *Salmogardenari* during early vitellogenesis, *Arch. Environ Contam Toxicol.* 15, 603-607
- Ruby S M D R Idler and P S Q Ying 1987 Changes in plasma , liver and ovary vitellogenin in landlocked atlantic salmon following exposure to sublethal cyanide, *Arch. Environ. Contam.Toxicol.* 16,507-510.
- Sarojini A and B Victor 1985 Toxicity of mercury on the ovaries of the candeanprawn. *Curt. Sci*, 54.398-340.
- Saxena P K Singh V P Kondal J K and Soni G I 1989 Effect of some pesticide on in vitro lipid and protein synthesis by the liver of the freshwater teleost *Channapunctatus* (Bl). *Environ. Pollut.* 58 (4) : 273-80
- SaxenaP K and M Garg 1978 Effect of insecticidal pollution on ovarionrecrudescence in the freshwater teleost *Channapunctatus*. *Ind. J. Exp. Biol.* 16.690-691.
- Shukla L A Srivastva D Meoxaoi and A KPandey A K 1984 Effect of sub-lethal malathion on ovarion histopathology in *Sarotherodonmossanbicus* *Comp. Physiol. Eco.* 9. 12.
- Singh S and Sahai S 1986 Toxic effect of BHC on the immature ovary of *Rasboradaniconius* (Ham) *Proc. Nat. Acad. Sci. India*, 56 (B), 106-110
- Sokal R Z C E Madding and R S Swerdloff 1985 Lead toxicity and the hypothalamic pituitary testicular axis. *Biol.Reprod.*, 33, 722-728.
- Tilak K S Veeraish K and KoteswaraRao D 2004 Toxicity and bioaccumulation of chlorphrifos in Indian carp *Catlacatla* (Hamilton), *Labeorohita* (Hamilton), *Cirrhinusmrigala* (Hamilton) *Bulletin Environ. Contami. Toxicol.* 3(5): 933-941.
- Yasuno M S Hatakeyama and M Miyashita 1980 Effect of reproduction in the Guppy (*Poeciliareticulatus*) under chronic exposure to temphos and fenitrothion. *Bull. Environ.Contam. Toxicol.* 25, 29-33.
- Zutshi B 2005 Ultrastructural studies on the effect of fenthion on pituitary (GTH Cells ) and testis of *Glossogobiousgiuris* (Ham) during breeding phase. *J Environ. Biol.* 26, 31-36.