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Histopathological Changes in Liver Tissue of *Heteropneustes Fossilis* Exposed Chlorpyrifos (20% Ec)

KEYWORDS: Chlorpyrifos, Heteropneustes fossilis, LC_{50}, behaviour, histopathology.

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ABSTRACT *The acute toxicity of Chlorpyrifos to Heteropneustes fossilis was assessed for 96 hrs. LC_{50} value was computed using probit method and found to be 1.2 µl/L. During acute toxicity test fish showed several behavioural changes like- gulping of air at water surface, swimming at the water surface, hanging vertically in water, increased mucus secretion and staying motionless at the bottom of the test chamber. For chronic study a sub lethal concentration of 0.12 µl/L (1/10th of LC_{50}) was choosen and histopathological changes in the liver was studied. The liver of treated fish showed congestion of central vein, degeneration of hepatocytes, cytoplasmic vacuolization, hepatocytes with pyknotic nuclei, and thrombosis in hepatoportal blood vessel. Haemorrhage around the central vein, necrosis and haemosiderin was also observed. These findings present the negative impact of the surrounding environment.*

INTRODUCTION

Pesticide is defined by United Nations Environment Programme (UNEP, 2005) as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides are routinely employed to protect crops from various pathogens. Widespread use of pesticide is now a worldwide phenomenon (Omitoyin et al 2006). It has been estimated that only about one percent of applied pesticides land on the target and that the rest contaminates the environment (Lawson et al 2011). Organophosphates are the most widely used class of insecticides. Exposure of aquatic ecosystems to these insecticides is difficult to assess because of their short persistence in water column due to low solubility and rapid degradation. However monitoring of these insecticides is important, because they are highly toxic to aquatic organisms (Halappa and David, 2009). Bioassays are used to determine the toxicity of chemical and to indicate which organisms are the most sensitive to such chemicals (Ndimele and Jenyo-oni, 2009). These data are used to rank chemicals, determine their water quality criteria and set standards for effluent discharges (Finney, 1971).Fishes represent the largest and most diverse group of vertebrates. They are excellent experimental models for toxicological research (Lacroix and Hontela, 2001; Law 2003 and Shiekh and Lee, 2008).Fishes are important component of the food chain. So any effect of toxicant may have adverse influence on the nutritive value of fish and on human being through their consumption (Gupta and Srivastava, 2006).Pesticides are widely applied in agricultural fields and tea plantation in Cachar district. Considering the rainfall pattern and the topography of the region the entry of pesticides in aquatic bodies is significant. The present paper tries to determine the LC_{50} of a widely used pesticide Chlorpyrifos on *Heteropneustes fossilis* and also chronic effects of it through histopathological changes in liver.

MATERIALS AND METHODS

Live specimens of *Heteropneustes fossilis* (10-12 cm in length & 4-6 gm in weight) were collected locally. Fishes were washed with 0.5% $KMnO_4$ solution for five minutes to remove external infections and then acclimated for 15 days. The physico-chemical characteristics of water which was used for experiment were pH 6.4±.04, dissolved oxygen 7.8±0.32 mg/l and temperature 29±0.47^{0}C.Commercial fish food was given to fishes but feeding was stopped 48 hrs before the acute test.

Static toxicity (96 h) was performed using OECD (guideline no. 203, 1992) and APHA (1998) test method. Stock solution of the toxicant was made by adding requisite amount of the chemical. Initially a stock solution of 1000µL/L of Chlorpyrifos (20% EC) was prepared. A small quantity of acetone (5 ml) was used for the preparation of stock solution and acetone was reported to be nontoxic to fish (Pickering et al, 1962). From this stock solution different concentrations were prepared for the experiment. Since a small quantity of acetone was used in stock solution, so two sets of controls were maintained- one containing only water and the other containing the highest concentration of acetone used during the test (APHA 1998). LC $_{50}$ for Chlorpyrifos on *H. fossilis* was calculated by probit analysis with the help of SPSS 17 statistical softwere. For chronic study a sub lethal concentration of 0.12 µl/L (1/10th of LC_{50}) was taken and the experiment was continued for 21 days. Fish were fed once a day and water exchange was made at three day intervals with fresh test solutions in each experimental aquarium. Ten fish were maintained in each aquarium. After 21 days both the control and test fish were sacrificed for histopathological study. Liver was dissected and kept in formaldehyde for 24 h. Then dehydrated, embedded in paraffin and sections cut at 5 µm thickness and stained with Haematoxylin and Eosin. Photographs were taken by a Olympus CX41 microscope

RESULTS AND DISCUSSION

LC_{50} OF CHLORPYRIFOS

With increasing concentrations of chlorpyrifos the number of mortality of fishes increases (Table 1). In this study the 96 hrs LC_{50} for *Heteropneustes* fossilis exposed to chlorpyrifos was 1.2 µl/L (Table 2). The LC_{50} of chlorpyrifos and other organophosphates vary considerably when different reports are compared. 96 hrs LC_{50} of Chlorpyrifos in *Channa punctatus*, Tilapia guineensis and Cyprinus carpio were 0.365ppm,0.002mg/L and 0.160mg/L as reported by Jaroli and Sharma (2005), Chindah et al (2004) Halappa and David (2009)

Table 1: Mortality of *Heteropneustes fossilis* (n=10) in different concentrations of Chlorpyrifos and LC_{50} value for three replicates

Conc (µl/L)	Mortality after 96 hrs		
	1st replicate	2nd replicate	3rd replicate
1	1	1	2
1.1	2	3	3
1.2	5	5	6
1.3	6	7	8
1.4	9	9	10
LC_{50}	1.2	1.2	1.1

Volume : 4 | Issue : 7 | July 2014 | ISSN - 2249-555X

Table 2: LC_{50} value with 95% confidence limit as estimated by probit analysis

LC_{50}(µl/L)	95% CL	
	lower	upper
1.2	1.1	1.3

respectively. Ramesh and Saravanan (2008) reported that 24 hrs LC_{50} of Chlorpyrifos in Cyprinus carpio is 5.28ppm. Different LC_{50} values were reported in previous studies for different organophosphate pesticides. Patil and David (2008) found 9µl/L as LC_{50} for malathion against *Labeo rohita*. Pugazhvendan et al (2009) reported that LC_{50} for malathion is 16µL/L for *Ophiocephalus punctatus*. 96 hrs LC_{50} of different organophosphate pesticides- monocrotophos, diazinon, nuvan and dimethoate in *Channa punctatus, Silurus glanis, Ctenopharyngodon idella and Heteropneustes fossilis* are 18.56ppm (Agrahari et al 2006), 4.142mg/L (Kopriicii et al 2006), 6.5mg/L (Tilak and Kumar 2009) and 11.34mg/L (Srivastava et al 2010) respectively. During acute toxicity study fish showed different behavioural changes like gulping of air at water surface, swimimming at water surface, hanging vertically in water and increased mucus secretion. Before death fishes were often found lying motionless at the bottom of the aquarium. The behavioural changes showed by the fishes are similar to those observed in other fishes exposed to organophosphate pesticides. Gulping of air at water surface, swimming at the water surface and increased mucus secretion were observed in *Labeo rohita* treated with malathion which has been reported by Patil and David (2008). In this study fishes were often found hanging vertically in toxic medium. Similar behavioural changes were observed by Halappa and David (2009) in Chlorpyrifos treated *Cyprinus carpio*. They also found increased secretion of mucus in Chlorpyrifos treated fishes. Kopriicii et al (2006) reported the behaviour- staying motionless on the aquarium bottom in fingerlings of *Silubus glanis* exposed to diazinon. Similar observation was also found in this study.

HISTOPATHOLOGY OF LIVER

Liver is the organ which is most associated with the detoxification and biomarker process. Due to its function, position and blood supply it is also one of the most affected organ by contaminants in water (Camargo and Martinez, 2007). Histological observation of liver from control fish in the present study showed normal homogenous mass of hepatocytes with no abnormalities. Sinusoids and central vein were systimetically arranged. After 21 days of experiment liver of *Heteropneustes fossilis* showed congestion of central vein, degeneration of hepatocytes , cytoplasmic vaculaization and a large number of hepatocytes appeared with pyknotic nuclei. Thrombosis was also observed in hepatoportal blood vessel. Haemorrhage around the central vein, necrosis and haemosiderin were also visible in Chlorpyrifos treated liver. Different authors noticed different toxicological changes in the liver of fish after exposing to different toxicants. Congestion of central vein in fish liver was reported by Ramesh(2009) and Kaoud et al (2010).Velisek et al (2009) and Hameid (2009) studied the impact of different toxicants on fish liver and they found degeneration of many hepatocytes. In this experiment cytoplasmic vaculaization was observed. This finding is in agreement with Indirabai et al (2010), Pugazhvendan et al (2009) and Sakr and lail (2005). Hepatocytes with pyknotic nuclei in liver were studied by Loganathan et al (2006) and Indirabai et al (2010) in *Labeo rohita*. Deka and Mahanta (2012) also observed pyknotic nuclei in malathion treated *Heteropneustes fossilis*. In our experiment thrombosis, haemorrhage, necrosis and haemosiderin were found in liver. These results are supported by the findings of Mohamed (2008), Kaoud et al (2010), Singh (2013), Kadry et al (2012), Parikh et al (2010) Sakr and Lail (2005) Loganathan et al (2006) and Indirabai et al (2010).

Fig 1. control liver showing central vein

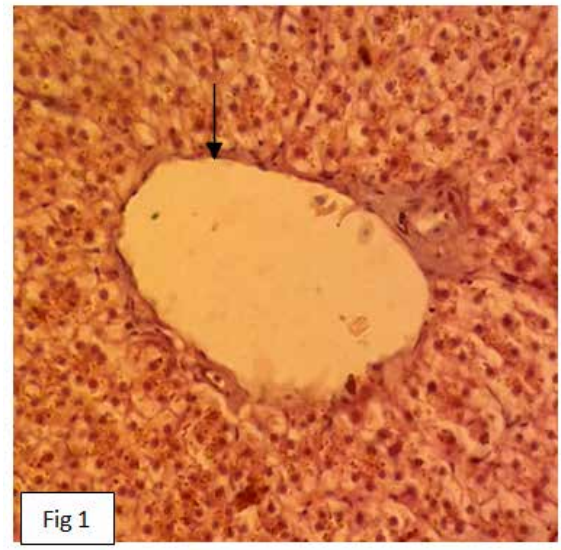


Fig 2. control liver showing hepatocytes and sinusoids (purple arrow)

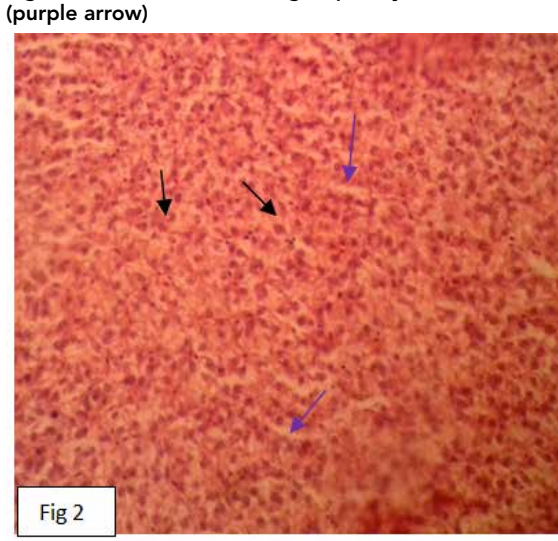
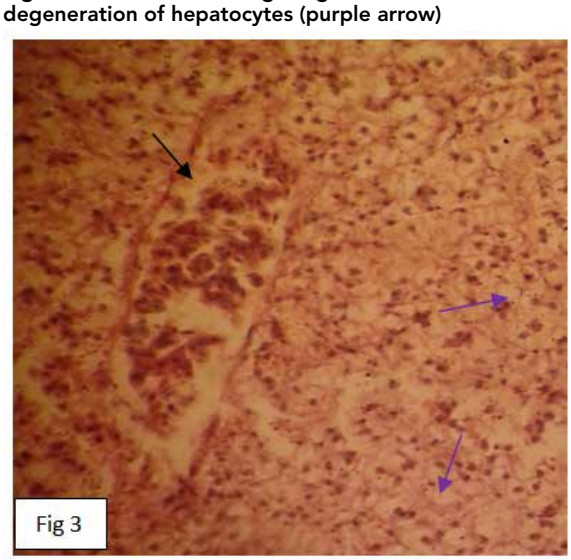


Fig 3. Treated liver showing congestion of central vein and degeneration of hepatocytes (purple arrow)



Volume : 4 | Issue : 7 | July 2014 | ISSN - 2249-555X

Fig 4. Treated liver showing cytoplasmic vaculaization and hepatocytes with pyknotic nuclei (purple arrow).

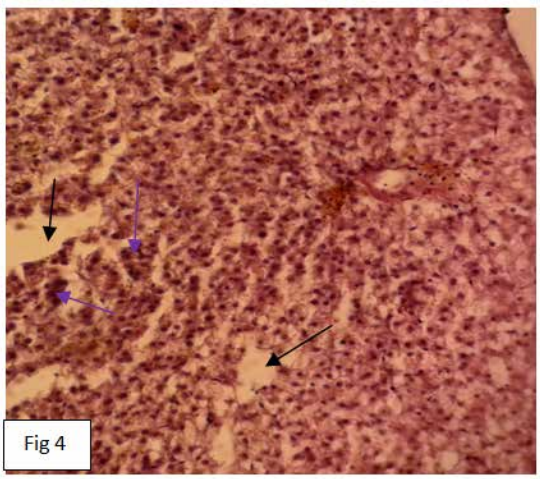


Fig 5. Treated liver showing thrombosis formation in hepatoportal blood vessel.

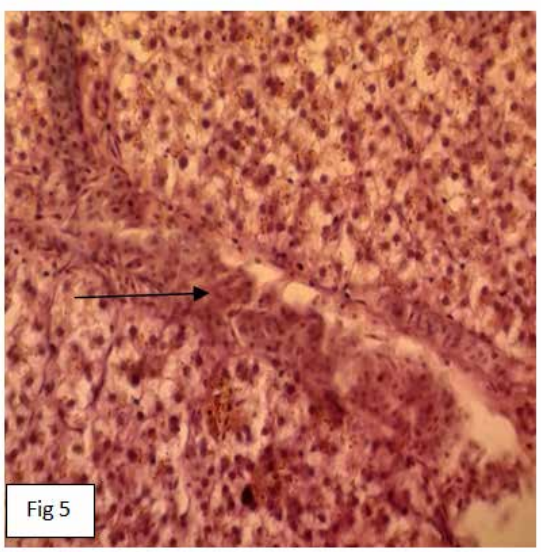


Fig 6. Treated liver showing haemorrhage around the central vein.

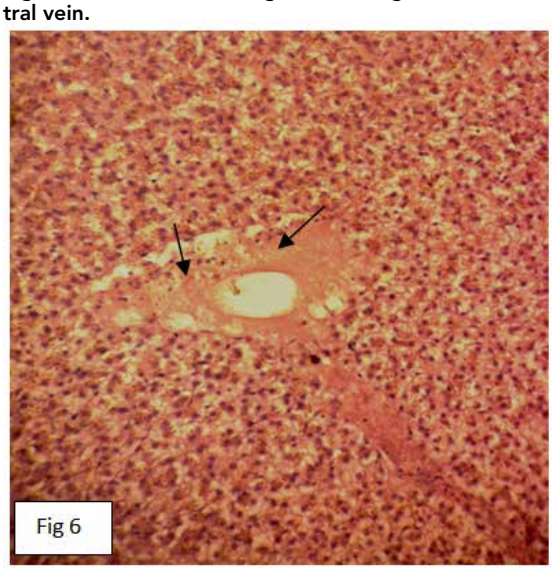


Fig 7. Treated liver showing necrosis.

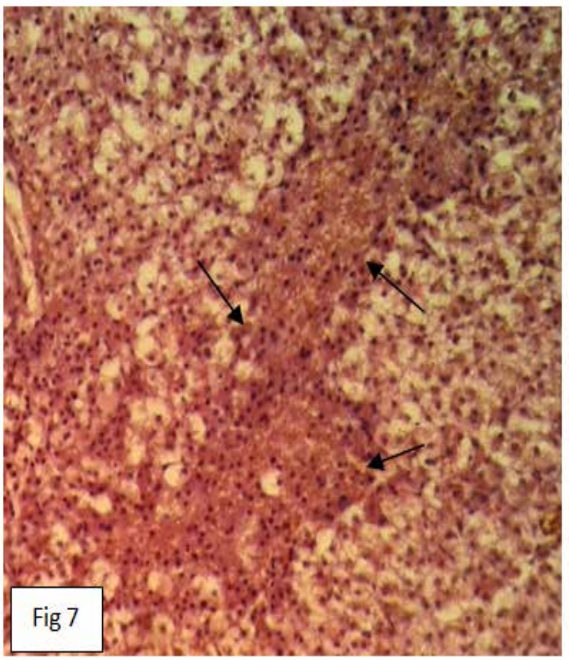
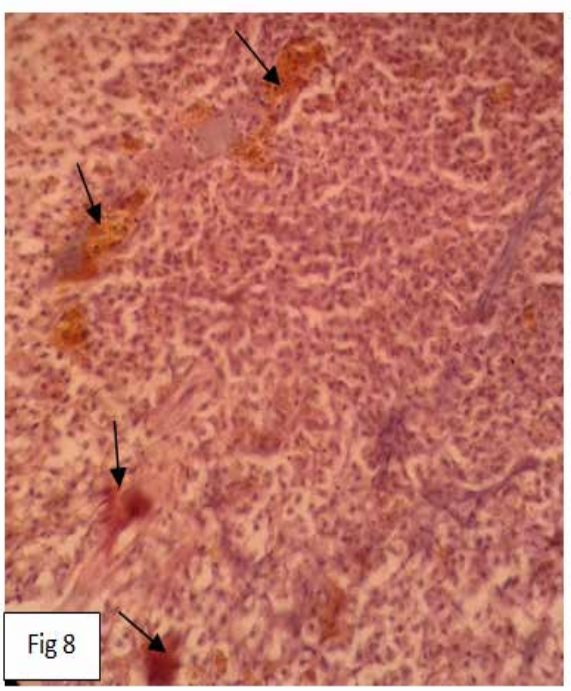


Fig 8. Treated liver showing haemosiderin.



CONCLUSION

From the present study it can be concluded that Chlorpyrifos is toxic even at very low concentration. Acute exposure of Chlorpyrifos to *Heteropneustes fossilis* adversely affects the behaviour. Chronic exposure causes significant histopathological changes in liver. So the use of this pesticide near the aquatic environment should be discouraged.

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Volume : 4 | Issue : 7 | July 2014 | ISSN - 2249-555X

REFERENCE

Agrahari,S Gopal, K and Pandey, KC 2006, Biomarkers of monocrotophos in a freshwater fish Channa punctatus (Bloch), J. of Environ. Biol., vol 27(2): pp 453-457. | APHA 1998 Standard methods for the examination of water and waste water.19th ed., American Public Health Association, Washington | | Camargo, MM and Martinez, CB 2007, Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream, Neotrop.Ichthyol, vol 5: pp 327-336 | Chindah, AC , Sikoki,FD , Vincent-Akpu, I 2004, Toxicity of an organophosphate Pesticide (Chloropyrifos) on a common Niger Delta Wetland fish – Tilapia guineensis (Blecker 1862), J. Appl. Sci. Environ. Mgt., vol 8(2): pp 11-17. | Deka,S and Mahanta, R 2012 A study on the effect of organophosphorus pesticide Malathion on Hepato-Renal and Reproductive organs of Heteropneustes fossilis (Bloch), The Science Probe, vol 1 (1): pp 1-13 | Finney,DJ 1971 Probit Analysis,Cambridge University Press, London. | Gupta,P and Srivastava,N 2006, Effects of sub- lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish Channa punctatus (Bloch), J. Environ. Biol.,vol 27: pp 211-215 | Halappa, R and David, M 2009, Behavioural responses of the freshwater fish, Cyprinus carpio (Linnaeus) following sublethal exposure to chloropyrifos, Turk. J. Fish. Aquat. Sci., vol 9 : pp 233-238 | Hameid , N HA 2009, A Protective Effect of Calcium Carbonate Against Arsenic Toxicity of theNile Catfish, Clarias gariepinus, Turkish Journal of Fisheries and Aquatic Sciences, vol 9: pp 191-200 | Indirabai,W P S, Tharani, G and Seetha, P 2010 Impact of sublethal concentration of Endosulfan on biochemicals and histology of organ tissues of freshwater fish, Labeo rohita (Hamilton,1822), The Bioscan, vol 5 (2) : pp 215-218. | Jaroli, DP and Sharma,BL 2005, Effect of organophosphate insecticide on the organic constituents in liver of Channa punctatus, Asian J. Exp. Sci.,vol 19(1): pp121-129. | Kadry, SM, Marzouk, MS, Amer, AM, Hanna, MI, Azmy, AH and Hamed, HS 2012. Vitamin E as antioxidant in female african catfish (Clarias gariepinus) exposed to chronic toxicity of atrazine, Egypt. J. Aquat. Biol. & Fish,vol 16 (2) : pp 83 - 98 | Kaoud, H A and El-Dahshan, A R 2010, Bioaccumulation and histopathological alterations of the heavy metals in Oreochromis niloticus fish, Nature and Science, vol 8(4): pp 147-156 | Kopriicii,SS, Kopriicii,K, Ural, M S, Ispir,U and Pala M 2006, Acute toxicity of an organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (Silurus glanis L.), Pesticide Biochemistry and Physiology, vol 86: pp 99-105. | Lacroix, M and Hontela, A 2001, Regulation of acute cortisol synthesis by cAMP-dependent protein kinase and protein kinase C in a teleost species, the rainbow trout (Onychorhynchus mykiss), J. Endocrinology, vol 169: pp 71-78 | Law, JM 2003, Issues Related to the Use of Fish Models in Toxicologic Pathology: Session Introduction, Toxicol Pathol, vol 31: pp 49-52 | Lawson, E O , Ndimele, PE, Jimoh, AA. and Whenu,OO 2011, Acute Toxicity of Lindane (Gamma Hexachloro-Cyclohexane) to African Catfish (Clarias gariepinus, Burchell, 1822), International Journal of Animal and Veterinary Advances , vol 3 (2): pp 63-68. | Loganathan, K , Velmurugan, B , Howrelia, J H, Selvanayagam, M and Patnaik,B B 2006, Zinc induced histological changes in brain and liver of Labeo rohita (Ham), J of Environ. Biol., vol 27 (1): pp 107-110 | Mohamed, FAS 2008, Bioaccumulation of Selected Metals and Histopathological Alterations in Tissues of Oreochromis niloticus and Lates niloticus from Lake Nasser, Egypt, Global Veterinaria, vol 2 (4): pp 205-218 | Ndimele, PE , Jenyo-Oni, A and Jibuike,CC 2010, Investigation of acute toxicities of Nigeria crude oil,dispersant, sodium dodecyl sulphate and a mixturecrude oil-plus-dispersant to Desmocaris trispinosa, Am-Eurasian J. Toxicol. Sci., vol 2(2): pp 100-107. | OECD 1992. Guidelines for testing chemicals. No.203. OECD,Paris. | Omitoyin,BO, Ajani,EK, Adesina,BT and Okuagu,CNF 2006, Toxicity of Lindane(Gamma Hexachloro-CycloHexane) to Clarias gariepinus (Burchell 1822),W.J.Zoology, vol 1(1) : pp 57-63 | Parikh,PH, Rangrez,A, Bagchi,RA and Desai,BN 2010, Effect of Dimethoate on some histoarchitecture of freshwater fish Orecromis mossambicus (Peters,1852), The Bioscan, vol 5 (1): pp55-58 | Patil,VK and David M 2008, Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, Labeo rohita (Hamilton), Turkish Journal of Fisheries and Aquatic Sciences,vol 8: pp 233-237 | Pugazhvendan,SR, Narendiran,NJ, Kumaran,RG , Kumaran, S and Alagappan, K M 2009, Effect of malathion toxicity in the freshwater fish Ophiocephalus punctatus- A histological and histochemical study, World Journal of Fish and Marine Sciences,vol 1(3): pp 218-224 | Pickering,QHC, Henderson and Lemke,AE 1962, The toxicity of organicphoshorus insecticides to different species of warm water fishes, Trans. Amer. Fish.Soc, vol 91(2): pp 175-184 | | Ramesh K 2011, Histopathological study on the effect of rice herbicides on grass carp (Ctenopharyngodan idella), African Journal of Biotechnology vol 10 (7) : pp 1112-1116 | Ramesh,M and Saravanan,M 2008 , Haematological and biochemical responses in a fresh water fish Cyprinus carpio exposed to Chlorpyrifos, International Journal Of Integrative Biology, vol 3(1): pp 80-83 | Sakr, S A and Lail,SMJ 2005, Fenvalerate induced histopathological and histochemical changes in the liver of the catfish Clarias gariepinus, Journal of Applied Sciences Research, vol 1 (3) : pp 263-267 | Shiekh R and Lee JS 2008, Fish models in impact assessment of carcinogenic potential of environmental chemical pollutants: an appraisal of Hermaphroditic Mangrove Killifish Kryptolebias marmoratus. Interdisciplinary studies on environmental chemistry-biological responses to chemical pollutants (eds) Y Murakami, K Nakayama, S I Kitamura, H Iwata and S tanabe (TERRAPUB) pp 7-15 | Singh, RN 2013, Effect of Dimethoate (30% EC), an organophosphate pesticide on liver of common carp, Cyprinus carpio,J. of Environ. Biol., vol 34: pp 657-661 Srivastava,AK, Mishra,D, Shrivastava,S, Srivastav,SK and Srivastav,AK 2010, Acute toxicity and behavioural responses of Heteropneustes fossilis to an organophosphate insecticide, Dimethoate, International Journal of Pharma and Bio Sciences,1(4): pp 359-363. | Tilak, KS and Kumari,R S 2009, Acute toxicity of Nuvan, an organophosphate to freshwater fish Ctenopharyngodon idella and its effect on oxygen consumption, J. of Environ. Biol. Vol 30(6): pp 1031-1033 | | United Nations Environment Programme (UNEP), 2005.Ridding the world of persistent organic pollutants: Aguide to the Stockholm convention on persistent organic pollutants. United Nations EnvironmentProgramme, 11-13, Chemin des Anemone, CH-1219,Chatelaine, Geneva, Switzerland, pp: 24. | Velisek ,J , Svobodova , Z, and Piackova, V 2009, Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (Oncorhynchus mykiss), Veterinarni Medicina, vol 54, 3 : pp 131–137 |