International Journal of Research in Applied, Natural and Social Sciences (IJRANSS)
Vol. 1, Issue 2, July 2013, 53-60

IMPACT OF QUINOLPHOS ON DNA AND RNA CONTENT OF THE FRESHWATER FISH *LABEO ROHITA* (HAMILTON)

V. VENKATA RATHNAMMA[1] & B. NAGARAJU[2]

[1]Department of Zoology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

[2]Department of Biochemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

ABSTRACT

Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish .The objective of the present study to investigate the toxic effect of quinolphos on DNA and RNA levels of Freshwater fish *Labeo rohita*. The fish were exposed to organophosphorus pesticides Quinolphos pesticide 25% EC to 96 hours LC50 technical $_{\text{lethal}}$ ((2.826 mg L^{-1}),) Technical sublethal (1/10 th of 96 hr LC50 value, 0.2826 mg L^{-1}), 25% EC Lethal (2.218 mg L^{-1}) and 25% EC sublethal (1/10 th of 96 hr LC50 value, 0.221 mg L^{-1}) concentrations for 8 days. The results observed in the present study reveals that quinalphos caused variability in the nucleic acid content in different tissues and the degree of variability by the quinalphos technical was less compared to 25% EC and was found to be dose dependent.

KEYWORDS: Pesticides, Chemicals, Fish, Nucleic Acid, Quinolphos

INTRODUCTION

The aquatic environment is subject to an ever increasing range of man-made (anthropogenic or xenobiotic) pollutants, reflecting the ever more rapid innovations of our technology to manufacture goods to satisfy a perceived increase in consumer demand on which our economy is based. Some of the pollutants that are now present in the tissues of fish, wildlife and humans also reflect past usage of chemicals, such as the organochlorine insecticides and PCBs, which have been banned or restricted in use for several decades. Measurements of tissue concentrations are, however, overwhelmingly limited to a range of pollutants such as pesticides, polyaromatic hydrocarbons (PAHs) and PCBs that are known to be present in the aquatic environment and for which measurement methods do exist. (R. E. Hester, R. M. Harrison and David E. Kime, 1999).

The natural physiological functioning of an organism gets disturbed on exposure to toxicant stress. It induces its effect first at cellular or even at molecular level, but ultimately causes physiological, pathological and biochemical alterations. It is, therefore necessary to focus attention on changes in biochemical composition of organisms, which are constantly under pollutant threat. When the pesticides come in contact with internal organs, irreversible changes in metabolic activities take place that eventually cause biochemical changes. Pesticide pollutants act as stress inducing agents, which affect the functional state of tissues of the exposed organisms, all pollutants are not toxic but all pesticides are toxicants. Many pesticides have been reported to produce a number of biochemical changes in fish both at lethal and more often at sublethal levels. Changes in ion concentrations, organic constituents, enzyme activity, endocrinal activity and chemoregulators in fish have been attributed to pesticides. Since aquatic environment is the ultimate sink for all pollutants, aquatic toxicity testing has became an integral part of the process of environmental hazard evaluation of the toxic

chemicals. The objective of the present study to investigate the toxicity of quinolphos on DNA and RNA levels of freshwater fish *Labeo rohita*.

MATERIAL AND METHODS

The fish *Labeo rohita* measuring 12 cm in length and 17 g in weight irrespective of the sex were used in the experiment. Fish were washed with 0.1% $KMnO_4$ solution to avoid dermal infection. All the precautions laid down by APHA *et al*., (1998) are followed, for maintaining the fish. The fish were exposed to organophosphorus pesticides Quinolphos pesticide 25% EC to 96 hours LC50 technical lethal ((2.826 mg L^{-1}),) Technical sublethal (1/10 th of 96 hr LC50 value ,0.2826 mg L^{-1}), 25% EC Lethal (2.218 mg L^{-1}) and 25% EC sublethal (1/10 th of 96 hr LC50 value., 0.221 mg L^{-1}) concentrations for 24 hrs and 8 days .if mortality occurred during the experimental period, dead fish were removed immediately to avoid depletion of dissolved oxygen (DO) level which adversely affects other fish (Schreck and Brouna, 1975). The vital tissues like muscle, brain, liver, and kidney of the fish were taken for the estimation of Nucleic acids (DNA&RNA).

RESULTS AND DISCUSSIONS

The calculated values of nucleic acids along with standard deviation and the percent change over the control were given in Table 1 & 2 and are graphically represented in Figure 1 and 2.

The DNA content in control fish *Labeo rohita* in different tissues are in the order of: Kidney > Liver > Brain > Muscle Under exposure to sublethal and lethal concentrations of quinalphos technical grade and 25% EC the DNA content in liver and kidney increased but was found to decrease in brain and muscle. The decreasing order of DNA content in different tissues is in the order of: Technical sublethal: Kidney > Liver > Brain > Muscle, Technical lethal: Kidney > Liver > Brain > Muscle, 25% EC sublethal: Kidney > Liver > Brain > Muscle, 25% EC lethal: Kidney > Liver > Brain > Muscle.

The RNA content in control fish *Labeo rohita* in different tissues are in the order of: Kidney > Liver > Brain > Muscle. Under exposure to sublethal and lethal concentrations of quinalphos technical grade and 25% EC it was found that the liver, kidney and muscle RNA content was decreased but the brain RNA content was found to increase. The decreasing order of RNA content in different tissues is in the order of: Technical sublethal: Kidney > Liver >Brain > Muscle, Technical lethal: Kidney > Liver > Brain > Muscle, 25%EC sublethal: Kidney > Liver > Gill > Brain > Muscle, 25% EC lethal**:** Kidney > Liver >Brain > Muscle, The results indicate heterogeneous levels of DNA and RNA in the tissues of brain, liver, muscle, and kidney. The level of DNA in different tissues indicates cell number (Goss, 1966) and it is constant for a species. In the present study, the DNA contents in brain decreased which may be due to reduction or absence of the essential factors controlling DNA synthesis which are the substrates (4-Deoxyribonucleoside triphosphates), enzymes (polymerase) templet activity of deoxyribonucleic-protein and activators like Mg++ and other divalent ions (Altman*al.*, 1970, Bharitya & Jaimala 1988). According to Holbrook (1980) thymine incorporation into hepatic DNA is markedly increased after 1-3 days administration of the various toxicants.The increase of DNA in gill region is due to hypertrophic nature of chloride cells leading to less transcription supporting the work of Natarajan (1981a), Durairaj and Selvarajan (1992) and Tilak *et al.* (2005) which reveal the enlargement of nuclei in the chloride secreting cell in *Channa striatus* exposed to metasystox, *Oreochromis mossambicus* to quinalphos and *Catla catla, Labeo rohita* & *Cirrhinus mrigala* to chlorpyrifos. But according to Das and Mukherjee (2000b), DNA levels were elevated in the tissues of Indian major carp, *Labeo rohita* when exposed to quinalphos for 15, 30 and 45 days. The alterations in DNA levels could be due to the disturbances in the normal synthesis and turnover rate of DNA besides degenerative changes.

Table 1: Change in the Amount of DNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in Different Tissues of Fish *Labeo rohita* Exposed to Sublethal and Lethal Concentrations of Quinalphos Technical Grade and 25%EC

Organs		Technical				25% E.C			
S. No: 1-5	Control	Sublethal	% Change	Lethal	% Change	Sub-Lethal	% Change	Lethal	% Change
Liver	6.74± 1.23	6.81± 1.84	+0.88	6.96± 1.77	+3.11	6.84± 1.42	+1.33	6.95± 0.28	+3.11
Kidney	8.36± 1.74	8.39± 1.32	+0.35	8.47± 1.70	+1.31	8.55± 1.71	+2.39	8.76± 1.33	+4.78
Brain	4.68± 1.90	5.57± 0.26	-1.84*	4.47± 1.31	-3.59*	4.51± 1.88	-3.62	4.46± 1.41	-4.70*
Muscle	0.69± 0.32	0.68± 0.26	-1.44*	0.67± 0.27	-2.21*	0.77± 0.45	-2.43	0.76± 0.59	-3.72*

Values are the mean of five observations, Standard Deviation is indicated as, and values are significant at $p < 0.05$,* indicates not significant.

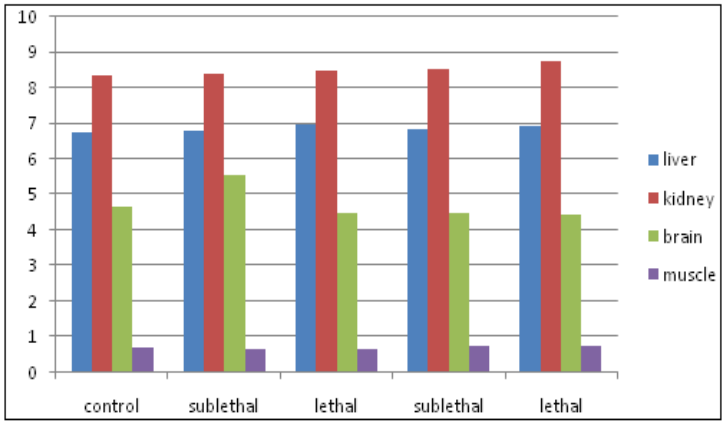

Figure 1: Change in the Amount of DNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in Different Tissues of Fish *Labeo rohita* Exposed to Sublethal and Lethal Concentrations of Quinalphos

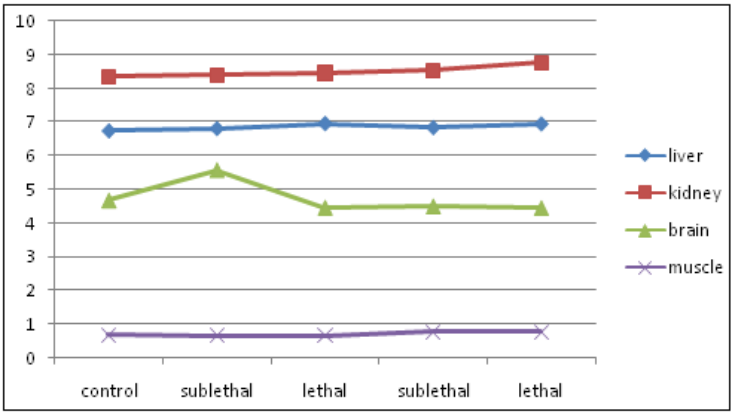

Figure 2: Change in the Amount of DNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in Different Tissues of Fish *Labeo rohita* Exposed to Sublethal and Lethal Concentrations of Quinalphos for 8 days, Mean± S.E,n=5,P < 0.05

Table 2: Change in the Amount of RNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in Different Tissues of Fish *Labeo rohita* Exposed to Sublethal and Lethal Concentrations of Quinalphos Technical Grade and 25%EC

Organs		Technical				25% E.C			
S. No: 1-5	Control	Sublethal	% Change	Lethal	% Change	Sub-Lethal	% Change	Lethal	% Change
Liver	2.75± 016	2.74± 0.92	-0.36	2.62± 0.28	-4.27	2.69± 0.47	-1.8	2.64± 0.29	-2.99
Kidney	3.37± 0.18	3.29± 0.72	-1.93	3.15± 0.36	-4.91	3.31± 1.58	-1.39	3.27± 1.20	-2.27
Brain	4.02± 0.18	3.88± 1.91	-2.78	3.95± 1.79	-1.69	3.87± 1.94	-2.99	3.78± 0.33	-4.84
Muscle	1.59± 0.36	1.61± 1.84	+1.56*	1.62± 0.35	+1.66	1.6± 0.57	+0.89*	1.61± 0.39	+1.98*

Values are the mean of five observations, Standard Deviation is indicated as (±), Values are significant at $p < 0.05$,* indicates not significant.

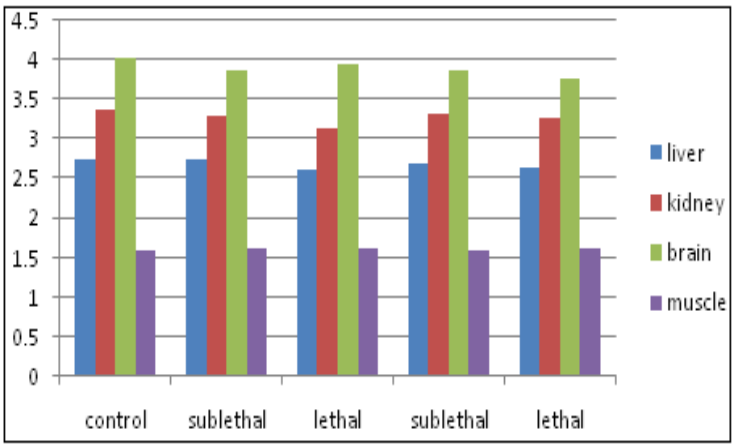

Figure 3: Change in the Amount of RNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in Different Tissues of Fish *Labeo rohita* Exposed to Sublethal and Lethal Concentrations of Quinalphos

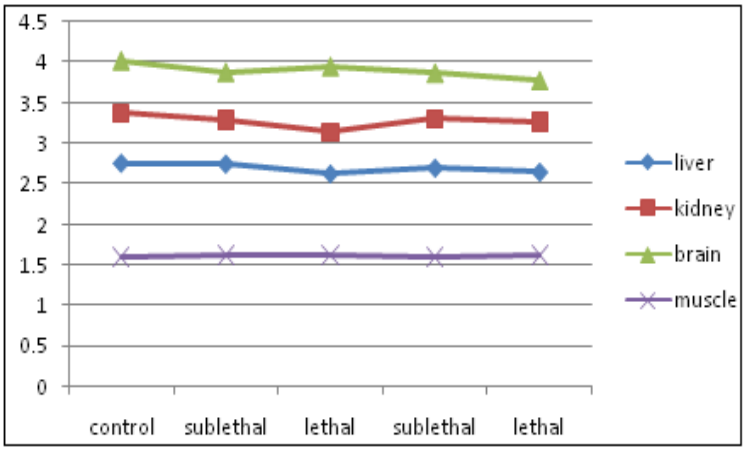

Figure 4: Change in the Amount of RNA (mg/gr Body Wet Weight of the Tissue) and % Change over the Control in different Tissues of fish *Labeo rohita* Exposed to Sublethal and Lethal Concentrations of quinalphos for 8 days, Mean± S.E,n=5,P < 0.05

Nucleic acid content is considered as an index of capacity of an organism for protein synthesis. Various studies on the effects of toxicants on the nucleic acid content in fishes have been reported. Significant decrease in RNA and DNA content in the fish, *Claria batrachus* exposed to endosulfan was recorded by Asfia Parveen and Vasanta (1986). Quinolphos induced significant decreases in RNA content of liver muscle and gill and DNA content of brain of fish *Oreochromis mossambicus* was observed by Durairaj and Selvarajan (1992). In the present study decrease in level of RNA was observed in all the tissues of fish exposed to sublethal and lethal concentrations of both technical grade and 25% EC, whereas RNA increased in brain.Organophosphorus compounds exhibit strong mutagenic and clastogenic potentiality (Patankar Nayana & Vaidya, 1980), which may be responsible for the alteration of DNA level. However the decrease of DNA is not very prominent when compared to RNA. The decrease may be attributed to the increased activity of DNAase as suggested by Tayyaba *et al*., (1981). Gautam *et al*., (2002) reported histo-chemical changes in nucleic acids (RNA & DNA) in the stomach and intestine of *Channa punctatus* after the treatment with endosulfan and diazinon pesticides and significant decrease in nucleic acids of gastrointestine tract was also reported. The depletion of RNA level suggests increased proteolysis and possible utilisation of the products of their degradation for metabolic purposes. The significant

decrease in both protein and nucleic acids would suggest that pollutant impair the process of protein synthesis in the tissues of fishes exposed to pesticides. Since RNA is the biochemical mid wife in the formation of proteins, the diminished RNA content also affects the cellular protein content. Clark and Eichhorn (1995) have also suggested that the depression in DNA synthesis is not energy dependent and may be due to the disruption of the replication process. the decrease of RNA may also be due to interference in the incorporation of precursor in the nucleic acid synthesis or inhibiting the function of RNA polymerase. Dawood (1986) and Benjamin (1990) have suggested that the decrement of RNA may also be due to the non-coding for the process of protein synthesis, thereby decrease in the RNA content, which in turn would have reduced the concentration of RNA. Maruthanayagam and Sharmila (2004) studied the effect of monocrotophos on *Cyprinus carpio* to understand the toxic effects of toxicant on the nucleic acids and concluded that the pesticide lead to several changes in the biochemical markers like DNA and RNA which may be due to the increased activity of the enzyme DNAase and the inhibition of RNA polymerase function. But during recovery period, the DNA and RNA levels increased progressively indicating a probable from the disruption of internal organs. According to Malla Reddy and Bashamohideen (1988) the role of nucleic acids particularly RNA/DNA and protein /DNA rations, which are used as an index of protein synthesis and cell size, are considered to be important and form an treatment with the pesticides causes variability in the nucleic acid content in different tissues and the degree of variability or extent of alterations caused by the pesticides is found to be dose dependent. The effects of cadmium and lead on DNA and RNA contents have been studied in gill, liver and brain of a common carp, *Cyprinus carpio* exposed to cadmium chloride and lead acetate by Muley *et al.,* (2000) and found that both the heavy metals decreased DNA content in all the tissues along with RNA content in liver and brain, but it was increased in gill due to cadmium and lead toxicity.

The estimation of percentage DNA damage by chemical induction clearly showed high genotoxicity by the herbicide 2,4-D. Similarly, 2, 4-D has shown higher mitotic index and higher Percentage of aberrations when compared to phosphamidon and sevin. The ring formation, number variation and gap formations were found predominantly in 2, 4-D and lindane treated samples than in phosphamidon and sevin compounds 2, 4 – D is reported to have mutagenic and cytotoxic effects on V 70 cells of Chinese hamster (Pavlica *et al*., 1991). 2, 4-D was also reported to cause genotoxicity to freshwater fish *Channa punctatus* (Abul Farah *et al.,* 2003),. A number of chemicals, associate with DNA damage, have been tested on live tested on live aquatic animal, isolated tissues or different cell types. These chemicals were grouped into four classes: (1) chemicals that act directly on DNA; (2) chemicals whose metabolites cause DNA damage; (3) chemicals that cause the production of reactive oxygen species that can damage DNA; (4) chemicals that inhibit DNA synthesis and repair. In addition, many chemical contaminants damage DNA by multiple mechanisms.The results observed in the present study reveals that quinalphos caused variability in the nucleic acid content in different tissues and the degree of variability by the quinalphos technical was less compared to 25% EC and was found to be dose dependent.

REFERENCES

1. Abou-Donia, M. B., Lapadul. D. M. and Carrington, C. D. (1988) Biochemical methods for assessment of neurotoxicity. In: *Perspectives in basic and applied toxicology* (Ed: B. Ballantyne), Butterworth & Co. Ltd., London, p.1-30.
2. Abul Farah, M., Ateeq, B., Niamat Ali, M., Ahmad, W. (2003). Evaluation of genotoxicity of PCP and 2,4-D by micronucleus test on freshwater fish *Channa punctatus. Ecotoxicol. Environ. Saf.* 54(1): 25-29.
3. Altman K., A.Southern,S.C. Uretsky,P.Zabos and G.Acs.(1972).Hydrocortisone induction of rat liver leucyl-transfer RNA and its synthetase,Pro,Natl.Acad.Sci.69:35-67.

4. Asfia Parveen and Vasanta, N. (1986) Effects of endosulfan on the nucleic acid content in different tissues of freshwater fish, *Clarias batrachus*. Proc. 7 th Nat. Symp. Ind. Soc. *Life Sci*. p. 31-38.

5. Asfia Parveen and Vasantha, N. (1994) Evaluation of pesticide endosulfan impact on respiratory metabolism of fish *Clarias batrachus*. *Indian J. Comp. Anim. Physiol.* 12(1): 83-89.

6. Asghari Marjanlo A, Mostofi Y, Shoeibi Sh, and Fattahi M (2009). Effect of Cumin Essential Oil on Postharvest Decay and Some Quality Factors of Strawberry. *Journal of Medicinal Plants* 8(31): 25- 43.

7. Benjamin, N. (1990) Biochemical changes due to learning of the brain of rat exposed to an Organophosphate pesticide,M.Phil thesis submitted to Madras University.

8. Brachet, J. (1955) In: The nucleic acids, Vol. 2, Academic Press, New York.

9. Das, B. K. and Mukherjee, S. C. (2000) Chronic toxic effects of quinolphos on some biochemical parameters in *Labeo rohita* (Hamilton), *Toxicol. Lett.* 3,114 (1-3): 11-8.

10. DAS, B. K., S. C. Mukherjee:(2000) A histopathological study of carp *(Labeo rohita)* exposed to hexachlorocyclohexane. *Vet. arhiv* 70, 169-180.

11. Dawood, N. (1986) Studies on the functional orgnization of the brain of rabbit,*Oryctalagus cuniculus* Ph.D thesis, University of Madras, India.

12. Durairaj and Selvarajan, V. R. (1992) Influence of quinalphos an organophosphorus pesticide,on the biochemical constituents of the tissues of fish, *Oreochromis mossambicus. J.Environ. Biol*. 13(3): 181-185.

13. Durairaj, S. and Selvarajan, V. R. (1992) Influence of quinalphos an organophosphorus pesticide, on the biochemical constituents of the tissues of fish, *Oreochromis mossambicus. J. Environ. Biol*. 13(3): 181-185.

14. Durairaj, S. and Selvarajan, V. R. (1992) .Influence of quinalphos an organophosphorus pesticide, on the biochemical constituents of the tissues of fish, *Oreochromis mossambicus. J. Environ. Biol.* 13(3): 181-185.

15. Gautam, R. K., Kalyana Gautam and Tejeshwarilal (2002) .Effects of pesticides on gastrointestinal nucleic acids in *Channa punctatus*. *J. Ecotoxicol. Environ*. Monit.12(1): 57-60.

16. Gracy A and Rajasekhar;Influence of cypermethrin on DNA and RNA ratio in gills of the freshwater fish *Channa striata*;Bioscience discovery,3:17-19 . *J Anim sci*, 38:1054-1070.

17. Health, D. F. (1961) In: Organophosphate Poisons, anticholinesterase and related compounds. p.186. Pergamon Press,London, New York.

18. Heath, A. G. (1987) .Water pollution and fish physiology. Boca Raton, CRC Press.

19. Heath, A. G. (1995). *Water pollution and fish physiology*. CRC. Press. Inc. Boca Raton, Florida.

20. Hester, R. E. Harrison, R. M. and David E. Kime.(1999) Environmentally induced endocrine abnormalities in fish,.ISBN**:** 978-0-85404-255-5 ,eISBN**:** 978-1-84755-074-3,**DOI:**10.1039/9781847550743-00027.

21. Malla Reddy, P. and Bashamohideen, Md. (1988) Toxic impact of fenvalerate on the protein metabolism in the branchial tissue of a fish, *Cyprinus carpio*. Curr. Sci**.** 57: 211-212.

22. Marc Andre ´ Meyers,, Po-Yu Chen, Albert Yu-Min Lin, Yasuaki.(2008). Seki Biological materials: Structur e and mechanical properties.,*Material science*.53:1-206

23. Maruthanayagam, C. and Sharmila, G. (2004) Biochemical variations induced by monocrotophos in *Cyprinus carpio* during the exposure and recovery period. *Nat. Environ. Poll*. 3(1): 1-9.

24. Mcilwain and Bachelard(1971).Biochemistry and the central nervous system 4th edition,Churchill Livingston,London.

25. Mukhopadhyay, P. K. and Dehadrai, P.V. (1980) Biochemical changes in the air breathing cat fish, *Clarias batrachus* (Linn.) exposed to malathion.*Environ.Poll*. 22(A): 149-158.

26. Muley, D. V., Kamble, G. B., Bhilave, M. P. (2000) Effect of heavy metals on nucleic acids in *Cyprinus carpio*. *J. Environ. Biol*. 21: 367–370.

27. Natarajan, G. M. (1981) Effect of lethal LC50 / 48 h Concentrations of metasystox on selected oxidative enzymes, tissue respiration and histology of gills of freshwater air breathing fish *Channa striatus*. **Curr. Sci.** 50(22): 985-991.

28. Patankar Nayana & Vaidya V.G. (1980) Evaluation of genetic toxicity of the insecticide phosphamidon using *in vitro* & *in vivo* mammalian test systems. *Indian J. Exp Biol*. 18: 1145-1147.

29. Pavlica, M., Papes, D., Nagy, N. (1991) 2, 4-Dichlorophenoxyacetic acid causes chromatin and chromosome abnormalities in plant cells and mutations in cultured mammalian cells. *Mut. Res*. 263: 77-81.

30. Pool-Zobel, B. L., Guigas, C., Klein, R., Neudecker, C., Renner, H. W., Schmezer, P. (1993)Assessment of genotoxic effects by lindane. *Food Chem. Toxicol*. 31: 271–283.

31. RavikiranK and .Kulkarni S,(2012). DNA AND RNA Content in some tissues of freshwater fish *notopterus notopterus* exposed to copper sulphate. *The Bioscan*,7(2) : 309-310, 2012.

32. Searchy and Maclinnis.(1970). Estimation of DNA & RNA standard method

33. Tayyaba, K., Hasan, M., Islam, F. and Khan, N. H. (1981) Organophosphate Pesticide metasystox induced regional alternation in brain nucleic acid metabolism. *Indian J. Exp. Biol.* 19: 688–690.

34. Tilak, K.S,P Wilson Raju, M S Butchiram,(2009) Effects of alachlor on biochemical parameters of the freshwater fish, Channa punctatus (Bloch). *Journal of Environmental Biology* 30(3):420-6.