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GENOTOXICOLOGICAL ASSESSMENT OF PYRETHROID INSECTICIDE BIOALLETHRIN IN FRESHWATER FISH *CHANNA PUNCTATUS*

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ABSTRACT: In this study micronucleus test was used to score the frequency of micronuclei in RBCs of fresh water fish *Channa punctatus* treated with bioallethrin. *Channa punctatus* were acclimatized in aquaria before experiments and divided in to control group and experimental group. Fishes of experimental group were exposed to sub lethal concentrations (0.0025ppm, 0.005ppm and 0.010ppm) of bioallethrin. The affected erythrocytes showed the occurrence of roundish micronucleus in the cytoplasm close to or completely free from the main nucleus. The results obtained reveal the differences in the frequency of micronuclei between these groups which are the clear indication of genotoxicity caused by bioallethrin in this fish.

KEYWORDS: Bioallethrin, Genotoxicity, Pyrethroid, Micronucleus test, *Channa punctatus*.

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1. INTRODUCTION

Fish is a valuable and easily accessible source of food. In the highly populated and developing countries like India, it can solve the ever-increasing demand of food for enormously growing population and the prevailing food scarcity. Fish provides high calorie diet to a large number of people all over the world but indiscriminate use of pesticides has reduced the fish growth and its nutritive value. Pesticides are extensively used to protect agricultural crops against the damages caused by pests. However, these chemicals may reach to lakes and rivers through rains and wind, affecting many

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other organisms away from the primary target. Only 0.1% reaches the specific target [1]. The significant increase of chemical emissions in the water resources has lead to harmful effects for aquatic organisms [2, 3]. A large proportion of these compounds are potentially genotoxic and carcinogenic to aquatic organisms [4, 5]. Recent evidence indicates that fish, are quickly becoming scarce. The interaction of genotoxic contaminants with DNA causes various genetic changes, which can be transmitted to the next generations [4, 6]. Fish are able to uptake and retain different xenobiotics dissolved in water. They can be used to detect pollutants released into their environment. Sub lethal concentrations of pesticides in aquatic environments cause structural and functional changes in aquatic organisms and this is more common than mortality [7]. The genotoxic effects of environmental pollutants can be monitored using a broad range of both in vitro and in vivo biomarker assays but the micronucleus test are gaining popularity over other assays due to their sensitivity for detecting cytogenetic and DNA damage and the short time needed to complete a study [8, 9, 10]. The formation of morphological nuclear abnormalities (NAs) was first described in fish erythrocytes by [11. NAs including blabbed nuclei (BL), lobed nuclei (LB), notched nuclei (NT) and binucleated cells (BN) have been used by several investigators as possible indicators of genotoxicity [12]. Micronucleus test (MNT) is a widely used cytogenetic technique for assessment of chromosomal damage induced by various genotoxicants. Schroder [13] first time studied the formation of micronuclei in mammalian bone marrow cells; subsequently this assay was developed by Schmidt [14] in mammalian systems. Like mammalian species, MNT has also been adopted to study genotoxicity in fishes. Fishes provide a suitable model for monitoring aquatic genotoxicity and waste water quality because of their ability to metabolize xenobiotics and accumulate pollutants [15] . Micronuclei in fish could be smaller in size than that suggested by Schmidt [14], because most fish chromosome are much smaller (e.g. 1/10 to 1/30 of the size of principal nucleus). The formation of micronuclei depends on fish species and target tissues and also on environmental conditions and the kind of pollution involved. Al Sabti and Metacalfe [10] have reviewed the literature on clastogenic effects of many chemicals and physical agents on fish cells, with emphasis on the induction of the micronuclei in teleost species. Svobodava *et al.* [16] studied the effect of Malachite green on *Cyprins carpio* by using micronuclei test. Campana *et al.* [17] evaluated genotoxicity of the pyrethroid λ-cyhalothrin using the micronuclei test in erythrocytes of the fish *Cheridon interruptus*. Sandra *et al.* [18] used MNT for the in situ mutagens in freshwaters in erythrocytes of *Barbus plebejus* from two natural environments. The present studies included the study of genotoxic effects of pesticides. It has provided valuable information about the changes in the genetic setup of the fish due to its exposure to pesticidal compounds which affect its overall development and in turn affect the human body, when taken as food.

2.MATERIALS AND METHODS

The common fresh water fish *Channa punctatus* were collected from the fresh water resources of Bareilly and adjoining places and acclimatized to laboratory conditions for few days in untreated soft water. After acclimation the fish were divided in to experimental and control groups. Control group of fishes was kept in an aquarium for few days in tap water and experimental fishes were exposed to sub lethal concentrations of Bioallethrin *i.e.* 0.0025ppm, 0.005ppm and 0.010ppm. Sampling was done at interval of 5, 10, 15, 20, 25 and 30 days at the rate of 5 fishes per duration. The blood samples were collected from the heart of the fish and a drop of blood from each fish was directly smeared on to the slides. Then the slides dried for 12hrs (overnight). Smears were subsequently fixed in absolute methanol for 10 minutes. After that the slides were stained in 6-10% Giemsa stain for 30 minutes. Then washed with DDW and air dried over night finally the slides were mounted with DPX. The frequency of micronucleation in erythrocytes was detected under binocular microscope (Olympus) using 100x oil immersion lens.

3. RESULTS AND DISCUSSION:

The persistence of pesticides in aquatic environment may be dangerous for the survival of fish [19, 20, 21]. The pesticides present in water reach the fish body directly through contact with the skin and gills and indirectly through water taken in with the food. They reach in blood circulation and finally accumulate in various organs of the body. Fishes are affected upon exposure to low level of pyrethroids. Due to liphophilic nature of them gills of fish can absorb these compounds even at very low concentrations in water. As the metabolizing ability of these compounds in the body of fish is very poor, they accumulate and prove to be lethal to fish. Some workers [22, 23, 24, 25, 26] have reported that toxicity of synthetic pyrethroids and some other pesticides to fish is dose and time dependent. Bioallethrin is a potent contact non-systemic and non-residual pesticide with rapid knockdown activity. It is highly toxic to fish. Its toxicity is more at lower temperature and thus more toxic to cold than warm water fishes. Nauck *et al.* [27] have reported that the toxicity of pyrethroids is least affected by pH of water and hardness.In present investigations micronucleated cells were scored at three sub lethal concentrations of 0.0025ppm, 0.005ppm and 0.010 ppm of bioallethrin. The results obtained are shown in Table 1.Micronucleus test is a test used to detect DNA damage. The DNA damage can be correlated with the clastogenecity of the mutagens. A large proportion of pesticides are potential toxic, genotoxic and carcinogenic to aquatic organisms [4, 5]. The interaction of genotoxic contaminants with DNA causes various genetic disturbances, which often are irreversible and can be transmitted to next generation [4, 6].

In the present study exposure to bioallethrin caused the formation of micronuclei in erythrocytes of *Channa punctatus* (Fig.1 and 2). The formation of micronuclei is an indication of the mutagenic

nature of these compounds. Results of present investigations show that the of micronuclei increased up to 10 days, after that the frequency of micronuclei started to decrease but even after 30 days of exposure the frequency was considerably higher than the frequency observed in control group of fishes.

The results obtained in this study clearly indicate the genotoxic effect of this compound in *Channa punctatus.*

Table-1: Percentage of micronucleated cells in blood of *Channa punctatus* exposed to sub lethal concentrations of Bioallethrin

Concentration of bioallethrin	**Period of exposure (in days)**						
	0	**5**	**10**	**15**	**20**	**25**	**30**
(Control) 0.0000ppm	0.0	0.01	0.03	0.05	0.07	0.11	0.19
0.010ppm	0.0	0.06	0.23	0.61	0.09	1.46	1.7
0.005ppm	0.0	0.14	0.82	1.36	1.85	2.18	2.81
0.0025ppm	0.0	1.28	1.97	2.74	3.26	3.81	4.1

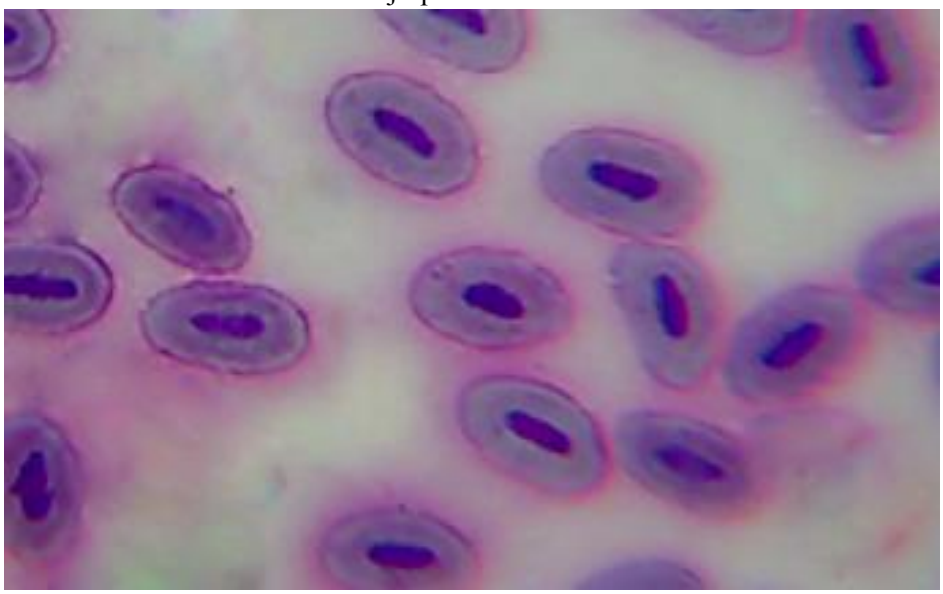

Fig 1. Photomicrograph showing erythrocytes in *Channa punctatus* before treatment of bioallethrin (control).

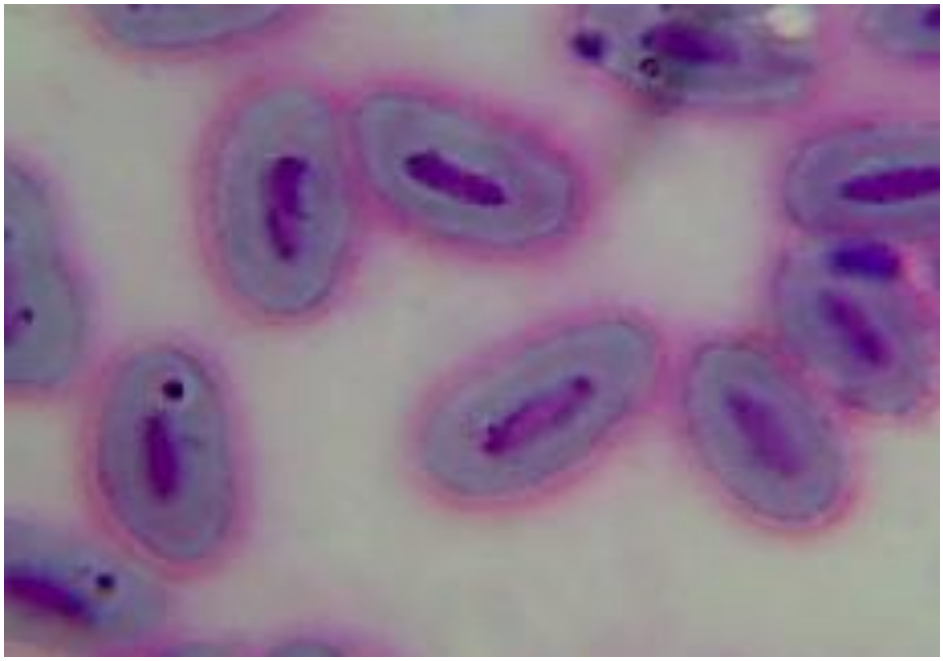

Fig 2. Photomicrograph showing micronucleated erythrocyte (MN) in *Channa punctatus* after treatment with 0.0025ppm.

4. CONCLUSION

The present findings indicate that micronucleus test is very useful for detecting the changes in the fish at genetic level. These studies also confirm the viability of this method as the powerful tool for measuring the relationship between genotoxicity and duration of exposure of fishes to pesticides and other pollutants. Therefore, this method can be successfully employed for *in vivo* genotoxic studies using fish as model species for environmental biomonitoring studies.

CONFLICT OF INTEREST

The authors declare that no competing financial interests exist.

REFERENCES

1. Rand G.M. and Petrocelli S.M. (1984). Fundamentals of Aquatic Toxicology: Methods and Applications. McGraw-Hill, New York, 666 pp.
2. Livingstone D.R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Bulletin of Marine Pollutants*, 42: 656-666.
3. Matsumoto S.T., Mantovani M.S., Malagutti M.I.A., Dias A.L. Fonseca, I.C. and Marin-Morales M.A. (2006). Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish, *Oreochromis niloticus* and chromosome aberrations in onion root-tips. *Genetics and Molecular Biology*, 29:148-158.
4. Jha, A.N. (2004). Review: Genotoxicological studies in aquatic organisms: an overview. *Mutation Research*, 552: 1-17.
5. Jha, A.N.(2008). Ecotoxicological applications and significance of the comet assay. *Mutagenesis*, 23(3):207-221.
6. Depledge, M.H. and Fossi, M.C. (1994). The role of biomarkers in environmental assessment (2).Invertebrates. *Ecotoxicology*, 3: 161-172.
7, Sancho, E., Fernandez-Vega C. Ferrando, M.D. and Andreu-Moliner E. (2003). Eel ATPase activity as biomarker of thiobencarb exposure. *Ecotoxicology and Environmental Safety*, 56:434-441.
8. Andrade, V.M., de Freitas T.R. and de Silva, J. (2004). Comet assay using mullet (*Mugil* sp.) and sea catfish (*Netuma* sp.) erythrocytes for the detection of genotoxic pollutants in aquatic environment. *Mutat. Res.*; 560:57-67.
9. Ateeq, B., Abul Farah, M. and Ahmad, W. (2005). Detection of DNA damage by alkaline single cell gel electrophoresis in 2,4-dichlorophenoxyacetic-acid- and butachlor-exposed erythrocytes of *Clarias batrachus. Ecotoxicol. Environ. Saf.*; 62:348-354..
10. Al-Sabti, K. and Metcalfe, C.D. (1995).Fish micronuclei for assessing genotoxicity in water. *Mutat.Res.*; 343:121-135.
11. Carrasco K., Tilbury, K L, and Myers M S (1990).Assessment of the piscine micronucleus test as an *in situ* biological indicator of chemical contaminant effects. *Can. J. Fish Aquat. Sci*.; 47:2123-

2136.

12. Cavas, T., and Ergene-Gozukara S. (2005). Micronucleus test in fish cells: a bioassay for *in situ* monitoring of genotoxic pollution in the marine environment. *Environ. Mol. Mutagen.*; 46: 64-70.
13. Schroder, T. M. (1966) Cytogenetische und cytologische befunde bei enzymopenischen panmyelopathienund pancytopanien (Familiare panmyelopathien fanconi glutathionreduktasema ngel-anamie und megalobelastare Vitamin B). *Humangenzetik.* 2, 287-316.
14. Schmid W. (1975).The micronucleus test. *Mutat. Res.* 31(1):9-15.
15. Grisolia, C. K. and Cordeiro, C. M. T. (2000) Variability in micronucleus induction with different mutagens applied to several species of fish. *Genet. Mol. Biol.* 23, 235-239.
16. Svobodova, Z., Flaj Shan, M., Vykusova, B. and Machova, J. (1997.) The effect of long term therapeutic batch of malachite green on common carp *(Cyprinus carpio.) Acta Veterinaria Brno Acta Vet. Brno.* 66 (2), 111-117.
17. Campana, M. A., Panzeri, A.M., Moreno, V.O. and Dulout, F. M. (1999). Genotoxic evaluation of the parathyroid λ- cyhalothrin using the micronucleus test in erythrocytes of the fish *Cheirdon interrtuptus. Mutat. Res. Genet. Toxicol. Environ. Mutat.*; 438 (2):155-161.
18. Sandra, S., Cicotti, E. and Rizzoni, M. (1996). Micronucleus test in erythrocytes of *Barbus plebejus* (Teleostei, Pisces) from two natural environments; a bioassay for the in- situ detection of mutagens in freshwaters. *Mutat. Res.* 367, 141-149.
19. Johnson D.W. (1968). Pesticides and Fishes: A review of selected literature. *Tram. Amer. Fish Sod.* 97: 398-424.
20. Saunders J.W. (1969). Mass mortalities and behaviour of brook trout and juvenile Atlantic salmon in a stream polluted by agricultural pesticides. *J. Fish Res. Ed. Canada.* 26: 695-699.
21. Chowdhary, B. P., Pandey, P.K. and Dubey, N. K. (1981). Effect of malathion on the behaviour and body composition of *Heteropneustes fossilis. J. Environ. Biol.* 2: 21-36.
22. Khan, N.Y. (1983). An assessment of the hazard of synthetic pyrethroid insecticies to fish and fish habitat, (In: *Pesticide chemistry, Human welfare and Environment,* Miyamoto, J. and Kearney, *P.C. Eds.)* 3: 437-450.
23. Kulshrestha, S.K., Srivastava R.S. and Arora, N. (1984).Toxicity of two pesticides to the kidney of a fresh water teleost *Channa striatus. Proc. Sem. Eff. Ag. Fam.* 63-70.

24. Jagan, P. M., Reddy, S. and Rao, A. P. (1989). Effect of certain insecticides on the fresh water fish *Cyprinus carpio* (L) *J. Environ. Biol.* 10: 35-13.

25.Saxena, K.K. and Seth, N. (2002). Toxic effect of cypermethrin on certain hematological aspects of fresh water fish *Channa punctatus. Bull. Environ. Contam.Toxicol.*64: 364-369.

26. Saxena, K.K. and Sirohi, V. (2005). Effect of λ-cyhalothrin on the metabolism of fresh water fish *Channa punctatus. Vegetos* 8: 135-138.

27. Nauck, W.L., Olsen, L.E. and Marking, L.L, (1976). Toxicity of natural pyrethrins and five pyrethroids to fish, *Arch. Environ. Contam.Toxicol.*4: 18-29.