Study of genotoxicity caused by bioallethrin in the freshwater fish *Channa punctatus*

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ABSTRACT

In this study genotoxicity of bioallethrin, a synthetic pyrethroid, was evaluated in kidney cells of *Channa punctatus* by using chromosomal aberration test system. *Channa punctatus* were acclimatized in aquaria before experiments and divided into control group and experimental group. Fishes of experimental exposed to sub lethal concentrations (0.0025ppm, 0.005ppm and 0.010ppm) of bioallethrin. Fishes were sacrificed after 5, 10 and 15 days. Kidney tissues were taken out from sacrificed fishes and Chromosomal spreads were prepared by standard techniques.

Key words: Bioallethrin, genotoxicity, pyrethroid, *Channa punctatus*

INTRODUCTION

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 other organisms away from the primary target.

In an ideal situation, a pesticide should fall exactly on the target and get degraded completely into harmless compounds. As per the estimate of Piemental and Leviton (1986) only less than 1% of the pesticides used reaches target and the remaining part spreads in the surroundings which in turn affects the non target organisms and cause pollution in the environment.

Fish provides a suitable model for monitoring aquatic genotoxicity because of their ability to metabolize xenobiotics and accumulate pollutants (Grisolia and Cordeiro, 2000). Fishes can also respond to mutagens at low concentration of toxicants in a manner similar to higher vertebrates (Al-Sabti and Metcalfe, 1995). The changes caused by genotoxins in the genetic material can be detected in fish at specific level by using various genotoxic assay. In fish kidney chromosomes the chromosome aberration test (CAT) is most suitable to study the genotoxic effect of pesticides. Synthetic pyrethroids are reported (Saxena and Seth, 2002; Seth and Saxena, 2003, Sirohi and Saxena, 2006 and Saxena and Sirohi, 2007) to be highly toxic to fish because of these compounds are strongly absorbed by the gills even at very low concentration in water due to their high lipophilicity. These compounds possess moderate mammalian toxicity, but they are extremely toxic to fish and aquatic invertebrates (Eisler, 1992). The study of genotoxic effect of pesticides and other pollutants in fishes is very important. Some workers (Rishi and Grewal, 1995; Arokia-Rita and Selvanayagam, 1998.

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.

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.

MATERIAL AND METHOD

Channa punctatus, a freshwater teleost fish was collected from the freshwater 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 for a period of 15 days and sampling was done after 5, 10 and 15 days at the rate of 5 fishes per exposure period.

Chromosomal preparations were made according to the method of Ojima (1982), Nagpure and Barat (1997) and Asano et al. (1998). After exposure to bioallethrin, the injection of 0.05% Colchicine (1ml per 100 gm body wt.) was given to the fishes, and they were left for 2 hrs. After this time duration the kidneys were excised from the treated fishes and were immediately transferred to separate Petri dishes containing freshly prepared 0.56% KCl (hypotonic solution). After processing the tissues, the suspension was prepared and centrifuged. After discarding the supernatant, remaining part was fixed with methanol acetic acid fixative. These slides were prepared by dropping method and after staining with Giemsa stain cells were covered with D.P.X. The chromosomes were observed by using 100x oil immersion microscope and their photographs were taken.

RESULTS AND DISCUSSION

The number of chromosomes in *Channa punctatus* was found to be 32. Out of these chromosomes the number of metacentric, submetacentric, acrocentric and telocentric chromosomes was 14, 8, 4 and 6 respectively. The number of structural chromosomal abnormalities were carefully observed.

During exposure to sublethal concentrations of bioallethrin (0.0025ppm, 0.005ppm and 0.010ppm), different structural chromosomal abnormalities were observed in kidney cells of *Channa punctatus*.

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.*, (1976) have reported that the toxicity of pyrethroids is least affected by pH of water and hardness. In respect of chromosomal aberrations, the observations in the present study revealed chromatid breaks, chromosome breaks, chromatid separations, fragmentation, condensation, acentric fragments, sticky plates and ring chromosomes which essentially confirm the findings of other reported in *Channa punctatus* treated with Dichlorovos (Rishi and Grewal, 1995) and *Oreochromis mossambicus* treated with pyrethroid fenvalerate (Arokia Rita and Selvanayagam, 1998). Manna (1984) reported that many chemicals produce similar aberrations though their mechanism may be different in

different cases.

The results of present study indicate that chromosomal aberrations in the kidney cells of *Channa punctatus* the aberrations increased for some period in the beginning of exposure and then decreased gradually. The dose dependent enhancement of chromosomal aberrations was also reported by Rishi and Grewal, (1995) who found regular decline in frequency of each type of aberration in *Channa punctatus* with increase in exposure period. The studies on genetic toxicity and mutagenecity synthetic pyrethroids indicated contradictory results, depending on the type of assay involved. The present findings suggest that pyrethroids interfere with cellular activities in fishes, even at genetic level including chromosomal aberrations.

The present work includes the study of toxicity assessment of synthetic pyrethroids bioallethrin on *Channa punctatus.* Definite changes in behaviour and genetic setup have been observed in present study.

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

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Table-1: Analysis of Chromosomal abnormalities induced in kidney cells of *Channa punctatus* after exposure to 0.010ppm of Bioallethrin

Time of exposure (in days)	No. of animals used	Total no. of spreads studied	Metaphase with chromosomal abnormalities	% of spread with chromosomal abnormalities	No. of spreads with three or more aberrations	No. of spreads with two aberrations	No. of spreads with one aberration	χ^2 calculated	$\chi^2_{(2)}$ 0.05	$\chi^2_{(2)}$ 0.01
Control	05	50	02	04	00	01	01	3.61*	5.99	9.21
5	05	50	11	22	04	04	03	3.43	5.99	9.21
10	05	50	23	46	16	02	05	2.35	5.99	9.21
15	05	50	16	32	11	03	02	0.22	5.99	9.21
20	05	50	28	56	14	09	05	2.50	5.99	9.21
25	05	50	31	62	26	02	03	5.94	5.99	9.21
30	05	50	36	72	22	10	04	1.27	5.99	9.21

* Significant at 5% level ($p \leq 0.05$), χ^2 calculated < $\chi^2_{(2)}$ 0.05. Therefore, null hypothesis is accepted, and the changes are significant at $\chi^2_{(2)}$ 0.05 and $\chi^2_{(2)}$ 0.01 levels

Table-2: Analysis of Chromosomal abnormalities induced in kidney cells of *Channa punctatus* after exposure to 0.005 ppm of Bioallethrin

Time of exposure (in days)	No. of animals used	Total no. of spreads studied	Metaphase with chromosomal abnormalities	% of spread with chromosomal abnormalities	No. of spreads with three or more aberrations	No. of spreads with two aberrations	No. of spreads with one aberration	χ^2 calculated	$\chi^2_{(2)}$ 0.05	$\chi^2_{(2)}$ 0.01
Control	05	50	04	08	02	01	01	0.49*	5.99	9.21
5	05	50	12	24	06	03	03	1.48	5.99	9.21
10	05	50	19	38	10	07	02	1.00	5.99	9.21
15	05	50	26	52	16	05	05	0.46	5.99	9.21
20	05	50	33	64	23	06	04	0.28	5.99	9.21
25	05	50	40	80	29	06	05	0.94	5.99	9.21
30	05	50	45	90	31	08	06	0.25	5.99	9.21

* Significant at 5% level ($p \leq 0.05$) , χ^2 calculated < $\chi^2_{(2)}$ 0.05. Therefore, null hypothesis is accepted, and the changes are significant at $\chi^2_{(2)}$ 0.05 and $\chi^2_{(2)}$ 0.01 levels

Table-3: Analysis of Chromosomal abnormalities induced in kidney cells of *Channa punctatus* after exposure to 0.0025ppm of Bioallethrin

Time of exposure (in days)	No. of animals used	Total no. of spreads studied	Metaphase with chromosomal abnormalities	% of spread with chromosomal abnormalities	No. of spreads with three or more aberrations	No. of spreads with two aberrations	No. of spreads with one aberration	χ^2 calculated	$\chi^2_{(2)}$ 0.05	$\chi^2_{(2)}$ 0.01
Control	05	50	04	08	01	02	01	3.61*	5.99	9.21
5	05	50	18	36	10	05	03	3.43	5.99	9.21
10	05	50	27	54	16	06	05	2.35	5.99	9.21
15	05	50	33	66	25	06	02	0.22	5.99	9.21
20	05	50	40	80	28	08	04	2.50	5.99	9.21
25	05	50	56	112	34	13	09	5.94	5.99	9.21
30	05	50	62	124	39	17	06	1.27	5.99	9.21

* Significant at 5% level ($p \leq 0.05$) ,χ^2 calculated < $\chi^2_{(2)}$ 0.05. Therefore, null hypothesis is accepted, and the changes are significant at $\chi^2_{(2)}$ 0.05 and $\chi^2_{(2)}$ 0.01 levels