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Research Article

EVALUATION OF BIFENTHRIN GENOTOXICITY ON *CHANNA PUNCTATUS*

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ABSTRACT

The frequency of chromosomal damage in *Channa punctatus* by using chromosomal aberration test when exposed to synthetic pyrethroid bifenthrin was investigated. The frequency was examined in metaphase spreads obtained from fishes exposed to different sublethal concentrations (0.016, 0.0347 and 0.0628 ppm) of bifenthrin. Control fishes were kept in tap water. Chromosomal preparations were made from kidney cells of *Channa punctatus* after 5, 10 and 15 days exposure. Different types of aberrations like chromosomal gap, sticky plates, chromatid separation and breaks were observed in the cells of fishes exposed to bifenthrin. These aberrations were found significantly higher as compared to that of control and were statistically significant at ($p<0.05$) level. These findings obviously indicated that bifenthrin caused genotoxicity in *Channa punctatus.*

Keywords: Genotoxicity, Bifenthrin, Chromosomal abnormalities, *Channa punctatus.*

INTRODUCTION

Bifenthrin is a member of the Pyrethroid family. Pyrethroids are synthetic analogues of natural pyrethrins which were originally derived from East African chrysanthemum flowers reported to possess insecticidal activity. Beginning in 1970s, synthetic pyrethroids came into the market for agricultural purposes to protect food grains and other agricultural products against pests and, later, used to control animal ectoparasites. Their use has increased rapidly in the past two decades (Bradbury and Coats, 1989a, 1989b; Wardhaugh, 2005). Pesticides have very important role in agriculture and in hygiene. On the other hand they are dangerous for the environment, nature and for the human beings. It is an important group of environmental pollutants and many of which are reported to be mutagenic (Sandhu *et al.* 1985; Waters *et al.*, 1982).

Bifenthrin is hardly soluble in water, so nearly whole of the bifenthrin will stay in the sediment, but it is very harmful for the aquatic life. Even in small concentrations, fish and other aquatic animals are affected adversely by bifenthrin. One of the reasons for the high sensitivity of fish is because of its slow metabolism. Bifenthrin will stay longer in the system of the fish. Another reason for the high sensitivity of fish is the effect of bifenthrin as ATPase-inhibitor. The gills need ATP to control the osmotic balance of oxygen. If the fish is not capable of taking up oxygen because ATP can no longer be used, the fish will die. In cold water, bifenthrin is even more dangerous.

Bifenthrin is an insecticide used for the control of termites and borers in timber, insect pests in agricultural crops and turf, and also for the control of spiders, ants, fleas, flies and mosquitoes (Australian Pesticides and Veterinary Medicines Authority, 2008). It is a third generation synthetic pyrethroid and it has low water solubility but binds strongly to sediment and has a relatively long environmental persistence (field dissipation half-life up to 345 days). It is extremely toxic to terrestrial and aquatic insects, crustaceans and fish, disabling the central and peripheral nervous systems by interfering with the sodium channels (Johnson *et al.*, 2010). It is more toxic to aquatic than terrestrial organisms because it inhibits enzymes required for osmoregulation and the maintenance of ionic balance in an aquatic environment, and is readily absorbed by gilled animals (Boyd *et al.*, 2002).

Fishes are excellent subject for study and monitoring of aquatic genotoxicity as they metabolize xenobiots and accumulate pollutants (Grisolia and Cordeiro, 2000). They can also respond to mutagens at low concentration of toxicants in manner similar to higher vertebrates (Goksoyr *et al.*, 1991; Al-Sabti and Metcalfe, 1995).

Chromosomal aberration analysis has been demonstrated as sensitive indicator of DNA damage caused by environmental pollutants by several workers using aquatic models (Klingerman *et al.* 1975; Alink *et al.*, 1980; Hooftman, 1981; Harrison *et al.*, 1986; Saxena and Rana, 2005; Yadav and Trivedi, 2006).

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The study of genotoxic effects of pesticides and other pollutants in fishes is very important as genotoxicity may affect the heredity process in fishes and in turn may affect the whole race and overall production of fishes.

The present studies include the study of genotoxic effects of pesticides. This study has provided valuable information about the changes in the genetic setup of the fish due to its exposure to pesticidal compounds which may affect its overall development and in turn may affect the human body, when taken as food.

MATERIALS AND METHODS

The fresh water fish *Channa punctatus* (Bloch, Family: Channidae and Order: Channiformes) was collected in living condition from local water bodies. The fishes were acclimatized for one week under laboratory conditions before exposing them to various concentrations of bifenthrin. After acclimation the fishes were divided into experimental and control groups. The experimental group of fishes was exposed to three sub lethal concentrations of bifenthrin for a period of 15 days. Tissue sampling was done after 5, 10 and 15 days at the rate of five fishes per exposure period. On each sampling day kidney were collected and immediately processed for chromosomal aberration test.

Chromosomal slides were prepared according to the method of Ojima (1982) and Asano *et al.* (1998). After exposure, the fishes were injected with 0.05% Colchicine (1 ml per 100 gm body wt.). These fishes were left for 2 hrs. After injection of colchicine fish specimen were anesthetized with clove oil and dissected to take out the kidney tissues and immediately transferred to petridishes containing freshly prepared 0.56% KCl (hypotonic solution) for swelling. The hypotonic action was stopped by adding 1.0 ml freshly prepared chilled Carnoy's fixative (Methanol acetic acid 3:1 ratio) slowly and cell suspension was centrifuged at 1200-1500 rpm for 10 min. After discarding the supernatant, chilled fixative was added to the tube and kept in refrigerator and after 30 min centrifuged for 10 min. The process was repeated 12-13 times till clear transparent cell suspension was obtained. Small quantity of cells suspension was taken in a Pasteur pipette and dropped on clean dry slide from a height of 1-1.5 feet. The slide was allowed to air/flame dry, stained with Giemsa stain and finally mounted with DPX. The slides were carefully observed under microscope and metaphase spreads were studied to identify chromosomal aberrations.

RESULTS

In *Channa punctatus* 32 chromosomes were observed in diploid cells in both sexes. No sex chromosomes were observed in this fish species (figure 1). Out of these 32 chromosomes the number of metacentric, submetacentric, acrocentric and telocentric chromosomes was 14, 8, 4 and 6 respectively.

The frequency of chromosomal aberrations like chromosomal gap, sticky plates, chromatid separation and breaks developed due to exposure to bifenthrin was observed in kidney cells of *Channa punctatus* after exposure to three sublethal concentrations i.e. 0.0161 ppm, 0.0347 ppm and 0.0628 ppm of the bifenthrin. Changes in chromosomes of *Channa punctatus* were observed after 5, 10 and 15 days of exposure which are shown in table 1 and figure 2. These aberrations were found significantly higher as compared to that of control and were statistically significant at ($p<0.05$) level.

Table 1. Frequency of Chromosomal aberrations induced by bifenthrin in kidney cells of *Channa punctatus.*

Conc. of Bifenthrin ppm)	Exposure period (days)	No. of fishes dissected	No. of Metaphase spreads studied	Total no. of Metaphase spreads with abnormalities	% of Metaphase spread with chromosomal Abnormalities
Control	0	5	50	0	0
	5	5	50	1	2
	10	5	50	2	4
	15	5	50	1	2
0.0161	5	5	50	2	4
	10	5	50	7	14
	15	5	50	11	22
0.0347	5	5	50	2	4
	10	5	50	9	18
	15	5	50	18	36
0.0628	5	5	50	5	10
	10	5	50	11	22
	15	5	50	26	52

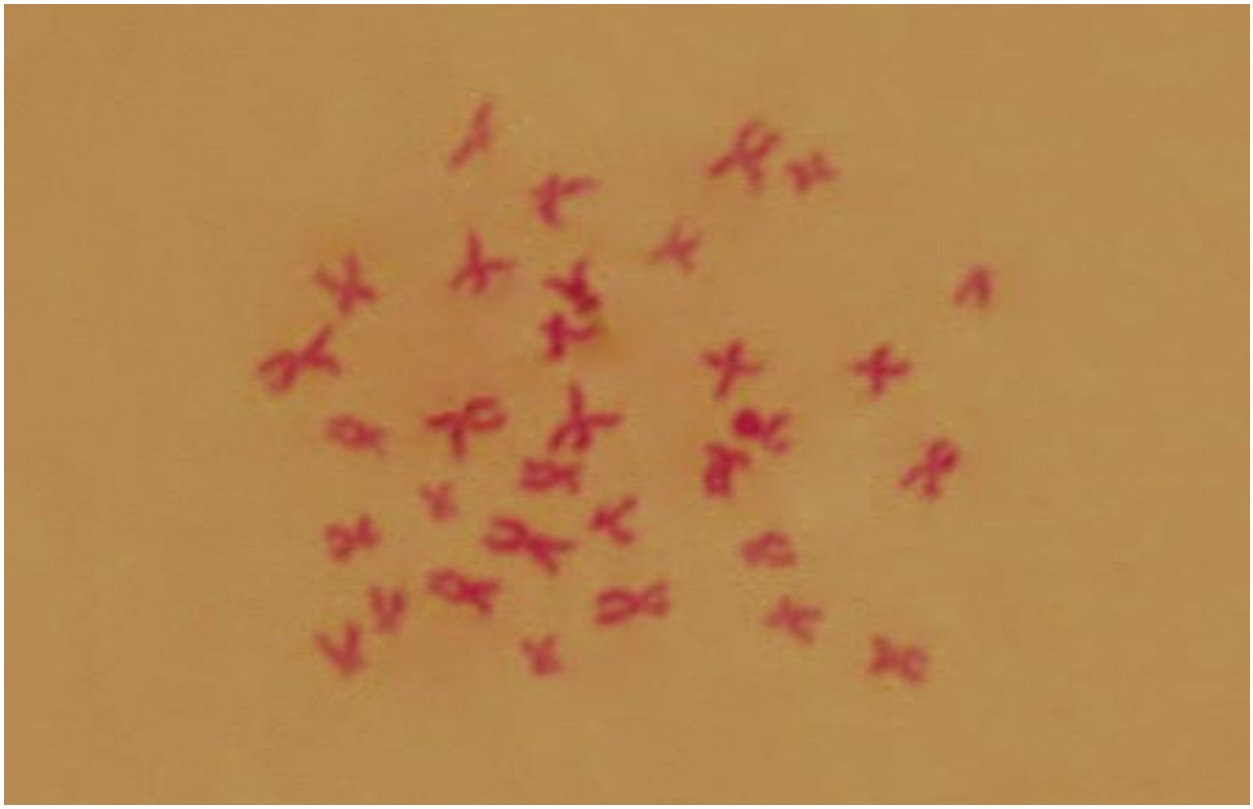

Figure 1. Normal metaphase spreads of kidney cells of *Channa punctatus* (Control).

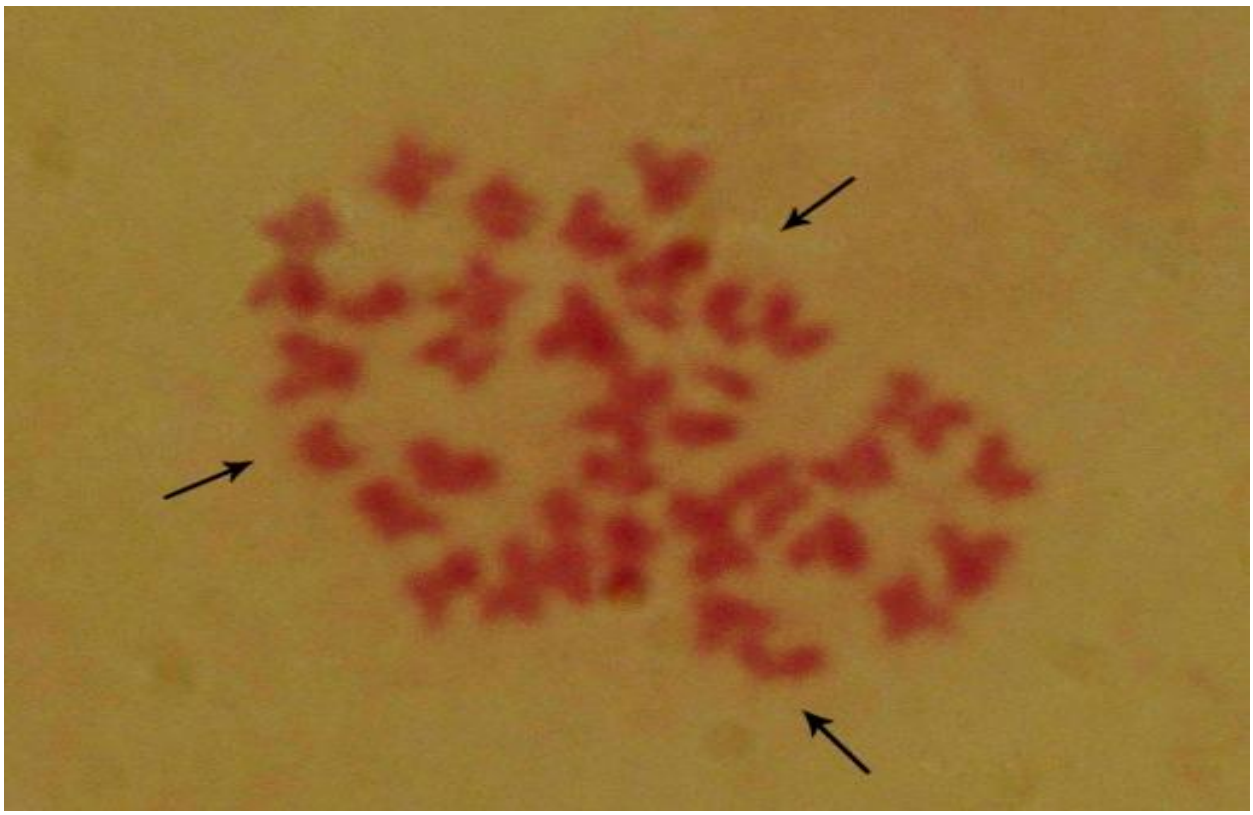

Figure 2. Changes in the chromosomes of kidney cells of *Channa punctatus* exposed to bifenthrin (changes are marked by arrows).

DISCUSSION

The chromosomes observed in present studies in *Channa punctatus* are similar to previous findings (Manna, 1983; Mathew and Jahageerdar, 1989). All the doses of bifenthrin increased the number of structural chromosomal aberrations. Exposure to sublethal concentrations of bifenthrin caused structural changes in chromosomes in *Channa punctatus* of experimental group. Present findings are similar to the findings of Rishi and Grewal (1995) who examined the effect of dichlorovas on fishes. The structural chromosomal aberrations were significantly higher than compared to control group at all the three exposure periods. It was found that if the exposure time is increased the percentage of structural chromosomal aberration (SCA) is also increased.

The results of the present study revealed that bifenthrin can cause genetic damage, when present in water even at sub lethal concentration. The results also indicate that the frequency of chromosomal aberrations in the fish serve as a tool to assess the existence of genotoxic pollutants.

CONCLUSION

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

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