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ORIGINAL ARTICLE

Genotoxic Effect of Cypermethrin in Muscles of *Channa punctatus*

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ABSTRACT

The speedy development of various sectors, industrial development in particular as a modern progress without a proper strategy, their effluent that have been reported to be the prime cause of environmental pollution. The ill-effects of which are constantly being faced by the inhabitants particularly the animal species, as it is most unfortunate that ecological and sociological problems are not often carefully considered and addressed properly while designing and locating many of the industrial projects, power generation plants, water dams, reservoirs and in the development and urbanization. The present study deals with genotoxic study in muscles of Channa punctatus.

Key words: *Genotoxic Profile, Muscle Channa punctatus, cypermethrin*

INTRODUCTION

Pyrethroids play an important role in modern agriculture by providing dependable, persistent and relatively complete control against harmful pests and insect with less expense and effort. They have no doubt, increased crop yield by killing different types of pests and insect. However, this positive trend in increased crop yield from use of pyrethroids has negative ramification for fish and other aquatic life inhabiting ponds, lakes, river, streams and other similar water bodies. The poisoning by pesticides from agricultural field is a serious water pollution problem and its environmental long term effects may result in the incidence of poisoning of fish and other aquatic life forms (Jyothi and Narayan, 1999). Pesticides toxicity in fishes has been investigated by several workers like Shafiq-ur-Rahman (2006), Velisek, *et al*. (2006) highlighting the extent of toxicity in fishes.

The toxicity of pesticides in the different organisms varies with the type of pesticide and the target organism. Some pesticides are extremely toxic to fish at a very low concentration and to aquatic invertebrates at even lower concentration. Some of those kill the organism relatively quicker while others have gradual effects on activity, feeding, general physiology and reproduction. In the former case the effect is quite obvious due to high mortality of organism while in later case it is difficult to detect in the beginning and effects are visible in the long term only. So it is necessary to assess the level of toxicity of a particular pesticide against the target species to make guidelines for safe use of pesticide.

The selection of synthetic pyrethroid (cypermethrin) in the present investigation is based on the fact that it is very common pesticide to control domestic as well as agricultural pests and is very toxic for fish and aquatic invertebrates. The toxicity ratio of the pyrethroids is much higher than that of the major classes of insecticides and this feature has promoted the wider application of pyrethroids in virtually all programs of insect control. In the last decade great progress have been made in development of more stable and highly active pyrethroids and in near future more widespread application of these compounds may be expected. Pyrethroids when applied on crop they also leached into soil and reach to water bodies. In water bodies they affect the aquatic life including fishes. The edible fishes are consumed by human and other livings. So they reached to next trophic level by biotransformation and causing adverse effects to living.

Genetic toxicity investigates the interaction of chemical and physical agents with genetic material in relation to subsequent adverse effect such as genetic disease in future generations. Those substances which produced alterations in genetic material at non-lethal, non-cytotoxic

concentrations are classified as 'genotoxins' changes in the genetic material due to the effect of genotoxic chemicals generally represent the first step (initiation) of the process of chemical mutagenesis/carcinogenesis. These changes in the genetic material of organisms can be detected at specific level by using various genotoxicity assay systems like Ames test, chromosomal aberrations, sister chromatid exchange, micronucleus assay and comet assay having different end points. These assays can detect relatively greater damage to genetic material manifested at cellular and chromosomal and DNA level. Genetic toxicology evolved from the initial studies of gene mortality demonstrated first by Muller in 1927 using radiation (Muller, 1927) followed almost 20 years later by Aurbach, *et al*. (1947) using chemicals. Both of these investigations concluded their studies using genetic changes in animals by radiation and chemicals were demonstrated. This work created some awareness that some of the 'hereditary disease' observed in human population might be environmental in origin. The period of time from 1953 to 1986 might be considered the first 'golden era' of molecular genetics. During this time, much of the basic information were developed regarding DNA structure and replication, the genetic code, mechanism of protein synthesis and reigned over this golden era and several received Nobel prizes for their contributions.

The muscles are the main edible parts of fish. Fish muscles provide proteins, fats and vitamins (A and D), a large amount of phosphorous and other elements are also present in it. The white muscles are highly nutritive and edible part of fish and on the other hand most of the pesticides are lipophilic in nature and stored in fish muscles.

At molecular level, primary DNA damage was quantified as strand breaks using the single cell gel electrophoresis assay (Comet assay) applied to fish erythrocytes. This work highlights the cypermethrin treatment can represent a genotoxic threat to fish. Therefore in the present study the effect of toxicity of these insecticide on biochemical and genotoxic alteration in white muscles and gills of a fish *Channa punctatus* have been observed.

In present study the aim is to assess effect of insecticide cypermethrin on a live fish *Channa punctatus*. The toxic effect was assessed based on the results of acute, sub-lethal, chronic testes and biochemical and the chief objective has been to notice DNA and RNA damage examination of fresh water fish *Channa punctatus* after exposure of insecticide on white muscles.

MATERIALS AND METHODS

COLLECTION OF MATERIAL AND TREATMENTS FOR LABORATORY EXPERIMENTS:

The live specimen of *Channa punctatus* commonly known as 'soli' were brought for the present study from ponds in surrounding vicinity of Agra and fish market of Agra. The selection of *Channa punctatus* as experimental fish went in for reason of its easy availability, its hardy nature in terms of survival despite pollutant treatments proposed which might indicate an advantage of long stay of toxic effects in soft tissues. Above all, fish has an economic food value. For experimental purpose fishes almost of the same size and weight so as to refer to similar age group as constant factor were used for noticing effects of treatments by several insecticides. The fishes were washed in 0.1% $KMnO_4$ solution to smear dermal infection if any. Then they were washed with ordinary water and smeared to aquaria filled with water. The latter was already equipped with sand and *Hydrilla* plants, overcrowding was avoided. The fishes were fed with readymade fish food after every 24 hrs. The water was changed to smear the faecal matter and excess food after every 24 hrs. If any mortality occurred the fish was removed immediately to avoid depletion of oxygen. Normally, the fish to be used for experiments were left for fifteen days. So they might acclimatize to the prevailing ecological conditions. For the analysis of insecticide toxicity, insecticide was used in commonly occurring chemical compound cypermethrin 25%EC is a synthetic pyrethroid insecticide.

Test compound	:	Cypermethrin 25%EC
CAS number	:	52315-07-8
Trade name	:	Super killer
Chemical formula	:	$C_{22}H_{19}Cl_{12}NO_3$
IUPAC number	:	(R,S)-alpha-cyano-3-phenoxylbenzylI(IRS)-cis,trans-3-(2,2dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate

Cypermethrin 25%EC is a synthetic pyrethroid insecticide used to control various pests.
The diluent water that was used for keeping experimental fishes was subjected to analysis for various physico-chemical characteristics as per procedure given in 'APHA (2000) standard methods for the examination of water and waste water'. The following data shows the physico-chemical parameters and their average values.
Nucleic Acid Estimation
The nucleic acid content (separately for DNA and RNA) was estimated by the method described by Burton (1956) and its further modification by Gendimaniko, *et al*. (1988) with some modifications. The tissues homogenized with ice cold 10% trichloroacetic acid and were centrifuged at 3000rpm. They were resuspended and recentrifuged. The precipitate was suspended in ethanol-ether mixture and centrifuged. Sodium hydroxide was added to the precipitate, mixed well and was left for eighteen hours at 37^0C. The supernatant containing protein and RNA was separated after centrifugation from precipitate containing protein and DNA.

1. DNA ESTIMATION:
The precipitate having most of DNA with some protein was suspended in perchloric acid, was heated in boiling water bath for one hour and then centrifuged at 300rpm. Supernatant was made up to known volume and freshly prepared diphenylamine reagent was added to it and heated in boiling water bath. It was cooled and extinction was read at 595nm against water as blank. Calf thymus DNA (Sigma Chemical Company, LTd, USA) solution (100mg/g) was used for standard graph.
Calculations were done as per following formula-

$$\text{DNA(mg/g)} = \frac{\text{Concentration of DNA from the standard graph}}{\text{Weight of tissue used for DNA separation}} \text{ x dilution}$$

2. RNA ESTIMATION:
The supernatant having most of RNA with some protein in hydrolyzed form was mixed with equal volume of 10% trichloroacetic acid (TCA) and was centrifuged at 3000rpm. The supernatant was made up to known volume for further estimation. Orcinol reagent was mixed to it and the tubes were kept in boiling water bath. It was cooled and extinction was read at 665nm against an orcinol blank. Pure yeast RNA (Sigma Chemical Company, LTd, USA) solution (100mg/g) was used for standard graph (Fig. 4).
Calculations were done as per following formula-

$$\text{RNA(mg/g)} = \frac{\text{Concentration of RNA from the standard graph}}{\text{Weight of tissue used for RNA separation}} \text{ x dilution}$$

3. DNA/RNA RATIO:
The DNA/RNA ratio was calculated by the following formula-

$$\text{DNA/RNA} = \frac{\text{Concentration of DNA}}{\text{Concentration of RNA}}$$

STATISTICAL CALCULATIONS:
In the present investigation, the formulae were used for different statistical calculations after Fischer and Yates (1950) using statistical software.

RESULTS AND DISCUSSION

The genetic constituents DNA and RNA with their ratio show altered results after treatment. DNA and RNA contents have been decreased with increased duration of exposure, however decrease was more in gill tissue and at recovery it comes to normal level. Similar to the present findings,

Schill (2008) revealed RNA-DNA ratio as an index of larval fish growth in the sea. These data demonstrate the importance of food availability in larval fish mortality and suggest that short-term growth under favorable conditions may be considerably higher than expected from long-term indicators. RNA-DNA ratio analysis offers new possibilities for understanding larval growth and mortality, and their relation to environmental variability.

Table 1: DNA (µg/dl) in white muscle of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

S.No.	Experimental set	No. of Fishes	(Mean±S.E.)
1.	Control	5	58.35±0.92
2.	Acute (4 days)	5	52.00±0.65 [a]
3.	Sub-lethal (20 days)	5	43.33±0.67 [b]
4.	Chronic (45 days)	5	40.13±0.52 [c]
5.	Recovery	5	59.50±0.22 [a]

Table 2: RNA (µg/dl) in white muscle of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

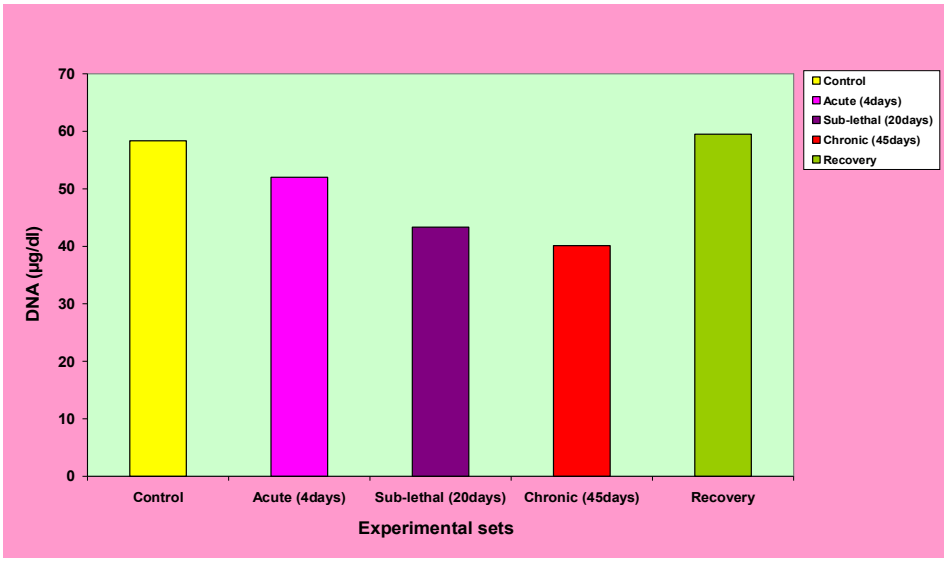
S.No.	Experimental set	No. of Fishes	(Mean±S.E.)
1.	Control	5	48.69±0.13
2.	Acute (4 days)	5	46.32±0.15 [a]
3.	Sub-lethal (20 days)	5	40.31±0.20 [a]
4.	Chronic (45 days)	5	34.25±0.13 [b]
5.	Recovery	5	48.00±0.10 [a]

Table 3: DNA/RNA ratio in white muscle of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

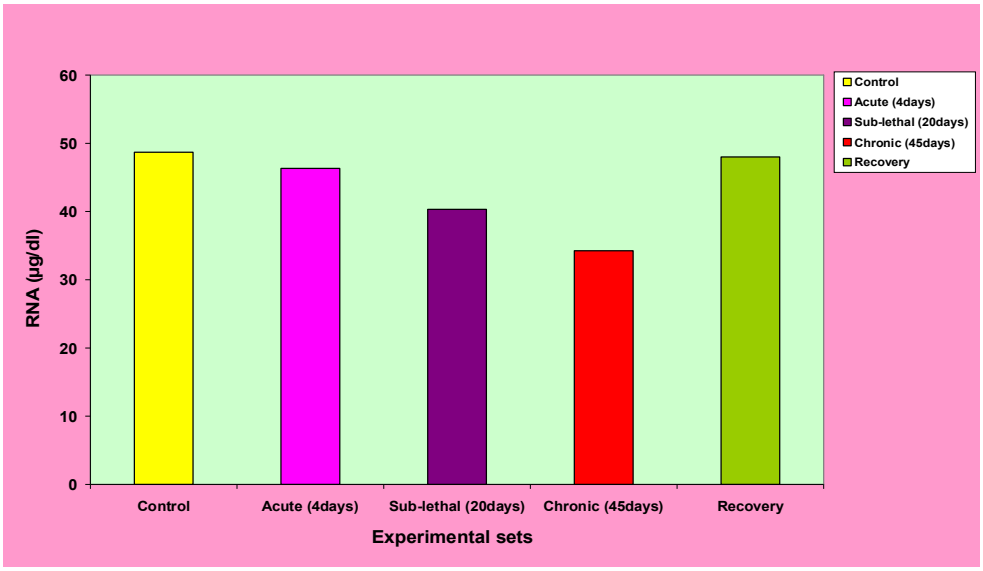
S.No.	Experimental set	No. of Fishes	(Mean±S.E.)
1.	Control	5	1.19±0.02
2.	Acute (4 days)	5	1.12±0.10 [a]
3.	Sub-lethal (20 days)	5	1.07±0.03 [b]
4.	Chronic (45 days)	5	1.17±0.03 [a]
5.	Recovery	5	1.23±0.06 [a]

a- Non-significant (P>0.05); b- Significant (P<0.05); c- Highly significant ; P<0.01); d- Very highly significant (P<0.001)

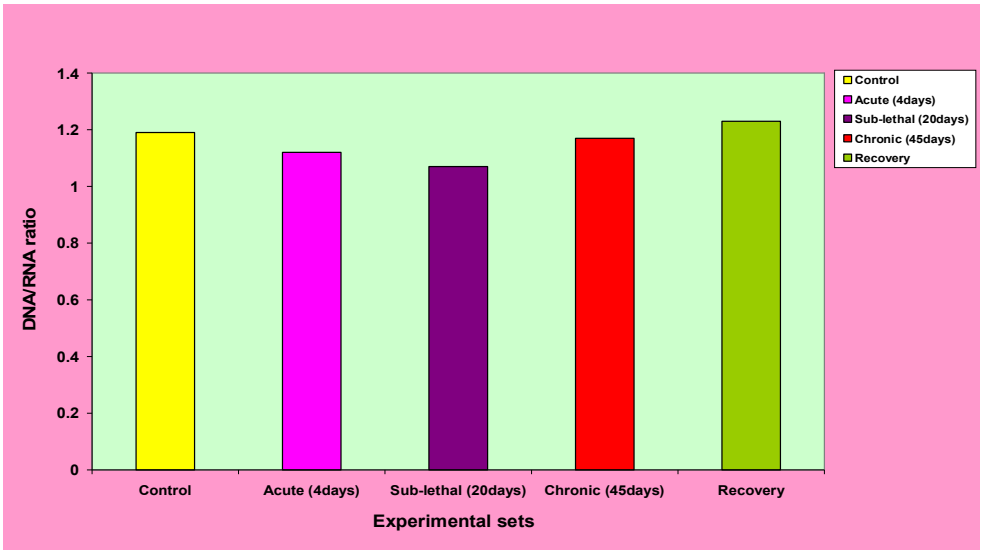
Graph 1: Showing DNA (µg/dl) in white muscle of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)



Graph 2: Showing RNA (µg/dl) in white muscle of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)



Graph 3: Showing DNA/RNA ratio in white muscle of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)



Taddei, *et al*. (2001) reported decline in DNA content and evaluated genotoxic hazard of pollutants in cetaceans via DNA damage and repair evaluated in the bottlenose dolphin (*Tursiops truncatus*) by the comet assay. Findings demonstrated that dolphin cells are characterized by higher efficiency in DNA repair when compared to human leukocytes. The observed resistance to methyl mercury toxicity in dolphins was hypothesized to be a defence strategy developed to combat high dietary exposure and compensate for limited capacity to excrete persistent pollutants. Sehgal and Goswami (2001) evaluated biochemical changes in the liver of the Indian freshwater murrel reported decrease in RNA and DNA content in *Channa punctatus* (Bloch) during estradiol-induced vitellogenin synthesis. Estrogen administration in *C. punctatus* increases RNA: protein and RNA: DNA ratios and depletes glycogen in the liver. Increase in glucose-6-phosphatase activity accounts for glycogen depletion whereas high activity of pyruvate kinase suggests stimulation of the glycolytic pathway at the pyruvate step to generate ATP and to provide carbon skeleton for the

vitellogenin molecule. Increase in the activity of glutamate pyruvate transaminase can be directly related to the synthesis to specific amino acids needed for the formation of vitellogenin whereas reduction in glutamate oxaloacetate suggests extra hepatic source of amino acids. Reduction in the activity of glutamate dehydrogenase may be due to catabolism.

In accordance to the present research work, Pandey, *et al*. (2006) did genotoxicity evaluation of acute doses of endosulfan to freshwater teleost *Channa punctatus* (Bloch) by alkaline single-cell gel electrophoresis and reported decreased DNA content. A comparison of DNA damage in both tissues at different doses indicated that the gill cells were more sensitive to the pesticide exposure than the kidney cells. This study explored the utility of the comet assay for in vivo laboratory studies using fish for screening the genotoxic potential of various agents. Shukla (2006) examined Malathion induced biochemical alterations in the liver of the fingerlings of *Channa punctatus* (BL.) including decrease in nucleic acid contents. 10 days of exposure brought non-significant alteration ($P < 0.05$) neither in the nucleic acids nor in protein content. However, significant quantitative decline in the liver's RNA ($P < 0.01$) and protein ($P < 0.001$) but not in the DNA content was observed after 20 days of exposure. Possible causes for such decline have been discussed. Schill, *et al*. (2008) detected DNA damage with single-cell gel electrophoresis in anhydrobiotic tardigrades. Ali and Kumar (2008) observed long-term genotoxic effect of monocrotophos in different tissues of freshwater fish *Channa punctatus* (Bloch) using alkaline single cell gel electrophoresis. In general, significant effects ($P < 0.01$) from both concentration and time of exposure were observed in exposed fish. It was found that the tissues at all concentrations exhibited the highest DNA damage on day 4, after which there was a decline in percentage tail DNA. The comparison of DNA damage among tissues at different concentrations indicated that the gill cells were more sensitive to the pesticide than the kidney cells and lymphocytes. This study also explored the utility of the comet assay for *in vivo* laboratory studies using fish for screening the genotoxic potential of various agents.

An important work has been done by Ali, *et al*. (2008) observed genotoxicity assessment of acute exposure of chlorpyrifos to freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Significant effects ($P < 0.01$) from both concentrations and time of exposure were observed in exposed fishes. It was found that the micronucleus induction was highest on 96 h at all concentrations in the peripheral blood. Similar trend was observed for the DNA damage measured in terms of the percentage of tail DNA in the lymphocyte and gill cells. This study explored the combined use of micronucleus assay and comet assay for *in vivo* laboratory studies using fresh water fish for screening the genotoxic potential of xenobiotics. Bony, *et al*. (2008) observed genotoxic pressure of vineyard pesticides in fish for field and mesocosm surveys. DNA damage in exposed fish erythrocytes recovered to unexposed level, suggesting possible involvement of both repair mechanisms and cellular turnover in this transient response. Ali *et al*. (2009) did assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. In general, significant effects for both the concentrations and time of exposure were observed in treated fish. It was found that MN induction in the blood was highest on day 14 at 203.0 g/l of Chlorpyrifos. The highest DNA damage was observed on day 5, followed by a gradual non-linear decline in the lymphocytes and gill cells. The study indicated MN and comet assays to be sensitive and rapid methods to detect mutagenicity and genotoxicity of chlorpyrifos and other pollutants in fishes.

Kumar, *et al*. (2010) investigated the genotoxicity of Malathion to freshwater teleost fish *Channa punctatus* (Bloch) using the micronucleus test and comet assay. The micronucleus formation in the peripheral blood cells was found to be significantly higher ($p < 0.05$) in the treated specimens at all sampling intervals compared to the control. Significant effects ($p < 0.05$) of both concentration and time of exposure were observed on DNA damage in the gill, kidney, and lymphocytes. All of the tissues exhibited a concentration-dependent increase in DNA damage up to day 3, followed by a nonlinear decrease with the duration of exposure. A comparison of the extent of DNA damage among the tissues showed the sensitivity of gill tissue to Malathion. Nwani *et al*. (2010) observed mutagenic and genotoxic effects of carbosulfan in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Significant effects ($P < 0.01$) from both concentrations and time of exposure were observed in exposed fishes. The MN induction was

highest on 96 h at all the concentrations in the peripheral blood. Similar trend was observed for the DNA damage measured in terms of the percentage of tail DNA in the erythrocyte and gill cells. This study confirmed that the comet and micronucleus assays are useful tools in determining potential genotoxicity of water pollutants and might be appropriate as a part of monitoring program.

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