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ASSESSMENT OF GENOTOXICITY IN GILL TISSUE OF *CHANNA PUNCTATUS* UNDER STRESS OF PYRETHROID CYPERMETHRIN

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ABSTRACT: ***Pyrethroids plays a significant role in contemporary agriculture by offering consistent, persistent, and mostly full control against dangerous pests and insects with less money and effort. There is little question that by eliminating certain pests and insects, crop productivity has grown. However, the fish and other aquatic species that live in ponds, lakes, rivers, streams, and other similar water bodies are negatively impacted by this good trend in improved agricultural output due to the usage of pyrethroids. Pesticide poisoning from agricultural fields is a severe concern for water pollution, and its long-term impacts on the environment may lead to the poisoning of fish and other aquatic species. The current study examines the genotoxicity of pyrethroids like cypermethrin on the gills of Channa punctatus..***

Keywords: ***Genotoxic Profile, Gill, Channa punctatus, cypermethrin.***

INTRODUCTION

The rapid growth of several industries—in particular—as a modern development without a clear plan, and their effluent, has been identified as the main contributor to environmental deprivation. The unfortunate fact that ecological and sociological issues are frequently not carefully considered and adequately addressed when designing and locating many industrial projects, power generation facilities, water dams, reservoirs, and in the development and urbanisation, means that the effects of which are constantly being faced by the inhabitants, particularly the animal species. According to Jyothi and Narayan, the pyrethroid family of insecticides is important in all respects, even for domestic usage (1999). Shafiq-ur-Rahman (2006) and Velisek et al. (2006) studied pesticide toxicity in aquatic fauna, focusing on the extent of pyrethroid toxicity in fishes.

Since the beginning of time, the world's population has been steadily growing, and so has the use of natural resources. As a result of the population crisis, which is becoming worse every day and is predicted to explode by the end of the 20th century, there will be an increased demand for food. Some of the synthetic substances created throughout this process not only aided humans but also caused him pain. However, a significant amount of chemicals in the form of pesticide residues over a considerable amount of time left several issues that were connected to human welfare.

Pesticides that are safer for flora and fauna are needed to tackle the issue of protecting crops and food products from pesticides, however no pesticide is free from negative side effects. Unfortunately, different pesticide effects vary depending on the particular target species. Injuries to non-target creatures, ecological imbalance, and environmental pollution by persistent pesticides are only a few of the unfavourable side effects that are widespread.

Every pesticide has a variable level of toxicity depending on how it works. Organophosphates and carbamates are contact poisons that usually cause pest mortality. Some repellents, such as pyrethroids, can potentially kill an animal if it takes too much of them. In flying insects, pyrethroids have a distinctive knockout action. Neem oil and nicotine are two effective natural insecticides that have little negative side effects.

Various genotoxicity testing approaches, such as the Ames test, chromosomal abnormalities, sister chromatid exchange, micronucleus assay, and comet assay with various end points, can be used to identify these genetic alterations in organisms at a specified level. These tests are able to identify substantially more severe genetic damage that manifests in DNA, chromosomes, and cells. This research raised some awareness about the possibility that some "hereditary diseases" found in the human population may have environmental causes. Much of the fundamental knowledge surrounding DNA structure and replication, the genetic code, and the process of protein synthesis emerged during this era. Many scientists contributed to this golden age and gained Nobel Awards for their work.

The purpose of the current study is to evaluate the impact of the pesticide cypermethrin on a live *Channa punctatus* fish. The toxic effect was evaluated using data from acute, sub-lethal, chronic, and biochemical tests. The main goal was to detect DNA and RNA damage in freshwater fish *Channa punctatus* after exposure to insecticide on the gills because the gills are the first organs to come into contact with any toxicant that enters the surrounding environment. The fish immediately consume it from the water, often via the gills. Then it causes a depressing effect on tissue respiration, which results in hypoxia-related mortality. Fish have a variety of highly vascularized respiratory appendages called gills. Water flows through gills in the opposite direction from blood flow through them. Such a process allows for rapid oxygen absorption and complete oxygen saturation of the circulation. Following that, blood enters muscles and other essential tissues, which are also crucial locations to evaluate the level of toxicity in the organism.

MATERIALS AND METHODS:

Laboratory experimentation

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. 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% KMnO4 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 (Finney, 1971).

Test compound: Cypermethrin 25%EC

CAS number : 52315-07-8
Trade name : Super killer
Chemical formula : C22H19Cl12NO3
IUPAC number : (R,S)-alpha-cyano-3-phenoxylbenzyl I(IRS)- cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclo propane-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 tissue 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 370C. 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-

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

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-

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

3. DNA/RNA ratio

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

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

Statistical Calculations

In the present investigation, the formulae were used for different statistical calculations after Fischer and Yates (1963) using statistical software.

RESULTS

The observations are tabulated and shown on graph as below-

Table- 1: DNA (µg/dl) in gill 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	84.99±0.10
2.	Acute (4 days)	5	81.30±0.50 [a]
3.	Sub-lethal (20 days)	5	72.34±0.19 [b]
4.	Chronic (45 days)	5	61.22±0.66 [d]
5.	Recovery	5	83.64±0.16 [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)

Table- 2: RNA (µg/dl) in gill 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	75.88±0.33
2.	Acute (4 days)	5	71.55±0.10 [a]
3.	Sub-lethal (20 days)	5	65.36±0.33 [b]
4.	Chronic (45 days)	5	59.51±0.64 [c]
5.	Recovery	5	75.10±0.15 [a]

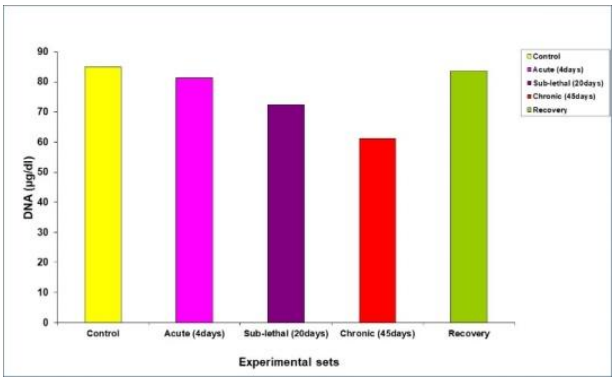
a- Non-significant (P>0.05); b- Significant (P<0.05); c- Highly significant (P<0.01); d- Very highly significant (P<0.001)

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

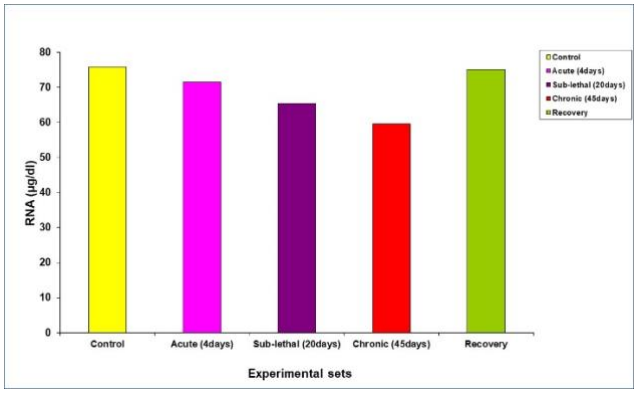
S.No.	Experimental set	No. of fishes	(Mean±S.E.)
1.	Control	5	1.12±0.02
2.	Acute (4 days)	5	1.13±0.05 [a]
3.	Sub-lethal (20 days)	5	1.10±0.03 [a]
4.	Chronic (45 days)	5	1.02±0.02 [b]
5.	Recovery	5	1.11±0.01 [a]

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

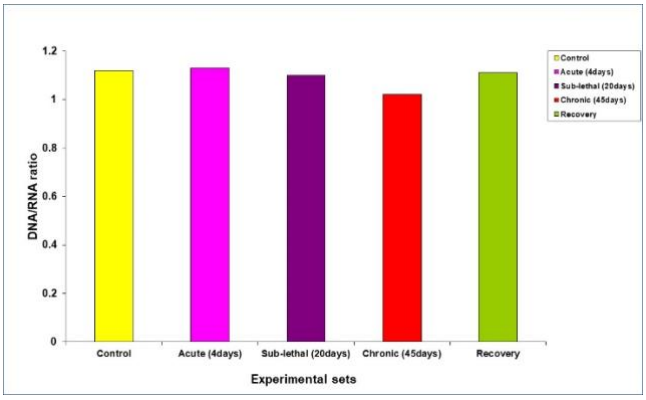
Graph- 1: Showing DNA (µg/dl) in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)



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



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



As research in the field of pesticides advanced, several new chemicals with particular features appeared and were widely employed in society. Companies who cared the least about their products' consequences and future in the environment performed the first and subsequent investigations. All of these chemicals, along with other types of pollution, will undoubtedly seep into ponds and rivers that have deep water. Ponds and rivers are the primary sources of our fish culture. Therefore, the health of the fish is very important to us. Utilizing computer software, statistical computations are performed on the data produced during the experimentation for the study. This will draw attention to how routinely used pesticides may cause pollution. These pesticides undermine fish health, and when humans consume these fish, they may be exposed to pesticide poisoning. These experimental insecticides' detrimental effects on fish can be extrapolated to other species, including people. Research on nucleotides, such as DNA and RNA, at the molecular level identifies the precise location at which genetic changes and variations that occur in species take place. Since each species has a specific amount of DNA in its cells and variations in this number indicate a trend towards adaptation or speciation of the species, any change in the transcriptional level will result in changes in information flow within the cells and in the gene flow among the individuals of the same species. Aquatic resources, including fisheries, are extremely valuable natural resources, as are ponds, lakes, rivers, streams, and oceans. Recreational boating, sport fishing, swimming, relaxation, and natural beauty are a few of the more indirect but still significant advantages of fish and aquatic habitats. Growing worries about the effects of expanding populations and human activities on aquatic life and water quality have been seen in conjunction with the assessment of fisheries and aquatic systems. Pesticides are a class of poisonous substances used by humans that have a negative impact on aquatic life and water quality.

A pyrethroid pesticide is cypermethrin; In 1974, it was first synthesized, similar to the pyrethrins found in pyrethrum extract, cyclomethrin is a synthetic compound (which comes from the *Chrysanthemum* plant). Longer-lasting than pyrethrins, pyrethroids like cypermethrin were created. The environment and water resources are contaminated by the over use of synthetic pyrethroids, harming both human and aquatic life. Because pyrethroids are lipophilic, fish may absorb these substances even at extremely low quantities in water. Pesticides may heavily contaminate water, which can result in cases of poisoning, oxygen deprivation, and catastrophic fish extinction. Farmers have been drawn to utilise synthetic pyrethroids for pest management because of its recently announced multifaceted benefits. But it has been shown that fish are extremely poisonous to these substances. A pyrethroid routinely used for home and agricultural pest management, cypermethrin was employed in this investigation.

After therapy, the genetic components DNA and RNA and their ratio exhibit different outcomes. With longer exposure times, DNA and RNA contents fell, although the decline in gill tissue was greater, returning to normal levels after recovery. Buckley (1984) discovered the RNA-DNA ratio as an indicator of larval fish development in the water, which is similar to the current findings. These findings highlight how crucial food availability is to fish larval mortality, and they also show that, given the right circumstances, short-term growth can be much higher than predicted by long-term indicators. Understanding the connection between environmental variation, larval development and mortality, and RNA-DNA ratio analysis opens up new possibilities.

According to the current research, Pandey et al. (2006) used alkaline single-cell gel electrophoresis to assess the genotoxicity of acute dosages of endosulfan to freshwater teleost *Channa punctatus* (Bloch) and found lower DNA content. The gill cells were found to be more vulnerable to pesticide exposure than the kidney cells when DNA damage in both tissues was compared at various dosages. In this investigation, we assessed the usefulness of the comet test for screening the genotoxic potential of several medications in in vivo laboratory investigations using fish. Shukla (2006) looked explored the biochemical changes caused by malathion in the liver of *Channa punctatus* (BL) fingerlings, including a drop in the amount of nucleic acids present. No significant change (P <0.05) was seen after 10 days of exposure in either the protein or nucleic acid content. However, after 20 days of exposure, there was a substantial quantitative drop in the liver's protein (P <0.001) and RNA (P 0.01) contents but not in the DNA content. There has been discussion of potential explanations for this drop. In anhydrobiotic tardigrades, Schill et al. (2008) used single-cell gel electrophoresis to identify DNA damage. Using the micronucleus test and comet assay, Kumar et al. (2010) examined the genotoxicity of malathion on the freshwater teleost fish *Channa punctatus* (Bloch). At all sampling periods, treated samples produced substantially more peripheral blood cell micronuclei than controls (p<0.05). DNA damage to the gills, kidneys, and lymphocytes was

shown to be significantly influenced ($p<0.05$) by both concentration and exposure period. By day 3, DNA damage was evident in all tissues, increasing in a concentration-dependent manner, followed by a non-linear decline as exposure time increased. The degree of DNA damage in various tissues allowed researchers to identify the gill tissue's vulnerability to malathion. Nwani et al. (2010) used micronucleus test and alkaline single-cell gel electrophoresis to detect the mutagenic and genotoxic effects of carbosulfan in freshwater fish *Channa punctatus* (Bloch). Both concentration and exposure period had a significant impact on exposed fish ($P < 0.01$) After 96 hours, MN induction was strongest in peripheral blood at all concentrations. In erythrocytes and gill cells, DNA damage as a proportion of tail DNA showed a similar pattern.

CONCLUSIONS

The compound show significant gill toxicity as evident in tissue damage parameters like protein profile which is altered due to tissue damage. Other researchers also show similar trend in protein profile. The comet and micronucleus tests were shown to be beneficial for assessing the potential genotoxicity of water pollutants and according to this study it may be appropriate as a component of a monitoring programme. The study suggests to minimize such harmful compounds in mass use and discover some less toxic pesticides.

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