DETECTION OF MICRONUCLEI IN HAEMOCYTES OF *PUNTIUS SARANA* EXPOSED TO LAMBDA-CYHALOTHRIN

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Abstract

Aquaculture is the farming of fish, crustaceans, mollusks, aquatic plants, algae, and other organisms. It involves cultivating freshwater and saltwater populations under controlled conditions, and it plays a significant role in providing food and employment opportunities globally.The organism may be cultured in various aquatic environments such as penned organisms of marine water, inland ponds, lakes, dams, and indoor tank systems. Fish toxicity refers to the harmful effects of various substances on fish species, including chemicals, heavy metals, and pollutants present in their aquatic environments. Understanding and monitoring fish toxicity is crucial for maintaining the health of aquatic ecosystems and ensuring the safety of fish populations. Pesticides were used very extensively in agriculture, forestry and veterinary practices. The insecticides control a wide variety of insectivorous and herbaceous pests increasing the quantity and quality of food production. Insecticides lead to decreased growth rate and increased reproductive disorders, spinal deformities and histopathological changes in gills, liver, and haematopoietic tissue such as spleen, kidney, renal tubules, endocrine tissues, nervous system, behavioural disorders and genetic defects. Lambda-cyhalothrin is a synthetic pyrethroid insecticide commonly used in agriculture, horticulture, and public health to control various pests. It has low water solubility and is known to bind tightly to soil particles, which minimizes the risk of direct exposure to aquatic organisms like fish. However, in some cases, lambda-cyhalothrin can enter water bodies through runoff, leading to potential impacts on freshwater fish. Micronuclei are tiny extra-nuclear bodies originating from acentric chromatid/chromosome fragments or whole chromatids/chromosomes that lag at the anaphase of dividing cells and are not included in the main nucleus during telophase. The genotoxic effect of Lambda-cyhalothrin on puntius sarana at different concentrations (0.5, 0.05 and 0.005 µl/l) of varying durations. The study was accompanied by a positive control (untreated fish). The frequency of abnormal nuclei was examined at different times (24 and 48 hrs) for the animals treated. The frequency of micronucleated erythrocytes was significantly increased while the nucleolar parameters were repressed by lambda-cyhalothrin treatment. It affects the nervous system of these organisms, leading to paralysis, hyperactivity, and even death. The severity of toxicity depends on factors such as concentration, exposure duration, and fish species sensitivity. Lambda-cyhalothrin can accumulate in the tissues of fish through the food chain. Predatory fish at the top of the food chain can accumulate higher levels of the pesticide due to the biomagnification of its residues. This can lead to long-term exposure and potential effects on higher trophic-level organisms. Our results demonstrated the existence of the genotoxic potential of pyrethroid lambda-cyhalothrin on Puntius sarana. Furthermore, nucleolar characteristics in fish fin cells seem to be an effective biomarker in the assessment of the cytogenetic potential of chemicals in the aquatic environment. Pesticides can directly interact with DNA molecules, leading to breaks, mutations, or other structural alterations in the genetic material. This DNA damage can interrupt normal cellular

processes and contribute to the formation of genetic mutations. Thus, we confirmed the conclusion that the use of morphologic NOR parameters in interphase nuclei as indicators of functional genomic changes, together with the MN test, a well-known indicator of structural genomic damage, may increase the sensitivity of genotoxicity test systems Our results confirmed that the use of nucleolar biomarkers on fish fin cells, in addition to micronucleus test, could provide valuable information in aquatic genotoxicity studies. To minimize the potential impacts on freshwater fish, it is crucial to follow proper pesticide application practices, including adhering to recommended dosage, application timing, and buffer zones near water bodies. Additionally, conducting environmental risk assessments and monitoring the water quality in areas where lambda-cyhalothrin is used can help mitigate potential negative effects on freshwater fish populations.

Keyword: Genotoxicity, Micronucleus, Lambda-cyhalothrin, *puntius sarana***.**

Introduction:

Aquaculture is the culture and management of aquatic animals in an aquatic system for human food, recreation, replenishing fish stocks, and other uses. The organism may be cultured in various aquatic environments such as penned organisms of marine water, inland ponds, lakes, dams, and indoor tank systems. Various commercial organisations are involved in this activity, such as large and discharging wastewater and agricultural drainage containing toxic chemicals into aquatic environments (Anonymous, 1991). These are responsible for multiple impacts on the biota of aquatic systems, such as organ function failure, reproductive ground, species survival, population size and ultimately loss of biodiversity. Aquatic organisms cultured in the contaminated water are prone to diseases due to toxic substances present in the aquatic system through their food and sheltered water. The industrial wastes of carcinogenic and mutagenic compounds may exert damage to all the individuals of a particular area and may have prolonged effects for several generations. Aquatic toxicology is the study of the effect of environmental pollutants on aquatic organisms. Pesticides were used very extensively in agriculture, forestry and veterinary practices. The insecticides are used for controlling a wide variety of insectivorous and herbaceous pests increasing the quantity and quality of food production. Unfortunately, it has great adverse effects that the long-term use of such chemicals in aquatic ecosystems may disrupt ecological relationships between organisms and the sheltered environment. Numerous studies have found that the insecticides at different concentrations were found to be toxic to aquatic organisms, especially fish species. The major insecticides usually applied are Organophosphate, Carbamates, Organochlorine, Pyrethroids, and Necotenoides. These are found to be highly toxic not only to fish but also to the other organisms which constitute the food chain in a particular ecosystem. Insecticides lead to decreased growth rate and increased reproductive disorders, spinal deformities and

histopathological changes in gills, liver, hematopoietic tissue such as the spleen, kidney, renal tubules, endocrine tissues, nervous system, behavioural disorders and genetic defects (Farid and El-Sayed, 2015). Micronucleus (MN) assay is an ideal monitoring system that uses aquatic organisms to assess the genotoxicity of water in the field and the laboratory. Micronuclei are tiny extra-nuclear bodies originating from acentric chromatid/chromosome fragments or whole chromatids/ chromosomes that lag at the anaphase of dividing cells and are not included in the main nucleus during telophase. Instead, they are enwrapped by the nuclear membrane and resemble the structure of the daughter nucleus, although they are way smaller in size (Sedelnikova *et al.,* 2007; Fenech *et al.,* 2011). Acentric chromatid/chromosome fragments usually originate after extensive DNA damage such as DSBs that if repaired result in asymmetrical chromosome rearrangements and exchanges. Whole chromatids or chromosomes in MN are Formed due to deficiencies in chromosome segregation during anaphase usually caused by mitotic spindle failure, kinetochore damage, centromeric DNA hypomethylation, and defects in the cell cycle control system (Mateuca *et al.,* 2006).

Lambda-cyhalothrin is a commonly used insecticide, highly active against a wide range of species of *Lepidoptera, Hemiptera, Diptera,* and *Coleoptera* (Bao *et al,* 2007). It belongs to a group of chemicals called synthetic pyrethroids. It has adulticidal, ovicidal and particularly larvicidal activity (Anonymous, 1991). It is a contact poison which affects the digestive system of the target organism. Pyrethroids are widely used in rice-cultivating countries (Bao *et al*., 2007, Anonymous 1998, 1991). In Turkey especially Turkish Thrace, the Lambda cyhalotrin has been used to control insect pests (*Lepidoptera, Hemiptera, Diptera* and *Coleoptera*) Lambda cyhalothrin runoffs from agricultural fields to aquatic environments and kill the fishes of various parts of the world. Various researches have documented the negative impacts of Lambda-cyhalothrin exposure at sublethal doses on behavioural and biochemical changes in fish (Bao *et al*., 2007, Anonymous 1998, 1991).

Puntius sarana is a tropical freshwater fish belonging to the minnow family. This species is commonly called an 'olive barb' which can be used as both food fish and ornamental fish. The generic status of the fish is still unclear and keeps flipping between *Barbodes* and *Puntius sarana* is a wide spread species with no known major wide spread threats. This barb is very widely distributed all over India in rivers and tanks. It attains a length of 31 cm. It breeds during monsoon in running waters amongst submerged boulders and vegetation. Spawning occurs in two stages once between May to mid-September but is prominent in June and the second spawning time in August and September. Currently,

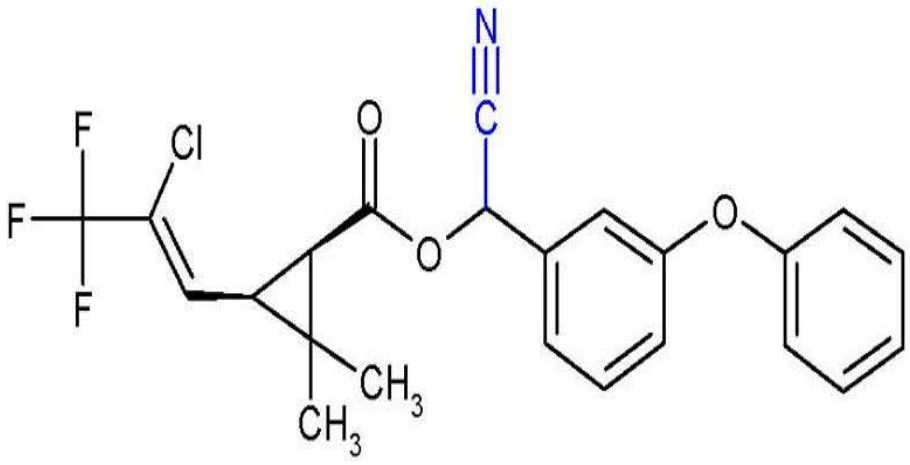
based on its wide distribution and apparent lack of threats it is taken for assessment for least concern. Though it is widely distributed in all aquatic environments, it is more affected by the insecticides released in sheltered aquatic environments. In recent years, the specificity of changes in nucleolar characteristics in plant and animal cells and the potential use of nucleolar parameters in the assessment of cytogenetic toxicity. In this study, a set of nucleolar biomarkers in interphase nuclei (the average number of nucleoli per cell; the volume of a single nucleolus; and the percentage of cells with heteromorphic paired nucleoli), together with the nuclear biomarker (MN test) were used to evaluate the functional and structural genotoxic effects of lambda-cyhalothrin on a *cyprinid* fish *Garra rufa* (Heckel, 1843), a common representative of fresh-water ecosystems in the Mersin region. Hence the current study was carried out to know the genotoxic effect of Lambda cyhalothrin on *Puntius sarana* at different concentrations of varying durations.

Materials and Methods

Chemical:

Lambda-cyhalothrin(5% EC); 3-(2-Chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-Cyano (3-phenoxyphenyl) methyl – cyclopropanecarboxylate. XYLO 5% EC is a pale-yellow coloured clear liquid free from extraneous matter (Figure 1).

Figure1: Chemical Structure of Lambda Cyhalothrin



Molecular formula **C23H19ClF3NO3**

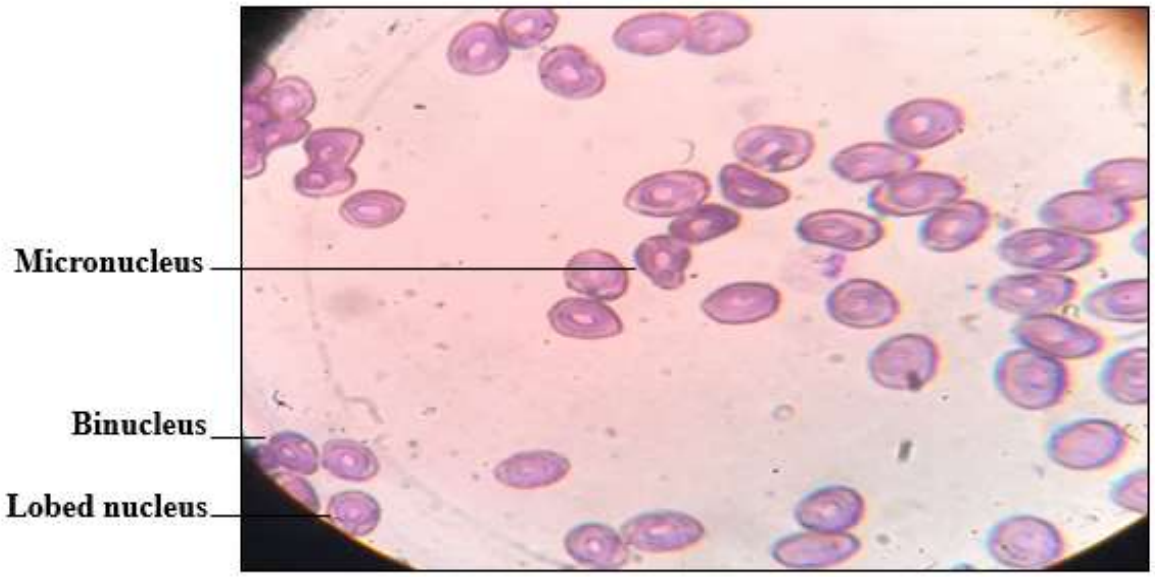
Molecular weight **449.90**

Experimental

The experimental fish, *Puntius sarana* was treated with 0.05 potassium permanganate (KMnO4) solution for 2 min to avoid any dermal infections. They were then acclimatized for 21 days under laboratory conditions with natural photoperiod and fed them with rice-bran oil cake. The faecal matter and other waste materials were siphoned off daily to reduce ammonia contamination in water. The fish were maintained in an aquarium of 50 L capacity. The systemic pesticide, Lambda-cyhalothrin emulsion was diluted in two different concentrations (0.5, 0.05 and 0.005 µl/l) with water and applied in the experimental aquaria. The water with the compound was replaced every two days. The fish was also fed at two-day intervals. Fish were not fed for 24 hours before testing. The study was accompanied by a positive control (untreated fish). The frequency of abnormal nuclei was examined at different times (24 and 96 hrs) for the animals treated.

The slides were prepared by smearing a drop of blood on clean microscopic slides fixed in methanol for 10 min and left to air-dry at room temperature and finally stained with 5% Giemsa for 20 minutes. A total of 1000 erythrocytes were examined for each specimen under the light microscope. The scoring of micronuclei was assessed based on the following criteria adopted by Fenech *et al.*, (2003). The diameter of the micronuclei (MN) of size less than one-third of the main nucleus, marginally overlapped nucleus as long as there is clear identification of the nuclear boundary. MN should have similar staining as the main nucleus.

Figure: 2 Micronuclei, Binuclei, lobed nuclei in *Puntius sarana* Erythrocytes



Result

Micronucleus constituted by lambda-cyhalothrin in erythrocyte cells, MN can originate during anaphase from lagging acentric chromosome or chromatid fragments caused by misrepair of DNA breaks or unrepaired DNA breaks. *Puntius sarana* erythrocyte cells are obtained in micronuclei, binuclei, and lobed nuclei. The fishes treated with 0.005 µl/l concentration of Lambda-cyhalothrin at 24 hours had 90 micronuclei and 121 abnormal nuclei. The total number of cells counted was 30000. At 0.05 µl/l concentration, the number of micronucleus observed was 1760 and abnormal cells were 480. Fish treated with 0.5µl/l concentration of Lambda-cyhalothrin for 24 hours had 1900 micronucleus and 510 abnormal nuclei (Table 1).

Table : 1 Number of micronucleus and abnormal cells examined at 24 hours

Treatment	Concentration (µl/l)	Number of cells (n×1000)	Abnormal Erythrocytes	
			MN	AN
Lambda Cyhalothrin	0.005	30000	90	121
	0.05	80000	1760	480
	0.5	70000	1900	510

MN-Micronucleus; AN – Abnormal cells

At 48 hours, the fishes treated with lambda-cyhalothrin at 0.005 µl/l concentration showed 138 micronucleus and 121 abnormal cells. The total number of cells examined was 23000. The fishes treated with 0.05 µl/l concentration had 560 micronucleus and 980 abnormal nuclei. The total number of cells counted was 70000. The fishes treated with 0.5 µl/l concentration had 680 micronuclei and 220 abnormal nuclei. The total number of cells counted was 80000 (Table 2).

Table : 2 Number of micronucleus and abnormal cells examined at 48 hours

Treatment	Concentration (µl/l)	Number of cells(n×1000)	Abnormal Erythrocytes	
			MN	AN
Lambda Cyhalothrin	0.005	23000	138	121
	0.05	70000	560	980
	0.5	80000	680	220

MN-Micronucleus; AN – Abnormal cells

Table: 3 Number of micronucleus *Puntius sarana* exposed to lambda-cyhalothrin

Exposure period (hrs)	control	Concentrations of Lambda cyhalothrin(µl/l)		
		0.005	0.05	0.5
24	0	1.43 ± 0.98	3±0.79	8±0.92
96	0	1.8 ± 0.13	2 ± 0.47	2.2 ± 0.92

The value indicates in mean ± SD

The result obtained from the micronucleus mean value increased in high concentration (Table 3). The exposure period low at micronucleus value increased.24 hours mean value was 8 ± 0.92 then 2.2 ± 0.92 in 96 hours, not signification ($p<0.5$) compared with the control. The lambda cyhalothrin concentration (0.5, 0.05 & 0.005 µl/l) signification ($p<0.5$) value at 96 hrs.The highest concentration of lambda-cyhalothrin was 0.5µl/l signification different from other concentrations 0.05 and 0.005µl/l. The signification same variant concentration (0.5, 0.05 & 0.005 µl/l) at 24 hrs and 96 hrs. The micronucleus decreased in consuming time exposed pesticide in fish samples. The more time exposed to pesticides formation of micronuclei and abnormal nuclei.

Discussion

The marine ecosystem is a vital source of nourishment for aquatic biota. The micronucleus (MN) assay is to estimate the biomagnifications of pollutants in the marine environment. The present study is similar to Vidhya and Radhakrishnan Nair, 2013 Genotoxic evaluation of lambda-cyhalothrin on brackishwater fish, *Etroplus suratensis* (Pearl spot) micronuclei (MN) formation increased significantly when the concentrations and time of exposure were increased on *E.suratensis*. It was observed from two-way ANOVA that there was a significant difference between the exposure durations concerning the micronuclei ($P<0.05$) and variations due to concentrations were statistically highly significant ($P<0.001$) compared with the control. According to Bhunya and Pati,1987 cypermethrin caused micronuclei in mouse erythrocytes. Miadokova *et al*., 1992, also reported that cypermethrin treatments gave positive results for gene conversion in *Saccharomyces cerevisiae* and frequency of aberrant anaphase–telophases in root tips of *Hordeum vulgare* and *Vicia faba*. However, allethrin was tested in the Drosophila wing spot test and reported to be unable to induce genotoxic effects. Micronuclei are formed by the loss of whole chromosomes or portions of chromosomes from daughter nuclei at mitosis and exist separately from the main nucleus of the cell. Micronuclei result either from chromosome breaks (clastogenic effects) or dysfunction of the spindle apparatus centromere kinetochore complexes, with

subsequent elimination of whole chromosomes (aneugenic effects). Compared to other cytogenetic assays, the several advantages of quantifying micronuclei include the speed and ease of analysis, and the lack of requirement for metaphase cells (Tucker and Preston, 1996). Micronuclei are formed by the loss of whole chromosomes or portions of chromosomes from daughter nuclei at mitosis and exist separately from the main nucleus of the cell. Micronuclei result either from chromosome breaks (clastogenic effects) or dysfunction of the spindle apparatus centromere kinetochore complexes, with subsequent elimination of whole chromosomes (aneugenic effects).In the previous study, an attempt was made to detect the Micronucleus Test (MNT) of *Etroplus suratensis* in blood erythrocytes after exposure to pyrethroid insecticide lambda-cyhalothrin. The fishes exposed to lambda-cyhalothrin at different sub-lethal concentrations of LC50 value for short-term exposure. The blood samples were obtained from a puncture to the caudal vein using heparinised syringes from control and pesticide-treated fishes at 24, 48, 72 and 96 hrs of exposure. From the result, the formation of micronuclei in blood erythrocytes increased from lower to higher concentrations of lambda-cyhalothrin and also the time of exposure was increased. However, the present study revealed that *E. suratensis* can be used as a good model to study the genotoxic effects of aquatic pollutants in fish. (Vidhya and Radhakrishnan Nair, 2013.) Campana *et al*., 1999 state the elevated response in the micronuclei .incidence differed from controls at 24 h ($p < 0.001$).48 h($p< 0.01$) and 72 h after exposure ($p < 0.01$) In vivo exposure of fishes to lambda-cyhalothrin showed the genotoxic effects of the compound on erythrocytes of C. i. interruptus. The highest response was induced 24 hours after exposure. Starting from this time, the incidence of micronuclei declined gradually over 23 days. Naqvi *et al.,*2016 report same this study, MN frequencies in the fish peripheral blood erythrocytes after exposure to different concentrations of OP pesticides (chlorpyrifos, malathion), SP pesticides (cypermethrin, lambda-cyhalothrin) and herbicide (buctril), The MN frequencies of the OP, SP and herbicide (buctril) treated fish are observed to increase significantly ($p<0.05$) with increase in concentration and time at all exposure periods.

According to Çava and Ergene-Gözükara ,2003 of the micronucleus test after 36 h, the frequencies of micronucleated erythrocytes were increased in all treatment groups. This increase was significantly different at the two highest doses 0.05 ($P < 0.01$) and 0.01 g/l ($P < 0.05$), compared with the negative control. The benzene treatment also caused a significant increase ($P < 0.01$) in the frequency of micronucleated erythrocytes. The frequency of micronucleated erythrocytes was significantly increased while the

nucleolar parameters were repressed by lambda-cyhalothrin treatment. This similar present study decrease was insignificant at the lowest concentration. Benzene, a well-known genotoxic agent, was used as the positive control in our experiments. It caused a significant increase in the MN frequency and a significant decrease in the nucleolar characteristics. Benzene-induced MN formation in fish erythrocytes, as was previously shown by (Al-Sabti, 1995). In this study, it was observed that the highest concentration (0.05 g/l)of lambda-cyhalothrin and 10 mg/l benzene had very similar effects on both micronucleus and nucleolar characteristics, although the difference in their concentrations is very large. In conclusion, our results demonstrated the existence of the genotoxic potential of pyrethroid lambda-cyhalothrin on *G. rufa.* Furthermore, nucleolar characteristics in fish fin cells seem to be an effective biomarker in the assessment of the cytogenetic potential of chemicals in the aquatic environment. Thus, we confirmed the conclusion that the use of morphologic NOR parameters in interphase nuclei as indicators of functional genomic changes, together with the MN test, a well-known indicator of structural genomic damage, may increase the sensitivity of genotoxicity test systems Our results confirmed that the use of nucleolar biomarkers on fish fin cells, in addition to micronucleus test, could provide valuable information in aquatic genotoxicity studies. Controlling fish toxicity involves monitoring water quality, minimizing pollutant discharge, and implementing measures to mitigate the impact of harmful substances on aquatic environments. It also involves regulatory efforts to limit the release of toxic substances and the development of sustainable aquaculture practices.

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