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RESEARCH ARTICLE

GENOTOXIC EVALUATION OF LAMBDA-CYHALOTHRIN ON BRACKISHWATER FISH, *Etroplus suratensis* (PEARLSPOT)

Vidhya, V. and *Radhakrishnan Nair, C.

Department of Zoology, S.T. Hindu College, Nagercoil-629002, Kanyakumari District, Tamil Nadu, India

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ABSTRACT

Every year, the increase of human population and the accompanying with the growth of industrialization results in the increase of pollution in aquatic ecosystems. Micronucleus (MN) assay is an ideal monitoring system that uses aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. In the present 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 a short-term exposure. The blood samples 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 were 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.

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INTRODUCTION

Every year, the increase of human population and the accompanying with the growth of industrialization results in the increase of pollution in aquatic ecosystems (Caussy *et al.*, 2003). The excess amounts of these pesticides and chemicals produce unwanted and unwarranted residues, which pose a great threat to aquatic organisms (Ramasamy *et al.*, 2007). Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, and homes and gardens (Oros and Werner, 2005). Pyrethroids are several orders of magnitude more toxic to fish than the organophosphate pesticides they are replacing in many agricultural, commercial and residential applications (Oros and Werner, 2005). Lambda-cyhalothrin is a pyrethroid insecticide. Micronucleus (MN) assay is an ideal monitoring system that uses aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. Howell (1891) and Jolly (1905) discovered the micronuclei in red blood cells. In early days, these micronuclei were, therefore, called Howell-Jolly bodies. Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division (Palhares and Grisolia, 2002; Fagr *et al.*, 2008). Because genetic damage that results in chromosome breaks or

spindle abnormalities leads to micronucleus formation, the incidence of micronuclei serves as an index of these types of damage (Fagr *et al.*, 2008). Manna *et al.* (1985) reported significant increase in the rates of micronuclei in peripheral erythrocytes of *Sarotherodon mossambicus* exposed to different genotoxicants. Das and Nanda (1986) reported induction of micronuclei in the peripheral erythrocytes of *Heteropneustes fossilis*. Genotoxicity is a deleterious action, which affects a cell's genetic material affecting its integrity (Environ Health Perspect, 1996). Fishes provide a suitable model for monitoring aquatic genotoxicity and waste water quality because of their ability to metabolize xenobiotics and accumulate pollutants (Grisolia and Corderio, 2000). In the present investigation has been made to detect the micronucleus under the influence of lambda-cyhalothrin on *E. suratensis*.

MATERIALS AND METHODS

The MN test was performed in erythrocytes of *E. suratensis*, according to the methods described by Grisolia and Cordeiro (2000) with some minor modifications. The blood samples obtained caudal vein using heparinised syringes from control and pesticide treated fishes at 24, 48, 72 and 96 hrs of exposure and smeared on 5 to 7 clean slides. Slides were kept in a dark place to avoid light reaction and allowed to dry over night. Smeared slides were fixed in methanol for 15 minutes. After fixation, the slides were allowed to air-dried and stained with 5% Giemsa solution for 20 minutes. Then these slides were washed with tap water. Five slides were selected for each fish, and 1000 cells were scored from each slide under 100 ×

*Corresponding author: Vidhya, V.

Department of Zoology, S.T. Hindu College, Nagercoil-629002,
Kanyakumari District, Tamil Nadu, India

magnification. The main criteria for scoring the Micronucleus (MN) were based on those of Al-Sabti and Metcalfe (1995), considering the small, nonrefractive, circular or ovoid chromatin bodies, absence of connections with the main nucleus and similar coloration. Finally 1000 cells per animal were screened for estimating the rate of MN formation which was expressed as MN per 1000 cells. Statistical analysis was carried out by two-way ANOVA and significant difference in pesticide treated group from control group for various time of exposure were represented along with mean \pm SD.

RESULTS AND DISCUSSION

In the present study, micronuclei (MN) formation increased significantly when the concentrations and time of exposure were increased on *E.suratensis* (Table 1 and Figure 1). It was observed from two way ANOVA that there was significant difference between the exposure durations with respect to the micronuclei ($P<0.05$) and variations due to concentrations were statistically highly significant ($P<0.001$) compared with control.

(1999) time variations in the MN incidence were observed in erythrocytes of *Cheirodon I. interruptus* after lambda-cyhalothrin exposure. Insecticides or pesticides have been reported to lead to DNA damage which appears in the form of micronucleus formation, chromosome aberrations and mitotic aberrations (Sankar *et al.*, 2010; Sharaf *et al.*, 2010). With the treatment of pyrethroids, micronucleus could result when the entire or chromosome fragments are not incorporated in the main nucleus after cell division (Sankar *et al.*, 2010). As a result of genetic damage, i.e., damage to the chromosomes, fragments lagging in the course of anaphase or lagging acentric chromosomes or cytoplasmic chromatin-containing bodies are failed to be incorporated into daughter nuclei (clastogenesis), results in the development of micronuclei in red blood cells (Sharaf *et al.*, 2010). Maximum number of micronuclei was observed in the *E. suratensis* exposed to highest dose of lambda-cyhalothrin (0.026 ppm) for 96 hrs. In other, *E. suratensis* exposed to lower concentration (0.005 ppm) with 24 hrs exposure the minimum number of micronuclei were observed higher than that of control fishes. Hose *et al.* (1987) stated the micronuclei frequency of

Table 1. Number of micronucleus (MN) in erythrocytes of *E. suratensis* exposed to lambda-cyhalothrin

Exposure period (hrs)	Control	Concentrations of lambda-cyhalothrin (ppm)				
		0.005	0.006	0.008	0.013	0.026
24	0	1.33 \pm 0.47	3 \pm 0.82	7 \pm 0	9 \pm 0	10.33 \pm 0.47
48	0.33 \pm 0.47	2.33 \pm 0.47	4 \pm 1.41	7 \pm 0.82	11 \pm 0.82	13.67 \pm 0.47
72	0.33 \pm 0.47	2 \pm 0.82	3.33 \pm 0.47	9.33 \pm 1.25	11.33 \pm 1.25	17.67 \pm 0.94
96	0.67 \pm 0.94	4.67 \pm 1.25	4.67 \pm 0.94	11.33 \pm 0.94	13.67 \pm 0.94	23.33 \pm 1.25

Values are expressed as mean \pm SD

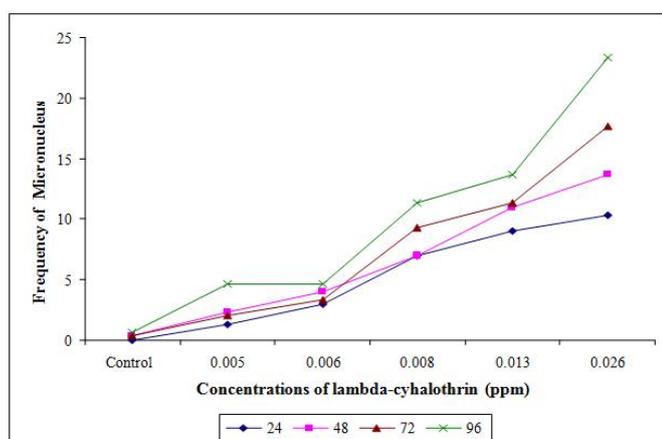
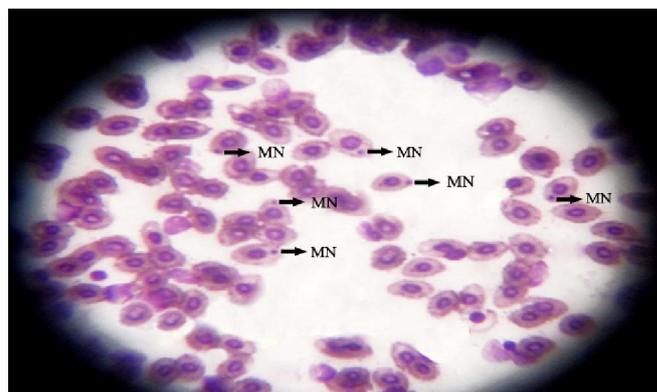


Figure 1. Variations of micronucleated erythrocytes in *E. suratensis* exposed to lambda-cyhalothrin for different exposure period

The results of this study coincide with the observations of Madhu (1995) who reported that the pesticides like methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion induced micronuclei formation in *Liza parsia* and *Mugil cephalus*. Nuzhat and Shadab (2011) studied the percentages of micronuclei increased with increase in the concentration of malathion on *Channa punctatus*. Marcela *et al.* (2003) reported that the frequency of micronuclei increased with increasing the concentrations of lambda-cyhalothrin on *Rana catesbeiana* tadpole. However, in some studies, both concentration- and time-dependent increases in MN induction have also been reported due to chemical exposure in fish (Bahari *et al.*, 1994). Similarly, in the study of Campana *et al.*

(1999) time variations in the MN incidence were observed in erythrocytes of *Cheirodon I. interruptus* after lambda-cyhalothrin exposure. Insecticides or pesticides have been reported to lead to DNA damage which appears in the form of micronucleus formation, chromosome aberrations and mitotic aberrations (Sankar *et al.*, 2010; Sharaf *et al.*, 2010). With the treatment of pyrethroids, micronucleus could result when the entire or chromosome fragments are not incorporated in the main nucleus after cell division (Sankar *et al.*, 2010). As a result of genetic damage, i.e., damage to the chromosomes, fragments lagging in the course of anaphase or lagging acentric chromosomes or cytoplasmic chromatin-containing bodies are failed to be incorporated into daughter nuclei (clastogenesis), results in the development of micronuclei in red blood cells (Sharaf *et al.*, 2010). Maximum number of micronuclei was observed in the *E. suratensis* exposed to highest dose of lambda-cyhalothrin (0.026 ppm) for 96 hrs. In other, *E. suratensis* exposed to lower concentration (0.005 ppm) with 24 hrs exposure the minimum number of micronuclei were observed higher than that of control fishes. Hose *et al.* (1987) stated the micronuclei frequency of 0.8% in fishes from control site. The significant increase in micronuclei with increase in exposure time might be due to the high number of damaged cells undergoing mitosis enabling production of micronuclei and its accumulation over a period of time. Increased MN incidences after lambda-cyhalothrin exposure might be due to the inability of the fish to detoxify the poison and the existing unexcreted toxic metabolites effect leading to the loss in integrity of nuclear membrane and membrane bound enzymes by enhancing lipid peroxidation. The tested pesticide lambda-cyhalothrin induced micronuclei formation in *E.suratensis* (Plate 1). This observation was in support with the study of Rahman and Khuda Bukhsh (1992) who reported that industrial effluents and chemical pollutants induced the formation of micronuclei in *O. mossambicus* and *C. punctatus*.

Plate 1. Micronucleated erythrocytes in *E.suratensis* exposed to lambda-cyhalothrin



MN- Micronucleus

Fagr *et al.* (2008) demonstrated the cyclophosphamide induced micronucleus in tilapia species *Oreochromis niloticus*, *Oreochromis aureus*, *Tilapia zilli* and the African catfish, *Clarias gariepinus*. The results of the present study are similar to those of Campana *et al.* (1999) who reported that lambda-cyhalothrin is a genotoxic agent in erythrocytes of the fish *Cheirodon interruptus interruptus*, and are in accordance with those of Cavas and Ergene-Gozukara (2003) who showed that lambda-cyhalothrin treatment caused an increase in the frequency of micronucleated erythrocytes in the fish *Garra rufa*. 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. The results of the present study intensely showed that the lambda-cyhalothrin induced genetic damage in the form of micronuclei in *E. suratensis*.

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